

Electron Microscopy of *Treponema pallidum* Occurring in a Human Primary Lesion

LEWIS M. DRUSIN,¹ GABRIELLE C. ROUILLER, AND GEORGE B. CHAPMAN

Department of Medicine, Georgetown University Medical School, Washington, D.C. 20007, National Communicable Disease Center, Atlanta, Georgia 30333, and Department of Biology, Georgetown University, Washington, D.C. 20007

Received for publication 1 November 1968

Early electron microscope studies of *Treponema pallidum* (5, 7, 12, 18, 19) were of no value in defining the morphological aspects of the host-parasite interrelationship because observations were made only of bacterial cells derived from suspensions. Such preparations, obviously, destroyed the anatomical relationships which existed between the host and the bacterium. Improvements in techniques, e.g., a combination of shadow-casting procedures with enzyme digestion, did enable several investigators (2, 3, 6, 14, 15) to describe more clearly the structure of certain spirochetes. Thus, for example, the association of a fibrillar bundle with the bacterial cells was established. It remained, however, for Ovchinnikov and Delektorsky (11) to locate convincingly the fibrillar bundle between the cell wall and the plasma membrane, to describe fine structural features of the fibrils, and to clarify their numbers.

Also, within the last 5 years, there have been several reports of electron microscope studies which included ultrathin sections of Nichols pathogenic or Reiter strains of *T. pallidum* either in situ or extracted from rabbit testis (1, 9, 10, 13). In an effort to extend our knowledge by describing the appearance in the electron microscope of ultrathin sections of *T. pallidum* in a human lesion, this study of a biopsy of a human chancre was undertaken.

A 22-year-old Negro male under treatment for sarcoidosis at the Walter Reed General Hospital noticed the onset of small papule which developed into a 1.5 cm in diameter, hard, indurated ulcer on the glans penis approximately 2 weeks following sexual intercourse while he was on convalescent leave. This was accompanied by well-developed firm, nontender, bilateral inguinal adenopathy. Dark-field examination and a qualitative venereal disease research laboratory test were both positive. After local anesthesia, a 4.0-mm biopsy was taken from the center of the

chancre, and this tissue was immediately fixed in 5.0% glutaraldehyde buffered at pH 7.4 with 0.2 M cacodylate buffer (8). Postfixation was accomplished in 1.0% OsO₄, similarly buffered. The samples were dehydrated in increasing concentrations of ethyl alcohol and embedded in Epon 812 (4). Ultrathin sections were cut with a DuPont diamond knife on an MT-2 Porter-Blum ultramicrotome, picked up on collodion-coated copper grids, stained with uranyl acetate (17), and examined in an RCA EMU-2D electron microscope.

It should be noted that, although the host tissue represents an inflammatory reaction, sarcoidosis is associated with alteration of the immune mechanism. Consequently, this lesion may not constitute a typical example of a true inflammatory reaction (16).

The helical form characteristic of *T. pallidum* is readily apparent (Fig. 1-4), although the frequency and amplitude of the sine wave pattern are variable. These changes are probably due to differences in the plane of the section and to differences in the degree of shrinkage of the specimens, as well as to real differences in these characteristics. In each section, the bacterial cells are located in the extracellular space. We have never observed them within the cytoplasm of a host cell. The bacteria are found both in amorphous extracellular substance (Fig. 1, 2) and in association with collagen fibrils (Fig. 3, 4). No definite preferential location for the bacterial cells could be determined. Occasionally (Fig. 1, arrow), one observes a clear area adjacent to the bacterial cell. Whether this represents a shrinkage space or an area from which some bacterial or host substance has been removed in the preparation of the material cannot now be determined.

A cell wall may be observed most clearly in one nearly longitudinal section (Fig. 4, arrow). The three-layered wall structure reported by Ryter and Pillot (13) could not be seen. The total wall thickness of about 14 nm did not fall within the range (7-9 nm) of wall thicknesses reported

¹ Present address: Department of Medicine, The New York Hospital, Cornell Medical Center, New York, N.Y. 10021.

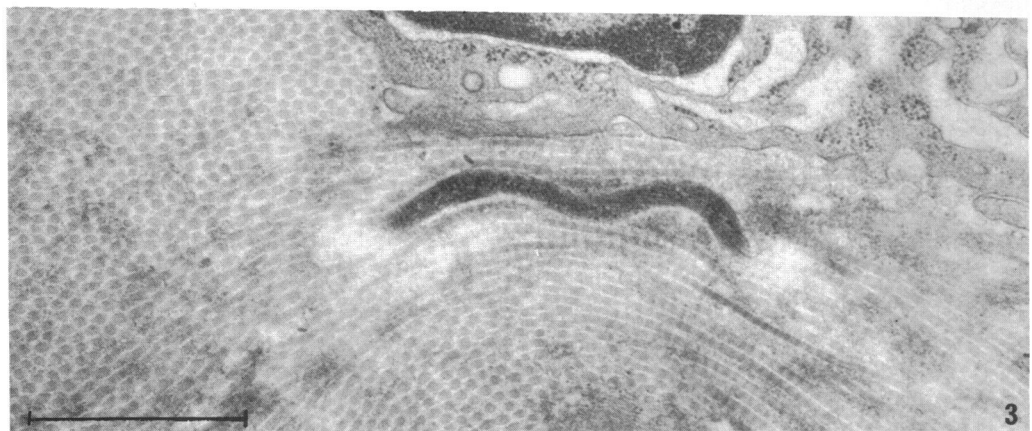


FIG. 1. *Treponema pallidum* located in amorphous extracellular ground substance. A clear space is discernible (arrow) adjacent to the cell. Three fibrils are visible (double arrow) and a bacterial and fibroblast cytoplasm (F) rich in ribonucleoprotein particles. $\times 28,000$. The magnification mark represents $1 \mu\text{m}$.

FIG. 2. *Treponema pallidum* located in amorphous extracellular ground substance, but proximal to areas of collagen. One prominent inclusion is seen (arrow) in the bacterial cytoplasm. $\times 28,800$. The magnification marker represents $1 \mu\text{m}$.

FIG. 3. *Treponema pallidum* located in a group of collagen fibrils. $\times 28,800$. The magnification marker represents $1 \mu\text{m}$.

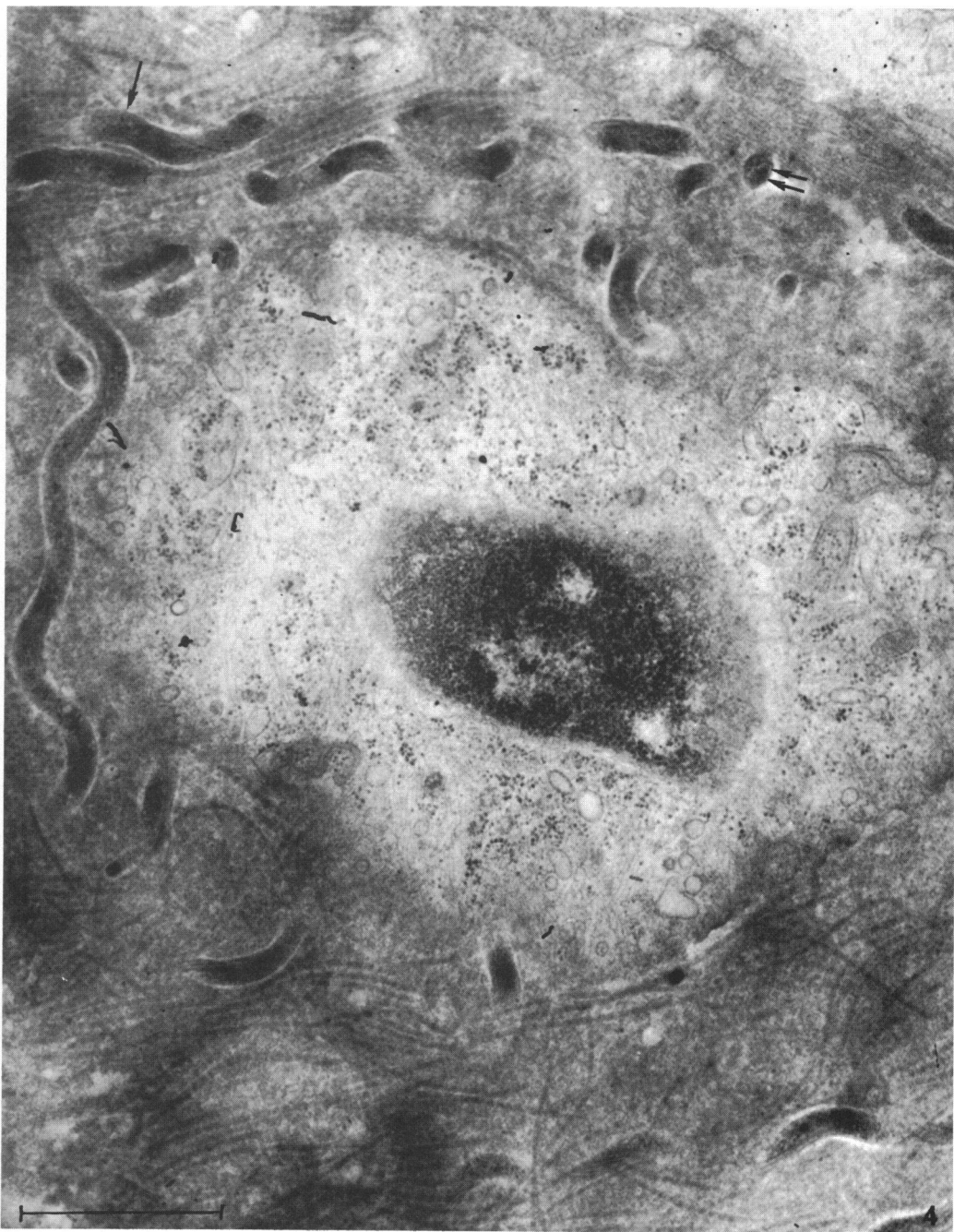


FIG. 4. Sections through several different *Treponema pallidum* cells. A cell wall may be seen (arrow) and a cytoplasmic membrane (double arrow). The bacterial cells are here located in an area of collagen fibrils. $\times 28,800$. The magnification marker represents $1 \mu\text{m}$.

by those authors, and it was appreciably less than that measured (20 nm) from Fig. 4 of Ovchinnikov and Delektorsky (9). A thin cytoplasmic membrane, reported by both Abe (1) and by Ryter and Pillot (13), may be seen (Fig. 4, *double arrow*). It is about 7 nm thick. Only one of our sections passed through a cell at a plane appropriate to reveal the three fibrils (Fig. 1, *double arrow*). This compares to the three to seven reported by Ryter and Pillot (13), the two to eight reported by Ovchinnikov and Delektorsky (9), and to the three to four reported by Swain (15). Abe (1), on the other hand, reported seeing no fibrils in the Nichols strain of *T. pallidum*. In our sections, the diameter of each fibril was about 14 nm. Ryter and Pillot (13) reported a fibril diameter of 20 nm, whereas Ovchinnikov and Delektorsky (9) reported a fibril diameter of 10 to 20 nm. Along with Ryter and Pillot (13), we could find no substructure in the fibrils. We were unable to confirm, in this material, the location of the fibrils between the cell wall and plasma membrane, as reported by Ryter and Pillot (13) and by Ovchinnikov and Delektorsky (11). However, in ultrathin sections of testis from a syphilitic rabbit, containing Nichols pathogenic strain of *T. pallidum*, the fibrils were found between the cell wall and plasma membrane (L. M. Drusin and G. B. Chapman, *unpublished data*).

The cytoplasm of the bacterial cells appears to be packed homogeneously with 10- to 15-nm diameter ribonucleoprotein (RNP) particles. Ovchinnikov and Delektorsky (9) report the same size range for RNP particles. The distribution of RNP particle sizes seems to be somewhat smaller in the bacterial cytoplasm than in the human fibroblast cells (F), appearing in the same microscope field (Fig. 1).

Apparent size differences noted in this report may be due to variations in preparative procedures. It is also possible that differences in appearances, noted below, may be attributed either to similar variations or to the fact that preservation of structures of bacteria fixed in situ in human tissue is a different matter from the preservation of comparable structures of bacteria fixed in suspension.

Although several inclusion bodies are present in some of the bacterial cells, we prefer not to consider even the one in Fig. 2 (*arrow*) as being a mesosome-like structure. Similarly differentiated structures were thus described by Ryter and Pillot (13) and by Ovchinnikov and Delektorsky (9). We, also, did not find structures which could be accurately described as basal granules or round, spore-like structures as they had been identified by Ovchinnikov and Delektorsky (9) and by

Ryter and Pillot (13). In reality, it appears that the basal granules, "b," in plate 6, Fig. 3 and 4 of Ovchinnikov and Delektorsky (9), are the same structures which Ryter and Pillot (13) called mesosomes. Furthermore, these structures, labeled "b," as noted above, are much larger than the similarly labeled structures in plate 4, Fig. 1 (9). The structures labeled "b" in plates 1, 3, 4 (9) appear more likely to be basal granules.

In none of our electron micrographs could we identify nuclear material in these bacteria, an observation shared by Abe (1). This phenomenon is in contrast with the obvious nuclear material found in the investigation of Ryter and Pillot (13) and material considered by Ovchinnikov and Delektorsky (9) to represent a nucleoid (plate 2).

This investigation was supported by Public Health Service training grant 5T01 GM 01268-04 from the National Institute of General Medical Sciences.

The assistance of Louis E. Harman, Jr., Richard L. Gardner, and Michael J. Merchant of the Dermatology Service, Walter Reed General Hospital, and Daniel H. Connor, Chief, Infectious Disease Branch, Geographic Pathology Division, Armed Forces Institute of Pathology is gratefully acknowledged.

LITERATURE CITED

- Abe, S. 1966. Electron microscopic observation of Syphilid. I. The ultrastructure of *T. pallidum* (Nichols Strain). *Bull. Pharm. Res. Inst. (Takatsuki)* 60:1-6.
- Bradfield, J. R. G., and D. B. Cater. 1952. Electron microscopic evidence on the structure of spirochaetes. *Nature* 169:944-946.
- Greifelt, A., and E. Mölbart. 1955. Elektronenmikroskopische Untersuchungen zur Morphologie des *Treponema pallidum*. *Hautarzt* 6:17-20.
- Luft, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9:409-414.
- Magerstedt, C. 1944. Ein Beitrag zur Morphologie der Syphilis spirochäete. *Arch. Dermatol. Syphilis* 185:272-280.
- Mölbart, E. 1956. Vergleichende elektronenmikroskopische Untersuchungen zur Morphologie von *Treponema pallidum*, *Treponema pertenue* und *Reiterspirochaetes*. *Z. Hyg. Infektionskrankh.* 142:510-515.
- Mudd, S., K. Polevitsky, and T. F. Anderson. 1943. Bacterial morphology as shown by the electron microscope. V. *Treponema pallidum*, *T. macrodentium*, and *T. microdentium*. *J. Bacteriol.* 46:15-24.
- Osinchak, J. 1964. Electron microscopic localization of acid phosphatase and thymic pyrophosphatase activity in hypothalamic neurosecretory cells of the rat. *J. Cell Biol.* 21:35-48.
- Ovchinnikov, N. M., and V. V. Delektorsky. 1966. Morphology of *T. pallidum*. *Bull. World Health Organ.* 35:223-229.
- Ovchinnikov, N. M., and V. V. Delektorsky. 1966. Ultrathin sections of *T. pallidum* in the electron microscope. *J. Hyg. Epidemiol. Microbiol. Immunol. (Prague)* 10:195-201.
- Ovchinnikov, N. M., and V. V. Delektorsky. 1968. Further study of the ultrathin sections of *Treponema pallidum* under the electron microscope. *Brit. J. Venereal Diseases* 44:1-34.
- Prokopchuk, A. Y., V. A. Prokopchuk, and A. G. Bondarovich. 1951. Causative agents of skin and venereal diseases as they appear in the electron microscope. *Observation I. Spirocheta pallida* as it appears in the electron microscope. *Vestn. Dermatol. i Venerol.* 3:20-23.
- Ryter, A., and J. Pillot. 1963. Etude au microscope électronique de la structure externe et interne du *tréponème Reiter*. *Ann. Inst. Pasteur* 104:496-501.

14. Schmerold, W., and B. Deubner. 1954. Elektronenmikroskopische Untersuchungen von *Reiter-Spirochaetales* und *Nichols-Treponemen*. *Hautarzt* 5:511-513.
15. Swain, R. H. A. 1955. Electron microscopic studies of the morphology of pathogenic spirochaetes. *J. Pathol. Bacteriol.* 69:117-128.
16. Turkington, R. W., and C. E. Buckley, III. 1966. Macrocryoglobulinemia and sarcoidosis. *Am. J. Med.* 40:156-164.
17. Watson, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* 4:475-478.
18. Wile, U. J., and E. B. Kearney. 1943. The morphology of *T. pallidum* in the electron microscope. Demonstration of flagella. *J. Am. Med. Assoc.* 122:167-168.
19. Wile, U. J., R. G. Picard, and E. B. Kearney. 1942. The morphology of *Spirochaeta pallida* in the electron microscope. *J. Am. Med. Assoc.* 119:880-881.