Scanning Electron Microscopical Observations on the Cytopathology of Porcine Enteroviruses in PK-15 Cells

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SUMMARY

Examination by scanning electron microscopy (SEM) of PK-15 cells infected with each of the two cytopathogenic types of porcine enterovirus revealed changes similar to those observed by light microscopy. However, differences between the two types could be detected at an earlier stage of the infection. Cells infected with T80 virus (type I) were rounded, with surface microridges, while V13 (type II) infected cells were characterized by cytoplasmic protrusions.

Although several papers (Betts, 1964; Rasmussen, 1969) describe the examination by light microscopy of the cytopathological changes provoked in pig kidney cell cultures by porcine enteroviruses, there is no report of the examination of cells infected with porcine enteroviruses or any other picornaviruses by SEM. Porcine enteroviruses are classified into two types on the basis of their cytopathic effects (c.p.e.) on pig kidney cells. Type I strains produce rounding and clumping of the affected cells while type II strains produce characteristic cytoplasmic protrusions (Rasmussen, 1969). This pattern of c.p.e. induced by porcine enteroviruses is a stable characteristic. The objective of the present study was to obtain information on the topography of cells infected with representative strains of each type of porcine enterovirus.

PK-15 cells obtained from the American Type Culture Collection were cultivated in Eagle's minimal essential medium containing 200 units of penicillin and 80 μg of streptomycin per ml, supplemented with 10% foetal calf serum. When confluent monolayers were formed on glass coverslips in Leighton tubes they were infected with a high multiplicity of either the T80 (type I c.p.e.) or V13 (type II c.p.e.) strain of porcine enterovirus. At intervals after infection, one coverslip for each virus was washed three times with pre-warmed (37 °C) 0.2 M-phosphate buffer at pH 7.4. The cells were then fixed with 2.5% gluteraldehyde in the same buffer for 30 to 60 min at room temperature. Following fixation the cells were rinsed with the same buffer containing 2% sucrose, and post-fixed with 1% osmium tetroxide in Millonig's buffer at pH 7.4 for 2-4 h at room temperature. After rinsing, the cells were dehydrated through a graded series of ethanol. Finally, after two changes in 100% ethanol the cells were air-dried at room temperature (Dalen & Scheie, 1969; deHarven, Lampen & Sato, 1973; Dutta, 1975). Premature drying of the cells was avoided by keeping the cells immersed during all fixation and dehydration steps. Non-infected PK-15 cells were similarly processed for control purposes.

The dried specimens were attached to specimen stubs with colloidal silver and coated with gold-palladium (60:40) alloy in a sputter coater. The thickness of the coating was about 20 to 25 nm. The coated specimens were stored in a desiccator and examined within one week in an ETEC Autoscan at 45° tilt to the primary electron beam at 10 or 20 kV. Kodak Panatomic X or Plus X Pan films were used for photography.

Normal PK-15 cells (Fig. 1) appeared spindle or polygonal in shape and they were attached
to the substrate by thin cytoplasmic fibrils. The cell surfaces were covered with numerous microvilli of varying length distributed evenly throughout.

The early changes observed in the T80 infection consisted of enlargement and retraction of the affected cells from the neighbouring cells, to which they remained attached by thin retraction fibrils. The microvilli were still visible. These changes were soon followed by rounding and clumping of the cells, whose retraction fibrils became distorted. A marked reduction in the number of microvilli was apparent as the infection progressed. The surface of the infected cells showed many microridges (Fig. 2a).

The initial changes in V13 infected cells were similar to those seen in T80 infected cultures, but in the former, the retraction fibrils were thicker and the cell surface showed a large number of small rounded protrusions (Fig. 2b). Another difference was the appearance of a sheet-like projection from the cellular surface, while some of the cells possessed spike-like projections which had slightly tapered tips and were thicker than the microvilli and thicker and shorter than the retraction fibrils. As the infection progressed, these changes advanced and during the later stages the cells showed a large number of cytoplasmic blebs of various sizes so that each cell presented the appearance of a bunch of grapes. There was no obvious rounding and clumping of the cells. As in the case of T80 infected cells, microvilli were absent at this late stage.

The above observations showed that SEM can be used successfully to study the cytopathic changes induced by porcine enteroviruses and represent the first application of this technique to the study of the topography of cells infected with a picornavirus. Although the resolution is only about 10 to 15 times that of the light microscope, the large depth of the focus and three-dimensional image formation help to demonstrate the alterations of the cellular surface. Characteristic differences in the c.p.e. of type I and type II porcine enteroviruses were recognized at an earlier stage of the infection than by light microscopy. The changes seen by SEM were similar to those observed in the light microscope, except that the surfaces of the cells infected with T80 virus, which appear quite smooth when examined by light microscopy, were shown to be covered with microridges.
Fig. 2. (a) Scanning electron micrograph of PK-15 cells infected with T80 virus. (b) Scanning electron micrograph of PK-15 cells infected with V13 virus.

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