

THE ELECTRON MICROSCOPY OF SODIUM DESOXYRIBONUCLEATE

by

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INTRODUCTION

The molecular characteristics of sodium desoxyribonucleate have been studied by a variety of methods¹⁻⁴. These studies have led to the conclusion that the shape of the elementary particle is a fiber of diameter 13–20 Å and a length of 4,000–7,000 Å, the length and molecular weight depending upon the method of preparation. It seems obvious that the diameter of the elementary fiber should be a constant irrespective of preparative treatment.

SCOTT⁵ examined nucleate obtaining photographs of fibers for which no diameter was given. NIXON AND OSTER⁶ reported measurements on bundles of elementary fibers 50 to 60 Å in diameter. BAYLEY⁷ obtained photos of material which formed spherical shaped bodies which were thought to be coiled up globules of nucleate. Our experiments on the other hand indicate a reasonable agreement between the diameter as obtained by electron microscopy and by indirect methods.**

MATERIALS AND METHODS

Three samples of nucleate prepared from calf thymus were studied; 1. A preparation using the GULLAND⁸ method by R. STEINER AND J. ROWEN; 2. A preparation using a modified HAMMERSTEN method⁹ by J. P. GREENSTEIN AND M. HOYER; 3. A sample prepared by N. SIMMONS, S. CHAVAS AND H. K. ORBACH using their method¹⁰.

Nucleo-protein, obtained as a first step in preparation (1) above was available and the sample was studied for comparison with the nucleate.

Dilute solutions made up in distilled water were either sprayed on to formvar coated metal screens or added to the coated screens in fine drops from a glass capillary. The specimen was then shadowed with a light coat of chromium, platinum or uranium ($\tan \alpha = 0.2$) and examined in the electron microscope at 23,000 ×. The microscope was fitted with an objective lens compensated for astigmatism.

RESULTS

Photographs of the fibers of nucleate are shown in Fig. 1, 2, 3. Since the thickness of accumulated metal on the particle is greater than the particle diameter there is little value in measuring the diameter of the coated particle. On the other hand, shadow length measurements and the deduction of the height of the fiber therefrom is a fairly reliable procedure. The height is $2R = \frac{2A}{M} \frac{\tan \alpha/2}{(1 - \tan \alpha/2)}^{11}$ where A is the shadow length from

* National Institutes of Health, U.S. Public Health Service Federal Security Agency.

** After this paper was sent to the publishers R. C. WILLIAMS, *Biochim. Biophys. Acta*, 9 (1952) 237, reported a diameter of 15 Å.

References p. 359.



Fig. 1. Photograph of a sprayed nucleate solution. Chromium shadowed. Preparation 2.

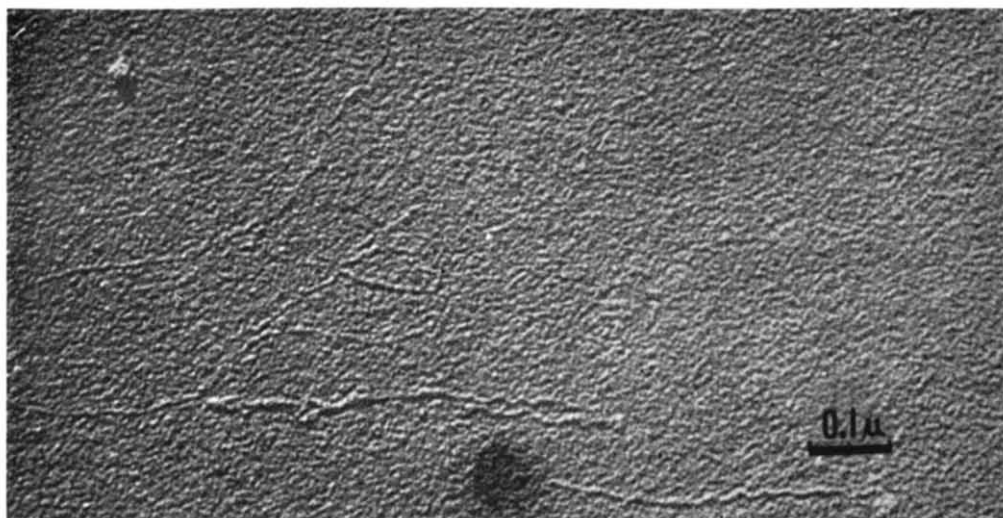


Fig. 2. Nucleate shadowed with chromium. Preparation 3.



Fig. 3. Fibers and globules of nucleate. Platinum shadowed. Preparation 3.

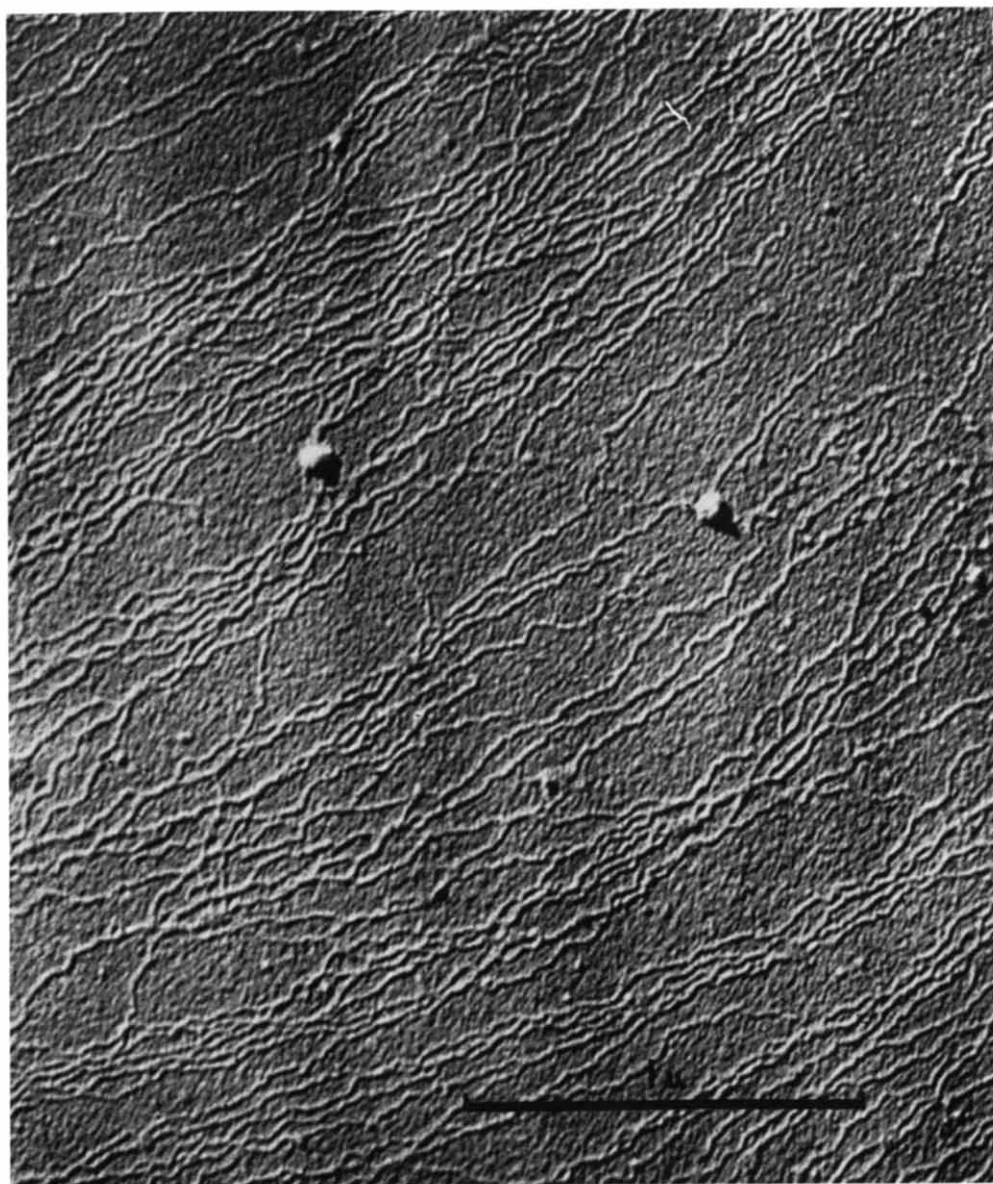


Fig. 4. Nucleo-Protein. Chromium shadowed.

the shadowed edge of the particle, M is the magnification and α is the shadowing angle. Such measurements gave a value 15 ± 5 Å agreeing with the results of other indirect methods. No significant difference could be detected between the three preparations studied. Frequently globules were seen similar to those described by BAYLEY. However, the impression is given from these and a large number of other photographs that the nucleate fibers are intermediate between a stiff rod and randomly coiled fibers. This agrees with the results of light-scattering studies^{12,13}.

A photograph of calf thymus nucleo-protein is shown in Fig. 4. These fibers appear uniform in diameter and appear somewhat larger than the nucleate fibers. The height of the fibers shown as calculated from the shadow length was 25 Å, this value being smaller than the value given by CARTER¹⁴. A difficulty in the interpretation of such photographs is in the question of the dissociation of the nucleate from the protein in the drying process.

SUMMARY

Electron micrographs of sodium desoxyribonucleate have been obtained from which a diameter of the fiber of 15 ± 5 Å has been deduced. Similar measurements on nucleo-protein from calf thymus were made from which a diameter of 25 Å was deduced. The nucleate fibers are intermediate in shape between a random coil and a stiff rod.

RÉSUMÉ

Nous avons obtenu, à l'aide du microscope électronique, des photographies de désoxyriboncléate de sodium et nous en avons déduit la valeur de 15 ± 5 Å pour le diamètre des fibres. Des mesures semblables, faites avec de la nucléoprotéine de thymus de veau, nous ont donné un diamètre de 25 Å. La forme des fibres de nucléate est intermédiaire entre celle d'une bobine irrégulière et d'un bâtonnet rigide.

ZUSAMMENFASSUNG

Es wurden Elektronenmikroskopaufnahmen von Natriumdesoxyribonukleat erhalten, aus denen ein Faserdurchmesser von 15 ± 5 Å abgeleitet worden ist. Ähnliche Messungen wurden mit dem Nukleoprotein aus Kalbsthymus durchgeführt, für das ein Durchmesser von 25 Å abgeleitet wurde. Die Form der Nukleatfasern liegt zwischen der einer unregelmässigen Spule und eines steifen Stäbchens.

REFERENCES

- ¹ R. CECIL AND G. OGSTEN, *J. Chem. Soc.*, (1948) 1382.
- ² H. KAHLER, *J. Phys. Colloid Chem.*, 52 (1948) 676.
- ³ W. T. ASTBURY, *Symp. Soc. Exptl Biol.*, 12 (1947) 66.
- ⁴ D. P. RILEY AND G. OSTER, *Biochim. Biophys. Acta*, 7 (1951) 526.
- ⁵ J. F. SCOTT, *Biochim. Biophys. Acta*, 2 (1948) 1.
- ⁶ Quoted by G. OSTER, *Trans. Faraday Soc.*, 46 (1950) 794.
- ⁷ S. T. BAYLEY, *Nature*, 168 (1951) 470.
- ⁸ J. M. GULLAND, D. O. JORDAN AND C. J. THRELFALL, *J. Chem. Soc.*, (1947) 1129.
- ⁹ B. TAYLOR, J. P. GREENSTEIN AND A. HOLLAENDER, *Arch. Biochem.*, 16 (1948) 19.
- ¹⁰ N. S. SIMMONS, S. A. CHAVOS AND H. K. ORBACH, *Federation Proc.*, 11 (1952) 390.
- ¹¹ H. KAHLER AND B. J. LLOYD, JR., *J. Appl. Phys.*, 21 (1950) 699.
- ¹² S. KATZ, *J. Am. Chem. Soc.*, 74 (1952) 2238.
- ¹³ J. ROWEN, M. EDEN AND H. KAHLER, *Biochim. Biophys. Acta*, In Press.
- ¹⁴ R. O. CARTER, *J. Am. Chem. Soc.*, 63 (1941) 1960.

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