Some Properties of Purified Molluscum Contagiosum Virus

By G. D. PIRIE*, PRUDENCE M. BISHOP, D. C. BURKE† AND R. POSTLETHWAITE

Departments of Bacteriology and Biochemistry,
University of Aberdeen, Aberdeen, Scotland

(Accepted 16 July 1971)

SUMMARY

Molluscum contagiosum virus was purified by two methods involving differential centrifugation; treatment with ribonuclease and sucrose density gradient centrifugation. When the final gradient centrifugation was preceded by two consecutive centrifugations through 36% sucrose, the percentage of DNA in the purified virus particles was reduced. The purified virus was similar to purified vaccinia virus in DNA content, in the absence of detectable RNA and in sedimentation characteristics. Treatment of secondary mouse embryo monolayers with purified molluscum virus caused a reduction in the number of viable cells and in the amount of DNA/culture but had little effect on the amounts of RNA and protein/culture. The depression in the rate of DNA synthesis was maximal at about 24 to 36 hr after infection.

INTRODUCTION

On electron microscopical and histopathological grounds, the virus of molluscum contagiosum is considered to be a poxvirus, but few studies have yet been made on the virus particle, since it cannot be grown outside the human host. This report is concerned with methods of purifying the virus and with some properties of the purified agent. The well-characterized vaccinia virus was used for comparison and for development of techniques for use with the less abundant molluscum material.

METHODS

Molluscum virus was extracted from clinically typical lesions by grinding with sterile sand in 0.01M-tris buffer, pH 9.0, or in 0.004M-McIlvaine's buffer, pH 7.2, and centrifuged at 1000g for 10 min. The pellet was re-extracted twice and the combined supernatant fluids constituted the 'crude' molluscum virus suspension which was stored at -70°. The amount of virus in such extracts varied considerably, and a particularly active preparation from 70 lesions showed a total interfering capacity of 10⁷ PDD 50 (see below) with more than 10¹² virus particles.

Vaccinia virus. A strain originally obtained from the Lister Institute, London, was grown in rabbit skin according to the method of Hoagland, Smadel & Rivers (1949). Skin scrapings, collected into tris or McIlvaine's buffer, were extracted by shaking with glass beads or in a mechanical homogenizer (Measuring and Scientific Equipment, London) and clarified by

* Present address: The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.
† Present address: Division of Biological Sciences, University of Warwick, Coventry, England.
centrifugation at 1000 \( g \) for 10 min. The sediment was re-extracted twice and the combined supernatant fluids were stored at \(-70^\circ\) as 'crude' virus. In a few experiments similar crude extracts were prepared from infected HeLa cell monolayers. Cultures showing a marked cytopathic effect were scraped into tris buffer, frozen and thawed, and treated by ultrasonic vibration for 1 min. (Mullard, 50 w apparatus) before centrifugation as above to yield the 'crude' virus suspension. The virus content of such rabbit and HeLa cell passaged vaccinia virus stocks ranged from \(10^8\) to \(10^{10}\) plaque-forming units (p.f.u.)/ml. as determined in mouse embryo monolayers. On the chorioallantoic membrane of hens' eggs the pock-forming activity was about ten times greater.

**Virus assays.** Vaccinia virus was assayed by plaque count and molluscum virus by its capacity to interfere with vaccinia virus in secondary mouse embryo monolayers (Postlethwaite & Lee, 1970).

**Purification of viruses.** Two methods were used, based on those described respectively by Zwartouw, Westwood & Appleyard (1962) and Joklik (1962a, b). In the first method (A) crude virus was treated by two cycles of differential centrifugation at 35,000 \( g \) for 30 min. and 1000 \( g \) for 10 min. in McIlvaine's buffer. The final clarified supernatant fluid constituted the 'partially purified' preparation which was then centrifuged on a 20 to 60 \% (w/v) gradient of sucrose in McIlvaine's buffer, pH 7.8, at 39,000 \( g \) for 20 min. In later preparations the partially purified virus was treated for 30 min. at 37\(^\circ\) with 25 \( \mu g./ml. \) ribonuclease (RNase) immediately before the density gradient run. Fractions were collected through a hole pierced in the bottom of the tube or by successive withdrawals from the top. In the second method (B) the crude virus was differentially centrifuged in 0.001 M-tris buffer, pH 9.0, alternately at 15,000 \( g \) for 40 min. and at 1000 \( g \) for 10 min. The third high-speed pellet was resuspended in McIlvaine's buffer, pH 7.8, treated for 30 min. at 37\(^\circ\) with RNase and then sedimented through 36 \% sucrose in tris buffer, pH 9.0, at 23,000 \( g \) for 2 hr. The resuspended pellet was further centrifuged through 36 \% sucrose in buffer before centrifugation on a 20 to 60 \% sucrose gradient in tris buffer at 39,000 \( g \) for 20 min. Fractions were collected as before.

To monitor the purification procedures, samples from all stages were assayed for biological activity and for protein content, and their specific activities were determined in terms of infectivity (p.f.u.) or interfering capacity (PDD 50) per mg. protein.

**Physical and chemical characterization of the purified virus particles.** Caesium chloride gradients were used to measure virus density (Pfau & McCrea, 1963). Samples of virus were mixed with CsCl adjusted to a density of 1.29 g./ml. in McIlvaine's buffer, pH 7.2. The suspension was centrifuged in a swing-out rotor at 100,000 \( g \) for 18 hr at 10\(^\circ\). Fractions collected from the bottom of the tube were used to measure (1) the refractive index, (2) the \( E_{260} \), (3) the infectivity of vaccinia virus. The interfering capacity of molluscum virus was inactivated by CsCl.

Sedimentation co-efficients of vaccinia and molluscum viruses, suspended at a concentration of 0.4 mg./ml., were measured in the Spinco Model E ultracentrifuge, by centrifugation at 6200 rev./min. Photographs were taken at 2 min. intervals using ultraviolet optics, and the calculated sedimentation coefficients were corrected for viscosity and temperature (Schachman, 1957).

Particles were counted by the method of Watson (1962), as modified by Postlethwaite, Watt & Hodgkiss (1967), with 1 \% potassium phoshotungstate or 1 \% ammonium molybdate as contrast stains.

Protein was assayed by the Miller (1959) modification of the method of Lowry et al. (1951) using bovine plasma albumin, fraction V (Armour Pharmaceuticals) as a reference standard. RNA was determined by a modification of the Bial reaction for pentoses (Volkin &
Properties of purified molluscum virus

Cohn, 1954) using hydrolysed yeast RNA (British Drug Houses, Poole, Dorset) as a reference standard. RNA values quoted for dialysed density gradient fractions were corrected for non-dialysable orcinol-reacting material. Vaccinia virus DNA was estimated by the Burton (1956) modification of the Dische (1930) diphenylamine reaction using hydrolysed salmon sperm DNA (Sigma, Missouri, U.S.A.) as a standard. Alternatively, and for assay of the small amounts of molluscum virus DNA, the fluorimetric method of Kissane & Robbins (1958) was used, fluorescence being measured in a Locarte fluorimeter.

Chemical analysis of secondary mouse embryo cells. Monolayer cultures were fractionated by a modification of the method of Salzman (1959) at 2 ° unless otherwise indicated. The monolayers were washed with phosphate-buffered saline, pH 7-2, and stored at - 70 °. They were subsequently thawed and, after extraction with 5 % trichloroacetic acid, the insoluble material was de-fatted with alcohol and ether, and the residue heated in 5 % trichloroacetic acid for 3O min. at 100 ° to extract nucleic acids. The residue was dissolved in 0.5N-NaOH and analysed for protein.

Incorporation of radioactive precursors into secondary mouse embryo cells. The rates of protein, RNA and DNA synthesis were determined using [aH]-DL-valine, [2-14C]-uridine and [Me-3H]-thymidine as precursors, as described by Skehel et al. (1967).

RESULTS

Purification of vaccinia virus

Preliminary results with vaccinia virus showed that virus grown in rabbit skin was purified up to 70-fold by two cycles of differential centrifugation but had a DNA content varying from 2.8 to 16 % (with respect to protein as 100 %) and an RNA content of 0.3 to 0.6 %.

Table 1. The effect of treatment of partially purified vaccinia virus with RNase on some properties of the finally purified virus

<table>
<thead>
<tr>
<th>Property measured after purification by methods</th>
<th>Value of measured property of purified virus after treatment of partially purified preparation with</th>
<th>RNase</th>
<th>No RNase</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA as % virus protein A</td>
<td>6.4 (4.9 to 7.8)* [2]†</td>
<td>6.0 (4.8 to 8.9) [4]</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.2 (3.6 to 4.6) [3]</td>
<td>4.8 (4.4 to 5.1) [2]</td>
<td></td>
</tr>
<tr>
<td>Specific activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.f.u./mg. of protein × 10^8</td>
<td>11.0 [1]</td>
<td>12.4 (3.8 to 34.0) [5]</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.6 (4.5 to 8.7) [2]</td>
<td>4.3 (2.7 to 5.8) [2]</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein content/particle (× 10^{-15} g.)</td>
<td>25.0 (7.9 to 47.0) [3]</td>
<td>17.5 (9 to 26) [2]</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.2 (3.4 to 13.0) [2]</td>
<td>8.4 (3.8 to 13.0) [2]</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Round brackets indicate range of measured values.
† Square brackets indicate number of measurements.

In attempts to remove contaminating nucleic acids by nuclease treatment, deoxyribonuclease was unsatisfactory since it was bound to virions and its optimal concentration of magnesium chloride caused flocculation of the virus. Treatment with 25 μg./ml. of ribonuclease for 30 min. at 37 ° removed all detectable RNA from the virus without any loss of infectivity or consistent change in other properties (Table 1) and was therefore adopted for routine use. Immediately after nuclease treatment, further purification by method A was achieved by density gradient centrifugation through sucrose but this was preceded, in method B, by centrifugation of the partially purified virus twice through 36 % sucrose. During this pro-
procedure visible bands of opacity and 30 and 13% of the total residual protein remained in the respective supernatant fluids, whilst more than 99% of the infectivity was pelleted.

Analysis of vaccinia virus purified by these two procedures showed (Table 2) that the longer procedure (method B) gave virus with a lower DNA and protein content/particle, although there was some loss of infectivity and consequent rise in the number of particles/p.f.u. The RNA analysis was complicated by the presence of orcinol-reacting material in the sucrose which could not be removed by dialysis, and the figure of 0·1% RNA is an upper limit. In general, the analytical figures are similar to those obtained by other workers although, in this study, the amounts of protein/particle show a wide range and a higher mean value. This may be attributed to errors in particle counting due to the ready aggregation of

### Table 2. Characterization of purified vaccinia and molluscum (MCV) viruses

<table>
<thead>
<tr>
<th>Property measured</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA vaccinia as % of mass of virus protein</td>
<td>6·1 (4·8 to 8·9)* [6]</td>
<td>4·4 (3·6 to 5·1) [5]</td>
</tr>
<tr>
<td>DNA MCV as % of mass of virus protein</td>
<td>6·7 (6·0 to 8·0) [3]</td>
<td>4·7 (4·6 to 4·8) [2]</td>
</tr>
<tr>
<td>RNA vaccinia as % of mass of virus protein</td>
<td>&lt;0·1 [1]</td>
<td>---</td>
</tr>
<tr>
<td>RNA MCV as % of mass of virus protein</td>
<td>&lt;0·1 [4]</td>
<td>&lt;0·1 [2]</td>
</tr>
<tr>
<td>Specific activity</td>
<td>Vaccinia (p.f.u./mg. protein × 10⁻⁸)</td>
<td>12·2 (3·8 to 34) [6]</td>
</tr>
<tr>
<td></td>
<td>MCV (PDD 5₀/mg. protein × 10⁻³)</td>
<td>6·0 [1]</td>
</tr>
<tr>
<td>Protein content/particle (× 10⁻¹ g.)</td>
<td>Vaccinia</td>
<td>22 (8 to 47) [5]</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
<td>4·2 (1·7 to 8·5) [3]</td>
</tr>
<tr>
<td></td>
<td>Particles/p.f.u. of vaccinia virus</td>
<td>58 (12 to 140) [4]</td>
</tr>
<tr>
<td></td>
<td>Particles/PDD 5₀ of MCV (× 10⁻²)</td>
<td>4·0 [1]</td>
</tr>
<tr>
<td>Density (g./ml.)</td>
<td>Vaccinia virus</td>
<td>1·287 (1·284 to 1·290) [2]</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
<td>1·288 [1]</td>
</tr>
<tr>
<td>Sedimentation coefficient (S₂₀w)</td>
<td>Vaccinia (as % of mass of virus protein)</td>
<td>177 (J); 177 (S); 5·35 (J)</td>
</tr>
<tr>
<td></td>
<td>MCV</td>
<td>4·0 (P); 4·0 (J); 4·0 (S); 4·0 (P)</td>
</tr>
</tbody>
</table>

DNA as % of mass of virus protein
RNA as % of mass of virus protein
Specific activity (p.f.u./mg. protein × 10⁻⁸)
Protein content/particle (× 10⁻¹ g.)
Particles/p.f.u.
Density (g./ml.)
Sedimentation coefficient $S_{20w}$

Other published data for vaccinia virus

<table>
<thead>
<tr>
<th>Property measured</th>
<th>A</th>
<th>D</th>
<th>H</th>
<th>J</th>
<th>Jo</th>
<th>K</th>
<th>M</th>
<th>P</th>
<th>P &amp; S</th>
<th>S</th>
<th>S, P &amp; S</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA as % of mass of virus protein</td>
<td>5·6 (H); 5·8 (J); 7·8 (A); 3·2 (Z)</td>
<td>5·6 (H); 5·6 (H); 5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td>5·6 (H)</td>
<td></td>
</tr>
<tr>
<td>RNA as % of mass of virus protein</td>
<td>&lt;0·26 (A); 0·1 (J, Jo); 0·1 to 0·2 (P); 0·1 (Z)</td>
<td>Assayed as pocks on chorioallantoic membrane</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Specific activity (p.f.u./mg. protein × 10⁻⁸)</td>
<td>177 (J); 177 (S); 5·35 (J)</td>
<td>Assayed on chick embryo monolayers</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Protein content/particle (× 10⁻¹ g.)</td>
<td>4·43 (S); 4·43 (J)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Particles/p.f.u.</td>
<td>2·4 to 9·2 (S); 12·3 to 97 (D); 12 (K); 10·6 (J); 47 (P)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Density (g./ml.)</td>
<td>1·16 (S, P &amp; S); 1·25 (S, P &amp; S); 1·27 (S, P &amp; S); 1·27 (J)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Sedimentation coefficient $S_{20w}$</td>
<td>4910 s (P &amp; S); 5000 s (M)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

---

* Round brackets indicate range of measured values.
† Square brackets indicate number of measurements.

1 As % dry weight
2 Assayed as pocks on chick embryo monolayers
3 Assayed as plaques in chick embryo monolayers
4 Calculated as 83% of mass of dehydrated particle
5 Assayed in rabbit skin
6 In dilute buffer solution
7 In 50% sucrose
8 Calculated for dehydrated elementary body
9 In caesium chloride
Properties of purified molluscum virus

The low p.f.u./mg. of protein and the high particle/p.f.u. ratio are due in part to the low efficiency with which vaccinia virus forms plaques in mouse embryo monolayers (pock/plaque ratio = 10/1).

Purification of molluscum virus

Differential centrifugation of the crude molluscum extract removed 85% of the contaminating protein, with only negligible loss of virus. Centrifugation of the virus through a sucrose density gradient showed a single band at a density of 1.16 g./ml., which contained 70 to 90% of the virus (Fig. 1). Partially purified vaccinia virus banded in a similar position. Centrifugation of partially purified molluscum virus through 36% sucrose before a final density gradient centrifugation led to further purification, and protein, but not virus, remained in the supernatant fluids.

![Density gradient centrifugation of partially purified vaccinia virus (V) and molluscum virus (M) on 20 to 60% gradients of sucrose in McIlvaine's buffer, pH 7.8, 0.004 M, at 39,000 g for 20 min.](image)

Properties of purified molluscum virus

The final product was free of particulate contamination as judged by electron microscopy (Fig. 2). Two types of particle were present corresponding to the M and C forms of Westwood et al. (1964). Re-centrifugation of the virus in a sucrose density gradient gave a single sharp band which contained $3 \times 10^{10}$ particles and 78% of the interfering activity applied to the gradient. The visible band coincided with a sharp peak in interfering activity and extinction at 260 and 280 nm. (Fig. 3).

The sedimentation characteristics of molluscum virus were similar to those of vaccinia
virus when examined under identical conditions (Table 2). Its interfering capacity was destroyed by caesium chloride. Chemical analysis of the purified virus particles showed no detectable RNA and closely similar percentages of DNA. In view of the observed similarities in sedimentation characteristics, the discrepant protein values recorded for vaccinia virus are considered to be spuriously high, as already noted. The lower values recorded for molluscum virus are considered more reliable, since this virus does not aggregate so readily.

Fig. 2. Electron micrograph of preparation of molluscum virus purified by method B. Negatively stained with 2% (w/v) potassium phosphotungstate.

**Effect of purified molluscum virus on secondary mouse embryo cells**

**Cell numbers.** Cells were infected with virus at a multiplicity of $6.6 \times 10^{-5}$ PDD$_{50}$/cell (corresponding to about 20 particles/cell), and cell numbers were determined at intervals. The results of a representative experiment (Table 3) showed that in cultures treated with virus
Properties of purified molluscum virus

Cell division proceeded but the number of viable cells was about 20\% lower than in untreated cultures.

Nucleic acid and protein estimations. Cells were infected with virus at a multiplicity of $40 \times 10^{-5}$ PDD 50/cell (corresponding to about 120 particles/cell), washed and incubated at 37°. At intervals the cells were harvested and the amounts of DNA, RNA and protein estimated in cells of the virus-infected and control cultures. The results (Fig. 4) showed that infection had little effect on the protein and RNA content of the cells, but that infected cells contained less DNA. Measurement of the rates of incorporation of [14C]-thymidine (Fig. 5) showed that the inhibitory effect of the virus on DNA synthesis was maximal at about 24 to 36 hr after infection.
Fig. 4. Synthesis of protein, RNA and DNA in molluscum-infected mouse embryo cultures. a, b and c indicate analytical values at indicated hours after treatment with approximately 120 particles/cell of purified molluscum virus. d indicates interference with the growth of vaccinia virus in replicate cultures challenged 24 hr after treatment by the same dose of molluscum virus. ○—○, control cultures; △—△, molluscum-treated cultures.

Fig. 5. Uptake of [H]-thymidine by molluscum-infected mouse embryo monolayers. In two experiments cultures were exposed to approximately 60 particles/cell of purified molluscum virus and examined at intervals after infection for the rate of DNA synthesis. Note different time scale: ○—○, control cultures; △—△, molluscum-treated cultures.
Properties of purified molluscum virus

Discussion

Molluscum virus, which on electron microscopical and histopathological grounds has been considered to be a poxvirus, has now been shown to be very similar to vaccinia virus in chemical composition and physical properties. This strengthens its classification as a poxvirus. Although the virus does not multiply in secondary mouse embryo cells, it induces the formation of interferon (Postlethwaite & Lee, 1970), and it was therefore of interest to measure the effect of virus treatment on cell metabolism. There was no effect on the overall rate of RNA and protein synthesis/culture, but the reduced number of cells in infected cultures suggested that RNA and protein values/cell may have been increased. The rate of DNA synthesis was somewhat depressed in accordance with the reduced amounts of DNA and numbers of viable cells per culture. These results may be attributed to a 'toxic' or cytopathic effect of virus on some cells in the cultures, as noted in other systems (Burnett & Neva, 1966; La Placa, 1966).

In preliminary experiments molluscum virus failed to induce the formation of the enzyme thymidine kinase in mouse embryo cells, as found by La Placa et al. (1967) in human amnion cells. Thus molluscum virus induces the formation of interferon without any marked effect on host cell metabolism and without induction of an enzyme which, characteristically though not obligatorily, appears early during other poxvirus infections. Whether treatment with molluscum virus stimulates the formation of a double-stranded RNA which acts as an interferon inducer (Colby & Duesberg, 1969) or whether this virus induces interferon by some novel mechanism remains to be determined.

The work was supported by grants to R. P. from the British Empire Cancer Campaign for Research. The help of Mrs H. C. Jeffery with electron microscopy and the technical assistance of Mrs J. Fallas and Miss P. Butler are gratefully acknowledged. Drs T. E. Anderson and R. A. Main kindly referred patients and Professor A. Macdonald and W. O. Kermack gave continued support.

References


(Received 15 March 1971)