A Study of the Heterogeneous 37s Ribonucleic Acid Induced by Foot-and-Mouth-Disease Virus

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(Received 2 October 1967)

1. The 37s RNA induced in baby-hamster kidney cells by infection with foot-and-mouth-disease virus was examined on sucrose gradients and by filtration through Sepharose 4B. 2. The RNA sedimented faster (37s) and as a broader band than the 35s RNA from purified virus. 3. Treatment with deoxyribonuclease, Pronase or amylase did not alter the sedimentation profile of the 37s RNA. 4. Treatment of individual fractions of the RNA with phenol, dimethyl sulphoxide or methylCellosolve did not decrease the sedimentation rate of the faster-sedimenting molecules. 5. Sedimentation in sucrose gradients of different ionic strengths or containing EDTA had no effect on the heterogeneous nature of the profile. 6. On filtration through Sepharose 4B columns, the 37s virus-induced RNA was eluted before viral RNA. 7. Only 20% of the rapidly sedimenting RNA was incorporated into complete virus particles.

Infection of BHK-cell† monolayer cultures with FMDV in the presence of actinomycin and radioactive precursors leads to the production of at least three species of labelled RNA molecules that can be separated by sucrose-density-gradient centrifugation (Brown & Cartwright, 1964). The two more slowly sedimenting peaks (16–20s) have low infectivity and are partially ribonuclease-resistant; the ribonuclease-resistant part of this fraction has a base composition corresponding to a double-stranded structure (Brown & Martin, 1965). The most rapidly sedimenting peak is considerably more infective and is completely degraded by ribonuclease (Brown & Cartwright, 1964). Although it was first thought likely that this rapidly sedimenting fraction represented viral RNA, it was noticed in some of our previous experiments that the peak appeared to be much broader than that obtained when RNA prepared from purified virus was sedimented under the same conditions (Brown & Martin, 1965). Further, resedimentation of the individual fractions comprising the broad peak showed that the RNA extracted from infected cells consisted of more than one molecular species. In the present paper we have extended our studies of the physical characteristics of this RNA and have also estimated the efficiency with which it is incorporated into mature virus particles.

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† Abbreviations: BHK cells, baby-hamster kidney cells; FMDV, foot-and-mouth-disease virus; SDS, sodium dodecyl sulphate.

MATERIALS AND METHODS

Materials. [¹⁴C]Uridine of specific radioactivity 486 mc/m-mole and carrier-free [³²P]orthophosphate were obtained from The Radiochemical Centre, Amersham, Bucks. Actinomycin D was a gift from Merck, Sharp and Dohme Inc., Rahway, N.J., U.S.A. SDS and amylase were obtained from British Drug Houses Ltd., Poole, Dorset. Sepharose 4B gel was obtained from Pharmacia (G.B.) Ltd., London, W. 13. Pronase was obtained from Calbiochem Ltd., London, W. 1, deoxyribonuclease from Sigma (London) Chemical Co. Ltd., London, S.W. 6, and ribonuclease from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex.

Cell cultures. BHK cells (BHK 21, clone 13), isolated by Macpherson & Stoker (1962), were grown as monolayers in either Roux bottles or 4 oz. medicine bottles, in Eagle's medium containing 10% (v/v) ox serum, 0.2% tryptose-phosphate broth, and antibiotics (penicillin, streptomycin and polymyxin, each at 100 units/ml.).

Virus production and assay. FMDV of strain 1, type 0, was grown in monolayers of BHK cells as described by Brown, Martin & Underwood (1966). For the preparation of [¹⁴C]uridine-labelled virus, the culture medium in which the virus was grown contained actinomycin D at a concentration of 1 µg./ml. Virus labelled with ³²P was obtained by growing the virus in cells maintained in modified Earle's saline containing 1 µg. of actinomycin D/ml. and carrier-free [³²P]orthophosphate. The Earle's saline had been modified by replacing the phosphate with 0.01 M-tris-HCl buffer, pH 7.6, and contained less than 0.1 mM-phosphate. Estimates of infectivity were made by the plaque-assay method (Mowat & Chapman, 1962).

Purification of virus. The virus was purified from cell harvests or ultrasonically treated cell extracts by the method described by Brown & Cartwright (1963). In this method the virus was first precipitated with 50%-saturated ammonium sulphate and the resuspended precipitate was

then centrifuged for 1 hr. at 30000 rev./min. in the no. 40 rotor of the Spinco model L ultracentrifuge. This pellet was resuspended in 1% (w/v) SDS and centrifuged in a 15–45% (w/v) sucrose gradient for 3.5 hr. at 25000 rev./min. at 4° in the Spinco SW 25.1 rotor. The overall recovery of virus by this procedure was about 50%.

Preparation of RNA. Purified virus suspensions in 0.04 M-phosphate ($\text{Na}_2\text{HPO}_4\text{--KH}_2\text{PO}_4$) buffer, pH 7.6, were extracted twice with an equal volume of cold phenol that had been equilibrated with the same buffer solution at 4°. The aqueous layer containing the viral RNA was mixed with RNA from BHK cells, precipitated with 2 vol. of cold ethanol and stored at -20°. The precipitate was dissolved in 0.1 M-sodium acetate buffer, pH 5.0, and filtered through a column of Sephadex G-25 to remove small molecules before examination by sucrose-gradient centrifugation.

RNA was extracted from infected cells by first washing the monolayers with cold Eagle's medium and then adding phenol saturated with phosphate buffer directly to the cell sheets. The aqueous layer obtained by centrifugation at 2000g was extracted a second time with cold phenol and then precipitated with 2 vol. of cold ethanol. The subsequent treatment was then the same as that described in the previous paragraph.

Sucrose-gradient centrifugation. A 2 ml. sample of the RNA solution was centrifuged in a 25 ml. gradient of 5–25% (w/v) sucrose in 0.1 M-sodium acetate buffer, pH 5.0, in the SW 25.1 rotor of the Spinco ultracentrifuge. Centrifugation was continued at 4° for 14 hr. at 20000 rev./min. At the end of this period, 25 1 ml. fractions were collected from the bottom of the tube and portions of these were used for the estimation of radioactivity and extinction at 260 m μ . Individual or pooled fractions were precipitated with ethanol in the presence of added BHK-cell RNA and stored at -20°.

Gel-filtration on Sepharose 4B columns. Columns of Sepharose 4B gel were prepared in 0.01 M-sodium acetate buffer, pH 5.0, containing 0.1% of SDS and 0.1 M- or 0.2 M-NaCl. The RNA fractions were filtered through the columns and the eluted fractions were tested for radioactivity and extinction at 260 m μ .

Determination of radioactivity. Radioactivity was determined by plating the samples on to aluminium planchets. The samples were dried, and counted in a Panax Ltd. (Redhill, Surrey) solid-scintillation counter.

RESULTS

Comparison of virus-induced and viral RNA. RNA was extracted with phenol from BHK cells that had been infected with FMDV for 3 hr. in the presence of actinomycin and [^{32}P]orthophosphate. After filtration through Sephadex G-25 followed by sedimentation in a sucrose gradient, the fastest-moving peak (peak A, Fig. 1a) was precipitated by 2 vol. of ethanol in the presence of added BHK-cell RNA. The precipitated RNA was dissolved in 0.1 M-acetate, pH 5.0, and resedimented through a second sucrose gradient. Individual 1 ml. fractions of this gradient were precipitated from the sucrose and resedimented in gradients in the presence of carrier ribosomal RNA. Fig. 1(b) shows that

Fig. 1. Sucrose-gradient sedimentation of RNA extracted from cells infected with FMDV. The RNA was centrifuged in a gradient of 5–25% sucrose in 0.1 M-acetate, pH 5.0, for 14 hr. at 4° in the SW 25.1 rotor of the Spinco model L ultracentrifuge at 20000 rev./min. Peak A was precipitated with 2 vol. of ethanol, dissolved in 0.1 M-acetate buffer, pH 5.0, and reprecipitated in a second gradient. Individual fractions (fractions 2, 4 and 6) from the gradient were precipitated with BHK-cell RNA and reprecipitated in separate gradients. (a) —, ³²P radioactivity of RNA from infected cells; ---, E₂₆₀. (b) ³²P radioactivity: ---, fraction 2; —, fraction 4; ·····, fraction 6.

fractions from the virus-induced RNA sedimented in their original positions. This indicated that the spread of radioactivity observed in the first gradient was not due merely to diffusion. In contrast, RNA prepared from purified virus sedimented more slowly (35s) than the induced RNA and, further, when fractions from the leading edge of the peak were recycled through fresh gradients, they took up the position of the original peak (Fig. 2). These two features point to a difference between the RNA prepared from infected cells and that isolated from purified virus.

Treatment of virus-induced RNA with enzymes. RNA was prepared from BHK cells infected for 3 hr. with FMDV in the presence of actinomycin and [^{14}C]uridine. Peak A, operationally referred to as 37s RNA, was purified as above. Separate portions of the RNA solution in 0.1 M-acetate were treated with deoxyribonuclease, Pronase, amylase and ribonuclease, and then sedimented in sucrose gradients. Fig. 3(a) shows that ^{14}C -labelled RNA from infected cells had a more heterogeneous and

Fig. 2. Sucrose-gradient sedimentation of RNA extracted from purified FMDV. The RNA was mixed with BHK-cell RNA and centrifuged as described in Fig. 1. Individual fractions (fractions 2, 4, and 6) from the gradient were precipitated with BHK-cell RNA and re-centrifuged. (a) —, ^{32}P radioactivity of RNA from purified virus; ----, E_{260} . (b) ^{32}P radioactivity: ----, fraction 2; —, fraction 4; ·····, fraction 6.

Fig. 3. Effect of enzymes on the sedimentation in sucrose gradients of RNA extracted from cells infected with FMDV. Cells were labelled with [^{14}C]uridine ($1\mu\text{C}/\text{ml.}$) for 3 hr. after infection in the presence of actinomycin D ($1\mu\text{g.}/\text{ml.}$), and fraction A of the induced RNA was isolated as described in the text. RNA labelled with [^{32}P]orthophosphate was isolated from the purified virus. (a) —, [^{14}C]Uridine radioactivity of fraction A of virus-induced RNA; ----, ^{32}P radioactivity of RNA extracted from purified virus; ·····, E_{260} . (b) —, [^{14}C]Uridine radioactivity after treatment of fraction A with deoxyribonuclease ($10\mu\text{g.}/\text{ml.}$); ----, [^{14}C]uridine radioactivity after treatment of fraction A with ribonuclease ($1\mu\text{g.}/\text{ml.}$); ·····, E_{260} . (c) —, [^{14}C]Uridine radioactivity after treatment of fraction A with amylase ($5\text{mg.}/\text{ml.}$); ----, [^{14}C]uridine radioactivity after treatment of fraction A with Pronase ($100\mu\text{g.}/\text{ml.}$); ·····, E_{260} .

slightly higher sedimentation rate (37s) than viral ^{32}P -labelled RNA (35s). Treatment with deoxyribonuclease had no effect on the sedimentation of the induced RNA, whereas treatment with ribonuclease resulted in complete degradation to low-molecular-weight components (Fig. 3b).

The result of treatment with Pronase and amylase is shown in Fig. 3(c). Although Pronase tended to sharpen the peak, the sedimentation rate was unchanged and was still higher than that of RNA from purified virus. Amylase had no effect on the profile. These experiments indicate that the induced RNA did not owe its slightly higher sedimentation rate to association with fragments of DNA, protein or carbohydrate (Bramwell & Harris, 1967). On the other hand, the small increment in sedimentation rate is unlikely to have resulted from dimerization of single-stranded 35s molecules.

Treatment with phenol, dimethyl sulphoxide and methylCellosolve. Individual fractions from a sucrose-gradient fractionation of [^{14}C]uridine-labelled RNA from infected cells were precipitated by ethanol in the presence of purified ribosomal RNA. Fraction 3 was dissolved in 0.1M-acetate buffer, pH 5.0, and a portion re-extracted with phenol. The treated and untreated portions of this

fraction were then sedimented in separate sucrose gradients and their radioactivity profiles compared with that of fraction 8 of the peak, which had a sedimentation value of 35s, equal to that of viral RNA (Fig. 4a). Fraction 6 was dissolved in 0.1M-acetate buffer, pH 5.0, containing 0.1% of SDS, and a portion was made to 85% dimethyl sulphoxide and shaken at room temperature for 10 min. The solution was then treated with 2 vol. of ethanol at -20° , and the precipitate was dissolved in acetate buffer, pH 5.0, containing 0.1% of SDS, and sedimented in a sucrose gradient (Fig. 4b).

Both the phenol and the dimethyl sulphoxide

Fig. 4. Effects of phenol, dimethyl sulphonyl and methyl-Cellosolve on the sedimentation of individual fractions of peak A of virus-induced RNA. (a) A sample of fraction 3 was extracted with an equal volume of phenol-acetate and the aqueous layer precipitated with 2 vol. of ethanol and BHK-cell ribosomal RNA. The precipitate was dissolved in 0.1M-acetate, pH 5.0, containing 0.1% of SDS, and centrifuged in a sucrose gradient as described in the text. —, Untreated; ----, phenol-treated;, untreated fraction 8 (approx. 35s). (b) Fraction 6 was precipitated with 2 vol. of ethanol and BHK-cell ribosomal RNA and the precipitate was dissolved in 0.1M-acetate buffer, pH 5.0, containing 0.1% of SDS. A sample of this was made to 85% dimethyl sulphonyl and kept at room temperature for 10 min. The solution was then treated with 2 vol. of ethanol at -20° . The precipitate was dissolved in SDS-acetate buffer, applied to a sucrose gradient and centrifuged as described in the text. —, Untreated; ----, treated with dimethyl sulphonyl;, untreated fraction 8. (c) Fraction 5 was precipitated with 2 vol. of ethanol and BHK-cell ribosomal RNA and the precipitate dissolved in 0.1M-acetate buffer, pH 5.0, containing 0.1% of SDS. A sample of this was made to 70% methyl-Cellosolve and shaken at room temperature for 10 min. The solution was then diluted with an equal volume of 0.1M-acetate buffer, applied to a sucrose gradient and centrifuged as described in the text. —, Untreated; ----, treated with methyl-Cellosolve;, untreated fraction 8.

treatment caused the RNA to give a more heterogeneous profile. Experiments that we carried out on RNA extracted from purified virus, which, as we

have shown above, gave a homogeneous profile in sucrose gradients (cf. Fig. 2), also gave a heterogeneous profile after a similar treatment with dimethyl sulphonyl. The production of heterogeneous fractions of slower-sedimenting material probably resulted from some minor fragmentation of the RNA during preparation, fractionation or treatment (Spirin, 1964). In the non-treated samples the chain fragments were probably held together by hydrogen bonding, but were released when the secondary structure was disrupted by these reagents. The observation that the major portion of the radioactivity continued to sediment more rapidly than the 35s RNA, however, indicated that the high-molecular-weight virus-induced RNA could not be directly converted into 35s RNA by changes in configuration alone.

In contrast with the above results, treatment of fraction 5 with 70% methyl-Cellosolve for 10 min. at room temperature followed by direct sedimentation in sucrose gradients (Fig. 4c) did not affect the sedimentation behaviour of the RNA. Since methyl-Cellosolve is known to dissociate hydrogen bonds (K. S. Kirby, personal communication), this result also supports the contention that the heterogeneity of the induced RNA (37s) was not merely caused by variations in configuration of the 35s viral RNA.

Effect of ionic strength on sedimentation. [^{14}C]-Uridine-labelled RNA from infected cells was sedimented through sucrose gradients prepared in 0.1M-, 0.01M- and 1mM-acetate buffer, pH 5.0. The sedimentation rate of the entire peak was lowered by the decrease in ionic strength as were those of the ribosomal RNA molecules (Fig. 5). This behaviour is typical of single-stranded molecules. When virus-induced RNA was sedimented in gradients prepared in 1mM-EDTA-0.1M-acetate, pH 5.0, or in gradients prepared in 0.01M-magnesium chloride-0.1M-acetate, pH 5.0, it sedimented at the same rate relative to ribosomal 28s RNA as in 0.1M-acetate gradients. These results indicated that the apparently higher sedimentation rate of the virus-induced RNA was unlikely to have been due to configurational differences or to aggregation caused by the presence of bivalent metal ions.

Gel-filtration of virus-induced RNA. Sepharose 4B columns equilibrated with buffers containing sodium chloride can be used to separate ribosomal RNA from the viral RNA (Oberg & Philipson, 1967). When peak A of FMDV-induced RNA was passed through a Sepharose column, the ^{14}C radioactivity was eluted before the ribosomal RNA. Fig. 6 compares the elution pattern in 0.1M- and 0.2M-sodium chloride. Although at 0.1M-sodium chloride the ^{14}C radioactivity was eluted as one main peak, at 0.2M-sodium chloride part of the induced RNA was delayed along with the ribosomal RNA.

Fig. 5. Effect of ionic conditions on the sedimentation of fraction A of virus-induced RNA. (a) Gradient buffered in 0.1M-sodium acetate, pH 5.0; (b) gradient buffered in 0.01M-sodium acetate, pH 5.0; (c) gradient buffered in 1mM-sodium acetate, pH 5.0. —, ^{14}C radioactivity of fraction A of virus-induced RNA; , E_{260} .

In another experiment, [^{14}C]uridine-labelled RNA from infected cells was mixed with ^{32}P -labelled RNA extracted from purified virus and the mixture was filtered through a Sepharose column equilibrated with 0.1M-sodium chloride. The peak of ^{32}P radioactivity was slightly retarded compared with the ^{14}C radioactivity peak (Fig. 7). This experiment provided further evidence that size of the RNA from purified virus was less than the average size of the RNA present in infected cells.

Incorporation of [^{14}C]uridine into viral and virus-induced RNA. The above results indicate that a large proportion of the RNA induced in cells by virus infection is different in both sedimentation and gel-filtration properties from the RNA of purified virus. The following experiment was carried out to estimate the efficiency of incorporation of the induced RNA into mature virus particles.

Eight monolayers, each containing 10^7 BHK cells

Fig. 6. Effect of ionic strength on the elution of virus-induced RNA (fraction A) from Sepharose 4B columns. Cells were labelled with [^{14}C]uridine in the presence of actinomycin D for 3 hr. after infection with FMDV, and the RNA was extracted by phenol treatment. Fraction A was prepared by sucrose-gradient sedimentation as described in Fig. 3(a), and precipitated from the sucrose gradient by 2 vol. of ethanol and ribosomal RNA. The precipitate was dissolved in 0.1M- or 0.2M-NaCl in 0.01M-acetate buffer, pH 5.0, and each sample was applied to a Sepharose 4B column equilibrated with the respective buffer. The total volume of these columns was 35 ml. (a) 0.1M-NaCl column: —, [^{14}C]uridine radioactivity; ----, E_{260} . (b) 0.2M-NaCl column: —, [^{14}C]uridine radioactivity; ----, E_{260} .

in 4 oz. bottles, were infected at 37° for 30 min. by using a multiplicity of 100. The cells were then washed and the incubation continued in 10 ml. of Eagle's medium containing $1\text{ }\mu\text{g}$. of actinomycin/ml. and $0.1\text{ }\mu\text{C}$ of [^{14}C]uridine/ml. The medium was removed from two of the monolayers 2.75 hr. after infection and the cells were extracted with cold phenol to release the RNA. At the same time, the cells were removed from two other monolayers and disintegrated by ultrasonic treatment to release the virus. The virus was then purified according to the procedure described above. The four remaining bottles were washed free from the medium containing [^{14}C]uridine and incubated for a further 5.25 hr. in Eagle's medium containing $1\text{ }\mu\text{g}$. of actinomycin/ml. and a 1000-fold excess of non-radioactive uridine. At this time (8 hr. after infection) the sheets had

Fig. 7. Gel filtration on Sepharose 4B of ^{14}C -labelled RNA from infected cells and ^{32}P -labelled RNA from purified virus. A Sepharose 4B column was equilibrated with 0.01 M-acetate buffer, pH 5.0, containing 0.1% of SDS and 0.1 M-NaCl. Samples of ^{14}C -labelled RNA from infected cells and ^{32}P -labelled RNA from purified virus were mixed and filtered through the same column. —, $[^{14}\text{C}]\text{Uridine}$ radioactivity of virus-induced RNA; ----, ^{32}P radioactivity of RNA from purified virus; ·····, E_{260} .

disintegrated owing to the multiplication of the virus. The medium from these four bottles was combined and centrifuged at 1000g for 5 min. to remove cell debris, and the supernatant was divided into two equal parts. One part was used for the estimation of the amount of radioactivity incorporated into virus particles. The other part was extracted with phenol to obtain the viral RNA. Both RNA samples were precipitated by adding 2 vol. of ethanol and stored overnight at -20° . The precipitates were then separated, dissolved in 0.1 M-acetate, pH 5.0, shaken with ether to remove any remaining phenol and then freed from ether in a stream of nitrogen. Each sample was then centrifuged in a 5–25% sucrose gradient and the amount of radioactivity sedimenting in the 37s region was measured.

In order to make an accurate estimate of the efficiency of transfer of activity from cellular induced RNA to virus RNA, a number of correction factors needed to be applied to the experimental results. (1) Allowance had to be made for the fact that only 50% of the virus was recovered by the purification procedure as measured by infectivity determination. (2) Estimates based on the direct experimental results would be high because of the continued incorporation of $[^{14}\text{C}]\text{uridine}$ into RNA for a period subsequent to the addition of non-radioactive medium. T. F. Wild (unpublished work) has shown that in this cell system incorporation of $[^{14}\text{C}]\text{uridine}$ is decreased to nil only after the first 15 min. of a chase period.

Table 1. Incorporation of $[^{14}\text{C}]\text{uridine}$ into virus-induced 37s RNA and virus particles

Source of RNA	Total ^{14}C radioactivity (counts/1000 sec.)	% of radioactivity (corrected)
37s RNA in cells at 2.75 hr.	1.23×10^6	100
Virus in cells at 2.75 hr.	8.2×10^4	14
37s RNA in medium at 8 hr.	3.04×10^5	25
Virus in medium at 8 hr.	1.47×10^5	20

Use of these corrections allows the results of the above experiment to be interpreted as follows: Table 1 shows that 14% of the radioactivity present in the 37s RNA from cells at 2.75 hr. after infection was already in mature virus particles. The radioactivity in the virus particles isolated from the medium 8 hr. after infection was about 20% of the total radioactivity present in 37s RNA in the cells at 2.75 hr. In agreement with this observation, the amount of radioactivity in 37s RNA extracted from the medium 8 hr. after infection was also in the region of 25% of that extracted from cells at 2.75 hr. after infection. It would therefore seem that a reasonable estimate of the amount of induced RNA that became incorporated into mature virus was approx. 20%.

DISCUSSION

The present paper considers two aspects of the relationship between RNA extracted from infected cells and that from purified virus particles. First, the RNA prepared from BHK cells infected with FMDV was characterized by sedimentation in sucrose gradients and by gel filtration on Sepharose 4B columns, and the main fraction (peak A) was shown to be greater in size than the RNA from purified virus. Secondly, the proportion of the 37s virus-induced RNA synthesized after infection that became incorporated into mature virus particles was estimated.

The main differences between the two RNA molecules were the higher sedimentation rate and heterogeneous nature of the 37s peak of the induced RNA. Treatment of this fraction with enzymes, such as deoxyribonuclease, Pronase or amylase, did not cause any decrease in sedimentation rate. Hence, the apparent increase in size was not caused by contamination of the fraction by DNA fragments, protein or carbohydrate.

The possibility exists that the heterogeneity of the 37s RNA was due to the presence of rapidly sedimenting replicative intermediates consisting of complexes of single strands and double strands, as have been shown to occur in HeLa cells infected

with poliovirus (Baltimore & Girard, 1966) and in *Escherichia coli* cells infected with R17 bacteriophage (Erikson, 1966). Whereas these authors labelled for extremely short intervals, in the present experiments labelling was allowed to proceed for 3 hr. and no evidence was found for the presence of ribonuclease-resistant RNA in our 37s RNA fraction. This result showed that the heterogeneity of the 37s RNA from FMDV-infected cells was not due to the presence of a mixture of replicative intermediates and viral RNA.

Resedimentation of individual fractions from the leading edge of the 37s peak after treatment with phenol, methylcellosolve or dimethyl sulphoxide showed that the fractions continued to sediment in approximately the same position. This is further evidence that the fractions could not readily be converted into 35s material, and that configurational differences were unlikely to account for the heterogeneity of the RNA. Both phenol and dimethyl sulphoxide treatments resulted in the formation of some slower-sedimenting RNA that at present can only be explained by assuming that some fragmentation occurred during the treatment or that fragments were released after dissociation of the secondary structure of the RNA chains (Spirin, 1964).

Gel filtration through Sepharose 4B has provided a useful method for the separation of large molecules (Oberg & Philipson, 1967). When a mixture of [¹⁴C]uridine-labelled RNA from infected cells and ³²P-labelled RNA from purified virus was passed through a Sepharose column, the latter was eluted after the ¹⁴C radioactivity. This double-labelling experiment made it highly probable that the main difference between the two RNA species was one of size rather than aggregation or configurational phenomena.

In the final experiment described above, the evidence suggests that only 20% of the virus-induced RNA was incorporated into new virus particles. This low efficiency of incorporation could be explained by the formation of progeny RNA chains that were too long for virus particles. It has previously been suggested by Brown & Martin

(1965) that a cyclic replicating mechanism would permit the synthesis of chains longer than the in-going primer. Such a cyclic mechanism would necessitate the existence of some means of cutting the long chains into pieces of the correct length for incorporation into virus particles. If this scission process was not perfect then there would be a tendency to get large pieces of RNA that might not be incorporated into virus particles. It has now become generally accepted that scission of long molecules of RNA might occur in the metabolism of cellular RNA, for example in the production of 28s and 16s ribosomal RNA from 45s RNA (Scherrer, Latham & Darnell, 1963), and it has also been suggested that rapidly labelled high-molecular-weight RNA in the nucleus might be segmented in a similar fashion (Warner, Saeiro, Birnboim, Girard & Darnell, 1966). The present results suggest that some type of scission process may be a general phenomenon associated with RNA metabolism.

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