Acta Derm Venereol (Stockh) 1986; 66: 423–430

Treponema pallidum in Human Chancre Tissue: An Electron Microscopic Survey

ASMUS POULSEN, TAKASI KOBAYASI, LENA SECHER and KAARE WEISMANN

Department of Dermatology and Venereology, University of Copenhagen, Rigshospital, Copenhagen, Denmark

Poulsen A, Kobayasi T, Secher L, Weismann K. Treponema pallidum in human chancre tissue: an electron microscopic survey. Acta Derm Venereol (Stockh) 1986; 66: 423-430.

In biopsies obtained from syphilitic chancres of varying ages in 10 patients, a total of 766 ultrathin sections of Treponema pallidum were studied by electron microscopy. The course and number of axial filaments observed reveal that one bunch of 3–4 filaments without interruption entwine the whole cytoplasmic body. In 9.2% of the sections a trilaminar or a fragmentary trilaminar periplastic membrane was observed outside the cytoplasmic membrane and the axial filaments. The occurrence of the periplastic membrane decreased with advancing ages of the chancres. A protective function of the membrane is discussed. A peritreponemal fine reticular halo demonstrable in most fragments is supposed to be due to fixation induced shrinkage of treponemal hyaluronidase-influenced semifluid glycosaminoglycans. Peritreponemal reticular halos were also observed in collagen tissue. A destructive effect of the treponemes on collagen fibres could explain how the organisms penetrate through the collagen rich meninges into the central nervous system. A surface associated narrow border of electron dense amorphous substance, probably originating from the host organism, yields to tangentially cut treponemes a spiny caterpillar-like appearance. *Key words: Primary syphilis; Axial filaments; Periplastic membrane; Peritreponemal amorphous substance.* (Received January 29, 1986.)

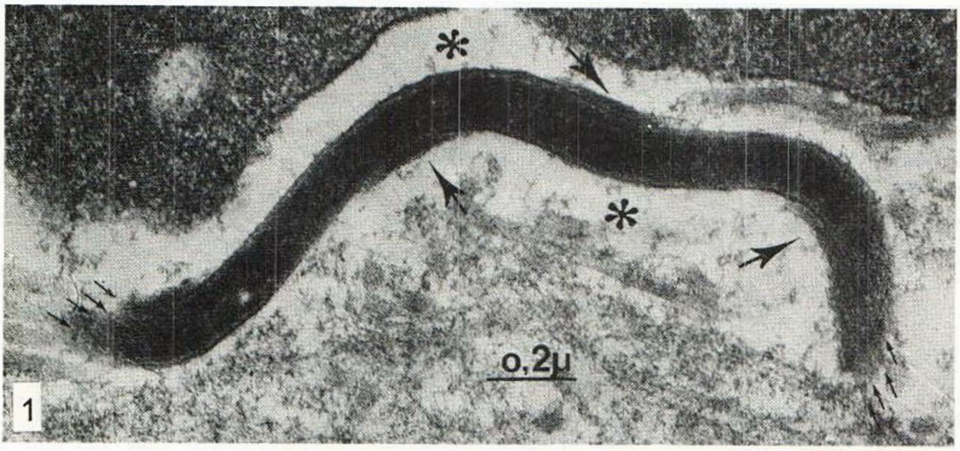
A. Poulsen, Department of Dermatology and Venereology, University of Copenhagen, Rigshospital, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark.

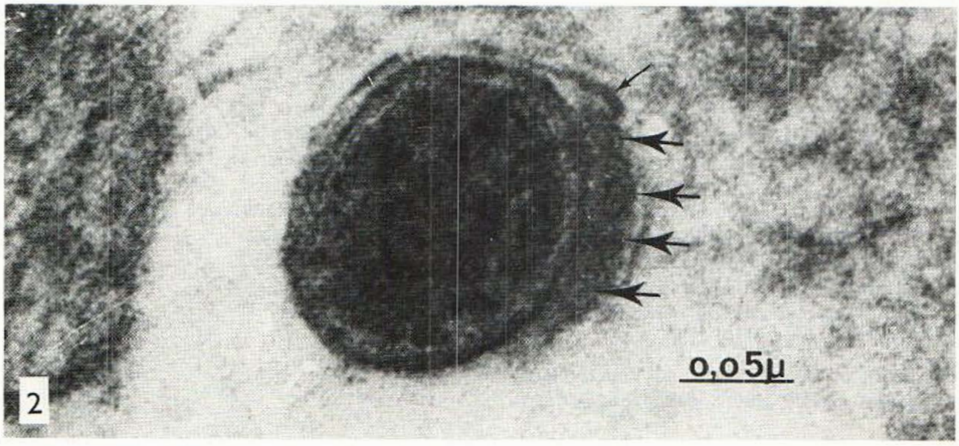
Since, in 1942, the electron microscope was introduced in the study of Treponema pallidum (1), several investigations have been published on isolated treponemes and ultrathin sections of treponemes in tissue from syphilis-infected rabbits and humans. These studies have shown that outside the cytoplasmic membrane the axial filaments, synonymously treponemal flagellae or periplastic fibres, entwine the cytoplasmic body in a helical way (2, 3, 4). The course of the axial filaments in the central part of the treponeme is uncertain. It is not known, whether one bunch of bipolarly attached filaments uninterruptedly entwine the whole cytoplasmic body (5, 6, 7) or whether two separate bunches, each of which is originating at one end of the treponeme encircle the cytoplasmic body up to the middle, where they terminate in an interdigitating pattern (8, 9, 10).

There are different opinions concerning the periplastic membrane, named outer envelope, outer sheats or outer coat. Does it cover the cytoplasmic membrane, or are the axial filaments and the cytoplasmic membrane in direct contact with surrounding host tissue? (2, 12).

Some studies indicate that in rabbit tissue the treponemes are embedded in a blurrily outlined substance (13, 14), and a peritreponemal amorphous substance has been observed in macular and papular secondary syphilis (15). Such a substance has not been described in human chancres.

The course of the axial filaments and the periplastic membrane as well as the peritrepon-





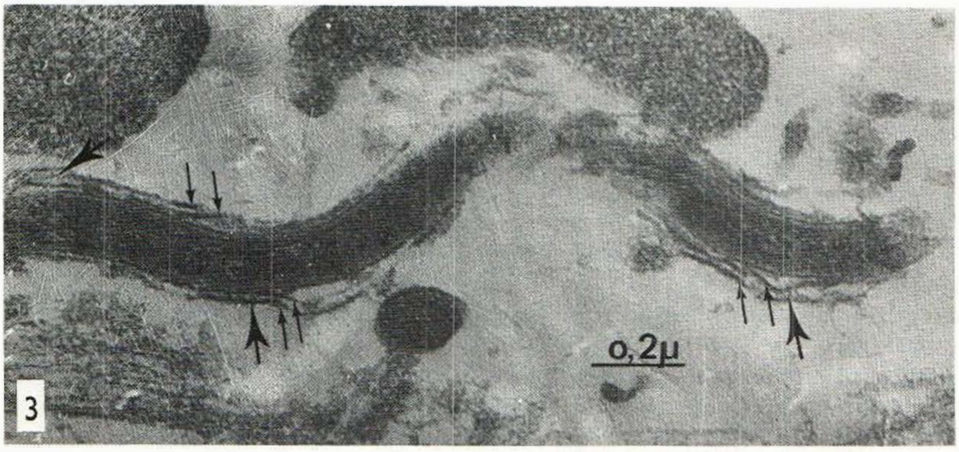


Fig. 1. Treponema pallidum (×60 000) longitudinally sectioned. The axial filaments appear as an electron dense substance at the cytoplasmic membrane of the concave aspects of the flections (→). The four individual filaments are detected at the end of the treponeme (→). The treponeme is surrounded by a wide transparent space (✻). No periplastic membrane is seen.

Fig. 2. Cross-sectioned treponeme (×300 000). Four tubular axial filaments (→) with an electron dense central core are observed between the cytoplasmic membrane and fragments of the periplastic membrane (→).

emal substance are the subjects of the present electron microscopical study of ultrathin sectioned treponemata pallida *in situ* obtained from human syphilitic chancres.

MATERIAL AND METHODS

Ethyl chloride spray served as anaesthetic to obtain 3 mm punch biopsies from the edge of syphilitic genital chancres of 10 patients, one female and 9 males. The chancres were classified into three age groups. Group I included chancres of less than 2 weeks' duration (4 patients). Two to four week- old chancres were classed into group II (3 patients); and, finally, chancres persisting for more than 4 weeks were referred to as group III (3 patients). Treponemes were demonstrated by dark field microscopy of chancre exudate, and in blood serum of all patients, except one in group I, antitreponemal antibodies (TPI, FTA-ABS) were reactive. After fixation in ice-cooled 6% glutaraldehyde in 0.5 M cacodylated buffer pH 7.3 with 7.5% sucrose the specimens were osmicated and dehydrated in a series of increasing concentrations of alcohol and finally embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 100 CX electron microscope at 80 kV.

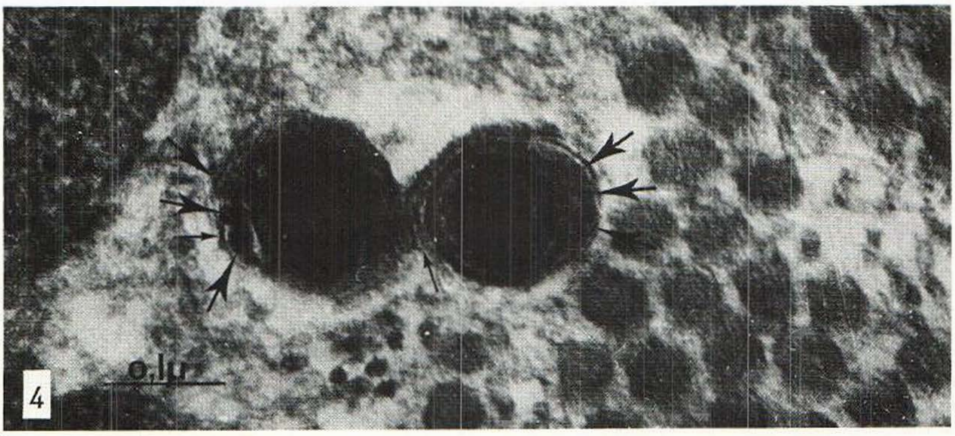
RESULTS

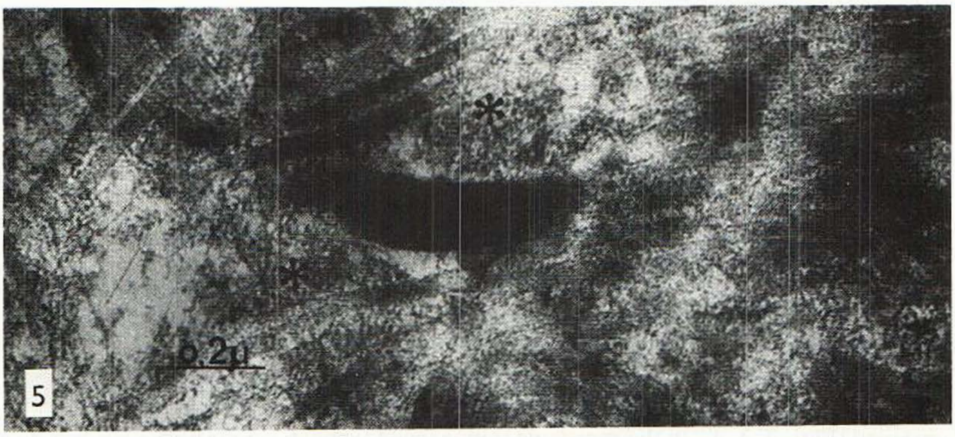
A total of 766 treponemal fragments were examined. In longitudinally sectioned treponemes the axial filaments were demonstrable in the concave parts of the cell body as a demarcated electron dense substance at the cytoplasmic membrane. At the end of the fragments, the individual filaments were demonstrated (Fig. 1). In tangentially cut sections the number of filaments crossing the cytoplasmic body never exceeded four. Cross-sectioned treponemes showed axial filaments, which, because of their helical course, were often tangentially cut so that a differentiation of single filaments was difficult. In the cases, where a differentiation was possible, the number of filaments was 3 or 4 (Fig. 2).

In 9.2% of the examined treponemal fragments, a trilaminar periplastic membrane or fragments of a periplastic membrane external of the cytoplasmic membrane and the axial filaments was observed (Figs. 2, 3, 4). In the remaining treponemal fragments no membranous structure was seen outside the cytoplasmic membrane. The thickness of the external and internal electron dense lamina was 30–75 Å, while the less electron dense central part varied from 200 to 500 Å. Sometimes the periplastic membrane was folded or duplicated and appeared loosely affixed to the cytoplasmic membrane (Fig. 3). At the areas covered by the periplastic membrane, the cytoplasmic membrane structure was often well-defined and well-marked. The periplastic membrane was less often observed in older chancres. Thus, in 11.5% of the treponemal fragments in group I, 10.8% of group II, and 5.2% of group III, a periplastic membrane could be found.

Enveloped and non-enveloped treponemes were frequently observed within a 0.2–1 μm wide, apparently empty, tissue space. In 1–2% of the treponemal fragments, spaces were absent and the treponemes were embedded in a fuzzily outlined amorphous substance (Fig. 5). A higher magnification of the spaces disclosed reticular arc-forming electron dense threads connecting the treponemal surface to the surrounding tissue. In this way, tangentially sectioned treponemes obtained a caterpillar-like appearance (Fig. 6). No differences in the peritreponemal zones were observed in the different chancre groups.

Fig. 3. Longitudinally sectioned Treponema pallidum in human chancre tissue (×60000). The trilaminar periplastic membrane with external and internal electron dense laminae (→) separated by a lucent zone (→), folded and apparently loosely attached to the cytoplasmic membrane, covers the treponeme. The areas covered by the cytoplasmic membrane appear more distinct than non-covered areas.





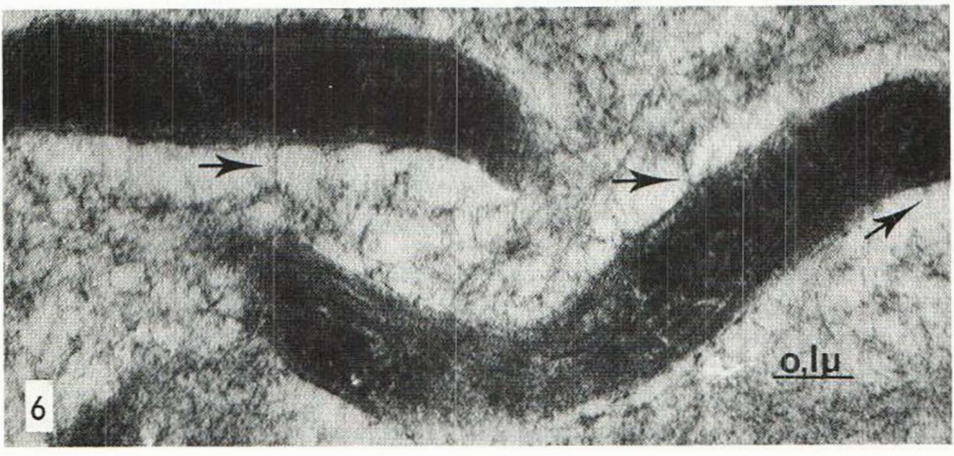


Fig. 4. Trilaminar, partly ruptured, periplastic membrane (→) (×150000) is enveloping cross-sectioned treponemes. Probably because the latter are tangentially sectioned, the outlines of a single axial filament are indistinct (→).

Fig. 5. Treponema pallidum between collagen fibres (×60 000). The microorganism is embedded in an amorphous electron dense substance ✻ covering the cytoplasmic membrane.

Fig. 6. Filamentous "amorphous" substance (→) (× 100000) of the peritreponemal reticular space links the treponemal electron dense surface to the surrounding tissue.

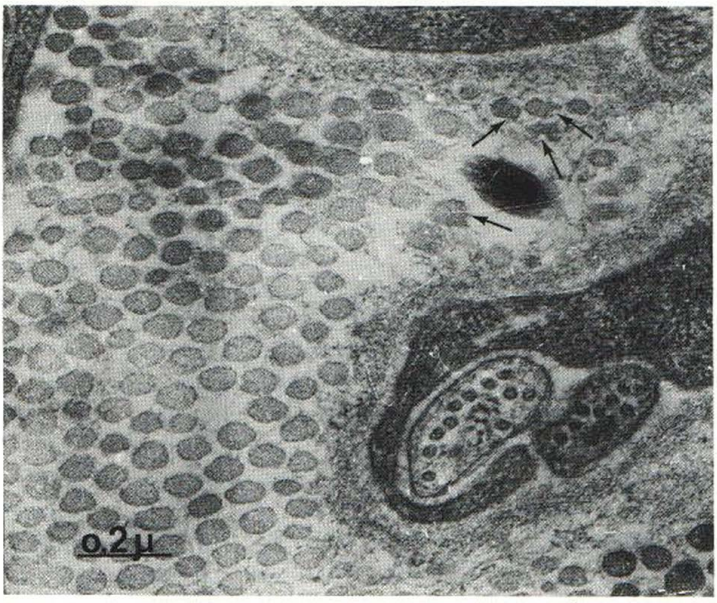


Fig. 7. Treponema pallidum (×60 000) in the centre of a reticular space in collagen fibril bundles of a nerve. The space is bounded by collagen fibrils which are irregular demarcated (→) indicating that these fibrils have been exposed to lytic influence. The treponeme shows three axial filaments, but no periplastic membrane.

Also within collagen tissue and in invaginations of the cell membranes of myelin nerve fibres, finely reticular peritreponemal spaces were seen (Fig. 7). In areas, where treponemes were close to the nerve fibres, the myelin sheath became lamellar losing electron density (Fig. 8).

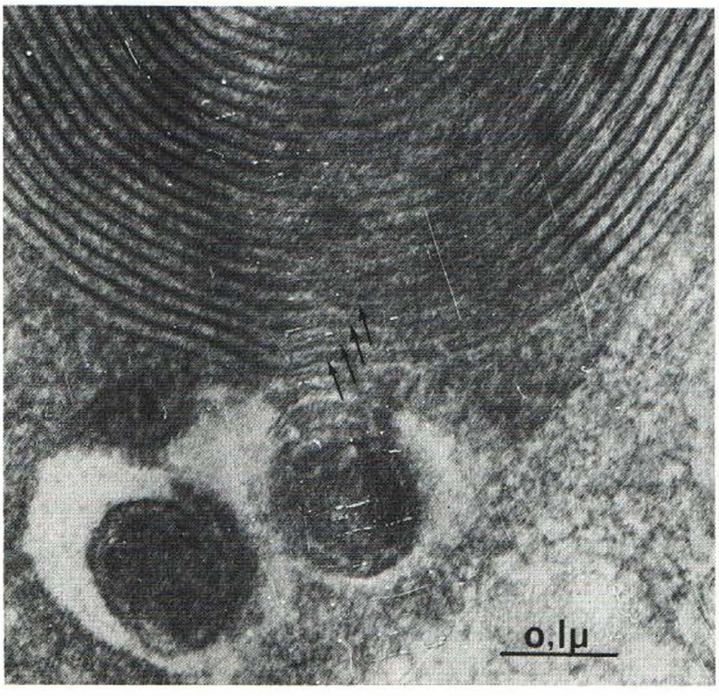


Fig. 8. Cross-sectioned treponemes (×150 000) in invagination of the cell membrane of a Schwann cell of a myelinated nerve fibre. Juxtatreponemal myelin sheaths show evidence of irregular lamination (→). The periplastic membrane is absent.

DISCUSSION

Presumably, the function of the axial filaments is related to the three-dimensional movements of the Treponema pallidum by fixing and contracting the spirally coiled cytoplasmic body (4, 16, 17). In order to contract the cytoplasmic body, the axial filaments must be fixed to this body in at least two areas. This is not compatible with another hypothesis based on studies of isolated treponemes suggesting that only one end of a bunch of 3–4 axial filaments inserts in the cytoplasmic body, while the opposite end terminates at the middle of the treponeme interdigitating with filaments originating from the opposite end of the treponeme (8, 9, 10). In this way, 6–8 parallel or nearly parallel arranged filaments should be demonstrable in the central part of the microorganism. In the present study of Treponema pallidum *in situ* no interdigitation was observed and the number of filaments never exceeded four, supporting the idea that only one bunch of 3–4 axial filaments, bipolarly attached, uninterrupted entwine the whole cytoplasmic body (7), thus being responsible for its contractility. The axial filaments are fragile and often disrupted during isolation of the treponemes. In at least some cases the phenomena of overlapping and interdigitation may be artefacts caused by shrinkage of the cytoplasmic body during fixation intermingling ruptured filaments and duplicating their numbers.

The presence of the periplastic membrane is inconsistently described. In some studies no membranous structure external of the cytoplasmic membrane was detectable in treponemes studied *in situ* or isolated (2). Sell & Norris (12) observed an outer membrane in treponemes obtained from rabbit orchitis. The structure was not seen in *in situ* preparations and consequently the investigators suggested that the external membranous structure seen in isolated treponemes was due to precipitates produced by preparation. A trilaminar external periplastic membrane has, however, been demonstrated in a few *in situ* studies of treponemes in human (18) and rabbit syphilis (11). The low and decreasing frequency of the periplastic membrane in chancres of increasing age in the present study of treponemes may settle the discussion of the existence of the membrane. Its function is unknown. Protective properties securing the survival of the treponeme in the host organism seems likely (19). As the syphilis infection becomes more advanced, the host organism should be supposed to be more capable of destroying the periplastic membrane. The membrane is seen only exceptionally in macular and papular secondary syphilis (15). The finding that areas of the cytoplasmic membrane covered by the periplastic membrane appears more well-defined supports a protection theory. The membrane is apparently not a prerequisite to avoid degenerative changes of a treponeme in the host organism (Fig. 8).

The existence of a treponeme-associated spreading factor in the syphilitic infection has been assumed for many years. Several recent studies indicate that the factor is the enzyme hyaluronidase uniformly present along the treponemal body surface (20, 21, 22). The enzyme hydrolyses hyaluronic acid, one of the components of intercellular matrix, and in this way the organism would be embedded in a lower molecular and less viscous substance derived from hydrolysed matrix facilitating treponemal penetration into tissues. Non-treponemal glucosaminoglycans have been observed to coat treponemes (23). The peritreponemal reticular halo reported here could be due to shrinkage of such a substance. The reticular halo surrounding treponemes invading collagen fibres could reflect that the microorganisms have a lytic, maybe collagenase provoked, effect on this tissue and explain, how the treponemes are able to penetrate collagen-rich meninges entering the cerebrospinal fluid.

A surface coat of host-derived serum proteins including IgM, IgG, and IgA has also been detected on the surface of treponeme isolates (24), and electron microscopy of isolated treponemes subject to the treponema pallidum inmobilisation (TPI) test (25–26) has

revealed bacteria covered by fuzzy materials. Immunological reactions on the treponemal surface could be expected to occur in a sensitized host. The narrow peritreponemal border of an electron dense amorphous material might represent deposits of immunoprecipitates and glycosaminoglycans giving the tangentially sectioned cells a spinous appearance.

REFERENCES

1. While VJ, Picard RG, Kearny EB. The morphology of spirochaeta pallida in the electron microscope. J Am Med Assoc 1942; 119: 880–881.
2. Sykes JA, Miller JN. Ultrastructural studies of treponemes: Location of axial filaments and some dimensions of Treponema pallidum (Nichols strain), Treponema denticula and Treponema Reiteri. Infect Immun 1973; 7: 100–110.
3. Swain RHA. Electron microscopic studies of the morphology of pathogenic spirochaetes. J Pathol Bact 1955; 69: 117–128.
4. Wiegand SE, Strobee PL, Glassman MS. Electron microscopic anatomy of pathogenic Treponema pallidum. J Invest Dermatol 1972; 58: 186–204.
5. Ovcimnikov NM, Delektorskij VV. Current concept of the morphology and biology of Treponema pallidum based on electron microscopy. Br J Vener Dis 1971; 47: 315–328.
6. Pillot J, Ryter A. Structure des spirochetes. Ann Inst Pasteur 1965; 108: 791–804.
7. Poulsen A, Kobayasi T, Secher L, Weismann K. The ultrastructure of Treponema pallidum isolated from human chancres. Acta Derm Venereol (Stockh) 1985; 65: 367–373.
8. Hovind-Horiger K. Determination by means of electron microscopy of morphologica criteria of value for classification of some spirochetes, in particular treponemes. Acta Pathol Microbiol Scand [B] 1976; Supplement 255.
9. Jepsen OB, Hovind-Horiger K, Birch-Andersen A. Electron microscopy of Treponema pallidum Nichols. Aca Pathol Microbiol Scand 1968; 74: 241–258.
10. Klingmüller G, Ishibashi Y, Radke K. Der elektronmikroskopische Aufbau des Treponema Pallidum. Arch Klin Exp Dermatol 1968; 233: 197–205.
11. Johnson RC, Ritzi DM, Livermore BP. Outer envelope of virulent Treponema pallidum. Infect Immun 1973; 8: 291–295.
12. Sell S, Norris SJ. The biology, pathology and immunity of syphilis. Int Rev Exp Pathol 1983; 24: 203–276.
13. Sell S, Baker-Zander S, Powell HC. Experimental syphilitic orchitis in rabbits. Lab Invest 1982; 46: 355–364.
14. Zeigler JA, Jones AM, Jones RH, Kubica KM. Demonstration of extracellular material at the surface of Treponema pallidum cells. Br J Vener Dis 1976; 52: 1–8.
15. Poulsen A, Kobayasi T, Secher L, Weismann K. Treponema pallidum in macular and papular secondary syphilitic skin eruptions. Acta Derm Venereol (Stockh), in press.
16. Musher DM. Spirochetes: Treponema and Borrelia. In: Baude AJ, ed. Medical microbiology and infectious diseases Philadelphia: WB Saunders Campany, 1981: 490-495.
17. Paster BJ, Canale-Parola E. Involvement of periplastic fibrils in motility of spirochetes. J Bacterial 1980; 141: 359–364.
18. Secher L, Weismann K, Kobayasi T. Treponema pallidum in peripheral nerve tissue of syphilitic chancres. Acta Derm Venereol (Stockh) 1982; 62: 407–411.
19. Fitzgerald TJ. Pathogenesis and immunology of Treponema pallidum. Ann Rev Microbiol 1981; 35: 29–54.
20. Fitzgerald TJ. Johnson RC. Mucopolysaccharidase of Treponema pallidum. Infect Immun 1979; 24: 261–268.
21. Fitzgerald TJ, Gannon EM. Further evidence for hyaluronidase activity of Treponema pallidum. Can J Microbiol 1983; 29: 1507–1513.
22. Fitgerald TJ, Johnson RC. Surface mucopolysaccharides of Treponema pallidum. Infect Immun 1979; 24: 244–251.
23. Strugnell RA, Handley CJ, Lowther DA, Farne S, Graves SR. Treponema pallidum does not synthesise in vitro a capsule containing glucosaminoglycans or proteoglycans. Br J Vener Dis 1984; 60: 8–13.
24. Alderete JF, Baseman JB. Surface-associated host proteins on virulent Treponema pallidum. Infect Immun 1979; 26: 1048–1056.
25. Hovind-Horiger K, Nielsen H Aa, Birch-Andersen A. Electron microscopy of treponemes subjected to the Treponema pallidum immobilization (TPI) test. I. Comparison of immuneimmo-

bilization (TPI) test. I. Comparison of immuneimmobilized cells and control cells. Acta Pathol Microbiol Scand [C] 1979; 87: 217–222.
26. Hovind-Horiger K, Birch-Andersen A, Nielsen H Aa. Electron microscopy of treponemes subjected to the treponema pallidum immobilization (TPI) test. II. Immunoelectron microscopy. Acta Pathol Microbiol Scand [C] 1979; 87: 263–268.