# *RNA SYNTHESIS IN REOVIRUS-INFECTED L929 MOUSE FIBROBLASTS**

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Reovirus contains double-helical RNA.[1, 2] Both single- and double-stranded reovirus-specific RNA's have been found within phenol extracts of reovirus-infected cells.[3–9] In the present study, analyses of extracts of reovirus-infected cells were made under conditions in which the double-stranded RNA within virus or subviral particles is not released. Three new viral-specific single-stranded RNA's have been found. These are associated with polyribosomes and are in part messenger RNA's (mRNA's).

No double-stranded RNA has been found free within virus-infected cells other than that in newly synthesized virus and subviral particles. The virus and subviral particles appear to be associated with virus-synthesizing factories.

*Materials.—Virus:* Dearing strain of reovirus 3, cloned,[1] was used as stock virus after two passages.

*Cell culture:* L cells, strain 929, were grown in suspension for the preparation of virus stocks and large quantities of RNA labeled with radioactivity. Monolayer cultures were used for titration of virus.[10]

*Other materials:* Ribonuclease-free (RNase-free) sucrose was further treated with the coarse Mg-bentonite fraction (20,000 × *g*), prepared as described by Petermann,[11] before preparing stock solutions of 15 and 30% sucrose in buffer containing 0.01 *M* Tris HCl, pH 7.4, 0.01 *M* KCl, 0.0015 *M* $MgCl_2$ (RSB). Sucrose not treated with bentonite was used in experiments for determination of the base compositions of RNA fractions. Sodium dodecyl sulfate (SDS) was recrystallized according to Mandel.[12] Polyoxyethylene (20) cetyl ether (BRIJ 58) was purchased from Atlas Chemical Company.

*Methods.—Preparation of cellular extracts:* L929 cells, in the logarithmic stage of growth, were collected by centrifugation from suspension cultures and inoculated with reovirus 3 at a multiplicity of 7–10 plaque-forming units per cell. After the 2-hr adsorption period, the cells were collected and resuspended at a concentration of 1–3 × $10^6$ cells/ml in reinforced Eagle's spinner medium[13] containing 2% fetal bovine serum. Controls were carried through the same procedure using growth medium without virus. Where indicated, actinomycin D was added after virus adsorption to a final concentration of 0.15 μg/ml and samples were then incubated at 37°C on a roller drum. At various times during the virus growth cycle, 0.33 μc/ml of $H^3$-labeled uridine, 18,000–30,000 mc/mmole (in the presence of 1000-fold thymidine), or 50 μc/ml of $P^{32}$-labeled orthophosphate was added for a chosen time interval at 37°C.

After the labeling period, the cells were washed one time with RSB, resuspended in RSB at 0°C, and ruptured with 25 strokes in a tight-fitting Dounce homogenizer. Cytoplasmic and nuclear extracts were prepared as described by Penman,[14] modified in that nuclear extracts were prepared in buffer containing 0.01 *M* Tris HCl, pH 7.4, 0.01 *M* sodium chloride, 0.0015 *M* $MgCl_2$. The cytoplasmic extracts contained from 70 to 80% of the total cellular protein, from 70 to 80% of the RNA, and less than 2% of the DNA.

*Analytical procedures:* Gradient centrifugations were done in the Spinco L2 ultracentrifuge. Fractions were collected from below. Absorbancy (at 260 mμ) of fractions was determined, and acid-insoluble radioactivity was obtained by precipitating the fractions with 5% trichloroacetic acid (TCA). The resulting precipitates were collected on Millipore filters, and the amount of radioactivity in the samples was determined in a Tri-Carb scintillation spectrometer.

*Determination of base composition:* Labeled RNA's in sucrose gradient fractions were precipitated with TCA or ethanol and collected by centrifugation. More than 90% of the radioactivity was recovered in the pellets. The pellets were dissolved in 0.3 *N* KOH and the RNA's

hydrolyzed for 18 hr at 37°C. Potassium ions were removed by chromatography on CM-82 paper, as described by Ingram and Pierce,[15] and the eluate, to which carrier mononucleotides were added, was spotted on Whatman no. 3 paper and subjected to electrophoresis for $4^1/_2$ hr at 68 v/cm. The buffers used were either 0.05 *M* ammonium formate, pH 3.5, or 20% acetic acid brought to pH 3 with concentrated ammonium hydroxide. The nucleotides were located on the paper by absorbance in ultraviolet light. The spots were cut out, and radioactivity was determined as described above. Of the counts spotted, 90–100% were located in spots absorbing in the ultraviolet.

*RNA annealing:* Fractions from sucrose gradients containing molecules labeled with radioactivity were collected by precipitation with ethanol and redissolved in 1/10 × KKC (0.015 *M* potassium chloride, 0.0015 *M* potassium citrate). Reovirus was purified as described before and its RNA extracted with phenol.[1] Reovirus RNA was preheated to 95°C for 5 min and quenched quickly in ice.

Samples were added to glass tubes in a final volume of 1 ml of 3.3 × KKC, tightly stoppered, heated to 98°C for 5 min, and placed in a water bath at 63°C for 5 hr. They were slowly cooled to 37°C during the next 12 hr and then brought to room temperature. One set of control samples was quenched quickly at 0°C after they had been heated to 98°C for 5 min, and a second set kept at 0°C during the whole procedure. The samples were divided. One half was left untreated and the second half, in RSB, was treated with 3.3 μg/ml of pancreatic RNase for 30 min at 37°C. Acid-insoluble radioactivity in all samples was determined as described above.

*Results.—RNA synthesis in cells infected with reovirus:* The growth of reovirus in L929 cells is characterized by a latent period of 7 hours, exponential growth from 7 to 11 hours, and maximum yield of virus at 15 hours.[10] RNA synthesis has been studied during various periods of the growth cycle, but especially from $11^3/_4$ to $13^3/_4$ hours when virus yields increase from 20 to 40 per cent of maximum.

Reovirus growth is inhibited by actinomycin D at a concentration of 2 μg/ml;[10] at lower concentrations of inhibitor, full yields of reovirus can be obtained from infected cells.[3, 4] In the present experiments, 0.15 μg/ml of actinomycin D was used. At this concentration, synthesis of ribosomal RNA was not detectable (see Fig. 1*C*), whereas the growth cycle of the virus was unchanged and full yields of virus were obtained.

The intent of this study was to analyze cytoplasmic extracts under conditions in which the double-stranded RNA would not be released from virus or subviral particles. The infectivity of purified virus labeled with radioactivity in its nucleic acid was not destroyed by treatment at pH 6.8–7.4 with 1.9 per cent SDS and 0.5 per cent BRIJ 58 at room temperature, nor was there any detectable radioactivity released from virus particles. In all experiments, 90–100 per cent of virus within SDS-BRIJ-treated cytoplasmic extracts was recovered in the pellet after sucrose density gradient centrifugation for separation of cytoplasmic RNA's (as for Fig. 1). Thus, to identify new RNA's within infected cells, with the virus remaining intact, cytoplasmic extracts were analyzed after treatment with SDS and BRIJ 58. To investigate the structures to which new RNA's are attached within infected cells, cytoplasmic extracts were treated with sodium deoxycholate (DOC) and BRIJ 58.

Figure 1 shows the results of sucrose density gradient centrifugation of SDS-BRIJ-treated cytoplasmic extracts. $H^3$-labeled uridine was added for 120 minutes, beginning at $11^3/_4$ hours after virus inoculation. There were three species of molecules labeled with $H^3$ uridine present in the actinomycin-treated, reovirus-infected cells (Fig. 1*A*) in addition to transfer RNA which was the only species labeled in cytoplasmic extracts from actinomycin-treated, uninfected cells (Fig. 1*C*). These three new species of RNA sedimented with *S* values of 24, 18, and 14.

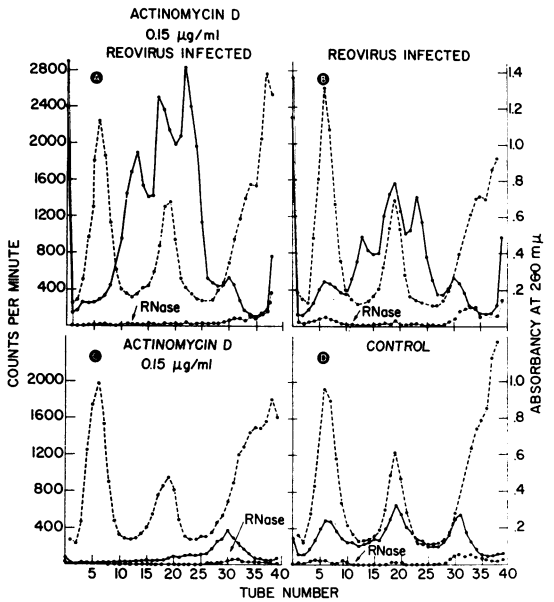



FIG. 1.—Distribution of radioactivity and absorbancy at 260 mμ after sucrose density gradient centrifugation of nucleic acid from cells (*A*) reovirus-infected, actinomycin D-exposed; (*B*) reovirus-infected, not exposed to actinomycin D; (*C*) not inoculated with virus, but exposed to actinomycin D; (*D*) not inoculated with virus and not exposed to actinomycin D. Cells were exposed to $H^3$-uridine from $11^3/_4$ to $13^3/_4$ hr after infection. Cytoplasmic extracts were prepared as described in *Materials and Methods*, treated at room temperature with SDS, BRIJ 58, and EDTA to final concentrations of 1.95%, 0.5%, and $5 \times 10^{-4}$ *M*, respectively. Treated samples were layered over a linear 15–30% sucrose gradient in RSB and centrifuged at 20°C for 18 hr in an SW 25.2 rotor at 24,000 rpm. Fractions were collected and absorbancy of each fraction determined. Serving as markers for the molecules labeled with radioactivity are the optical densities of the two ribosomal RNA's, 32 and 18*S*,[16] and the radioactivity in the transfer RNA, 4*S*.

The fractions were divided into two sets: in the first set, the total acid-insoluble radioactivity was determined as described in *Materials and Methods;* fractions in the second set were exposed to 3.0 μg/ml of pancreatic RNase at 37°C for 30 min, and then the remaining acid-insoluble radioactivity determined.

Molecules with *S* values of 24 and 14 were also discernible in virus-infected cells in the absence of actinomycin D (Fig. 1*B*). However, there was continuing synthesis of ribosomal RNA's, and the new species of 18*S* was contaminated with newly formed 18*S* ribosomal RNA. In control cultures, neither infected with reovirus nor exposed to actinomycin D, there was labeling with radioactivity of the two ribosomal RNA's in addition to the 4*S* RNA (Fig. 1*D*).

Treatment with pancreatic RNase in solutions of KCl varying from 0.15 to 0.50 *M* rendered the radioactivity in the 24, 18, and 14*S* RNA's completely acid-soluble (Fig. 1*A*, *B*). There has been no evidence of RNase-resistant molecules within the gradients. Thus, under conditions where reovirus RNA, double-stranded, is RNase-resistant,[1] these three new RNA's were sensitive to hydrolysis and are evidently single-stranded RNA.

Analyses of cytoplasmic extracts from actinomycin-treated, reovirus-infected cells revealed the same three viral-specific RNA's with $H^3$-labeled adenine or $P^{32}$ as precursors as were seen with $H^3$-labeled uridine (Fig. 1). The three viral-specific RNA's were first detectable from six to eight hours after infection, and throughout the exponential period they were produced in increasing amounts but in constant proportion to each other.

To study the kinetics of syntheses of the three viral-specific RNA's and possibly to detect a species of RNA made but rapidly degraded, labeling with radioactivity

was shortened from 120 to 15 minutes at 7, 9, and 11 3/4 hours after infection. The three new species of RNA of $S$ values 24, 18, and 14 were again evident. They can thus be completed or made *in toto* in a maximal time interval of 15 minutes, and their cytoplasmic location suggests that they are cytoplasmic in origin. When the $H^3$-labeled uridine was removed after 15 minutes and incubation at 37°C was allowed to occur for another 30 minutes in the presence of excess unlabeled uridine and thymidine, there was almost a twofold increase in the amount of radioactivity of each of the three species of RNA molecules.

During the exponential phase of virus growth, there was an increasing amount of RNase-resistant material labeled with radioactivity that sedimented as a pellet during the centrifugation of SDS-BRIJ-treated cytoplasmic extracts from infected cells (Fig. 1*A* and *B*). This material is at least in part present in infectious virus and subviral particles (see below).

Sucrose density gradient analyses of nuclear extracts from cells labeled with $H^3$ for various two-hour periods during the viral growth cycle did not reveal any difference in nuclear RNA synthesis between the actinomycin-treated reovirus-infected cells and respective control. There were thus no new viral-specific single-stranded or double-stranded RNA's detectable in nuclei of reovirus-infected cells.

*Base compositions of reovirus-specific RNA's from infected cells:* Table 1 shows the base compositions of the RNA within reovirus 3, Dearing strain, reported before,[17] and of the three viral-specific RNA's made from 11 3/4 to 13 3/4 hours postinfection (p.i.). The base compositions of the 24, 18, and 14*S* are the same within experimental error and have a slightly higher mole per cent of cytidine 5′-phosphate (CMP) and slightly lower guanosine 5′-phosphate (GMP) than that present within reovirus RNA itself. The base compositions of the RNA's made during 2-hour periods earlier than 11 3/4 to 13 3/4 hours were not significantly different from those reported in Table 1.

*Annealing of complementary RNA's from infected cells to viral RNA:* To ascertain that the new RNA's in reovirus-infected cells were virus-specific, the ability of these molecules to hybridize with parental reovirus RNA was determined. All three RNA's, 24, 18, and 14*S*, annealed to heat-denatured reovirus RNA. Maximal values were obtained at about 6–10 μg of reovirus RNA. Under these conditions, 70–80 per cent of the 24*S*, 60–70 per cent of the 18*S*, and 50–60 per cent of the 14*S* material was capable of annealing to parental RNA. These three RNA's did not detectably anneal to L-cell ribosomal RNA, to heat-denatured L-cell DNA, nor to each other.

The 24, 18, and 14*S* RNA's, which on recentrifugation retain their original $S$

TABLE 1

BASE COMPOSITION OF $P^{32}$-LABELED RNA FROM REOVIRUS-INFECTED L929 MOUSE FIBROBLASTS

| Reo RNA from virus | 24*S* | 18*S* | 14*S* |
|---|---|---|---|
| C 0.223 | 0.247 | 0.240 | 0.232 |
| A 0.280 | 0.279 | 0.282 | 0.288 |
| G 0.220 | 0.189 | 0.190 | 0.198 |
| U 0.279 | 0.281 | 0.285 | 0.281 |
| | (Av. 11) | (Av. 12) | (Av. 12) |

$P^{32}$-labeled viral-specific RNA's were obtained from cytoplasmic extracts of actinomycin-treated, reovirus-infected cells labeled from 11 3/4 to 13 3/4 hr after infection as for Fig. 1. The RNA's were collected and base compositions determined as described in *Materials and Methods*. The data for base composition of viral RNA are those reported before.[18]

values, are distinct from each other also in base sequence. When denatured reovirus RNA is limiting in annealing experiments, 24, 18, or 14S RNA molecules labeled with $P^{32}$ competed effectively with $H^3$-labeled RNA molecules of the same sedimentation value for binding sites on the denatured reovirus RNA. The percentage of $P^{32}$ or $H^3$-labeled RNA molecules bound to reovirus RNA in an RNase-resistant configuration was 40–50 per cent lower when the $P^{32}$ and $H^3$ RNA's were present in the same annealing mixture than when either was added alone to reovirus RNA. Table 2 shows that under these conditions, there is no competition between virus-specific RNA's of different S values. Thus, the 24, 18, and 14S RNA's do not contain identical long sequences of nucleotides.

*Location of the 24, 18, and 14S RNA's within the infected cell:* The functional unit of protein synthesis within cells appears to be polyribosomes.[18–21] With the assumption that the 24, 18, and 14S RNA's in reovirus-infected cells were in part mRNA's, analyses of the RNA's attached to ribosomes were made. L cells were exposed to $H^3$-labeled uridine in the presence of actinomycin D for two-hour periods (Fig. 2). During the progression into and out of the exponential phase of virus growth, there were increasing amounts of material labeled with radioactivity associated with structures sedimenting not only with single ribosomes, but also more rapidly and more slowly (Fig. 2D). The patterns of sedimentation presented in Figure 2 are similar to those seen in reovirus-infected cells which were not exposed to actinomycin D.

Analyses of the RNA's associated with polyribosomes and with single ribosomes showed that the 24, 18, and 14S RNA's in reovirus-infected cells were at least in part associated with both polyribosomes (Fig. 3A) and single ribosomes (Fig. 3B) and probably function as mRNA's. The large amount of molecules labeled with radioactivity sedimenting more slowly than the reovirus-specific RNA's (Fig. 3A and B) is at least in part due to hydrolysis of those molecules. Material labeled with radioactivity and sedimenting more slowly than single ribosomes but more quickly than 24, 18, and 14S (Fig. 2D) when analyzed after SDS-BRIJ treatment revealed that it also contained the 24, 18, and 14S RNA's.

TABLE 2

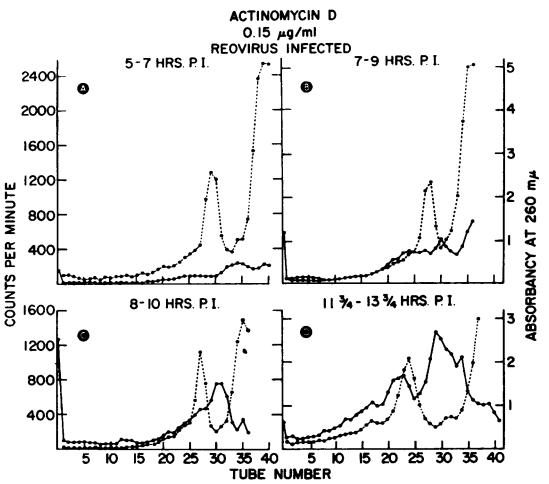
4, 18, AND 14S REOVIRUS-SPECIFIC SINGLE-STRANDED RNA'S: NONIDENTITY OF NUCLEOTIDE SEQUENCES

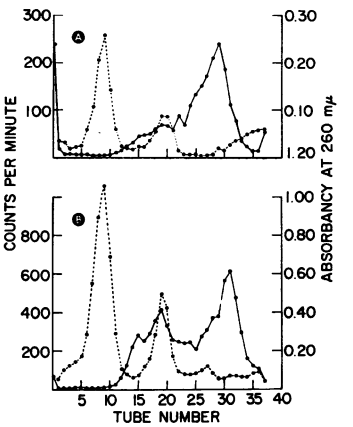
| Total Cpm Added $P^{32}$ | Total Cpm Added $H^3$ | RNase-Resistant after Annealing (% of total) $P^{32}$ | RNase-Resistant after Annealing (% of total) $H^3$ |
|---|---|---|---|
| 18S | 24S | 18S | 24S |
| 0 | 719 | — | 45.5 |
| 600 | 719 | 35.7 | 44.0 |
| 1204 | 719 | 25.2 | 44.9 |
| 600 | 0 | 30.0 | — |
| 1204 | 0 | 25.7 | — |
| 14S | 24S | 14S | 24S |
| 0 | 719 | — | 45.5 |
| 404 | 719 | 36.2 | 49.6 |
| 865 | 719 | 26.1 | 46.7 |
| 404 | 0 | 34.5 | — |
| 865 | 0 | 29.4 | — |
| 18S | 14S | 18S | 14S |
| 0 | 2238 | — | 23.8 |
| 600 | 2238 | 27.2 | 20.0 |
| 1204 | 2238 | 22.0 | 21.2 |
| 600 | 0 | 30.0 | — |
| 1204 | 0 | 25.7 | — |

The 24, 18, and 14S RNA's were prepared by pulse labeling actinomycin-treated, reovirus-infected cells from $10^1/_2$ to $11^1/_2$ hr p.i. with $H^3$-labeled uridine or $P^{32}$ as in *Methods*. The RNA's were collected and purified, and annealing conditions were as described in *Materials and Methods*. The amount of denatured reovirus RNA present in each annealing mixture was 1 μg.

*Location of virus within the infected cell:* During the exponential phase of growth of virus, reovirus and subviral particles appear in the cytoplasm within developmental foci which are discrete, compact, and have a dense granular matrix.[22–24]

As suggested by the morphological data, it appears as if reovirus in cytoplasmic extracts is also present in some




(*Left*) FIG. 2.—The association of labeled cytoplasmic RNA in reovirus-infected cells with polyribosomes. Cytoplasmic extracts were prepared from actinomycin-treated reovirus-infected cells which had been labeled with $H^3$-uridine from (*A*) 5–7 hr p.i.; (*B*) 7–9 hr p.i., (*C*) 8–10 hr p.i., and (*D*) $11^3/_4$ to $13^3/_4$ hr p.i. The cytoplasmic extracts were treated with DOC and BRIJ, and EDTA to final concentrations of 1.0%, 0.5%, and $5 \times 10^{-4}$ *M* EDTA, respectively. They were then layered on sucrose gradients and analyzed as in Fig. 1. The time of centrifugation was, however, for 135 min at 24,000 rpm and the temperature at which centrifugation occurred was 4°C.

———, Cpm per tube. – – – –, absorbancy at 260 mμ per tube.

(*Right*) FIG. 3.—Sedimentation analysis of rapidly labeled RNA from reovirus-infected cells associated with polyribosomes (*A*) and single ribosomes (*B*). Cytoplasmic extract was prepared from an actinomycin-treated, reovirus-infected cell culture labeled with $H^3$-uridine for 30 min at $11^3/_4$ hr after infection and treated with DOC-BRIJ, as described for Fig. 2. Aliquots of the collected fractions were analyzed and showed a spectrum of radioactivity and absorbancy similar to that shown in Fig. 2*D*. The fractions which sedimented more quickly than ribosomes were pooled, as were those under the single ribosomes. These two pooled samples were further centrifuged for $5^1/_2$ hr at 78,000 × *g* in an S30 rotor. The pellets were resuspended in RSB containing bentonite, treated with SDS, BRIJ 58, and EDTA, and analyzed as in Fig. 1.

———, Cpm per tube; – – – –, absorbancy at 260 mμ per tube.

larger structure or aggregates. These foci do not appear to be surrounded by lipid membranes. Of the total virus present in the *released* virus preparation, 10–15 per cent sediments as a pellet at 20,000 × *g* for 30 minutes. When RNA in infected cells was labeled with radioactivity during the exponential phase of growth and cytoplasmic extracts not treated with detergents prepared, 90 per cent of the *cell-associated* virus and 50 per cent of the material labeled with radioactivity sedimented as a pellet at 20,000 × *g* for 30 minutes. When this pellet was resuspended and treated with either DOC or with SDS and the resulting suspension recentrifuged at 20,000 × *g* for 30 minutes, 85 and 70 per cent, respectively, of the total viral infectivity sedimented as a pellet. In addition to some virus, after the DOC or SDS treatment, most of the 24, 18, and 14*S* RNA's were released into supernatant.

The virus within the developmental bodies is extracted with fluorocarbon. Figure 4*A* shows the equilibrium centrifugation of material from the fluorocarbon-treated pellet which had sedimented from SDS-BRIJ-treated cytoplasmic extracts (e.g., see Fig. 1). The peak of viral infectivity is at a density of 1.382.[1] Newly

made material, labeled with radioactivity, equilibrated at 1.415 with a shoulder at the density of complete virus.

Reovirus has an outer and an inner protein shell.[23, 25] Treatment with sodium pyrophosphate has been shown to result in preferential removal of the outer capsid and release of inner shells.[23] Figure 4*B* shows that when purified virus, labeled in its nucleic acid with $P^{32}$, was treated with pyrophosphate[23] subviral particles containing nucleic acid within the inner shells were produced which equilibrated at a density of 1.40 away from the peak of viral infectivity at 1.382. Thus, there are present in the SDS pellet of cytoplasmic extracts virus and subviral particles with a density similar to that of reovirus inner shells.

Most of the newly labeled material shown in Figure 4*A* sedimented to the pellet in this gradient, as would be expected for single- and double-stranded RNA's. When this pellet from the cesium chloride gradient shown in Figure 4*A* was analyzed by equilibrium centrifugation in cesium sulfate, there was a sharp peak of radioactivity at a density of 1.667, and over 90 per cent of the material in this peak was sensitive to hydrolysis with pancreatic RNase and is single-stranded RNA. It thus appears that there was no double-stranded RNA found free in the infected cells other than that in subviral particles or virus.

*Discussion and Summary.*—In reovirus-infected cells, there appear three new virus-specific, single-stranded RNA's which are at least in part mRNA's. The base compositions of these are similar to that of parental virus, and they anneal specifically with viral RNA. They have been found associated with polyribosomes, single ribosomes, and lying free of ribosomes in the cytoplasm.

The viral-specific RNA's can be detected first at the beginning of exponential growth of reovirus. They are produced in amounts constant to each other through the exponential growth of virus, and during this time they double in amount in a maximal interval of 30 minutes. The doubling time of reovirus growth during

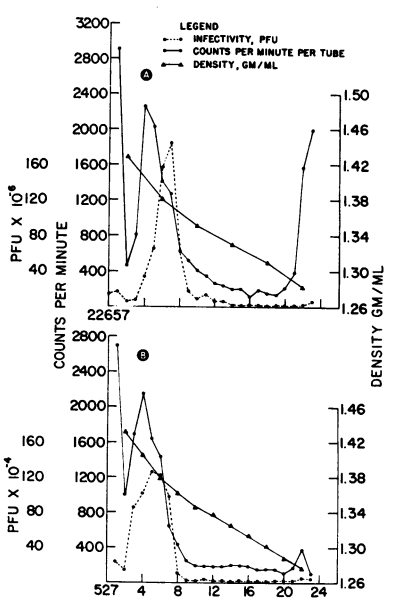



FIG. 4.—Equilibrium density gradient centrifugation of reovirus particles from cytoplasmic extracts and also pyrophosphate-treated purified reovirus.

(*A*) Actinomycin-treated reovirus-infected cells were labeled with $P^{32}$ for 2 hr from 9 to 11 hr p.i., and cytoplasmic extracts prepared as described in *Materials and Methods*. The extract was treated with SDS, BRIJ, EDTA, and centrifuged as described in Fig. 1. The resulting pellet was resuspended and homogenized for 3 min at 0°C in the presence of $^1/_2$ vol of the fluorocarbon, genetron 113. The genetron was removed by centrifugation, and the aqueous layer brought to a density of 1.37 by the addition of solid cesium chloride. The suspension was centrifuged in an SW50 rotor for $17^1/_2$ hr and fractions collected from below. Density of the fractions was determined by weighing aliquots, the infectivity in each fraction determined by means of the plaque assay,[10] and radioactivity determined in each fraction as described in *Materials and Methods*.

(*B*) Reovirus was grown during its whole growth cycle in the presence of $P^{32}$-labeled orthophosphate, 15 $\mu$c/ml. At 18 hr p.i., the cells were sedimented and cell-associated virus collected and purified as described before.[1] The purified virus samples were treated with 0.3 *M* sodium pyrophosphate at pH 8.1 for 30 min at room temperature and then the mixture brought to a density of 1.37 by the addition of solid cesium chloride. The suspension was centrifuged for $17^1/_2$ hr and density, infectivity, and radioactivity were determined as described in (*A*).

this period is also about 30 minutes.[10] The possibility exists that the viral-specific, single-stranded RNA's, in addition to their mRNA function, serve as components of the RNA within reovirus itself, perhaps as templates for the synthesis of their complementary strands to form double-stranded RNA. If this is the case, multiples of double-stranded molecules formed from the 24, 18, and 14*S* RNA's linked in linear sequence would form the genetic material of reovirus RNA. Since no free pools of molecules have been detected complementary to the 24, 18, and 14*S* viral-specific RNA's, formation of double-stranded RNA would have to be followed quickly by formation of virus.

As shown before, reovirus develops within large virus-synthesizing factories.[23] These factories, which remain as discrete bodies in cytoplasmic extracts from infected cells untreated with detergents, contain the bulk of the virus, subviral particles, all the double-stranded RNA, and also the three new single-stranded RNA's described above. The factories are probably the site of synthesis of these single-stranded RNA's which then migrate out to become attached to ribosomes. The double-stranded RNA within these virus-synthesizing bodies is sensitive to extraction with phenol, and it is suggested that the double-stranded RNA found previously within cytoplasmic extracts[3, 4] may have come from extraction with phenol from subviral particles and virus. The double-stranded RNA within the infected cells appears to be coated in a very short time to subviral particles. No double-stranded RNA has been found other than that in virus or subviral particles.

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