

The Growth of Varicella-Zoster Virus in Guinea Pig Embryo Cells (37587)

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The growth of varicella-zoster virus (V-Z) in nonprimate cell cultures has not been extensively studied. Weller, Witton and Bell (1) reported partial success in adaptation of V-Z to rabbit kidney cells, and Söltz-Szöztz (2, 3) described the susceptibility of guinea pig cells to V-Z, which was confirmed by Sefcovicova (4). Cell-free virus was not obtained by these investigators. This communication deals with the propagation of V-Z in embryonic guinea pig (GP) cells, and some of the biologic properties of this system.

Materials and Methods. *Virus.* Two strains of virus, designated 1991 and Webster, were isolated from the vesicular fluid of varicella patients and propagated in WI-38 cells. The viruses were characterized as V-Z virus by cytopathic effect (CPE), electron microscopy (EM), cell susceptibility and serological identification. The viruses were passed 9 times in WI-38 using infected cells, before being used for infection of GP cells. Cell-free virus was prepared by ultrasonic disruption (2 min, Raytheon Sonifier) or an infected cell pack in MEM with 2% FCS.

Cells. GP cells from skin and muscle of 4 wk old embryos were prepared by trypsinization and used between the 1st and 50th passages. Primary rabbit kidney cells from adult rabbits, were prepared by trypsinization.

WI-38 cells were obtained from Dr. Hayflick (Stanford University) and from Flow Laboratories.

All cells were grown in Eagle's minimal essential medium (MEM) supplemented with fetal calf serum (FCS) (10% for growth, 2% for maintenance) and antibiotics.

Assay of virus. Viral infectivity titers were determined by the end point dilution method of Reed and Muench (5) and by plaque

assay in WI-38 (6) and guinea pig cells.

Plaque titration. Plaque assays were performed by inoculating virus onto WI-38 or guinea pig cell monolayers in 35 mm petri dishes. The virus was allowed to adsorb for 1 hr at 37° before adding the agarose overlay. A second agarose overlay was applied on Day 5. Plaques were counted on Day 12 with the aid of a light microscope.

Serological tests. Plaque reduction neutralization tests were performed in monolayers of GP and WI-38 cells in 35 mm petri dishes. Acute and convalescent phase sera were used for this technique (7). The same sera and rabbit antihuman γ -globulin conjugated with fluorescein were used for the indirect fluorescent antigen staining.

Complement fixing (CF) and precipitin antigen preparation. Infected cells were removed by treatment with 0.25% trypsin and 0.1% EDTA (ethylenediaminetetraacetic acid). The cells were sedimented at 1000 rpm for 10 min and resuspended in phosphate buffered saline (PBS) (pH 7.2) at a 5% concentration. This suspension was sonicated for 2 min. The resultant material was used for CF and precipitin antigens. Complement fixing antigen was assayed by the microtiter technique (8).

Gel precipitin test. Molten agarose (0.4%) dissolved in Ca- and Mg-free PBS (pH 7.2) containing 0.01% Merthiolate was added to 9 cm glass petri dishes. Wells 8 mm in diameter were made with centers 12 mm apart. The center well was filled with human antiserum, the adjacent wells were filled with antigen. Petri dishes were incubated in moist boxes at 4° for 1 wk. Precipitin lines were observed with the positive antigen used as a control, after 2 days incubation.

Animal infection. Groups of 5 newborn guinea pigs (48 hr) were injected either with 0.5 ml of infected cell suspension intraperitoneally (ip) or with 0.05 ml intracerebrally (ic).

Histopathology of guinea pig organs and cytopathology of virus infected cells. Guinea pig organs were fixed in Bouin's solution for hematoxylin and eosin staining. Infected cell cultures were fixed in methanol and stained with May-Grunwald-Giemsa or fixed in Carnoy's fluid and tested with Feulgen reagent.

Electron microscopy. Monolayers of cells on 35 mm plastic petri dishes were fixed with 2% glutaraldehyde in 0.05 M phosphate buffer at pH 7.4 and 1% OsO₄ in Millonig's phosphate buffer (9), then bits of plastic were embedded in Epon 812 *in situ* as described previously (10). Thin sections were obtained from the cells showing CPE and stained with uranyl acetate and lead citrate (11).

Results. Guinea pig embryo cell cultures were infected with two strains of V-Z virus in WI-38 cells. Both strains produced focal areas of syncytia and rounded cells with intranuclear inclusions. The areas remained focal for at least 10 days. Following 3 to 4 passages of these viruses using intact cells, the CPE became more generalized. When GP cultures were infected at a high m.o.i. (>1), small syncytial cells with intranuclear inclusions were seen within 24 to 48 hr. At Day 3 post-infection (pi) the CPE involved the whole monolayer which consisted of giant cells with many nuclei arranged in a ring and containing inclusion bodies (Fig. 1). During the following days big vacuoles appeared inside the cytoplasm and cells began to detach from the monolayer at Days 4 to 5. Complete degeneration of the monolayer occurred around Day 10 pi. Feulgen staining was lightly positive on Days 1 and 2 pi, becoming strongly positive 3 days pi and thereafter.

The susceptibility of GP cells to V-Z virus infection was first tested by comparing the viral infectivity titer obtained in GP cells to the titer obtained in WI-38 cells, by injecting both cell systems with 10-fold dilutions of inocula containing intact WI-38 infected

cells. The titers detected by the end point method were identical.

The growth cycle of 1991 virus was investigated after 40 passages in GP cells. GP monolayers in milk dilution bottles were infected with 2 ml of infected cell suspension (5×10^4 PFU/ml). After 90 min adsorption at 37° the inocula were removed, the monolayers were washed and incubated in fresh medium at 37°. The viral infectivity titers of media and cells before and after sonic disruption were detected by plaque assay in both GP and WI-38 cells at different time intervals pi. In these experimental conditions full CPE occurred in GP cells 3 days pi. The results of a representative experiment are shown in Fig. 2. The viral titers obtained in GP and in WI-38 cells were the same. New viral progeny appeared at Day 2 and maximal infectivity was detected at Day 3. Thereafter viral titer decreased slowly. Cell-free virus could not be demonstrated, either in the media or in the disrupted cells before or after Day 3. Cell-free virus was detected in small amounts (50 PFU/ml) when the infectivity titer of the intact cells reached the peak (4×10^5 PFU/ml).

The antigens of V-Z virus were sought in GP cells by neutralization, complement fixation, gel precipitation and fluorescent antibodies tests. Cell-free virus (10 PFU/culture) was totally neutralized by convalescent serum; but not at all by acute serum. The titer of V-Z CF antigen obtained from infected GP cells was $\frac{1}{8}$ as against a titer of $\frac{1}{32}$ obtained in WI-38 cells. No precipitin antigens could be detected with the virus grown in GP cells, whereas the virus obtained from WI-38 cells showed precipitin antigen even when diluted 1:16. GP infected cultures, stained 3 days pi for fluorescent antigens with convalescent human serum, showed a strong cytoplasmic fluorescence which was stronger in the perinuclear area, and positive intranuclear inclusions. Acute serum was negative for FA. Controls consisting of infected cells stained with antihuman γ -globulin and infected cells were also negative.

Attempts to infect primary rabbit kidney cells with V-Z virus grown either in GP cells or in WI-38 cells by using intact infected

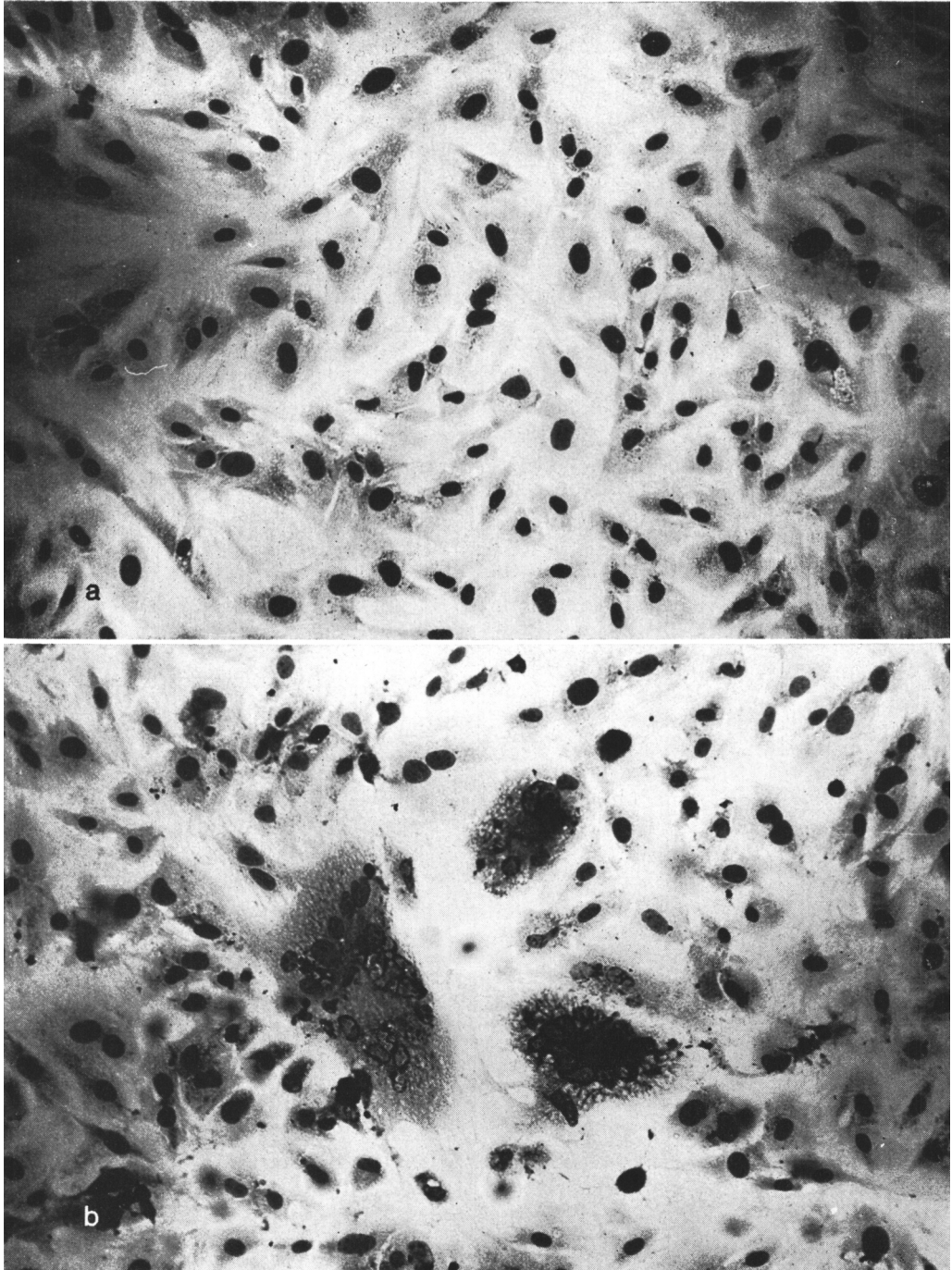


FIG. 1a. Normal guinea pig cells. (b) Varicella infected guinea pig cells 3 days postinfection.

cells were without success.

Newborn guinea pigs were injected either ip or ic with a suspension containing GP

infected cells. Control animals were injected with uninfected cells. Animals were observed daily and sacrificed after 1 mo. Brain, lung,

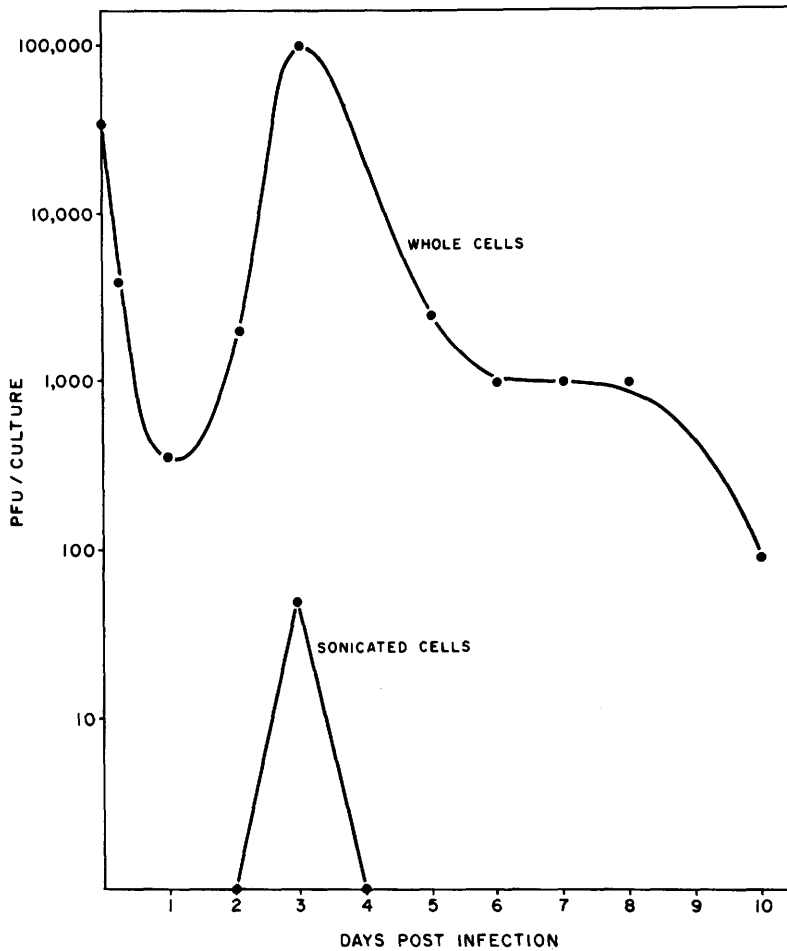


FIG. 2. Growth of varicella virus in guinea pig cells.

liver, spleen, and kidney were removed and examined for the presence of intranuclear inclusions in their cells. Brain, lung and spleen were also examined for the presence of virus. For this purpose WI-38 cells and GP cells grown in tubes were inoculated with small pieces of the organ and cultures were observed for CPE as long as possible (4 wk). Examination of the organ sections and attempts at virus isolation using GP and WI-38 cells did not reveal any virus. The sera were negative for V-Z and CF antibodies. Electron microscopic observation on the second day pi revealed the presence of intranuclear aggregation of herpes-type virus capsids with or without cores. Besides these intranuclear particles, numerous particles were encountered in the cytoplasm. The majority of the intracyto-

plasmic particles were contained in the vacuoles and occasionally, these cytoplasmic particles were seen to be encased in an osmophilic substance. Pleomorphism of virions in the cytoplasm and in the extracellular space indicated the similarity of this virus to varicella-zoster viruses rather than to herpes simplex.

Discussion. V-Z virus grows in guinea pig cells. The evidence for this statement is as follows: The CPE was syncytial and there were Feulgen-positive Cowdry type A inclusions. Herpes-type virus particles were seen on electron microscopic observations. Passage of GP virus to WI-38 resulted in a CPE typical of V-Z. Convalescent serum neutralized the guinea pig virus, and revealed fluorescent antigens when used in an indirect

fluorescent antibody test. V-Z CF and antigen was demonstrated in GP cells, although for unknown reasons precipitin antigen was lacking. Finally, the growth curve of the virus in GP cells was similar to that in WI-38 cells, and GP cells and WI-38 were similar in sensitivity to the virus.

The inability to transmit the GP virus to rabbit cells differentiates it from the herpes-like virus isolated by Hsiung, Kaplow and Booss (12) from guinea pigs, or herpes simplex virus. Newborn guinea pigs resisted infection.

Söltz-Szöetz (2, 3) and Sefcovicova (4) failed to demonstrate cell-free virus in infected guinea pig cells, but by sonication we produced cell-free virus from infected guinea pig cells 3 days pi.

The change in appearance of the CPE after passage might be due to the presence of variants in the original population and the selection of one by growth in GP cells. Nii and Maeda (13) noted this occurrence with V-Z virus in Vero cells.

Summary. Varicella-zoster virus was shown to replicate and to cause a cytopathic effect in guinea pig embryo cell culture, characterized by syncytia and intranuclear inclusions. Small amounts of cell-free virus were

released from the cells, which also produced CF antigen. Electron microscopy showed herpes-type virus particles in the infected cells.

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