

The Ultrastructure of *Treponema pallidum* Isolated from Human Chancres

Morphologic Variations from Nichols' Strain

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The ultrastructure of *treponema pallidum* obtained directly from human chancres by biopsy was studied by electron microscopy. The treponemes were enveloped by a trilaminar cytoplasmic membrane and a trilaminar periplastic membrane. The central part of the periplastic membrane corresponds to the protective mucoid layer. In undamaged organisms bunches of axial filaments were seen to entwine the whole cytoplasmic body without any disruption or overlapping. The number of axial filaments varied between three and four. Identical nose pieces were demonstrable in both ends of the treponemes. Axial filaments and nose pieces seem to differ from those of Nichols' strain. *Key words:* *Chancre; Electron microscopy; Nichols' treponema pallidum; Syphilis.* (Received January 28, 1985.)

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Nichols' strain of *treponema pallidum* is widely applied in experimental syphilis. The strain was isolated in 1912 from the spinal fluid of a patient with recurrent syphilis (1). Since then it has been maintained in the laboratory in rabbit testicles for investigative purposes. In 1982, Penn & Clay isolated an additional strain from human chancres and found that it was considerably less virulent in rabbits than the Nichols' strain (2).

This study presents the ultrastructure of freshly isolated *treponema pallidum* from human syphilitic chancres. The morphologic features are compared to those of *treponema pallidum* of Nichols' strain.

MATERIAL AND METHODS

Specimens were obtained from penile chancres of two patients by a 3 mm punch biopsy using ethyl chloride as surface anaesthesia. The material was minced in physiologic saline solution with a scalpel. Then the tissue suspension was shaken well in a well-stoppered flask and left until coarse tissue fragments had settled. After removal of these fragments, the suspension was centrifuged at 2000 rpm for 10 min. The pellet were resuspended in one or two drops of distilled water and mixed with the same volume of 1% phosphotungstic acid adjusted to pH 7.0 by 2 N KOH. The mixture was then placed on coated grids and studied in a JEM-100 CX electron microscope at 80 kV with liquid nitrogen cooling to reduce contamination.

RESULTS

The treponemes studied were 8-16 μm in length. The wave length was about 0.9 μm with an amplitude of 0.2 μm . The cytoplasmic body had a diameter of an average of 0.13 μm which was rather constant for the single treponeme except at the terminal parts where the diameter gradually decreased to half that of the central part. An acorn-like nose piece with

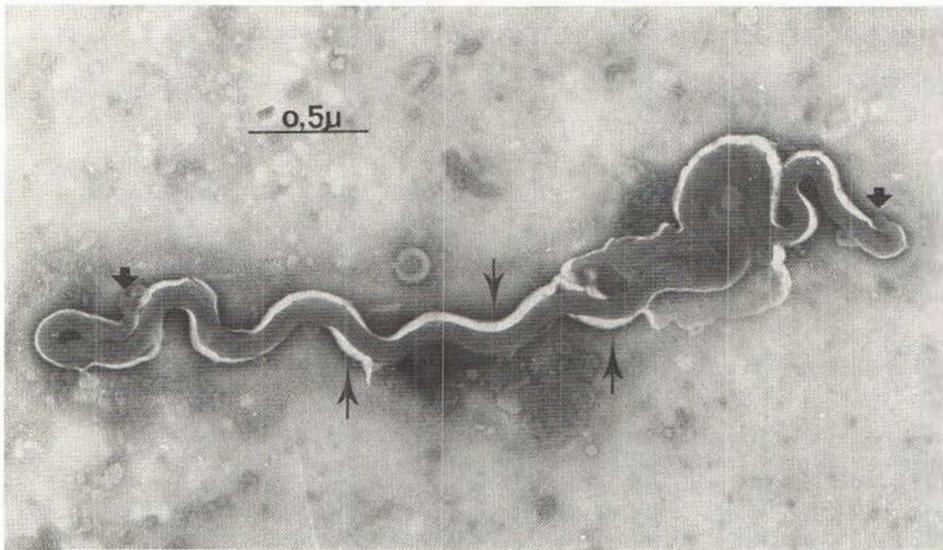
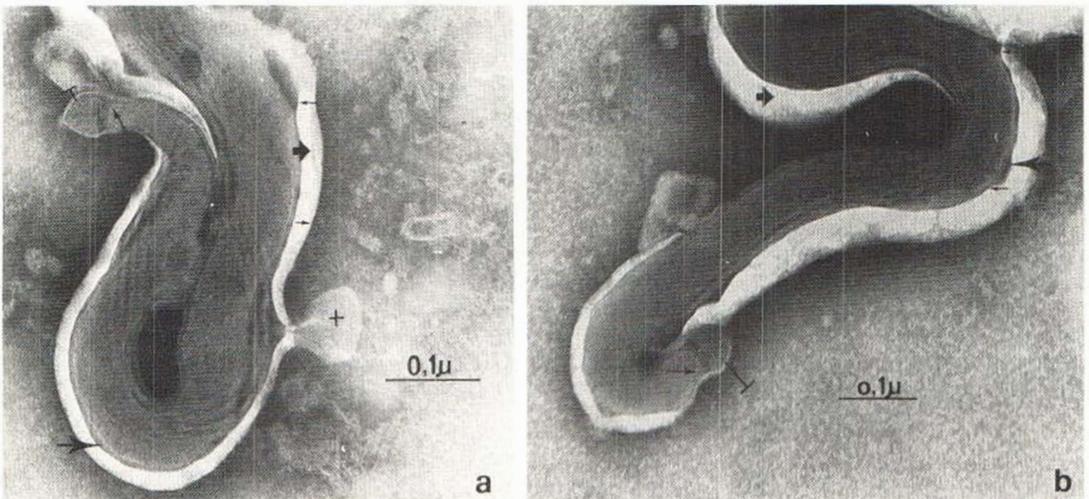


Fig. 1. *Treponema pallidum* ($\times 36\,000$). The periplastic membrane (\Rightarrow). Acorn-like nose pieces (\blacklozenge).

a length of about 600 \AA and overall width of 500 \AA formed the terminal parts of the treponeme (Fig. 1). The cytoplasmic body was enveloped in a 70 \AA thick triple-layered cytoplasmic membrane (Fig. 2a, b). Next to the cytoplasmic body this membrane consisted of a lucid zone followed by a dark zone and, next, another lucid zone was demonstrable. Each of the three layers was of equal thickness. At the base of the nose piece the membrane appeared to be split. The internal and possibly the central layer continued as a



Figs. 2a, b The two microphotographs represent the identical nose pieces of *treponema pallidum* ($\times 100\,000$). Outer and inner layer of the trilaminar periplastic membrane (\rightarrow). Central amorphous layer of the periplastic membrane (\blacklozenge). Triple layered cytoplasmic membrane (\Rightarrow). Modified granular external layer of the cytoplasmic membrane enclosing the nose piece (\vdash). The internal layer of the cytoplasmic membrane covering the blunt tip of the cytoplasmic body (\rightarrow). Bulge of the periplastic membrane (+).

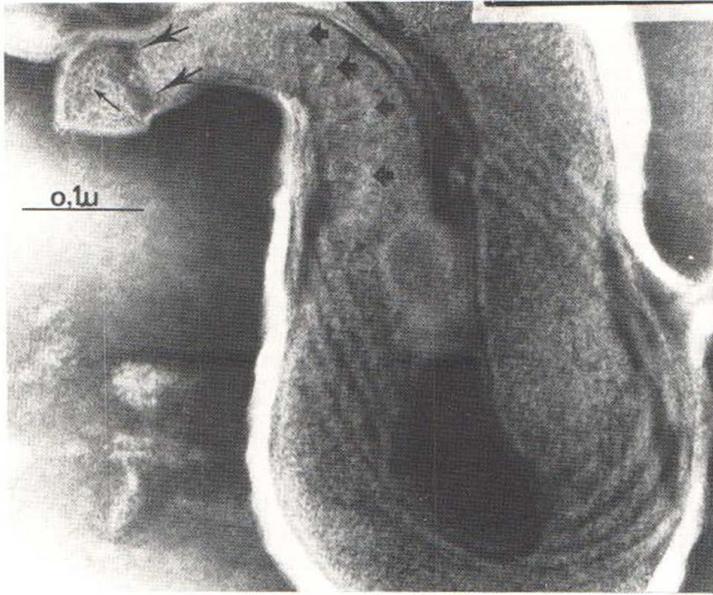


Fig. 3. *Treponema pallidum* ($\times 200\,000$). Four axial filaments situated between the cytoplasmic membrane and the periplastic membrane insert to the cytoplasmic body in electron dense circular areas surrounded by a lucid zone (\blacklozenge). Palisade like rows of granules of the head of the nose piece (\rightarrow). Two electron dense circular areas in the neck of the nose piece (\blacktriangleright).

bag around the blunt tip of the cytoplasmic body, while the external layer, as a granular structure, covered the nose piece. Both nose pieces of the single treponeme were identical. A nose piece was composed of a head and a neck (Fig. 3). The central part of the head appeared as a dark, electron dense granular structure. In the periphery the granules formed two to three layers of palisade-like rows. The head of the nose piece was separated from the cytoplasmic body by a neck of dark, homogeneous structure, in which two circular electron dense areas could be demonstrated.

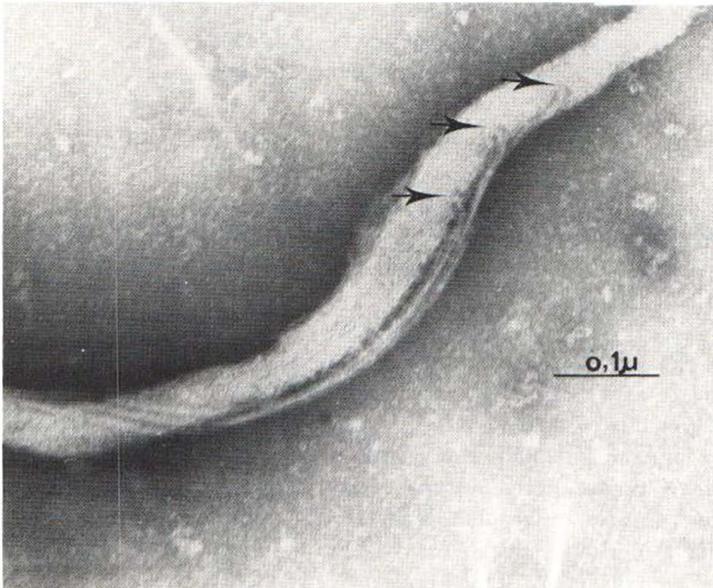


Fig. 4. Insertion of three axial filaments in the subterminal region. The hooks by which the filaments are attached are demonstrated (\blacktriangleright) ($\times 100\,000$).

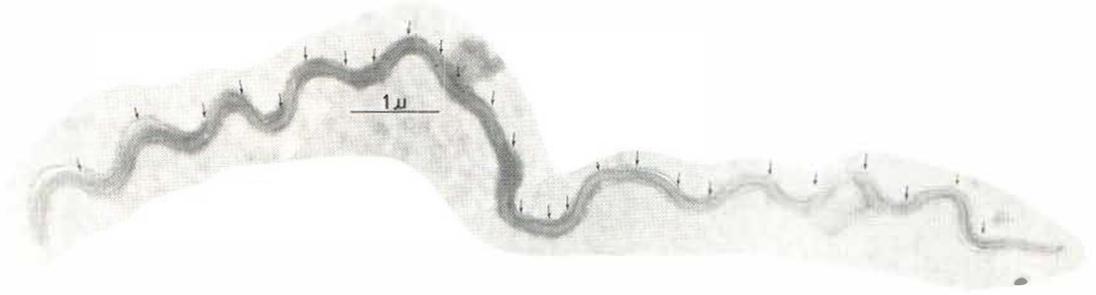


Fig. 5. *Treponema pallidum* ($\times 20\,000$). Four microphotos of different areas of one and the same treponeme are stuck together. A bunch of axial filaments uninterruptedly entwine the cytoplasmic body from one subterminal region to the other. The arrows illustrate where the axial filaments can be seen crossing the cytoplasmic body. (\rightarrow).

The cytoplasmic body with its membrane and the nose pieces were mantled in a trilaminated periplastic membrane (Fig. 2*a, b*). The internal and external dark, delicate laminae of this envelope had an overall thickness of 25 \AA , while the central lucid amorphous layer varied from 60 \AA (at the nose piece) to 500 \AA . In all treponemes examined large areas had no periplastic membrane and in some the membrane was scant. At the periplastic membrane a few bulges without cytoplasm or cytoplasmic membrane were observed (Fig. 2*a*).

Outside the cytoplasmic body, between the cytoplasmic membrane and the periplastic membrane parallel lined spirally coiled axial filaments were demonstrable (Fig. 3). Close to the nose pieces the axial filaments were inserted by hooks in electron dense, circular areas in the cytoplasmic body. Half of the treponemes had four and half had three axial filaments attached in this way (Figs. 3 and 4). When putting together microphotos of various regions of the intact *Treponema pallidum* bunches of three to four axial filaments were observed to uninterruptedly entwine the whole cytoplasmic body from one subterminal region to the other (Fig. 5). In the cytoplasmic body parallel to the cytoplasmic membrane 6–8 deep filaments were demonstrated (Fig. 6). The diameter of the deep filaments was about half the diameter of the axial filaments. They could not be demonstrated beyond the site of the insertion of the axial filaments and we did not succeed in demonstrating how they ended.

DISCUSSION

The ultrastructure of *Treponema pallidum* Nichols isolated from experimental syphilis has been studied repeatedly (3–13), whereas few investigations have been carried out on the nature of the ultrastructure of *Treponema pallidum* in or isolated from human syphilis (14–17).

The nose piece of Nichols' strain is described as a zonal region outside the cytoplasmic membrane consisting of granular cell wall material only (5). The microphotos of the present study illustrate the nose piece as a well-defined structure tied to the tip of the cytoplasmic body, surrounded by a modified external layer of the cytoplasmic membrane and a triple-layered periplastic membrane. Orčinnikov & Delektorskij (15) described morphologic differences between the two nose pieces of treponemes isolated from rabbit testicles and implied a cephalic and an excretorial function of the nose pieces. In the present study the nose pieces of the treponemes appeared identical. The function of the nose piece is presumably connected with the ability of the treponemes to adhere to tissue.

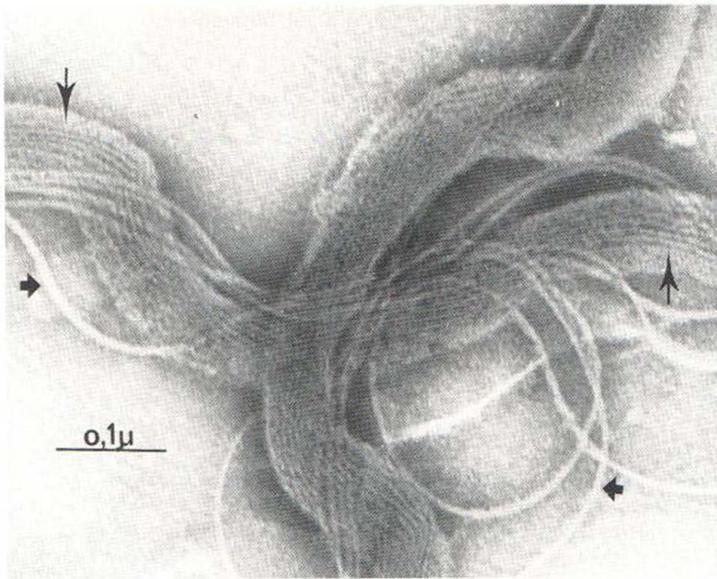


Fig. 6. *Treponema pallidum* ($\times 100\,000$). The deep filaments are lined parallel to the axis of the cytoplasmic body (➤). Partly detached axial filaments (◈).

This was demonstrated by scanning electron microscopy of *treponemata pallida* attached to cultured cells derived from normal rabbit testes or human skin epithelium which showed that the treponemes usually adhere to the cells with one and sometimes both endpoints (18).

The outer limitation of the treponemes is the periplastic membrane (15, 16, 19). In earlier studies (17), the outer limitation is supposed to consist of an enveloping structure outside which an amorphous mucoïd layer—also named the slime layer—possibly derived from the surrounding host tissue is present (20, 21, 22, 23). The mucoïd layer is presumed to protect the *treponema pallidum* from a host immune response (20). This study indicates that the amorphous layer is the central part of the periplastic membrane, which, externally and internally, is limited by a distinct lamina. The amorphous mucoïd layer therefore probably forms an integral part of treponemes as isolated from a human syphilitic chancre.

In several hundred treponemes of Nichols' strain, Jepsen et al. and Hovind Hougen (4, 5) examined the topography of the attachment of the axial filaments concluding that invariably three axial filaments were inserted at the terminal ends. This could not be confirmed by the present study, in which the number of attached axial filaments varied from three to four. The number of axial filaments of the Nichols' strain then seems to differ from naturally occurring treponemes. The course of the axial filaments around the cytoplasmic body has been a matter of debate. A *treponema pallidum* has been stated to have two bunches of axial filaments (4, 24, 25). Each bunch originates at one of the two subterminal regions of the cytoplasmic body and in the middle of the treponeme the two bunches are overlapping or intermingling. In the intact organism we observed that one bunch of axial filaments uninterruptedly entwines the whole cytoplasmic body from one subterminal part to the other. It seems reasonable to assume that the axial filaments are responsible for the motility of the cell (26, 27), but, so far, it has not been proven.

According to Turner (28) the pathogenic treponemes become adapted to laboratory animals with continuous passage. The Nichols' strain is used in about 90% of all laboratory studies of *treponema pallidum* (29) and, therefore, it is essential that the Nichols' strain still owns the same characteristics as the pathogenic treponemes of human syphilitic

lesions. The present findings, however, indicate that nose pieces and axial filaments of treponemes of human chancres are unlike those of Nichols' strain.

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