ASSEMBLY OF 229E VIRIONS IN HUMAN EMBRYONIC LUNG FIBROBLASTS AND EFFECTS OF INHIBITION OF GLYCOSYLATION AND GLYCOPROTEIN TRANSPORT ON THIS PROCESS

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Assembly of virions in 229E infected cells were studied along with the effect(s) of an inhibitor of glycosylation (tunicamycin) and an inhibitor of glycoprotein transport (monensin) on this process. The results of these studies are summarized herein.

There were no significant differences observed between untreated and monensin- or tunicamycin-treated cells early in the infectious cycle. One hour post infection (PI) virus particles were seen in close proximity to the plasma membrane and projections from the viral envelope, presumably viral glycoproteins, appeared to be making membrane contact. A few particles were seen to be in various stages of being engulfed by a process of viropexis. By two hours PI a small number of intact spherical enveloped particles (80-150nm) were seen within intracytoplasmic vacuoles, some, however, were oval or dumb-bell shaped. All particles, consisted of a double membrane surrounding a core of varying electron density. Densely stained viral particles were observed in lysosomes in untreated and treated cells, but lysosomal activity was more pronounced in treated cells.

At 6 hours PI when viral protein synthesis was first detected (1) and before viral assembly was observed, roughly circular, electron lucent structures with dense limiting membranes (2) were seen in untreated and treated cells.

Budding of virus particles from the intracytoplasmic membranes was first observed in all cells at 8hr PI. In tunicamycin treated

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cells, aggregation of ribosomes and increased numbers of virions within the golgi complex were observed. Virions assembled in tunicamycin treated-cells were enveloped but the particles appeared smooth, whereas, in untreated or monensin-treated cells delicate projections radiated from the viral membrane.

Viral particles assembled in monensin-treated cells contained translucent cores. Similar particles were observed in untreated and tunicamycin-treated cells but they were far less numerous. Particles having a translucent core are not devoid of nucleic acid since the ribonucleoprotein complex was seen underlying the capsid structure. It is probable that the translucent particles are immature virions.

Vacuolization of all cells increased with time. But, monensin caused the formation of large dilated vesicles, which did not contain any viral particles.

At 12hr. PI cytoplasmic inclusions similar to those described by Oshiro et al (3), composed of densely staining particles within a granular matrix and enclosed by a double membrane were seen in cells.

As early as 12hr PI in untreated cells, large virus containing vacuoles could be seen to be fusing with the plasma membrane and in the process of rupturing, thereby, releasing virions. Numerous virions were observed in extracellular spaces surrounding untreated cells. But, in treated cells membrane bound aggregates of virions were observed in the extracellular spaces. By 24hr PI many untreated cells were lysed thus releasing virus. Of particular interest was the large accumulation of spherical and dumb-bell shaped particles in the perinuclear space late in the infection.

From these studies it may be concluded that glycosylation and glycoprotein transport effect the agress of 229E virions but not the assembly.

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