Electron Microscopic Studies on the Development of Vesicular Stomatitis Virus

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With 12 Figures

(Received July 17, 1967)

Vesicular stomatitis virus (VSV) is essentially an unclassified virus. Suggestions have been made by some (Mussgay and Suárez, 1962) to group it with the arboviruses, while others (McCombs et al., 1966) have, on the basis of biophysical studies, emphasized certain similarities between VSV and typical myxoviruses. Simpson and Hauser (1966) have prepared a list of animal as well as plant viruses that share similar morphological properties with VSV.

One of the areas of study that needs further clarification is on the mode of development of VSV in infected cells, since previous reports on the subject have been contradictory. Reczko (1960) reported virus maturation in delimited cytoplasmic granular areas in cells of the chorioallantoic layers of the developing chick embryo. In HeLa cells Stone et al. (1961) demonstrated virus particles within cytoplasmic vesicles and also free in the cytoplasm, while Howatson and Whitmore (1962) stated that the principal site of virus maturation in L cells is at the cell membrane, although they were able to demonstrate a few virus particles in cytoplasmic vesicles also. In KB cells the principal site of VSV maturation was reported to occur inside cytoplasmic vacuoles and in cytoplasmic membranes (Mussgay and Weibel, 1963).

The discrepancies in the above reports may be due to differences in the cell types used.

In the present study, directed towards clarification of the mode of replication of VSV, chick embryo fibroblasts (CEF) were the selected

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cultures. This choice was made because of the higher susceptibility of CEF to VSV infection as compared to other cell types (McClain and Hackett, 1958) and promised to provide more favourable conditions for VSV replication, resulting, it was expected, in a clearer picture of the developmental events of the virus.

The results obtained in this study provide evidence for a close similarity between the development of VSV and that of rabies virus.

**Materials and Methods**

**Virus.** The Indiana strain of VSV was kindly provided by Dr. M. S. Mahdy of this laboratory. The virus was originally obtained through the courtesy of Dr. G. F. Whitmore of Princess Margaret Hospital, Toronto, as L cell preparation. It has been maintained in CEF and purified by the plaque technique.

For the study of the development of the virus the harvested culture fluids were titrated in primary CEF cultures. Serial dilutions of each fluid were made in HB-597 (Farrell and Reid, 1959) and 0.2 ml amounts of each dilution were inoculated into 4 culture tubes. In order to minimize the inactivating effects of light (Skinner and Bradish, 1954) all the operations were carried out under subdued light. The TCID\(_{50}\) was calculated by the Kärber's method after 48 hours incubation at 37°C in a roller drum incubator. Plaque assays in CEF monolayer cultures were set up concurrently with the same volume of inoculum.

**Tissues.** Chick embryo fibroblasts were prepared from 11-day-old embryos. The growth medium consisted of Medium HB-597 supplemented with 4% fetal calf serum. Falcon plastic bottles were seeded with 7 ml of cell suspension containing 6 \( \times \) 10\(^5\) cells per ml, while culture tubes received 1 ml each of the suspension. Complete cell sheets were formed after 24 hours incubation at 37°C. However, 48-hour-old cultures were used as a routine.

A few tests were also performed with African green monkey kidney cells (AGMK), using 2 \( \times \) 10\(^5\) cells per ml.

**Plaque assay.** The overlay medium consisted of 2% lactalbumin hydrolysate in 4 \( \times \) concentrated Hank’s balanced salt solution (25.0 ml), fetal calf serum (4.0 ml), 1:1000 neutral red solution (3.0 ml), 4% solution of sodium bicarbonate (7.0 ml), penicillin stock solution containing 1 \( \times \) 10\(^5\) units per ml (0.4 ml), streptomycin stock solution with 5 \( \times \) 10\(^4\) \( \mu \)g per ml (0.2 ml). The volume was made up to 50.0 ml by adding 10.4 ml of sterile distilled demineralized water. To this was added an equal volume of a solution of Difco Noble agar to give a final agar concentration of 1.0%. After virus adsorption at 37°C for 1 hour, 8.0 ml of the complete overlay medium was added to each bottle. The plastic covers were replaced with rubber stoppers and the bottles were incubated at 37°C. Though plaques usually appeared after 24 hours, they were only counted after 48 hours incubation.

With AGMK monolayers, a second overlay was usually needed, inspite of this the system did not prove as successful as the CEF monolayers. The use of AGMK was thus discontinued.

**Infection of chick embryo cell monolayers.** Monolayer cultures of chick embryo fibroblast cells were infected at a multiplicity of infection (m.o.i.) of about 1. After adsorption at 37°C for 1 hour the cell sheet was washed