

Ultrastructural Changes of *Treponema pallidum* Isolated from Secondary Syphilitic Skin Lesions

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Treponema pallidum was isolated from various types of secondary syphilitic skin lesions. From moist genital papules and from condylomata lata several treponemes were isolated whereas few were isolated from dry papules of the trunk. One third of the observed treponemes were morphologically different from treponemes isolated from human chancres. Especially the nose-piece structures of the terminal parts of the treponemes were deviating. Some nose-pieces were coated by a fuzzily outlined electron dense substance, whereas others were degenerated or nearly separated from the cytoplasmic body. Other treponemes were missing their nose-piece as avirulent saprophytic treponemes. Recent studies have indicated that the nose-pieces are essential for the tissue attachment of treponemes and the treponemal virulence. The significance of the altered nose-piece structure observed is discussed. *Key words: Syphilis; Nose-piece deviations; Virulence.* (Received September 29, 1986.)

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The morphology of *Treponema pallidum* isolated from syphilitic tissue of rabbits and humans has been subjected to electron microscopy in several studies. In the study of treponemes isolated from rabbit syphilis the microorganism is usually "harvested" 1–2 weeks after inoculation, as, at that time, the number reaches a maximum (1). This implies that only treponemes being exposed to the immune defense of the host organism for a limited period of time are examined. In electron microscopic studies of treponemes obtained from humans, microorganisms mostly have been isolated from syphilitic chancres and are thus of a relatively young age (2).

In disseminated dry secondary syphilitic skin lesions the treponemes are scarce (3, 4), whereas moist hypertrophic papules harbour numerous treponemes. These efflorescences, occurring in florid secondary syphilis, have become unusual to-day because of the marked decrease in syphilis in the last years (5, 6). Our previous electron microscopic studies of *Treponema pallidum* in primary and secondary syphilitic lesions indicated that the ultrastructure of treponemes in these lesions is varying (7). The ultrastructural variations were further examined in the present study of isolated *Treponema pallidum* obtained from human secondary syphilitic skin lesions.

MATERIAL AND METHODS

With ethyl chloride spray used as local anaesthetic 3 mm punch biopsies were obtained from secondary syphilitic lesions in three patients, one male and two females. Before the biopsies were taken, the lesions were cleaned by swabs saturated with 70% isopropyl alcohol and chlorhexidine 0.5%.

In the male patient, aged 21 years, a moist papule presented for 6–8 weeks on the glans penis was

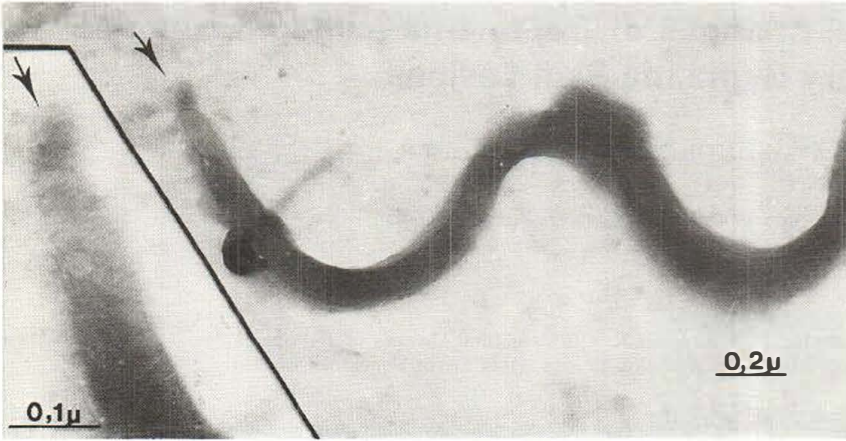


Fig. 1. *Treponema pallidum* (48 000 ×) isolated from a secondary syphilitic papule with a well-defined acorn-like electron dense nose-piece outside the cytoplasmic body (inset 120 000 ×) (→).

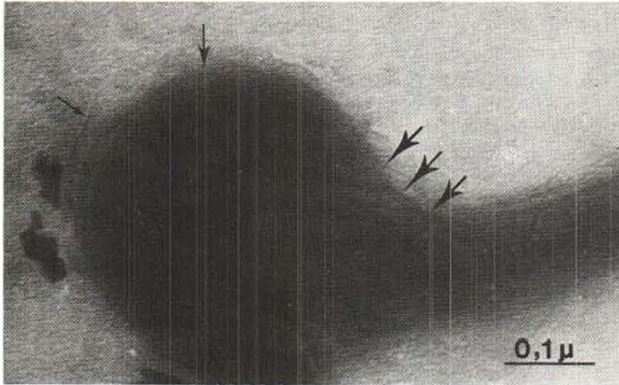


Fig. 2. Part of a *Treponema pallidum* with 3 axial filaments crossing the cytoplasmic body (120 000 ×) (→). A trilaminar cytoplasmic membrane (→) and a fragmented and partly detached periplastic membrane are indicated (→).

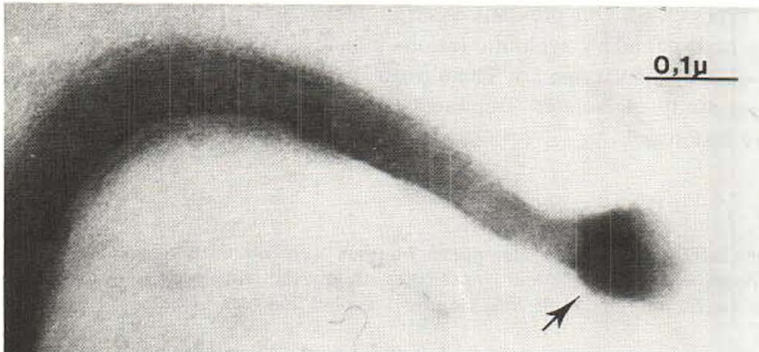
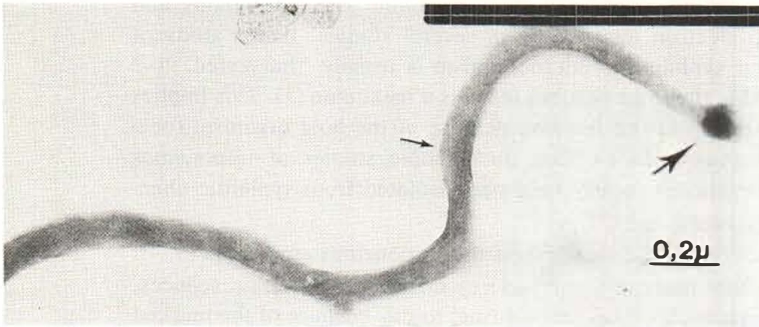


Fig. 3. *Treponema pallidum* (48 000 ×). No periplastic membrane is present. The surface is irregular, indicating a damaged cytoplasmic membrane (→). The treponeme ends with a tuft-like structure (→) having a centrally located nose-piece coated by amorphous substance (inset 120 000 ×).

biopsied. The patient also presented papules of the palms. In serum, Wassermann Reaction (WR) was 9, Automated Reagin Test (ART) 8 dil, Antiflagellar Antibodies IgG (AF-G) 13, *Treponema pallidum* Immobilization (TPI) test 3+, and Absorbed Fluorescent Treponemal Antibody (FTA-ABS) test 4+. In the first female patient, who was 23 years old, a biopsy was taken from a dry, scaly, annular syphilitic persisting for 7–8 months at the right hip. In this patient was also noted leucoderma on the trunk and extremities. In serum, WR was 7, ART 16 dil, AF-G 10, TPI 3+, and FTA-ABS 4+. The second female patient, aged 37 years, demonstrated dry, scaly papules on the trunk, palms and soles lasting for 4–6 weeks, and condylomata lata in the anogenital region. In serum, WR was 13, ART 8 dil, AF-G 12, FTA-ABS 4+, and TPI 2+. One of the condylomata lata was biopsied.

The biopsies were minced into fine particles with a scalpel, put into physiological saline solution and shaken well for 10 minutes. To allow tissue fragments and the blood cells to settle and in order to minimize centrifugation damage to the treponemes the suspension was stored for 8 hours at 4°C. The supernatant was then centrifuged at 2000 rpm for 20 minutes. After removing the supernatant the sediment was resuspended in a drop of distilled water and mixed with an equal volume of 1% phosphotungstic acid adjusted to pH 7 by 2N KOH. Drops of this suspension were placed on coated copper grids and, to prevent desiccation, the grids were kept in a plastic box for 20 minutes during which the treponemes were allowed to settle. The excess of suspension was removed by filter paper. Examination of the treponemes was performed in a JEOL 100 CX electron microscope at 80 kV.

RESULTS

From moist genital papules and condylomata lata several treponemes were isolated, whereas few were extracted from the dry skin syphilitic. The length of the treponemes examined varied between 8 and 18 μm . Including the lamina externa of the trilaminated cytoplasmic membrane the width of the treponemes varied from 0.09 to 0.13 μm . The wavelength varied from 0.9 μm to 1.3 μm with an amplitude varying from 0.16 to 0.3 μm . The amplitude was approximately 1/5 of the wave length (Fig. 1). The periplastic membrane, synonymously the outer envelope, was rarely intact. If present, the membrane was seen fragmented and split with only one of the laminae detectable with certainty (Fig. 2). The morphologic features mentioned above were present in 3/4 of treponemes examined. Some treponemes terminated in a round, highly electron dense, fuzzily outlined substance in the centre of which the contours of a nose-piece structure was visible (Fig. 3). Nose-pieces were occasionally seen as rudimentary and ill defined electron dense substances located externally to the cytoplasmic body (Fig. 4). In few treponemes the nose-pieces were apparently separated from the cytoplasmic body, only attached to the body with a membrane structure (Fig. 5). A few treponemes shown no nose-pieces (Fig. 6). In a single treponeme the terminal part of the cell body was split so the organism ended with a Janus head-like nose-piece (Fig. 7).

DISCUSSION

The present study corroborates that condylomata lata and moist secondary syphilitic papules are suitable for isolation and identification of *Treponema pallidum*. It is unclear why this type of secondary syphilitic efflorescence predominatingly involves the anogenital region. Outer factors such as climate influence the type of skin lesions caused by treponemal infections (8). In tropic climate areas the profuse treponeme induced skin lesions often become succulent and rich in treponemes (9). It is believed that the human skin in the cooler and dryer temperated climate zones offers less favourable conditions for the treponemes, and the secondary syphilitic lesions appearing in these zones are often of the dry maculo-papular type with few treponemes present (9). An essential step in the pathogenesis of the syphilitic lesions is the treponemal attachment to the host cells. In the laboratory, a maximum attachment of treponemes to cells was reached at 37°C, decreasing with falling temperature (10). On the human body surface a temperature favouring trepo-

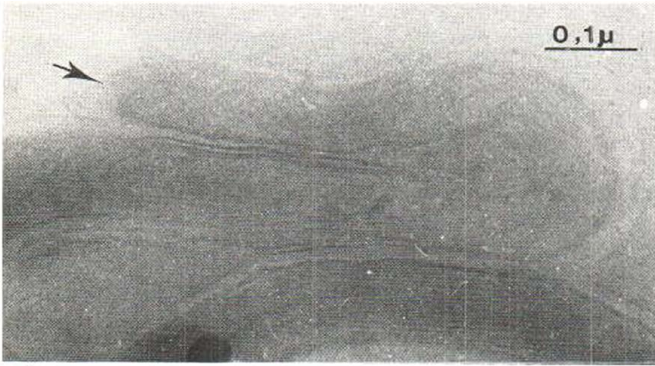


Fig. 4. *Treponema pallidum* (120 000 \times) terminating with an indistinctly outlined small nose-piece structure, probably degenerated (\rightarrow).

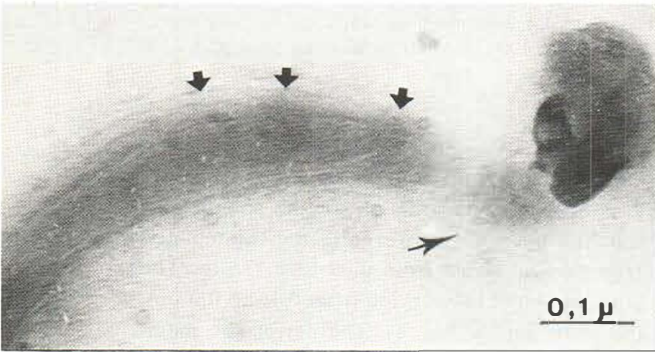


Fig. 5. A nose-piece of *Treponema pallidum* is detached from the cytoplasmic body. It is surrounded by a membrane-like structure, possibly the periplastic membrane (\rightarrow). Insertion regions of three axial filaments (\blacklozenge).

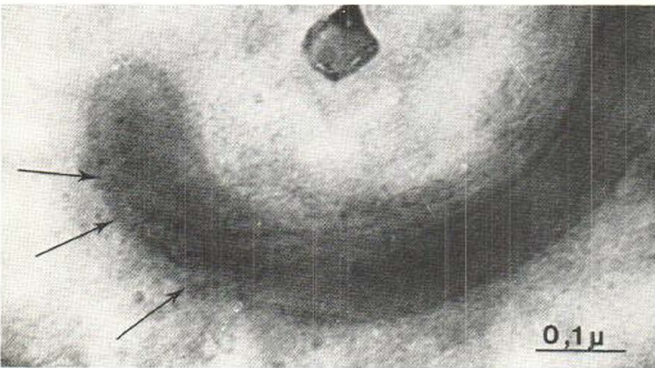


Fig. 6. *Treponema pallidum* with three axial filaments (\rightarrow) crossing the cytoplasmic body outside of which no nose-piece structure is visible. Loss of the nose-piece characteristics renders the *Treponema pallidum* indistinguishable from avirulent treponemal strains.

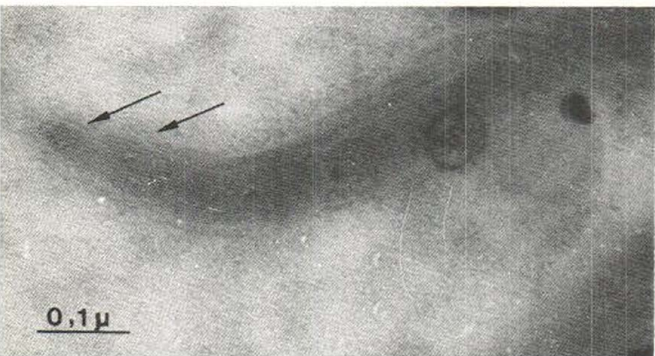


Fig. 7. A two terminal and region of a treponeme. In one end Two-nose-piece structures are seen (\rightarrow). Whether this Janus head-like configuration represents a treponemal variant or whether it is a step in a longitudinal fission is speculative. Ordinarily, the fission of *Treponema pallidum* is concluded as being transversal.

nemes to attach to cells and to grow should be expected especially to exist in the anogenital area. This could explain why syphilitic lesions in this region are particularly rich in treponemes. These factors could possibly also favour the existence of the chancre in this region.

The avirulent *Treponema phagedenis*, biotype Reiter, which originally may be derived from human pathogenic treponemes (11) is enveloped by a periplastic membrane sensitive to the osmotic pressure in NaCl solutions (12). In hypo-osmotic solutions the membrane becomes swollen. An analogous phenomenon is occasionally noticed, when isolated *treponemata pallida* during negative staining are suspended in distilled water, in order to avoid crystallization of NaCl (13). In the subsequent electron microscopic examination the intermediate lamina of the periplastic membrane sometimes appears swollen (2), differing from the intermediate layer of the periplastic membrane observed *in situ* in preparations of sectioned treponemes (7), while the external and the internal lamina appear unaffected. In the present study such swelling of the periplastic membrane was not remarkable, possibly because, in contrast to treponemes isolated from primary syphilitic lesions (2), the membrane was rarely intact with all three laminae preserved. The infrequently occurring and often fragmented periplastic membrane corresponds well with *in situ* studies of *Treponema pallidum* in secondary syphilitic skin lesions, in which the membrane is usually absent (7). It has previously been discussed, if the structure has a protective function for the treponeme and whether it is eliminated by the host immune response during the syphilitic infection (14, 15).

The treponemal attachment to the host cells, a critical point for initiating the syphilitic infection, seems mediated by the terminal nose-pieces (16, 17). Usually, the attachment is established at one, occasionally at both treponemal endpoints, but it is not observed along the entire length of the surface of the treponeme (17). Killed or inactivated or avirulent treponemes do not attach to tissue cells (10, 18, 19, 20). Syphilitic rabbit serum prevents the attachment of *Treponema pallidum*, but does not influence the treponemal motility indicating the existence of a specific reaction directed against the nose-pieces of the treponeme (10, 18, 21). The treponemal attachment blocking effect can be demonstrated in rabbit serum at the earliest 30 days after infection (21). The morphologic differences in the nose-piece structures described in this report may reflect distinct stages of a degeneration process caused by a "host-versus-treponemal-nose-piece-reaction" which becomes increasingly evident with increasing age of the syphilitic infection. The initial step in this process could be a precipitation of blocking anti nose-piece antibodies, which leaves the treponemal nose-pieces coated by a fuzzy substance (Fig. 3). The nose-pieces probably then degenerate (Fig. 4) or become loosened from the cytoplasmic body (Fig. 5). In this way, the ability of *Treponema pallidum* to attach to tissue could be lost. Loss of the nose-piece characteristics may render the *Treponema pallidum* indistinguishable from avirulent treponemal strains (Fig. 6). A substantial morphologic electron microscopic difference between these treponemes and the *Treponema pallidum* is probably their terminal ends. The avirulent treponemes terminate with a blunt or somewhat tapered end of the cytoplasmic body without the electron dense nose-piece structure described in pathogenic treponemes (22).

The final elimination of *Treponema pallidum* in syphilis is most likely mediated by phagocytosis (23, 24) which may be facilitated by a preceding destruction of the periplastic membrane (15) or by precipitation of antibodies directed against the treponemal surface (7). Recent studies have indicated that the syphilitic tissue damage is mainly caused by the specific treponemal cell attachment and not by toxins (25). It is conceivable that avirulent but motile *Treponemata pallida* with destructed nose-pieces may survive as saprophytes in sites where host reactions cannot be activated. If the treponemes keep their genetic codes

and ability for dividing, a subsequent reduction of the host defense directed against the nose-piece may facilitate new generations of nose-piece intact, virulent treponemes. Perhaps, in part this could explain the latency and late lesions of syphilis.

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