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# Electron Microscopic Observations of the Vascular Endothelial Cells in the Central Nervous System of Piglets Infected with Porcine Enterovirus Serotype 3

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# Porcine Enterovirus 感染仔豚의 中樞神經系 血管內皮細胞의 電子顯微鏡的 觀察

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**抄錄** : 國內에서 分離된 porcine enterovirus(serotype 3)를 初乳를 攝取하지 않은 仔豚에 經口感染시킨 후 灰白腦脊髓炎을 나타내는 時期에 中樞神經系 血管內皮細胞를 電子顯微鏡的으로 觀察하였던 바 다음과 같은 結果를 얻었다.

脊髓 및 小腦 血管內皮細胞에서는 picornavirus의 特徵的인 virus結晶體가 出現하였고 virus結晶體는 rough ER과 밀접하게 연결되어 있었으며 髓膜의 血管內皮細胞에서도 電子密度가 높은 virus樣 粒子들의 凝集體가 觀察되었다. 細胞小器官의 變化로는 mitochondria의 擴張과 cristae의 消失, rough ER의 擴張과 ribosome의 脫落이 현저하였다. 또한 血管周圍腔의 擴張과 淋巴球의 浸潤, 血管內皮細胞의 細胞膜과 基底膜의 破壞가 認定되었다.

## Introduction

Porcine enteroviruses have been associated with various conditions including polioencephalomyelitis, female reproductive disorders, enteric disease and pneumonia by certain serogroups(Derbyshire, 1986).

Since the isolation of porcine enteroviruses from the affected pigs in Korea(Kwon *et al.*, 1978), two serotypes of porcine enterovirus were identified by complement fixation test(Shin, 1987). The clinical and histopathological characteristics associated with the pathogenic strain of porcine enterovirus isolated in Korea have been described in detail in previous publications(Shin, 1987; Chang and Lee, 1986; Shin and Lee, 1985). Although there were some variations in time of onset of various clinical manifestations between different strains of pathogenic porcine enteroviruses, the characteristic histopathology of the central nervous system(CNS) of porcine polioencephalomyelitis consisted of meningitis, neuronophagia and perivascular cuffings(Shin and Lee, 1985).

Since Watanabe *et al.* (1971) suggested by the fluorescent antibody technique that the pigs infected with Teschen disease virus showed fluorescence in

the capillary endothelial cells of the brain, there are few reports on the electron microscopic studies of the CNS vessels in pigs with polioencephalomyelitis.

The object of this report was to study the ultrastructural changes of the vascular endothelial cells in the CNS of the colostrum-deprived piglets infected with porcine enterovirus serotype 3.

## Materials and Methods

**Inoculum**: The porcine enterovirus used in this experiment was originally isolated from the brain of a pig with polioencephalomyelitis. It was identified as porcine enterovirus serotype 3(Shin, 1987).

**Experimental Animals**: One litter of colostrum-deprived piglets was selected to an experimental group(6 piglets) and a control group(2 piglets). The experimental piglets were orally inoculated with 1ml of tissue culture suspension containing $10^{7.5}$ $TCID_{50}$/0.1ml. The 2 control piglets were given 1ml of virus-free tissue culture suspension. Each group of piglets was maintained in a separate isolator.

**Collection of Tissues**: The experimental piglets developed a sudden rise of body temperature and paralysis during 4 to 7 days postinoculation. Two piglets infected with porcine enterovirus were necropsied at 7 days postinoculation and were processed for electron microscopic observation. The cerebellum and spinal cord were minced and fixed in 2% paraformaldehyde-2.5% glutaraldehyde(0.075M cacodylate buffer), postfixed in 1% osmic acid, dehydrated and embedded in Epon 812. Ultrathin sections of the specimens were stained with uranyl acetate and lead citrate and examined by Hitachi 800 electron microscope.

## Results

The clinical and histopathological characteristics of polioencephalomyelitis of piglets infected with porcine enterovirus serotype 3 had been published in a previous report(Shin, 1987).

Ultrastructurally crystalline arrays characteristic of picornaviruses were found in the vascular endothelial cells of the spinal cord(Figs. 1~4). The rough ER was deprived of ribosomes, irregularly dilated and closely associated with crystalline arrays of viral particles(Figs. 2, 4). Numerous ribosmes were scattered throughout the cytoplasm and some ribosomes were found in the from of cluster. There were also mitochondrial swelling and loss of mitochondrial cristae. Abundant cytoplasmic filaments were observed around viral crystals and intimately associated with the viral crystals. There were dilation of perivascular space, perivascular cuffing and partial disruption of endothelial cell basal lamina(Figs. 1, 3).

In the meninges, the aggregates of electron-dense particles, presumably immature virions, wene occasionally observed in the vascular endothelial cells. There were loss of mitochondrial cristae, dilation of rough ER and detachment of ribosomes(Fig. 5).

Crystalline arrays of viral particles were also present in the vascular endothelial cells of cerebellum (Fig. 6). In some capillaries, there were increase in lipid droplets and clusters of ribosomes.

## Discussion

Porcine enterovirus infection occurs by ingestion of the virus, and it is well established that initial replication occurs in the tonsil and intestinal tract(Long, 1985; Baba *et al.*, 1966). Viremia follows leading to infection of the CNS, although it occurs less regularly with the less virulent strains(Derbyshire, 1986). An important question in relation to the pathogenesis of viral multiplication and persistence is which forms of viral antigens are localized on porcine enterovirus infection in piglets. Because picornaviruses resemble ribosomes both in size and structure, it is difficult to identify viral antigens by routine electron microscope unless they are compartmentalized within the cytoplasm such as in crystalline arrays(Rosenthal *et al.*, 1986; Dal Canto and Lipton, 1982). It was not possible to crystallize some enteroviral strains including ECHO virus(Godman *et al.*, 1964), the O3b strain of porcine enterovirus(Koestner *et al.*, 1966) and the Brunhilde and Mahoney strains of human polio type I(Mayor and Jordan, 1962) *In vitro*, and mouse poliovirus(Nelson *et al.*, 1964) *In vivo*. That explains the enteroviral strains not always crystallize easily. Koestner *et al.*, (1966) assumed that capillary endothelial cells might be not only transient

stores and pathways of the virus but also important locations of viral multiplication within the CNS of germfree pigs infected with a O3b strain of porcine enterovirus. The possible mechanisms of viral crystallization in the endothelial cells were not clearly established.

In this study, crystalline arrays of viral particles in the vascular endothelial cells were found in the spinal cord and cerebellum. These results were generally comparable with those of Koestner *et al.*, (1966) working with a different serotype of porcine enterovirus. Cytoplasmic filaments were also rich around the viral crystals. These findings explain that immature virions originated from the rough ER might adhere to the rearranged cytoplasmic filaments and hereby mature to infective viral antigens. In addition, Lank and Penman(1979) concluded that rearrangement of the cytoskeleton and virus-filament interaction were necessary for virus replication. A similar association has been also observed in Theiler's virus infections by peroxidase-antiperoxidase methods(Dal Canto and Lipton, 1982).

Rescently it has been reported that CNS endothelial cells could express MHC class II molecules and present antigens *In vitro*(Male *et al.*, 1987), in experimental allergic encephalomyelitis(Sobel *et al.*, 1984) and localize viral antigens on the luminal surface and in the cytoplasm of endothelial cells in subacute sclerosing panencephalitis using immunoperoxidase labelling techniques(Wisniewski *et al.*, 1983). It would be also nteresting wheather MHC antigens might be localized in the CNS endothelial cells of pigs infected with porcine enterovirus.

Pitts *et al.* (1987) suggested that the retrovirus-associated ultrastructural changes in the endothelial basal lamina may alter normal physiological functions and thus play a primary role in the pathogenesis of retrovirus-induced spongiform disease. In this study there were no definite viral particles in the endothelial basal lamina contrary to the murine retrovirus infections, partial disruption of basal lamina would interfere the normal function associated with the increase of perivascular space.

## Summary

In the course of studying porcine enterovirus infection in piglets, the vascular endothelial cells in the CNS of colostrum-deprived piglets with polioencephalomyelitis were investigated by electron microscope. The experimental piglets were orally infected with the porcine enterovirus serogroup 3 isolated in Korea and necropsied at 7 days postinoculation.

Crystalline arrays of viral particles were found in the vascular endothelial cells of the spinal cord and cerebellum. Aggregates of immature viral particles were occasionally observed in the vascular endothelial cells in the meninges. The rough ER was deprived of ribosomes, irregularly dilated and associated with viral crystals. There were abundant cytoplasmic filaments, dilatation of perivascular space, perivascular cuffing, and the partial distruptions of endothelial cell membrane and basal lamina.

### Legends for Figures

**Fig. 1.** Spinal cord of a piglet with polioencephalomyelitis at 7 days postinoculation. A vascular endothelial cell contains crystalline arrays of viral particles. Red blood cell(RBC). ×10,000.

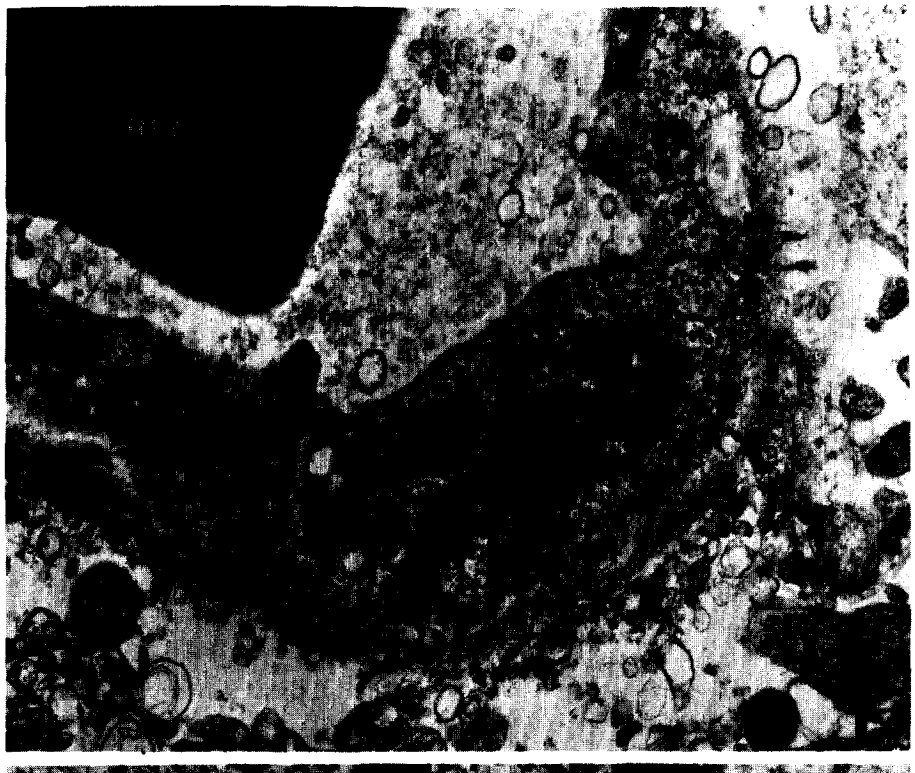
**Fig. 2.** Higher magnification of crystalline arrays of the viral particles in Figure 1. ×60,000.

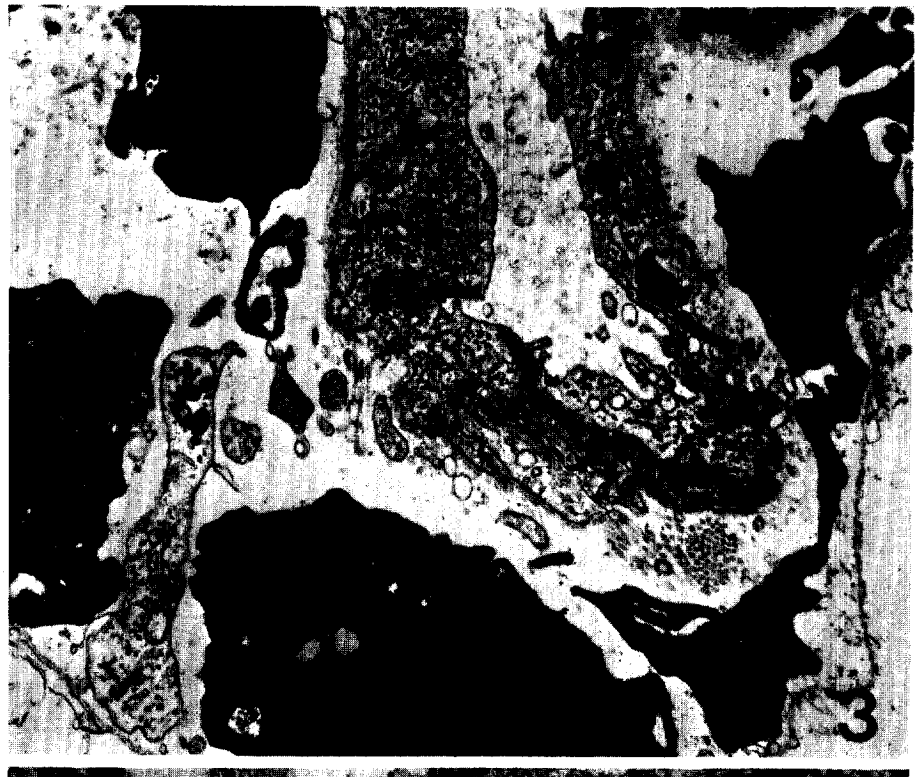
**Fig. 3.** Spinal cord. There are perivascular cuffing and appearance of viral crystals(arrow) in the vascular endothelial cells. Capillary endothelial cells(EC). ×10,000.

**Fig. 4.** Higher magnification of the arrow area in Figure 3. ×60,000.

**Fig. 5.** Vascular endothelial cells in the meninges show dilation of rough ER, loss of mitochondrial cristae, and aggregation of electron-dense particles, presumably immature virions. ×10,000. Inset:Higer magnification of arrow area. ×40,000.

**Fig. 6.** Cerebellum. A vascular endothelial cell contains viral crystals(arrow). ×10,000.

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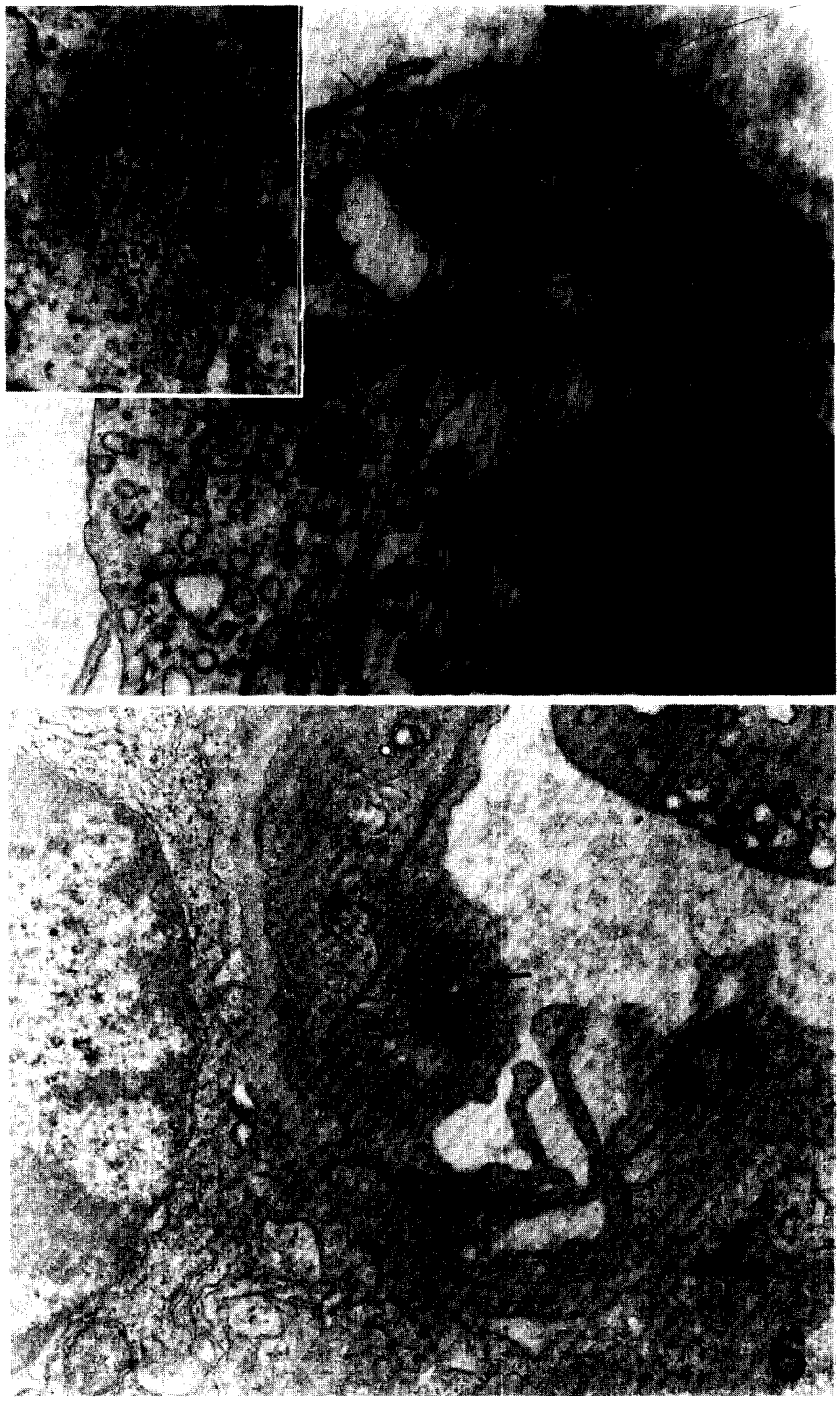

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