Replication of Bacteriophage Ribonucleic Acid: Some Physical Properties of Single-stranded, Double-stranded, and Branched Viral Ribonucleic Acid

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Replicative intermediate (RI) is considered to be the double-stranded ribonucleic acid (RNA) template for synthesis of viral RNA, with bound nascent single-stranded viral RNA. A theoretical description of RI is based on the analysis of a steady state of biopolymerization on a template which determines not only nucleotide sequence, but also chain length. The hydrodynamic properties of RI isolated from *Escherichia coli* infected with bacteriophage R17 are compared with those of RNA isolated from R17 (single-stranded RNA) and of replicative form (RF) isolated from *E. coli* infected with R17. RF is double-stranded RNA template without any single-stranded component. Whereas S for R17 RNA is a function of the ionic strength ($\Gamma/2$) of the solvent, S is almost invariant with $\Gamma/2$ for RF. By contrast $\bar{S}$ for RI lies between the sedimentation constants for R17 RNA and RF and $\bar{S}$ varies with $\Gamma/2$ as does R17 RNA. The weight distribution of S for RI demonstrates the heterogeneity of this material, and the variation in the weight distribution with ionic strength demonstrates the duality of structure in RI. Using $\bar{S}$ and $[\eta]$, the $\bar{M}\text{w}$ for RI is estimated to be 2.6×10^6 daltons, as compared with the theoretical value of 2.9×10^6 daltons.

Several unique species of RNA have been demonstrated in cells infected with small ribonucleic acid (RNA) viruses (6). Besides the single-stranded RNA of the virus, which may have a molecular weight or sedimentation characteristics which differ from those of any normal cellular RNA, one finds double-stranded RNA (replicative form = RF) which is composed of one strand of viral RNA (+ strand) and one strand of RNA complementary to the viral RNA (− strand; 33). Also, one finds a population of molecules with both a single-stranded and a double-stranded component (replicative intermediate = RI; 6, 7).

A semiconservative mechanism of viral RNA replication, based on the biochemical properties of RF and RI, was independently proposed by Weissman and his co-workers (34) and by Fenwick, Erikson, and Franklin (7). Although a hypothetical conservative model of the replicative process can be proposed (6), a recent experiment on the turnover of double-stranded RNA is best interpreted by a semiconservative mechanism of replication in which viral RNA can rapidly recycle into replicative structures (21). According to this model, RF acts as the template for new viral RNA synthesis, and RI is a population of

RF with bound nascent single-stranded viral RNA. The length of the single-stranded component of RI varies according to the degree of completion of the polynucleotide chain (6). Newly synthesized single-stranded RNA, when displaced from RI, could again enter into the cycle of RNA synthesis as RF and then as RI.

RF and RI, although intimately related in the biosynthetic processes involved in viral RNA synthesis, can be expected to have quite different physicochemical properties. RI may be a branched polymer with very unusual properties since the branch is single-stranded and the main chain is double-stranded. To develop a theoretical treatment of such molecules, it was convenient to compare their physicochemical properties with those of single-stranded RNA and double-stranded RNA. The single-stranded RNA used was that isolated from the virion of R17 bacteriophage. This RNA has been studied by several groups of biochemists and physical chemists. It has a molecular weight of $1.1 \pm 0.1 \times 10^6$ (13, 26) and has an unusual degree of secondary structure. The double-stranded RNA used in this study was RF isolated from cells infected with bacteriophage R17. It should have a molecular weight of 2.2×10^6, since it is composed of

one complete viral strand and one complementary strand (1, 10, 33). In the present study, a theoretical model is developed for the structure of RI, and some of the hydrodynamic properties of R17 RNA, RF, and RI are compared. The effect of ionic strength on the sedimentation constant is described in detail. From known solution properties of single-stranded RNA (14, 19) and single-stranded and double-stranded deoxyribonucleic acid (DNA; 5, 30), there should be a marked difference between the effect of ionic strength on single- and double-stranded RNA.

Theoretical treatment of the structure of RI. RI is considered to be template RNA with nascent viral RNA. In the semiconservative model of RNA replication, the displaced "+" strand of RI forms the component which behaves hydrodynamically like a single-stranded RNA polyelectrolyte. Since the single-stranded component of the displaced strand should be of approximately the same length as the newly synthesized "+" strand, these will be formally equivalent in a theoretical treatment (*see* model B in Fig. 4 of reference 6). Although there must be an enzyme associated with the complex during synthesis, this is removed during the isolation of the RNA, since there is no protein or polypeptide associated with the phenol-extracted replicative intermediate, as shown by use of ^{14}C-amino acids and ^{3}H-uridine in attempts to double-label RI (R. M. Franklin, *unpublished data*).

The population of RI will be considered as a double-stranded template of a molecular weight of 2.2×10^{6} with a *steady-state* population of nascent single strands. This may be compared with the analogous classical problem of the steady-state distribution in double-bond polymerization (12). RI may be treated as a steady state either when isolated in trace amounts uniformly labeled with an isotope (such as ^{3}H-uridine) or when isolated in amounts measurable by optical density. In contrast to double-bond polymerization, in this biopolymerization, termination will occur only when the polymer reaches a well-defined length by virtue of the template. The steady-state hypothesis predicts equal amounts of all stages of polymerization, as can be readily shown by the following formal treatment.

Consider RNA to be synthesized on a template by stepwise addition of nucleotides. Thus, in one step a chain of length $\lambda - 1$ nucleotides is transformed into a chain of length λ nucleotides. The chain is initiated with one nucleotide and terminated when the chain length is λ, the number of nucleotides in viral RNA. The three processes of chain initiation, growth, and termination must be considered separately.

Initiation. In the initiation process, an enzyme, RNA-dependent RNA polymerase, interacts with the "starting end" of the double-stranded RNA template. The chain is then initiated when the first nucleoside-5'-triphosphate binds to this site. We are not now concerned with details of this reaction, such as the problem of separate enzymes for the synthesis of the double-stranded and single-stranded RNA (20), but rather with the special features of initiation compared with chain growth. Assuming the concentration of all four ribonucleoside-5'-triphosphates to be the same and equal to B_o, then for initiation:

$$\frac{dP_i}{dt} = K_i B_o - K_P P_1 B_o \qquad (1)$$

where P_1 = concentration of the polymer of chain length 1; K_i = rate constant for initiation, a complex term including an expression for the interaction of the enzyme(s) with the template as well as an expression for the binding of the first substrate, the base of the 5' end of the chain, to the enzyme and template; and K_P = rate constant for polymerization, assumed to be independent of the type of base or chain length. This is a reasonable first approximation since the catalytic action of the enzyme is probably the overriding factor in the K_P term, owing to the lowering of the activation energy for the condensation step.

Growth process. This is the stepwise addition of nucleotide units to the chain with no possibility for chain termination.

$$\frac{dP_r}{dt} = K_P P_{r-1} B_o - K_P P_r B_o$$

for

$$r = 2, 3, \cdots, \lambda - 1, \qquad (2)$$

and

$$\text{Prob } (P_r \rightarrow M_r) = o \qquad (3$$

where M_r = concentration of a terminated chain of length r.

Chain termination. This occurs only when chains have reached length λ. In the general case, the final step of polymerization is considered to be distinct from the displacement of the completed chain from the template.

$$\frac{dP_\lambda}{dt} = K_P P_{\lambda-1} B_o - K_t P_\lambda \qquad (4)$$

$$\text{Prob } (P_\lambda \rightarrow M_\lambda) = 1 \qquad (5)$$

where K_t = rate constant for removal of completed chains from the template. In the steady

state:

$$\frac{dP_1}{dt} = \frac{dP_r}{dt} = \frac{dP_\lambda}{dt} = 0 \qquad (6)$$

for $r = 2, 3, \cdots, \lambda - 1$. Then

$$P_1 = K_i/K_p \qquad (7)$$

$$P_r = P_{r-1} \qquad (8)$$

for $r = 2, 3, \cdots, \lambda - 1$

$$P_\lambda = (K_p/K_t)(B_o P_{\lambda-1}) \qquad (9)$$

Equation 8 is most important for our present analysis. Since the sequence r is very long, to a first approximation $P_r = P_{r-1}$ for all r from 1 to λ, thus simplifying our further calculations.

The average molecular weight of this population of polynucleotides can be easily calculated. As an example, we calculate the weight-average molecular weight:

$$\overline{M}_W = \frac{\sum\limits_{i=1}^{\lambda} N_i M_i^2}{\sum\limits_{i=1}^{\lambda} N_i M_i} = \frac{\sum X_i M_i^2}{\sum X_i M_i} \qquad (10)$$

where N_i = number of molecules of molecular weight M_i, and X_i = mole fraction of molecules of molecular weight M_i. But for a polynucleotide with a monomer unit of average molecular weight M_o, $M_i = i M_o$ and, therefore,

$$\overline{M}_W = M_o \frac{\sum i^2 X_i}{\sum i X_i} \qquad (11)$$

But $X_i = X_j = 1/\lambda$ for all values of i and j and, therefore,

$$\overline{M}_W = M_o \frac{\sum\limits_{1}^{\lambda} i^2}{\sum\limits_{1}^{\lambda} i} = \frac{M_o \frac{1}{6}\lambda(\lambda+1)(2\lambda+1)}{\frac{1}{2}\lambda(\lambda+1)} \qquad (12)$$

$$= \frac{1}{3} M_o(2\lambda + 1)$$

For R17 bacteriophage, $\lambda = 3{,}342$, and $M_o = 321$ (26). Then $\overline{M}_W = 321 \times 2{,}228 = 7.2 \times$

10^5. For replicative intermediate, then, $\overline{M}_W = 2.2 \times 10^6 + 0.72 \times 10^6 = 2.92 \times 10^6$.

Similar calculations can be made for other average molecular weights, and these are summarized in Table 1.

The results presented here will be referred to in this paper, as well as in several future papers on the structure of replicative intermediate.

MATERIALS AND METHODS

Preparation of RNA. Single-stranded viral RNA was isolated by phenol extraction from bacteriophage R17 RNA purified as previously described (32). Replicative form and replicative intermediate were purified by two cycles of adsorption-elution chromatography on cellulose (11).

Sedimentation analysis. Sedimentation coefficients were determined with a Spinco model E analytical ultracentrifuge by use of the ultraviolet-absorption system. All runs were performed at 20.0 C. A 12-mm path-length aluminum cell was used for most measurements. Some studies on the variation of S value with concentration were performed with 3- and 30-mm path-length aluminum cells. In some studies with a 12-mm path-length Kel-F cell, there was no change in sedimentation patterns or S values when compared with the majority of runs performed with the aluminum cells. The photographic record was made on Kodak commercial sheet film, developed in Kodak D-11 developer for 6 min at 25 C. The usual exposure time was 5 sec. Densitometer tracings were made on a Spinco model R Analytrol equipped with a microdensitometer accessory. Sedimentation coefficients were corrected to water at 20 C, as usual.

Weight distributions of sedimentation coefficients were calculated according to the method of Schumaker and Schachman (25). The effective time of sedimentation was calculated from an extrapolation to the meniscus of the plot of ln (distance of 50% point from rotor center) versus time. This plot was also used to calculate $\overline{S}$ for RI. Then ΔC_i^{obs} (incremental change in concentration of RNA) was measured for a fixed ΔX (incremental change in distance from rotor center) for a series of X_i values (distance from rotor center). Since ΔX and X_o (distance of meniscus from the rotor center) were constant, then

$$q_i(S) = \frac{(\Delta C_i^{obs}/\Delta X)(X/X_o)_i^3}{\Delta S_{20,w}^\circ \sum (\Delta C_i^{obs}/\Delta X)(X/X_o)_i^3}$$

$$= \frac{\Delta C_i^{obs} X_i^3}{\Delta S_{20,w}^\circ \sum (\Delta C_i^{obs})(X_i)^3}$$

TABLE 1. *Theoretical values of molecular weight averages of replicative intermediate*

Avg	Avg mol wt of single-stranded component of maximal length λ	Avg mol wt of single-stranded component for R17 RNA ($\lambda = 3{,}342$)	Avg mol wt of replicative intermediate of bacteriophage R17
$\overline{M}_N$	$\frac{1}{2} M_o (\lambda + 1)$	5.4×10^5	2.74×10^6
$\overline{M}_W$	$\frac{1}{3} M_o (2\lambda + 1)$	7.2×10^5	2.92×10^6
$\overline{M}_Z$	$\frac{3}{2} M_o \frac{\lambda(\lambda+1)}{2\lambda+1}$	8.0×10^5	3.00×10^6

The latter equation was programmed on the LOCI-2 computer (Wang Laboratories, Inc., Tewsbury, Mass.).

The absorbancy index of R17 RNA at 260 mμ was determined, by the method of Mitra, Enger, and Kaesberg (22), to be 23.9 cm²/mg, compared with their value of 23.3 cm²/mg. Since this value depends on the initial optical density at 260 mμ of the RNA and therefore can vary considerably, these values are in very close agreement. The same method was used to determine the absorbancy index of RF and RI, and these were found to be within experimental error of the value of R17 RNA. Therefore, for consistency, an absorbancy index of 23.3 cm²/mg was used for all three types of RNA, merely as a convenient means of estimating nucleic acid concentration.

Viscosity. Viscosity was determined with a Cannon-Ubbelohde semi-micro viscometer. As is well known, this suspended-level viscometer requires no kinetic energy corrections, at least in the range of viscosities dealt with here. The temperature was held to 20.0 C with a Haake circulating water bath.

Solvents. The properties of the solvents used in this study are described in Table 2. All of the solvents contained 10^{-3} M sodium ethylenediaminetetraacetate (neutralized) and the concentrations of NaCl and potassium phosphate buffer listed in Table 2. The *p*H was determined with a Radiometer model TTT1 titrator with a model PHA 630 scale expander. The density of the solutions was calculated from the empirical relationship between density and refractive index (3): $\rho^{25} = 4.23061\ n_D^{25} - 4.64125$, a relation determined for NaCl for $1.00 < \rho^{25} < 1.38$.

Refractive index was measured at 25 C with the Bellingham and Stanley high-accuracy model Abbe "60" refractometer. Viscosity of the solvents was compared to that of water in a Cannon-Ubbelohde viscometer at 20.0 C.

TABLE 2. *Solvents used in the studies on the sedimentation and viscosity of RNA*

Composition of solvents (moles/liter)		*p*H	$\Gamma/2$	$\rho^{25°}$	$\eta_{rel}^{20°}$
Potassium phosphate buffer	NaCl				
0.001	0	6.60–6.80	.012	0.997	1.0015
0.005	0	6.68–6.85	.020	0.997	1.0018
0.0066	0	6.80	.022	0.997	1.0033
0.009	0	6.81–6.87	.028	0.999	1.0037
0.01	0	6.87–6.92	.030	0.999	1.0037
0.01	0.01	6.88–6.92	.040	1.000	1.0040
0.01	0.02	6.81	.050	1.000	1.0048
0.01	0.03	6.85	.060	1.000	1.0052
0.01	0.05	6.87–6.88	.080	1.000	1.0063
0.01	0.07	6.79–6.84	.100	1.002	1.0081
0.01	0.10	6.80	.130	1.002	1.0085
0.01	0.20	6.74–6.75	.230	1.007	1.0103
0.01	0.30	6.65–6.67	.330	1.010	1.0136
0.01	0.40	6.54–6.59	.430	1.014	1.0192
0.01	0.60	6.49–6.50	.630	1.024	1.0288

RESULTS

Sedimentation velocity analysis. Sedimentation velocity studies were carried out at very low concentrations of nucleic acid, usually between 3×10^{-3} and 3.9×10^{-3} g/dl. The sedimentation patterns obtained with ultraviolet-absorption optics are shown in Fig. 1 (R17 RNA), 2 (RF), and 3 (RI). R17 RNA was composed of a homogeneous major component and a variable
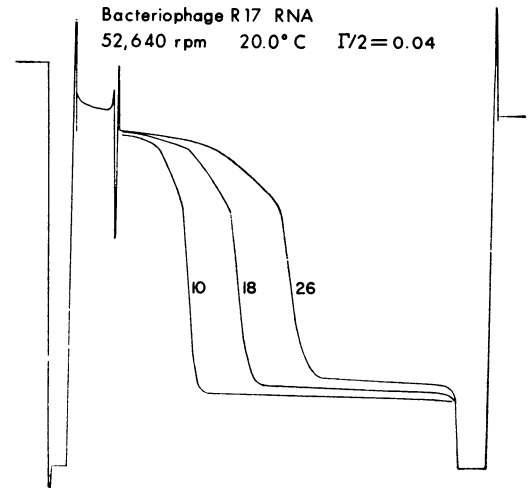

FIG. 1. *Densitometric tracings of an ultraviolet absorption ultracentrifuge pattern of R17 RNA, run at OD (260 mμ) = 0.922. The patterns were photographed at 10, 18, and 26 min after reaching speed (52,640 rev/min). The run was at 20.0 C in a solution of ionic strength 0.04. The direction of sedimentation is from left to right.*
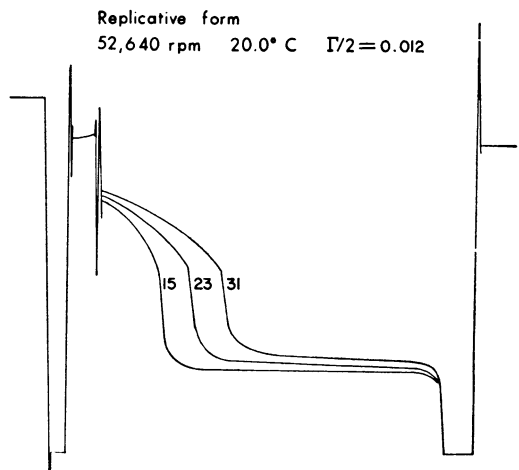

FIG. 2. *Same as Fig. 1 for RF. The OD (260 mμ) was 0.741; the patterns were photographed at 15, 23, and 31 min after reaching speed (52,640 rev/min); the temperature was 20.0 C; and $\Gamma/2$ was 0.012.*

amount of a heterogeneous population of molecules of S value lower than the major component. When the weight distribution of S values was calculated (25) and analyzed by planimetry, the homogeneous component comprised about 80% of the population in one typical preparation. Replicative form was less homogeneous than R17 RNA, but there was a well-defined homogeneous component comprising approximately 60% of the population. The heterogeneous population of RF was also variable in amount and of an S value lower than the homogeneous component. Measurements of RF S values were only made on the homogeneous component. The heterogeneous components of RF and R17 RNA are probably
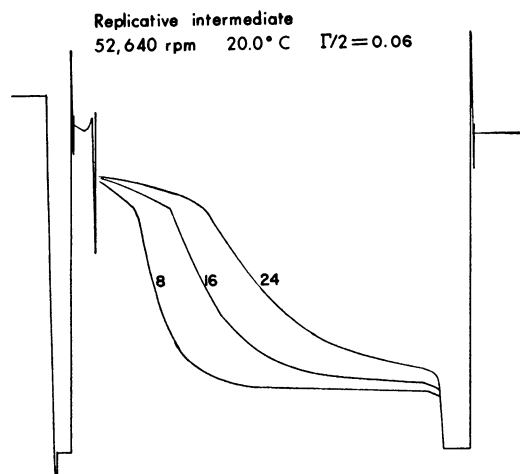
degradation products (22). In contrast, RI was a completely heterogeneous population of molecules, as might be expected from the pattern of RI obtained by sucrose density gradient centrifugation (11).

The effect of ionic strength on S was measured for the homogeneous components of R17 RNA and RF. For RI, an "average" S value was calculated from the slope to the 50% points of the sedimentation pattern. The values calculated are assumed to be $S°$ values. No consistent variation of $S_{20,w}$ with concentration was noted for R17 RNA in the low concentrations used. In a more extensive study on RF, the value of $S_{20,w}$ remained constant in the concentration range of 7.2×10^{-4} to 1.6×10^{-2} g/dl. The results of this study of variation of S with $\Gamma/2$ are shown in Fig. 4 where $S°_{20,w}$ is plotted versus $\Gamma/2$ on log-log coordinate paper.

R17 RNA will be considered first. The log-log plot of S versus $\Gamma/2$ is linear between $\Gamma/2 = 0.022$ and 0.14 M. Above $\Gamma/2 = 0.14$ M, $S°_{20,w}$ varied only slightly, the increase in value from 0.14 to 0.62 M being from 28.0 to 28.5S. Between 0.020 and 0.022 M, there was a discontinuity in the S values. This effect was not studied in any detail in the present work. For the present study, the lowest value of $\Gamma/2$ used was 0.012 M. The changes in $S°_{20,w}$ were completely reversible. For example, a sample in a solvent of $\Gamma/2$ of 0.020 with $S°_{20,w}$ of 13.2 to 13.7 was adjusted to $\Gamma/2$ of 0.060 by addition of NaCl. The $S°_{20,w}$ value increased to 22.1, which fitted on the log-log plot of $S°_{20,w}$ versus $\Gamma/2$.

In contrast to the behavior of R17 RNA, the $S°_{20,w}$ of RF remained constant over the range of $\Gamma/2 = 0.028$ to 0.43 M. Measurements were not made at values of $\Gamma/2$ higher than 0.43 M. The
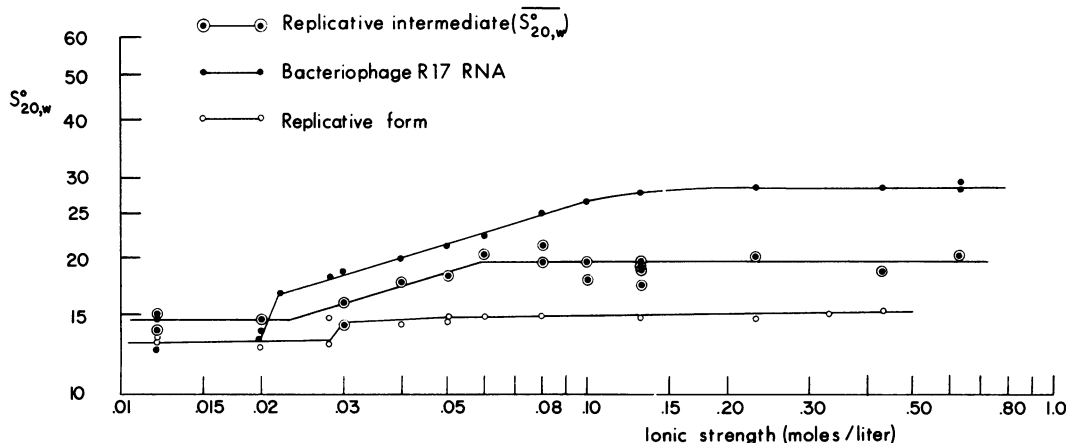
Replicative intermediate
52,640 rpm   20.0° C $\Gamma/2 = 0.06$

FIG. 3. *Same as Fig. 1 for RI. The OD (260 mμ) was 0.832; the patterns were photographed at 8, 16, and 24 min after reaching speed (52,640 rev/min); the temperature was 20.0 C; and $\Gamma/2$ was 0.06.*

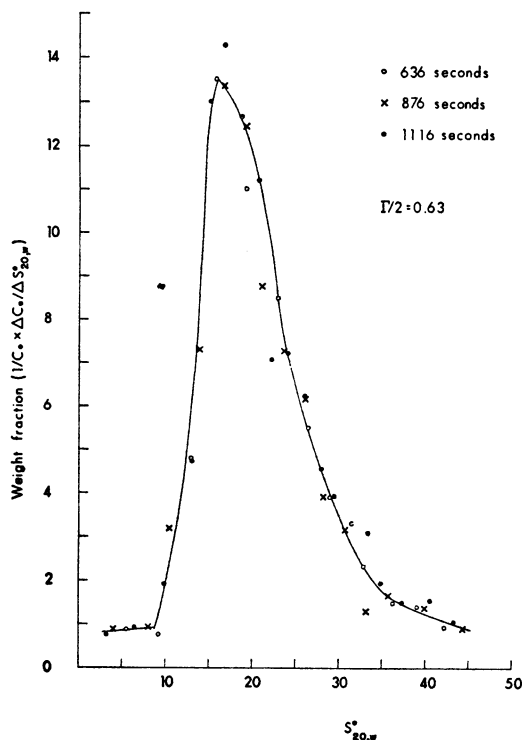FIG. 4. *Variation of $S°_{20,w}$ with ionic strength:* ●, *R17 RNA;* ○, *RF;* ◉, *RI.*

FIG. 5. *Weight distribution of S values of RI, calculated from material run at 52,640 rev/min for:* ○, *636 sec;* ✕, *876 sec; and* ●, *1,116 sec. The ionic strength was 0.63, and the temperature was 20.0 C.*

range of $S_{20,w}^{o}$ values between $\Gamma/2 = 0.08$ and 0.33 M was 14.8 to 14.9S. The average value was 14.8. There was a slight discontinuity in the log-log plot; this occurred at approximately 0.03 M, as compared with the discontinuity at 0.02 M for R17 RNA. The significance of this discontinuity is not clear.

The "average" S values of RI were between those of R17 RNA and RF. The variation in S with $\Gamma/2$ occurred over a smaller range of ionic strengths (0.023 to 0.06 M) than in the case of R17 RNA. Also, the overall variation was only 4.0 S units, as compared with 11.5 S units for R17 RNA.

Of far greater importance for an analysis of RI was the weight-distribution of S values. Some typical results are shown in Fig. 5 for a solvent of ionic strength 0.63 M. The distribution extended from 10S to over 30S, but that part of the distribution between 30 and 45S represented measurements of extremely small slopes ($\Delta C^{obs}/\Delta X$) and therefore was not very meaningful. The distribution remained invariant over the three times analyzed, which represented a total time interval of 480 sec. This indicated that the distribution was due to heterogeneity of sample rather than to some other effect such as convective disturbances within the cells (25). The effect of variation in the centrifugal field on the distribution function was not studied.

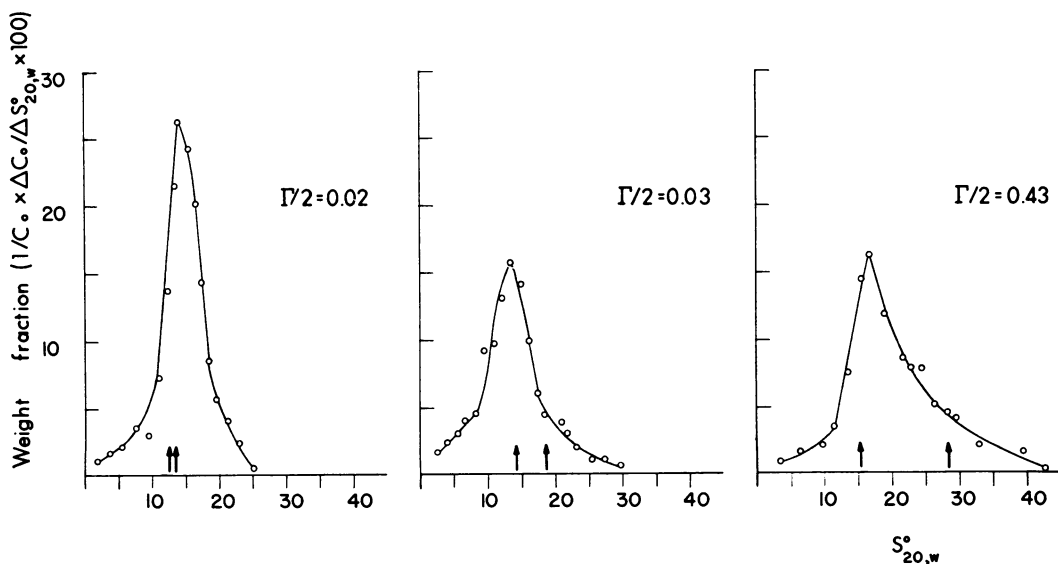Variation in ionic strength of the solvent resulted in systematic changes in the distribution

FIG. 6. *Weight distribution of S values of RI run in solvents of ionic strength 0.02, 0.03, and 0.43. The arrows indicate the sedimentation constants of RF (left arrow) and R17 RNA (right arrow).*
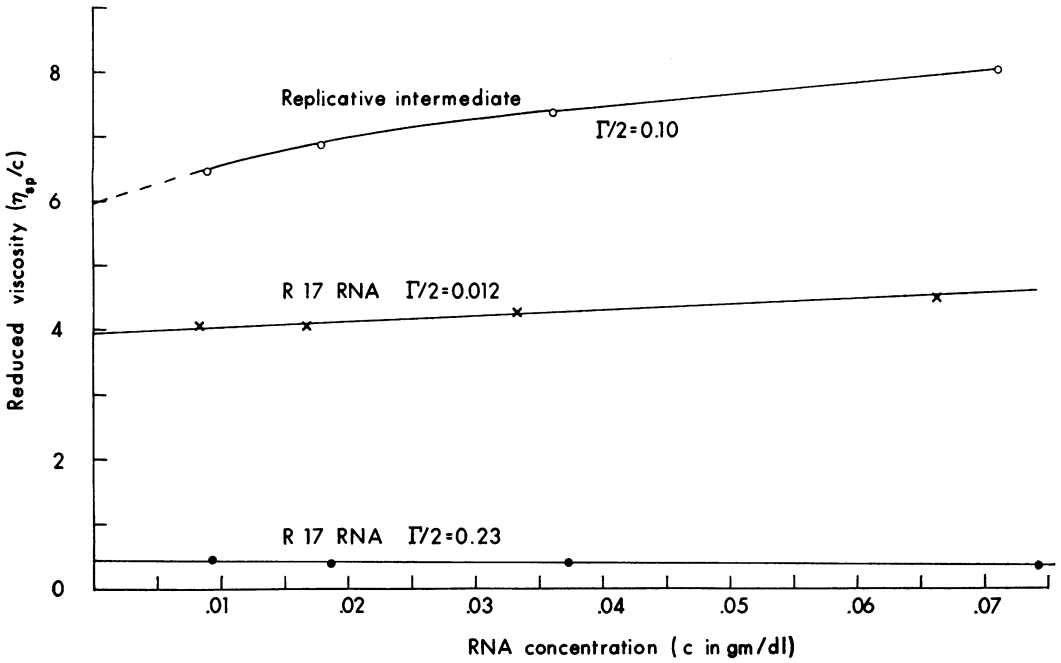
Fig. 7. *Concentration dependence of the relative viscosity of R17 RNA in* $\Gamma/2 = 0.012$ ($\times$) *and* $\Gamma/2 = 0.23$ ($\bullet$); *and of RI in* $\Gamma/2 = 0.10$ ($\bigcirc$).
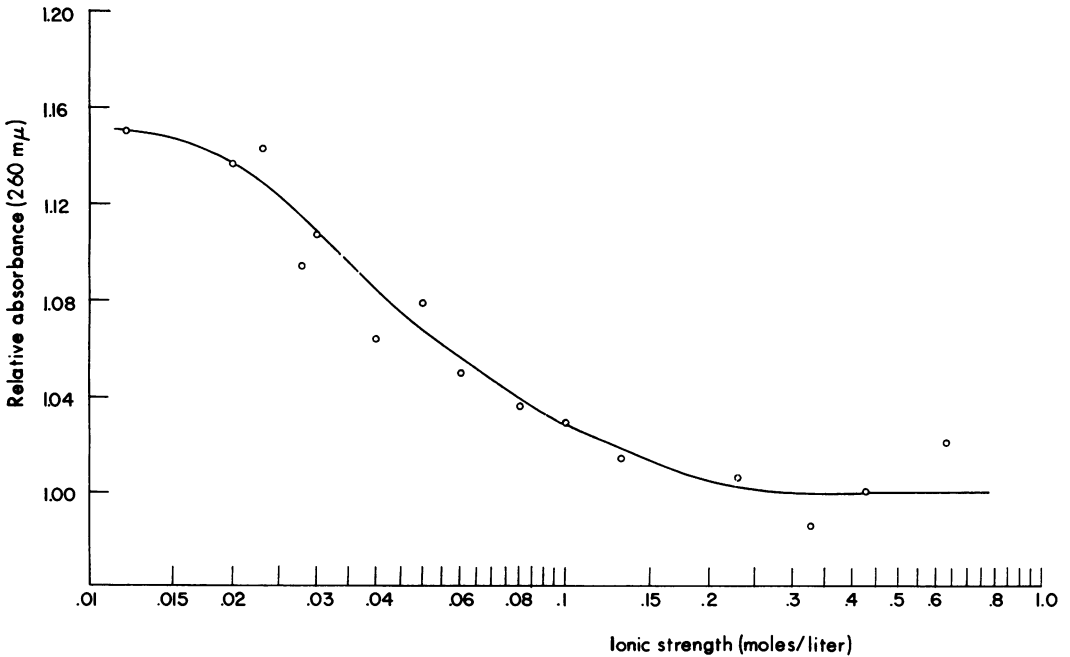

Fig. 8. *Variation of OD* (260 mμ) *of R17 RNA with ionic strength.*

function (Fig. 6). At low ionic strength the distribution was narrow, and with increasing ionic strength the distribution broadened and skewed in the direction of higher S values. This change could be correlated with the variation of the S value of R17 RNA with $\Gamma/2$ and the relative constancy of S value of RF (Fig. 4, 6).

Viscosity. The reduced specific viscosity

$$\frac{1}{c}\frac{\eta - \eta_0}{\eta_0}$$

of R17 RNA at $\Gamma/2$ of 0.012 and 0.23 and RI at $\Gamma/2$ of 0.10 is plotted versus concentration of RNA in Fig. 7. For both values of $\Gamma/2$ used, η_{red} was a linear function of c for R17 RNA. At $\Gamma/2$ of 0.23 $[\eta]$, the intrinsic viscosity, was 0.44 dl/g, and at $\Gamma/2$ of 0.012 it was 3.95 dl/g. The relation between η_{red} and c for RI departed from linearity. The intrinsic viscosity was estimated to be 6.00 dl/g.

Variation of optical density with ionic strength. The variation with ionic strength of $S_{20,w}$ and $[\eta]$ for the single-stranded RNA suggested configurational changes in the molecule in solutions of different ionic strength. These could also be indicated by the change in optical density at 260 mμ with ionic strength. R17 RNA was dialyzed against a solvent of $\Gamma/2 = 0.012$. The optical density was measured on one portion and other portions were carefully diluted in micro-volumetric flasks into solutions of varying ionic strength; then the optical densities of these solutions were determined, all at ambient temperature (25 C). The results, shown in Fig. 8, demonstrated the expected variation of optical density with ionic strength.

DISCUSSION

Some comments on the preparations of replicative intermediate. The preparations of replicative intermediate used in the present study had a single-stranded component amounting to 20 to 30% of the mass. This was determined by measuring the resistance of uniformly labeled RI to digestion with pancreatic ribonuclease and by determining the relative height of the single-stranded and double-stranded plateau regions on the curve of hyperchromicity (11). From the theoretical analysis presented in this paper, it is possible to utilize this information to calculate the average number of single strands per double strand. For 20 to 30% single-stranded component, this is 1.0 to 1.2 single strand of any length per double strand of molecular weight 2.2×10^6. At present, it is not possible to determine whether the functioning intracellular replicative intermediate has a higher ratio of single strands per double strand. RI is certainly altered during phenol extraction, since the polymerase, presumably associated with the double-stranded template, is completely removed. This might result in increased H bonding of the RNA to repair the gap left by the polymerase. Whatever the true intracellular structure, we must be content for the present to study the properties of purified RI

and attempt to correlate these properties with the intracellular complex involved in viral RNA synthesis.

Properties of the single-stranded RNA. Although single-stranded RNA is a polyampholyte, the major charge is negative in the pH range of 6.5 to 6.9 used in this study. Many studies have demonstrated the alterations in shape of this flexible polyelectrolyte with changes in temperature or ionic strength (27, 28). The detailed study of the dependence of S on ionic strength, as reported in this paper, is but a further example of the conformational changes in single-stranded RNA.

The theoretical relationship between S and $\Gamma/2$ for a flexible polyelectrolyte was developed by Harris and Rice (16, 17, 23) by considering the variation in radius of gyration (R_G) as a function of the electrostatic free energy. The expression derived is extremely complicated, but does indicate an exponential relationship between R_G and $\Gamma/2$. This relationship is clearly demonstrated for R17 RNA for values of ionic strength between 0.022 and 0.14 M. Empirically, this relationship may be written as $S^0_{20,w} = A(\Gamma/2)^b$ and, from Fig. 4, $b = 0.301$ for R17 RNA.

The effects of ionic strength on the optical density and on the intrinsic viscosity of R17 RNA are but further reflections of the conformational changes. In solutions of high ionic strength, the charge due to the dissociated groups, particularly the phosphate group, is neutralized by the Gegenion cloud, and the molecule can have a conformation representing a minimal internucleotide distance and also have a very compact form due to the extensive H bonding between base pairs. As the ionic strength decreases, the charged phosphate groups repel each other, possibly resulting in an increase in internucleotide distance and, more importantly, in a dissociation of H bonds as the electrostatic energy becomes greater than the H-bond energy. The discontinuity in the S versus $\Gamma/2$ curve may represent a cooperative effect due to dissociation of critically placed groups of H bonds which result in a change from a compact structure to an extended structure. The value of S at ionic strengths lower than at the point of discontinuity are close to those for the double-stranded RNA of twice the molecular weight (RF). Thus, the single-stranded RNA, in solutions of low ionic strength, may not be as rigid as double-stranded RNA. If both types of molecules had exactly the same degree of rigidity, then they should form a homologous series, and the S value for R17 RNA should be less than that for RF under conditions of low ionic strength. A further indication of the similarity of structure is the equality

of internucleotide distance for single- and double-stranded RNA when measured by a modified Kleinschmidt technique (15).

In solutions of ionic strength greater than 0.14 M, where S is constant, the S value does not fit into the homologous series formed by almost all other types of single-stranded RNA since it does not fit the two similar empirical relationships between S and M, the relationship $M = 1,100\ S^{2.2}$ derived by Gierer (14) and the relationship $M = 1,550\ S^{2.1}$ derived by Spirin (28). This phenomenon, already noted by Strauss and Sinsheimer for RNA from bacteriophage MS2 (29) and by Gesteland and Boedtker for RNA from bacteriophage R17 (13), leads one to suppose that the RNA from this group of RNA bacteriophages can assume an unusually compact structure in solutions of relatively high ionic strength.

Properties of double-stranded RNA (RF). The remarkable difference between the S versus $\Gamma/2$ relationship for R17 RNA and RF can be attributed to the relative rigidity of the double-stranded RF. Thus, the electrostatic forces developed in Gegenion-poor solutions are neither sufficient to denature the double strand nor to cause any striking change in the conformation. This demonstration of the behavior of RF can be cited as further evidence for the double-stranded nature of this molecule. A similar difference between native and denatured DNA was described by Studier (30).

With the use of the published value of translation per nucleotide residue of 3.03 to 3.05 A for double-stranded RNA (18, 31), the number of nucleotide pairs per intact RF molecule can be calculated from the length of the molecule to be approximately 3,500 (15). The molecular weight of such a molecule would be 2.4×10^6. On the other hand, the best estimate of the molecular weight of R17 RNA is 1.1×10^6 (26), so that the molecular weight of RF should be 2.2×10^6. Thus, the molecular weight of RF should be between 2.2×10^6 and 2.4×10^6. The $S^o_{20,w}$ value at ionic strengths of 0.14 or more is 14.7, and it is interesting to calculate the molecular weight of a DNA molecule with this S value by use of the several empirical equations available (Table 3). In every case, the resulting value of molecular weight is too high, suggesting that double-stranded RNA may be somewhat more flexible than double-stranded DNA. This flexibility would result in a smaller coefficient of friction and therefore a higher S for the RNA molecule. A similar conclusion has been reached by Gomatos and Stoeckenius (14a), who compared the molecular weight (determined by electron microscopy) with S value for fragments of double-stranded reovirus RNA. The data of Gomatos and Stoeckenius (14a) and the present data can be fitted to the relation $S = 0.86\ M^{0.19}$ or $M = 2.24S^{5.16}$.

The sedimentation constant of biologically active double-stranded RNA isolated from cells infected with bacteriophage M12 was determined by sedimentation in a sucrose gradient (10). The value of 15S is in good agreement with that reported here. On the other hand, double-stranded RNA isolated from cells infected with bacteriophage MS2 after a preliminary extensive treatment of the phenol-extracted RNA with pancreatic ribonuclease had a sedimentation constant of 8.0 to 9.5S (2). This discrepancy is due to the partial degradation of double-stranded RNA by ribonuclease, as already discussed by Billeter et al. (2). Two preparations of RF, having $S^o_{20,w} = 14.7$, were treated with ribonuclease (0.1 μg/ml, 10 to 15 min at 37 C, $\Gamma/2 = 0.14\ M$). The sedimentation constant was reduced to 8.8 to 9.6, and the sedimentation pattern indicated an increase in heterogeneity, confirming the observations and speculations of Billeter et al. (2).

Properties of replicative intermediate. The "average" S values for RI lie between the values for R17 RNA and RF. The theoretical weight average molecular weight of the single-stranded component was calculated to be 7.2×10^5, which would represent a molecule three-fourths the length of the double-strand in the fully extended state. Thus, RI has a higher S than RF by virtue of its higher molecular weight *and* the contribution of the flexible single-strand to the hy-

TABLE 3. *Molecular weight of native DNA of* $S_{20,w} = 14.7$

Reference	A^*	b^*	B^*	a^*	M
Eigner and Doty (5)[a]............	0.116	0.325	756	3.08	2.96×10^6
Eigner and Doty (5)[b]............	0.034	0.405	4,225	2.47	3.23×10^6
Burgi and Hershey (4)...........	0.080	0.350	1,361	2.86	2.95×10^6
Studier (30)....................	0.0882	0.346	1,116	2.89	2.64×10^6

* From $S = AM^b$ derive $M = BS^a$.
[a] For molecular weights from 3×10^5 to 4×10^6 daltons.
[b] For molecular weights greater than 4×10^6 daltons.

drodynamic properties. This can be seen in the variation of S with $\Gamma/2$. Although this variation occurs over a smaller range of $\Gamma/2$ values and the overall variation is less than for R17 RNA, the exponential dependence is the same for the two types of molecules ($b = 0.303$ for RI).

The hybrid hydrodynamic properties are more strikingly demonstrated by the change in the pattern of weight distribution of S values with ionic strength. In all cases, the width of the distribution can be correlated with the difference between the sedimentation constants of R17 RNA and RF (Fig. 6). This observation clearly demonstrates that RI molecules have hydrodynamic properties depending on both the single-stranded and double-stranded component, and supports the original proposal that the sedimentation heterogeneity in RI may be due to the variable length of the single-stranded component (7).

The intrinsic viscosity of RI is much higher than that of R17 RNA at low ionic strength. This may be due to the rigidity of the double strand and to the higher molecular weight of RI. With the use of the Flory-Fox equation (9) and molecular weights of 1.1×10^6 and 2.9×10^6 for R17 RNA and RI, respectively, the ratio R_G (R17)$/R_G$ (RI) is 3.3 for R17 RNA at high ionic strength (0.23) and is 1.6 for R17 RNA at low ionic strength (0.012). Both values indicate that the volume occupied by RI molecules is very large compared with R17 RNA, probably due to the branched structure of RI.

The molecular weight of RI can be estimated by use of $[\eta]$, $S^\circ_{20,w}$, and the Scheraga-Mendelkern equation (24):

$$M^{2/3} = \frac{S^\circ_{20,w}[\eta]^{1/3}\eta_o N}{\beta(1 - \bar{v}\rho)}$$

The first problem is to choose a value for the constant β. By comparison with values of β determined for other macromolecules or calculated theoretically, β should be between 2.12×10^6 and 3.00×10^6. With the values of $[\eta]$ and $S^\circ_{20,w}$ for R17 RNA, the value of β which best fits with the known molecular weight of 1.1×10^6 is 2.5×10^6 for R17 RNA in solvents of high *or* low ionic strength (Table 4). This suggests that β is not very sensitive to the shape of the RNA molecule and indicates that we may possibly use the same value of β for RI. Indeed, the value of β which yields a molecular weight closest to $\overline{M}_W$ (theoretical) $= 2.9 \times 10^6$ is again 2.5×10^6. Mitra et al. (22) pointed out that the value of β should not be greater than 2.5×10^6 for R17 RNA and Eigner and Doty (5) showed that (i) $\beta = 2.5 \times 10^6$ for native DNA, and

TABLE 4. *Molecular weight estimates with the Scheraga-Mandelkern equation*

Sample	$\Gamma/2$	$S^\circ_{20,w}$	$[\eta]$ (dl/g)	$\beta \times 10^{-6}$	$M \times 10^{-6}$
R17 RNA	0.23	28.2×10^{-13}	0.44	2.12	1.5
				2.50	1.17
				2.70	1.04
				3.00	0.89
R17 RNA	0.012	13.2×10^{-13}	3.95	2.12	1.43
				2.50	1.12
				2.70	1.00
				3.00	0.85
RI	0.10	20.0×10^{-13}	6.00	2.12	3.32
				2.50	2.60
				2.70	2.32
				3.00	1.98

(ii) $\beta = 2.3 \times 10^6$ for denatured DNA (although the values varied between 1.6×10^6 and 2.6×10^6 in this case). Furthermore, β has been shown to have the value of 2.5×10^6 for many synthetic polymers (8). Thus, the present best estimate of $\overline{M}_W$ for RI is 2.6×10^6, with $\beta = 2.5 \times 10^6$.

Some further comments concerning the estimate of RI molecular weight are in order. First of all, $[\eta]$ has not been corrected to zero shear, which may slightly increase the value of $[\eta]$ and therefore the value of $\overline{M}_W$. It should also be pointed out that the same value of $\bar{v} = 0.53$ cm³/g (22) was chosen for single-stranded and branched double-stranded RNA, i.e., R17 RNA and RI. Since the partial specific volumes of all nucleotides are almost identical (J. Witz, Ph.D. Thesis, Faculté des Sciences de l'Université de Strasbourg, Strasbourg, France), there should be little difference in $\bar{v}$ for single- and double-stranded polynucleotides due to differences in these ratios. In practice, $\bar{v}$ is also considered to be the same for native and denatured DNA (5).

In conclusion, these preliminary comparisons of the hydrodynamic properties of RI with those of single- and double-stranded RNA have clearly indicated the hybrid character of RI. These molecules are of interest not only because of their presumed key role in viral RNA synthesis but also because of their unusual polymeric structure. A second approach to a description of the structure of RI is to analyze the population of single-stranded RNA resulting from denaturation of RI. This, as well as further hydrodynamic properties of intact RI, will be described in a subsequent paper (R. M. Franklin, *in preparation*).

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