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Treponema pallidum in Macular and Papular Secondary Syphilitic Skin Eruptions

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The ultrastructure of biopsies from dry macular and papular secondary syphilitic skin lesions of 10 patients were studied by electron microscopy. In all biopsies few diffusely distributed treponemes were observed. This may explain the difficulties in demonstrating treponemes by darkfield examination of tissue fluid from dry secondary syphilitic skin lesions. The outlines of treponemes were less distinct as compared to those of primary syphilis. The periplastic membranes were almost invariably absent and the cytoplasmic membranes appeared in close contact with an enclosing layer of irregularly demarcated, electron dense amorphous substance. This substance may be a manifestation of the immune reaction of the host cells to the treponemes. Degenerations were noted in both unmyelinated and myelinated nerve tissue. This accounts for the fact that skin lesions in secondary syphilis are usually wihtout symptoms. Also in the vessel walls treponemes were demonstrated. The vascular endothelial cells were proliferating and the basement membranes were multilaminated and split. *Key words: Nerve degeneration; Periplastic membrane; Peritreponemal amorphous substance.* (Received September 5, 1985.)

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Several electron microscopic studies have been performed on the nature of experimental syphilis in rabbits (1–4), and primary syphilitic infection in man (5–8). However, the knowledge of ultramicroscopic details of the skin lesions of secondary syphilis is limited. It is not possible to employ the rabbit models ordinarily used for ivnestigative purposes of syphilis, since rabbits do not develop secondary syphilitic skin eruptions (9). In the literature we found six studies dealing with ultrastructure of secondary syphilitic manifestations (10–15). In these, no differences in the morphology of treponemes in primary and secondary syphilis were noted. In the present study, such differences as well as degenerative changes in vessels and nerves are illustrated.

MATERIAL AND METHODS

Skin specimens from 10 patients (8 males, 2 females) with secondary syphilitic efflorescences of 3 to 12 weeks' duration were examined. In two patients roseola was diagnosed, whereas 8 patients had dry, papular lesions. In all patients both Wassermann test and antitreponemal antibodies in serum were strongly reactive. Three mm punch biopsies were removed from typcial lesions on the trunk. Ethyl chloride spray served as local anaesthetic. The specimens were immediately fixed in ice-cooled 6% glutaraldehyde in 0.5 M cacodylated buffer pH 7.2 with 7.5% sucrose. The samples were osmicated, dehydrated in a series of alcohol solutions and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by a JEOL 100 CX electron microscope.

RESULTS

Treponemes were found in all biopsies, but present in a modest number. In the individual field of view, at a magnification of 2000, the number of treponeme fragments observed did

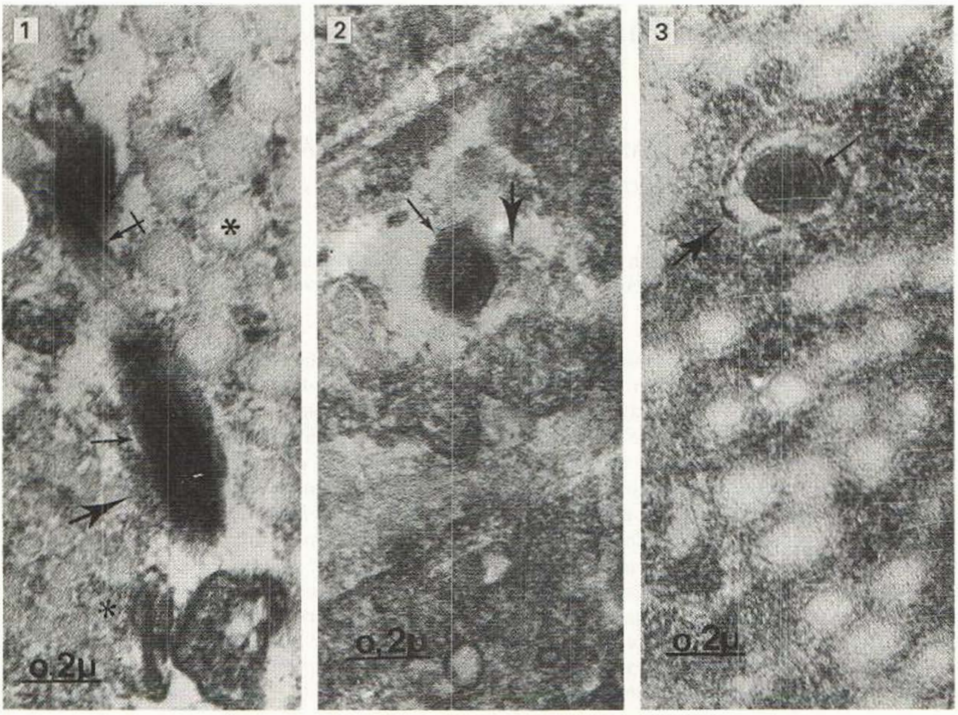


Fig. 1. Treponema pallidum (×60 000) embedded in an amorphous substance (➔) irregularly delimited from surrounding collagen fibres (*). No periplastic membrane is visible. The cytoplasmic membrane (→) and the axial filaments (↔) are seen in intimte contact with the amorphous substance.

Fig. 2. Cross-sectioned Treponema pallidum (×60 000). The 3–4 axial filaments are diffusely demarcated from each other (⟶). The treponeme, partly surrounded by amorphous substance (➔), is delimited from the surrounding tissue by a structureless zone. This empty space is probably due to shrinkage of oedematous tissue around the treponeme during the fixation process.

Fig. 3. Treponema pallidum (×60 000) with well demarcated cytoplasmic (→) and periplastic membrane (➔) embedded in an amorphous and granular substance.

not exceed 4, and, in most cases, they were solitary. The spirochetes were seen in the epidermis and the dermis both extracellularly between infiltrating cells and intracellularly in macrophages as well as in vessel walls and in nerve fibres. The cytoplasm of the treponemes appeared electron dense and homogeneous without well defined cytoplasmic structurs. The trilaminar cytoplasmic membrane was just recognizable, but especially the external outline of the membrane was poorly demarcated. When tangentially cut the axial filaments of the treponemes were recognized externally to the cytoplasmic membrane (Fig. 1). In transverse sections, the outlines of the axial filaments were blurred (Fig. 2). In exceptional cases the cytoplasmic membrane and the axial filaments were surrounded by a membranous structure, probably the periplastic membrane (Fig. 3). In most cases, such a membrane was not detectable. The cytoplasmic membrane and the axial filaments were coated by an electron dense amorphous substance, irregularly surrounded by a light zone without structural components. The cellular infiltrate in the present 9 biopsies was dominanted by mononuclear cells, mainly histiocytes, lymphocytes and plasma cells, did not differ from what has been described elsewhere in histopathologic studies of secondary syphilis (16, 17). In a few histiocytes, treponeme-like electron dense structures with a

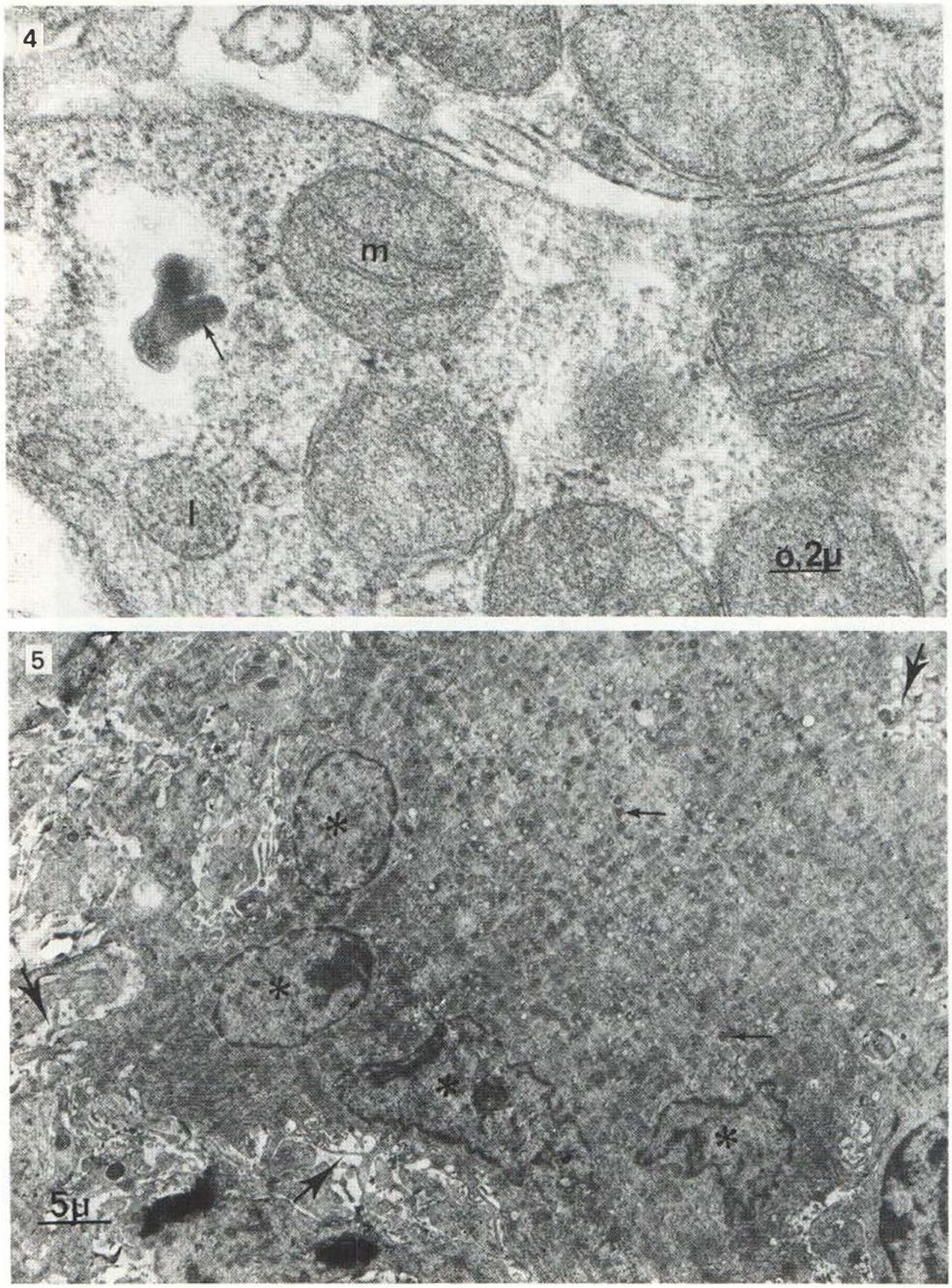


Fig. 4. Intracellular treponeme (×60 000). Mitochondria (*m*). Lysosome (*l*). The microorganism is surrounded by a bright lucid zone. The central part of the treponeme appears electron dnse, whereas the periphery is less electron dense. The axial filaments are scarcely recognizable (⟶).

Fig. 5. Giant cell in a secondry syphilitic papule (×4 000). The nuclei are scattered along the periphery of the cell (*) from which villous proceses protrude (➔). In the centre of the cell numerous lysosomes are seen (⟶).

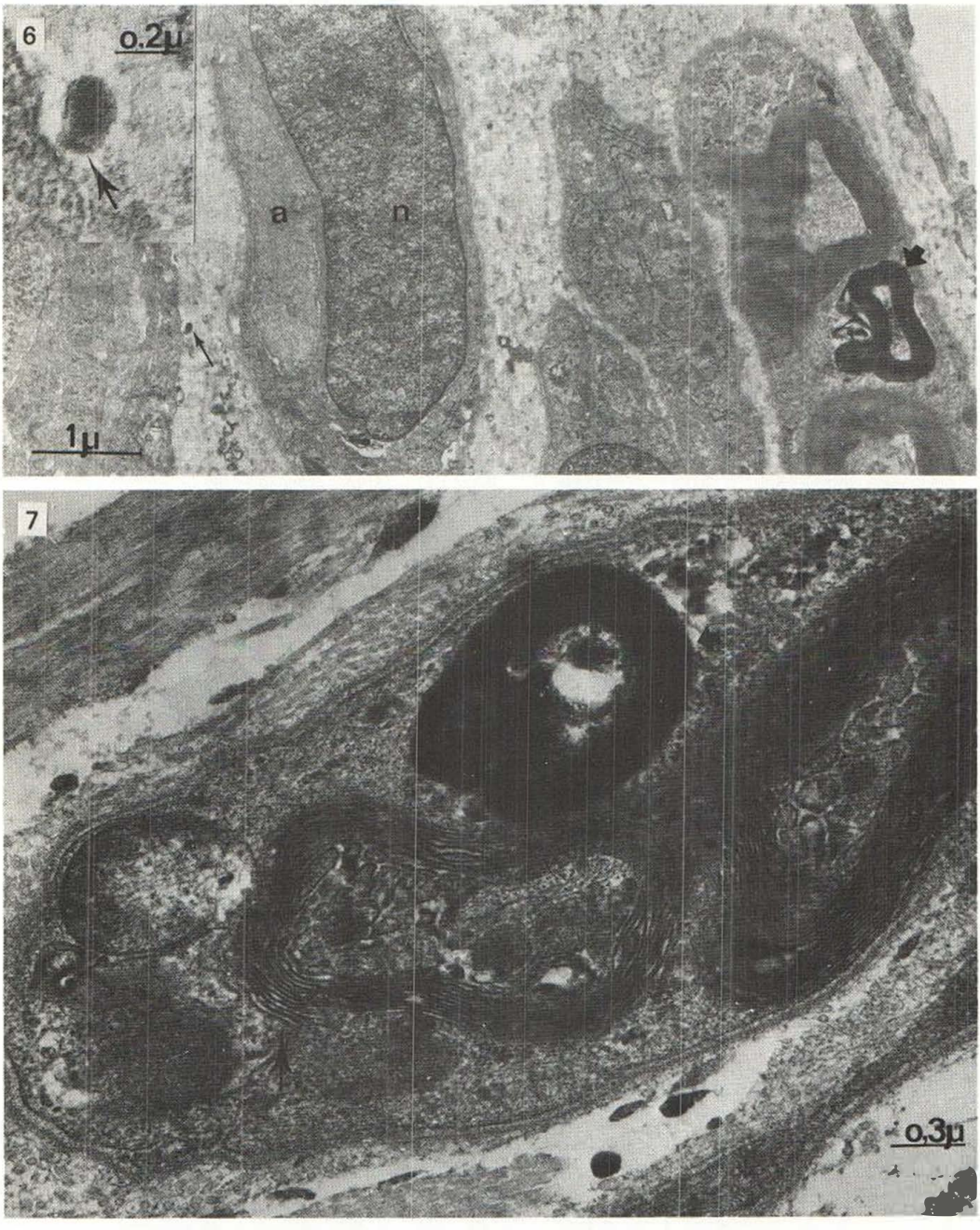
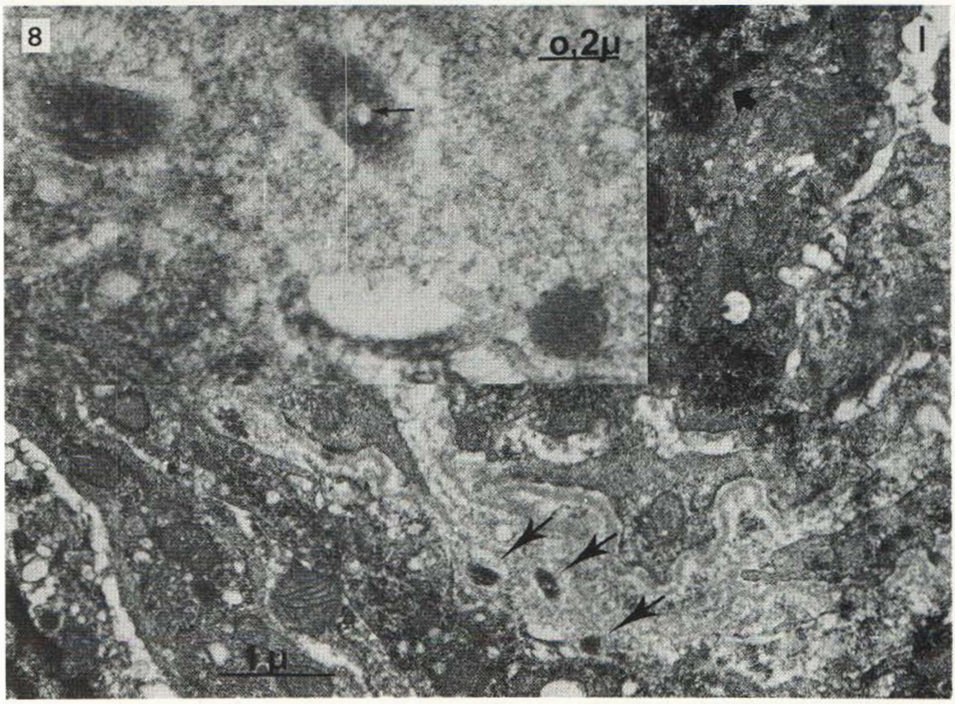


Fig. 6. Treponema pallidum (→) in endoneurium of a cutaneous nerve fibre (×10 000). At the inset (×60 000) the axial filaments of the partly tangentially sectioned treponeme are demonstraed (➔). Nucleus of umyelinated nerve fibre (*n*). Axons of unmyelinated nerve fibres (*a*). One of the myelin sheaths shows higher degree of electron density when compared to the others (◗).

Fig. 7. Degenerative processes in a myelinated nerve fibre in a biopsy from a syphilitic papuel (×30 000). In two of the myelin sheaths the laminae are split and fragmented losing their electron density (➔) and their axons are granular and vacuolized. The myelin around one axon (◗) appears electron dense and almost homogeneous.



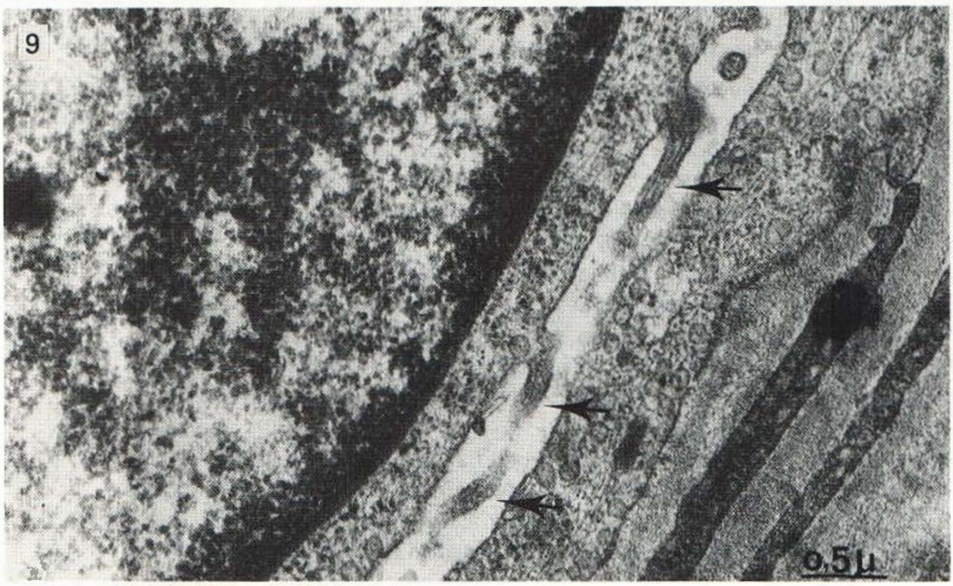


Fig. 8. Treponema pallidum (➔) in a multilaminated, fragmented and split basement membrane of a vessel (×15 000). At the inset (×60 000) the treponeme shows evidence of degeneration with blurred outlines and vacuoles in the cytoplasmic body (→). Lumen of the vessel (*l*). Nucleus of endothelial cell (◗).

Fig. 9. Wavy villous processes from the surface of an endothelial cell (×40 000) are protruding into the lumen of a capillary (➔). Such processes may be mistaken for treponema pallida. In these organisms, however, the axial filaments spirally entwining the cytoplasmic body give the treponeme fragment a cross-striped appearance. A lamellar configuration as seen in the villus demonstrated is not found in the treponemes.

lighter periphery were noted in vacuoles. Because of recognizable axial filaments such structures could be identified as phagocytized treponemes (Fig. 4).

In two biopsies from papular lesions multinuclear giant cells of Langhans type were observed. Several nuclei were distributed along the periphery of the cells from which villous dytoplasmic protrusions were radiating. Numerous mitochondria and lysosomes were dispersed in the cytoplasm. Treponemes could never be demonstraed in the giant cells (Fig. 5).

Treponemes were observed in perineurium and in endoneurium of peripheral nerves (Fig. 6). Degenerative processes were predominantly observed in myelinated nerve fibres. Some myelin sheaths were split or fragmented losing their electron density, whereas others were thinned showing a high degree of electron density. Vacuoles were observed in myelinated and unmyelinated axons. In Schwann cells granulated and vacuolated cytoplasm was noted (Fig. 7). Proliferating endothelial cells with numerous, partly swollen mitochondria, abundant smooth endoplasmic reticulum and large pale nuclei, were observed almost occluding the lumen of the capillaries. The basement membrane of vessels was thickened, here and there multilaminated and fragmented. Treponemes could be demonstrated in invaginations of endothelial cells as well as between the laminas of the vascular basement membranes and around vessels. In the vessel walls some treponeme fragments presented vacuoles in their cytoplasm (Fig. 8). When projecting into the lumen, wavy villous processes of endothelial cells imitated treponemes, but could be discerned from these by the absence of crossing axial filaments (Fig. 9).

DISCUSSION

Electron microscopic studies of syphilitic chancres have demonstrated numerous, often accumulated, treponemes in the tissues, especially nerves and vessels (5–8). No such places of pre-dilection were observed in the present study and the scanty treponemes present explain why it may be difficult to demonstrate treponemes by dark field examination of tissue fluid from dry macular and papular secondary syphilitic efflorescenses (18–19). However, moist secondary syphilitic skin eruptions, such as condylomata lata, contain numerous treponemes.

The fine structure of the treponemes was less distinct as compared to the findings in primary syphilis (6) and in experimental syphilis (3, 4), but characteristic features such as the cytoplasmic membrane and the axial filaments were regularly recognized. Dense amorphous substance on the treponemal surfaces has been noted in experimental syphilitic lesions of rabbits (4). Recent studies have indicated that this substance is not synthesized by the treponemes but is probably produced by the host organism (10).

In treponemes isolated from human primary syphilis (21) and from rabbit syphilitic orchitis (22) as well as treponemes in ultrathin sections from human chancres (23) an enveloping periplastic membrane external to the axial filament and a cytoplasmic membrane are observable. Such structures were only exceptionally seen in the present study. In most cases, the cytoplasmic membrane appeared in direct contact with the surrounding electron dense amorphous substance. In the secondary stage of syphilis various antitreponemal antibodies are produced (24). The periplastic membrane almost invariably absent and the indistinct outlines of the treponemes studied as well as the strong electron density and the vacuolization of the cytoplasmic body may be explained as defects caused by host immune reactions against treponemes. The reactions might also precipitate the surrounding amorphous substance and herebty inhibit the movements and facilitate the phagocytosis of the treponemes. The amorphous substance has previously been observed around phagocytized treponemes (4).

Degenerative processes in axons in human chancres have been reported (8). Treponemes have been demonstrated in epi-, peri-, and endoneurium of peripheral nerves in human and rabbit chancres (3, 6). In cultured sensory neurons from rat embryos the treponemes caused perforations of the cell surfaces and an electrophysiologic dysfunction of the cells (25). The findings of this study illustrate that, in secondary syphilis axons, myelin sheaths and Schwann cells may be subject to severe degenerative processes. The phenomena may explain why the cutaneous lesions in secondary syphilis are usually neither itchy nor painful.

Following infection the treponemes immediately enter blood stream and disseminate to all parts of the organism (26). In primary syphilis the treponemes have been demonstrated to accumulate in the subendothelial spaces of capillaries (8) and have also been found in the lumen of capillaries and lymph channels (7, 11). The skin reaction in secondary syphilis is due to treponemes which have left the vascular system and once again have penetrated into the tissue. Virulent treponemes attach to cells with their nose pieces, i.e. their fine-tipped endpoints (27). The mechanisms of penetration is suggested to be correlated to hyaluronidase produced by the treponemes (28). This enzyme may degrade intercellular hyaluronic acid separating the endothelial cells, thus leaving the treponemes room to enter the surrounding tissue (29). The degenerative changes in the vessels appear similar to those described in primary syphilis (8).

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