Adaptation of Swinepox Virus to an Established Cell Line

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Swinepox virus was successfully adapted to the PK-15 cell line, but not the MDBK cell line. Virus titers obtained in the PK-15 host cell system were comparable to diploid swine kidney cell cultures.

Swinepox virus is highly host-specific, and cultivation has been limited to the pig or primary tissue cultures of swine origin. To provide a host cell system more amenable to laboratory manipulations than either the intact pig or primary porcine cell cultures, attempts were made to adapt the swinepox virus to an established cell line of swine (PK-15) and bovine (MDBK) origin. Both cell lines were obtained from the Cell Respository, American Type Culture Collection, Rockville, Md.

Inoculum was prepared by triturating skin lesions from gnotobiotic piglets infected with swinepox virus. A 1:10 suspension was prepared in sterile Hanks balanced salt solution containing 200 units of penicillin per ml and 200 mg of dihydrostreptomycin per ml and cleared of debris by centrifugation.

Cover slip cultures in disposable glass tubes (16 by 150 mm) were inoculated with 0.1 ml of the virus suspension and allowed to absorb for 1 hr at 37 C. The maintenance medium for the cell cultures consisted of 5% fetal calf serum in Eagle's minimal essential medium. Cover slips were removed on days 4, 5, 6, and 7 postinoculation. Cells were fixed in absolute methyl alcohol, stained with May-Grünwald-Giemsa, and examined under a microscope for viral cytopathic effects (CPE).

Four serial blind passages of the virus in PK-15 cells (1) were required before any consistant indication of viral adaptation and growth was obtained in this host cell system. In the fifth passage, a few cells showed margination of the nuclear chromatin and cellular degeneration. In the sixth passage, a typical

CPE characteristic of swinepox virus infection was observed: nuclear vacuolation, formation of acidophilic granular intracytoplasmic inclusion bodies, cytoplasmic stranding, and cell death (Fig. 1). Similar alterations have been described in primary swine kidney cells infected with swinepox virus (2, 5). Control cells showed none of these changes.

When the sixth passage of swinepox virus adapted to PK-15 cells was inoculated intradermally into a susceptible 6-week-old SPF pig, small, flat, grayish macules were observed at the inoculation sites 4 days postinoculation. Distinct developmental stages (papule to vesicle to pustle), however, were not observed. This is not uncommon in experimental swinepox. On day 8 postinoculation, encrusted lesions were excised for histopathologic examination and for attempts to reisolate the virus with PK-15 cell cultures.

Histologic sections through skin lesions, stained with hematoxylin-eosin, revealed hydropic degenerative changes involving all the layers of the epidermis, especially the superficial layers of the stratum malpighii (Fig. 2). A few areas were necrotic with marked polymorphonuclear infiltration extending to the dermis. The nuclei of the affected epithelial cells usually contained one large vacuole or occasionally two or three smaller vacuoles. A few cells were found to have compact eosinophilic round bodies, but most of the cells had irregular acidophilic granular masses. These changes and similar cytoplasmic inclusion bodies have been described previously in sections of swinepox skin lesions and are consistent with naturally occurring cases (4). It was thus concluded that the passage of swinepox virus in PK-15 cells apparently did not alter its capacity to produce disease. Virus was

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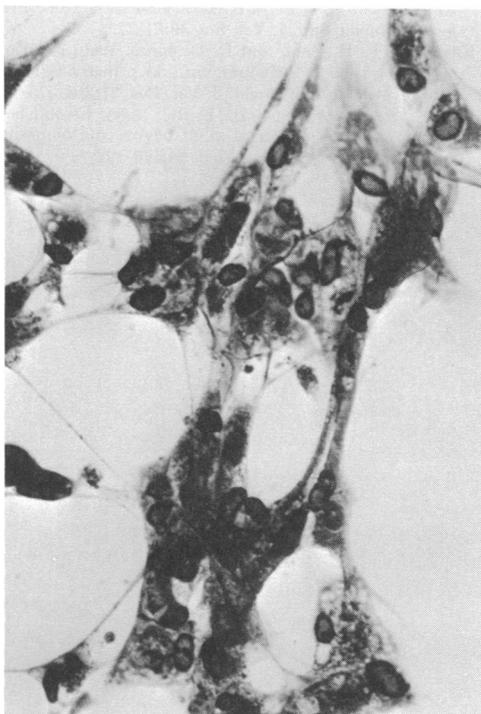
FIG. 1. *Swinepox-infected PK-15 cell culture 96 hr postinoculation. Stained with May-Grünwald-Giemsa.* ×250.

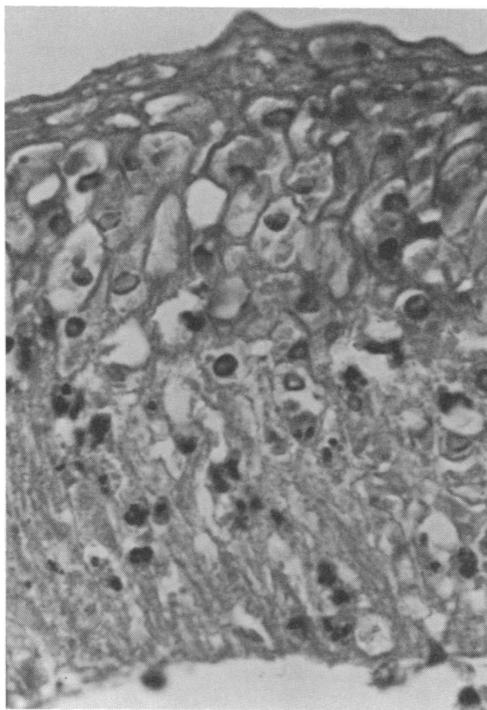

FIG. 2. *Section through swinepox skin lesion. Stained with hematoxylin-eosin.* ×400.

readily reisolated from the above lesions and demonstrated by cell culture inoculation.

The virus in question, propagated in PK-15 cells, was used in cross-protection tests with known swinepox virus. It not only conferred protection against a swinepox virus challenge, but also was unable to initiate an infection in swinepox-immune SPF pigs. This fact, plus the characteristic CPE in cell cultures, the induced histopathology, and the origin of the original virus inoculum, leave little doubt that the virus is indeed that of swinepox.

PK-15-adapted swinepox virus grew equally well in PK-15 and primary swine kidney cells as evidenced by comparable virus titers in both kinds of cells as determined by standard TCD_{50} and plaque titrations (e.g., 11×10^6 PFU/ml for primary swine kidney culture and 8.5×10^6 PFU/ml for PK-15 cells). Plaque formation, however, was observed only when the inoculum was mixed with diethylaminoethyl-dextran (3).

PK-15-adapted swinepox virus was passed serially up to 12 blind passages in the MDBK cell line (6) without any indication of adaptation to, or replication in, this host cell system.

Similar unsuccessful attempts to cultivate swinepox virus in primary bovine kidney cells and rabbit kidney cells had been reported earlier by Kasza et al. (5). However, they found that, although material from their first passage in primary bovine kidney cells produced no CPE in bovine cells, virus survival (but not necessarily replication) was indicated by the fact that first-passage material was still infectious for primary swine cells. In our studies, the virus failed to replicate in MDBK cells, and after 12 blind passages neither produced a CPE in MDBK cells nor proved infectious when cells and culture fluid were introduced into susceptible primary swine kidney or PK-15 cell cultures.

This virus-host cell system is being used in our laboratory to study the replication of this agent and host cell interactions.

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