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REFERENCES

1 R. C. VALENTINE AND A. C. ALLISON, *Biochim. Biophys. Acta*, 34 (1959) 11.
2 A. C. ALLISON AND R. VALENTINE, *Biochim. Biophys. Acta*, 40 (1960) 393.
3 R. DULBECCO, *Proc. Natl. Acad. Sci. Wash.*, 38 (1957) 747.
4 J. S. PORTERFIELD AND A. C. ALLISON, *Virology*, 10 (1960) 233.
5 H. J. ROGERS AND P. C. SPENSLEY, *Biochim. Biophys. Acta*, 13 (1954) 293.
6 A. C. ALLISON, unpublished observations.
7 J. S. YOUNGNER, *J. Immunol.*, 76 (1956) 288.
8 A. S. KAPLAN, *Virology*, 4 (1957) 435.
9 T. T. PUCK, *Cold Spring Harbor Symposia Quant. Biol.*, 18 (1953) 149.
10 L. J. TOLMACH, *Advances in Virus Research*, 4 (1957) 63.
11 J. DIRKX, J. BEUMER AND M. P. BEUMER-JOCHMANS, *Ann. inst. Pasteur*, 93 (1957) 340.
12 T. T. PUCK AND L. J. TOLMACH, *Arch. Biochem. Biophys.*, 51 (1954) 229.
13 E. J. W. VERWEY AND J. TH. G. OVERBEEK, *Theory of the Stability of Lyophobic Colloids*, Elsevier, Amsterdam, 1948.
14 L. H. FROMHAGEN, N. K. FREEMAN AND T. C. A. KNIGHT, *Virology*, 5 (1958) 173.
15 G. L. MILLER, M. A. LAUFFER AND W. M. STANLEY, *J. Exptl. Med.*, 80 (1944) 549.
16 A. D. BANGHAM, B. A. PETHICA AND G. V. F. SEAMAN, *Biochem. J.*, 69 (1958) 2.
17 R. M. HERRIOTT, *Advances in Protein Chem.*, 3 (1947) 170.
18 A. ROTHSTEIN AND R. MEIER, *J. Cellular Comp. Physiol.*, 38 (1951) 245.
19 H. HEYMANN, Z. R. GULICK, C. J. DE BOER AND G. DE STEVENS, *Arch. Biochem. Biophys.*, 73 (1958) 366.
20 H. NOGUCHI, *Biochim. Biophys. Acta*, 22 (1956) 459.
21 A. BIERMAN, *Proc. Natl. Acad. Sci. Wash.*, 41 (1955) 245.
22 O. STERN, *Z. Elektrochem.*, 30 (1924) 508.

LIGHT-SCATTERING STUDIES ON ASCITES TUMOR CELL RNA

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SUMMARY

The molecular parameters of the high molecular weight, ribonucleic acid, prepared from Ehrlich ascites tumor cells have been determined. This RNA appears to consist of two main components of molecular weights of $2.3 \cdot 10^6$ and $3.2 \cdot 10^5$. Under conditions close to physiological, these molecules can be described best as compact rods 40–45 Å in thickness.

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INTRODUCTION

The discovery of GIERER AND SCHRAMM[1] that TMV ribonucleic acid (RNA) carries the viral infectivity has precipitated a number of studies on the molecular state of this material. As a result, GIERER has shown that the molecular weight of the infective RNA is close to two million[2], and that TMV-RNA is a monodisperse substance. Further, GIERER has found that with a change in ionic strength of the medium this RNA can undergo a reversible transition from a compact to a loosely coiled structure[2].

Since the preparation by COLTER AND BROWN[3] of high molecular weight RNA from the cytoplasmic fraction of Ehrlich ascites tumor cells, it became of interest to characterize these molecules which are mammalian in origin. In preliminary reports[3,4], it has been shown that the high molecular weight fraction of this RNA is paucidisperse. It is the purpose of this paper to present a detailed description of studies on the physical-chemical properties of these RNA molecules in solution.

EXPERIMENTAL

The RNA was prepared by the phenol extraction technique according to the method of COLTER AND BROWN[3]. Following the phenol extraction, the high molecular weight RNA was precipitated in each case with 1 *N* NaCl. Sample I was lyophilized, sample II was frozen and stored at —40°, being thawed only a few hours before use. Before light scattering measurements these samples were dissolved in 0.1 ionic strength buffer (0.02 Na phosphate, 0.08 NaCl) of pH 6.8 (sample I) and 7.0 (sample II), dialyzed overnight against it in the cold, and the dialyzate was used as diluent in the light scattering measurements, carried out at 25°. In the case of sample III, the pellet obtained on centrifugation of the salt precipitate was immediately resuspended in a cold pH 7.0 buffer (ionic strength: 0.01 Na phosphate, 0.14 NaCl), dialyzed for 2 h against the same buffer at 6° with stirring, and promptly used for the light scattering measurements at 10–12°.

The light scattering measurements on samples I and II were carried out at 25° in a 20-ml WITNAUER type[5] cell on the BRICE photometer[6] with 2 mm slit optics between 25° and 135°. The solutions were clarified by preliminary centrifugation in a Spinco Model L* centrifuge followed by filtration through an ultra-fine sintered glass filter of special design[7,8]. Dilutions were carried out using a modified DINTZIS technique[9]. Successive concentrations were measured in a single cell, starting with pure solvent, using a microburette for addition of a concentrated stock solution and mixing with a magnetic stirrer. In the case of sample III, all manipulations and measurements were made at 10–12° unless otherwise stated. Solutions were prepared by quantitative dilution of the stock solution. Light scattering measurements were made between the angles of 19° and 140° with 1 mm entrance slits in the photometer, using a recently designed cell[10]. The latter, which consists of a cylindrical cell[5] fused to an ultrafine filter, permits rapid angular measurements on as little as 8 ml of solution without the necessity of transferring solution from filter to cell. By using a separate cell-filter for each solution it was possible to clarify six individual solutions and to measure their angular envelopes in less than 2.5 h.

* Mention of this company does not constitute an endorsement of its product to the possible detriment of other companies not mentioned.

Concentrations were measured by u.v. absorption at 260 mμ using the absorbtivity[3] of 21.0 l g^{-1} cm^{-1}. The value of dn/dc used was 0.1716[4]. Sedimentation distribution curves were derived from u.v. absorption data obtained with the Spinco Model E ultracentrifuge.

RESULTS AND DISCUSSION

Molecular weight and size of RNA

The light scattering data were plotted in all cases in ZIMM[11] and YANG[12] plots and showed a weak angular dependence. The presence of some polydispersity was attested to by the slight curvature of the zero concentration envelopes. These results are presented in Figs. 1, 2 and 3 for Samples I, II and III, respectively. The radius of gyration, the molecular weight obtained and the partial specific volume of RNA (0.528)[13] are inconsistent with a spherical model. The rod model, found by BROWN in electron microscopic studies[14] is in reasonable agreement with the data. In the data analysis, multi-component effects[9] were neglected. Molecular parameters calculated from the light scattering data for the rod-shaped molecules are presented in the Table. The values of the weight average molecular weight ($\overline{M}_w$) were obtained from the extrapolation of the ZIMM plot to zero angle and zero concentration, the slope of the YANG plot at zero concentration, and the extrapolation to zero concentration of the 90° data, corrected for intrinsic dissymmetry (z) at 45 to 135°. In each case, all three values are found to be in good agreement. The weight average length, $\overline{L}_w$, was obtained from the intrinsic dissymmetry, and the Z-average length, $\overline{L}_z$ from the limiting slope of the zero concentration curve of the ZIMM plot and the intercept of the YANG plot.

Since it has been reported that heavy metals[15] have a strong influence on the shape of the RNA molecule, a comparison was made of light scattering from solutions containing 0.01 % Versene with that from Versene-free solutions. The results shown in Fig. 3 indicate that any trace metals that might be present have no discernible effect on the scattered intensity and hence on $\overline{M}_w$ or the size of RNA.

BROWN[14] has shown in electron microscope studies on similar preparations of

TABLE I

MOLECULAR PARAMETERS OF EHRLICH ASCITES TUMOR CELL RNA

	Sample I	*Sample II*	*Sample III*	*Sample III (calculated)**
$\overline{M}_w$, ZIMM	$1.40 \cdot 10^6$	$1.18 \cdot 10^6$	$1.49 \cdot 10^6$	$1.6 \cdot 10^6$
$\overline{M}_w$, YANG	$1.32 \cdot 10^6$	$1.17 \cdot 10^6$	$1.48 \cdot 10^6$	
$\overline{M}_w$, Dissym	$1.34 \cdot 10^6$	$1.09 \cdot 10^6$	$1.37 \cdot 10^6$	
Z (Dissym)	1.24	1.14	1.20	—
$\overline{L}_w$	890 Å	690 Å	810 Å	920 Å
Lim slope (ZIMM)	$6.2 \cdot 10^{-7}$	$4.2 \cdot 10^{-7}$	$(4.0–7.5) \cdot 10^{-7}$	—
Lim slope (YANG)	$4.1 \cdot 10^{-7}$	$3.0 \cdot 10^{-7}$	$7.5 \cdot 10^{-7}$	—
ϱg^2	$(9.71–17.6) \cdot 10^4$ Å^2	$(7.10–10.3) \cdot 10^4$ Å^2	$(11.2–22.4) \cdot 10^4$ Å^2	—
$\overline{L}_z$	1,060–1,500 Å	925–1,110 Å	1,160–1,640 Å	1,310 Å
$\overline{M}_z$, calc.	$(2.14 \pm 0.36) \cdot 10^6$	$(1.69 \pm 0.15) \cdot 10^6$	$(2.34 \pm 0.42) \cdot 10^6$	$2.18 \cdot 10^6$

* Calculated from bimodal distribution of 63 % of 34-*S* and 37 % of 18-*S* component.

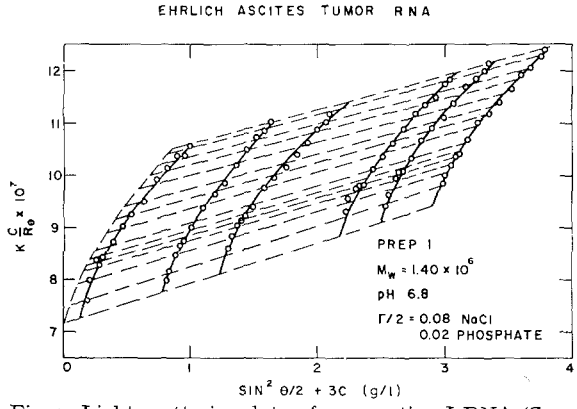

Fig. 1. Light scattering data of preparation I RNA (ZIMM plot).

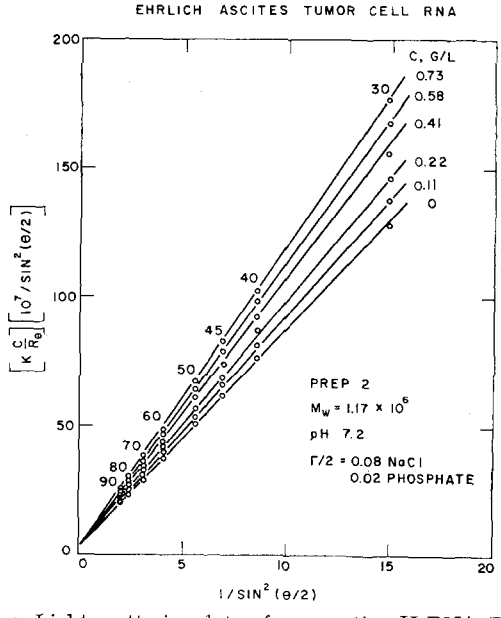

Fig. 2. Light scattering data of preparation II RNA (YANG plot).

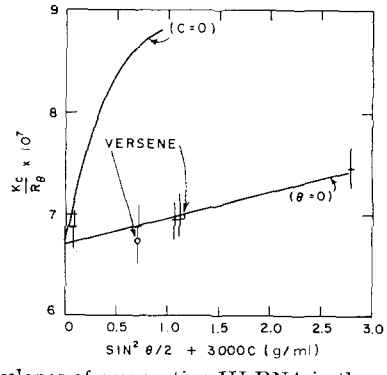

Fig. 3. Light scattering envelopes of preparation III RNA in the presence and absence of versene.

RNA that the RNA molecules are rods 40–45 Å thick. This thickness remains constant for particles of varying lengths. Considering the RNA molecules to be cylinders with a diameter of 43 Å, the molecular weight must then be proportional to the particle length. In this manner, the Z-average molecular weights, $\overline{M}_z$, given in the last line of the Table, were calculated from the experimental $\overline{L}_z$.

The light scattering data can be further analyzed with the help of electron microscopic and ultracentrifugal information. In the case of sample III, a cylindrical molecule with a weight average molecular weight of $1.49 \cdot 10^6$, $\overline{V}$ of 0.52_8 and a base diameter of 43 Å has a calculated weight average length of 890 Å. This is in very good agreement with the observed value of $\overline{L}_w$ of 810 Å, and can serve as further verification of the electron microscopic observations on the uniformity of particle thickness.

Ultracentrifugally, sample III is found to contain essentially two components: 63 % of a 34-S component and 37 % of a 18-S component, as shown in Fig. 4. The shape of the molecules and the values of the sedimentation constants of the two components are found to be compatible with molecular weights of $2.3 \cdot 10^6$ (63 %) and $3.2 \cdot 10^5$ (37 %)[14]. The weight average molecular weight ($\overline{M}_w$) calculated for such a bimodal distribution is $1.6 \cdot 10^6$, while the weight average length, $\overline{L}_w$, is 920 Å, both of which are in good agreement with experimental values by light scattering. Shown in the last column of the table are molecular parameters calculated for such a distribution. Comparison of the calculated values with the experimental data, shown in column 4, point to good agreement between the light scattering and ultracentrifugal data.

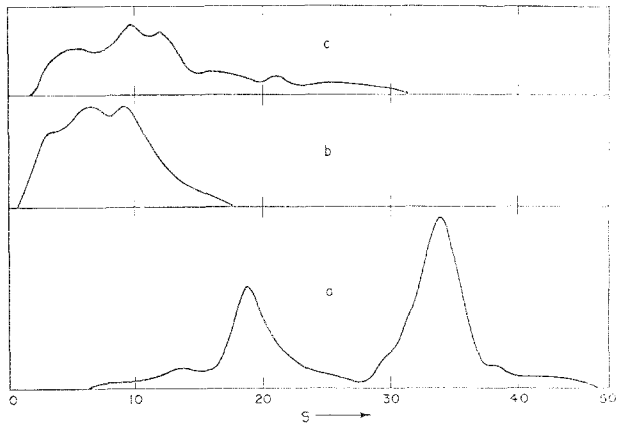

Fig. 4. Ultracentrifugal diagrams of preparation III RNA. a, starting material; b, 2.9 g/l solution stored three days at 25°; c, 0.25 g/l solution stored three days at 25° in presence of versene.

Similar RNA preparations were found to have an intrinsic viscosity of 0.40[8]. Assuming an axial ratio of 18, use of the SCHERAGA-MANDELKERN equation[16] results in a value of 34-S for the sedimentation constant of the hypothetical RNA molecule represented by the weight average light scattering data on Sample III. The experimental value of the weight average sedimentation constant is 31-S. This can be compared further with the weight average sedimentation constant of 27-S calculated according to the method of GIERER[2] for $\overline{M}_w = 1.5 \cdot 10^6$.

Degradation and aggregation of RNA

A comparison of the three samples shows good agreement between samples I and III, while sample II shows signs of degradation, its weight average molecular weight and particle dimensions being smaller. This indicates that freezing and thawing results in depolymerization, while lyophilization seems to have no pronounced effects on the molecules.

In order to determine if significant changes occur in the state of aggregation of RNA during the interval between its preparation and the time of measurement, time studies were carried out at 10–12° and 25°. Shown in Fig. 5 are representative light scattering data obtained over about 68 h for RNA solutions (sample III) in the presence and absence of 0.01 % Versene. Zero time was taken to be the time during the preparation when the dialysis was initiated.

As Fig. 5 illustrates even at 10–12° there is a slow degradation of RNA to the extent of a change in $\overline{M_w}$ of about 10 % in 26 h. Changes in $\overline{M_w}$ occurring during the interval between preparation and initial measurements are thus minor, particularly since during 40 % of this time the solutions were kept at 6°. As Fig. 5 illustrates, when the temperature was raised to 25°, the rate of degradation increased but in Versene-free solutions was not accompanied by aggregation. This is most evident from the data taken at an angle of 19°.

The magnitude of the aggregation can be best appreciated by comparing angular envelopes for Versene-free and Versene-containing solutions. Such plots, shown in Fig. 6, indicate that in the absence of Versene very large aggregates are formed, the maximum in the Kc/R_θ *vs.* $\sin^2 \theta/2$ plot being characteristic of very large spherical particles[17]. The asymmetry of the 68-h Versene curve as compared to the 8-h Versene-free curve indicates that aggregation probably occurs to some extent even in the presence of Versene.

The fact that Versene inhibits aggregation indicates that in spite of the fact that

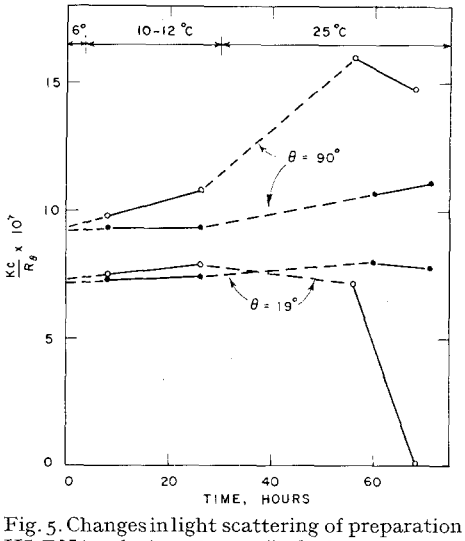

Fig. 5. Changes in light scattering of preparation III RNA solutions (0.25 g/l) during storage at various temperatures. O—O, no Versene; ●—●, in the presence of 0.01 % Versene.

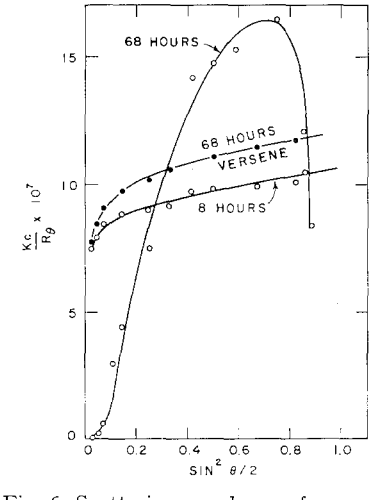

Fig. 6. Scattering envelopes of preparation III RNA solutions (0.25 g/l) after various durations of storage. O—O, no Versene; ●—●, in the presence of 0.01 % Versene.

RNA was prepared with redistilled phenol the final solutions contain some metal ions. Nonetheless, as Fig. 3 illustrates these metal ions have no effect upon the scattering of light from RNA solutions at high ionic strength and thus could have had no marked effect on either the molecular weight or size. These results contrast with the findings of HASCHENMEYER, SINGER AND FRAENKEL-CONRAT[15], who showed that TMV-RNA prepared in the presence of metals such as Ca^{2+} had a more compact configuration in solutions of low ionic strength than that of metal-free preparations. It would appear that this is simply a polyelectrolyte effect, *i.e.*, at low ionic strength the bound metal ions reduce the net charge and the electrostatic repulsion, thereby decreasing the average molecular end to end distance.

While the light scattering data emphasize the aggregative processes, and in the case of the Versene-containing RNA sample, point to an apparent absence of gross changes, sedimentation measurements indicate the contrary. Shown in Fig. 4 are sedimentation distribution curves of samples kept for three days at 25°. Extensive degradation has occurred at both high (2.9 g/l) and low (0.25 g/l) RNA concentrations. In the case of the 0.25 g/l samples, when Versene was absent, degradation proceeded to a point where no sedimentable material could be observed. Similar degradative changes of RNA have been observed by GAVRILOVA *et al.*[18] and by LITTAUER AND EISENBERG[19], the latter authors having demonstrated that metal ions can promote RNA degradation.

The above data show that while RNA is capable of aggregating under the influence of metal ions, the more important process is a degradation, also apparently promoted by metal ions.

CONCLUSION

The high molecular weight fraction of cytoplasmic Ehrlich ascites tumor cell RNA appears to consist of two main components with molecular weights of $2.3 \cdot 10^6$ and $3.2 \cdot 10^5$. Under conditions close to physiological these molecules can be described best by compact rods, 40–45 Å in thickness.

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REFERENCES

1 A. GIERER AND G. SCHRAMM, *Nature*, 177 (1956) 702.
2 A. GIERER, *Z. Naturforsch.*, 13b (1958) 447.
3 J. S. COLTER AND R. A. BROWN, *Science*, 124 (1956) 1077.
4 S. N. TIMASHEFF, R. A. BROWN, J. S. COLTER AND M. DAVIES, *Biochim. Biophys. Acta*, 27 (1958) 662.
5 L. P. WITNAUER AND H. J. SCHERR, *Rev. Sci. Instr.*, 23 (1952) 99.
6 B. A. BRICE, M. HALWER AND R. SPEISER, *J. Opt. Soc. Am.*, 40 (1950) 768.
7 M. BIER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Academic Press, N.Y., 1957, p. 165.
8 F. F. NORD, M. BIER AND S. N. TIMASHEFF, *J. Am. Chem. Soc.*, 73 (1951) 289.
9 S. N. TIMASHEFF, H. M. DINTZIS, J. G. KIRKWOOD AND B. D. COLEMAN, *J. Am. Chem. Soc.*, 79 (1957) 782.

[10] M. J. KRONMAN AND S. N. TIMASHEFF, *J. Polymer Sci.*, 40 (1953) 573.
[11] B. H. ZIMM, *J. Chem. Phys.*, 16 (1948) 1099.
[12] J. T. YANG, *J. Polymer Sci.*, 26 (1957) 305.
[13] L. DELCAMBE AND V. DESREUX, *Bull. soc. chim. Belges*, 59 (1950) 521.
[14] R. A. BROWN, unpublished experiments.
[15] R. HASCHENMEYER, B. SINGER AND H. FRAENKEL-CONRAT, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 313.
[16] H. A. SCHERAGA AND L. MANDELKERN, *J. Am. Chem. Soc.*, 75 (1953) 179.
[17] W. B. DANDLIKER, *J. Am. Chem. Soc.*, 72 (1950) 5110.
[18] L. P. GAVRILOVA, A. A. SPIRIN AND A. N. BELOZERSKII, *Doklady Akad. Nauk, S.S.S.R.*, 124 (1959) 933.
[19] U. Z. LITTAUER AND H. EISENBERG, *Biochim. Biophys. Acta*, 32 (1959) 320.

Biochim. Biophys. Acta, 40 (1960) 410–417

STUDIES ON DEOXYNUCLEOSIDIC COMPOUNDS

I. A MODIFIED MICROBIOASSAY METHOD AND ITS APPLICATION TO SEA URCHIN EGGS AND SEVERAL OTHER MATERIALS*

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SUMMARY

1. By modifying HOFF-JØRGENSEN's microbiological assay method for deoxynucleosides, *viz.* by digesting samples to be assayed with a snake venom enzyme, it was possible to detect a new group of deoxynucleosidic compounds. This group of deoxynucleosidic compounds, differing from either simple deoxynucleosides or deoxynucleotides, were tentatively designated as "masked" deoxynucleosidic compounds.

2. "Masked" deoxynucleosidic compounds were detected in various tissues such as sea urchin eggs and embryos and mammalian tissues including tumors.

3. The relative amounts of "masked" deoxynucleosidic compounds compared with DNA or simple deoxynucleosidic compounds in eggs and early embryos were markedly higher than those in adult tissues. In this connection, the biological significance of "masked" deoxynucleosidic compounds was discussed.

Abbreviations: DNA, deoxyribonucleic acid; PCA, perchloric acid.

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