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Comparison of Proliferation and Cytopathogenicity of Swine Vesicular Disease Virus and Coxsackievirus B5[1]

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Sequential appearance of both swine vesicular disease virus and Coxsackievirus B5 antigens in a pig kidney cell line was studied by immunofluorescence and electron microscopy. The replication cycle of each virus was approximately 3–4 h. Viral antigens were demonstrable in the cytoplasm 2 h after inoculation. A compact mass of fluorescence was seen when cells showed cytopathogenic effect at 5.5 h. After 3 h, a few viral particles, seen by electron microscopy, were in the cytoplasm. Morphological changes of cells occurred at the same time. Cytoplasmic crystalline arrays of virus were first detected at 7 h.

Swine vesicular disease virus (SVDV) is closely related serologically to Coxsackievirus B5 (CB5) as indicated by neutralization,[1] complement-fixation (P. D. McKercher, unpublished data) and immunodiffusion test.[2] However, differences between these viruses were shown by immunodiffusion, ribonucleic acid (RNA) hybridization and neutralization.[8]

Studies relating to animal inoculation with SVDV and CB5 have been reported. The close serological relationships between these two viruses and pathogenesis in mice suggest the possibility that SVDV might be the result of transfer of CB5 from man to swine.[1] However, pigs inoculated with the Faulkner strain of CB5 developed no clinical disease, but developed non-suppurative meningoencephalomyelitis.[4,5,6]

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In single cycle growth of SVDV on IBRS-2 cells, infectivity was detected as early as 2.5 h; growth was maximum at 5 h.[7,8] Specific viral antigen in the cytoplasm was detected by immunofluorescence within 4-5 h after infection[7].

The purpose of this study was to compare the cytopathogenicity of SVDV and CB5 in a pig kidney cell line (MVPK)[9] by electron microscopy and immunofluorescence.

MATERIALS AND METHODS

Methods used for electron microscopy were similar to those previously described.[10] Two bottles of MVPK cells infected at a multiplicity of 10 plaque-forming units per cell were removed at 1 h intervals up to 10 h. Cells were collected with a rubber policeman, and the suspension was centrifuged for 5 min at 150 × g. The cell pellet was fixed with 1% (v/v) glutaraldehyde in Sorenson's phosphate buffer. Cells were post-fixed in osmium tetroxide, and Epon-embedded thin sections were examined on Formvar carbon-coated grids in a Philips EM 201 electron microscope.

Hyperimmune sera against SVDS or CB5 were produced in guinea pigs, subcutaneously inoculated with crude antigen preparations extracted from SVDV- or CB5- infected mouse brains. Preparation of fluorescent antibody conjugate and the staining technique have been described previously.[11] For immunofluorescence studies, monolayers of MVPK cells grown on coverslips in closed Leighton tubes were inoculated with either SVDV or CB5 at the same multiplicity as that used in electron microscopy studies. The cells were examined for cytopathic effect (CPE) before staining. Pairs of coverslips were removed after 30 and 60 min of adsorption at 37°C, rinsed and stained with appropriate corresponding fluorescent antibody conjugate. After adsorption for 1 h, additional tubes were rinsed three times with phosphate buffered saline and overlayered with 2 ml of 1% methylcellulose in minimum essential medium and incubated at 37°C. Two coverslips were examined for CPE and processed for staining at half-hour intervals up to 5 h then hourly to 10 h postinoculation. Specific fluorescence was examined with a Leitz ultraviolet microscope with the appropriate filters.

RESULTS

The formation of virus particles and structural changes in virus-infected cells as observed by electron microscopy were essentially similar in most respects. However, virus synthesis progressed more slowly with CB5 than with SVDV. The description, therefore, applies to both viruses except where noted.

The infected cells harvested at 1 and 2 h after infection showed no significant difference from normal cells. However, in SVDV-infected cells harvested at 2 h, ribosomes were more pronounced and aggregated, and cytoplasmic vesiculation was observed. Mitochondria had indistinct cristae (Fig. 1a). At 3 h after infection, dense inclusions, where few virus particles were evident, were seen in the cy o-plasm of infected cells (Fig. 1b). Complete viral particles in tubules at the margins of condensed viroplasm

were seen in infected cells harvested between 4-6 h after infection (Fig. 1c). Infection by both viruses produced cytoplasmic vacuoles of varying sizes. Cytoplasmic viral crystalline arrays were visible at 7 h after infection with SVDV (Fig. 1d) and 8 h after infection with CB5 (Fig. 1e). At later (10 h) stages of infection with both viruses, vesiculation of the cytoplasm was accompanied by very large cytoplasmic arrays of virus particles (Fig. 1f).

The appearance and localization

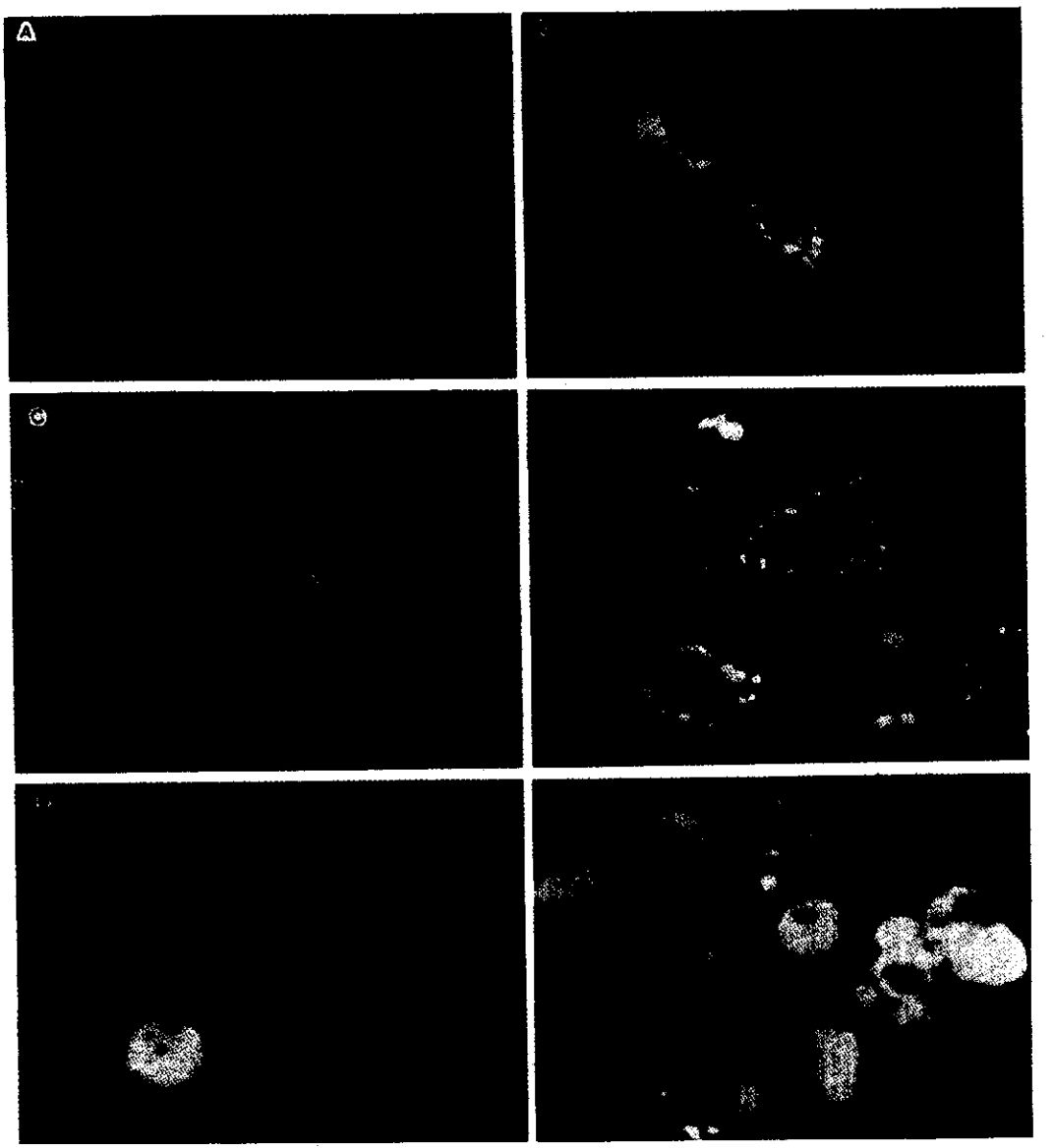

Fig. 1. Electron micrographs of MVPK cells infected with either swine vesicular disease virus (SVDV) or Coxsackievirus B5 (CB5) at the indicated h postinfection. (*a*) SVDV, 2 h; (*b*) CB5, 3 h; (*c*) SVDV, 5 h; (*d*) SVDV, 7 h; (*e*) CB5, 8 h; (*f*) CB5, 10 h. Magnification mark—1 μm.

of the viral antigens were studied by the direct immunofluorescent method after growth of the viruses in MVPK cells. No great difference was found between the results for the two viruses, but the intensity of the fluorescence was slightly greater in the SVDV-infected cells as seen at the same period. The description, therefore, applies to both viruses except where noted. At 2 h postinoculation, the specific fluorescence was detected at one polar region of the cytoplasm of a few cells (Fig. 2a). As the cells

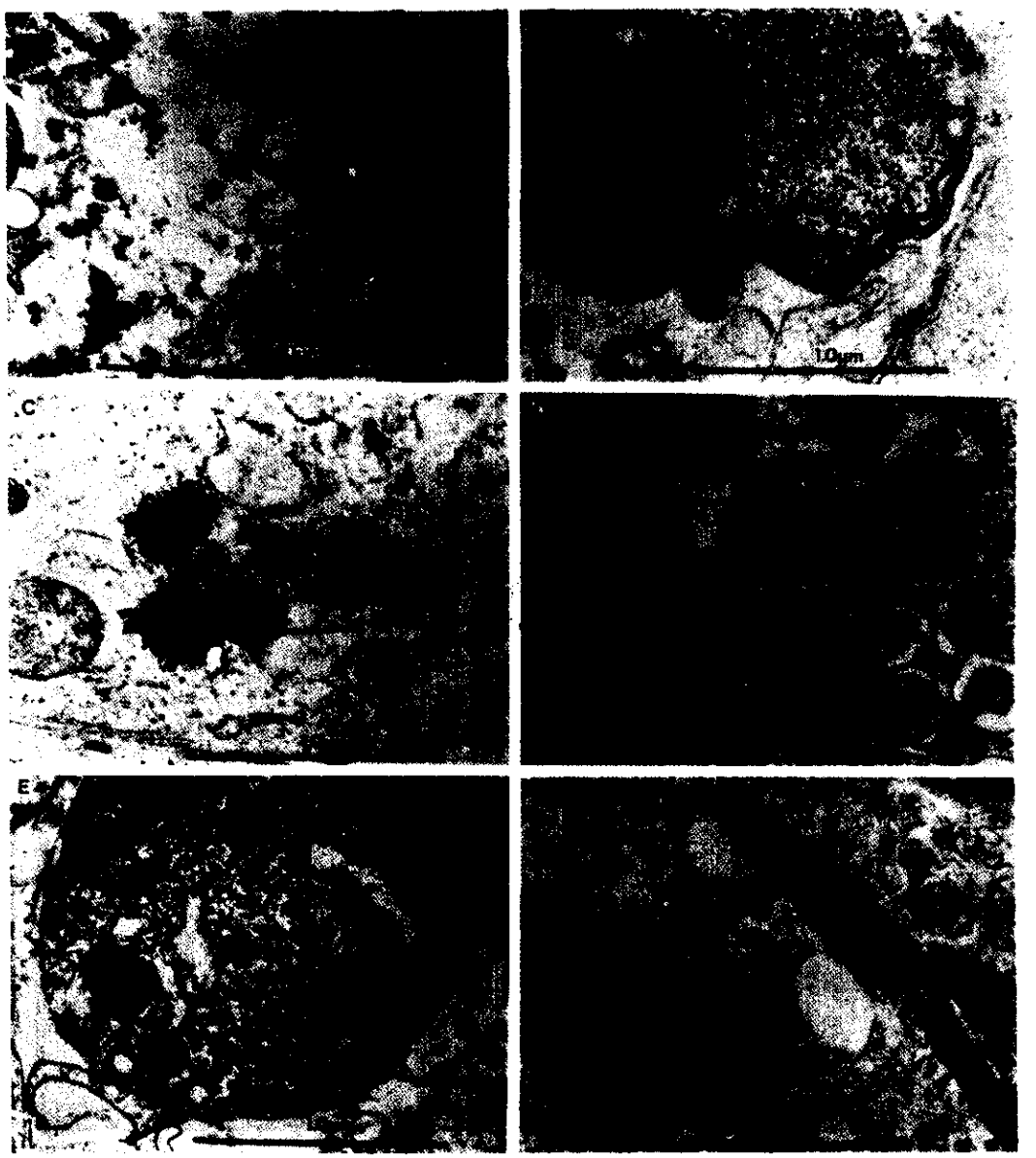

Fig. 2. Immunofluorescent staining of MVPK cells infected with either swine vesicular disease virus (SVDV) or Coxsackievirus B5 (CB5) at the indicated h postinfection. (*a*) SVDV, 2 h; (*b*) SVDV, 3 h; (*c*) CB5, 3 h; (*d*) SVDV, 4.5 h; (*e*) CB5, 5.5 h; (*f*) CBS, 7 h.

containing viral antigens increased, strong bright granular fluorescence was seen in about 10% of the cells at 3 h postinoculation with SVDV (Fig. 2b) and CB5 (Fig. 2c). At 4.5 h postinoculation (Fig. 2d), cells showed increased fluorescence with SVDV infection, and similar development occurred at about 5.5 h in the CB5-infected cells (Fig. 2e). However, CPE was not detected at this period in either virus system in active cultures. The intensity and the size of the granular fluorescent inclusions increased with time until the compact fluorescence indicating CPE was seen. The number of cells showing CPE and strong fluorescence increased at 7 h postinoculation with both viruses (Fig. 2f).

DISCUSSION

Immunofluorescence and electron microscopy both show that the temporal development of SVDV and CB5 in MVPK cells is very similar. Immunofluorescence indicates that antigen is formed somewhat earlier than discernible virus particles are seen with the electron microscope. By the later stages, when fluorescence becomes intense, large crystalline arrays of virus particles are also found. These studies support previous findings that indicate that SVDV and CB5 viruses are closely related.

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猪水疱病毒及柯沙奇 B_5 型病毒之增殖及細胞病變機序之比較

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以電子顯微鏡及螢光抗體法比較檢出猪水疱病毒及柯沙奇 B_5 型病毒抗原連續在猪腎細胞內出現的情形。兩者病毒之增殖情形極爲類似，增殖週期均爲3～4 小時 ，在感染後2小時病毒抗原可由螢光抗體法在細胞質內檢出，感染後3小時完整的病毒顆粒可以電子顯微鏡在細胞質內檢出，同時可見細胞的顯微病變。感染後5小時半可見細胞病變及均勻之特異螢光抗原物質。感染後7小時在細胞質內可見病毒之結晶排列，本試驗結果證實該兩種病毒關係極爲密切。