RIBONUCLEIC ACID FROM *ESCHERICHIA COLI*: ELECTRON MICROSCOPICAL STUDY

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SUMMARY

The dimensions of high-molecular weight RNA from *E. coli* were measured by electron microscopical methods. Filaments 10 to 13 Å in diameter, and 2000–4000 Å in length, could be observed in preparations obtained from salt-free aqueous solutions of RNA. When ammonium acetate solutions were used, individual fibers became rare, and granules and various forms of aggregates dominated the picture.

INTRODUCTION

RNA from *E. coli* has been previously characterized by viscometry, streaming birefringence and potentiometry[1,2]. These studies indicated that this high molecular weight RNA is much more flexible in solution than DNA, as would be in keeping with a single stranded structure.

In the present study an attempt has been made to determine the dimensions of *E. coli* RNA molecules, using electron microscopical methods.

Electron micrographs of TMV RNA have been prepared by SHUSTER *et al.*[3] and HALL[4], using an improved technique for visualizing macromolecules, has compared electron micrographs of calf liver and TMV RNA and salmon sperm DNA.

MATERIALS AND METHODS

RNA from *E. coli* was prepared as previously described[2]. A suspension of "protoplasts" was extracted with a phenol–water mixture, the RNA was precipitated by ammonium sulfate, dialyzed against dilute sodium chloride solution, and then lyophilized. The RNA revealed two boundaries in the ultracentrifuge, having sedimentation constants of 16.5 and 23.7 S (as determined using the u.v. absorption optical system). RNA was dissolved in distilled water (1 mg/ml). Complete solution was obtained after 30 to 60 min. Aliquots were diluted with water or with 0.1 *M* ammonium acetate (pH 6.8) to a final concentration of 0.1 mg/ml.

DNA from T_2 bacteriophage was prepared by osmotically shocking a phage suspension. The bacteriophage particles were suspended in 3 *M* ammonium acetate

Abbreviations: DNA, deoxyribonucleic acid; RNAase, ribonuclease; RNA, ribonucleic acid; TMV, tobacco mosaic virus.

($2 \cdot 10^{11}$ particles/ml) and then diluted fifty fold with 0.1 M ammonium acetate. The suspension was clarified by centrifugation for 40 min at 105,000 $\times$ g.

Crystalline pancreatic RNAase was an Armour and Company product.

Electron microscopy

Freshly cleaved mica was cut into rectangles of the size of a microscope slide. The mica rectangles were dipped in a 0.5 % parladion (Mallinckrodt Chemical Works) solution in redistilled amyl acetate. After drying at room temperature in an erect position under a cover, the film from the freshly-cleaved mica surface was floated onto glass doubly-distilled water. The grids were deposited on the floating film, and taken up on a glass slide[5]. Solutions were spread as fine droplets onto the film covering the grids on the slide, using, as the case may be, laboratory made low and high velocity spray guns. The preparations were dried at room temperature in a slightly open petri dish. In some experiments, a drop of the solution was deposited on the film on the grid, the preparation frozen on a copper block previously immersed in liquid-air and freeze dried in a Virtis apparatus.

The dried preparations were shadow-cast with either a chromium–nickel alloy, or platinum at shadow to height ratios 6:1 to 10:1. The films were backed with a thin supporting layer of SiO (see ref. 6). The platinum was evaporated from a tungsten spiral[7]. An aqueous suspension of polystyrene spheres of 0.34 μ diameter was added to the solution, to aid in the location of the droplets and in the exact determination of the shadow-casting angle and the direction of shadow. An RCA EMU 2A Electron Microscope was used with a locally made improved specimen stage, with a 50 μ objective aperture and with apertures at the intermediate and condenser lenses.

RESULTS AND DISCUSSION

Serious difficulties were encountered in stripping off the film from the mica surface, if the shadow casting and reinforcement with SiO were done directly on the mica as described by HALL. This was overcome by floating the collodion film first and then spraying the sample onto the surface of the film which had been in contact with the mica. This surface, being a true replica of the smooth surface of the mica, was structureless and suitable for the purpose intended. The shadow casting and reinforcing with SiO were done directly on the film.

Chromium–nickel alloy was used in some experiments because better contrast was attained with this alloy than with platinum. The larger grain, however, limited its use for more precise measurements. For this purpose platinum had to be employed.

Hydrodynamic measurements in solution have indicated that RNA molecules fold up into more compact structures when salt is added to the solution[2]. An attempt was therefore made to visualize RNA in both the more compact and the more unfolded state by this means and indeed the electron micrographs of RNA obtained from water solution showed molecules in the form of threads. A strong tendency for aggregation was noticed, especially at the center and border of the micro drops, probably due to the surface tension of the drying droplets. Using the high pressure gun single threads, 10–13 Å in diameter and 200–2000 Å in length (with a maximum in the distribution at between 200–600 Å), were generally found outside the periphery of the droplet (Fig. 1). Using the low pressure gun, the average length was higher (1200–4000 Å).

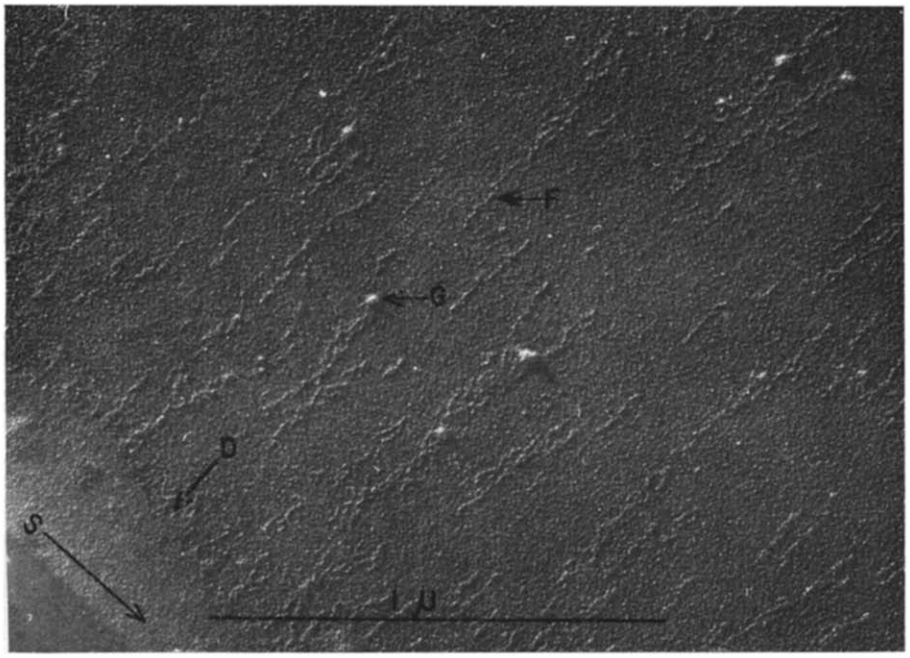

Fig. 1. *E. coli* RNA in water solution sprayed from a high pressure gun and air-dried at room temperature. Shadow cast with Pt at a shadow to height ratio of 6:1. S, direction of the shadow; D, border of the micro-drop; G, granule; F, fiber.

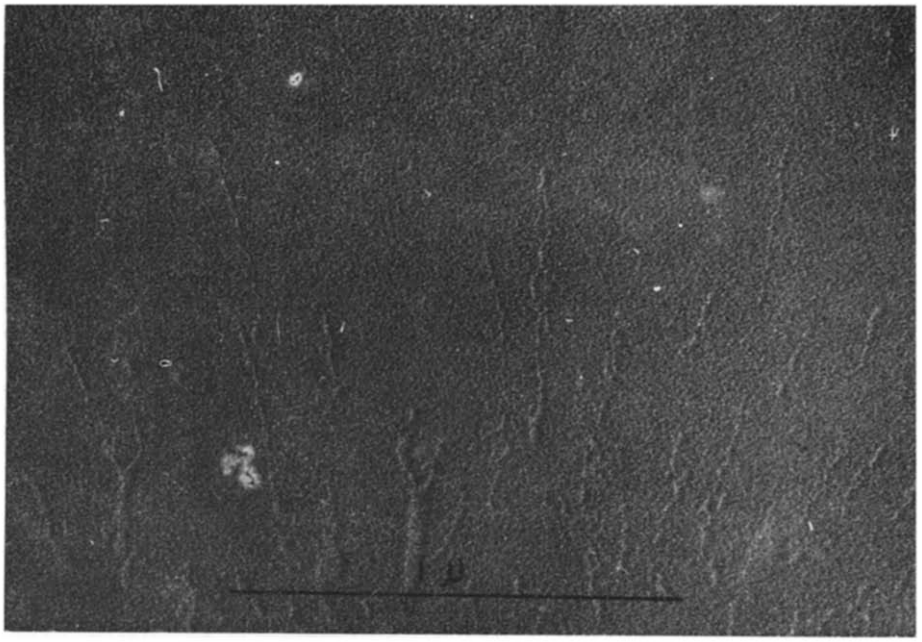

Fig. 2. *E. coli* RNA in water solution sprayed from low pressure gun and air-dried at room temperature. Shadow cast with Pt at a shadow to height ratio of 6:1.

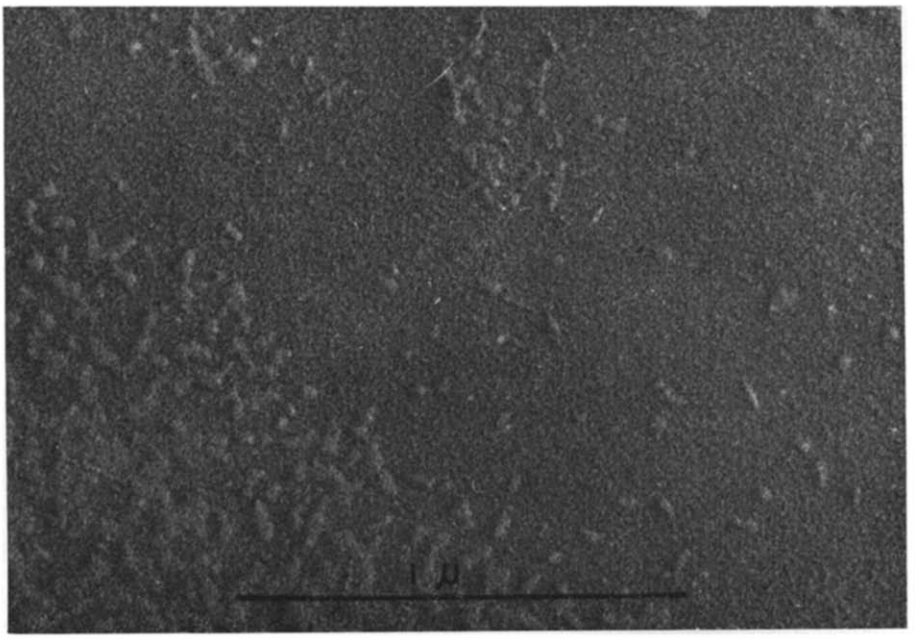

Fig. 3. *E. coli* RNA in 0.1 *M* ammonium acetate solution sprayed from a high pressure gun and air-dried at room temperature. Shadow cast with Pt at a shadow to height ratio of 10:1.

The same diameter values were observed (Fig. 2). Occasionally small flat granules, 400–600 Å in diameter, were noticed.

When RNA deposited from ammonium acetate solution was examined, individual threads became rare while granules and various forms of aggregates dominated the picture (Fig. 3).

In freeze-dried preparations, the material was found to be more homogeneously dispersed on the surface of the film. In preparations made by this technique from water solutions individual fibers were much more difficult to locate, due to the absence of microdroplets. The filaments measured from 1500–4000 Å in length (Fig. 4). A particular form of aggregation was revealed in these preparations that was not observed when the material was sprayed and air-dried at room temperature (Figs. 4 and 5). This aggregation was both longitudinal and lateral and showed a high degree of organization. The latter may be attributed to crystallization phenomena, apparently due to imperfections in the freeze-drying technique.

No difference in the form of the molecules, or in the nature of this aggregation could be revealed by varying the pH of the solution from 6 to 9.

When RNAase was introduced into the test tube containing the RNA, prior to preparation of the specimen, neither filaments nor granules could be detected.

DNA fibers appeared as long smooth strands, about 20 Å in diameter (Fig. 6).

The values 10–13 Å found for the RNA diameter are lower than the 15 Å estimated by SHUSTER *et al.*[3] for the finest fibers they could observe in TMV RNA preparations. They are also considerably lower than the values published by HALL for calf liver and TMV RNA[1]. In HALL's study, RNA was seen as threads with an apparent thickness of about 30 Å. According to HALL the molecules were thicker

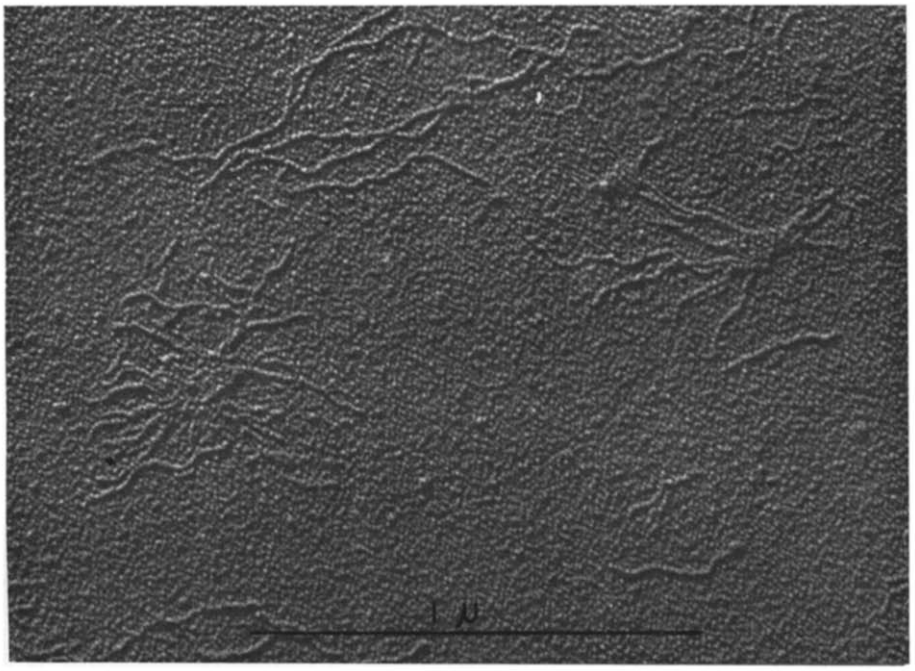

Fig. 4.

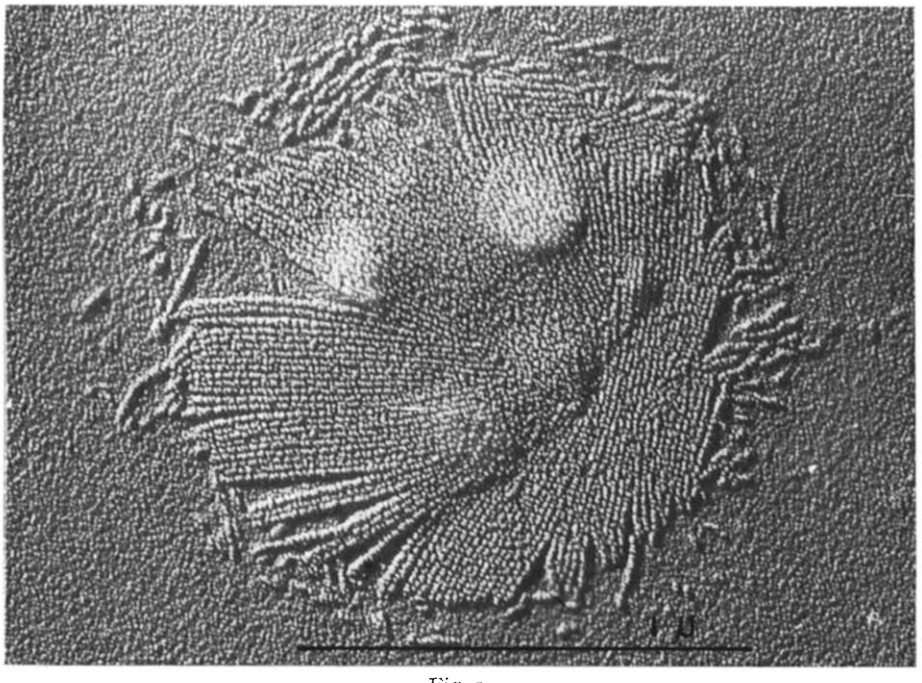

Fig. 5.

Figs. 4 and 5. *E. coli* RNA freeze-dried and shadow cast with chromium–nickel alloy at a shadow to height ratio of 6:1.

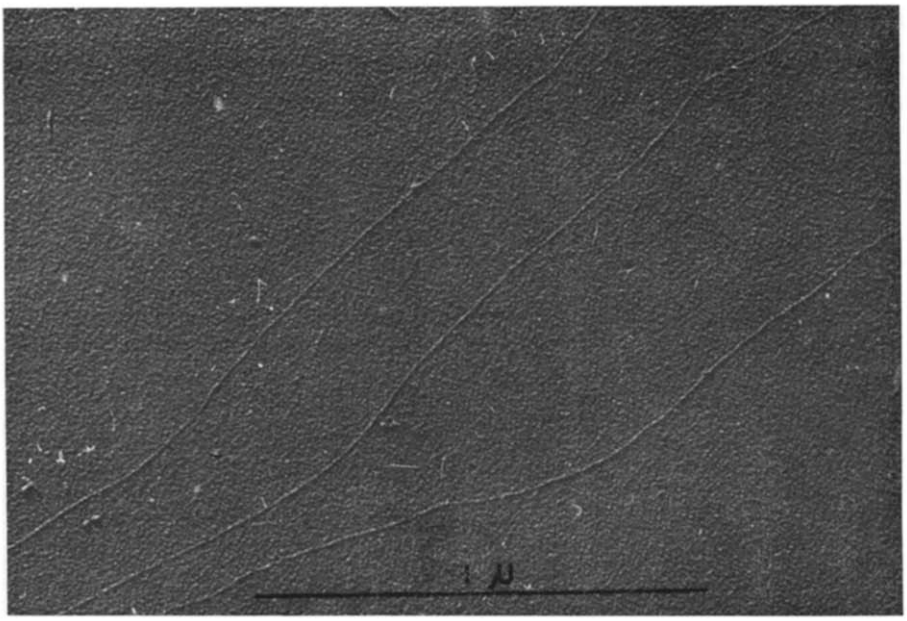

Fig. 6. Bacteriophage T_2 DNA in 0.1 *M* ammonium acetate solution sprayed from a low pressure gun and air-dried at room temperature. Shadow cast with Pt at a shadow to height ratio of 6:1.

than expected, and he thought that they were "probably partially coiled up on themselves". The present result for *E. coli* RNA would indicate that these molecules are being observed practically unfolded, probably due to the fact that aqueous solutions were used. Whenever salt solutions were used in our study granules appeared and practically no fine filaments were observed. These findings are in accordance with information derived from the hydrodynamic measurements[2], and support the idea that RNA is a molecule capable of great degree of coiling.

The RNA chain of *E. coli* appeared to be granular in nature, in contradistinction to the smooth and rigid DNA strands. This granular character of the fibers made it somewhat difficult to distinguish between a single long fiber and shorter ones, longitudinally aggregated. However, the majority of the fibers measured were found to be between 2000 and 4000 Å when the low pressure gun was used for spraying, or when using the "freeze-drying" technique. When the high pressure gun was used, the majority of the fibers were between 200 and 600 Å, and were apparently broken down by the treatment. This was confirmed by an ultracentrifuge and viscosity investigation of an RNA solution before and after passage through the high pressure gun. Fig. 7 shows the change in sedimentation pattern. The high molecular weight component has almost disappeared and a broad shoulder is in evidence at the low molecular weight end. The viscosity number η_{sp}/c fell from 1400 to 590 ml/g after spraying (viscosity measured at a concentration of $1.3 \cdot 10^{-1}$ g/ml in 10^{-3} *M* NaCl).

From the agreement between the pictures obtained by the freeze-drying technique and the low-pressure spray technique there is reason to believe that no degradation of the RNA molecules took place. Having regard to the high molecular weight of the molecules the strand lengths observed are much too short if considered as fully

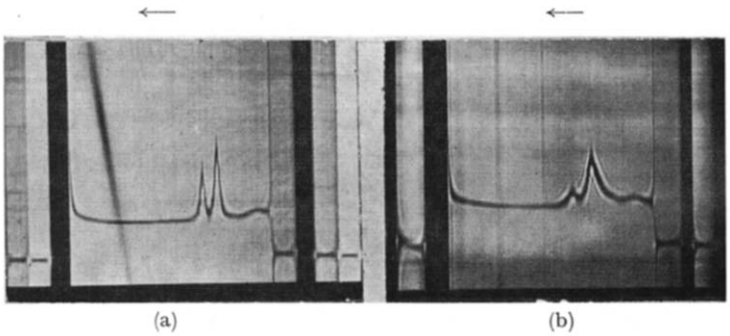

Fig. 7. Effect of spraying on the sedimentation diagram of *E. coli* RNA. (a) RNA solution in 0.2 *M* NaCl. Picture taken at bar angle of 60° after 16 min at 52,640 rev./min and 23°. RNA concentration about 5 mg/ml. (b) RNA solution in 0.2 *M* NaCl after spraying from high pressure gun. Picture taken at bar angle of 60°, after 16 min at 56,000 rev./min and 21°. RNA concentration about 5 mg/ml.

stretched chains. Similarly the diameters of 10–13 Å are too large for an extended single chain. It is not unreasonable to suppose therefore that in the state visualized the single strand of RNA twisted itself up perhaps in helical fashion in which case both the diameter and length will be in agreement with the molecular weight. It is interesting to note that measurements of optical rotation[8], viscosity, sedimentation and potentiometric titration[9] have indicated the possibility of a high degree of secondary structure of *E. coli* RNA even in solution.

ACKNOWLEDGEMENT

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REFERENCES

[1] H. EISENBERG AND U. Z. LITTAUER, *Bull. Research Council Israel*, 7A (1958) 115.
[2] U. Z. LITTAUER AND H. EISENBERG, *Biochim. Biophys. Acta*, 32 (1959) 320.
[3] H. SHUSTER, G. SCHRAMM AND W. ZILLIG, *Z. Naturforsch., B* 11 (1956) 339.
[4] C. E. HALL, *Proc. IVth International Congress of Biochemistry*, Vol. 9, 1958, p. 90.
[5] C. E. HALL, *Introduction to Electron Microscopy*, McGraw-Hill Book Company, 1953, p. 315.
[6] C. E. HALL, *J. Biophys. Biochem. Cytol.*, 2 (1956) 625.
[7] H. MOOR, *J. Ultrastructure Research*, 2 (1959) 393.
[8] R. A. COX AND U. Z. LITTAUER, *J. Mol. Biol.* in the press.
[9] R. A. COX AND U. Z. LITTAUER, *Nature*, 184 (1959) 818.