

## ELECTRON MICROSCOPY OF LEDNICE VIRUS IN CHICK EMBRYO CELLS

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*Summary.* — Replication of Lednice virus in chick embryo cells was studied for 72 hr after inoculation by infectivity titration and the indirect immunofluorescence technique. At 24 and 48 hr after inoculation, electron microscopy revealed spherical virions of uniform morphology, 80–105 nm in diameter, which were localized mostly extracellularly.

*Key words:* Lednice virus; M'Poko virus; electron microscopy; immunofluorescence

Lednice virus, repeatedly isolated in South Moravia from *Culex modestus* mosquitoes, belongs to the Turlock group of arboviruses and is antigenically closely related to M'Poko virus (Catalogue, 1978) which was originally designated Yaba 1 and under this name reported in our previous papers (Málková *et al.*, 1972, 1974).

Data of filtration experiments (Málková, 1972) suggested that the virus is large rather than small. The aim of the present study was to obtain more information about the size of the virus and its relationship to the cell.

*Virus and cell cultures.* As starting material, a lyophilized 20% suckling mouse brain suspension infected with Lednice virus strain 6118 Ms 3 (Málková *et al.*, 1974) was used. Two experiments were carried out on 3 days old chick embryo cell (CEC) monolayers grown in 300-ml Roux bottles. The cell counts for the calculation of the multiplicity of infection (MOI) were determined immediately before inoculation. The MOI in experiments 1 and 2 was 0.0006 and 0.0004 LD<sub>50</sub> per cell, respectively. After removal of growth medium, inoculum was added to the cultures for 1 hr at 37 °C, after which it was removed and the cultures washed three times with phosphate buffered saline, supplied with maintenance medium and incubated further at 37 °C.

*Virus assay.* Virus in pools of culture fluids harvested at a given interval was titrated intracerebrally (0.01 ml) in 1–2 days old suckling mice. A litter of 10 mice was used for each dilution. After an observation period of 14 days, the titres were calculated according to Reed and Muench. Virus in the cells was assayed by the indirect immunofluorescence (IF) technique in CEC monolayer tube cultures (Marhoul *et al.*, 1976).

*Electron microscopy.* Cells from infected and control cultures were scraped off mechanically from the bottle walls at 24, 48 and 72 hr after inoculation (p. i.) in exp. 1 and at 12, 24 and 48 hr in exp. 2. Cells from each bottle separately were centrifuged and the pellets fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 for 60 min at 4 °C, washed for 15 min in the same buffer and post-fixed in 1% OsO<sub>4</sub> in the same buffer for 60 min at room temperature. The cell pellets were dehydrated with acetone and embedded in Durcupan ACM (Fluka). Sections cut on an LKB III ultramicrotome were stained with uranyl acetate and lead citrate and examined in a JEM 7A electron microscope at 80 kV.

At 12 hr p. i., no virus was demonstrated in the culture fluid. Virus titres (log LD<sub>50</sub>/0.01 ml) in exp. 1 (exp. 2) were 1.6 (2.32) at 24 hr, 3.57 (4.41) at 48 hr and 3.15 (3.4) at 72 hr p. i.

First cytopathic changes appeared at 24 hr p. i., when islets of degenerated cells in exp. 2 were more numerous than in exp. 1. The situation was similar at 48 hr p. i., when about half of the monolayer in exp. 2 was destroyed. At 72 hr p. i., almost the whole monolayer was destroyed in either experiment.

Visualization of viral antigen was attempted only in exp. 1. At 12 hr p. i. the finding was negative (Fig. 1), at 24 hr p. i. 1% of cells showed fluorescence (Fig. 2), at 48 hr there occurred foci of 17–33% of fluorescent cells (Fig. 3) and at 72 hr the majority (80% or more) of the cells showed fluorescence (Fig. 4).

Electron microscopy revealed mostly spherical or slightly oval virus particles. These contained a highly electron dense material forming a nucleoid 60–74 nm in diameter, demarcated by a narrow light halo against the bounding membrane, the surface of which usually was not smooth. The size of the whole virions was 90–105 nm.

These virus particles of uniform morphology and size, never observed in control cultures, were detected in both experiments. In exp. 1 we found them at 24 and 48 hr p. i. mostly extracellularly and occasionally in what could be taken for cytoplasmic vesicles (Figs 5–7). But the possibility that these actually were extracellular virions among microvilli could not be excluded. In exp. 2 the virions were only found at 48 hr p. i., exclusively extracellularly. We never found them in cell nuclei. The virions usually occurred in groups of 2 to 6 particles (Fig. 7), but occasionally there were places with several dozens of particles (Fig. 8).

The finding of virions in CEC at 48 hr p. i. in both experiments coincided with the highest virus titre in the culture fluid. The finding at 24 hr p. i. in exp. 1 may be considered accidental in view of the virus titre in the culture fluid and the IF finding at this interval. At 72 hr p. i. the cells were already damaged so much that they were unsuitable for electron microscopic examination. To reach virus titres that would offer a chance for its demonstration in the electron microscope we had to use a low MOI in view of the marked autointerference phenomena observed with Lednice virus (Marhoul *et al.*, 1976; Málková, unpublished results).

#### References

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*Explanation of Micrographs (Plates III–V):*

*Figs 1–4.* Indirect immunofluorescence staining of Lednice virus-infected CEC.  $\times 360$ .

1 – Negative finding at 12 hr p. i.

2 – Occasional fluorescent cells, 24 hr p. i.

3 – Foci of fluorescent cells, 48 hr p. i.

4 – The majority of cells showing fluorescence at 72 hr p. i.

*Figs 5–8.* Electron micrographs of ultrathin sections of Lednice virus-infected CEC.  $\times 66\ 250$ .

5 – Two virions between microvilli, 24 hr p. i.

6 – Virus particles in cytoplasmic vesicles, 24 hr p. i.

7 – Virus particles localized extracellularly and between microvilli, 48 hr p. i.

8 – Part of an area with numerous extracellularly localized virus particles, 48 hr p. i.