

Helical Structures in the Nucleus of *Amoeba proteus*. BY GEORGE D. PAPPAS.*
(From the Department of Anatomy, New York University-Bellevue Medical Center,
New York.)†

While *Amoeba proteus* has long been the subject of extensive cytological and cytochemical studies, investigations with the electron microscope have been limited principally to the nuclear membrane (1-3). The observations reported herein were made in the course of studies intended to correlate cytochemistry (4) with knowledge of fine structure obtained with the electron microscope. It is felt that the unique character of these observations justifies this brief note.

The amebas were first washed free of other protozoa, and then fixed with 1 per cent OsO₄ in a veronal-acetate buffer, pH 8.6, containing 0.01 per cent CaCl₂. Fixation time varied from 10 to 20 minutes. After dehydration and embedding in *n*-butyl methacrylate, thin sections were cut and subsequently studied in the electron microscope (RCA, EMU-2E).

The characteristic nuclear structure is seen in Fig. 1. Just within the unusually thick and complex nuclear membrane (NM) are numerous dense bodies (D) which range from 0.4 to 1.7 μ in size. Such bodies, as described by Chalkley (5), stain intensely with hematoxylin but are Feulgen-negative. The more interior regions of the nucleus, beyond these peripheral bodies, show a diffusely Feulgen-positive reaction. In these regions, we have found clumps of helical structures. These helices are single and not paired. It is difficult to trace their origins. The longest spirals are between 2500 and 3000 A in length and radiate irregularly from a diffuse central region

(Fig. 2). The diameter of the coil of the helix is between 250 and 280 A and the distance between repeating turns is about 140 A. The diameter of the helix and the distance between adjacent turns are about the same in all the helices. The thickness of the filament making up the helix is approximately 70 A.

The fact that unequivocal helices have been found *in situ* in a region known to contain DNA is of particular interest. As is well known, a helical structure has been proposed for both the molecule of desoxyribonucleic acid (6) and chromosomes. Although typical chromosomes have not been described in *Amoeba proteus* (7), Chalkley, in his study of mitosis, has found an "equatorial plate" made up of tiny "chromatin granules." As far as dimensions are concerned, the helices described in this note occupy an intermediate position between nucleic acid molecules and chromosomes, differing from both by an order of magnitude. Nevertheless, as suggested by their central, intranuclear location, these helices may be related to both structures mentioned, and accordingly further study of their nature seems desirable.

BIBLIOGRAPHY

1. Bairati, A., and Lehmann, F. E., *Experientia*, 1952, **8**, 60.
2. Harris, P., and James, T. W., *Experientia*, 1952, **8**, 384.
3. Greider, M. H., Kostir, W. J., and Frajola, W. J., *J. Protozool.*, 1955, **2**, suppl., 7, abstract.
4. Pappas, G. D., *Ohio J. Sc.*, 1954, **54**, 195.
5. Chalkley, H. W., *J. Morphol.*, 1936, **60**, 13.
6. Watson, J. D., and Crick, F. H. C., *Nature*, 1953, **171**, 737.
7. Hughes, A., *The Mitotic Cycle*, New York, Academic Press, Inc., 1952, 75.

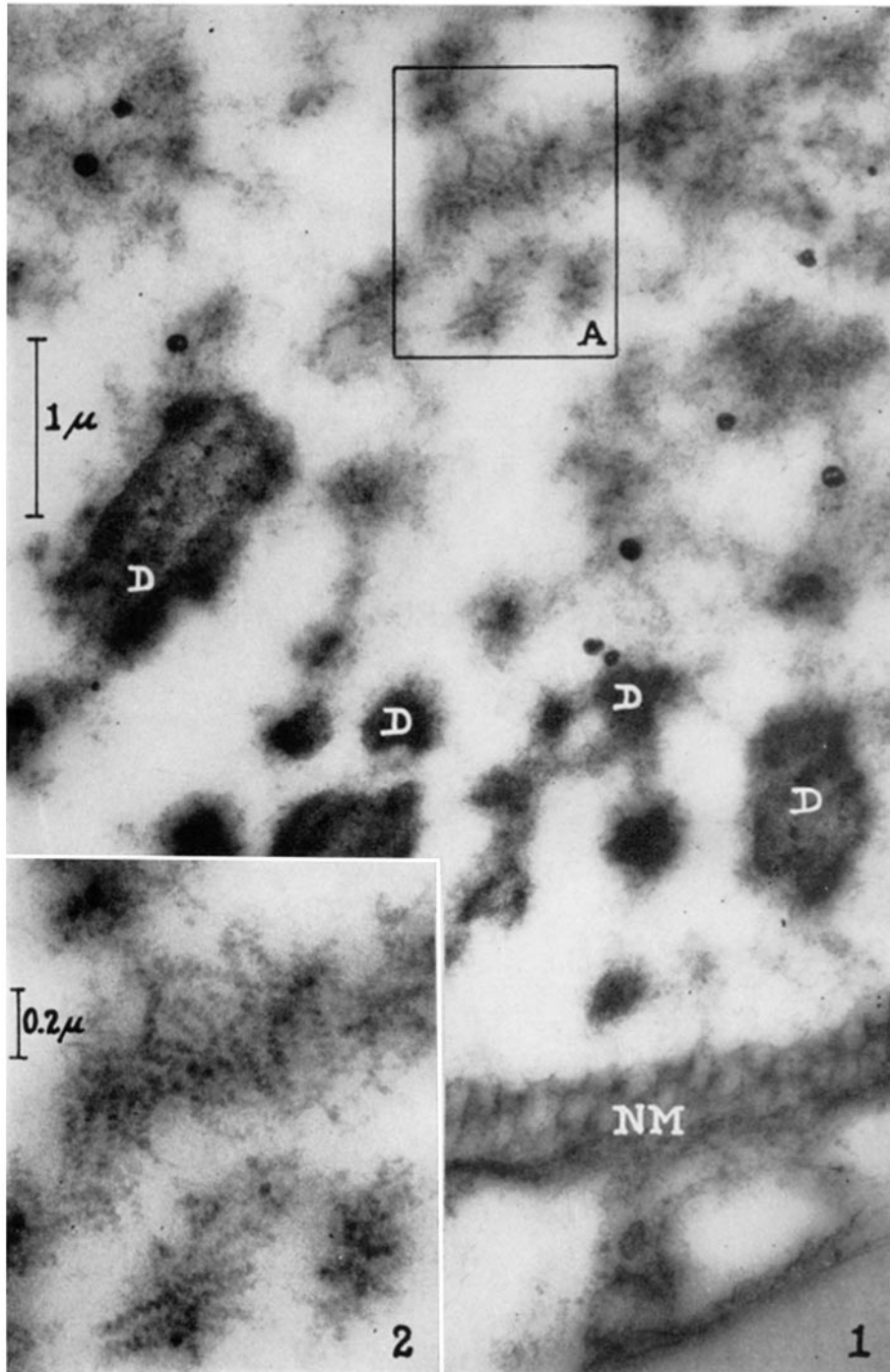
* Fellow of the Arthritis and Rheumatism Foundation.

† Received for publication, January 26, 1956.

EXPLANATION OF PLATE 37

FIG. 1. Electron micrograph of a section through the nuclear membrane of *Amoeba proteus* showing within the nucleus peripheral dense bodies (*D*). Area *A* contains helical structures. $\times 23,400$.

FIG. 2. An enlargement of area *A* showing helices. The length of these spirals is 2500 to 3000 A. The diameter of the coil of the helix is 250 to 280 A, while the thickness of the filaments is about 70 A. $\times 44,500$.



(Pappas: Helical structures in nucleus of *Amoeba proteus*)