Replication of Rhinovirus RNA

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SUMMARY

We have studied the synthesis of virus RNA in human embryo lung cells infected with rhinovirus type 2. The three species of RNA in extracts of infected cells are, in order of decreasing electrophoretic mobility, single-stranded RNA, replicative form and replicative intermediate. The kinetics of synthesis of these RNA species were investigated. The electrophoresis of mixtures of single-stranded RNA synthesized early and late in infection showed no differences in size. This observation was confirmed with a bovine enterovirus. The native form of poliovirus type 1 single-stranded RNA migrated faster on polyacrylamide electrophoresis than rhinovirus single-stranded RNA.

INTRODUCTION

The 89 different serotypes (Collaborative Report, 1967, 1971) of rhinoviruses form the largest subgroup of the picornaviruses. Although rhinoviruses cause a typically mild upper respiratory infection, they are collectively an important group since their effect upon the general population is widespread (Tyrrell, 1968).

Despite their importance, the fundamental properties of rhinoviruses have not been widely investigated. Rhinoviruses have been characterized as a group in terms of their general properties (Tyrrell & Chanock, 1963) and are distinguished from other picornaviruses by optimal growth at 33 °C (Tyrrell & Parsons, 1960; Stott & Heath, 1970), by destruction of their infectivity below pH 6.0 (Ketler, Hamparian & Hilleman, 1962; Dimmock & Tyrrell, 1964) and by a high buoyant density in cesium chloride (Chapple & Harris, 1966; Dans, Forsyth & Chanock, 1966). Recently the virus particle proteins have been investigated by Medappa, McLean & Rueckert (1971) and Korant et al. (1972) who reported that the polypeptide composition resembled that of other picornaviruses.

This paper is concerned with the replication of rhinovirus RNA but differs in several ways from recent studies by Sethi & Schwerdt (1972) and Yin & Knight (1972). Sethi & Schwerdt (1972) analysed the RNA synthesized in HeLa cells by rhinovirus type 20 by centrifuging on sucrose gradients, and Yin & Knight (1972) assayed infected cells for virus polymerase activity. We have studied the replication of a different serotype in different cells, that is rhinovirus type 2 in human diploid cells. The RNA was analysed by electrophoresis in polyacrylamide gel, which permits higher resolution than that obtained by velocity sedimentation. We have confirmed some of the observations of Sethi & Schwerdt and have obtained further results on the kinetics of synthesis of virus RNA's. In addition, we have examined single-stranded RNA synthesized at different times during replication and have not found molecules larger than the virus RNA. This is discussed in relation to the hypothesis of Brown & Martin (1965) of a "rolling-circle" model for the replication of picornavirus
RNA. We have also established, by electrophoresis of mixtures of RNA labelled with different isotopes, that rhinovirus native single-stranded RNA is larger than that of poliovirus.

METHODS

Viruses. Rhinovirus type 2 was obtained from Dr E. J. Stott, poliovirus type 1 (LSc 2ab) from Dr M. S. Pereira and bovine enterovirus (VG-5-27) from Dr S. J. Martin.

Cells. Monolayers of diploid human embryo lung (HEL) cells of the MRC 5 line were grown in Eagle’s medium supplemented with 10% calf serum, 0.1% sodium bicarbonate and antibiotics. These cells of pass 18 to pass 35 were used for experiments when RNA was to be extracted. Rhinovirus stocks were prepared in L132 cells and titrated in HeLa cells which were both obtained from Dr Stott and grown as described by Stott & Heath (1970). Baby hamster kidney cells (BHK21, clone 13) were used to grow bovine enterovirus as described by Martin, Johnston & Clements (1970).

Infectivity titrations. The infectivity of all virus samples was titrated by plaque assay; rhinovirus samples on monolayers of HeLa cells (Stott & Heath, 1970), poliovirus samples on monolayers of HeLa cells, with an overlay containing Eagle’s medium and 4% calf serum and bovine enterovirus samples on monolayers of BHK cells with an overlay without calf serum.

Preparation of virus RNA from infected cells. HEL cells were used for the majority of these experiments. Monolayers of about 5 x 10^7 cells on 130 cm^2 were infected with 5 to 10 p.f.u./cell at 33°C. After 1.5 h virus was removed and replaced with 25 ml medium containing 2% calf serum and 25 µg actinomycin D. Following incubation for the required time, 100 µCi of [3H]-uridine (specific activity 24 Ci/mmol) or 20 µCi of [14C]-uridine (specific activity 62 mCi/m-mol) (Radiochemical Centre, Amersham) were added. At the end of the pulse of radioactivity the monolayer was washed twice with extraction buffer solution containing 100 mM-NaCl, 50 mM-tris and 2.5 mM-EDTA, pH 7.5, and the cells scraped from the glass into the same buffer solution containing 1% (w/v) sodium dodecyl sulphate (SDS). RNA was extracted by shaking at room temperature with two volumes of phenol saturated with extraction buffer solution. The aqueous phase was separated by centrifuging and extracted three times with ether; the RNA was then precipitated with ethanol at -20°C. RNA was finally redissolved in extraction buffer solution in preparation for electrophoresis.

Enzyme treatment. RNA was dissolved in 300 mM-sodium chloride containing 30 mM-sodium citrate, pH 8.45 (2 x SSC) and incubated with 0.1 µg/ml pancreatic ribonuclease (RNase) (Sigma) for 10 min at 35°C. Treatment with deoxyribonuclease (DNase) necessitated further purification by ethanol precipitation to remove traces of SDS which inhibited this enzyme. The reaction mixture contained 10 µg/ml DNase and 10 mM-MgCl₂ in extraction buffer solution, and was incubated for 30 to 60 min at 37°C.

Polyacrylamide electrophoresis. The system described by Loening (1969) was used. Polyacrylamide gels of 1.7 or 2.0% were supported by 0.5% agarose and polymerized in 10 cm tubes of internal diameter 0.7 cm. Gels were subjected to normal electrophoresis before use in experiments. Up to 60 µg of RNA as estimated by absorption at 260 nm was loaded onto each gel and electrophoresed at 50 V for 3 to 5 h. Subsequently gels were extruded and analysed for material absorbing at 260 nm on a Joyce-Loebl ‘Chromoscan’ densitometer. The same gels were then frozen and cut into approximately 1 mm slices. The gel slices were dissolved in hydrogen peroxide at 80°C for 3 h before adding scintillation fluid consisting of toluene containing 33% triton X100. Radioactivity was determined on a Packard scintillation counter.
**Replication of rhinovirus RNA**

Fig. 1. Sedimentation of concentrated [\(^3\)H]-uridine labelled virus on a 15 to 45\% sucrose gradient at 65,000 g for 3 to 5 h. Fractions of 1 ml were collected and the radioactivity of 100 \(\mu l\) samples was determined (○—○). Only the infectivity (○—○) of the peak fractions is shown. Inset: RNA was extracted from gradient fractions 6, 7 and 8 and electrophoresed on polyacrylamide gel. Ribosomal RNA markers from HeLa cells were co-electrophoresed and detected by optical density measurements.

**Preparation and purification of infectious rhinovirus.** Monolayers of L132 or HEL cells in rolled Winchester bottles were infected with 0.01 to 0.1 p.f.u./cell. Cells were maintained at 33 °C in medium containing 2\% calf serum. When the cytopathic effect extended to 80\% of the cells, the cells were shaken into the medium and cell-associated virus released by freezing and thawing. The fluid was clarified and virus stocks stored at −65 °C.

When radioactive virus was required [\(^3\)H]-uridine was added at the onset of the cytopathic effect. Virus was harvested as described above usually after a labelling period of about 5 h. Purification (Brown & Cartwright, 1963) involved precipitation of the virus with 50\% (v/v) saturated ammonium sulphate, pelleting the redissolved virus by centrifuging, and sedimentation of the resuspended virus in a 15 to 45\% sucrose gradient (Fig. 1). RNA was extracted from purified virus as described above for infected cells.
RESULTS

Multiplication of virus in HEL cells

Confluent monolayers were inoculated at a multiplicity of 5 to 10 p.f.u./cell and placed immediately at 33 °C. Virus was allowed to adsorb for 90 min and residual virus was removed by washing cell sheets three times. At intervals after infection, cells were examined for cytopathic effects and were then scraped into the culture fluid and frozen and thawed to release cell associated virus. After clarification at 1000 g the fluids were titrated for infectivity (Fig. 2). Infective virus was first detected at 4 h after infection and showed a plateau of infectivity at 11 h. The separate titrations of cell associated and released virus indicated that over 90% of infectivity was not released from the cells. These conditions of virus growth were used in the following experiments on the synthesis of virus RNA.

Synthesis of virus RNA

RNA was extracted from cells labelled with [³H]-uridine from 5 to 7 h after infection and analysed on polyacrylamide gel (Fig. 3). Three RNA species were identified clearly which were absent from extracts of uninfected cells.

The fastest (1) had a mobility identical with that for virus RNA (Fig. 4) and migrated to a position between the 28S ribosomal RNA and a 'marker' of cellular DNA which was frequently observed in the optical density trace. The species of intermediate mobility (2) migrated close to the DNA marker, and the slowest (3) entered the gel to a variable extent, usually no further than fraction 5.

Treatment of the extract with DNase before electrophoresis did not alter the position of
Fig. 3. Electrophoresis of RNA extracted from infected HEL cells labelled with $[^{3}H]$-uridine from 5 to 7 h after infection. ●, untreated RNA; ○, RNA treated with RNase (0.1 μg/ml). The arrows are taken from the $E_{260}$ trace, indicating the position of the ribosomal RNA and the 'marker' DNA.

Fig. 4. Co-electrophoresis of RNA extracted from HEL cells labelled with $[^{14}C]$-uridine (○) from 9 to 11 h after infection and virus RNA labelled with $[^{3}H]$-uridine (●). The continuous line is the $E_{260}$ trace, indicating the position of the 28S ribosomal RNA and the 'marker' DNA.
Table 1. Treatment with ribonuclease of rhinovirus RNA from infected cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicative intermediate</th>
<th>Replicative form</th>
<th>Single-stranded</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>254 (100%)</td>
<td>76 (100%)</td>
<td>938 (100%)</td>
</tr>
<tr>
<td>0.1 μg RNase/ml</td>
<td>47 (19%)</td>
<td>164 (216%)</td>
<td>0 (0%)</td>
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RNA in 2 × SSC was treated with RNase and then electrophoresed on polycrylamide gel.

any of the virus RNA's and served only to remove the peak of cellular DNA referred to above.

Further information on the virus RNA species was obtained by ribonuclease treatment.

Ribonuclease resistance of virus RNA

RNA extracted from infected cells was dissolved in 2 × SSC and incubated with 0.1 μg/ml RNase. Electrophoresis of the RNase-treated extract showed that the fastest and slowest moving peaks had been degraded, while the middle peak had increased in size (Fig. 3 and Table 1). The RNase resistant peak was therefore designated as the
Replication of rhinovirus RNA

Replicative form (RF). Since the RNase-sensitive fast-moving peak migrated to the same position as virus RNA it was designated single-stranded (SS) RNA. Since the slowest moving peak was heterodisperse, was RNase sensitive and appeared to be degraded by RNase treatment to the same mobility as RF, it was designated as replicative intermediate (RI).

Time course of the synthesis of virus RNA’s

(a) With [3H]-uridine present continuously

RNA was extracted from cells labelled with [3H]-uridine continuously during the multiplication cycle, and analysed by electrophoresis on polyacrylamide gel (Fig. 5). The mean radioactivity above background in peaks of RI, RF and SS RNA was calculated and normalized by reference to the optical density of the ribosomal RNA present in each preparation (Fig. 6). Radioactivity at the origin suggested that RI could be detected at 3 h after infection, RF was not detected until 5 h and SS RNA until 7 h. RI and RF increased exponentially from 3 h and SS RNA from 5 h up to the end of the experiment at ~3 h. This was surprising since by 11 h, the production of virus particles had reached a plateau and the majority of cells showed cytopathic effects. A small peak of high mol. wt. RNA, which migrated between RI and RF, was occasionally observed in these experiments and in the pulse experiments described below (Fig. 7). Its significance is not known.

(b) With pulses of [3H]-uridine

RNA was analysed as described above from cells which had been pulsed for consecutive periods of 2 h with [3H]-uridine (Fig. 7). The area of radioactivity in peaks of virus RNA was calculated and normalized as described above (Fig. 8). Synthesis of virus RNA was first detected during the 5 to 7 h pulse. RF and SS RNA continued to increase exponentially even during the last pulse from 11 to 13 h. The synthesis of RI increased until the 7 to 9 h pulse and then remained constant.
Fig. 7. Synthesis of virus RNA in HEL cells. Cells were pulsed with \[^{3}H\]-uridine for consecutive periods of 2 h throughout the growth cycle. The arrows indicate 28S ribosomal RNA. RNA was electrophoresed under standard conditions.

Over a large number of experiments using different concentrations of radioactive uridine, different batches of virus and cells of different passage numbers, the proportion of each RNA species expressed as a percentage of a sum of virus RNA’s was remarkably constant (Table 2). The predominant species was SS RNA. From its first appearance until late in infection, SS RNA comprised approximately 80% of the total. RF remained constant at about 10% while the percentage of RI declined from 10 to 2.5%.

Undine uptake by infected cells

It can be argued that the incorporation of uridine into virus RNA depends upon the efficiency with which the cell is able to metabolize the \[^{3}H\]-uridine. Consequently, to determine the variation in pool size (Fig. 9), we measured the acid-soluble \[^{3}H\]-uridine present in non-infected cells and in infected cells throughout the growth cycle. It was evident that while the cells remained free of cytopathic effects, the pool of \[^{3}H\]-uridine varied by
Replication of rhinovirus RNA

Fig. 8. Rate of synthesis of rhinovirus RNA. Each point represents the middle of a 2 h pulse of \[^{3}H\]-uridine and is the sum of each peak of radioactivity above the background level of the gel. Different preparations were normalized with reference to the optical density of ribosomal RNA markers. \(\triangle\), RI (replicative intermediate); \(\bullet\), RF (replicative form); \(\circ\), SS (single-stranded RNA).

Table 2. Percentage of total rhinovirus RNA synthesized as RI, RF or SS species during infection of HEL cells

<table>
<thead>
<tr>
<th>Pulse time (h)</th>
<th>RI</th>
<th>RF</th>
<th>SS</th>
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<tr>
<td>5–7</td>
<td>10.0*</td>
<td>9.3</td>
<td>80.8</td>
</tr>
<tr>
<td>7–9</td>
<td>6.1</td>
<td>12.7</td>
<td>81.2</td>
</tr>
<tr>
<td>9–11</td>
<td>2.5</td>
<td>13.9</td>
<td>83.6</td>
</tr>
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</table>

* Cells were pulsed with \[^{3}H\]-uridine during the times indicated in the presence of actinomycin D. The extracted RNA was electrophoresed and the radioactivity in each peak, above the background level in the gel, was estimated. Virus RNA was not detected during the 3 to 5 h pulse.

only 20%. At the onset of the cytopathic effect, the size of the radioactive uridine pool fell gradually to 50% of the control value. This probably reflected the numbers of cells which were detached from the monolayer, since only cells remaining attached were assayed.

Size of SS RNA synthesized early and late in infection

Evidence has been provided that early in infection some picornaviruses synthesize SS RNA which is larger than the RNA of the virus (Brown & Martin, 1965; Clements & Martin, 1971). With this evidence these authors proposed a ‘rolling-circle’ model for the synthesis of picornavirus RNA. Since this matter is fundamental to theories of RNA replication we examined the size of SS RNA synthesized by rhinovirus and by the bovine enterovirus used by Clements & Martin (1971).

(a) Rhinovirus in HEL cells

Rhinovirus RNA was labelled at 5 to 7 and at 9 to 11 h postinfection with \[^{3}H\]- or \[^{14}C\]-uridine, respectively, and analysed by electrophoresis on a single polyacrylamide gel (Fig. 10). The SS RNA labelled at either time migrated to the same position in the gel and in peaks of similar width.
Fig. 9. Comparison of the acid-soluble uridine pools in cells infected with rhinovirus and non-infected cells. The extent of cytopathic effect is indicated as an internal marker of the extent of infection from 10% (±) to over 80% (+++) of cells.

Fig. 10. Co-electrophoresis of RNA from rhinovirus-infected HEL cells labelled from 5 to 7 h with [3H]-uridine (○-○) or from 9 to 11 h with [14C]-uridine (○-○). The continuous line is the $E_{260}$ trace, indicating the position of 28s ribosomal RNA and the ‘marker’ DNA.
Replication of rhinovirus RNA

Fig. 11. Co-electrophoresis of RNA from bovine enterovirus-infected cells (a) HEL cells labelled from 0.5 to 4 h with [14C]-uridine (●——●) or from 4 to 7 h with [3H]-uridine (○——○). (b) BHK cells labelled from 0.5 to 4 h with [3H]-uridine (●——●) and from 4 to 6 h with [14C]-uridine (○——○). The continuous line is the trace at E$_{260}$. 
Fig. 12. Co-electrophoresis of RNA from cells infected with rhinovirus or with poliovirus. Both were labelled with uridine from 9 to 11 h after infection. The rhinovirus RNA was [14C] labelled (○—○) and poliovirus RNA [3H] labelled (■—■). The continuous line is the trace at $E_{600}$.

(b) Bovine enterovirus in HEL cells

In HEL cells the exponential production of virus ended at 7 h after infection. Accordingly, RNA was labelled from 0.5 to 4 h and from 4 to 7 or 9 to 11 h post-infection with [3H]- or [14C]-uridine, respectively. Co-electrophoresis showed that SS RNA extracted at either time migrated identically (Fig. 11a).

(c) Bovine enterovirus in BHK cells

The occurrence of SS virus RNA larger than virus particle RNA has only been reported in BHK cells. Since we failed to find such RNA in infected HEL cells we examined the system described by Clements & Martin (1971), that of BHK cells infected with bovine enterovirus. In BHK cells the exponential production of virus particles started at 3 h and reached a plateau at 5 h after infection. Accordingly, we labelled from 0.5 to 4 h and from 4 to 6 h after infection with [3H]- or [14C]-uridine, respectively (Fig. 11b). Co-electrophoresis showed that the peaks of virus SS RNA coincided and had similar distributions. SS RNA obtained when cells were labelled from 0.5 to 2.5 h was also of the same size.

Comparison of SS virus RNA from HEL cells infected with rhinovirus or with poliovirus

By sedimentation, native or denatured poliovirus RNA was found to be larger than rhinovirus RNA (Nair & Lonberg-Holm, 1971; Sethi & Schwerdt, 1972), but no comparison by polyacrylamide electrophoresis has been reported.

Parallel cultures of HEL cells were infected with rhinovirus or poliovirus at a multiplicity of 10 p.f.u./cell. The rate of growth of both viruses was similar as judged by the
Replication of rhinovirus RNA

appearance of cytopathic effects. Both cultures were labelled from 9 to 11 h; that infected with rhinovirus was labelled with [14C]-uridine and that infected with poliovirus with [3H]-uridine. RNA extracts were electrophoresed together (Fig. 12). Rhinovirus single-stranded RNA was clearly larger than that of poliovirus. The mol. wt. of rhinovirus RNA was estimated as 2.8 x 10^6, by reference to ribosomal RNA's (mol. wts. of 0.67 x 10^6 and 1.64 x 10^6, Petermann & Pavlovec, 1966) and poliovirus RNA (mol. wt. of 0.67 x 10^6, Granboulan & Girard, 1969; Tannock, Gibbs & Cooper, 1970). Close confirmation of this value was obtained when rhinovirus RNA was co-electrophoresed with HeLa cell nucleolar RNA. Since the mol. wt. of the markers ranged from 0.65 x 10^6 to 4.1 x 10^6 no extrapolation from the calibration curve was required in this experiment to obtain the mol. wt. of the rhinovirus SS RNA.

DISCUSSION

We have shown that cells infected with rhinovirus type 2 synthesize a species of SS RNA which has an electrophoretic mobility identical to that of virus RNA. The details of synthesis are not known but evidently it is a closely regulated process since SS RNA molecules synthesized in infected cells during exponential increase of virus and during the plateau of virus production showed no detectable variation in size. This feature is of particular interest since other picornaviruses, foot-and-mouth disease virus (Brown & Martin, 1965) and a bovine enterovirus (Clements & Martin, 1971), are reported to synthesize SS RNA larger than that of the virus RNA, and bovine enterovirus to synthesize RNA of different mol. wts. at different times during the multiplication cycle. We have repeated these experiments with bovine enterovirus and found that SS RNA synthesized in HEL or BHK cells at different stages of the multiplication cycle also showed no detectable variation in size. It is still possible to argue that molecules of SS RNA larger than virus RNA, were present in the rhinovirus-, bovine enterovirus- or poliovirus-infected cells represented in the electrophoretograms above. However, we were unable to resolve such molecules and the area where such material would be expected in the gel showed radioactivity indistinguishable from the background scatter. It is also possible that our experimental conditions favoured the labelling of SS RNA of the same size as virus RNA. However, we would have expected the high resolving capacity of polyacylamide gels to distinguish between the range of SS RNA molecules found by Wild & Brown (1970) and Clements & Martin (1971). However, we were able to resolve only one species of SS RNA which, whether we labelled early or late in the infectious cycle, was indistinguishable from virus RNA. We conclude that our data provide no evidence to support the ‘rolling-circle’ hypothesis for picornavirus RNA replication, and do not conclusively disprove it.

In addition to the SS RNA, two other species of RNA were detected in rhinovirus-infected cells. The species of intermediate electrophoretic mobility formed a discrete peak on electrophoresis and was resistant to degradation by RNase. On this evidence it was similar to the replicative form (RF) of poliovirus RNA described by Noble, Kass & Levintow (1969). The third species did not form a discrete peak and migrated only a short distance into the gel upon electrophoresis. Treatment with RNase degraded this species and resulted in a corresponding increase in the peak of RF. For these reasons this species seemed very similar to poliovirus replicative intermediate (RI) described by Noble et al. (1969).

The dynamics of synthesis of the three species of rhinovirus RNA in HEL cells are of some interest since there are differences in the results obtained with poliovirus by pulse or continuous labelling (Noble & Levintow, 1970) and with total uridine incorporation by
rhinovirus type 20 in HeLa cells where the exponential increase is not maintained late in infection (Sethi & Schwerdt, 1972). However, the replication of poliovirus RNA in HEL cells closely resembled the replication of rhinovirus RNA in HEL cells: despite poliovirus incorporating 100-fold more uridine than rhinovirus the proportion of virus RNA species (4% RI, 11% RF and 85% SS RNA (Fig. 8)) was very similar to that found in rhinovirus-infected cells. Although a direct comparison of poliovirus replication in HEL and HeLa cells is not available, we infer that the host cell may be exerting an effect on virus RNA synthesis.

The proportion of each species of rhinovirus RNA synthesized during a 2 h pulse of [³H]-uridine clearly varied with time. Surprisingly, however, there was a constant proportion of SS RNA synthesized at all times which accounted for about 81% of the total virus RNA synthesized. The proportion of RF synthesized increased from 9 to 14% of the total while the proportion of RI decreased from 10 to 2.5%.

The pattern of RNA synthesis was not very different when followed by continuous labelling except at 1 to 5 h when virus RNA was first observed. At this early time there was a slight excess of RF over RI and very little SS RNA. With longer periods of labelling the proportion of the total RNA contributed by RI fell by about tenfold and the proportion of RF by about threefold. Over the same period the proportion of SS RNA increased very rapidly and was close to maximum after labelling from 1 to 7 h.

Our results are generally consistent with the hypothesis that SS RNA and RF are end-products of RNA replication and show RI in its postulated role as an intermediate in RNA replication (Girard, 1969). Although only RI was observed during the earliest pulses there are other features which are difficult to reconcile with this theory of RNA replication. First, SS RNA was the most rapidly labelled species. This is hardly consistent with an end-product but may be accounted for by the long (2 h) pulse interval. Secondly, with continuous labelling to 5 h the major virus RNA species was RF. It is unlikely that so much end product RNA should be synthesized unless RF can have some intermediate role in replication, as suggested by Noble & Levintow (1970). Thirdly, we find that with continuous labelling all virus RNA's increase exponentially, yet on pulse labelling we find that only SS RNA increases exponentially while RF and RI increase at a slower rate or not at all. We cannot reconcile for RI the results by pulse- and continuous-labelling, since the rate of RI synthesis seems insufficient to account for the total RI present in the cell. However, this would be explained if uridine was selectively incorporated into SS RNA during relatively short labelling periods. The increase in RI, RF and SS RNA with continuous labelling indicates that all virus RNA species are stable.

Earlier reports have compared the size of SS RNA of rhinovirus with poliovirus: McGregor & Mayor (1971), Nair & Lonberg-Holm (1971) and Sethi & Schwerdt (1972) found that both native and denatured poliovirus type 1 RNA sedimated faster on sucrose gradients than did rhinovirus RNA from type 14, type 2 and type 20, respectively. However, the co-electrophoresis of native RNA from cells infected with rhinovirus type 2 or poliovirus type 1 showed that the rhinovirus SS RNA was larger. Clearly there is a discrepancy which indicates that movement of these RNA molecules in sucrose gradients or polyacrylamide gels cannot be determined solely by the mol. wt. of the RNA. Such an observation has already been made with the ‘26s’ RNA of Semliki Forest virus, which appears larger than 28s ribosomal RNA under certain conditions of electrophoresis (Kennedy, 1972).

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REFERENCES


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