

NOTES

Fusion of Vesicular Stomatitis Virus with the Cytoplasmic Membrane of L Cells

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At an early stage in infection, vesicular stomatitis viruses were attached to the surface of L cells by fusion of the viral and cell membranes.

It has been proposed by Morgan et al. (3, 4, 5) that membrane-enclosed viruses such as herpes, influenza, and Sendai viruses infect cells by fusing with the cytoplasmic membrane and releasing their nucleoprotein core into the cytoplasm of the cell. In view of these reports, we decided to examine this process with vesicular stomatitis virus (VSV), since this is a membrane-enclosed virus of quite different structure.

The Indiana serotype of VSV was grown in cultures of L cells, and the number of virus particles was estimated by electron microscopy counts, as previously described (2). The cells were infected at 4 C at a multiplicity of 10^8 by sedimenting virus-cell mixtures at $18,500 \times g$ for 15 min onto a flat agar surface (1, 8). The infected cells were suspended in cold minimal essential medium (Gibco) without serum and samples were placed in 100- μ liter capillary tubes which were sealed at one end. Cells were incubated by placing the tubes in a water bath set at 37 C for various time periods (2 to 30 min). The incubation period was terminated by placing the tubes in an ice bath; the contents were then transferred to microcentrifuge tubes half filled with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). The tubes were then centrifuged for 10 sec in a model 512 Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) operated at 70 v to reduce the speed, and the pellets were removed and allowed to stand in fresh glutaraldehyde solution for 2 hr. They were then washed repeatedly and fixed in 2% OsO_4 in the same buffer for 2 hr. The pellets were then stained for 2 hr with 0.5% uranyl acetate in acetate-Veronal buffer (7) to increase the contrast of the virus and cellular membranes. The pellets were embedded in Epon 812, and sections were poststained with saturated uranyl acetate in 1% sodium borate for 7 min at

60 C and with lead citrate. Electron micrographs were taken with a Siemens Elmiskop IA at an initial magnification of 46,000.

The electron micrographs serve to illustrate the adsorption and membrane fusion steps which we believe precede the entry of viral nucleoprotein into the cell. Figures 1 and 2 show the appearance of the virus particle in transverse and longitudinal sections; the morphology of the particle is essentially as described by Nakai and Howatson (6). In samples which were not incubated at 37 C (Fig. 3-4), virus particles were often observed in close proximity to the cell membrane. At this stage, prior to incubation at 37 C, no particular orientation of the virus particle was observed, although in these micrographs the virus seems to be adsorbed at its blunt end. The viral and cell membranes are separated by a minimal gap of 13 nm, which is slightly greater than the length of the spikes (10 nm) of the virion.

The micrographs shown in subsequent figures (Fig. 5-12) are of samples which were incubated at 37 C for 8 to 15 min as described in the figure legends. In Fig. 5, a virion appears to be in contact with the cell membrane in such a fashion that the outer, dense layers of the cell and virus membranes appear fused. The virions shown in Fig. 6-7 appear to be connected to the cell membranes by a bridge of material, and neither the cellular nor the viral membranes are well defined. Similar fusion stages were described by Palade and Bruns (7) for the fusion of vesicular membranes. The origin of this bridge may be the membranous extensions which are often observed at the blunt end of virus particles (Fig. 5) and are presumed to originate during the synthesis of the virus. Figures 8-11 illustrate complete fusion of the viral and cell membranes. The viral membrane is continuous with, and completely indistinguishable from,

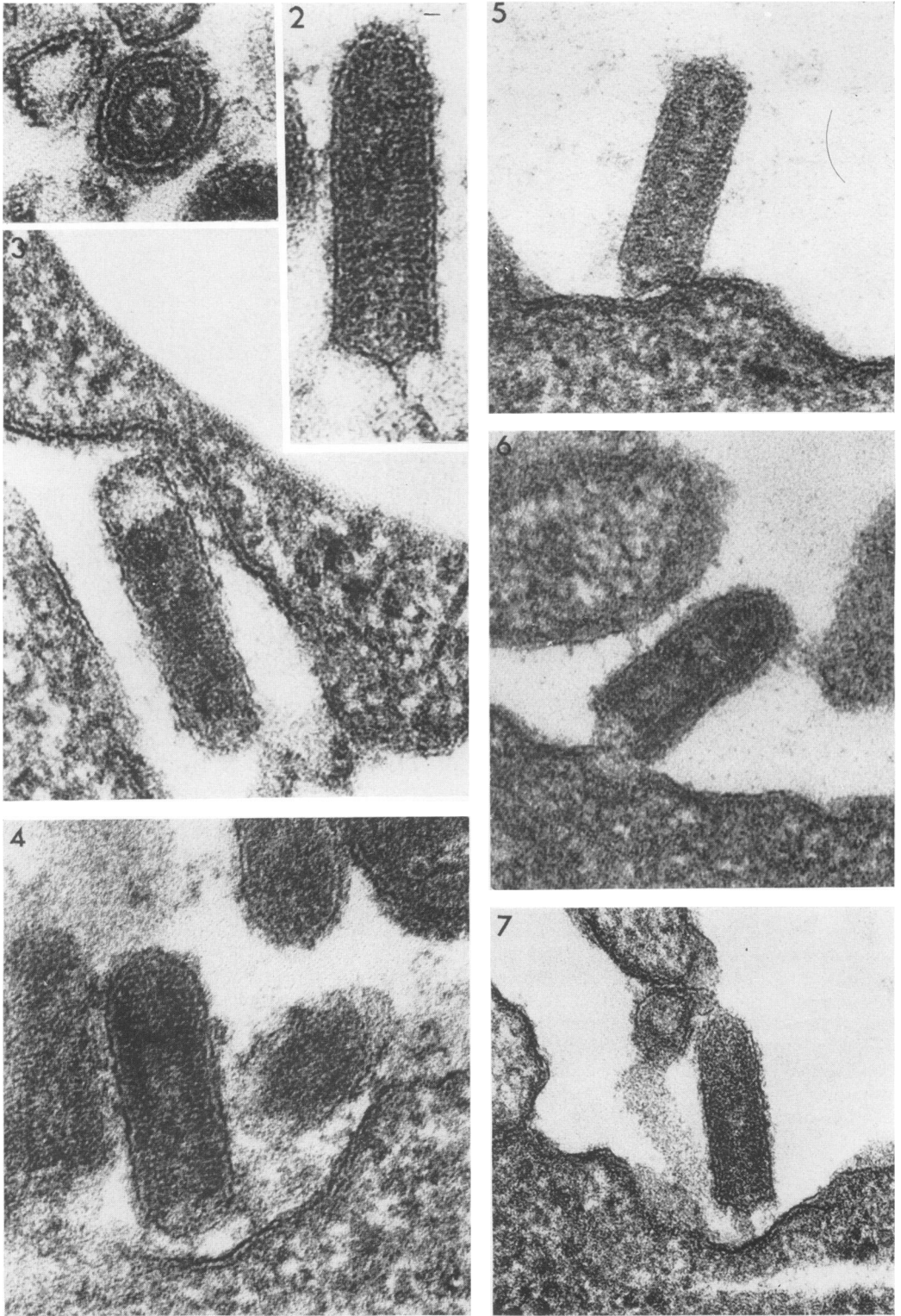


FIG. 1-7. Transverse and longitudinal section of VSV. $\times 240,000$ (Fig. 1 and 2). Virus was sedimented onto L cells at 4 C, no incubation. $\times 207,000$ (Fig. 3 and 4). Virus treated as in Fig. 3 and 4, but incubated at 37 C for 10 min. $\times 184,000$ (Fig. 5 and 6). $\times 138,000$ (Fig. 7).

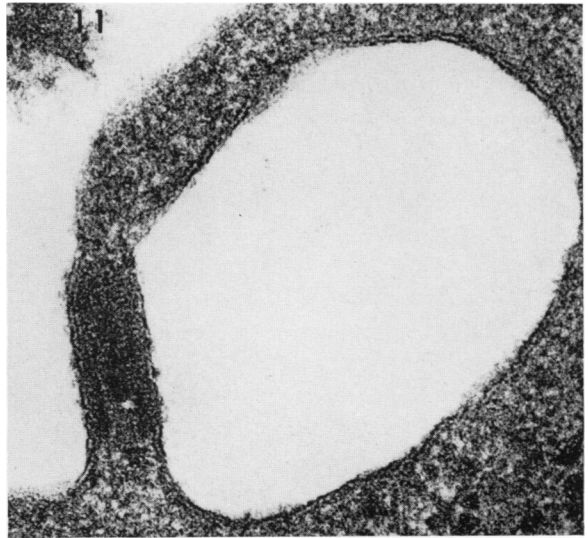
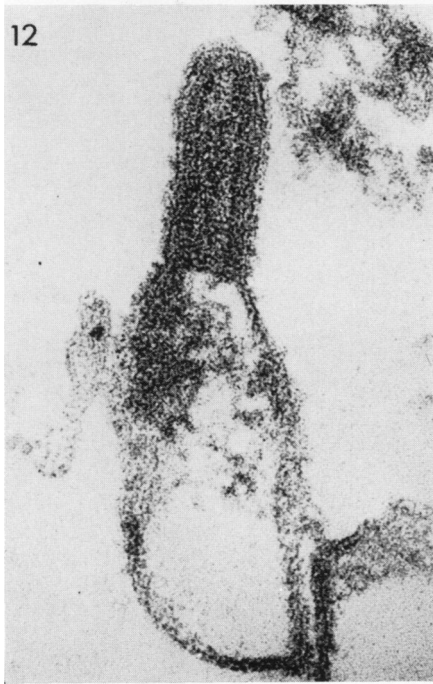
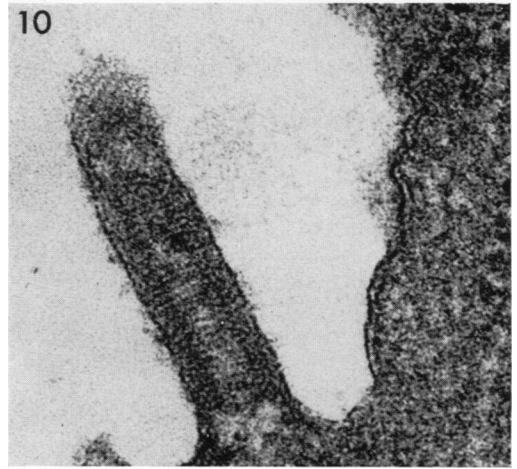
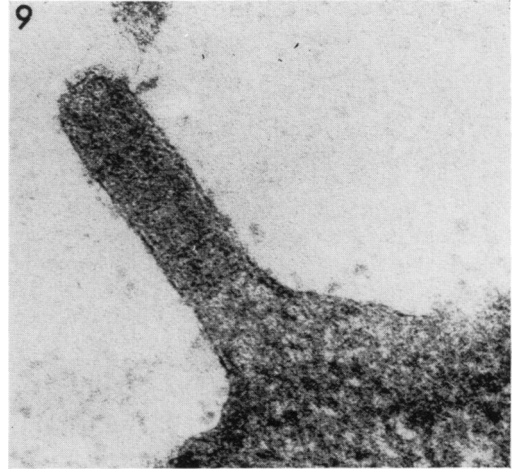


FIG. 8-12. Virus sedimented onto L cells at 4 C and incubated at 37 C for 15 min. $\times 230,000$ (Fig. 8). $\times 161,000$ Fig. 9 and 10. Virus treated as in Fig. 8, but incubated for 8 min. $\times 161,000$ (Fig. 11). Virus was treated as in Fig. 11; note fusion with membrane of dead cell. $\times 161,000$ (Fig. 12).

the cell membrane. The transverse striations of the virus make it easily recognizable. Double fusion (Fig. 11) was frequently observed, but fusion at the rounded end alone was never observed. We believe the final step of penetration to be the release of the nucleoprotein into the cytoplasm. This could not be observed because the density of the cytoplasm is similar to the density of the viral nucleoprotein. No empty shells of attached virions were observed extending from the surface of the cell. We noted the fusion of a virus with a fragment of a lysed cell (Fig. 12) in which no release of nucleoprotein is apparent. This has also been observed with influenza virus by Morgan and Rose (3).

No membrane fusion of the type shown in Figures 8–12 was observed in preparations which were not incubated at 37 C after infection at 4 C. Fusion was observed most frequently in cell-virus preparations which were incubated from 8 to 15 min at 37 C but was also observed at 2 min, the shortest incubation period tested.

In a recent study employing the same virus-cell system, Simpson et al. (9) observed phagocytosis of virus particles but found no fusion of viral and host-cell membranes at the surface of the cell. These authors employed somewhat different conditions, in that infection was carried out in suspension and serum was included in the infection medium. These factors may favor phagocytosis in this cell system.

We have also observed virus particles in what appeared to be phagocytic vacuoles, although this was observed less frequently than the fusion de-

scribed above. The virus particles observed in these vacuoles did not appear to be disrupted. In some cases, the viral and vacuole membranes appeared to be fused similarly to that shown in Figures 8–11. Since membrane fusion under these circumstances might be more efficient, phagocytosis may have played some role in the infectious process.

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LITERATURE CITED

- Galasso, G. J. 1967. Enumeration of VSV particles and a demonstration of their growth kinetics by electron microscopy. *Proc. Soc. Exp. Biol. Med.* **124**:43–48.
- Heine, J. W., and G. J. Galasso. 1968. Effects of X-irradiated aqueous solutions on vesicular stomatitis virus. *J. Virol.* **2**:1147–1153.
- Morgan, C., and H. M. Rose. 1968. Structure and development of viruses as observed in the electron microscope. VIII. Entry of influenza virus. *J. Virol.* **2**:925–936.
- Morgan, C., H. M. Rose, and B. Mednis. 1968. Electron microscopy of herpes simplex virus. I. Entry. *J. Virol.* **2**:507–516.
- Morgan, C., and C. Howe. 1968. Structure and development of viruses as observed in the electron microscope. IV. Entry of parainfluenza I (Sendai) virus. *J. Virol.* **2**:1122–1132.
- Nakai, T., and A. F. Howatson. 1968. The fine structure of vesicular stomatitis virus. *Virology* **35**:268–281.
- Palade, G. E., and R. R. Bruns. 1968. Structural modulation of plasmalemmal vesicles. *J. Cell Biol.* **37**:633–649.
- Sharpe, D. G., and K. O. Smith. 1960. Rapid adsorption of vaccinia virus on tissue culture cells by centrifugal force. *Proc. Soc. Exp. Biol. Med.* **104**:167–169.
- Simpson, R. W., R. E. Hausek, and S. Dales. 1969. Viropexis of vesicular stomatitis virus by L cells. *Virology* **37**:285–290.