The Virus Envelope in Cell Attachment

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The mechanism of penetration and uncoating of enveloped animal viruses has recently been the subject of renewed controversy. Electron microscopic studies have led a number of investigators to conclude that penetration takes place by cellular engulfment (viropexis) of intact virus particles (Dales & Choppin, 1962; Mussgay & Weibel, 1962; Dales, 1963; Simpson, Hauser & Dales, 1969). Other investigators have concluded that enveloped animal viruses penetrate by fusion of the virus envelope with the host cell plasma membrane (Morgan & Howe, 1968; Morgan, Rose & Mednis, 1968a, b; Heine & Schnaitman, 1969). We now report on the role of the Sindbis virus envelope in the attachment of virus to host cells.

Friedman & Berezesky (1967) and Sreevalsan & Alien (1968) noted that nucleocapsid components obtained from cytoplasmic extracts of arbovirus-infected cells or by detergent treatment of intact virus particles were non-infective for chicken embryo cells. However, the RNA released from arboviruses by detergent treatment is infective when assayed appropriately (Richter & Wecker, 1963; Nakamura & Eguchi, 1968). To determine why the nucleocapsid was non-infective, the ability of the nucleocapsid component of the virus particle to attach to chick embryo cells was studied.

To prepare labelled virus with the majority of the $^{32}$P in virus RNA, chick embryo cell monolayers were incubated for 2 hr with phosphate-free Eagle's medium containing 5 $\mu$g./ml. actinomycin D (Merck, Sharp and Dohme). Cultures were infected, virus adsorbed for 1 hr, and then overlaid with phosphate-free Eagle's medium containing 25 $\mu$C/ml. of carrier-free phosphoric acid ($^{32}$P; New England Nuclear). Culture fluid containing virus was harvested 12 hr after infection. The virus was purified by differential centrifugation and zonal sedimentation through a 15 to 30 % (w/w) linear sucrose gradient (Strauss, Burge, Pfefferkorn & Darnell, 1968). The purified virus preparation was diluted in 0.005 M-tris, 0.1 M-NaCl buffer, pH 7.4, and then treated with a final concentration of 0.2 % sodium deoxycholate (DOC) for 20 min. at 4 °. This preparation was then centrifuged again through a 15 to 30 % sucrose gradient to separate the nucleocapsid fraction (Strauss et al. 1968).

The nucleocapsid component of Sindbis virus obtained by DOC treatment of intact particles was not infective when tested in the conventional plaque assay (Pfefferkorn & Hunter, 1963) in chick embryo cells. DOC treatment reduced the infectivity of the purified virus preparation from $2 \times 10^9$ to less than $10^2$ p.f.u./ml. Nucleocapsid preparations obtained from extracts of infected chick embryo cells and purified by sedimentation through linear sucrose gradients were also not infective. To determine why the nucleocapsid was non-infective, attachment was studied with $^{32}$P-RNA-labelled nucleocapsid and whole virus preparations. Monolayer chick embryo cultures were washed and 0.2 ml of the sucrose gradient fraction containing $1 \times 10^4$ counts/min. of $^{32}$P-RNA-labelled virus or of nucleocapsid was added to duplicate cultures. At different times after infection, the cells were washed ten times with Eagle's medium, collected, washed by low-speed centrifugation and disrupted with a glass Dounce homogenizer. The amount of radioactivity precipitable by trichloracetic acid from the total cell homogenate was determined. At the end of the 60 min. adsorption period, 17 % of the added virus label was cell-associated, compared with attachment of $1.5$ % of the labelled nucleocapsid (Fig. 1).

To demonstrate that the RNA was not damaged by DOC treatment, infectious RNA was
extracted from Sindbis virus particles and nucleocapsids by cold phenol sodium dodecyl sulphate (Wecker, Hummeler & Goetz, 1962) and assayed by the plaque technique on chick embryo monolayer cultures treated with hypertonic saline (Richter & Wecker, 1963). The amounts of infectious RNA obtained from nucleocapsids and from particles were equivalent. This is consistent with the observations of Richter & Wecker (1963) and Nakamura & Eguchi (1968) who used DOC and phenol to extract infectious RNA from arboviruses.

Fig. 1. Attachment of chick embryo cells of $^{32}$P-labelled, partially purified Sindbis virus and $^{32}$P-labelled nucleocapsid preparations; ▲—▲, attachment of unlabelled virus; ●—●, $^{32}$P-RNA-labelled virus; ▼—▼, $^{32}$P-RNA-labelled nucleocapsid.

A direct comparison of the ability of infectious RNA and of nucleocapsids to penetrate chick embryo cells could not be made since the isolated nucleocapsid component obtained by DOC treatment of extracellular Sindbis virus or extracted from infected cells was structurally unstable. When the osmotic strength of the suspending sucrose buffer was reduced, the majority of the nucleocapsid components ruptured and released the virus RNA.

The nucleocapsid component of the Sindbis virus particle was unable to attach to host cells and was therefore unable to penetrate and to initiate infection. Since the RNA inside the nucleocapsid was still infective it appears that the virus envelope was necessary for attachment to host cells. Host range studies with defective particles of Rous sarcoma virus encapsulated with different helper virus envelopes have also indicated the importance of the envelope in attachment to host cells (Vogt & Ishizaki, 1965).

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