A COMPARATIVE ELECTRON MICROSCOPICAL STUDY OF RNA FROM DIFFERENT SOURCES

D. DANON, M.D., Y. MARIKOVSKY, and U. Z. LITTAUER, Ph.D.

From the Weizmann Institute of Science, Rehovoth, Israel

ABSTRACT

Electron micrographs of ribosomal RNA from *Escherichia coli*, microsomal RNA from calf, rat, and chick liver, *Bacillus cereus* RNA and *E. coli* soluble RNA are presented. Filaments of about 10 A in diameter could be observed in preparations obtained from aqueous solutions of high molecular weight RNA. When ammonium acetate solutions were used a tendency for coiling and aggregation was observed. *E. coli* soluble RNA appears as small, sometimes elongated particles the smallest diameter being of about 10 A.

In a previous communication electron micrographs of *E. coli* ribosomal RNA were presented (1). The RNA fibers were described as having a diameter of about 10 A and a variable length of 1500 to 4000 A. These fibers demonstrated a tendency to coil up on themselves and aggregate in the presence of salt. In view of the fact that RNA fibers from other sources were described as having a larger diameter (2, 3), a comparative study of RNA from five different sources was undertaken.

MATERIALS AND METHODS

E. coli RNA was prepared as previously described (4). RNA from *Bacillus cereus* 569/H was prepared by a slight modification of the procedure used for the isolation of *E. coli* RNA. Cells (10 gm.) were frozen with liquid air and ground under liquid air in a mortar; the crushed cells were vigorously stirred with 80 ml. of phenol-water mixture, (1 volume of Tris buffer (0.01 M, pH 7.4) containing EDTA (10^{-4} M) and 1 volume of 90 per cent phenol solution). The suspension was homogenized in an all glass homogenizer and stirred at 20° for 60 minutes. The homogenate was chilled and centrifuged for 3 minutes at 10,000 *g* at 2°. The aqueous phase was removed and allowed to stand. The phenol phase was mixed with 40 ml. Tris-EDTA buffer and, after 5 minutes in the cold, the aqueous phase was separated by centrifugation. The aqueous supernatant solutions containing the RNA were combined and centrifuged for 20 minutes at 10,000 *g*. The RNA solutions were precipitated from the supernatant by addition of 2 volumes of cold 96 per cent ethanol, containing 2 per cent K-acetate (4), washed with 75 per cent ethanol, dialyzed against 10^{-3} M sodium chloride solution and then lyophilized.

Microsomal RNA from calf, rat, and chick liver was extracted from the microsomal fraction with a phenol-water mixture and precipitated with NaCl using a modification of a previously described method (8).

Livers were homogenized in 0.25 M sucrose solution containing 0.005 M $MgCl_2$ and 0.01 M potassium phosphate buffer pH 6.8 and centrifuged for 10 minutes at 10,000 *g*. The supernatant was removed and centrifuged for 45 minutes at 78,000 *g*. The microsomal pellet thus obtained was suspended in 10^{-4} M cold EDTA solution and to this was added an equal volume of 90 per cent freshly redistilled phenol. The mixture was warmed to 20°C., stirred at this temperature for 60 minutes, and then centrifuged at 10,000 *g* for 3 minutes at 0°C. The RNA was isolated from the top aqueous phase by NaCl (1 M) precipitation (details of the technique to be published (5)).

Soluble RNA from *Escherichia coli* was prepared as described elsewhere (6). A suspension of "protoplasts" was extracted with a phenol-water mixture and precipitated with ethanol. The dissolved precipitate contained both the soluble and ribosomal RNA; the latter was separated from the soluble RNA by ammonium sulfate precipitation. For this purpose, the RNA (300 mg.) was dissolved in 25 ml. of Tris buffer (0.01 M, pH 7.4) and small amounts of insoluble

matter were removed by centrifugation for 20 minutes at 10,000 *g* and discarded. To the clear solution $(NH_4)_2SO_4$ (0.365 g/ml.) was added. After 30 minutes at 4° the solution was centrifuged for 10 minutes at 10,000 *g*. The supernatant, which contained the soluble RNA, was dialyzed 36 hours against four changes of NaCl (10^{-3} M) and then lyophilized.

Sedimentation in the Ultracentrifuge: Sedimentation analyses were made in a Spinco model E ultracentrifuge with phase-plate schlieren optics. Sedimentation studies were performed on 0.3 to 0.5 per cent solutions of RNA in 0.2 M NaCl, at room temperature and corrected to 20°.

Calf, rat, and chick liver microsomal RNA each revealed two major boundaries in the ultracentrifuge. The sedimentation constants (S) were: 17, 28; 18, 26; and 19, 24 respectively. *E. coli* ribosomal RNA gave two boundaries (S = 16.5 and 23.7) while soluble RNA gave one boundary (S = 4.1). *B. cereus* RNA revealed four boundaries, S = 4, 8, 12, and 22.

Electron Microscopy: A modification of Hall's method for visualizing macromolecules (7) imposed by the quality of the available mica was used as described previously (1).

Freshly cleaved mica rectangles were dipped in a 0.5 per cent parlodion (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. Solutions were sprayed as fine droplets onto the film covering the grids on the slide, using a low pressure gun. All the samples of RNA (2 ml.) were centrifuged for 20 minutes at 105,000 *g*.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; TMV, tobacco mosaic virus; Tris, tris(hydroxymethyl)aminomethane.

The upper portion (1.5 ml.) was removed and used for spraying on the grids.

The air dried preparations were shadow-cast with platinum at shadow-to-height ratios of 8 to 1. The films were backed with a thin supporting layer of SiO (7). An aqueous suspension of polystyrene spheres of 0.34 μ diameter was added to the solution, to aid in the location of the microdroplets and in the exact determination of the shadow-casting angle and the direction of shadow. Measurements of the length of the shadow were made on filaments perpendicular to the direction of the shadow and in close vicinity to one of the latex spheres. In rare cases when the film was stripped successfully following exactly the method of Hall (7), similar results were obtained.

RCA EMU-2A with an improved home-made specimen stage, 25 μ objective aperture, and a back focal plane projector aperture, was used.

RESULTS AND DISCUSSION

Calf Liver Microsomal RNA: Appears rarely as a single long fiber; it has a strong tendency for lateral aggregation and is sometimes coiled up as irregular grains (Fig. 1). The diameter of the single fiber is about 10 A which is similar to that of *E. coli ribosomal RNA* (Fig. 7). However, in regions of aggregation larger diameters were observed. In *rat liver microsomal RNA* the single fibers are more frequent, presenting here and there a thicker diameter as if they were coiled up on themselves (Fig. 3). *The chick liver microsomal RNA* shows a particular form of fibers frequently attached to a granule at one end, as if the fiber was drawn out of a granular aggregation. The fiber is thinner as it goes away from the granular body (Fig. 6). The *B. cereus RNA* revealed numerous long fibers of about 10 A in diameter (Fig. 5).

Explanation of Figures

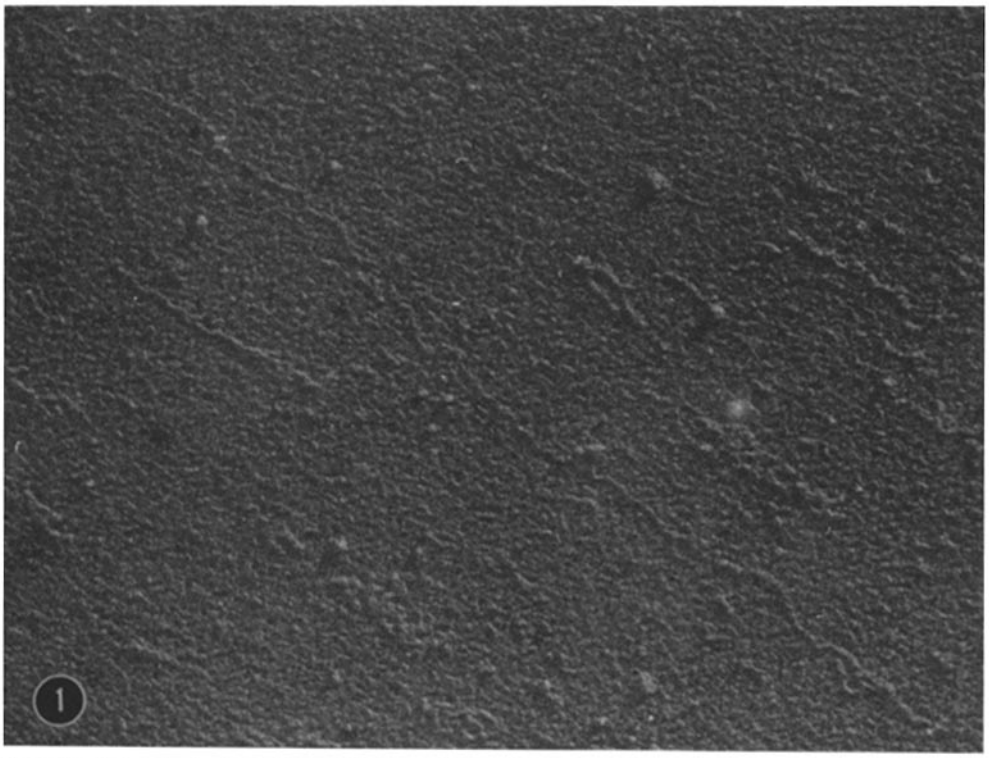
All the preparations were sprayed from a low pressure gun and air dried at room temperature. Shadow-cast with Pt at a shadow-to-height ratio of 8 to 1. Electron micrographs were taken at an electronic magnification of 20,000 and photographically magnified to 100,000.

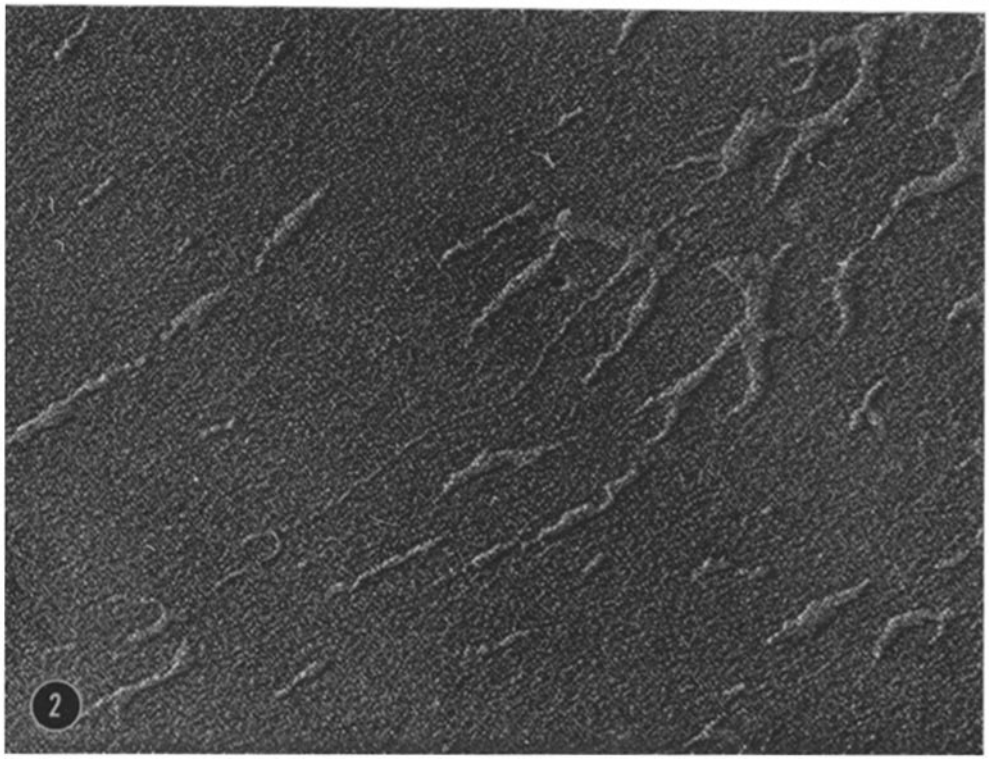
FIGURE 1

Calf liver microsomal RNA sprayed from a water solution.

FIGURE 2

Calf liver microsomal RNA sprayed from 0.1 M ammonium acetate solution.


1


2

Soluble RNA from E. coli appears as very small, sometimes elongated particles or grains, longitudinally and laterally aggregated; the smallest diameter observed was about 10 A (Fig. 8). In view of the low molecular weight of this material (about 30,000) it is difficult to state if the elongated particles are single molecules or a longitudinal aggregation of smaller subunits.

The length of the fibers of all the high molecular weight RNA preparations, is much more variable than would have been expected from the sedimentation data. This variability in length of the fibers was observed in RNA preparations of various sources and to a lesser degree in different samples of the same source. This variability seems to be due to a certain amount of longitudinal aggregation, which makes the measurements of the length of the molecule a very approximate one. The fact that the sedimentation constants did not vary to such an extent might suggest that, during the preparation of the electron microscopical specimens, the various RNA preparations were differently affected by the spraying and the drying of the micro droplets, thus showing different degrees and forms of aggregation as well as coiling. The effect of the spraying on the *E. coli* RNA was demonstrated in a previous study (1). Although the sedimentation constants of the calf liver RNA present the highest values, the length distribution of the fibers is considerably lower as compared to the other RNA preparations.

Effect of Salt: Hydrodynamic measurements of *E. coli* ribosomal RNA (4), rat and calf liver microsomal RNA (5, 8) have indicated that RNA molecules fold up into more compact structures when salt is added to the solution. These findings were recently supported by an electron microscopical study of *E. coli* ribosomal RNA, where it was shown that when RNA was deposited from ammonium acetate solutions, granules and various forms of aggregates dominated the picture while individual threads became rare. On the other hand RNA deposited from aqueous solutions showed this kind of aggregation to a much lesser degree, and single strands were observed. The effect of salt on the appearance of rat and calf liver microsomal RNA was therefore studied. With both RNA samples it was observed that the addition of ammonium acetate resulted in a strong tendency of the fibers to aggregate (Figs. 2 and 4), usually laterally, causing the appearance of relatively thick threads, whereas in *E. coli* ribosomal RNA granular forms of aggregation were more frequent. These findings are in accordance with the hydrodynamic measurements and support the idea that both rat and calf liver RNA are molecules capable of coiling. The fact that some RNA threads appear to be coiled up upon themselves and aggregated even when deposited from aqueous solutions (containing less than 10^{-4} M salt), might be explained by the considerable increase of the local salt concentration during the drying of the micro droplets, occasionally reaching sufficiently high values (greater than 10^{-2} M) to cause the observed effect. The present study with calf liver RNA might explain the observations made by Hall (3) who, using ammonium acetate solutions, reported relatively short threads of an apparent thickness of about 30 A. The use of a different method for the preparation of the RNA might also contribute to the morphological difference.

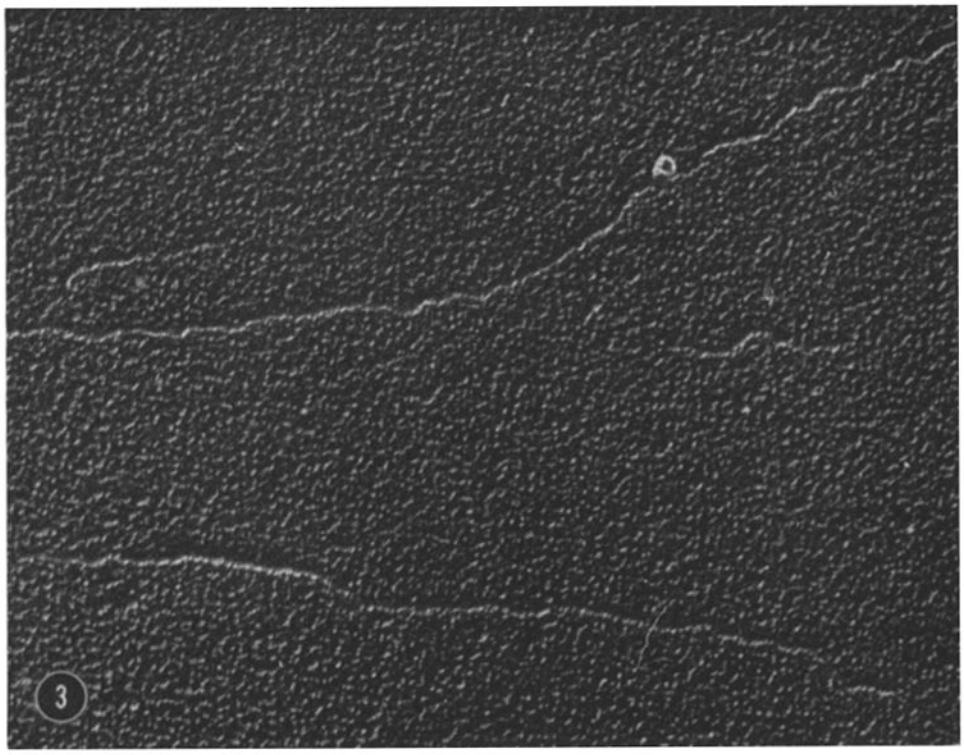
Hart (9) observed that RNA fibers which were attached to the rod end of TMV, were extended when sprayed from a salt solution. However, as stated by him the RNA fibers "are resolvable in the micrographs only because of adhering contaminant material (perhaps detergent or denatured protein)." It will be hard to evaluate the effect of the presence or absence of salt on the association of contaminants with RNA molecules.

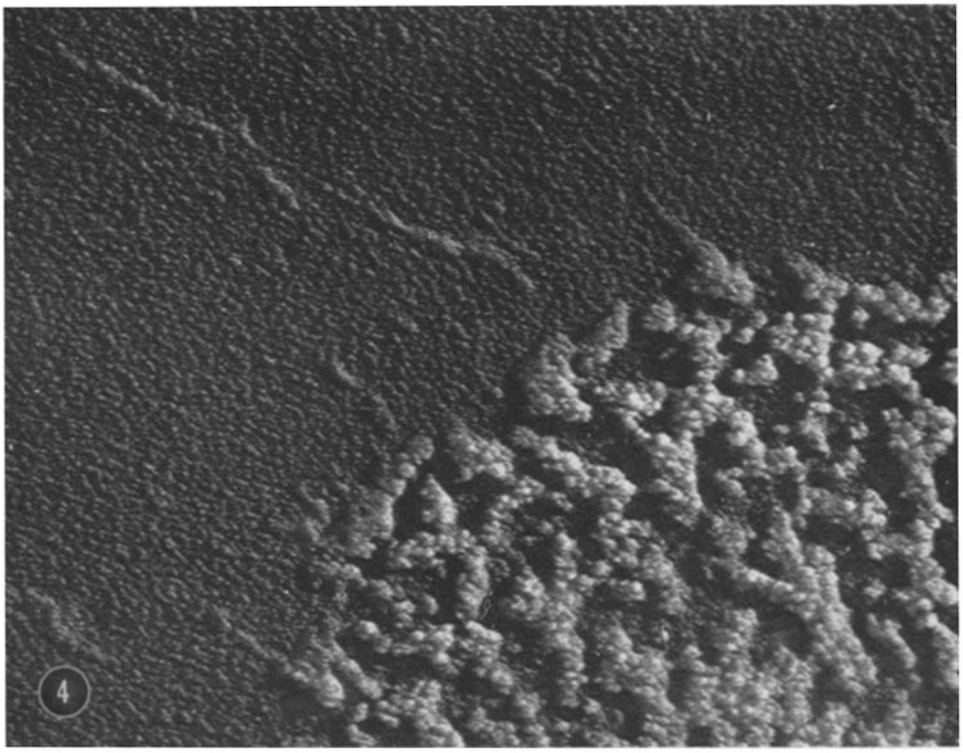
FIGURE 3

Rat liver microsomal RNA sprayed from a water solution.

FIGURE 4

Rat liver microsomal RNA sprayed from 0.1 M ammonium acetate solution.


3


4

All the RNA fibers studied present a diameter of about 10 A, which is similar to that found for *E. coli* ribosomal RNA and is about half the diameter reported for DNA (20 A). The RNA fibers are wavy and granular in appearance as compared to the smooth and rigid DNA strands (1).

The authors are indebted to Dr. M. R. Pollock for providing us with *Bacillus cereus* 569/H.

The authors wish to thank D. Givon for his technical assistance.

This investigation was supported in part by a United States Public Health Service grant RG-5217.

Received for publication, July 30, 1960.

REFERENCES

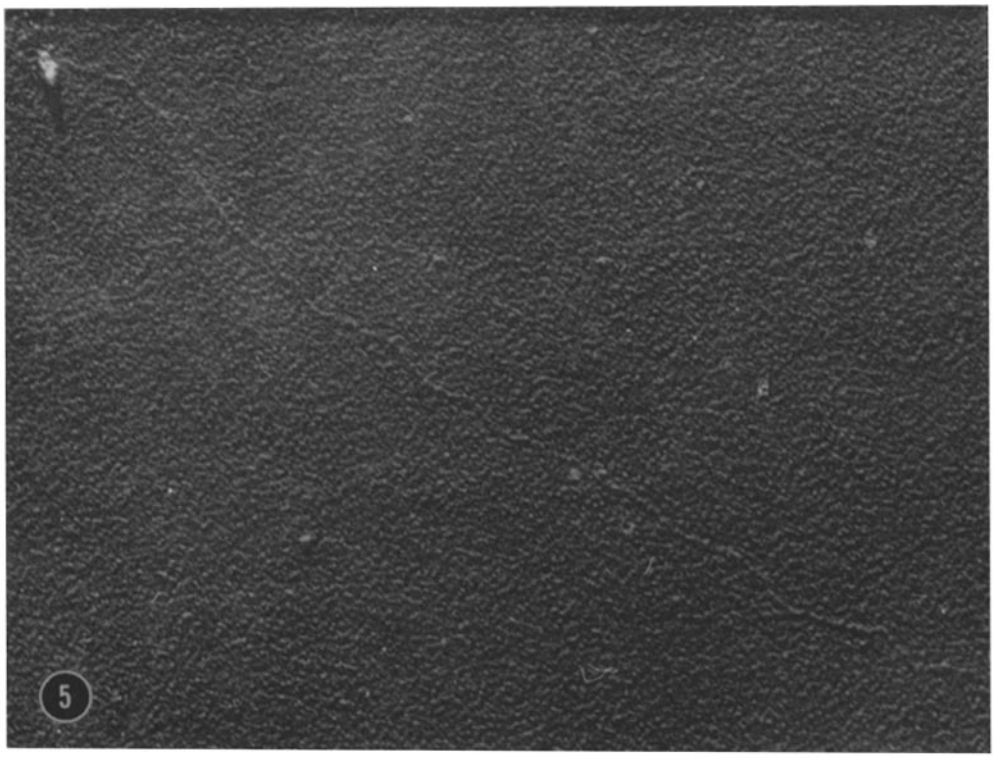
1. LITTAUER, U. Z., DANON, D., and MARIKOVSKY, Y., *Biochim. et Biophysica Acta*, 1960, **42**, 435.
2. SHUSTER, H., SCHRAMM, G., and ZILLIG, W., *Z. Natürforsch.*, 1956, **B11**, 339.
3. HALL, C. E., *Proc. IVth Intern. Cong. Biochem.*, 1958, **9**, 90.
4. LITTAUER, U. Z., and EISENBERG, H., *Biochim. et Biophysica Acta*, 1959, **32**, 320.
5. LITTAUER, U. Z., data to be published.
6. COX, R. A., and LITTAUER, U. Z., *J. Molec. Biol.*, 1960, **2**, 166.
7. HALL, C. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 625.
8. LASKOV, R., MARGOLIASH, E., LITTAUER, U. Z., and EISENBERG, H., *Biochim. et Biophysica Acta*, 1959, **33**, 247.
9. HART, G. R., *Biochim. et Biophysica Acta*, 1958, **28**, 457.

FIGURE 5

Bacillus cereus RNA sprayed from a water solution.

FIGURE 6

Chick liver microsomal RNA sprayed from a water solution.


5

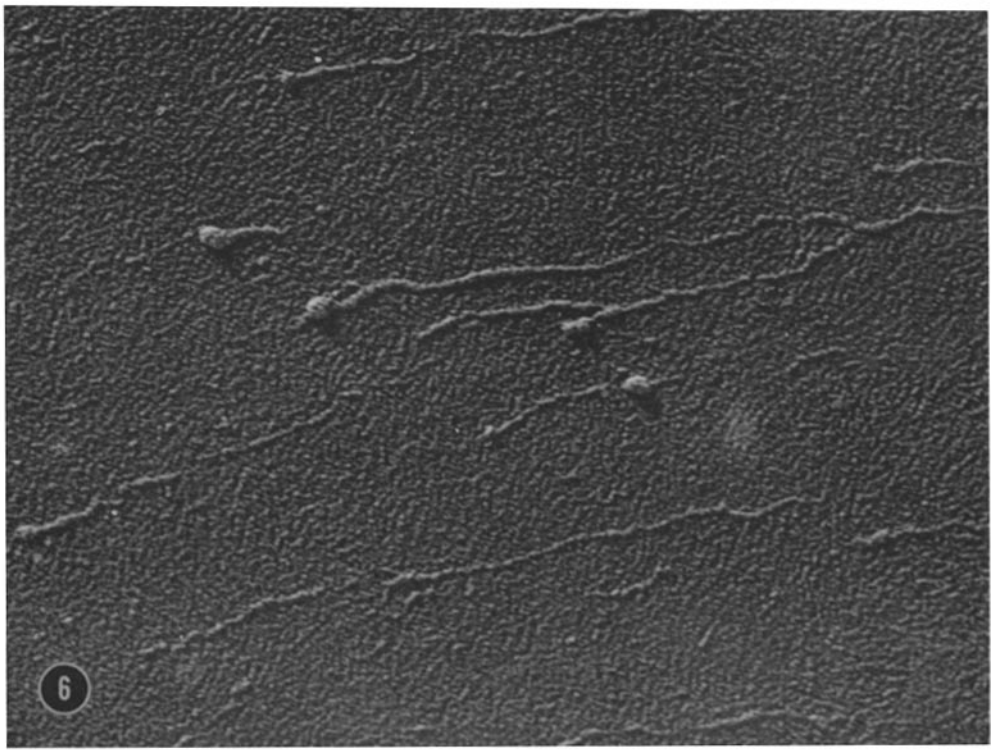
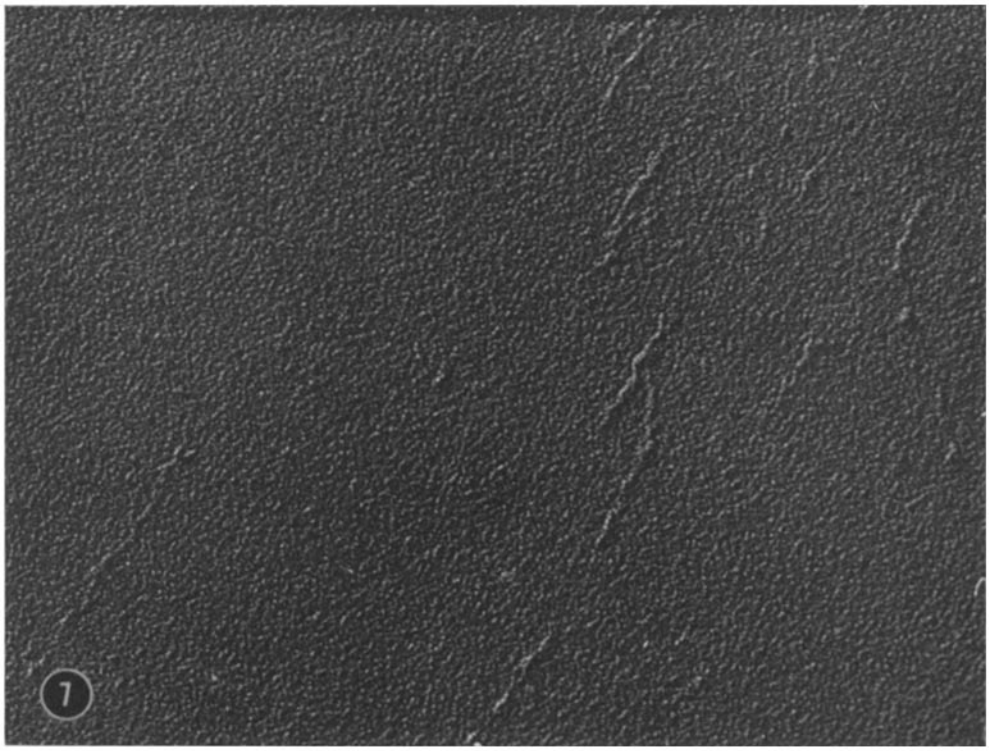

6

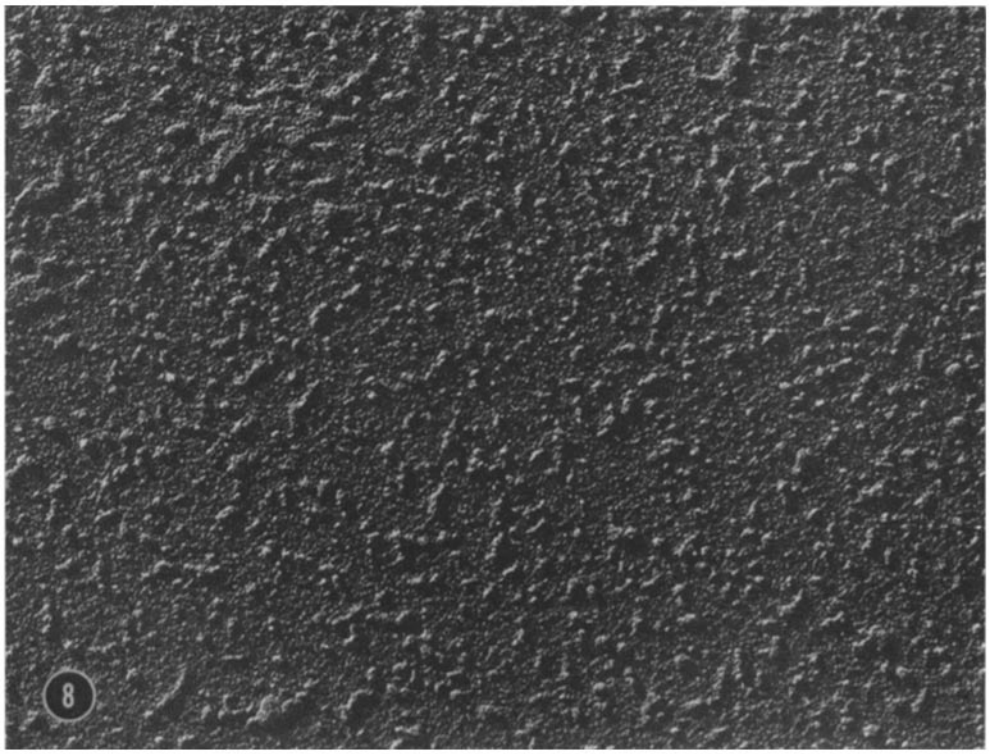
FIGURE 7

E. coli ribosomal RNA sprayed from a water solution.

FIGURE 8

E. coli soluble RNA sprayed from a water solution.


7


8