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Intracellular Development of Vaccinia Virus.* (19509)

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The cytoplasmic inclusions formed in cells infected with vaccinia virus have been recognized for 60 years and extensively studied with the optical microscope. It is generally accepted that the inclusions consist of masses of elementary bodies, but the manner in which they are formed is still uncertain and several conflicting views are held by various authors. The literature on this subject has been reviewed by Van Rooyen and Rhodes(1).

Recent technics of making ultra-thin tissue sections(2-5) have been utilized by Bang(6) and Wyckoff(7), who have each published electron micrographs made from thin sections of infected chorioallantoic membrane. They clearly show the inclusions to be aggregates of elementary bodies. The development of the inclusion bodies has not been reported, however, and it is with this phase of the problem that the present paper is concerned.

Methods. Ten to 12-day embryonated eggs were inoculated on the chorioallantoic membrane with approximately 10^8 infectious doses of virus in order to have as many cells infected as possible. The suspensions of virus were prepared and the inoculations were carried out according to the method of Briody and Stannard(8). The virus was a commercial calf lymph strain of vaccinia (Lederle) passed once in rabbit skin and 3 times in eggs. Membranes were harvested 48 hours after inoculation and fixed for 24 hours in 4% formalin buffered with M/10 phosphate at pH 7.0. The tissue was dehydrated in graded alcohols, starting at 10%, and imbedded in 1:8 methyl:butyl methacrylate. Polymerization was carried out at 60°C overnight using 2-4 dichlorobenzoyl peroxide as catalyst(3). Sectioning was performed on a Spencer microtome modified with a 1:20 wedge and driven by a motor as described by Hillier and Gettner(4). A glass knife(5) was used for cutting. The sections were lifted from the water with collodion covered grids and the methacrylate was removed from the sections with toluene before examination with an RCA electron microscope, Model EMU.

Results. In the ectodermal layer of 48-hour membranes nearly every cell examined was infected with virus and in any one group of cells, inclusions ranging from a few particles to those occupying most of the cytoplasm could be observed. In almost every cell containing a small or medium size inclusion, the inclusion was located at the nuclear membrane and consisted of particles of varying sizes enmeshed in a dense matrix (Fig. 1-5). The matrix appeared less dense in the larger inclusions. Recognizable virus was never seen within the nucleus.

Occasionally, cells were seen to be ringed with virus particles which filled the intercellular spaces (Fig. 5). Cells in such areas contained little and, occasionally, no detectable virus. In other areas, cells appeared to have

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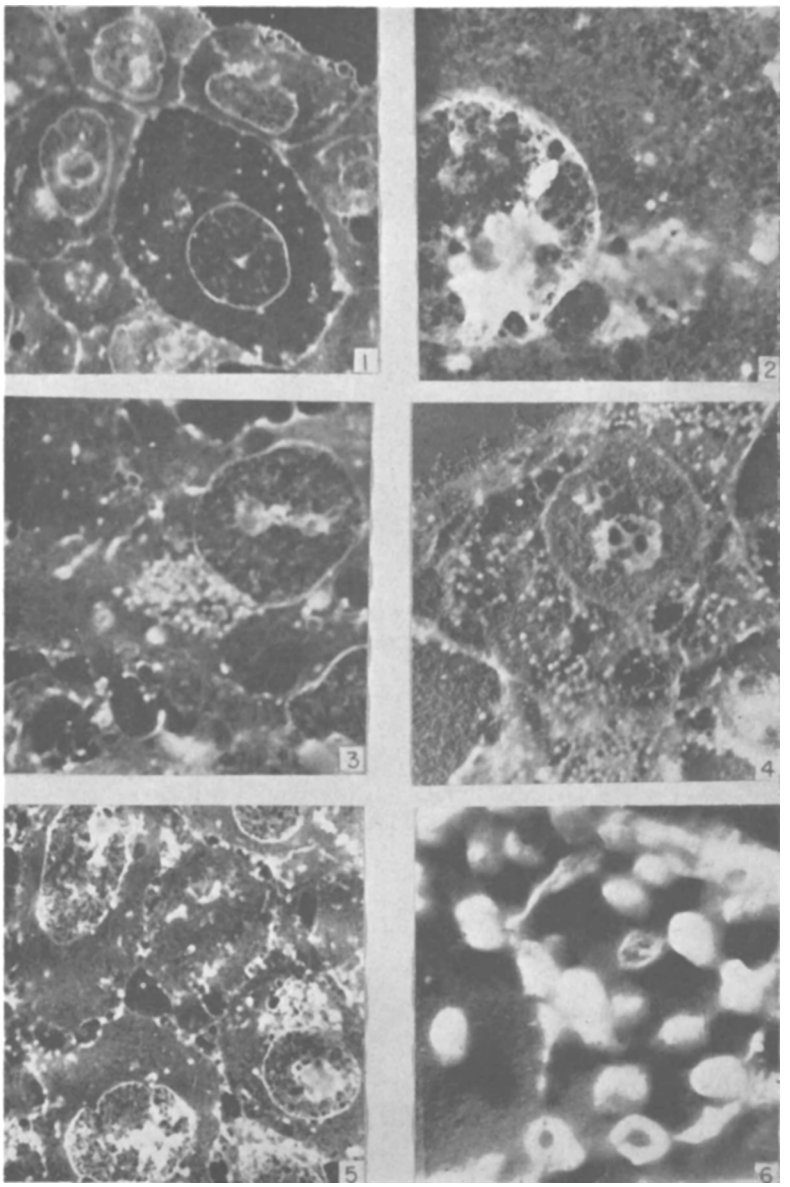

FIG. 1. Ectodermal cells of the chorio-allantoic membrane infected with vaccinia virus. The large cell in the center has only a few particles of virus and is probably recently infected. Other cells show varying stages of development of inclusion bodies. × 2300.

FIG. 2. A small inclusion showing definite attachment to nucleus. × 6000.

FIG. 3. A fairly large inclusion. The surrounding matrix is less dense. Note mitochondria in both filamentous and swollen forms. × 4000.

FIG. 4. A cell filled with virus and apparently rupturing. Palladium shadowed. × 3000.

FIG. 5. General view of ectodermal cells most of which are surrounded by virus particles. This is possibly the result of the liberation of quantities of virus from neighboring cells. One cell to the right appears to be infected in the next cycle of virus growth, for a perinuclear inclusion is present. × 2300.

FIG. 6. Virus particles some of which have been sliced by the knife. Note dense wall of two particles and apparent internal structure in one. Nuclear membrane at upper right. Palladium shadowed. × 30000.

several isolated virus particles in the cytoplasm but no real inclusion bodies. (See large cell of Fig. 1). Multiple inclusions were seen infrequently, with the majority of cells containing only one.

The general appearance of the infected cells gave the impression that no obvious cytopathologic changes had taken place. Occasional swollen cells, as in Fig. 1, were rare and the vacuolation shown at the border of the same group of cells could be seen in normal tissue. Although the cells seen in Fig. 5 pulled away from each other leaving large intercellular spaces, the effect could not be ascribed to the infectious process *per se* because other infected areas had closely packed cells. Few recognizable mitochondria have been observed. The nucleoli of normal and infected cells varied considerably in size and shape but a role in infection was not apparent.

In tissue sections, the elementary bodies of vaccinia appeared spherical to ovoid but never brick-shaped with sharp angles (Fig. 6). In any one cell, particle size varied, ranging from 0.23 μ to nearly 0.28 μ in length. Many tore-like or doughnut shapes were seen and were interpreted as particles which had been twice sliced by the knife removing segments from both sides. Occasionally dense material was observed within such structures (Fig. 6). Central protuberances appeared on some particles suggesting the presence of a central mass similar to those found by Dawson and McFarlane after treating elementary bodies with pepsin(9).

Discussion. It is generally assumed that viruses are adsorbed to cells, penetrate the cell wall, multiply, and the new generations are released to infect other cells. The pictures presented here would support this theory. In Fig. 1 only a few particles are seen scattered through the cytoplasm. In Fig. 2 and 3, there are single inclusions of different sizes. Fig. 4 shows a cell filled with virus and apparently rupturing. In Fig. 5 the large amount of extracellular virus in an area in which cells contain only early inclusions, suggests an early phase of a second round of infection. It is possible to observe all phases of the development of the inclusion in a late lesion because vaccinia infection induces marked ectodermal proliferation and as new ectodermal cells are formed they become exposed to virus liberated from older cells.

The constant finding of inclusions in contact with the nuclear membrane suggests that their formation is in some way influenced by the nucleus. Ewing(10) concluded from his study of sloughed cells of vaccinia-infected rabbit cornea that the inclusion was continuous with the nucleus and was a result of the outward diffusion of nuclear material. Goodpasture, Woodruff, and Buddingh(11) emphasized the perinuclear position of inclusions in their classical work demonstrating that the inclusions consist of masses of elementary bodies.

The fact that the matrix in which the virus particles were imbedded was dense in small inclusions and almost absent in larger ones may be significant. If the nucleus is supplying nucleic acids, it might be expected that the loss of matrix material could be correlated with staining reactions. Several authors (12,13) have noted that the reactions of inclusions change with age from basophilia to eosinophilia and that there is also a progressive diminution in the intensity of the Feulgen reaction.

Bang(6) has suggested that there might be a relationship between mitochondria and vaccinia virus. Few mitochondria were seen in the present study (Fig. 1, 3, 4) but filamentous, swollen, and club-shaped intermediates occurred and no significant association with virus was noted. The plaque-like areas described by Wyckoff(7) in chorioallantoic cells infected with vaccinia were never seen, although somewhat similar areas have recently been described by Banfield *et al.*(14) in *Molluscum contagiosum*. Occasionally, particles about the size of mature virus could be seen in normal cells but virus could be clearly distinguished because of its greater electron density and uniformity of size and shape.

Summary. 1. Electron micrographs of tissue sections of chorioallantoic membrane infected with vaccinia virus were examined. Some cells were found to be almost filled with virus, others contained the virus particles grouped in single inclusions of varying sizes. Only a few virus particles were seen scattered

throughout the cytoplasm of cells in certain areas, and in other areas extra-cellular virus was found in large numbers. 2. Small or medium sized inclusions were always located at the nuclear membrane and in contact with it. The inclusions consisted of masses of virus particles imbedded in a matrix which was dense in small inclusions and less dense in larger inclusions. The virus particles were ovoid and varied in length from 0.23 μ to 0.28 μ. Sectioned virus particles indicate that all have a dense outer wall, and some could be observed to have an internal structure. 3. The possible relationship of these observations to the growth cycle of vaccinia virus has been considered.

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Concentration of Influenza Virus (PR8 Strain) by a Cation Exchange Resin.* (19510)

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The application of ion exchange resins to the separation of closely related chemical substances by adsorption or exchange of ions has proven in the past few years to be a valuable adjunct in the purification and concentration of biological products. Ion exchange resins have been used previously to purify partially a number of neurotropic viruses(1). The resins in this case were so adjusted that when suspensions of infected chick embryos and mouse brains were passed through columns of resin, inert nitrogenous material was removed and the virus appeared in the percolate fluid. Poliomyelitis and Theiler viruses have also been partially purified by adsorption of the viruses and inert nitrogenous material on anion exchange resins followed by selective elution of the viruses(2).

In the present experiments a method has been developed to concentrate rapidly and to purify partially the PR8 strain of influenza virus. It was observed that the virus was selectively adsorbed on columns of a specially prepared cation exchange resin. At the same time a large proportion of nitrogenous impurities in the starting material passed through the resin columns into the percolates. The virus could be subsequently eluted from the resin in a fraction of the original volume of

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