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Arterial Viral Infection in Restricted−Ovulator Chickens with Endogenous Hyperlipidemia

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Abstract: Electron microscopic observations of aortas and coronary arteries from laying and non−laying hens which appeared to be endogenously infected with a virus such as retro C were described. The arteries of non−laying hens contained greater numbers of virus−like particles than those of laying hens. Viral matrix areas were most frequently observed in medial smooth muscle cells of arteries from non−laying hens. Affected smooth muscle cells were accompanied by degenerative changes. Interlamellar connective tissue cells contained abundant mature viral particles which budded from the in host medial smooth muscle cells.

Key words: Viral infection, Artery, Non−laying hens

INTRODUCTION

Paterson and co−workers (1948) originally reported that coronary arterial lesions of chickens were caused by infectious lymphomatosis, a disease which is common in chickens under one year of age. The experiments with chickens also suggested that inoculation with Marek's disease virus results in arterial lesios containing foam cells and lymphocytes (Minick *et al.*, 1979).

In a previous study, we reported that focal infiltrations of lymphocytic cells were prevalent in the epicardium or myocardium of chickens with endogenous hyperlipidemia (Toda *et al.*, 1980). This report ultrastructurally describes the presence of virus−like particles which shows the evidence of viral infection of the arterial cells in laying and non−laying hens with endogenous hyperlipidemia.

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Materials and Methods

Fifty nine chickens (35 non−laying and 24 laying hens)1 to 2 years of age, were used in this experiment. The chickens were housed in individual cages and fed with a commercial chicken mash with a trace aount of cholesterol as libitum throughout the experiment. The hens were sacrified by decapitation after which blood samples were collected. Plasma total cholesterol concentrations were enzymatically determined using a commercial cholesterol reagent (Allain *et al.*, 1974). Triglycerides concentrations were determined by the Foster and Dunn method (1973). The arteries were manually perfused with 1.4% glutaraldehyde in Sörensen's phosphate buffer (pH 7.4) and both left and right coronary arteries, proximal thoracic aorta and abdominal aorta were collected. Tissue blocks were post−fixed in 1% phosphate buffered osmium tetroxide, dehydrated in ethanol, and embedded in EM bed 812 epoxy resin. Sections were cut with glass knives on an AO−Reichert OMU−3 ultramicrotome. Thick sections for light microscopy were stained with alkaline toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HU−12 electron microscope.

Results

Chemical and Gross Findings

The mean levels of plasma triglyceroides and cholesterol were 3709±240.3 and 653.8±89.4 mg/100ml, respectively, in non−laying hens, which these values for laying hens were 1477.9±88.4 and 202.7±65.9 mg/100ml, respectively. Lipid accumulation in the arteries was obvious in most of the non−laying hens. Fatty dots and yellow fatty streaks were obseved in the proximal aorta. The abdominal aorta had fibrous plaques and lipid−rich lesins as reported in our previous study (Toda *et al.*, 1981). Occasionl pinpoit−sized lipid−rich nodules were observed in the proximal portion of the extramural coronary arteries.

Proximal Thoracic Aorta

The endothelial cells in lipid−rich aortic lesions showed remarkable structural changes including enlarged cells and crenated nuclei with prominent nucleoli. The cytoplasm of these endothelial cells contained much rough endoplasmic reticulum as well as many free ribosomes. The Golgi complexes and picnocytic vesicles increased in number and size. Occasional dense particles were observed on the luminal surface and in the plasmalemal vesicles and in intercellular junctions of the endothelial cells (Fig. 1) These dense particles were approximately 900−10,000Å in size and had a virus−like particles with electron dense core and envelope. Some subendothelial spaces were enlarged and contained many dense virus−like particles, various sized cell debris, abundant extracellular lipid granules, and lipid−containing cells.

Cellular components of the middle layer of thoracic aorta including smooth muscle cells and interlamellar connective tissue cells. The interlamellar connective tissue cells

contained more lipid vacuoles than did the smooth muscle cells. In addition to extracellular lipid granules, numerous clusters of dense enveloped virus-like particles were observed in the intercellular spaces of the media and adventitia. These dense particles were observed in 4 out of 35 non-laying hens and in 2 out of 24 layers. Similar virus-like particles were seen in the interlamellar connective tissue cells (Fig. 2 and Fig. 3).

Concerning the distribution of the virus-like particles, a sharp contrast was noted between smooth muscle cells and interlamellar connective tissue cells. Many dense viral matrix areas were observed in smooth muscle cells, whereas very few dense matrix areas were observed in the interlamellar connective tissue cells (Fig. 4). The cells with numerous dense matrix areas containing many naked cores had the characteristics which were identical to smooth muscle cells with fusiform densities, myofilaments, pinocytotic vesicles, and basement membranes. Some dense, naked and virus-like particles appeared to be released by budding from originally infected smooth muscle cells into the stroma after they received an envelope from a part of the cytoplasmic membrane of the host cells(Fig. 5). These dense matrix areas were observed in 3 out of 35 non-laying hens, while none were noted in laying hens. The virus core-like and mature virus-like particles might be identified same virus because of these particles size.

Infected smooth muscle cells were frequently accompanied by pyknotic changes in the nuclei as well as cytolytic changes. The advantitia contained degenerated cells and lymphoid cells. Numerous virus-like particles were seen in the intercellular space. Some of these particles were also observed in the intercellular junctions and plasmalemal vesicles of endothelial cells of the vasa vasorum(Fig. 6)

Coronary Artery and Abdominal Aorta

The coronary artery and the abdominal aorta have a similar muscular type arterial structure, in which the smooth muscle cell is the only cellular component of the media(Moss and Benditt, 1970). Ultrastructural findings were similar in these two arteries. The coronary artery is described in order to avoid duplication of cytological features. In the coronary artery, virus-like particles were occasionally observed in the intercellular junctions of endothelial cells, as shown in the proximal thoracic aorta. Abundant cell debris and virus-like particles were observed in the stroma of the thickened intima(Fig. 7). Matrix areas were found in medial smooth muscle cells(Fig. 8), although their frequency was lower in the coronary artery and the abdominal aota than in the proximal thoracic aorta.

Some smooth muscle cells with viral matrix areas displayed a degeneration of their nuclei and cytoplasmic organellae. Other smooth muscle cells adjacent to degenerated cells showed reactive changes characterized by prominent nuclei, decrease in heterochromatin, and increase in rough endoplasmic reticulum and polysomes. Numerous virus-like partticles were seen in the stroma of the media and adventitia.

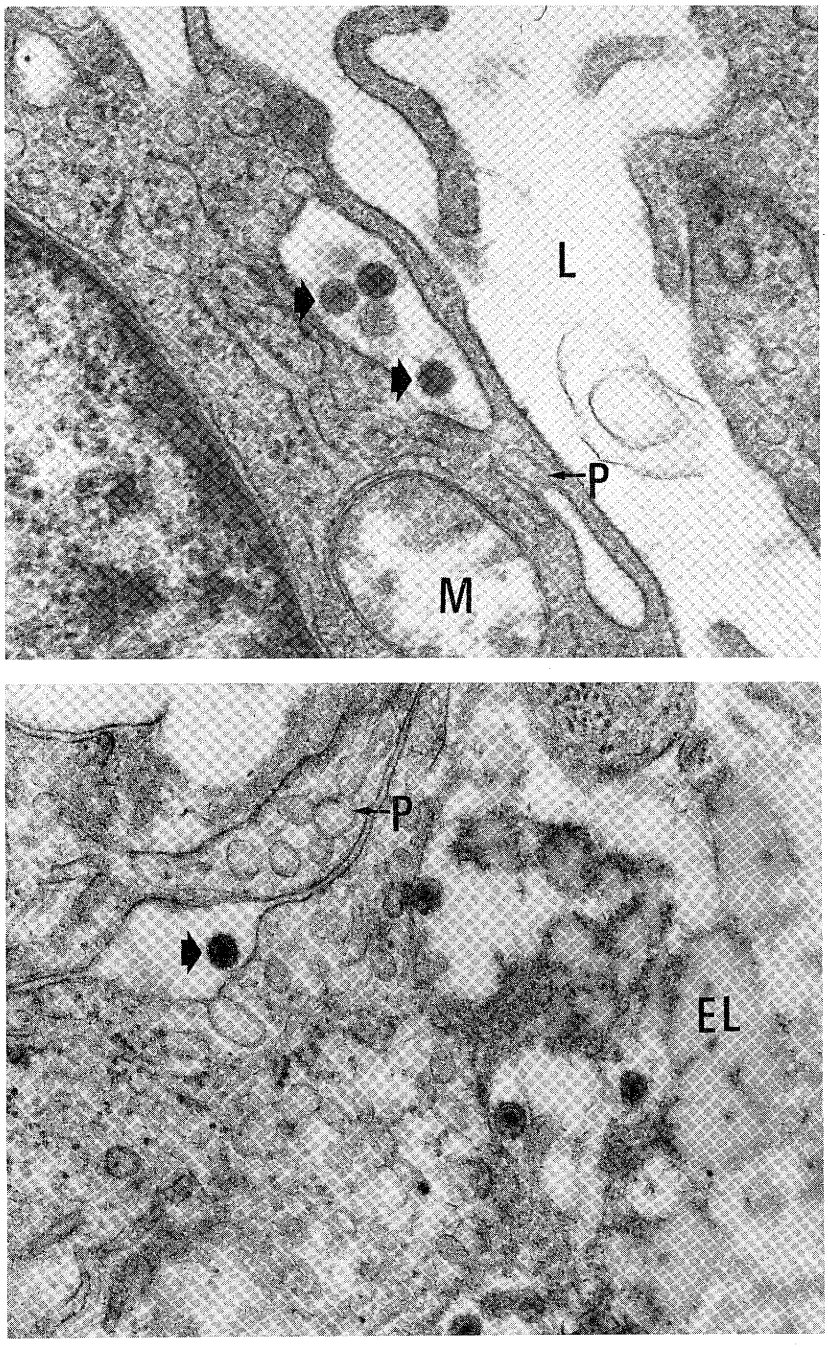

Fig. 1. Mature virus-like particles in endothelial cells.
A: The particles (arrow) in plasmalemal space of an endothelial cell (X 61,000).
B: The particles (arrow) in the intercellular junction and subendothelial space (X 46,000).
EL: elastic fiber, L: lumen, M: mitochondria,
P: pinocytotic vesicle

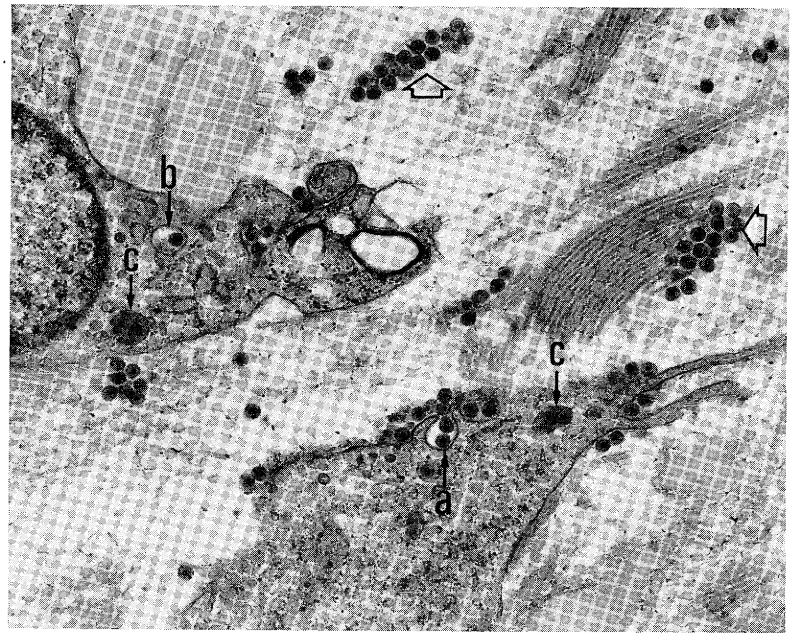

Fig. 2. Virus−like particles in the interlamellar connective tissue cells. Mature viral particles in cytoplasmic concavity (a), and in cytoplasm (b) are shown. Clusters of viral particles (open arrow) are also visible in the stroma (X 22,000).

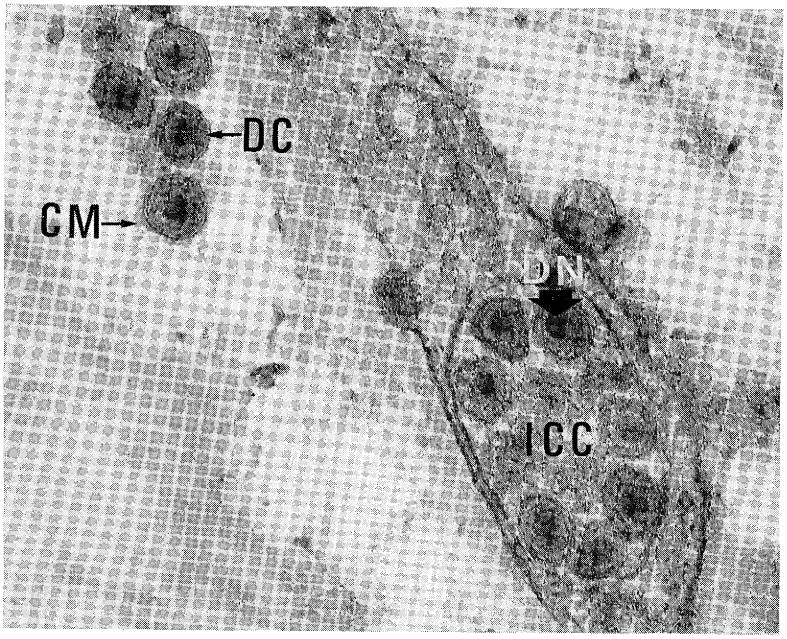

Fig. 3. Highter magnification of mature virus−like particles in interlamellar connective tissue cells (ICC) and stroma. Note dense nucleoid (DN), double capsid membrane (DC), and capsomeres (CM) (X 96,000).

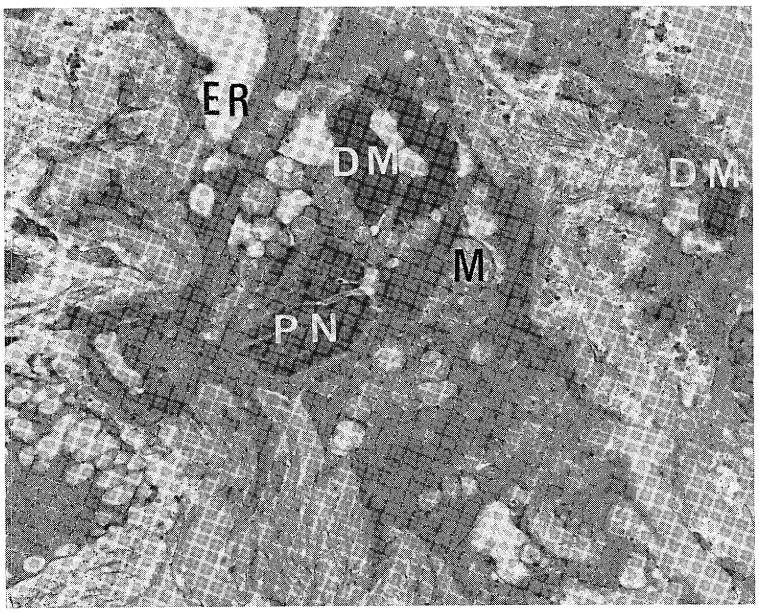

Fig. 4. Degeneration of smooth muscle cells apparently infected with viruses. Several dense matrix areas of virus-like particles (DM), are visible in smooth muscle cells with swollen mitochondria (M) and dilated endoplasmic reticulum (ER) (X 63,000).
PN: pyknotic nucleus

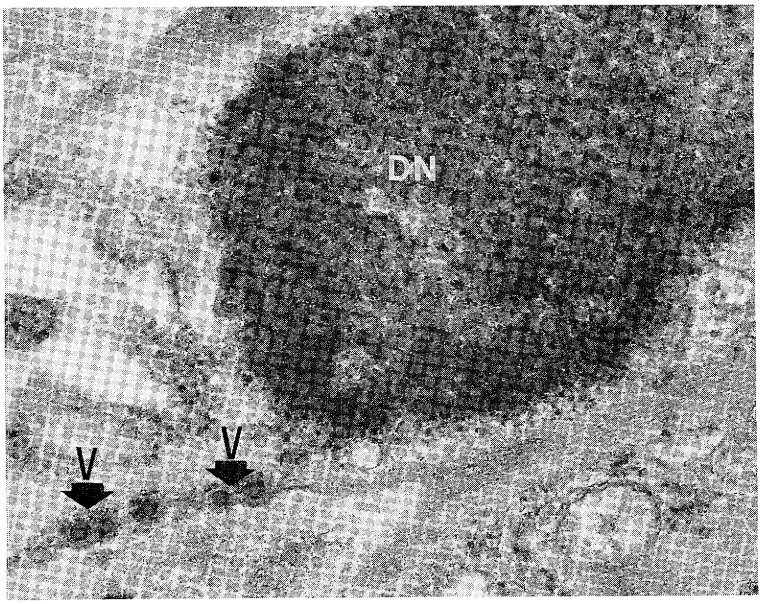

Fig. 5. Higher magnification of dense matrix area of virus-like particles in smooth muscle cells. Dense matrix areas (DN) consists 500Å sized dense core particles. New virus-like particles (V) are shown leaving the host smooth muscle cell by budding outward from the cell membrane (X 60,000).

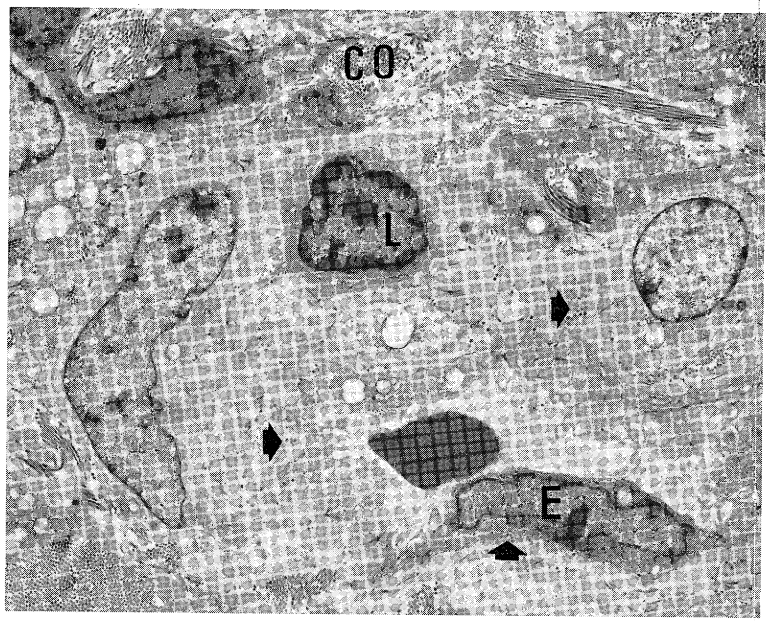

Fig. 6. Vasa vasorum in the adventitia. Groups of dense dots (arrow) seen in this picture were identified as virus−like particles at a higher magnification (X 4,800).
CO: collagen fibers, E: endothelial cell,
L: lymphocytoid cell

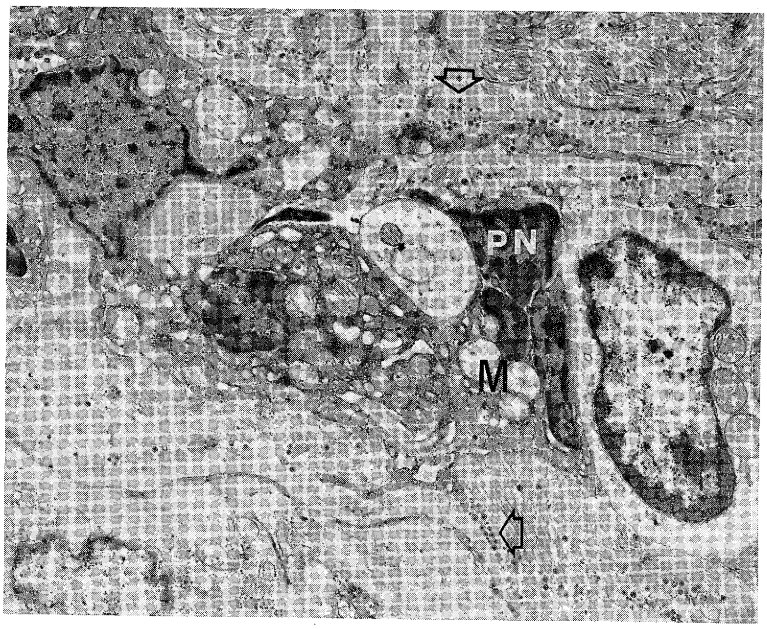

Fig. 7. Degeneration of smooth muscle cells in the thickened intima. Note pyknotic nucleus (PN) and swollen mitochondria (M). Numerous mature virus−like particles (open arrow) are visible in the stroma even at this level of magnification (X 7,500).

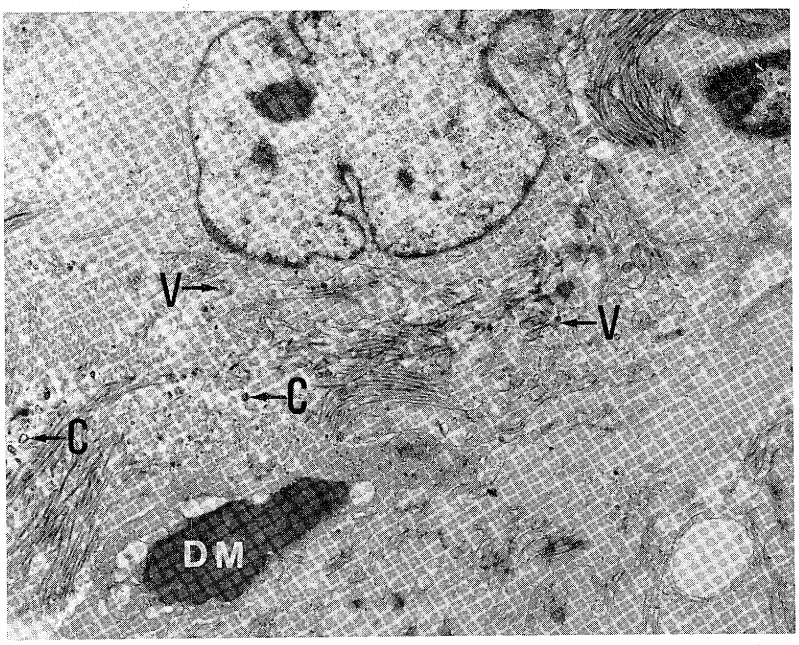

Fig. 8. Dense matrix (DM) containing smooth muscle cells in the media. Note dense matrix area of virus−like particles (DM) in an atrophied medial smooth muscle cell (X 7,100).
C: cell debris, V: virus−like particle

Discussion

Karrer (1974) indicated that the aorta and liver of chick embryo served as foci for virus storage rather than synthesis because only mature viral particles were seen in these tissue. However, the presence of numerous viral matrix areas was observed in the arterial smooth muscle cells of our chickens. This result suggested that arterial tissue can be viral replication site. It is speculated that the presence of mature virus−like particles in the kidney, liver and spleen are indicative of systemic viremia rather than organ specific replication, since no viral matrix areas were observed in these tissue (unpublished data). Viral particles were frequently seen in the plasmalemal vesicles and intercellular junctions of endothelial cells from the intima and the vasa vasorum. Their presence is in accordance with the mode of nutrient transport via passive intercellular transport, endocytosis and exocytosis in the arterial wall.

Burch (1974) reported that the encephalomyocarditis virus to be highly infective to fibroblasts in the mouse aorta. The virus−like particles observed in this study are the same size and shape as various chicken tumor viruses reported by other investigators (Benedetti and Bernhard, 1958; Bernhard, 1958). In the present study, mature virus−like particles were seen in fibroblast−like cells in the thoracic aorta of chickens. We have also found similar particles and matrix areas in arteries from 2 month−old cockerels of normal Japanese domestic white leghorns (Toda *et al.*, 1983a), but the fre-

quency was much lower than in non−laying hens. The arteries of non−laying hens contained greater numbers of virus−like particles than laying hens. A possible explanation may be that hyperlipidemia, peroxide and estrogen may play an important role in the suppression of host defence in non−laying hens(Toda *et al.*, 1983b). Further studies using immunohistochemistry are necessary to clarify the relationship of the virus infection to the development of atherosclerosis.

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内因性高脂鳥大動脈のウイルス感染について

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産卵性雌鳥と不産卵性雌鳥の大動脈および冠動脈に内因性ウイルス様粒子を電子顕微鏡を用いて見出した．産卵性雌鳥より不産卵性雌鳥の大動脈の中に多くのウイルス様粒子が存在していた．不産卵性雌鳥の大動脈の平滑筋細胞の中にウイルス様粒子がしばしば見られた．なお，感染した平滑筋は変性していた．多くの成熟したウイルス様粒子が宿主の平滑筋より結合組織の層状構造の中に飛び出していた．

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