

THE STRUCTURE OF HIGH MOLECULAR
WEIGHT RIBONUCLEIC ACID IN SOLUTION
A SMALL-ANGLE X-RAY SCATTERING STUDY

SERGE N. TIMASHEFF, J. WITZ, and V. LUZZATI

From the Centre de Recherches sur les Macromolécules, Strasbourg, France. Dr. Timasheff's present address is Eastern Regional Research Laboratory, United States Department of Agriculture, Philadelphia

ABSTRACT Small-angle x-ray scattering studies on an absolute scale have been carried out on isotropic solutions of high molecular weight RNA obtained from ascites tumor cells, *E. coli*, and yeast. It was found that in all three cases the RNA is composed of short rigid rods (50 to 150Å in length) joined by small flexible regions. The rods account for almost the entire structure (at least 90 per cent); their radius of gyration about the axis and their mass per unit length are similar to those of DNA, suggesting a double-stranded helical structure. The rods are joined in an array forming the compact RNA molecule. On thermal degradation, the molecular superstructure disappears while the rods persist.

Since Watson and Crick's (1a) successful approach to the structure of DNA, (1b, 1c) the study of the structure of RNA has been undertaken by similar procedures (2). But, in spite of the similarity of chemical composition, the crystallographic properties of the two substances are quite different: while DNA gels can be spun into well oriented crystalline fibers, which give excellent x-ray diffraction pictures, RNA fibers are hardly oriented, and little information is contained in the x-ray pictures, although some common features can be noticed in the x-ray pictures of both substances (3). Synthetic polyribonucleotides have been extensively investigated (3), since they are expected to provide a convenient model for the structure of RNA. More recently (4) a gel of low molecular weight RNA has been successfully oriented, and the x-ray pictures resemble those of DNA. All these results suggest that in drawn fibers the structure of RNA bears some similarity to that of DNA.

The hydrodynamic behavior of high molecular weight RNA (henceforth referred to as RNA) is consistent with the concept of a non-rigid particle capable of assuming various conformations as a result of changes in environment (5-7). Since under identical conditions DNA is rigid, the assumption has been made that RNA

is single-stranded and that its structure is of the random coil type (6, 8). On the other hand ultraviolet and thermal denaturation experiments have been interpreted in terms of a secondary structure similar to that of DNA (8). Thus, it has been suggested that the RNA molecule consists of a chemical single strand capable of bending back on itself, with the result that short segments of two strands become joined by adenine-uracil and guanine-cytosine hydrogen bonds. In this proposed model about 40 per cent of the bases would be involved in the regular double-stranded segments, the rest forming loops and disordered regions which allow for the flexibility of the molecule (8-11).

Studies on the structure of RNA are further complicated by the fact that most preparations display the presence of two principal ultracentrifugal components with $s_{20,w}$ values of about 30 and 17 S (5, 6), and molecular weights of 2×10^6 and 3 to 5×10^5 (12, 13). In the case of ascites tumor cell RNA, the distribution of components is highly reproducible from one preparation to another, and it seems that they represent, at least in part, a dynamic system in a rather slow equilibrium (14).

Since the main features of RNA structure depend on short-range configuration (in the 10 to 500 Å region), the x-ray small-angle scattering technique one of us has recently developed (15, 16) is ideally suited to the study of RNA in solution (17). This has been particularly well demonstrated by recent studies of DNA (16) and some proteins (18, 19). It is the purpose of this paper to present preliminary results of such studies on isotropic solutions of high molecular weight RNA from various sources.

THEORY AND TECHNIQUE

The x-ray apparatus and the technique have been described elsewhere (15, 16); we shall briefly summarize the essential features.

The x-ray set-up is of the Guinier type. The monochromatic beam is focused by a bent quartz monochromator on the entrance slit of a Geiger counter: the samples are examined by transmission. The intensity is recorded as a function of the diffraction angle during the continuous rotation of the counter around the sample. In the focal plane, the beam is long and narrow, so that the correction of the collimation distortions can be dealt with by the limiting case of an infinite slit. The energy of the direct beam, absorbed by a set of carefully calibrated filters, is measured and is directly compared with the intensity of the diffracted beams. The sample holder is a flat cell, provided with thin mica windows: its thickness is measured by a double microscope.

As a result of each experiment, and after some simple calculations a normalized function $j_n(s)$ is obtained, which only depends on the structure of the sample ($s = 2 \sin \theta/\lambda$).

In the case of an isotropic solution of identical and very long rods, sufficiently dilute for all correlation effects to disappear, we have shown that the expression of $j_n(s)$, for s small, is:

$$j_n(s) = A \frac{1}{2} \exp(-\pi^2 R_c^2 s^2) K_0(\pi^2 R_c^2 s^2) \quad (1)$$

$$A = \mu C_o (1 - \rho_o \psi)^2 \quad (2)$$

where μ is the linear (electronic) mass of the rods, *i.e.* number of electrons per unit length ($\text{el } \text{\AA}^{-1}$), C_o is the concentration (the number of electrons of the solute divided by the number of electrons of the solution), ρ_o is the electron density of the solvent ($\text{el } \text{\AA}^{-3}$), ψ is the (electronic) specific volume of the solute in the solvent ($\text{\AA}^3 \text{el}^{-1}$), R_c is the radius of gyration around the rod axis of the difference between the electron density of the rods and the electron density of the solvent.

By comparing the experimental curves and the theoretical function (equation 1) it is possible to determine A and R_c . This operation can be easily performed in a

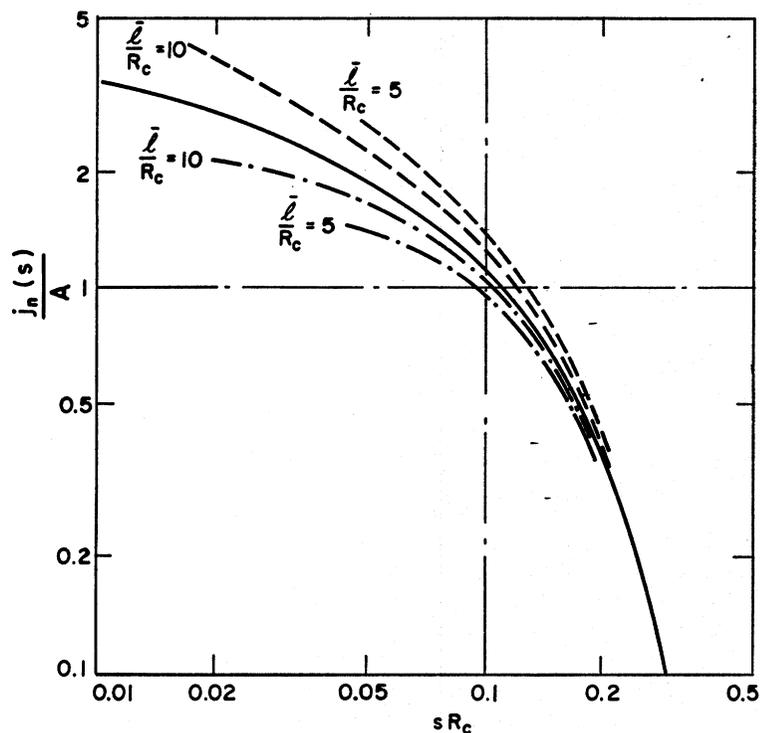


FIGURE 1 Theoretical curves for rod-like particles.

—————: infinitely long rods.
 - - - - -: Gaussian coil of rigid rods.
 - . - . - .: independent rods of finite length.
 \bar{l} is the average length of the rods.

doubly logarithmic plot. The experimental curve $\log j_n(s)$ versus $\log s$ can be superimposed on the function $\log [\frac{1}{2} \exp(-\pi^2 s^2) K_0(\pi^2 s^2)]$ versus $\log(R_0 s)$ by a translation, without rotations: the shifts of ordinates and abscissae provide the values of A and R_0 .

The theoretical curve (equation 1) is derived for infinitely long rods. In the case in which the solute is made of rods of finite length (all having the same μ and R_0 values), either separate from one another or somehow linked together, it can easily be shown that for s large enough, $j_n(s)$ coincides with the curve of infinitely long rods, and that the difference between the two curves becomes significant only for s small. Luzzati and Benoit (20) have dealt with this problem recently, and have analyzed the asymptotic behavior of $i_n(s)$ (for point collimation) as a function of some geometric parameters of the distribution of the rods. These results can be easily transformed to the case of "infinite slit" collimation. We have drawn in Fig. 1 the theoretical asymptotic curves $j_n(s)$ for a random coil of rigid rods, and for a set of independent rods of finite length. The calculations will be given elsewhere.

The curves of Fig. 1 have been calculated for simple models, and show the general trend of the experimental curves: $j_n(s)$ is below the curve of infinitely long rods if the rods are short and independent, and is above it if the rods are linked together and are folded into a rather compact particle (20).

The asymptotic curves of Fig. 1 depend only on the *average* length of the rigid segments: near the origin $j_n(s)$ becomes a complex function of the over-all configuration of the molecule. It can be shown that the $j_n(s)$ versus s curve becomes steeper for decreasing s , as the molecule becomes more compact.

EXPERIMENTAL

RNA samples from three sources have been examined. (a) Ascites tumor cell, prepared by Colter and Brown (5) according to their method and containing only material precipitated by 1 M NaCl. This was ultracentrifugally identical with previous samples of the same material. (b) *E. coli* prepared by Professor J. P. Ebel according to the method of Littauer and Eisenberg (6). This sample was highly aggregated. (c) Yeast, prepared by Ebel; this sedimented in a broad band with an $s_{20,w}$ distribution between 4 and 15S.

The x-ray experiments were carried out as described previously. Since HMW-RNA is known to be labile at ordinary temperatures, the sample holder was provided with a temperature-controlling attachment and most experiments were carried out at 3°C.

The concentrations varied between 10 and 40 gm/liter. Concentrations were determined by ultraviolet absorption at 260 m μ using absorptivity values of 21.0 (5, 12), 23.3,¹ and 18.2¹ liter \times gm³cm⁻¹ for RNA (a), (b), and (c), respectively. These concentrations were subsequently checked by nitrogen analyses of the entire contents of the x-ray cells, which had a capacity of ca. 0.35 cm³.

Ultracentrifuge analyses were carried out with a Spinco Model E analytical ultracentrifuge at 35,600 RPM. using ultraviolet absorption optics.

¹ Absorptivity values determined by J. P. Ebel.

RESULTS AND DISCUSSION

The results of experiments on ascites tumor cell RNA are given in Fig. 2*a*. These experiments were performed under two sets of conditions: (a) pH 6.8, $\Gamma/2 = 0.15$ (0.14 NaCl, 0.01 Na phosphate buffer); (b) samples dissolved in the first solvent were dialyzed *versus* distilled water for 3 hours to reduce the ionic strength; although the exact salt concentration in the second series is not known, it can be estimated to be below $\Gamma/2 = 10^{-8}$. In Fig. 2*a* the results are plotted as $\log j_n(s)$ *versus* $\log s$; the points were taken from the continuous recordings at arbitrary intervals.

The points shown in Fig. 2*a* are seen to fall on a series of curves which approach the theoretical curve for infinite rods (shown by the solid lines in Fig. 2) as s increases. This difference can be decreased further if a curve for an array of rods as in Fig. 1 is used (dashed lines in Fig. 2). For the smallest values of s , $j_n(s)$ rises considerably above all the theoretical curves. Experiments carried out at $\Gamma/2 = 0.3$ and 0.5 gave results essentially identical with those at $\Gamma/2 = 0.15$. It should be noted that the curves obtained after dialysis against distilled water are superimposable on those obtained at $\Gamma/2 = 0.15$ in the higher range of s values. At small s , there is again a positive deviation from the theoretical curve for infinite rods, but this deviation is smaller than at $\Gamma/2 = 0.15$.

Results obtained with *E. coli* and yeast RNA display essentially the same pattern, as is shown in Figs. 2*b* and 2*c*: the structure of all these samples is, thus, essentially the same.

The shape of the $j_n(s)$ curves is consistent with a structure composed of a set of rigid rods, somehow linked together to build a rather compact particle. Assuming that the molecule is a random coil of rigid rods, the average length of the rods is 100 to 150 Å, if the dashed line of Fig. 2 is taken as the asymptotic form of the $j_n(s)$ curves, although shorter rod lengths are quite possible. The concept of a compact array of rods is quite compatible with light scattering (12, 13), sedimentation (5), and electron microscope (21) data obtained with similar preparations of RNA. We shall not discuss at this time, however, the detailed over-all configuration of the particle, which depends on the interpretation of the inner part of the diffraction diagram. Let us remark only that the decreased deviation of $j_n(s)$ from the theoretical curve for infinitely long rods in low salt solutions can be due to stretching of the particle, which is perfectly compatible with hydrodynamic data in the literature (5, 6). The failure of this decrease to be observed in *coli* RNA is probably due to the highly aggregated state of this material.

The values of R_0 and A can be determined as described previously; they are listed in Table I. The values of A/C_0 are constant for all runs, within the limits of experimental error; the average is 15.9.

It is quite remarkable that R_0 and (A/C_0) have very close values in RNA and

FIGURE 2 Experimental results:

Medium: I: pH = 6.8, $\Gamma/2 = 0.15$ (0.14 NaCl, 0.01 Na phosphate)
 II: I dialyzed versus distilled H₂O.

RNA: (a) ascites tumor cell, (b) *E. coli*, (c) yeast
 Open and filled circles represent experimental points.

—: theoretical curve for infinitely long rods.
 - - -: theoretical curve for a Gaussian coil of rigid rods: $\bar{l} = 120 \text{ \AA}$.
 The concentrations are given in the table.

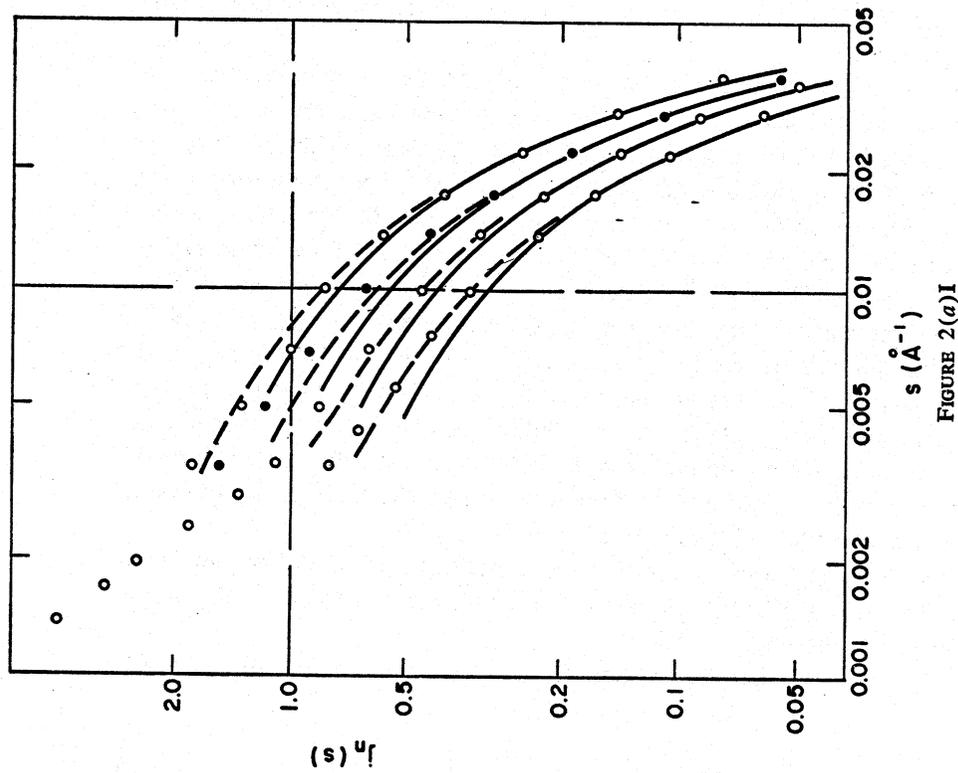


FIGURE 2(a)I

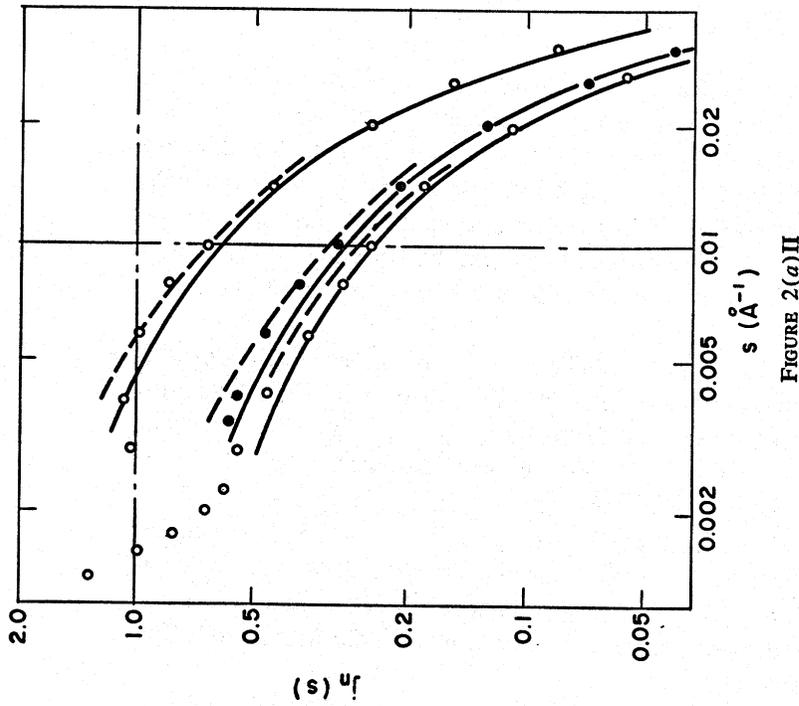


FIGURE 2(a)II

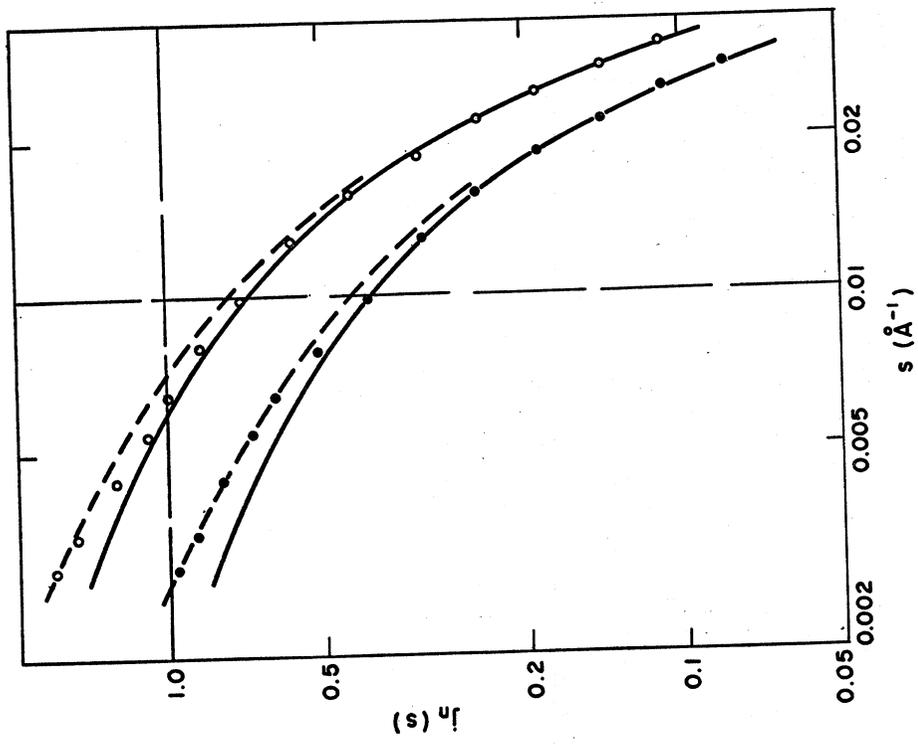


FIGURE 2(b)II

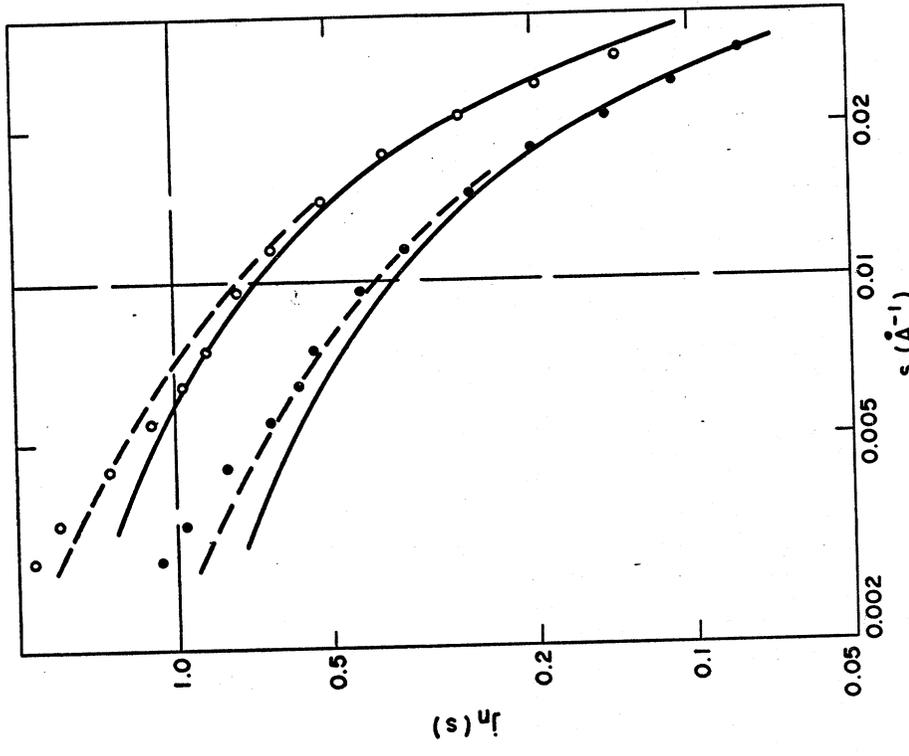


FIGURE 2(b)I

(Figs. 2(c)I and 2(c)II on following page)

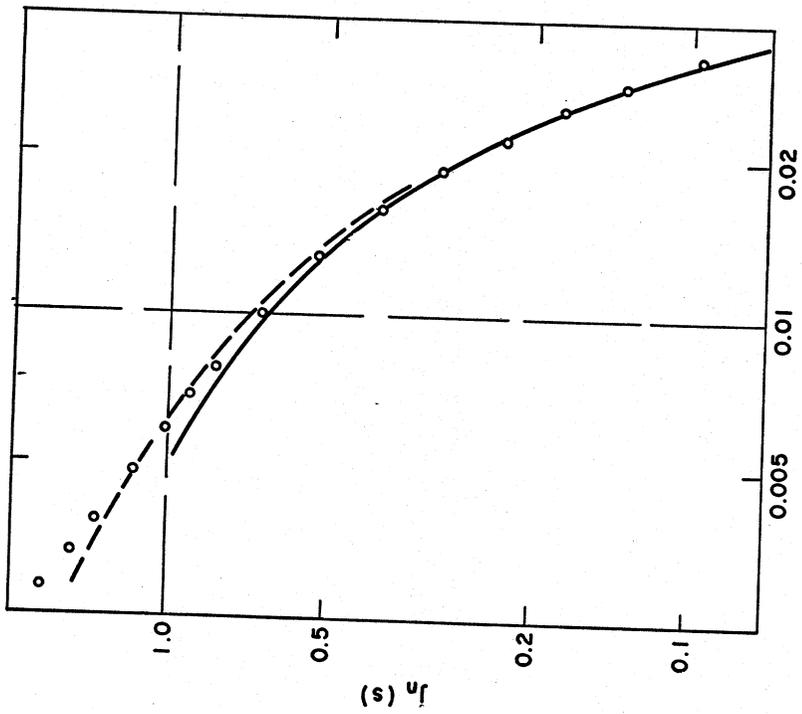


FIGURE 2(c)I

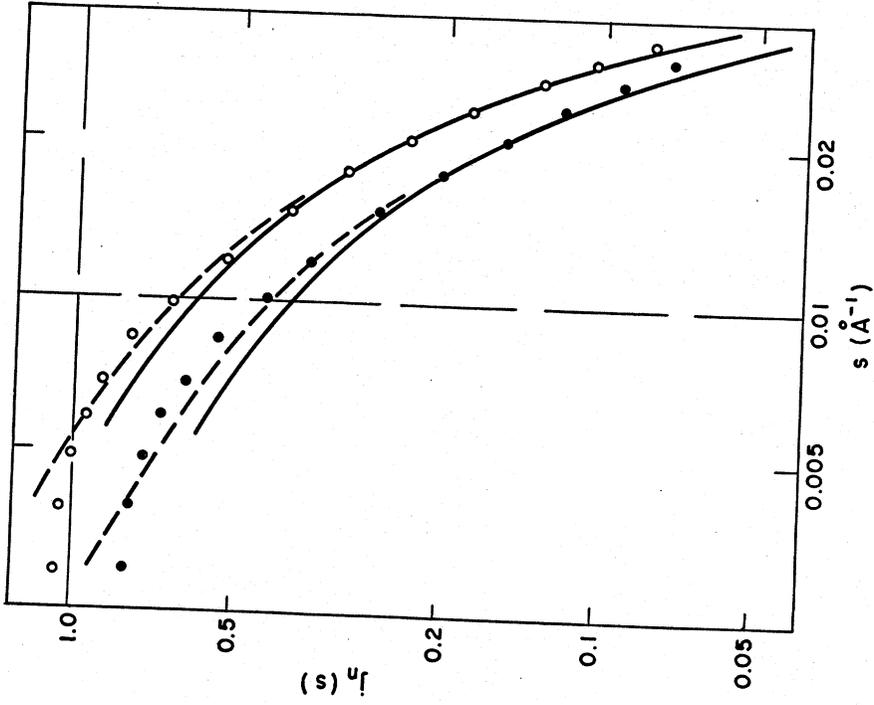


FIGURE 2(c)II

TABLE I
EXPERIMENTAL RESULTS

RNA Source	$\Gamma/2$	Concentration	C_0	R_0	A	A/C_0
		<i>gm/liter</i>	<i>per cent</i>	\AA		
Ascites tumor cells	Dialyzed <i>versus</i> distilled H ₂ O	12.3	1.14	8.3	0.185	16.23
" " "	" " " "	14.9	1.38	"	0.222	16.09
" " "	" " " "	30.5	2.82	"	0.455	16.13
" " "	0.15	16.5	1.53	8.0	0.230	15.03
" " "	0.15	20.6	1.90	"	0.308	16.21
" " "	0.15	28.5	2.64	"	0.408	15.45
" " "	0.15	36.7	3.39	"	0.555	16.37
<i>E. coli</i>	Dialyzed <i>versus</i> distilled H ₂ O	32.6	3.02	8.3	0.535	17.72
" " "	0.15	19.8	1.83	8.0	0.278	15.19
" " "	0.15	36.0	3.33	"	0.513	15.41
Yeast	Dialyzed <i>versus</i> distilled H ₂ O	20.5	1.89	8.3	0.299	15.82
" " "	" " " "	29.6	2.74	"	0.445	16.24
" " "	0.15	32.1	2.97	8.0	0.476	16.03

DNA (16) (for DNA in 0.15 M NaCl, $(\overline{A/C_0}) = 15.0$, $R_0 = 8.4 \text{ \AA}$). If the partial specific volumes of RNA and DNA are the same (and indeed there seems to be no reason why they should differ to a large extent (22)) this coincidence is consistent with the concept that the very largest fraction of RNA is rod-like, and that the linear mass and the radius of gyration of the rods are the same in RNA and in DNA.

The simplest interpretation² of these results is that the RNA molecules in solution consist of an assemblage of rigid rods joined together by flexible regions. The structure of these rods is similar to that of DNA, the rod-like segments of the molecule being probably double-stranded helices with the bases approximately perpendicular to the rod axis and hydrogen-bonded by pairs. In this interpretation at least 90 per cent of the RNA molecule is in the rod form and the disordered regions amount only to a minor fraction of the molecule.

The model which we propose is in some respects similar to the one suggested by Fresco *et al.* (11). Our data suggest, however, that the fraction of the molecule in an ordered state as well as the lengths of the rigid segments are much greater than proposed by these authors. Furthermore, our model would require that the RNA molecule accommodate base pairs other than adenine-uracil and guanine-cytosine. It should be pointed out that Donohue and Trueblood (23) have shown that a num-

² An alternate interpretation would be to assume that the coincidence is fortuitous: the values of R_0 and A obtained are average ones and polydispersity in these parameters cannot be detected by this technique alone. Thus, only a small fraction of RNA might be rod-like, the linear mass of the rods being higher than in DNA. The similarity of the results obtained with different RNA's and even with thermally degraded samples (see below) makes this interpretation unlikely.

ber of non-complementary base pairs could be introduced into the DNA structure without causing considerable distortion.

The tendency to adopt double-stranded helical structures seems to be a general property of polynucleotides. The extreme case is DNA where the base sequence of the two strands is highly specific, the two-stranded structure is very stable, and the macromolecule remains in the shape of a rod over the entire range covered by low-angle x-ray scattering and even light scattering (24). The question comes up: what is the relation between the structure of RNA which we have observed in solution and that which exists *in vivo*? At present, no general answer can be given (25). In one case, at least, the structure of RNA has been shown to be different in solution

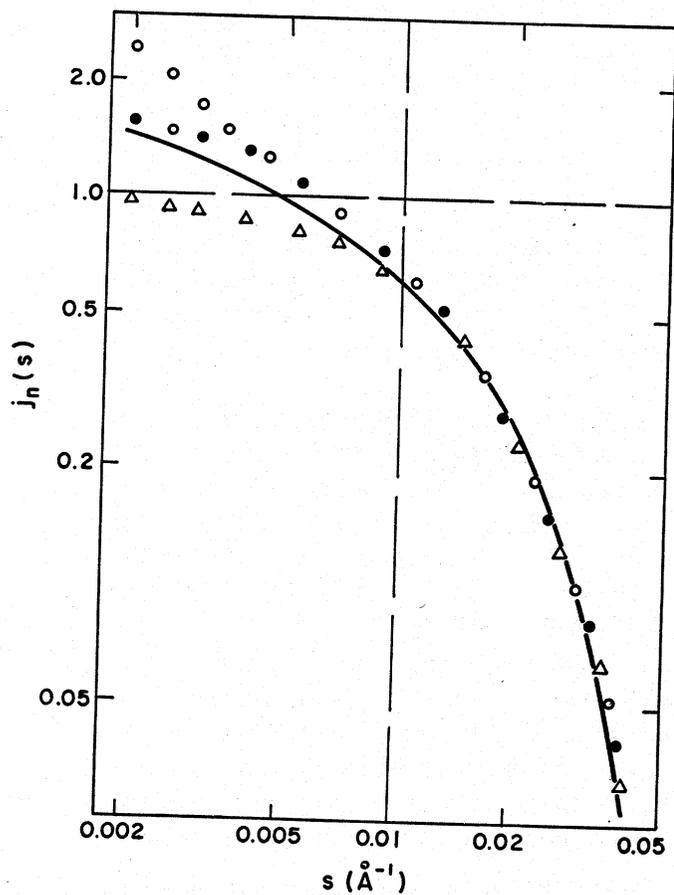


FIGURE 3 Thermal degradation experiments.

Ascites tumor cell RNA; 33.6 gm/liter in pH = 6.8, $\Gamma/2 = 0.15$ (0.14 NaCl, 0.01 Na phosphate).

○: fresh solution at 3°C.

●: same solution kept at 25°C for 48 hours, x-ray experiments at 25°C.

△: same solution kept at 25°C for 18 days, x-ray experiments at 25°C.

from that *in situ*: TMV-RNA in solution assumes a rod array structure similar to that described above (26). This is known to be different from the structure existing in the virus (27). It is conceivable, therefore, that once the RNA has been separated from the proteins with which it is associated *in vivo*, it will tend to become organized in a double helical structure which would satisfy the general tendency of polynucleotides in solution, although it is not excluded that, in certain cases, the RNA exists *in vivo* in a conformation analogous to the one which we have observed.

The stability of the rigid rod regions of RNA has been examined in thermal degradation experiments. Solutions of RNA were examined at 3°C; they were then brought to room temperature and examined as a function of time. Typical results are presented in Fig. 3. Two effects can be seen:

1. In the course of time the region above $s \sim 0.01 \text{ \AA}^{-1}$ remains constant for all practical purposes, indicating that the small rods remain essentially intact even after 18 days in solution at room temperature.

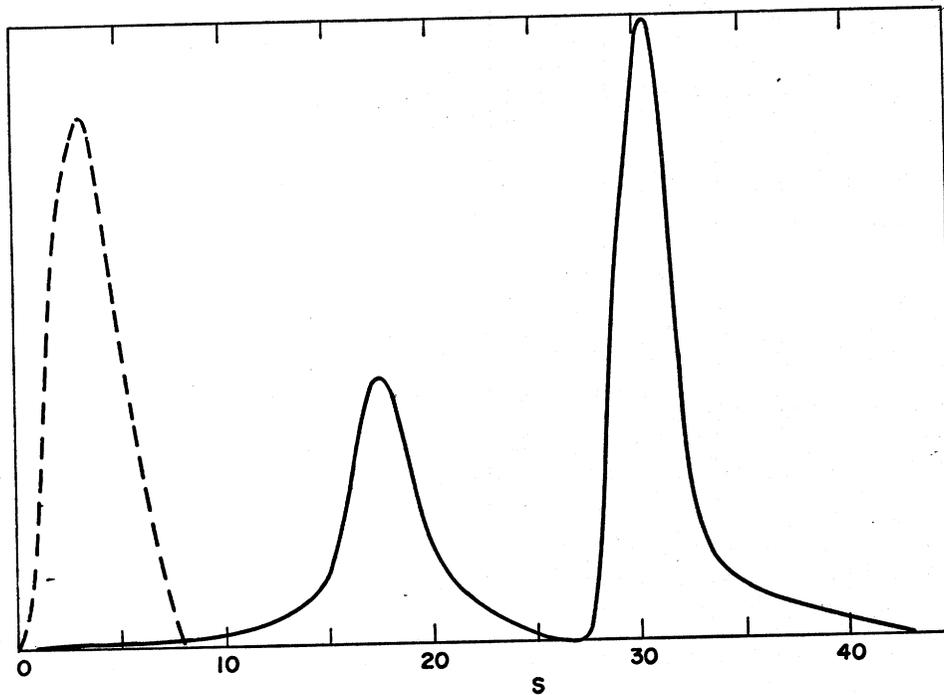


FIGURE 4 Sedimentation distribution of ascites tumor cell RNA.

—————: fresh solution.

— — — — —: same solution, kept at 25°C for 18 days. The concentrations of the two solutions are identical at *ca.* 0.05 gm/liter. The curves have not been corrected for diffusion effect. For quantitative comparison of the two patterns, the dashed curve pattern should be multiplied by 2.5 along the ordinate.

2. In the region below $s \sim 0.01 \text{ \AA}^{-1}$ a sharp change in scattering occurs. The $j_n(s)$ curves fall with time, eventually assuming values below the theoretical curve for infinite rods. This indicates a breakdown of the assemblage of rods and a disappearance of the large compact particle.

At the end of the experiments, aliquots of the RNA solutions were withdrawn from the cell, diluted, and examined in the ultracentrifuge. In all cases the sedimentation constant had fallen sharply, with no material higher than 8S present. The sedimentation distribution of the solution removed from the x-ray scattering cell after 18 days at 25°C (x-ray experiment shown in Fig. 3) is presented in Fig. 4, along with the original distribution. It can be seen that extensive degradation had taken place, the RNA sedimenting in a single peak with a sharp maximum at 3.3 Svedberg units. The degradation mechanism, however, is such that the rod-like configuration and the structure of the rods are preserved. It is further of interest to note that in the course of such dissociations (28) there is no change in the ultraviolet absorption of the RNA at 260 $m\mu$.

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