Replication of Vesicular Stomatitis Virus: Characterization of the Virus-induced RNA

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SUMMARY

Four major species of single-stranded RNA (38s, 30s, 19s and 10 to 16s) and one double-stranded species of RNA (13s) were found in BHK cells infected with vesicular stomatitis virus. A portion of the 38s and 19s was associated with ribonucleoprotein particles sedimenting at 140s and 80s respectively. Some of the 38s RNA and the 10 to 16s RNA could be isolated from the polyribosome region of fractionated cells. The latter RNA could be fractionated further into at least five (possibly eight) peaks by electrophoresis on polyacrylamide gels. The molecular weight of the RNAs in these peaks ranged from 0.24 to 1.0 x 10^6. Hybridization studies revealed that the 10 to 16s RNA was complementary to the RNA extracted from purified virion, suggesting that the different sizes represent monocistronic (negative) messengers. A high molecular weight RNA complex was also isolated from virus-infected cells. Denaturation of the complex revealed the constituent strands to consist of 38s, 19s and 10 to 16s RNA. This may be the active transcription complex.

INTRODUCTION

Expression of genetic material of the RNA-containing viruses in animal cells has been shown to use one of a number of pathways: (1) The infecting parental (positive) RNA specifies a RNA-dependent RNA polymerase which synthesizes, via a complementary strand, more positive strands. This RNA can then either code for virus proteins or be incorporated into virus particles. This mechanism is operative in picornaviruses (for review, see Erikson, 1968). (2) The infecting (positive) genome synthesizes negative strands which act directly as messenger RNA for protein synthesis. Such a mechanism has been postulated for Newcastle disease virus (Bratt & Robinson, 1967) and Sendai virus (Blair & Robinson, 1968). (3) The infecting genome can act as a template for DNA synthesis which can then be incorporated into the host DNA and so use the normal cell DNA → RNA → protein pathway. Such a mechanism has been postulated for RNA tumour viruses (Temin & Mizutani, 1970; Baltimore, 1970).

In this paper the operative mechanism has been studied in BHK cells infected with vesicular stomatitis virus (VSV). The results presented suggest that VSV replicates by mechanism 2. The size and polarity of the messenger RNAs have been correlated with proteins induced by VSV.

METHODS

Viruses and cells. The Indiana strain of vesicular stomatitis virus (VSV) was used throughout. This had been plaque-purified and grown in BHK 21 cells (Crick, Cartwright & Brown, 1969). Virus was titrated either by intracerebral inoculation of 7-day-old mice or by the
plaque assay method. BHK cells were grown as monolayers in Roux bottles in Eagle's medium containing 10% (v/v) ox serum, 0.2% tryptose phosphate broth and antibiotics (penicillin, streptomycin and polymyxin, each at a concentration of 100 units/ml). BHK cells in monolayer culture were infected in Eagle's medium at the required multiplicity of infection for 15 min. at 37°. The cells were then washed in prewarmed Eagle's medium and incubated in the same medium containing actinomycin D, 1 μg/ml. Fifteen min. later 5 to 25 μc./ml. final concentration of [3H]-uridine (29 c/m-mole: Radiochemical Centre, Amersham, Buckinghamshire) was added.

Purification of virus, interfering component and ribonucleoprotein. These three particles were separated by the method of Crick et al. (1969). Virus was separated from the interfering component and ribonucleoprotein by centrifugation through a 15 to 45% sucrose gradient (in 0.04M-phosphate, pH 7.6) at 20,000 rev./min. for 2 hr in the SW 25 Spinco rotor. The ribonucleoprotein and interfering components were separated by centrifugation on a 4.5 ml. potassium tartrate gradient (1.1 to 1.3 g./ml.) at 30,000 rev./min. for 2 hr in the SW 39 Spinco rotor.

Preparation of cytoplasmic extracts. The cells in monolayer culture in Roux bottles were washed in cold Eagle's medium, drained and 2 ml. of 0.5% Nonidet P40 (v/v) in PS buffer (0.1M-NaCl, 0.002M-MgCl2 and 0.01 M-tris, pH 7.6) added (Blair & Robinson, 1970). The sheets were rocked gently at room temperature until they left the glass. The cytoplasmic extract was then centrifuged at 2000 g for 10 min. to remove cell nuclei before sedimenting in a 5 to 30% sucrose gradient (in PS buffer) at 24,000 rev./min. for 2 hr in the SW 25 Spinco rotor.

Extraction and fractionation of RNA. Cell sheets were extracted twice with phenol buffered in STE (0·15M-NaCl, 0·001M-EDTA, 0·01M-tris, pH 7·6). The RNA was precipitated from the aqueous phase with two volumes of ethanol at −20°. RNA was also extracted from cytoplasmic extracts by adjusting to 0·1% sodium dodecyl sulphate and then extracting with phenol. Double-stranded RNA was separated from single-stranded and RNA containing single-stranded regions by precipitation with 1·5M-NaCl (Ammann, Delius & Hofschneider, 1964). RNA suspended in STE (about 500 μg./ml) was adjusted by the addition of 4·5 M-NaCl to contain 1·5M-NaCl and held at 4° for 18 hr. The precipitate and supernatant fractions were separated by centrifugation at 5000 g for 10 min.

Column chromatography. After fractionation in 1·5M-NaCl, the supernatant fraction was filtered through a Sephadex G-200 column (30 × 1·5 cm.) in equilibrium with STE and eluted in the same buffer at 13 ml./hr at room temperature. The 1·5M-NaCl precipitate was resuspended in STE and filtered through a Sepharose 2B column (90 × 1·5 cm.) in equilibrium with the same buffer. The RNA was eluted at 17 ml./hr at room temperature.

Sucrose gradient centrifugation. One ml. samples of RNA were centrifuged on 5 to 25% sucrose gradients in 0·1 M-NaCl at 18,000 rev./min. for 15 hr in the Spinco SW 25 rotor. Gradients were fractionated into 1 ml. samples and acid-insoluble radioactivity determined by precipitation with an equal volume of 10% trichloroacetic acid in the presence of carrier bovine serum albumin. Where fractions were to be treated with ribonuclease, the fraction was diluted into STE and incubated with 1 μg./ml. pancreatic ribonuclease (Armour Pharmaceutical Co Ltd, Eastbourne, Sussex) at 37° for 10 min. before precipitation with 10% trichloroacetic acid.

Acrylamide gel electrophoresis. RNA was analysed in acrylamide gels by the method of Loening (1967). RNA suspended in 20 to 50 μl. of electrophoresis buffer containing 18% sucrose and 0.2% sodium dodecyl sulphate was electrophoresed in 9 cm. gels at 5 mA/gel for 3 to 4 hr. The gels were frozen and sliced into 2 mm. fractions with a gel slicer (Mickle...
Laboratory Engineering Co, Gomshall, Surrey). The fractions were dissolved in NCS solubilizer (Amersham/Searle) at 60° for 1 hr and then counted in a toluene scintillant in the Packard liquid scintillation counter. For molecular weight determinations \[^{32}P\]-BHK ribosomal RNAs were used as markers. The molecular weights of the 28s and 18s RNAs were taken as \(1.64 \times 10^6\) and \(0.67 \times 10^6\) respectively (Peterman & Pavlovec, 1966). Foot-and-mouth disease virus RNA, \(2.8 \times 10^6\) (prepared as previously described by Wild & Brown, 1970) and R17 RNA (a gift from Dr M. L. Fenwick), \(1.1 \times 10^6\) (Sinha, Fujimura & Kaesberg, 1965) were also used to calibrate the gels.

Density determinations. Volumes of 0.2 ml of RNA were centrifuged on 4 ml. pre-formed caesium sulphate gradients (1.4 to 1.8 g./ml.) at 30,000 rev./min. for 64 hr in the SW 39 Spinco rotor. Density was determined by refractometry.

Hybridization. Samples of \[^3H\]-RNA were hybridized with 3 to 10 \(\mu\)g. of unlabelled RNA (extracted from purified virus) by the method of Baltimore (1966).

RESULTS

Kinetics of virus RNA synthesis

BHK cells in monolayer culture (about 10^7 cells/bottle) were infected at a multiplicity of either 3 or 30 for 30 min. at 37° and were then incubated with actinomycin D (1 \(\mu\)g./ml.) and \[^3H\]-uridine (5 \(\mu\)c./bottle). Samples were taken at hourly intervals. Cells and medium were separated and estimated for acid-precipitable radioactivity and the medium also titrated for infectivity (Fig. 1). At a multiplicity of 30, virus-induced RNA synthesis reached a maximum in the cell by 4 hr, whereas virus was released into the medium up to 6 hr. Infection at the lower multiplicity caused a slight delay in the growth cycle. Studies of RNA synthesis at a multiplicity of 300 revealed incorporation rates only just greater than controls. The virus products formed by 6 hr after infection were fractionated into virus, interfering component and ribonucleoprotein by centrifugation on sucrose gradients, followed by centrifugation in a potassium tartrate gradient (Crick et al. 1969) (Table 1). Not more than 9 % of the radioactivity was associated with interfering component even at a multiplicity of 30.

Examination of the virus-induced RNA

BHK cells were infected at a multiplicity of 30 and incubated with Actinomycin and \[^3H\]-uridine. After 3½ hr the RNA was extracted with phenol and a sample centrifuged on a sucrose gradient (Fig. 2a). Specific peaks of RNA sedimented at 38s, 30s, 19s and there was a broad band at 10 to 16s.

The total RNA was subjected to fractionation in 1.5 M-NaCl at 4° to separate the RNA containing single-stranded regions from the completely double-stranded molecules (Ammann et al. 1964). The precipitate and supernatant fractions were analysed on sucrose
gradients (Fig. 2b and c). The precipitate fraction contained the same major RNA species as the unfractionated RNA. The 1·5 M-NaCl supernatant fraction contained a ribonuclease-resistant 13s peak with smaller material sedimenting at the top of the gradient.

![Growth curve of vesicular stomatitis virus in BHK cells.](image)

**Fig. 1.** Growth curve of vesicular stomatitis virus in BHK cells. •••• m.o.i. of 3; △△△△ m.o.i. of 30; ○○○○ uninfected. (a) supernatant; (b) cells; (c) infectivity of supernatant.

**Characterization of 13s ribonuclease-resistant RNA**

For these experiments the 13s RNA was purified from the 1·5 M-NaCl supernatant fraction by passing it through a column of Sephadex G-200 buffered in STE. The 13s RNA eluted in the void volume, whereas the rest of the RNA was retarded. The properties of the 13s RNA were examined as follows:

1. The density of [3H]-13s RNA together with [32P]-RNA extracted from purified virus
was determined in a preformed cesium sulphate gradient (Fig. 3a). Virus particle RNA had a density of 1.665 g./ml compared with a value of 1.60 g./ml for the 13s molecule. This latter value was similar to values obtained for other double-stranded RNAs (Szybalski, 1966).

Although resistance of RNAs to digestion by ribonuclease can be used as a criterion for double-strandedness, the degree to which they are resistant can vary. To test its relative ribonuclease-resistance, [3H]13s-RNA was suspended in 10^{-3} M-EDTA, 0.01 M-tris, pH 7.6 or in this buffer containing 0.15 M-NaCl and incubated with different concentrations of

\[
\begin{array}{c}
\text{Counts/min. x 10^{-2}} \\
\text{Fraction}
\end{array}
\]

Fig. 2. Centrifugation on a 5 to 25% sucrose gradient of [3H]-virus induced RNA: (a) unfractionated; (b) 1.5 M-NaCl precipitate; (c) 1.5 M-NaCl supernatant. ○——○, [3H]-RNA; •——•, [3H]-RNA after ribonuclease digestion. The arrows indicate the positions of the large and small ribosomal RNA.
Fig. 3. Centrifugation on a preformed cesium sulphate gradient of vesicular stomatitis virus-induced RNA: (a) [3H]-RNA from 1.5 M-NaCl supernatant, ●...●; (b) [3H]-RNA eluting in void volume of Sepharose 2B column (RI - Fig. 6), ●...●. [32P]-RNA extracted from the purified vesicular stomatitis virus was used as a marker in each gradient, ○—○. Density is the continuous line.

Table 2. Relative ribonuclease-resistance of RNA

<table>
<thead>
<tr>
<th>Ribonuclease (µg/ml.)</th>
<th>[3H]13S RNA</th>
<th>[3H]-RI</th>
<th>[3H]-virus RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>31.4</td>
<td>15.5</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>30.8</td>
<td>2.6</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>23.6</td>
<td>2.3</td>
</tr>
<tr>
<td>100</td>
<td>8.6</td>
<td>4.6</td>
<td>—</td>
</tr>
<tr>
<td>0.1/No NaCl</td>
<td>8.6</td>
<td>2.5</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Each sample contained about 2000 counts/min. and was incubated for 10 min. at $37^\circ$ in STE buffer, except for the no NaCl sample which contained $10^{-3}$ M-EDTA and 0.01 M-tris, pH 7.6.
pancreatic ribonuclease for 10 min. at 37°. The residual radioactivity was estimated by precipitation with 10% trichloroacetic acid (Table 2). The RNA was completely resistant to digestion by ribonuclease up to a concentration of 1 μg./ml. Under the same conditions,

![Graph](image)

**Fig. 4.** Centrifugation on a 5 to 25% sucrose gradient of [3H]13s RNA. O—O, untreated; ⋄—⋄, heated in 90% dimethyl sulphoxide for 3 min. at 60° before centrifugation. The arrows indicate the positions of the large and small ribosomal RNA.

**Table 3. Hybridization of [3H]13s virus-induced RNA**

<table>
<thead>
<tr>
<th>Unlabelled virus particle RNA (μg./assay)</th>
<th>Percentage hybridization</th>
<th>[3H]13s RNA</th>
<th>[3H]-virus particle RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>8.5</td>
<td>4.0</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>47.0</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>47.0 to 52.5</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Approximately 2000 to 5000 counts/min./assay were annealed with the unlabelled virus particle RNA. The control samples were not incubated with ribonuclease.

single-stranded virus particle RNA was reduced to an acid-precipitable residue of 2%. Increasing the ribonuclease concentration above 1 μg./ml led to a greater digestion of the molecule. In the absence of NaCl, the RNA was digested by the lowest concentrations of ribonuclease.
(3) A sample of \([^3H]\)13s-RNA in water was denatured by suspending it in 90% dimethyl sulphoxide and heating to 60°C for 3 min. NaCl was added to 0.15 M and the RNA precipitated with two volumes of ethanol. After resuspending in STE, the RNA was centrifuged on a sucrose gradient (Fig. 4). Denaturation increased the sedimentation of the RNA from 13s to 19s. The RNA was then ribonuclease-sensitive.

Fig. 5. Electrophoresis of ribonuclease-resistant \([^3H]\)-RNA in 2-2% acrylamide gels.

\(\cdots\cdots\), \([^3H]\)13s RNA; \(\bigcirc\cdots\bigcirc\), \[^3P\]-ribosomal RNA.

(4) Hybridization experiments showed that 50% of 13s-RNA was complementary to the RNA from purified virus (Table 3). Recently, however, Robinson (1970) reported that Newcastle disease virus and Sendai virus contained both positive and negative strands in the virion which would make the above experiment invalid if this was so for VSV. To test whether or not VSV contains both positive and negative strands, increasing amounts of unlabelled virus particle RNA were annealed with a constant amount of \([^3H]\)-virus particle RNA (Table 3). No increase in the amount of hybridizable material was found, showing that all the RNA strands in the virus particle had the same polarity.

(5) The electrophoretic mobility of the 13s RNA in acrylamide gels (Fig. 5) suggested a
molecular weight of about $1.75 \times 10^6$. However, this value is based upon the relative mobility of the 13S RNA compared with that of single-stranded RNAs; double-stranded RNAs may have different electrophorectic properties.

Isolation and characterization of the high molecular weight RNA

High molecular weight complexes involved in virus replication – replicative intermediate (RI) – can be isolated by chromatography on agarose columns (Erikson & Gordon, 1966). To isolate such complexes from virus-induced RNA, the 1.5 M-NaCl precipitate fraction (see Fig. 2b) was passed through a Sepharose 2B column buffered in STE (Fig. 6). The RNA eluting in the void volume was designated ‘RI’. Centrifugation of samples of RI on a sucrose gradient (Fig. 7a) gave rise to a polydisperse pattern with molecules sedimenting from about 13S to material pelleting on the bottom of the tube (45% of total).

To investigate the nature of these rapidly sedimenting complexes, a sample of RI in STE was heated to 55°C for 3 min. before centrifugation on a sucrose gradient (Fig. 7b). Under these conditions the major peak sedimented as a broad band at about 13 to 17S. Very little RNA pelleted on the bottom of the tube. A ribonuclease-resistant core was associated with
all the molecules, although molecules in the 25 to 40S region were more resistant. In order to examine the size of the individual strands of RNA in the complex, RI was denatured in 90 % dimethyl sulphoxide before centrifugation on a sucrose gradient (Fig. 7c). After denaturation the RNA sedimented at 38S, 19S and a broad band was found at 10 to 16S.

The relative ribonuclease resistance of the RI preparation was compared with RF and single-stranded RNA (Table 2). At low ribonuclease concentrations the RI had a resistant core of about 30 %.

Fig. 7. Centrifugation on a 5 to 25 % sucrose gradient of [3H]-RNA induced by virus, isolated from the void volume of Sepharose 2B column (Fig. 6). (a) untreated, (b) heated in STE at 60° for 3 min., (c) denatured in 90 % dimethyl sulphoxide at 60° for 3 min. O—O, acid-insoluble radioactivity; •---•, treated with ribonuclease before precipitation.
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Intracellular distribution of virus-induced RNA

BHK cells infected at a multiplicity of 30 were labelled with [3H]-uridine in the presence of actinomycin D. After 3 hr the cells were washed with cold medium and a cytoplasmic extract prepared by the Nonidet method (Blair & Robinson, 1970). The extract was then

Fig. 8. Centrifugation of cytoplasmic extracts of virus-infected BHK cells on 5 to 30% sucrose gradients; (a) untreated, (b) incubated with 1 μg/ml. ribonuclease for 10 min. at room temperature before centrifugation. ○—○, [3H]-RNA; ——, [3H]-RNA after digestion with ribonuclease; △—△, ε_{max}. 
either centrifuged on a sucrose gradient without further treatment or first incubated with 1 µg./ml. ribonuclease at room temperature for 10 min. In the untreated extract radioactivity was dispersed throughout the gradient. Two major peaks sedimenting at about 140S and 80S were partially ribonuclease-resistant. The RNA sedimenting in the polysome region was sensitive to ribonuclease. Pretreatment of the cytoplasmic extracts with ribonuclease left only the 140S and 80S peaks (Fig. 8). These two peaks were identical in sedimentation and ribonuclease-sensitivity with the ribonucleoproteins released from purified virus and interfering component respectively by treatment with sodium deoxycholate (unpublished observation).

Fig. 9. Sucrose gradient analysis (5 to 25 % sucrose) of RNA extracted from regions A-D of polysome gradient (Fig. 8). (a) O—O, RNA extracted from region A; ——, region B; (b) O—O, region C; ——, region D. The arrows indicate the positions of the large and small ribosomal RNA.
The fractions labelled A to D in Fig. 8 were extracted with phenol + 0.1% sodium dodecyl sulphate and the RNA subsequently analysed on sucrose gradients (Fig. 9). The RNA extracted from the faster sedimenting polysome region (A) consisted of a peak at 38s with a major band at 10 to 16s (Fig. 9a). Region B which included the 140s ribonucleoprotein contained RNA sedimenting at 38s, 30s, 19s and 10 to 16s (Fig. 9a). Region C which contained the 80s ribonucleoprotein consisted mainly of 19s molecules, whereas region D contained only the 10 to 16s band (Fig. 9b).

Fig. 10. Polyacrylamide (2-2 %) gel electrophoresis of VSV-induced RNA. (a) O---O, [3H]-RNA extracted from purified VSV and interfering component; --- ---, [32P]-BHK ribosomal RNA. (b) O---O, [3H]38s VSV-induced RNA from Fig. 9a; --- ---, [32P]-RNA extracted from purified VSV and BHK ribosomal RNA. (c) O---O, [3H]-VSV-induced RNA from region C, Fig. 8. (d) O---O, [3H]-VSV-induced RNA from peak A, Fig. 8; --- ---, peak D, Fig. 8. The arrows indicate the positions of the large and small ribosomal RNA.

Properties of RNA fractionated from cytoplasmic extracts

RNAs isolated from the polysome preparations of Fig. 8 and 9 were hybridized with unlabelled RNA from purified VSV (Table 4). Virus particle [3H]-RNA and [3H]-interfering component were used as controls. Neither of these RNAs hybridized with virus particle RNA. The 10 to 16s RNA isolated from the polysome and 50s region of the gradient was completely complementary to virus particle RNA. The 19s RNA found in the 140s region of the polysome gradient was 79 to 83% hybridizable, whereas the 19s extracted from the 80s region (ribonucleoprotein peak) was 43 to 60% hybridizable. A low level of complementarity with virus particle RNA was also found in the 38s RNA (12 to 31%).
The size of the RNAs was estimated by acrylamide gel electrophoresis (Loening, 1967) and the molecular weights calculated from their mobility relative to BHK ribosomal RNA (Fig. 10 and Table 5). Foot-and-mouth disease virus RNA and R17 RNA were also used to standardize the gels. The molecular weights of the RNAs of VSV and interfering component were calculated to be $3.9 \times 10^6$ and $1 \times 10^6$ respectively. The 38s RNA extracted from polysomes co-electrophoresed with virus particle RNA in electrophoresis. The 10 to 16s RNA contained five distinct peaks and possibly at least 8 species of RNA with molecular weights in the range 0.24 to $1 \times 10^6$.

### Table 4. Hybridization of virus-induced RNA

<table>
<thead>
<tr>
<th>RNA</th>
<th>Percentage hybridization when annealed to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 µg. RNA</td>
</tr>
<tr>
<td>Virus particle</td>
<td>4</td>
</tr>
<tr>
<td>Interfering component</td>
<td>6</td>
</tr>
<tr>
<td>38s-induced</td>
<td>3</td>
</tr>
<tr>
<td>19s from 80s ribonucleoprotein</td>
<td>6</td>
</tr>
<tr>
<td>19s from polysome region</td>
<td>7</td>
</tr>
<tr>
<td>10 to 16s from polysome region</td>
<td>8</td>
</tr>
<tr>
<td>38s-induced RNA</td>
<td>5</td>
</tr>
</tbody>
</table>

[1H]-RNAs were isolated from the gradients of Fig. 8 and 9. Each assay contained about 1000 to 3000 counts/min. The results are shown as a range of at least four experiments.

### Table 5. Molecular weight determinations of RNA as determined by electrophoretic mobility in polyacrylamide gels

<table>
<thead>
<tr>
<th>RNA</th>
<th>Calculated molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV</td>
<td>$3.9 \times 10^6$</td>
</tr>
<tr>
<td>Interfering component</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>FMDV</td>
<td>$2.8 \times 10^6$</td>
</tr>
<tr>
<td>R17</td>
<td>$1.1 \times 10^6$</td>
</tr>
<tr>
<td>19S virus-induced RNA</td>
<td>$1.0 \times 10^6$</td>
</tr>
<tr>
<td>10 to 16s virus-induced RNA</td>
<td>$10 \times 10^5$ (a)</td>
</tr>
<tr>
<td></td>
<td>$8.4 \times 10^5$ (b)</td>
</tr>
<tr>
<td></td>
<td>$5.4 \times 10^5$ (c)</td>
</tr>
<tr>
<td></td>
<td>$3.4 \times 10^5$ (d)</td>
</tr>
<tr>
<td></td>
<td>$2.4 \times 10^5$ (e)</td>
</tr>
</tbody>
</table>

(a) to (e) refer to peaks in Fig. 10 (d).

### DISCUSSION

Previous studies on the multiplication of vesicular stomatitis virus in a number of different cell lines have shown that several single-stranded RNAs and up to two double-stranded RNAs are involved in its replication (Schaffer, Hackett & Soergel, 1968; Newman & Brown, 1969; Stampfer, Baltimore & Huang, 1969; Schincariol & Howatson, 1970; Huang, Baltimore & Stampfer, 1970; Mudd & Summers, 1970a). My work confirms the existence of several single-stranded RNAs and a double-stranded RNA in cells infected with vesicular stomatitis virus. The 10 to 16s RNA extracted from polyribosomes appears to be similar in polarity and size to the 14s messenger RNA described by Huang et al. (1970). These authors also found much 28s RNA in the polyribosome region but in my study this represented only a minor species. The number of species (at least five) and range of molecular weights (0.24 to $1 \times 10^6$) of the messenger RNA induced in BHK cells by vesicular stomatitis...
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Vesicular stomatitis virus differs from the messenger RNA induced in HeLa cells (Mudd & Summers, 1970a). The latter workers have described three major species with molecular weights of 3.3 to 6.8 x 10^6. Proteins induced by vesicular stomatitis virus and extracted from BHK cells have molecular weights of 2.5 to 9.0 x 10^4 (T. F. Wild & P. Talbot, unpublished observations) and from HeLa cells of 6.7 x 10^4 (excluding polypeptide I) (Mudd & Summers, 1970b). This suggests that the 10 to 16S RNA is probably a monocistronic messenger. It would be possible for the larger RNAs to specify more than one protein, but, against such a view, Mudd & Summers (1970b) have been unable to show cleavage of precursor proteins.

Relatively large amounts of 80S ribonucleoprotein could be extracted from virus-infected cells. Although the sedimentation and ribonuclease-resistance properties of this particle and its extracted RNA were similar to the ribonucleoprotein and RNA extracted from purified interfering component, the RNA from the cell-associated particle could be partially hybridized with virus particle RNA (43 to 60 %), whereas interfering component RNA could not. 'Pulse-chase' experiments have shown that the major portion (about 80 %) of the 80S ribonucleoprotein could not be 'chased' into interfering component in the medium (T. F. Wild, unpublished observations). This observation also agrees with the work of Petric & Prevec (1970) who were unable to demonstrate the passage of intracellular ribonucleoprotein into mature particles in the medium.

The high molecular weight RNA isolated by fractionation on Sepharose 2B contained RNA strands which would be expected from both replicative and transcriptive processes. The presence of the 10 to 16S RNA as a major constituent of the complex suggests that a large proportion of the molecules are involved in transcription. However, this may depend on the part of the growth cycle which the infection has reached when the RNA is extracted. Studies are now in progress to determine if there is a changeover from one type of RNA synthesis to the other.

In contrast to RNA bacteriophages and picornaviruses, the replication of vesicular stomatitis virus in BHK cells involves a number of species of RNA which are smaller than the complete genome. This implies that the RNA of vesicular stomatitis virus must have internal initiation sites for RNA synthesis. A similar replicative mechanism is exhibited by Newcastle disease virus (Bratt & Robinson, 1967) and Sendai virus (Blair & Robinson, 1968) but in them the messenger RNA is probably polycistronic because of the size of the induced RNA.

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REFERENCES


(Received 3 May 1971)