An Electron Microscope Study of Virus-like Particles in Chick Embryo and L Cell Cultures

(Accepted 29 August 1967)

Latent virus infections in chickens have been recognized for several years. Many investigators have seen spherical, virus-like particles in normal chicken cell cultures, and have suggested that they might have been the virus of avian lymphomatosis, which occurs frequently without producing disease symptoms and is transmitted trans-ovarially.

Virus-like particles were also observed during the present study of the ultrastructure of primary monolayers from 10-day-old chick embryos cultured in Eagle's (1955) medium supplemented with 3% calf serum. Cultures were prepared according to the method of Dulbecco & Vogt (1954) from a Heisdorf and Nelson hybrid White Leghorn commercial breed of chickens; 56% of the cells had virus-like particles either at their periphery or internally. Pl. 1, fig. 1 shows a group of extracellular particles in a chick embryo culture incubated for 4 days; their mean diameter was 1060 Å (Table 1). Each particle consisted of a dense central nucleoid surrounded by a membrane, then a clear area finally bounded by an outer membrane. Both the chicken and murine virus-like particles (discussed later) were fixed with either OsO₄ or glutaraldehyde followed by OsO₄ post-fixation, and then studied with a Siemens Elmiskop I electron microscope. Sections were cut on a Servall MTI Porter-Blum ultramicrotome. The particles were almost exclusively extracellular (Pl. 1, fig. 2). Occasionally they were seen in cytoplasmic vacuoles near the cell surface, and, rarely, in cytoplasmic vacuoles near the nucleus (Pl. 1, fig. 3). When chick embryo cultures were incubated for 4 days before fixation instead of one, the particles present appeared to have increased greatly in number, although cytopathic effects did not occur.

In the chick cells, the production of three different strains of Western equine encephalomyelitis (WEE) virus was not inhibited by the particles. For example, titration data from one strain gave a total WEE virus titre of 4.2 × 10⁶ p.f.u./ml for 13 hr after infection. The electron-microscopic observation of WEE virus multiplying to high titres in chick embryo cells containing these virus-like particles has not been previously reported. Experiments with WEE virus were not done using chick cells or L cells devoid of virus-like particles. However, Morgan, Howe & Rose (1961) studied WEE virus development using chick embryo cells in which no virus-like particles were observed. In Pl. 1, fig. 4, WEE virus and virus-like particles are seen together extracellularly, WEE virus having a mean diameter of 480 ± 3.3 Å (n = 99 WEE particles).

Febvre & Benedetti (1958) examined normal White Leghorn, as well as Brown Leghorn, cells and saw virus-like particles only in the former; their Brown Leghorns were supposed to be free from avian lymphomatosis virus. Their particles were similar in morphology to those described here, most of the particles were extracellular, and no cytopathic effects were observed.

In the second part of this study, a morphologically different type of virus-like particle was seen in two lines of cultured strain L 929 mouse cells. Lockart (1963) has
described culture methods for the cells. The particles are identical to those observed by Dales & Howatson (1961) in strain L mouse cells. Kindig & Kirsten (1967) also reported seeing them after the present work had been completed. One type of murine particle, resembling a spherical 'doughnut', is found both within and outside the cell. According to Bernhard's & Guérin's (1958) morphological classification of murine leukaemia particles, it is of type A. Dales & Howatson (1961) also observed type A virus-like particles in 10 to 20% of their cells. Intracellular and extracellular particles of this type described here consisted of an electron transparent nucleoid and a dense outer area (Pl. 2, fig. 5). In addition, the extracellular type A particles possessed two concentric membranes outside the dense area, and sometimes contained a nucleoid of intermediate density. Intracellular particles averaged 810Å in diameter, while extracellular ones averaged 1030Å. The smaller particles budding from the cell membrane in Pl. 2, fig. 5 are WEE virus. Clusters of immature WEE viruses free in the cytoplasm, and ribosome clusters associated with WEE virus development, also were observed in other cells. The electron microscopic observation of WEE virus multiplying to high titres in L cells containing virus-like particles has not been previously reported.

The other type of murine particle seen rarely in L cells was of type C, according to Bernhard's & Guérin's (1958) classification. Similar particles were reported by Dales & Howatson (1961), but they could not determine any biological activity for either type of particle. The spherical type C particles averaged 1000Å in diameter and contained a dense central nucleoid and an outer region of intermediate density surrounded by a membrane (Pl. 2, fig. 8).

Since the L cells were a relatively homogeneous population, an electron microscopic cell-sampling technique was used in conjunction with titration data. WEE virus samples

### Table 1. Measurements of virus-like particles

<table>
<thead>
<tr>
<th>Type of virus-like particle</th>
<th>Particles</th>
<th>Mean diam. measured (Å)</th>
<th>No. measured</th>
<th>Nucleoids</th>
<th>Mean diam. measured (Å)</th>
<th>No. measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick embryo</td>
<td></td>
<td>1060 ± 19*</td>
<td>50</td>
<td></td>
<td>410 ± 16*</td>
<td>48</td>
</tr>
<tr>
<td>L-cell intracellular type A</td>
<td></td>
<td>810 ± 24</td>
<td>40</td>
<td></td>
<td>250 ± 17</td>
<td>40</td>
</tr>
<tr>
<td>L-cell extracellular type A</td>
<td></td>
<td>1030 ± 36</td>
<td>18</td>
<td></td>
<td>390 ± 28</td>
<td>15</td>
</tr>
<tr>
<td>L-cell extracellular type C</td>
<td></td>
<td>1000 ± 76</td>
<td>11</td>
<td></td>
<td>580 ± 38</td>
<td>11</td>
</tr>
</tbody>
</table>

* 95% confidence interval of the mean.

### Table 2. Quantitative data from titrations and electron microscopic cell-sampling of uninfected and WEE virus-infected L cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total WEE virus titre (p.f.u./ml.)</th>
<th>Mean no. of type A virus-like particles*</th>
<th>Mean no. of WEE viruses†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr uninfected</td>
<td>—</td>
<td>6·6</td>
<td>—</td>
</tr>
<tr>
<td>48 hr uninfected</td>
<td>—</td>
<td>7·7</td>
<td>—</td>
</tr>
<tr>
<td>7 hr after infection</td>
<td>1·4 x 10⁸</td>
<td>10·6</td>
<td>1·9</td>
</tr>
<tr>
<td>24 hr after infection</td>
<td>1·0 x 10⁹</td>
<td>4·2</td>
<td>108</td>
</tr>
</tbody>
</table>

* Virus-like particles within and budding from cell per median cell section.
† WEE viruses within cell and adhering to cell membrane per median cell section.
7 hr and 24 hr after infection were chosen for statistical comparison using quantitative electron microscopy. Seven hours after infection the total WEE virus titre was $1.4 \times 10^8$ p.f.u./ml., contrasted to $1 \times 10^{10}$ p.f.u./ml. 24 hr after infection (Table 2). The significant increase ($0.002 < P < 0.01$) between these samples in the mean number of WEE viruses within the cell and adhering to the cell membrane per median cell section was determined from counts made from electron micrographs of the cells. Concurrently, there was a significant decrease ($P = 0.05$) in the mean number of type A particles seen within and budding from the cell per median cell section. There was no significant difference ($P \geq 0.4$) in the mean number of type A particles in the 0 hr and 48 hr uninfected L cells (Table 2). A modified $t$-test for small samples ($n < 30$) (Bailey, 1959) was used for statistical comparisons of the WEE virus and type A murine particles. Such an interaction between L cell virus-like particles and another virus has not been previously reported.

The type A particles were visible with the electron microscope after fixation with the two fixatives already mentioned or with LiMnO$_4$. Most of the intracellular type A particles were located within, budding into (Pl. 2, fig. 6), or in chains (Pl. 2, fig. 7) within endoplasmic reticulum cisternae. The second largest number of type A particles was observed to be budding from cytoplasmic processes (Pl. 2, fig. 6). Finally, a few type A particles occurred between the nuclear membranes (Pl. 2, fig. 7).

None of 36 samples from L cells in continuous subculture over a 2-year period was found to be devoid of type A virus-like particles; where counts were made, 91% of the cells contained these entities. These particles are similar in morphology, size and location in the cell to viruses found in mice having leukaemia, a disease of known viral aetiology (de Harven & Friend, 1958). They also strongly resemble those seen in diseases of uncertain viral aetiology, such as the development of plasma cell tumours (Parsons et al. 1961), renal carcinomas (Claude, 1962), or Ehrlich ascites tumours (Adams & Prince, 1957).

The viral nature of the chicken and murine virus-like particles is strongly suggested by their small dimensions, uniform size, and observation of them with the electron microscope in preparations fixed by several methods. Although biological activity has not been established, it is believed on morphological grounds that both types of particles are respectively chicken and murine oncogenic viruses.

The author wishes to thank Professors W. Gordon Whaley and Kenneth M. Smith for valuable suggestions and for reviewing the manuscript. Acknowledgement is also due to Drs R. Z. Lockart, Jun., and T. Sreevalsan for help and advice. Dr P. T. Allen kindly supplied one of the WEE virus strains. This work was supported in part by a United States National Defense Education Act Title IV predoctoral fellowship.

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REFERENCES


(Received 22 June 1967)

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Extracellular virus-like particles in a chick embryo cell culture incubated for 4 days. N = nucleoid, IM = inner membrane, OM = outer membrane. Bar = 0.1 μ.

Fig. 2. Extracellular particles (V) among collagen fibrils (CF) and along cell membrane of chick embryo cell. C = cytoplasm. Bar = 0.5 μ.

Fig. 3. Virus-like particle (V) within a cytoplasmic vacuole near the nucleus (N) of a chick embryo cell. C = cytoplasm. Bar = 0.5 μ.

Fig. 4. Extracellular particles (V) and WEE virus (W) 18 hr after infection with WEE virus in a chick embryo cell culture. Bar = 0.1 μ.

PLATE 2

Fig. 5. Intracellular and extracellular type A virus-like particles 18 hr after infection with WEE virus in an L-cell culture. C = cytoplasm, N = nucleoid, DR = dense region, OM = outer membranes, W = WEE virus budding from the cell. Bar = 0.1 μ.

Fig. 6. An intracellular type A particle (V) and type A particles budding (VB) in an L cell culture. ER = endoplasmic reticulum cisterna, ES = extracellular space. Bar = 0.5 μ.

Fig. 7. Intracellular type A particles (V1) between the nuclear membranes and two particles (V2) forming a chain within an endoplasmic reticulum cisterna in an L cell. N = nucleus. Bar = 0.5 μ.

Fig. 8. Extracellular type C virus-like particles 17 hr after infection with WEE virus in an L cell culture N = nucleoid, OR = outer region. Bar = 0.5 μ.