

MORPHOLOGY OF SPIROCHAETA MYELOPHTHORA IN MULTIPLE SCLEROSIS

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In a recent paper (1) the findings of specific spirochetes in the brain of a newly examined subacute case of multiple sclerosis were reported and evaluated. The purpose of the present paper is to give a detailed description of these spirochetes, their classification, their reproduction, and disintegration.

Four cases of multiple sclerosis, including the case to be reported, elicited abundant numbers of specific spirochetes in the central nervous system to warrant the publication of this paper.

1. *Further Evidence of Spirochetal Nature:* It has been said that the reported spirochetes represent only spirochete-like structures of the tissue proper, such as reticulin fibrils, neurofibrils, or axis cylinders.* To disprove these objections the following experiments were done: Sections from brains of general paresis containing numerous spirochetes in the cortex and those from lungs in congenital syphilis were stained with my silver technique II (1), then desilverized with potassium permanganate and oxalic acid (A. J. Wilson (2)), and restained for reticulin fibers (Wilder's method). They showed clearly the complete absence of spirochetes in these restained sections, at regions where many spirochetes were formerly seen. Reticulin fibrils were easily demonstrable. Such sections could be desilverized again and restained for spirochetes with positive results. In sections stained for axis cylinders (Bielschowsky's axis cylinder method for paraffin sections), no spirochetes were seen in places where previously masses of treponemas were present. Desilverizing and restaining with technique II brought the spirochetes out again. By the same procedure, applied to sections of brains of the cases of multiple sclerosis, reticulin fibrils in vessel walls appeared and no spirochetes were seen, while in these sections, desilverized again and stained with technique II, the spirochetes reappeared as before. This is definite proof that the "spirochete-like structures" are not related in any way to fibrillar reticulin elements, neurofibrils, or axis cylinders. This method of desilverizing sections of brain tissue containing the spirochaeta myelophthora following technique II, photographing the area in question, restaining these sections with different methods (Mahon stain for myelin sheaths, inflammatory reactions etc.), allows also a much better insight into the histopathogenetic sequence of events.

2. *Morphology and Polymorphism of Spirochaeta Myelophthora:* The polymorphic appearance is limited to a comparatively small amount of differences in shape, length, thickness or other irregularities. A common form is, what I call, the bayonet form characterized by a bend in the longitudinal axis in the middle portion of the individual spirochete so that the more peripheral portions run almost parallel. Figures 1a to e show this fairly characteristic type. Variations in length are regularly found. Probably the entire substance of the individual spirochete may be shortened by contraction in the longitudinal axis and by this process a short, thick, and rather straight, rigid form may appear (fig. 1f). In these rigid forms occasionally shallow spirals are seen (fig. 1g). The short and thick forms are seen often in the vicinity of haptocytes, but are entirely extracellular (fig. 1h). Such forms have been reported by Austregesilo (3) and Blackman (4). One of the important characteristics are the rather regular shallow spirals as seen in Figures 1i, j and j₁. In Figure 1k, a com-

* An interesting historical parallel is that the first demonstrations of treponema pallidum in fixed tissues (Bertarelli-Volpino's, Levaditi's silver techniques) met the same unjustified objection of being reticulin fibers and not spirochetes.

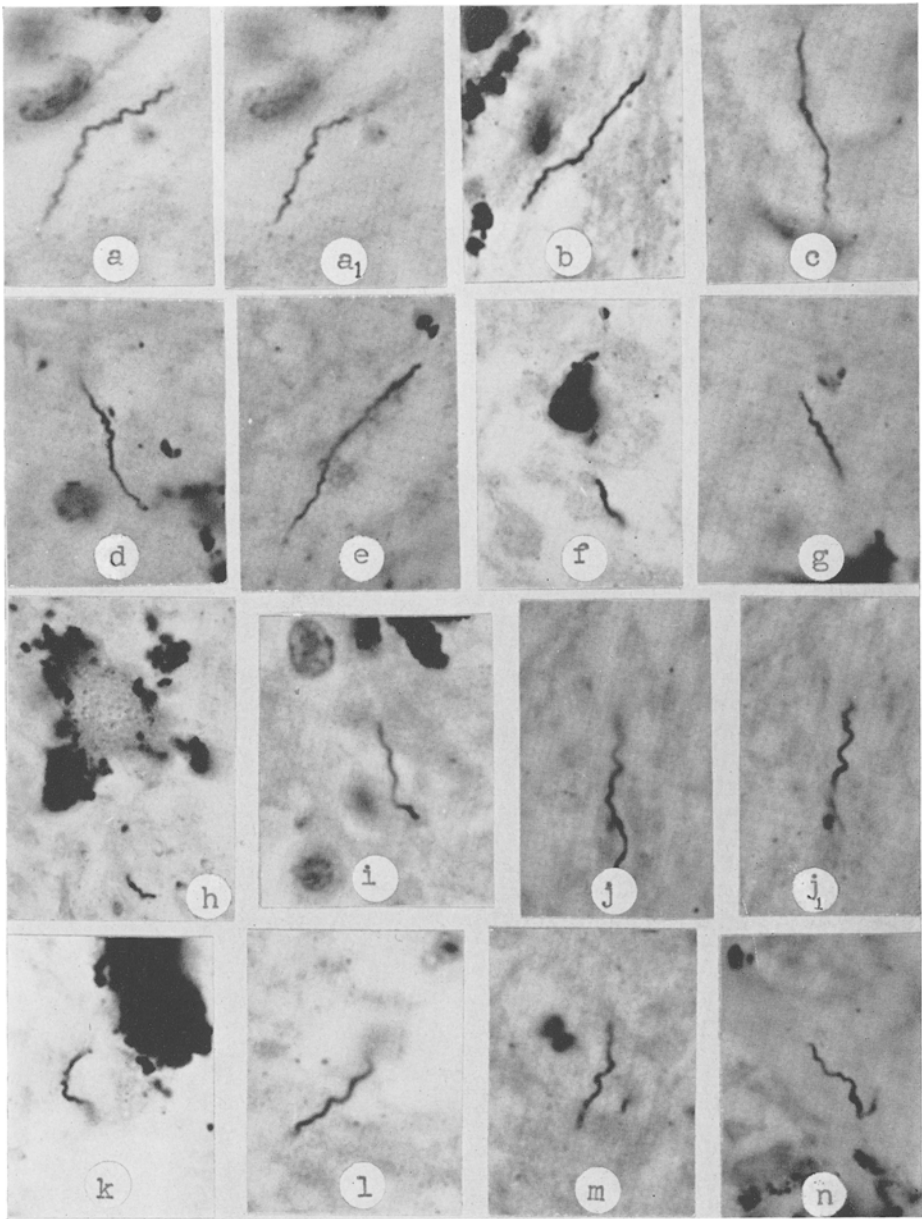


FIG. 1. a to e, Typical "bayonet" forms: a and a₁, bayonet form of spirochaeta myelophthora in two different focuses of the same microscopic field; f, short, thick, straight and rigid form; g, rigid form with shallow spirals; h, short and thick spirochetal form in the vicinity of a haptocyte; i to m, regularly coiled spirochetes: i, spirochete with shallow spirals; j and j₁, two different focuses of the same field; k, rather short spirochete near nerve cell of substantia nigra with 7 regular spirals; l and m, regularly coiled forms; n, large hook at one end; o and o₁, the same microscopic field photographed in two different focuses to show two spirochetes; p, spirochete with terminal hook and unusually narrow spirals.

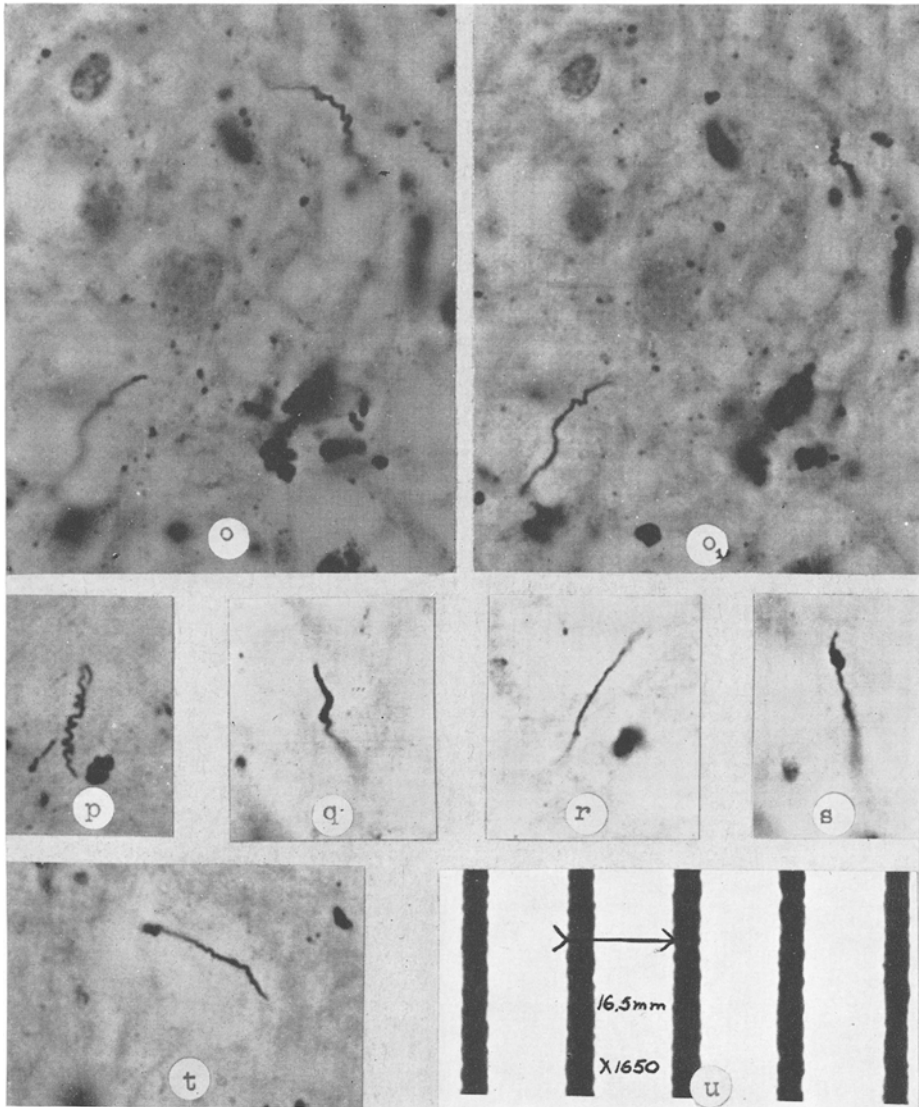


FIG. 1. (*continuation*) q to t, beginning disintegration of *spirochaeta myelophthora*: q, thickening in the middle portion of a spirochete as a sign of beginning of disintegration; r, s, t, knob formation in terminal portion or in peripheral portions of the spirochetes; t, the spirochete is located in a vacuole; u, micrometer photograph one unit: 10 microns.

The photomicrographs are taken from sections stained with Steiner's silver technique. Due to reproduction in print and reduction in size, the original magnification has changed. Original optical magnification, $900\times$. The magnification can be measured using the micrometer unit in Figure 1 u, representing 10 microns.

paratively short individual spirochete, near a nerve cell of the substantia nigra with at least 7 rather regular spirals, is seen. Somewhat irregular sizes of spirals are shown in Figures 1l and m. Most characteristic is the corkscrew-like regularly coiled form with 5 to 12 single shallow spirals and with always rounded, never pointed, crests and roots.

Occasionally, a single spirochete may exhibit one regularly coiled and another more straight portion. There can also be an irregular variation in the angle of the spiral, but the angle is always rounded. The individual spirochete tapers off at both ends to a thinner portion with a sharply pointed end. Not rarely a larger hook-like appearance at one end is seen (fig. 1n); the hook at the end shows a round, arc-like bending and is entirely different from the characteristic whip-like ends of the leptospiras. Loops, incomplete, nearly complete or totally complete rings are occasionally seen. The variation in size and coiling as well as the difficulties photographing spirochetes in sharp focus can be seen in Figure 1o and o₁, in which 2 individual spirochetes are reproduced in the same microscopic field at 2 different focuses. In Figure 1p the hook-like appearance and unusually narrow spirals with more acute angles are shown. Thickening in the middle portion of the spirochete is seen occasionally, which might possibly be already a sign of beginning disintegration (fig. 1q). Knobs in one of the peripheral portions of the individual spirochete or at one end of it are found occasionally (fig. 1, r, s, t). Spirochetes with knobs are usually more rigid, straight or irregularly coiled, indicating perhaps the beginning of disintegration. For an exact measurement of length, width of the individual spirochetes and its spirals a micrometer unit 0.01 mm. was used (fig. 1u).

Measurements: Length of longitudinal axis: 3–16 microns. There are coarse, flat, regular and also irregular spirals. Number of spirals: inconstant, 3 to 6 or more. Spiral amplitude: rather regular, 1.2 to 1.5 microns. Crests and root angles always roundly curved. Spiral depth of 0.2 to 0.6 microns.

The limited polymorphism of micro-organisms is nothing unusual in microbiology. Especially in old cultures or in chemically and antibiotically treated cases micro-organisms very often exhibit bizarre forms.

3. *Classification:* The classification of spirochaetales is still not very satisfactory. Up to the present time 3 genera are recognized among the family of Treponemataceae (Bergey (5)): treponema, borrelia and leptospira. The leptospiras differ from the other 2 genera by their very minute, regular primary spirals, their sharply pointed crests and roots, and their whip-like arrangements on both ends. The movements of the individual leptospiras, as seen in darkfield observation, differ from those of treponemas and borrelias. Morphological characteristics do not permit a classification or differentiation of single species (types, subspecies), since it is well known that in each of the 3 morphologically distinguishable genera, treponema, borrelia and leptospira, *morphologically identical*, but pathogenetically and antigenetically different spirochetes are known; for example, treponema pallidum, treponema pertenue, treponema cuniculi are identical in size and shape. In the borrelia group all the various agents of relapsing fever look very much alike. In the leptospira genus leptospira icterohaemorrhagiae, canicola, pomona, grippotyphosa and numerous others are morphologically identical, and the differentiation is based entirely upon their pathogeneticity for different animals, including man, upon the production of a specific clinical symptomatology, if pathogenic, and especially upon their specific antigenic property. It would be too early, and at present entirely inadequate, to attempt a definite classification of the spirochaeta myelophthora. For this purpose cultures of these microbes in artificial media would be necessary, which are not available. In case of a culture, the movements of the live spirochete could be examined in darkfield preparations and the antigenic properties could be investigated. What can be said now, with all reservation, is that the spirocheta myelophthora, taken from its morphological appearance in fixed central nervous system tissues, seems to belong to the genus borrelia of the spirochaetales, family of Treponemataceae.

4. *Reproduction:* Spirochetes reproduce by transverse fission. The division is initiated by a longitudinal growth of the individual spirochete. Elongated spirochetes mean older individuals, mature for fission. In young leptospiral cultures the elongation of short forms to longer individuals can be easily followed insofar as in young cultures the majority of spirochetes are of short length, and with increasing time more and more numbers of longer individuals are seen.

The division of spirochetes is preceded by a reduction in thickness at one place of the longitudinal axis into a very delicate fine filament which indicates the place of final separation into 2 individuals.

The process of *final separation* of one spirochete into 2 must be instantaneous. Consequently, it is not seen often in darkfield observation of young cultures of living treponemas, leptospiras, and borrelias. In permanent and stained preparations of smears, made from tailblood of mice 5 to 10 minutes after intraperitoneal inoculation with borrelia duttoni, beginning and completed transverse divisions can be demonstrated (6) (fig. 1a, b). In cultures, to which sublethal doses of antibiotics are added, the transverse division is often inhibited with the result of extreme elongation of organisms, showing long irregular filaments which may be 10 or more times longer than the average individual spirochete. Grotesque forms of giant length may be seen in cultures of culturable spirochetes. A close parallel to this inhibition of transverse fission and the evolution of bizarre elongated forms has been observed in many bacterial organisms. In Fleming's opinion these elongated bacterial filaments are due to the failure of bacterial division.

Theoretically there are at least 2 different modes of transverse fission possible, namely simultaneous reproduction of 2 (one separation) or 3 daughter spirochetes (2 separations) with complete disappearance of the mother spirochete. Another possibility could be *successive* reproduction into 3 or more daughter spirochetes with the mother spirochete remaining intact.

Interpretation of reproduction of well known spirochetes in live cultures is difficult. For this reason one can not expect to find any indication of spirochetal reproduction in sections prepared from *fixed tissues* in multiple sclerosis. Many years ago I studied treponema pallidum in numerous brains of cases of general paresis and other syphilitic tissues, particularly of congenital syphilis and leptospiras in kidneys of rats containing numerous organisms. The massive multiplication of treponema pallidum in innumerable individual spirochetes in tissue agglomerations may be called *colonies*. By comparing the spirochetes in acute cases of general paresis with those in less recent cases of the same disease, a reconstruction of the mode of invasion shows that the treponemas migrate from the leptomeninges by way of vascular *walls* (capillaries and larger vessels). They grow in long and parallel chains in the endothelial and adventitial tissues along the longitudinal axis of the blood vessels. The next step is the migration of the spirochetes through the barrier membranes, intima piae and limitans gliae perivascularis into the parenchyma proper, where they may appear in large colonies. Vascular walls serve as pathways for the invasion of treponemas from the surface of the brain, of the optic nerves and of the posterior roots (Sven Ingvar) into deeper structures. Moreover, these vascular walls probably also serve as favorable tissue media for growth and reproduction of the treponemas. Therefore, in general paresis a definite regional succession of invasive and reproductive phases of treponemas can be recognized: 1. in the leptomeninges; 2. in the vascular walls, in the pial funnels and the intracortical capillaries and other blood vessels; 3. penetration into the parenchyma proper with production of colonies; 4. diffuse distribution in parenchyma, and finally, 5. disintegration. Our chance to obtain post mortem an early preclinical or early clinical invasive phase (leptomeninges, pial funnels, and superficial intracortical blood vessels) in cases of general paresis is minimal, since our successful therapeutic measures prevent fatal outcome in such early stages. However, there is enough evidence from our former studies of general paresis and a recent acute, untreated, case for the establishment of this chronological sequence in reproduction and invasion of treponemas into the brain of general paretics. *Individual growth and reproduction in vascular walls precedes the parenchymal distribution.*

In *multiple sclerosis*, as in other chronic spirochetal infectious diseases, there is no continuous reproductive activity of the organisms. Their propagation may occur at regular or irregular intervals of time. No cultures of the spirochaeta myelophthora are available at present. Consequently, the facts concerning the morphological identification must be properly evaluated so that mistakes can be avoided as much as possible.

1. The first fact is the presence of enormous masses of extracellular and intracellular argyrophilic granular bodies in *recent* plaques of multiple sclerosis. This is nothing unusual in comparison with other acute or chronic spirochetel diseases, such as relapsing fever and syphilis in which first an extracellular breakdown of spirochetes and after that an intracellular ingestion of spirochetel debris is found. The pictures of extracellular disintegration of spirochetes in syphilis reported by Warthin and Olsen (7) are not different from the breakdown in other spirochetel diseases. They are the same in multiple sclerosis. If the granular bodies in multiple sclerosis are developing from broken-up spirochetes, and there is much evidence for it, the possibility of previous presence of countless numbers of actively multiplying spirochetes in the tissues is not far fetched.

2. In areas of massive breakdown of spirochetes comparatively few, but well preserved, spirochetes are frequently seen; they represent stragglers, left over from innumerable spirochetes which have disintegrated. These few stragglers may be the parents of new generations of spirochetes.

3. Reproduction of spirochetes may be recognized in the involved tissues. One may expect forms of unusual giant length. In Figure 2a, a₁ such a giant form is photographed in 2 focuses. Figure 2b, is illustrating the transverse division of the spirochaeta myelophthora. The fission is indicated by the 2 spirochetes retaining the same longitudinal axis. The neighboring ends of both spirochetes are tapering off and pointed, leaving only a short distance between them. The length of both spirochetes differs, one is only $\frac{1}{2}$ of the length of the other. Another indication of fission, Figure 2c and c₁, is suggested by the appearance of 2 spirochetes with their longitudinal axis arranged in nearly parallel direction to each other. In such a case the division may have occurred at a point where both portions of the mother-spirochete were bent to an inclination of nearly 180°.

In our 4 cases with abundant numbers of spirochetes in brain or spinal cord tissues, pictures, indicating transverse fission, are rather rare. My estimation is 1 spirochete in fission to 1200 without indication of fission.

4. Colonies of spirochetes are present in the case of very active spirochetel reproduction in the organs and tissues of congenital syphilis or in the cortex of brains of cases of general paresis (8). Agglomerations of spirochetes were seen, in rare instances, in vascular walls inside recent periventricular plaques in the 4 studied cases of multiple sclerosis. An accumulation of rather straight spirochetes in the spinal cord and parenchyma proper was found in another case of multiple sclerosis. It is, however, doubtful whether or not these accumulations represent reproductive phases or accumulations preliminary to the beginning of disintegration.

5. There is not the least evidence for an *intracellular* reproductive activity of spirochetes in nerve cells or other cells of the human tissue. The cells are not even used as shelters by the spirochetes. This is in accordance with the behavior of other classified spirochetes. In multiple sclerosis the spirochetes are located only in *extracellular* regions of the cerebral and spinal cord tissues. The process of individual growth and reproduction does not involve any intracellular phase and is exclusively extracellular.

6. The question also arises as to whether or not permanent inactive forms, such as spores, do exist. One may also ask, whether there are other forms of the spirochaeta myelophthora which are not visible with the light microscope which, however, could represent one particular ultravisible phase in the life and reproductive cycle of these organisms. It seems useless to speculate about such a possibility, since no morphological facts favor such a theory. The granular bodies, as in other spirochetel organisms, represent in all probability, remnants of disintegrated spirochetes. Many attempts to establish an evolutionary cycle of the granular bodies into spirochetel forms in many other well known spirochetel genera have failed to demonstrate convincing evidence.

5. *Disintegration*: There is a definite sequence of events in disintegration of the spirochaeta myelophthora. Breaking-up starts with the appearance of loops, rings (fig. 2d), knobs, (fig. 1 r, s, t), partial thickening and the formation of granules of different sizes. These special morphological appearances represent probably the beginning and final

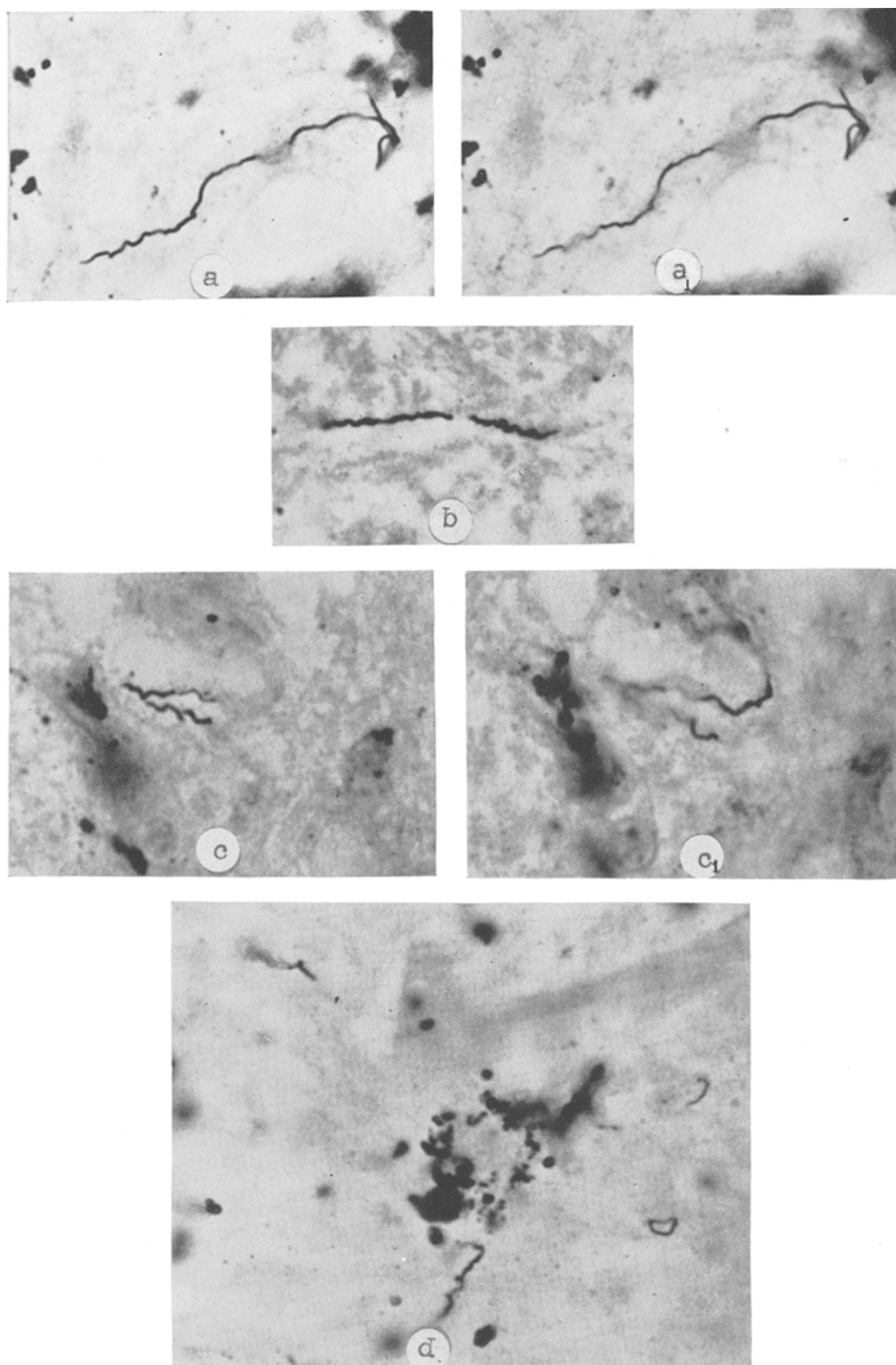


FIG. 2. a and a₁, *spirochaeta myelophthora* of giant length in two different optical focuses, probably shortly before transverse fission; b, transverse fission of *spirochaeta myelophthora* retaining the same longitudinal axis; c and c₁, probable transverse fission of *spirochaeta myelophthora*, the parent spirochete apparently was bent into a very acute angle so that the daughter spirochetes run nearly parallel, two different focuses of the same object; d, several spirochetes in the beginning of disintegration; ring form close to haptocyte.

disintegration of the spirochetes. Two chronological sequences may be established: a first phase is the extracellular location of intact, active and probably motile spirochetes, followed by a second phase of extracellular disintegration in granular form. The intracellular ingestion of spirochetal debris seems to be a later phase of the pathological process. There is a positive chemotaxis between astrocytes and intact spirochetes, so that extracellularly located spirochetes are seen occasionally close to hypertrophic astrocytes, so-called haptocytes. Intracellularly located intact spirochetes in astrocytes or microglial cells were never seen.

It should be mentioned here that the disintegration of the spirochaeta myelophthora proceeds in the tissue rather rapidly. In all our cases tissue blocks, containing abundant numbers of spirochetes, were less numerous than blocks containing extracellular and intracellular spirochetal debris without spirochetes. In old plaques, there is no chance to find argyrophilic granules or intact spirochetes.

6. *Pathogenesis of Tissue Damage*: The parallelism between the clinical manifestations, inflammatory reactions and their locations in regard to the presence of the spirochaeta myelophthora in acute lesions certainly indicates the pathogenetic significance of these spirochetes. Our finding of extracellular granules is leading to the disclosure of intact spirochetes especially by using serial sections through entire tissue blocks. The heterophasic nature of the disease process should be, however, emphasized. It is characterized by the presence of histologically old, more recent and of fresh plaques; the variation of inflammatory reactions in relation to the stage of the plaque and the number of well preserved spirochetes and extracellular spirochetal granular debris in or near acute plaques is impressive.

A detailed histopathogenetic analysis is still a postulate of the future. The spirochetes appear in complete detachment from any tissue element proper, often surrounded by a small halo. This micro-halo has been mentioned by several observers concerning the appearance of *treponema pallidum* in stained sections of the central nervous system. In some of my sections the spirochetes were surrounded by a definite microscopic vacuole in which no tissue elements were found (fig. 1t). This may or may not indicate a tissue-liquefying power of the spirochetes.

It is certainly premature to speculate about effects of exotoxins, endotoxins, or other chemical noxious substances derived from the spirochetes. Mechanical injuries to the tissues by these rapidly self-movable micro-organisms have to be considered also. There is finally a possibility that an indirect stimulation of neuroglial cells, such as astrocytes or microglial cells or even oligodendroglial cells (Lumsden (9)) by these micro-organisms may take place as a result of which these cells develop a myelolytic activity.

SUMMARY

Further evidence of the spirochetal nature of specific structures in brains and spinal cords of Multiple Sclerosis is presented. The morphology and the polymorphism of the spirochaeta myelophthora is discussed. Classification, reproduction and disintegration of these organisms in tissues are reported. The pathogenesis of tissue damage is outlined.

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