SYMBIOSIS (2009) 47, 51–58 ©2009 Balaban, Philadelphia/Rehovot ISSN 0334-5114

Position paper.

Spirochete round bodies
Syphilis, Lyme disease & AIDS: Resurgence of "the great imitator"?

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(Received August 18, 2008; Accepted August 31, 2008)

Abstract
We advocate investigation of spirochete cyclical symbioses (e.g., *Borrelia* sp*., Leptospira sp., Treponema* sp.) given the newly established verification of a developmental history in these gram-negative motile helical eubacteria, both in pure culture and in mammals. Symbiotic spirochetes can be compared to free-living relatives for their levels of integration (behavioral, metabolic, gene product or genetic levels). Detailed research that correlates life histories of symbiotic spirochetes to changes in the immune system of associated vertebrates is sorely needed. Genome analyses show that in necrotrophic symbioses (*Borrelia* and *Treponema* sp.) of humans and other primates, integration of the bionts occurs at the gene product and genetic level. Spirochete round bodies (also called cysts, L-forms and sphaeroplasts) can be induced by many types of unfavorable conditions (e.g., threats of starvation, desiccation, oxidation, penicillin and other antibiotics). Reversion to familiar helical, motile active swimmers by placement of pure cultures into favorable environments in some cases can be controlled. These observations are supported by a European literature, especially Russian, apparently unknown to American medicine and medical research.

Keywords: Spirochete cysts, *Treponema pallidum*, *Borrelia burgdorferi*, AIDS co-factor, immune suppression, STD, spirochetoses, *Spirosymplokos*, fossil spirochetes, spirochete life histories, *Mixotricha paradoxa*, round body reversion

1. Introduction

At a small meeting, *Spirochaete Co-evolution in the Proterozoic Eon: Ecology, symbiosis, and pathogenesis (an excursion into environmental immunology)* organized by Prof. Dr. Wolfgang E. Krumbein and Prof. Lynn Margulis held in the Museum für Naturkunde (Berlin, May 1–2, 2008) we scientists, medical researchers, historians and physicians endorsed this statement.

> *Powerful new techniques of microbiology, including molecular ecology and evolution inspire us to urge reinvestigation of the natural history of mammalian, tick-borne, and venereal transmission of spirochetes in relation to impairment of the human immune system.*

We, the signatories of this paper, limit ourselves to four issues. First, that current medical discussions of two spirochetoses (spirochete-associated infirmities, e.g., Lyme disease, syphilis) omit mention of "round bodies" or state that they have no clinical relevance (Feder et al., 2007). Round bodies are viable, motile, slowly reproductive morphologies assumed by spirochetes when they are

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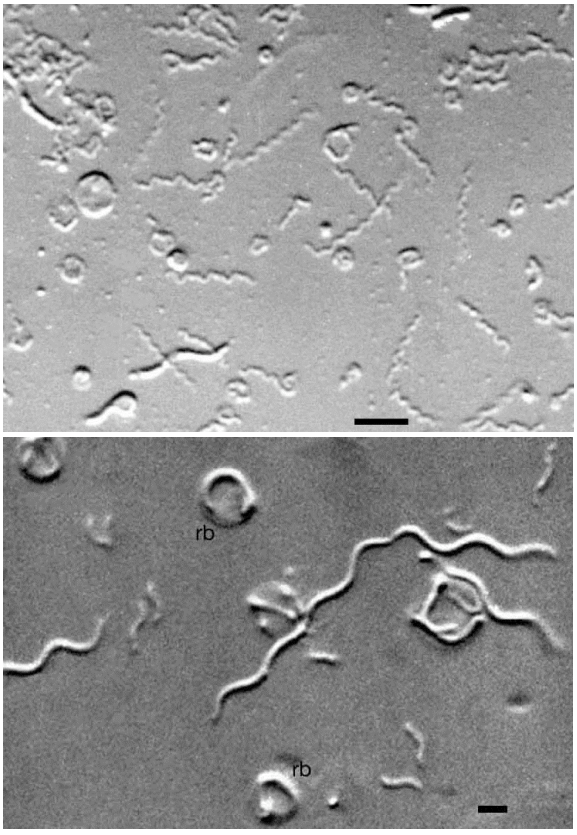
threatened by environmental insult such as changes in solution chemistry: acidity-alkalinity, salts, gas composition (i.e., oxygen, hydrogen sulfide); changes in chemical concentrations (i.e., antibiotics, antibodies, carbohydrates, amino acids, vitamins); or changes in viscosity or temperature. Both starvation and threat of desiccation induce round body formation. Some cultures of spirochetes (e.g., *Leptonema, Perfilievia*, Dubinina et al., 2008) seem to persist more as round bodies than as typical spirochetes. Round bodies, often called by other names such as "cysts", granular bodies, L-forms, non-growing bodies, sphaeroplasts, vesicles, etc. revert to the active helical swimmers when conditions favorable to growth return (Fig. 1).

Second, that infections by spirochetes in humans, when seen in their evolutionary and ecological context, are examples of cyclical symbioses that have evolved over geologic time. Certain symbioses have been shown to be associated with viral-like particles capable of synthesis of reverse transcriptases (Fig. 2). These are posited by Ryan (2007) based significantly on the work of Luis Villareal to be part of the integration process between the symbiont partners (i.e., in this case human and spirochete).



A.

B.

Figure 1. Live spirochete reversible round bodies in mixed culture. (The largest are *Spirosymplokos deltaeiberi*, from seaside microbial mats, Alfacs peninsula, Catalunya, Spain). Nomarski differential contrast light microscopy. A: Note several sizes of round bodies come from different kinds of spirochetes. B: Higher magnification shows the largest round bodies (rb) contain live, motile *Spirosysmplokos*. Scale bars = 5 μm.

Third, we caution that antibiotic treatment may be effective only in the earliest stages of these spirochetoses. Indeed antibiotics such as penicillin and its derivatives induce round body formation and quiescence of symptoms rather than cure. Suspension of round bodies in growth media causes rapid, days to weeks, reversion to helical swimmers as the Norwegian investigators have shown (Fig. 3; Brorson and Brorson, 2004).

Fourth, we question the accuracy of screening tests and clinical diagnoses for either of these infections, *Treponema pallidum* (the syphilis "germ") or *Borrelia burgdorferi* (the Lyme disease "germ"). Particularly vulnerable to misinterpretation are immunological tests in cases of re-infection, later secondary or tertiary syphilis.

2. Spirochetes: Past and Present

Most spirochete species live freely, are unrelated to any disease and therefore are unfamiliar to clinicians. We have studied or taught cell biology, environmental science, evolution, genetics, geobiology, natural history or microbiology. Our interest, perhaps summarized around the question "*What is the consequence of life's evolution on the Earth as a planet?"* has led us to scientific investigation of

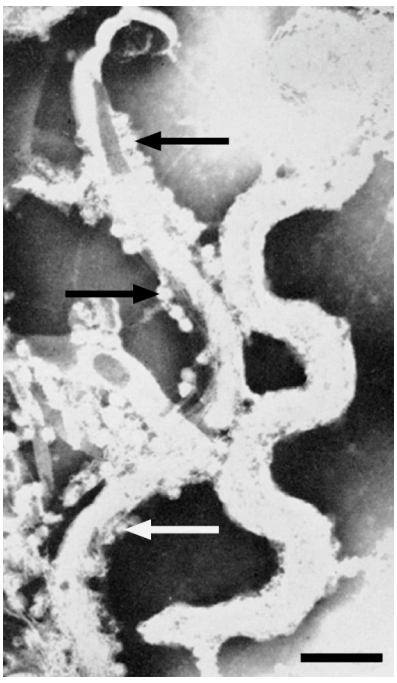


Figure 2. Detail of Fig. 4. of Hoogenraad et al. (1967) captioned: Electron micrograph of phage-infected spirochaetes, negatively stained as in Fig. 1 and using the agar stripping technique (3). The edge of the organism is lined with many small phages. The fibrillar structure of the spirochaetes is clearly shown. Also present in the lower section of the micrograph is a bacterial cell wall typical of those found in large numbers in sheep tureen contents. Scale bar = 0.5 μm.

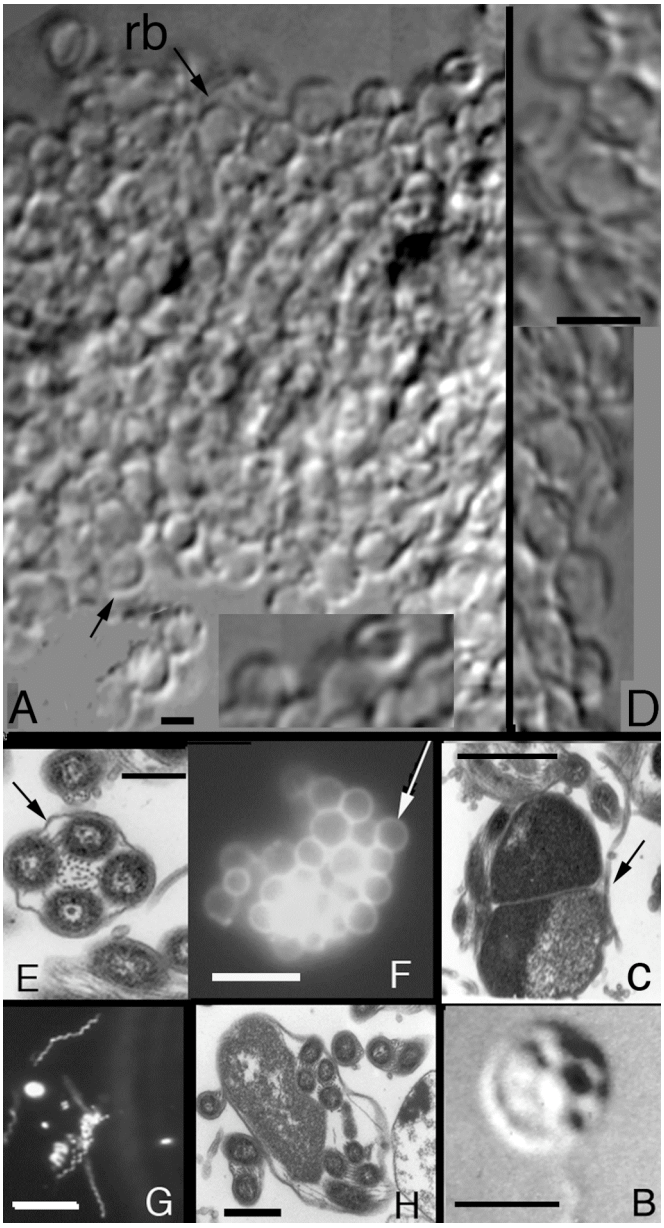


Figure 3. Formation and reversion of round bodies (rb=cysts, arrows) of *Borrelia burgdorferi* in pure culture. A, D: Conversion to round bodies induced by "unfavorable conditions" most rapidly by suspension in distilled water (starvation), spinal fluid or antibiotics. B: Rbs formed after three weeks starvation, some delayed, correspond to electron micrographs (by Sverre-Henning Brorson). C, E, and H: Round bodies can reproduce as in C. Fluorescein-conjugated polyclonal antibodies to *B. burgdorferi* stains spirochete rb membranes. F: Reversion of rbs to mobile spirochetes induced by resuspension in complete medium with rabbit serum (for 2–6 weeks) shown in G. Scale bars: A, C, E, H = 2 µm; B, D = 3 µm; F, G = 10 µm.

spirochetes in nature. Our comments here are generated by an abundant international literature and years of our own work much of it on harmless spirochetes. We have observed and occasionally isolated spirochetes from ponds rich in vegetation, digestive organs of marine mollusks (the "crystalline style" of oysters and clams) and intestines of wood-feeding termites and cockroaches. In these anoxic or low-oxygen habitats spirochetes swim and proliferate. Population densities often quickly reach over a thousand million per milliliter.

Margulis's laboratory explores an evolutionary hypothesis. She and her colleagues posit that a certain spirochete genome provided an ancestral component to the earliest nucleated cells (eukaryotes). Spirochete remnant DNA hypothesized to be present in all nucleated organisms should be detectable in the proteomes of fully sequenced genomes. Simply stated, spirochete ancestors of *Perfilievia russae* free-living spirochetes presented at the Berlin meeting by Galina Dubinina (Institute of Microbiology of the Russian Academy of Sciences, Moscow, Dubinina et al., 2008) by hypothesis are the closest co-descendants of the cytoskeleton of our nucleated cell lineage (Margulis et al., 2006). We envision these (sulfide-oxidizing, 0.25 µm diameter spirochetes) are related to ancestors of cilia, sperm tails, haptonemes and myriad other organelles of motility in nucleated organisms. If the evidence is correctly interpreted spirochete remnants have dwelled in stable symbioses in eukaryotes since their origin in the Proterozoic eon over 1000 million years ago (mya) (Hall, 2008). The most ancient intestinal spirochete symbiont in the fossil record is much younger (Miocene c. 20 mya). A *Pillotina* sp. large spirochete was discovered inside *Mastotermes electrodominicus,* a kalotermitid (dry wood-feeding termite) embedded in amber (Wier et al., 2002; 2007). Intestinal spirochetes lived as symbionts in insects long before the appearance, fewer than 0.5 million years ago, of any human animal on Earth.

Spirochetes are motile helical Gram-negative eubacteria. As heterotrophs, at optimal temperatures for growth, they require moisture and abundant food. Most ferment sugar in the absence of oxygen. They form a cohesive taxon detectable by the DNA sequence that corresponds precisely to the 16 Svedberg-unit ribosomal RNA (16S rRNA) component of the small 30S ribosomal subunit. Spirochetes with their Gram-negative cell walls and periplasmic (internal) flagella between their inner and outer membranes are distinctive at the level of thin section-electron microscopy (EM) (Fig. 2). The inner or plasma membrane is universal in all prokaryotic (bacterial) and eukaryotic cells. However the presence of an outer lipoprotein membrane typifies Gram-negative bacteria. To assign them to a lower taxon, a "species" or "genus", spirochete morphology is definitively discerned in EM thin section and less well by negative stain images that permit assessment of their flagella insertions and numbers. Spirochetes share a distinguishable flagellar pattern summarized as n: 2n:n where n=number of flagella at one end, 2n (or zero for leptospiras) refers to the overlap of

flagella in the middle and n=number of flagella at the opposite end. The flagella pattern of *Treponema pallidum* (1:2:1; 2:4:2), *Borrelia burgdorferi* (4:8:4/5:10:5), or any other spirochete can be detected in three ways: (1) morphology, especially active motility behavior in tissue, (2) thin section transmission EM and (3) negative stain whole mount EM. No reliable definitive tests for the presence of *T. pallidum* in patients exists short of quality high magnification observation of the spirochetes and/or their round bodies in affected tissue by an experienced expert microscopist. Reported cures of either infection, the syphilis *Treponema* or the Lyme disease *Borrelia*, lack this level of verification. Spirochetoses (e.g., leptospiroses, yaws, syphilis, Lyme disease) are bacterial diseases correlated with continued presence in the body of specific spirochetes (i.e., obey Koch's postulates).

3. Chronic Infections as Symbioses

The likelihood that these two spirochete infections, syphilis and Lyme disease, correlate with the establishment of permanent human-spirochete symbioses soon after entry of the bacteria into tissue has been insufficiently investigated. It is reported that reverse transcriptases and virus-like particles are generally abundant in cyclical symbioses and it is suggested that they may facilitate the integration of the association of the partners (Ryan, 2007). Our intent is to improve and expand awareness of the relationship between spirochetoses and symptoms associated with immune suppression. We posit that the spirochete disease syphilis persists in the human population where its signs and symptoms may be overlooked or misinterpreted for those of AIDS.

There may be many new drugs, but these two spirochetoses, syphilis and Lyme disease, are not new. Long-term association of symbiotic bacteria in animal tissue tends toward massive gene loss when compared to related bacteria that live freely in water, sand or mud. The fact that *Treponema pallidum* and *Borrelia burgdorferi* are no longer free-living and have lost many genes implies that these spirochetes have long co-evolved with mammals (and arthropods in the case of tick-borne *Borrelia burgdorferi* Lyme disease). Contrast these integrated symbionts to strains of *Leptospira* that live freely in rivers, streams and coastal ocean waters that cause acute infection. Compared to the fully genome-sequenced *Leptospira interrogans* spirochete, over 80% of the genome of *T. pallidum* when cultivated in vitro is absent. Dependency of *T. pallidum* on the gene products of the human has rendered it incapable of independent survival, growth or reproduction. Indeed *Borrelia burgdorferi* has lost relatively even more of its genophore (prokaryote "chromosome") genes than *T. pallidum*.

4. Mistaken for Dead

An extensive round body (=cyst) literature exists in Russian, but has remained relatively unknown even to spirochete experts elsewhere. In many spirochetes formation and reversion of round bodies has been documented by video microscopy techniques. The approximate number of genes of the entire sequenced genome of each genus in which round bodies are seen is listed in parenthesis: *Borrelia* (950), *Brachyspira* (?), *Leptospira interrogans* (4300), *Perfilievia* = aerotolerant "*Thiodendron*" spirochetes of Dubinina et al. (2008) (?), *Spirosymplokos deltaeiberi* (?), *Treponema pallidum* (1100), and the *Treponema*-like spirochetes epibiotic in the cortex of the giant trichomonad *Mixotricha paradoxa* (?). Both *T. pallidum* and *Borrelia burgdorferi* are anaerobic heterotrophs that require complex organic food under anoxic conditions. They die if exposed to ambient oxygen. The round bodies, propagules that, until they revert to swimming helices, seem incapable of at least rapid growth by reproduction, form quickly. Within less than an hour, under adverse conditions round bodies develop in large population numbers when the spirochete's needs are not met. They survive for extended periods of time. They revert to helical swimmer populations that grow vigorously when food, salt, temperature, acidity, media viscosity and other conditions become adequate. The controlled formation and reversion of round bodies of *Borrelia* are seen in many studies by the Brorsons. Negative stain EM images show canonical treponemes with the 1-2-1 flagellar pattern: "Borrelia"-type (4-8-4) or higher number flagella-pattern-spirochetes recognizable in tissue preparations, even in the same thin section from a rabbit syphiloma (Ovcinnikov and Delektorskij, 1968; 1975).

5. Spirochetoses and AIDS

Human tissue provides food and other conditions for growth for both *Treponema pallidum* and *Borrelia burgdorferi* spirochetes. Electron micrographic samples, in principle, could verify the persistence of round bodies in patients with symptoms, including Alzheimer's-type dementia. Examination of biopsies from AIDS patients or autopsies of brains from people who showed sudden personality disturbance could test the hypothesis. Round body formation in the test tube is induced by penicillin especially in the presence of glycine (a protein amino acid, read "food"). This discovery formed the major contribution of the PhD dissertation (and accompanying patent application) of Andrei Belichenko (2006). Dr. Belichenko was a student of a well-known medical microbiologist Dr. Igor Bazikov, Stavropol, Russia (Bazikov et al., 200). Belichenko reports that decrease in penicillin concentration induces reversion of round bodies to active hungry

spirochetes. Russian research and that by Brorson and Brorson (2004) on spirochetal life histories lead us to think that both the presentation and the course of syphilis, Lyme disease and other spirochetoses are altered by penicillin, other antibiotics and possibly by "anti-retroviral" or "protease inhibitor" drugs.

"Far from eradicating syphilis, antibiotics are driving the disease underground and increasing the difficulty of detection. Although the incidence of disease has more than tripled since 1955, the chancre and secondary rash no longer are commonly seen. Undoubtedly, some of these lesions are being suppressed and the disease masked by the indiscriminate use of antibiotics. The ominous prospect of a widespread resurgence of the disease in its tertiary forms looms ahead" (Pereyra and Voller, 1970).

We recommend studies to demonstrate or negate the hypothesis that relapse, grave illness or death may ensue from the reversion of round bodies to active spirochetes. Please see bibliography, 254 references on 49 pages compiled by Joanna Rubel "Spirochetal Cysts, L-forms, and Blebs, Observations from 1905 to 2005", at http://www.lymeinfo.net/medical/LDBibliography.pdf.

A three-decade-long gap ushered in by the touted "cure of penicillin" separates physicians today from the bulk of medical literature on "the great imitator". *T. pallidum* symbiosis may help explain the high correlation of the presence of viruses, pneumonias, other opportunistic infections and the general symptoms of immune suppression so well described in the "old syphilology" medical literature (Colman Jones www.cbc.ca/ideas/features/Aids/aidsspin.html). We suspect that many patients carry the latent disease that has become invisible because of the "syphilization effect" and misdiagnosis.

T. pallidum spirochetes that cover themselves with human proteins to which people make antibodies (Radolf and Lukehart, 2006) cause "autoimmune diseases".

The vigorous antigenic response of early infection fades to the classical secondary-to-tertiary symptoms of paresis. "PARESIS", a mnemonic, refers to a coherent and varying set of symptoms, a syndrome. "Personality disturbances, Affect abnormalities, Reflex hyperactivity, Eye abnormalities, Sensorium changes, Intellectual impairment and Slurred speech. PARESIS may begin with a dramatic delusional episode (e.g., Nietzsche January 1889 in the Turin Plaza; Margulis, 2004 and 2007). However, over the years, dementia may alternate with periods of such clarity that there seems to have been a cure" (Hayden, 2003).

Since the research group of Luc Montagnier first described LAV "virus-like particles" (later called "HIV-1") from "Patient 1", a close connection has been shown between AIDS and a history of syphilis in multi-partner men (Barre-Sinoussi et al., 1983). "Patient 1" sought medical consultation for swollen lymph nodes, muscle weakness without fever or weight loss, and for episodes of gonorrhea. He did not have AIDS. He had been previously treated for syphilis, but was he cured? Patient 1 tested positive for antibodies to three viruses: cytomegalovirus (CMV), Epstein-Barr virus and Herpes simplex. The first "HIV isolate" reported by Montagnier's group was from Patient 1. Since Montagnier's work, many centers that used immunological tests not sensitive for all stages of syphilis have documented a close relationship between a history of treponematoses and HIV/AIDS (Veugelers et al., 1992; Renzullo et al., 1991; Blocker et al., 2000). Chronic syphilitics and AIDS patients, those unmistakably ill and immune suppressed, do not succumb to HIV or syphilis directly. They die of reactivation tuberculosis (TB) and ubiquitous mycobacterium avium intracellulare (MAI group) diarrhea, and emaciation associated with refractory bowel infections in emaciated homosexuals and in immune compromised patients generally. TB and other myco-bacteria correlate with amoebic dysentery. Death records report causes as *Pneumocystis carini* pneumonia, *Entamoeba histolytica*, *Candida albicans* or other "opportunistic infection" (Coulter, 1987). In sub-Saharan Africa, the historic overuse of antibiotics and malnutrition also contribute to immune suppression. One of us (John Scythes) reports that he has not found a single documented case of an immune suppressed patient, whether HIV-positive or -negative, who has died of complications of syphilis since HIV records began being maintained in the early 1980s. Is it possible that the narrow focus on "HIV as the cause of AIDS", an example of scientific "misplaced concreteness" typical in explanation of evolution (Cobb, 2008), has facilitated missed diagnosis of syphilis?

Indeed, investigators in Toronto and San Francisco found an inverse relationship between treponemal antibody and AIDS symptoms that could be interpreted as the immune deficiency typical of disseminated syphilis (MacFadden et al., 1989; Haas et al., 1990; Fralick et al., 1994).

Contrary to the statements on many official government and medical websites that "syphilis is easily curable by antibiotics", the disease is often refractory to antibiotic and other treatments except perhaps in very early immuno-responsive stages (Musher et al., 1990). It has not been adequately shown that *T. pallidum* infection in its secondary and later stages is curable after any therapy. Because the lesions of secondary and tertiary syphilis are autoimmune, there is often an inability to react to a skin test of the delayed type. A loss of specificity against syphilis antigens is noted. Chronicity, or changes in immune response with time well established in spirochetoses is common to other infections: herpes viruses, tuberculosis and symptoms attributed to HIV. Syphilis, both early ulcers and the later immunoregulation problems, seems to facilitate acquisition of opportunistic bacteria, viruses, fungi and the progression to full-blown collapse of the immune system (Scythes and Jones, 2006).

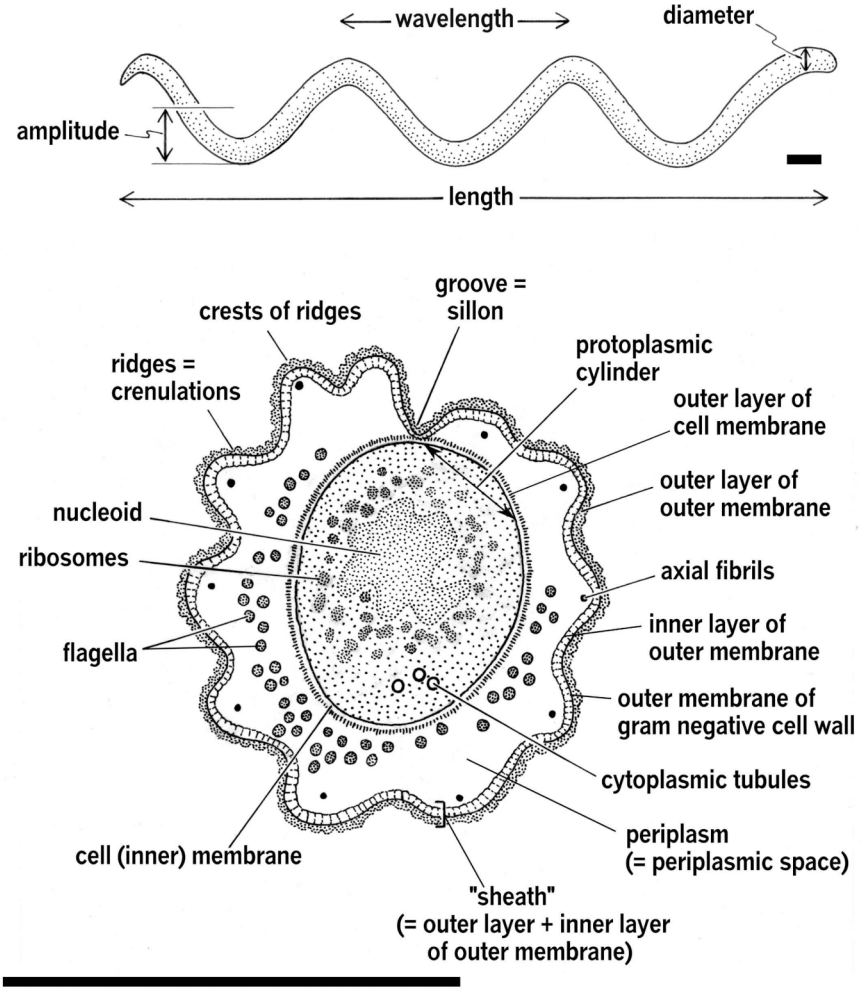


Figure 4. Spirochete structure as basis for morphometric analysis (Margulis, 2000). This drawing of a transverse section of a large, structurally complex spirochete, *Pillotina* sp., is based on many electron micrographs. Member of the family Pillotinaceae reside in wood-ingesting termites (kalotermitids); they tend to range from 0.5 to 3 μm in diameter. (*T. pallidum* of syphilis, by contrast, have diameters of 0.09 μm.) The flagella pattern here is at least 30:60:30. (Based on original drawing by Christie Lyons.) Scale bars = 3 μm.

"Numerous inconsistencies have been noted in HIV epidemiology between the various risk groups. Clinical signs of HIV infection seem to appear much later in previously healthy heterosexuals. Marked differences in both expression and progression of HIV disease between the sexually and non-sexually acquired forms have been reported. Extensive historical data supports the role of an STD as a co-factor allowing significant viral expression. Certain aspects of HIV epidemiology may therefore be explained by concomitant infection by treponemes and HIV" (MacFadden et al., 1989).

Most laboratories in North America use RPR or VDRL for the detection of all stages of syphilis. Investigators in Toronto and in Budapest have used improved immunological tests (i.e., TrepChek™, TrepSure™, Inno LIA syphilis™ , Behring Enzygnost syphilis™, and Abbott Architect syphilis™) and molecular screening (Talha et al., 2003; Scythes et al., 2004) using PCR (in development) to identify syphilis in patients who were missed using the standard of care currently practiced in most STD and HIV clinics. Modern technology now makes it very affordable for a large urban medical center to organize a comparison of nucleic acid testing (NAT)/PCR to results of immunological tests (i.e., RPR, FTA-Abs, TrepChek, TrepSure, Abbott, Behring, MardX western blotting/LIA Innogenetics, etc.) (Liu et al., 2001; Ballard et al., 2001). We urge investigation into the extent to which undetected latent syphilis overlaps with AIDS.

The correlation of a positive HIV test tends to indicate an enhanced likelihood of immune failure. To date, all attempts to produce an effective HIV vaccine have failed. Robert Gallo characterized the failure of the latest STEP vaccine trial, as a "catastrophe". Ronald C. Desrosiers, a molecular geneticist at Harvard University stated that "none of the products currently in the pipeline has any reasonable chance of being effective in field trials." Anthony S. Fauci, head of the National Institute of Allergy and Infectious Diseases that sponsored the trial, comments, "There is something very, very peculiar going on in the vaccine trials…. We've got to rethink these things" (Brown, 2008).

John Moore, an HIV virologist at Weill-Cornell Medical College stated "This was the first AIDS vaccine clinical trial in history where most people thought they'd at least see something positive"(Cohen, 2007). Kevin de Cock, head of the World Health Organization's HIV/AIDS department reports that AIDS is largely confined to high-risk groups (men who have sex with men, injecting drug users, and sex workers). "It is very unlikely there will be a heterosexual epidemic in countries (outside sub-Saharan Africa)" (O'Neill, 2008).

We agree that spirochetoses need to be reevaluated. Is the situation better described as an obligate and ancient symbiosis where the bionts (spirochetes and humans) are integrated at the behavioral, metabolic and genetic level rather than a new viral infection such that HIV equals AIDS? We think symbiosis analysis is appropriate here and in conclusion we advocate a necessary first step. We urge that the possible direct causal involvement of spirochetes and their round bodies to symptoms of immune deficiency be carefully and vigorously investigated.

Acknowledgements

We acknowledge with gratitude aid from Celeste Asikainen, E.L. Blunt, Sverre Henning Brorson, James di Properzio, Michael F. Dolan, Galina Dubinina, Victor Fet, Andre Khalil, Charles Geshekter, Ricardo Guerrero, Colman Jones, Georges Miklos, Renate Radek, Elwood Root, Juliana Sacher, Dorion Sagan, Melishia Santiago, Simon Silver, Gabriel Trueba, A.I. Tauber, Jessica H. Whiteside and Andrew M. Wier in the preparation of this statement comment. We also wish to acknowledge the financial support from the Rockefeller Foundation (Bellagio Study Center), The Tauber Fund, Mr. Abraham Gomel and the University of Massachusetts Amherst Graduate School, the Alexander von Humboldt Stiftung and the Deutsche Forschungsgemeinschaft (DFG) grant Gz. 4851/112/08.

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