Morphological Features of DNA Macromolecules as Seen with the Electron Microscope

By CECIL E. HALL, PH.D., AND MICHAEL LITT*

(From the Department of Biology, Massachusetts Institute of Technology, and the Department of Chemistry, Harvard University, Cambridge)

PLATES 1 AND 2

(Received for publication, September 20, 1957)

ABSTRACT

Desoxyribosenucleic acid molecules isolated from salmon sperm were studied with the electron microscope. The essential step in the technique which makes it possible to visualize the individual molecules consists in a preparative step wherein the materials are supported on the extremely smooth surface of cleaved mica where they are shadow-cast with platinum, which is then backed with a supporting film and stripped for observation in the usual manner. The DNA, which was originally about 8 million molecular weight, was also examined after fragmentation by sonic vibration. The fragments show a certain degree of rigidity and the ends generally terminate abruptly, indicating that the double helices of the Watson-Crick model both break close to the same place. DNA molecules heated to temperatures between 90 and 100°C. coil up into amorphous patches, although a few apparently unaltered molecules survive such heating.

In the interval since the first electron micrographs were obtained (1, 2) showing strands of individual DNA molecules, we have made further observations with improved methods and materials and wish to report here the principal morphological features of such molecules as we have observed them with the electron microscope. In the course of this work fair quantitative correlations have been obtained between the lengths of these and other macromolecules as obtained from electron microscopy and from physicochemical methods, which results are being reported in more detail elsewhere (3).

Materials and Methods

Samples were prepared of salmon sperm DNA by first isolating the sperm heads and then using the Simmons procedure for removal of the proteins.[1] The sperm heads are isolated from frozen salmon testes by repeated homogenization and washing with cold 0.15 M NaCl, 0.015 M Na-citrate, followed by repeated washing with distilled water. After each distilled water rinse, the sperm heads were collected by means of a Sharples supercentrifuge. The DNA is released from the sperm heads by addition of 5 volumes of a 37.5 per cent (weight/volume) solution of sodium-xylene sulfonate to the well stirred suspension of sperm heads in 0.15 M NaCl, 0.015 Na-citrate. Reduction of the xylene sulfonate concentration to 10 per cent, by dilution and adjustment of the pH to 4.3, results in quantitative precipitation of the protamines and other proteins in the cationic state. Proteins are completely removed by filtration with the use of graded celites. DNA is precipitated with isopropyl alcohol, redissolved in 0.03 M Na-acetate at pH 7, and reprecipitated with 0.54 volumes of isopropyl alcohol in the presence of 0.03 Na-acetate at pH 7. Sodium acetate is removed with 95 per cent ethanol. From physicochemical studies the weight average molecular weight in solution is calculated to be 8 million.

The DNA for electron microscopy was suspended in a buffer containing 0.05 M ammonium carbonate and 0.1 M ammonium acetate. Some salt is necessary to prevent denaturation, and this pair of volatile salts was chosen since they give better results than either used alone. This is presumably due to the maintenance of a more nearly neutral pH during evaporation. Sonically degraded DNA produced by exposure to frequencies of 5 or 9 kc. as described by

* Predoctoral Fellow of the National Cancer Institute.

[1] Personal communication from Dr. N. S. Simmons, Atomic Energy Project, University of California at Los Angeles.

Bunce, Doty, and Rice (4) were also examined. One such sample was reduced to a molecular weight of about 1 million, and another to about 0.25 million. Samples of denatured DNA were prepared by heating solutions at a concentration of 4 mg./ml. in 0.15 M NaCl, 0.015 M sodium citrate for 15 minutes at temperatures between 90 and 100°C. This treatment has been shown by physicochemical methods to cause a collapse of the DNA molecules at constant molecular weight (5). Samples were kept cold and examined with the electron microscope soon after they had been prepared and studied by physicochemical methods.

Specimens for electron microscopy were prepared essentially as previously described (1, 2). DNA in a concentration of about 0.002 per cent, to which polystyrene latex had been added, was kept cold, but sprayed at room temperature from either a high pressure spray gun (6) or a low pressure throat nebulizer onto the surface of freshly cleaved mica.[2] In most cases the surface was shadowed with Pt (2.5 cm. of 0.1 mm. wire) at a distance of 7 cm. and a shadow-to-height ratio of 10:1. Micrographs were recorded at a magnification of about 15,000 with an RCA type EMU3 microscope.

OBSERVATIONS

Soon after the first micrographs of DNA (from calf thymus) were reported, it became evident that by the time the materials were examined by electron microscopy they had become partially denatured. In these first micrographs one almost never saw a free end of the 20 A thick molecule, though there were many long segments, with the expected diameter of the double helix, passing between polystyrene spheres or terminating in flat amorphous patches. These patches we now feel reasonably sure represent denatured DNA in which the double helix has become dissociated so that the flexible chains have merged into a somewhat fluid state.

In contrast to the partially degraded morphology shown in earlier DNA micrographs, the DNA shown in Fig. 1 displays numerous free ends, indicating an integrity of the double helical structure out to the tip. Furthermore, where the concentration is high, as it is in Fig. 1, the individual molecular units can easily be discerned in the tangled areas in contrast to the structureless patches occurring in partially denatured material.

In Fig. 2 are shown segments of two well defined DNA molecules on a cleaner and less crowded area. Under these circumstances the molecules lie relatively straight for long distances and where they come together, they can be resolved sufficiently well that it may be seen that the fork is due to two separate molecules, rather than to a dissociation of the double helical structure. With the exception of rare instances noted below, it appears that when the double helical structure dissociates, the ends coil up rather than fraying out as two well defined strands.

In Fig. 3 are shown two DNA molecules from the same material as for Figs. 1 and 2 except that in Fig. 3 the lengths are very much shorter than the average. The chief feature of interest in these particles are the nodules, which we interpret as the result of partial denaturation at the ends. This feature appears at both ends of the molecule on the left, but only on the right end of the one on the right. Nodules of this sort nearly always appear at the ends of molecules and rarely in the middle, which probably indicates that dissociation usually starts at the ends and works inward along the length.

In Figs. 4 and 5 are shown segments of DNA which has been broken down by sonic vibrations to an average molecular weight of about 1 million. The weight average from physicochemical data is 970,000 for this sample, and the corresponding average from electron microscopy is 700,000 on the basis of 200 units per A. When the particles are lying free of one another, they are generally quite straight as in Fig. 5, but may be sinuous when crowded or associated with debris as in Fig. 4. Similar differences are noted between Fig. 1 and Figs. 2 and 3. Ends of fragmented molecules are broken squarely and generally do not show the nodules that are associated with partial denaturation. We have looked in these micrographs for evidence of the double nature of the DNA helical structures at broken ends, and although features which could be interpreted thus are very scarce, there are occasional particles which show an abrupt decrease to a smaller diameter near the end, and others where two apparently smaller threads project from the end. It appears that when one of the helices breaks, the other one usually breaks close to the same place.

Preparations of DNA denatured by heat have also been examined. These show patches of particulate and amorphous-appearing material and also intact threads that have survived, as shown in

[2] A-1 clear India uncut mica, Huse-Liberty Mica Company, Peabody, Massachusetts.

Figs. 6 and 7. The electron microscopy methods used up to this point have not been capable of giving a statistically significant estimate of the relative amounts of denatured material present in such preparations. Intact threads are considerably more scare than might be construed from relative amounts in Figs. 6 and 7. That few molecules are observed which appear to combine sequences of native and denatured material suggests that heat denaturation of the single DNA molecule is an "all or none" phenomenon.

The ultimate fine structures observed in micrographs made by this method may be: (1) real structure of the macromolecules under study, (2) overlying extraneous particulate materials or impurities, or (3) granularity of the metal or supporting film. It is not always easy or possible to distinguish between these possibilities, but it is a help if the edge of a drop pattern can be located to provide a comparison with the clean mica surface. The background between DNA molecules in many of the micrographs is exceptionally clean. In these instances the DNA molecules are very smooth, as would be expected from the Watson-Crick model. There is visible, however, an ultimate quasi-periodic structure which we interpret to be a graininess artifact, since it bears no obvious relation to the known structure of DNA.

Ultimate granularity due to metal and supporting films is somewhat variable from one preparation to another and very probably limits the dimensions of intramolecular structure which can be seen at present to a value which is considerably larger than the smallest dimensions of isolated particles which can be detected by the method. Apparent granularity is exaggerated at underfocused settings. Fig. 5, for example, which even in the original shows vanishingly small graininess of background, was recorded very close to focus. Fig. 6, on the other hand, is appreciably underfocused. The ability of the photographic emulsion to resolve the ultimate metallic structure can also have a bearing on the subjective evaluation of micrographs. For example, plates recorded at × 30,000 in which the plate grain can resolve very small particles may present an unpleasing granular background as compared with a similar field recorded at, say, × 10,000 and viewed at the same print magnification. Resolution of ultimate graininess which does not add to the pertinent information to be seen in an image is analogous to the effect produced when a rather coarse halftone reproduction is viewed with a magnifying glass.

SUMMARY

1. Improved electron micrographs have been obtained of DNA extracted from salmon sperm. The fresh material consists of well defined macromolecules having a diameter of about 20 A which is indistinguishable from that of DNA from calf thymus, and is consistent with the diameter deduced from x-rays.

2. DNA fragmented by sonic irradiation down to a molecular weight of about 1 million is also shown. Ends of broken fragments are clean and mostly square, indicating that when one of the double helices breaks, the other one breaks close to the same place. Observed lengths are in reasonable agreement with physicochemical results on the same materials.

3. Undegraded DNA manifests a degree of stiffness, but when denatured, the helices apparently dissociate and coil up into amorphous patches. Denaturation by heat with temperatures between 90 and 100°C. produces such effects, with the dissociation apparently most readily proceeding from the ends inward. At those temperatures some apparently intact molecules survive.

The investigation was supported in part by a research grant C-2171 (C5) from the National Cancer Institute of the National Institutes of Health, Public Health Service. We wish to acknowledge the helpful discussions contributed by Prof. Paul Doty and the technical assistance of Mr. A. Q. Dickson and Mr. H. Slayter in the electron microscopical part of the work.

REFERENCES

1. Hall, C. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 625.
2. Hall, C. E., *Proc. Nat. Acad. Sc.*, 1956, **42**, 801.
3. Hall, C. E., and Doty, P., *J. Am. Chem. Soc.*, 1957, to be published.
4. Bunce, B. H., Doty, P., and Rice, S. A., *J. Am. Chem. Soc.*, 1957, to be published.
5. Rice, S., and Doty, P., *J. Am. Chem. Soc.*, 1957, **79**, 3937.
6. Backus, R. C., and Williams, R. C., *J. Appl. Physics*, 1950, **21**, 11.

Explanation of Plates

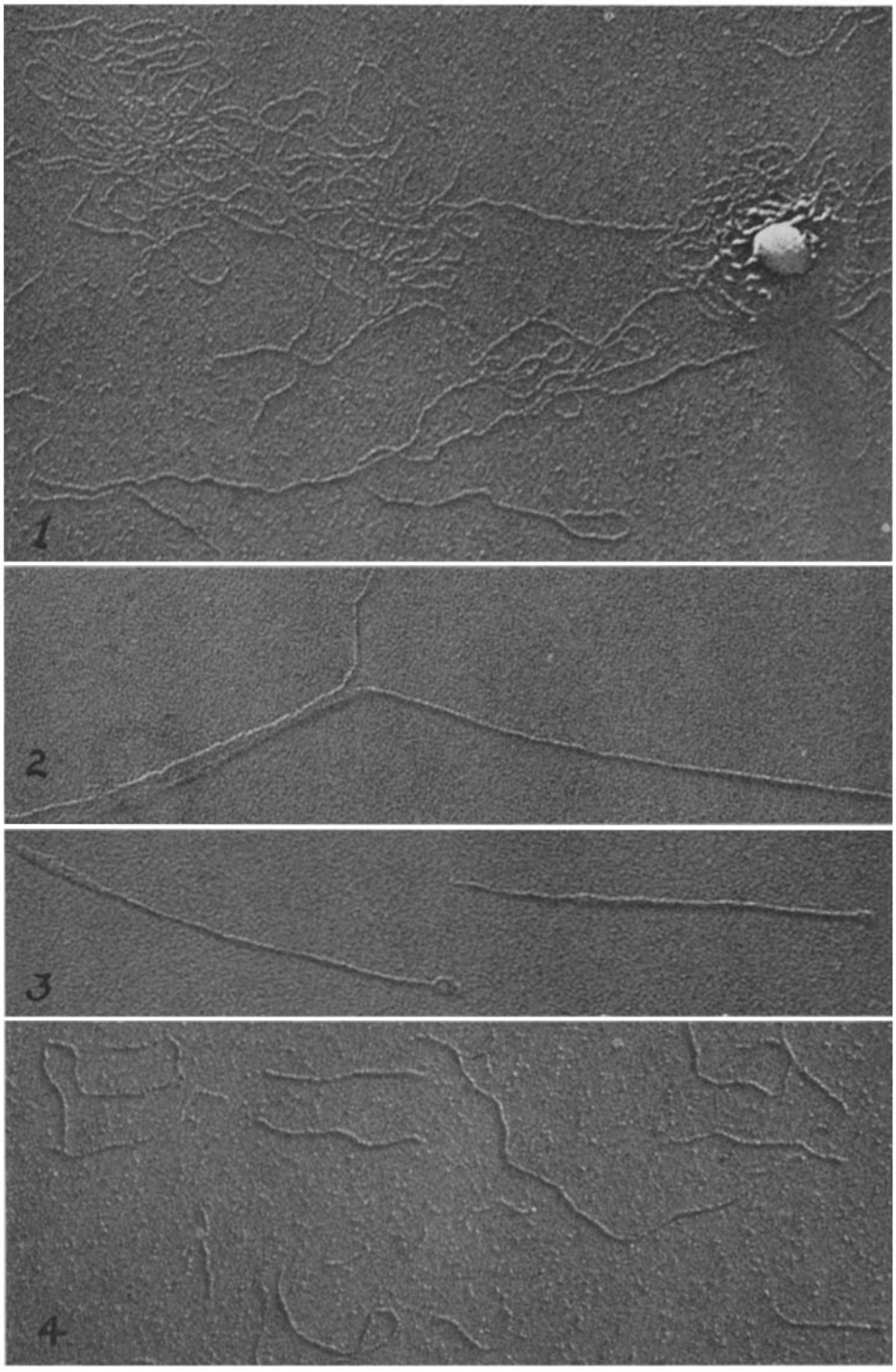
Plate 1

Fig. 1. DNA from salmon sperm in an area of relatively high concentration. Specimens for this and subsequent micrographs are from salmon sperm, were shadow cast at an angle of 10:1, and are reproduced at a magnification of about 100,000.

Fig. 2. Portions of two branching DNA molecules in a relatively clean area.

Fig. 3. Two DNA molecules showing thickening at the ends associated with partial denaturation.

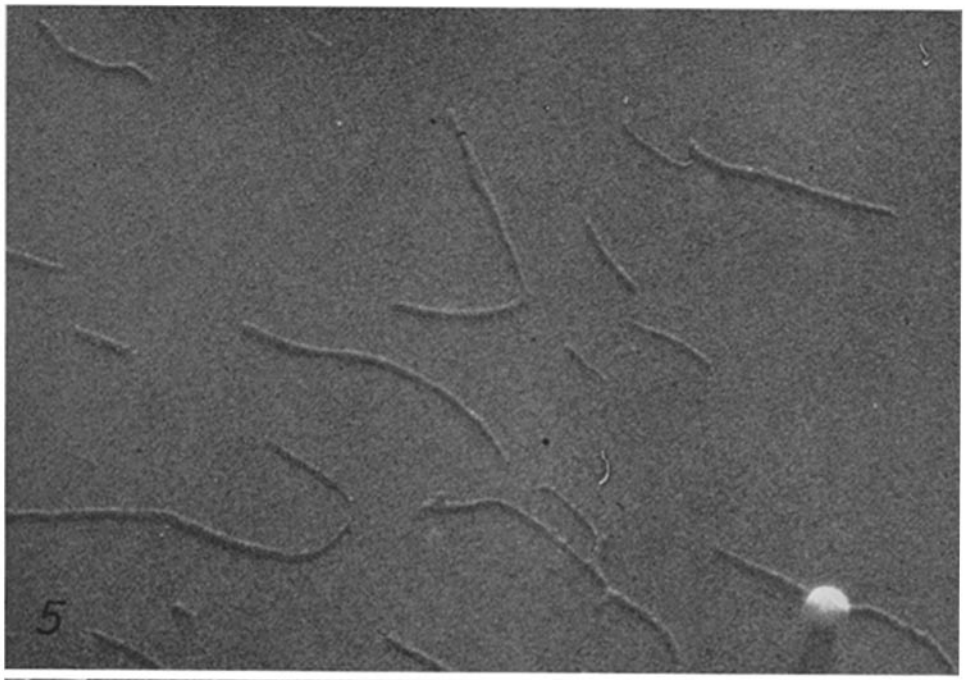
Fig. 4. DNA after fragmentation by sonic irradiation to a reduced molecular weight of about 1 million.

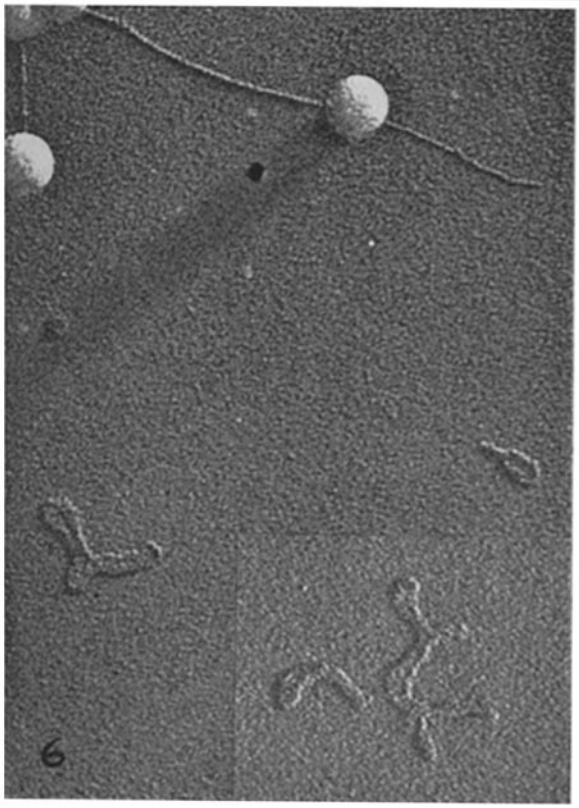


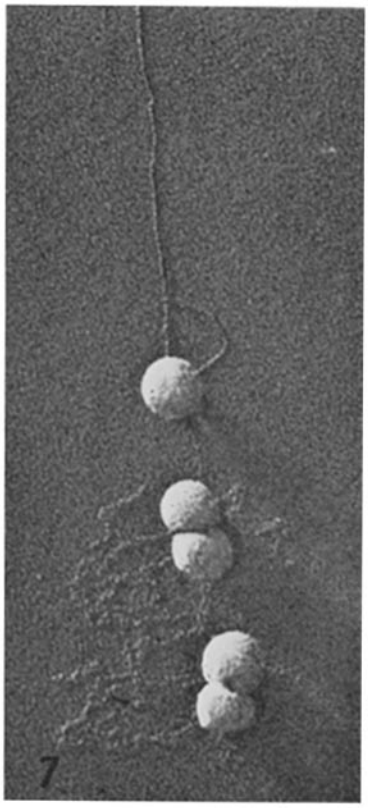
(Hall and Litt: Morphology of DNA macromolecules)

PLATE 2

FIG. 5. DNA treated as for Fig. 3, but located on a relatively clean surface.
FIG. 6. Typical appearances of DNA after heating to 94°C. for 30 minutes.
FIG. 7. Typical appearances of DNA after heating to 100°C. for 15 minutes.







(Hall and Litt: Morphology of DNA macromolecules)