Morphological Transformation in Borrelia burgdorferi and Other Spirochetes: Observations of Round Forms & Blebs, 1905-2010

262 Studies (63 on Lyme disease; 199 on other spirochetes)
Last Updated: 26 November 2010

	Author	Year	Title	Journal

Borrelia burgdorferi, afzelli, garini *(Lyme disease agents)*

1. Al-Robaly S; Dihazi, H; Kacza J; Seeger, J; Schiller, J; Huster, D; Knauer, J; Straubinger, RK. 2010 **Metamorphosis of Borrelia burgdorferi organisms - RNA, lipid and protein composition in context with the spirochetes' shape.** J Basic Microbio, Oct 21. (Epub)

Borrelia burgdorferi, the agent of Lyme borreliosis, has the ability to undergo morphological transformation from a motile spirochetal to non-motile spherical shape when it encounters unfavorable conditions. ...it seems that shape alteration begins with membrane budding followed by folding of the protoplasmatic cylinder inside the outer surface membrane. ...it was shown that the spirochetes' and spheres' lipid compositions were indistinguishable. ...no changes in differential protein expression were detected during this process. ...minimal degradation of RNA and a reduced antigen-antibody binding activity were observed with advanced age of the spheres. The results of our comparisons and the failure to generate mutants lacking the ability to convert to spheres suggest that the metamorphosis of B. burgdorferi results in a conditional reconstruction of the outer membrane. The spheres, which appear to be more resistant to unfavorable conditions and exhibit reduced immune reactivity when compared to spirochetes, might allow the B. burgdorferi to escape complete clearance and possibly ensure long-term survival in the host.

2. de Oliveira A; Fonseca AH; da Costa CM; Mantovani E; Yoshainari NH. 2010 **Growth, cysts and kinetics of Borrelia garinii (Spirochaetales: Spirochaetacea) in different culture media.** Mem Inst Oswaldo Cruz, 105(5), 717-19

[From the abstract:] "...the cultures developed cysts of different sizes, isolated or in groups, with a spiraled portion of variable sizes, mainly in unfavorable culture media. Brazilian Lyme disease-like illness, also known as Baggio-Yoshinari syndrome (BYS), is a new and interesting emerging tick-borne disease, caused by Borrelia burgdorferi sensu lato spirochetes, only during its cystic forms."

[From the article:] "Antibiotic efficiency requires active bacterial metabolism. Cystic form microorganisms are generally not pathogenic, but are resistant to antibiotic treatment because most of these drugs act at the bacteria cell wall. According to Brorson and Brorson (1997), this phenomenon explains why some LD patients are difficult to treat at the later stage of the disease."

3. Brorson O; Brorson SH; Scythes J; Macallister J; Wier A; Margulis L. 2009

Destruction of spirochete Borrelia burgdorferi round-body propagules (RBs) by the antibiotic tigecycline.

Proc Natl Acad Sci,106(44):18656-61

[From the abstract:] Spirochete RBs (reproductive propagules also called coccoid bodies, globular bodies, spherical bodies, granules, cysts, L-forms, sphaeroplasts, or vesicles) are induced by environmental conditions unfavorable for growth. Viable, they grow, move and reversibly convert into motile helices. Reversible pleiomorphy was recorded in at least six spirochete genera (>12 species). Penicillin solution is one unfavorable condition that induces RBs. This antibiotic that inhibits bacterial cell wall synthesis cures neither the second "Great Imitator" (Lyme borreliosis) nor the first: syphilis. ...We report in vitro inhibition and destruction of B. burgdorferi (helices, RBs = "cysts") by the antibiotic Tigecycline..."

[From the article:] "Antibiotics such as penicillin and its derivative doxycycline induce round body formation and quiescence of symptoms rather than cure." ... "Little, if any, evidence for cure of late Lyme borreliosis exists in the scientific literature."

4. Margulis L; Maniotis A; MacAllister J; Scythes J; Brorson O; Hall J; Krumbein WE; Chapman MJ. 2009

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

Symbiosis, 47:51-8

[From the abstract:] "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."

[From the article:] "In many spirochetes formation and reversion of round bodies has been documented by video microscopy techniques."

5. Miklossy J; Kasas S; Zurn AD; McCall S; Yu S; McGeer PL. *In Vivo (human brain)* 2008

Persisting atypical and cystic forms of Borrelia burgdoferi and local inflammation in Lyme neuroborreliosis.

J Neuroinflammation Sep 25;5:40

[From the abstract:] ...we observed atypical cystic, rolled and granular forms of these spirochetes. We characterized these abnormal forms by histochemical, immunohistochemical, dark field and atomic force microscopy (AFM) methods. The atypical and cystic forms found in the brains of three patients with neuropathologically confirmed Lyme neuroborreliosis were identical to those induced in vitro. ...

Conclusion: The results indicate that atypical extra- and intracellular pleomorphic and cystic forms of Borrelia burgdorferi and local neuroinflammation occur in the brain in chronic Lyme neuroborreliosis. The persistence of these more resistant spirochete forms, and their intracellular location in neurons and glial cells, may explain the long latent stage and persistence of Borrelia infection. The results also suggest that Borrelia burgdorferi may induce cellular dysfunction and apoptosis. The detection and recognition of atypical, cystic and granular forms in infected tissues is essential for the diagnosis and the treatment as they can occur in the absence of the typical spiral Borrelia form."

[From the article:] "Borrelia burgdorferi cultured in harmful conditions and in infected cell cultures where virtually all spirochetes showed pleomorphic and cystic forms were resuscitated under appropriate conditions in BSK-II medium, where apparently all spirochetes showed the typical spiral morphology. This suggests that these atypical forms may be viable Borrelia forms."

6. Brorson O; Brorson SH. 2007 **Grapefruit seed extract is a powerful in vitro agent against motile and cystic forms Borrelia burdorferi sensu lato.** Infection, 35(3):206-8

7. Brorson O; Brorson SH. 2006 **An in vitro study of the activity of telithromycin against mobile and cystic forms of Borrelia afzelli.** Infection, 34(1):26-8

In this study the new ketolide telithromycin was tested in vitro against motile and cystic forms of Borrelia afzelii, one of the species of Borrelia burgdorferi sensu lato. ... The activity was unexpectedly high, 0.0003 microg/ml < MBC < or = 0.0006 microg/ml for the mobile forms after 7 days of incubation at 34 degrees C. MIC was < 0.00015 microg/ml. It is likely that the agent works bacteriostatically and kills in a time-dependent and concentration-independent way, by binding tightly to the ribosomes. The agent was not able to prevent cyst formation, and the cysts were not affected at an in vivo achievable concentration.

8. Taylor RS; Simpson IN. 2005 **Review of treatment options for Lyme borreliosis.** J Chemotherapy Sep;17 Suppl 2:3-16

[From the abstract:] "Regardless of therapeutic agent, there appears to be a small minority of patients (<10%) who do not respond; such cases may be due to persistence of borrelial cysts..."

9. Zajkowska J; Hermanowska-Szpakowicz T; Rubel J. 2005 **Atypical Forms of Borrelia burgdorferi—Clinical Consequences.** Pol Merkuriusz Lek, 18(103):115-119

[Abstract:] "Borrelia burgdorferi utilizes a variety of mechanisms to counteract eradication by its host and establish chronic infection. We discuss several of these mechanisms, including plasmid encoded genes, morphologic variants, cysts formation, colonies formation, antigenic variation, and resistance to iron deprivation. These mechanisms, as well as the possible survival of Borrelia burgdorferi in forms with low metabolic activity, may explain relapsing Lyme disease, and may also account for the difficulties with eradicating this pathogen."

10. Murgia R; Cinco M. 2004 **Induction of cystic forms by different stress conditions in Borrelia burgdorferi.** APMIS, 112(1):57-62

[Abstract:] "Cystic forms of Borrelia burgdorferi might represent a low metabolic activity state or phase of B. burgdorferi cells that allows the spirochete to survive in a hostile environment until conditions are favourable to multiply again. In this study we evaluated the rate of cyst formation induced by oxidative stress, pH variations, and heating, reconversion of cysts to vegetative forms, and some aspects of their metabolic activity. We observed cyst formation in the presence of extreme pH values, and at high temperature, but the best production of cystic forms was observed in the presence of H2O2. When transferred to BSK II medium, the cystic forms reconverted to spirochetes in relation to their age and type of induction treatment. Furthermore, we demonstrated a low metabolic activity of cystic forms by measuring amino acid incorporation. Overall, these data suggest that the phenomenon of conversion to cysts by B. burgdorferi provides a limited survival potential. This short-term survival, however, gives borreliae an additional chance to overcome unfavourable environmental conditions."

11. Embers ME; Ramamoorthy R; Philipp MT. 2004 **Survival strategies of Borrelia burgdorferi, the etiologic agent of Lyme disease.** Microbes and Infection, 6:312-318

"It has been suggested that B. burgdorferi may avoid immune surveillance mechanisms altogether by seeking a refuge within a host cell or by encapsulating itself inside a cyst membrane… Several studies in vitro have motivated the suggestion that B. burgdorferi may transform into cysts in vivo… cyst formation also has been shown to occur in body fluids such as the cerebrospinal fluid and in response to the addition of β-lactam antibiotics in vitro. The reasonable, yet unproven notion that an inability to resolve infection with antibiotics in some patients could result from such cyst formation may follow."

12. Brorson O; Brorson SH. 2004 **An in vitro study of the susceptibility of mobile and cystic forms of Borrelia burgdorferi to tinidazole.** Int Microbiol, 7(2):139-40

[Abstract:] "The susceptibility of mobile and cystic forms of Borrelia burgdorferi to tinidazole (TZ) was examined. The minimal bactericidal concentration (MBC) of TZ against the mobile spirochetes was >128 microg/ml at 37 degrees C in micro-oxic atmosphere when incubated for 14 days. TZ significantly reduced the conversion of mobile spirochetes to cystic forms during incubation. The MBC for older (10-months-old) cysts at 37 degrees C in a micro-oxic atmosphere was >0.5 microg/ml, but >0.125 microg/ml for young (1-day-old) cysts. Acridine orange staining, dark-field microscopy and transmission electron microscopy revealed that, when the concentration of TZ was > or = MBC, the contents of the cysts were partly degraded, core structures did not develop inside the young cysts, and the amount of RNA in these cysts decreased significantly. When cysts were exposed to TZ, both the spirochetal structures and core structures inside the cysts dissolved, and the production of blebs was significantly reduced. These observations may be valuable in the treatment of resistant infections caused by B. burgdorferi, and suggest that a combination of TZ and a macrolide antibiotic could eradicate both cystic and mobile forms of B. burgdorferi."

13. Oliveira A; Fonseca AH; Ishikawa MM; Yoshinari NH. 2004 **Cinética de crescimento de *Borrelia burgdorferi* (Spirochaetaceae) em diferentes meios de cultivo.** Pesqui Vet Bras 24:61-4
(Kinetics of growth of Borrelia burgdorferi (Spirochaetaceae) in different culture media.)

14. Brorson O; Brorson SH. 2002 **An in vitro study of the susceptibility of mobile and cystic forms of Borrelia burgdorferi to hydroxychloroquine.** Int Microbiol, 5(1):25-31

[Abstract:] "In this work the susceptibility of mobile and cystic forms of Borrelia burgdorferi to hydroxychloroquine (HCQ) was studied. The minimal bactericidal concentration (MBC) of HCQ against the mobile spirochetes was > 32 microg/ml at 37 degrees C, and > 128 microg/ml at 30 degrees C. Incubation with HCQ significantly reduced the conversion of mobile spirochetes to cystic forms. When incubated at 37 degrees C, the MBC for young biologically active cysts (1-day old) was > 8 microg/ml, but it was > 32 microg/ml for old cysts (1-week old). Acridine orange staining, dark-field microscopy and transmission electron microscopy revealed that the contents of the cysts were partly degraded when the concentration of HCQ was > or = MBC. At high concentrations of HCQ (256 microg/ml) about 95% of the cysts were ruptured. When the concentration of HCQ was > or = MBC, core structures did not develop inside the cysts, and the amount of RNA in these cysts decreased significantly. Spirochetal structures inside the cysts dissolved in the presence of high concentrations of HCQ. When the concentration of HCQ was > or = MBC, the core structures inside the cysts were eliminated. These observations may be valuable in the treatment of resistant infections caused by B. burgdorferi, and suggest that a combination of HCQ and a macrolide antibiotic could eradicate both cystic and mobile forms of B. burgdorferi."

15. Murgia R; Piazetta C; Cinco M. 2002 **Cystic forms of Borrelia burgdorferi sensu lato: induction, development, and the role of RpoS.** Wiener Klinische Wochenschrift, 114(13-14):574-9

[Abstract:] "It has been demonstrated recently that cells of Borrelia burgdorferi sensu lato, the etiological agent of Lyme disease, transform from mobile spirochetes into nonmotile cystic forms in the presence of certain unfavourable conditions, and that cystic forms are able to reconvert to vegetative spirochetes in vitro and in vivo. The purpose of this study was to investigate the kinetics of conversion of borreliae to cysts in different stress conditions such as starvation media or the presence of different antibiotics.

Using the same experimental conditions we also investigated the possible role in cyst formation of RpoS, an alternative sigma factor that controls a regulon in response to starvation and transition to stationary phase. We observed that beta-lactams penicillin G and ceftriaxone, the antibiotics of choice in Lyme borreliosis treatment, favoured the production of cysts when used with serum-depleted BSK medium. In contrast, we observed a low level of cyst formation in the presence of macrolides and tetracyclines. In order to elucidate the role of the rpoS gene in cyst formation we analyzed the reaction of the rpoS mutant strain in comparison with its wild-type in different conditions. Under the same stimuli, both the wild-type borrelia and the rpoS knock-out isogenic strain produced cystic forms with similar kinetics, thus excluding the participation of the gene in this phenomenon. Our findings suggest that cyst formation is mainly due to a physical-chemical rearrangement of the outer membrane of Borrelia burgdorferi sensu lato leading to membrane fusion and controlled by different regulation mechanisms."

16. Zajkowska JM; Hermanowska-Szpakowicz T. 2002 **New aspects of the pathogenesis of Lyme disease.** Przegl Epidemiol, 56 Suppl 1:57-67

[From the abstract:] "Morphological changes of B. burgdorferi as well as changes in expression of surface proteins caused by environmental determinants are essential in pathogenesis of Lyme disease. Cysts, spherical form (spheroplasts, L-form) and 'blebs' (gemmae) can be responsible for long lasting antigenic stimulation, signs of chronic borreliosis, and even probably connected with MS and Alzheimer disease."

17. Gruntar I; Malovrh T; Murgia R; Cinco M. 2001 **Conversion of Borrelia garinii cystic forms to motile spirochetes in vivo.** APMIS,109(5):383-8

In Vivo

[Abstract:] "Cystic forms (also called spheroplasts or starvation forms) and their ability to reconvert into normal motile spirochetes have already been demonstrated in the Borrelia burgdorferi sensu lato complex. The aim of this study was to determine whether motile B. garinii could develop from cystic forms, not only in vitro but also in vivo, in cyst-inoculated mice. The cysts prepared in distilled water were able to reconvert into normal motile spirochetes at any time during in vitro experiments, lasting one month, even after freeze-thawing of the cysts. Motile spirochetes were successfully isolated from 2 out of 15 mice inoculated intraperitoneally with cystic forms, showing the infectivity of the cysts. The demonstrated capacity of the cysts to reconvert into motile spirochetes in vivo and their surprising resistance to adverse environmental conditions should lead to further studies on the role and function of these forms in Lyme disease."

18. Brorson O; Brorson SH. 2001 **Susceptibility of motile and cystic forms of Borrelia burgdorferi to ranitidine bismuth citrate.** Int Microbiol, 4(4):209-15

[Abstract:] "Gastrointestinal symptoms accompanying Lyme disease have not been considered in the treatment of Lyme patients yet. Here we examine the effect of ranitidine bismuth citrate (RBC) on motile and cystic forms of Borrelia burgdorferi in vitro, to determine whether it could cure this bacterial infection in the gastrointestinal tract. When motile forms of B. burgdorferi were exposed to RBC for 1 week at 37 degrees C, the minimal bactericidal concentration (MBC) was > 64 mg/ml. At 30 degrees C, the MBC was > 256 mg/ml. When the incubation lasted for 2 weeks at 37 degrees C, the MBC dropped to > 2 mg/ml. Bismuth aggregates were present on the surface of B. burgdorferi when RBC > or = MBC, as shown by transmission electron microscopy (TEM). Cystic forms of B. burgdorferi, exposed to RBC for 2 weeks at 37 degrees C, were examined by cultivation in BSK-H medium (Sigma B3528). They were stained with acridine orange (pH 6.4, pH 7.4) and studied by TEM. The MBC for RBC for young cystic forms (1 day old) and old cysts (8 months old) was estimated to be > 0.125 mg/ml and > 2 mg/ml, respectively. Bismuth aggregates were attached to the cysts and, in some, the pin-shaped aggregates penetrated the cyst wall. The bismuth aggregates also bound strongly to blebs and granules of B. burgdorferi when RBC > or = MBC. When B. burgdorferi is responsible for gastrointestinal symptoms, bismuth compounds may be candidates for eradication of the bacterium from the gastrointestinal tract."

19. Brorson O; Brorson SH; Henriksen TH; Skogen PR; Schoyen R. *In Vivo* 2001 **Association between multiple sclerosis and cystic structures in cerebrospinal fluid.** Infection, 29(6):315-9

"Cystic structures were observed in CSF of all ten patients by AO and TEM. DF revealed eight cyst-positive patients out of nine. One of five control persons had such structures in the CSF; this person had suffered from erythema migrans. Spirochete or rod-like structures emerged after culturing two of the MS patient CSF samples and these structures could be propagated. CONCLUSION: A significant association of CSF cysts and MS was identified in this small study among residents in a coastal area of southern Norway. The cysts could be of spirochetal origin."

20. Alban PS; Johnson PW; Delson DR. 2000 **Serum-starvation-induced changes in protein synthesis and morphology of of Borrelia burgdorferi.** Microbiology, 146 (Pt 1):119-27

"In a recent study, Brorson & Brorson (1997) demonstrated that B. burgdorferi cells transform from vegetative spirochaetes into spherical 'cyst-forms' when incubated in BSKII medium lacking rabbit serum (BSKII-S). We confirmed these observations. ...Within 24 h, cells started of serum were completely non-motile and 30-40% had begun to encyst. After 48 h incubation in RPMI, ~90% of serum-starved cells had formed cysts (Fig. 1). ...In contrast to typical helical vegetative cells, most 48 h serum-starved cells were coiled within a membrane. ...

When rabbit serum or BSK was added to RPMI containing 48 h serum-starved cells, the cysts opened within 10 s to yield intact, but non-motile spirochaete cells (Fig. 2). ...Cells began to regain motility 12-15 h after emerging from the cysts.

...the Western blots displayed consistent differences between the protein antigens recognized in vegetative cells and cysts. ...By forming cysts, it is also conceivable that B. burgdorferi cells evade detection by the immune system.

Cyst formation is an active cellular response to serum starvation. The addition of tetracycline inhibits cyst formation, demonstrating that cyst formation requires protein synthesis and that cysts are not merely degenerative forms."

21. Amosova LI. 2000 **An electron microscopic study of Borrelia in the body of the female ixodid tick Ixodes persulcatus.** Parazitologiia, May-Jun;34(3):234-40

[From the abstract:] "Borrelia burgdorfery s. lato in naturally infected females of tick Ixodes persulcatus were examined by transmission electron microscopy. The Borreliae were found in midgut and ovary. ...Two morphological types of borreliae were observed."

22. Beermann C; Wunderli-Allenspach H; Groscurth P; Filgueira L. 2000 **Lipoproteins from Borrelia burgdorferi applied in liposomes and presented by dendritic cells induce CD8(+) T-lymphocytes in vitro.** Cell Immunology, May 1;201(2):124-131

"We could document invasion of Bb into the dermis and shedding of Bb-blebs into the tissue under in vitro conditions (Fig. 1b). ...We show with electron microscopy that shedding of blebs by Bb also takes place in the tissue which confirms earlier observations (34). Bb-liposomes were used as a model for Bb-blebs to study uptake by cells. "...we studied the uptake of Bb-liposomes by human DC, fibroblasts, and B- and T-lymphocytes. All tested cells incorporated Bb-liposomes, as visualized by immunofluorescence microscopy. ...we could document that Bb-liposomes were incorporated within seconds."

23. **Zajkowska JM;** Hermanowska-Szpakowicz T et al. 2000 **[No title available].** Pol Merkuriusz Lek, 9(50):584-8

[From the abstract:] "Spheroplast L-form of borrelia could be responsible for difficulties with their eradication."

24. Zajkowska JM; Hermanowska-Szpakowicz T; Pancewicz SA; Kondrusik M. 2000 **[Selected aspects of immunopathogenesis in Lyme disease].** Pol Merkuriusz Lek, 9(50):579-83

[From the abstract:] "In pathogenesis of chronic and recurrent cases difficult to treat is essential is survive [sic] of metabolic inactive bacteria, antigens B. burgdorferi "blebs", cystic L-form or insoluble complexes antigen-antibody or possibility of intracellular survive [sic] of B. burgdorferi."

25. Filgueira L; Beermann C; Groscurth P. 2000 **Liposome-like vesicles from Borrelia burgdorferi modulate the function of human dendritic cells.** J Invest Dermatol, 114(1):23

[From the abstract:] "For Bb a high turn-over of lipoproteins and lipids has been reported. Since Bb is not able to recycle these components, huge amounts of liposome-like blebs are shed from the outer bacterial membrane. The aim of this study was to show that Bb-blebs influence the immune response.

With a Bb-liposome model we show for different cell populations with confocal and transmission electron microscopy that Bb-blebs can penetrate through the cell membrane into the cytoplasm, accumulate in the cytosol and enter the nucleus. Bb-blebs abrogate the T-cell stimulatory capacity of dendritic cells. In addition, by introducing foreign antigens into the MHC class I pathway, they transform cells to become targets for Bb-specific CTL. The symptoms of Lyme borreliosis may thus be explained by the impact of Bb-blebs on the immune system.

26. Benach JL. 1999 **Functional heterogeneity in the antibodies produced to Borrelia burgdorferi.** Wiener Klinische Wochenschrift, Dec 10; 111(22-23):985-9

[From the abstract:] "Upon contact with Borrelia burgdorferi, CB2 causes lysis of the outer membrane and the formation of a spheroplast."

27. Brorson O; Brorson SH. 1999 **An in vitro study of the susceptibility of mobile and cystic forms of Borrelia burdoferi to metronidazole.** APMIS, 107(6):566-76

B. burgdorferi cysts were found to degrade upon incubation with metronidazole (MZ). Mobile spirochetes did not convert to cysts in the presence of MZ.

"B. burgdorferi has the ability to make cystic forms both in vivo and in vitro, e.g. when exposed to antibiotics commonly used for treating Lyme borreliosis (19-24). This phenomenon, combined with the ability of the cysts to reconvert to normal mobile spirochetes (25-27), may explain a reactivation of the disease after an illusory cure -- and not a "post Lyme syndrome" as postulated by other researchers. ...

Our findings show that MZ had no significant effect against mobile spirochetes, but sufficient presence of MZ in distilled water reduced the creation of cystic forms. MZ disrupted the structure of cystic forms of B. burgdoferi and decreased their biological activity. ...

An important observation is the temperature-dependent influence of MZ on the cysts. A higher amount of MZ is needed to disrupt the cysts when the temperature is 30ºC than at 37-38ºC. This is the same for other antibiotics (39), and may be important when the cysts are located in the dermis. ...

Helicobacter pylori is also capable of transforming to coccoid (cystoid) forms and reversing to normal mobile forms (41), and for this bacterium treatment with three or more antibiotics has been established. Therefore, dual medication with MZ as one of the antibiotics could be of value, also for curing infections caused by mobile and cystic forms of B. burgdorferi."

28. Burgdorfer W. 1999 **Keynote Address - The Complexity of Vector-borne Spirochetes. 12th International Conference on Lyme Disease and Other Spirochetal and Tick-Borne Disorders.** www.medscape.com/medscape/cno/1999/lyme/Story.cfm?story_id=534.

"This relatively large Borrelia [Borrelia burgdorferi] is not readily detectable in blood smears or thick drops of Lyme disease patients and susceptible host animals, yet engorgement on infected hosts results in up to 100% infected ticks.... RML [NIH's Rocky Mountain Lab] scientists Dave Dorward and Claude Garon using silver staining, transmission and scanning electron microscopy investigated the nature of naturally elaborated membrane blebs on the surface of cultured B. burgdorferi or free in the medium, and found both linear and circular DNA (Fig.13)... These most recent findings [of RML researchers and others] do confirm the development of membrane-derived cysts, blebs, spherules, vesicles and the potential transformation to motile, helical spirochetes...as a "survival mechanism" of spirochetes to overcome or escape unfavorable conditions."

[Willy Burgdorfer, Ph.D., of the National Institutes of Health, is the discoverer of Borrelia burgdoferi.]

29. Brorson O; Brorson SH. 1998 **A rapid method for generating cystic forms of Borrelia burgdorferi, and their reversal to mobile spirochetes.** APMIS, 106(12):1131-41

Motile B. burgdorferi spirochetes converted to cysts within 1 minute when placed in distilled water. The cysts reverted back to spirochetes after transfer to a growth medium. "Dark field microscopy demonstrated that >95% of the normal mobile borreliae were converted to cystic forms after 1 min exposure to distilled water. It seemed as if the cysts were produced by the bacterium whirling into its own membrane-encapsulated space. ...Four hours after inoculation no normal spirochetes were observed in the distilled water, and all cysts were globularly shaped. ...The conversion to cystic forms occurred more rapidly if the temperature of the distilled water added was 4ºC than if it was 22ºC. ... Cysts transferred to the BSK-H medium became irregular and their volume shrank, possibly due to different osmolarity in the BSK-H medium than in distilled water. Daily observations of the cysts in BSK-H medium revealed one to five thin filamentous structures fastened to the envelope of the cysts. In the beginning, these structures were hypermobile and their shape was rectilinear or slightly curved (Fig. 2a). Subsequently, these filamentous forms grew both in length and diameter, and many of them acquired the shape of normal spirochetes that finally detached from the cysts. ... The biological activity of the cystic forms was confirmed by the step by step development to normal mobile spirochetes in BSK-H medium, and also indicated by the presence or RNA in 5-week-old cysts due to red-orange staining with acridine orange (pH 6.4) (Fig. 4b). ... The observation by TEM that blebs transformed into thin filaments leads us to speculate that these filaments develop to normal mobile spirochetes. If so, the blebs have to contain enough genetic material to synthesize a new bacterium (22). ...Similar cystic forms may occur in the human organism (11, 14, 15), and they may explain the long periods or latency, resistance to antibiotics, negative serological results (3-7, 10, 12, 13, 25), and low PCR sensitivity (5,8,10)."

30. Brorson O; Brorson SH. 1998 **In vitro conversion of Borrelia burgdorferi to cystic forms in spinal fluid, and transformation transformation to mobile spirochetes by incubation in BSK-H medium.** Infection, 26(3):144-50

[From the abstract:] "B. burgdorferi transformed into cysts (spheroplast L-forms) within 1-24h of inoculation into spinal fluid. When transferred to a growth medium, the cysts converted back to normal spirochetes after 9-17 days of incubation. "When neuroborreliosis is suspected, it is necessary to realize that B. burgdorferi can be present in a cystic form, and these cysts have to be recognized by microscopy. This study may also explain why cultivation of spinal fluid often is negative with respect to B. burgdorferi."

[From the article:] "The formation of cysts was somewhat different depending on the concentration of protein in the spinal fluid. ...Slower conversion was observed in spinal fluid with a higher concentration. ..The time of generation for spirochetes was up to 50% shorter when they were produced from cysts than when produced from normal, mobile spirochetes. However, the time of generation from cysts depends largely on the composition of the growth medium. ...

The biological activity of the cysts was manifested by their ability to reconvert to normal, mobile spirochetes. ...According to our estimates, about 50% of the cysts reconverted to normal, mobile spirochetes. The cysts observed in our study seem to resemble the spheroplast L-forms observed by other researchers (8,21) which appear to have defects in their cell wall manifested by resistance towards B-lactam antibiotics (22).

The conversion to cystic forms may explain why cultivation of spinal fluid often gives negative results with respect to B. burgdorferi... The antigenic variation in B. burgdorferi (32,33) may occur inside the cyst while the microbe is protected against external stress. Cystic forms of B. burgdorferi may be created both extra- and intracellularly (34,35) if the spirochetes are treated with antibiotics (22,36,37) or if antibodies are present (32)."

[Diagnosis:] "It is not known whether cystic forms of B. burgdorferi can be detected by PCR, but if we assume that cysts cannot be detected by PCR, this may explain why PCR on spinal fluid is negative even when the patient has the diagnosis of neuroborreliosis."

31. Phillips SE; Mattman LH; Hulinska D; Moayad H. 1998 **A proposal for the reliable culture of Borrelia burgdorferi from patients with chronic Lyme disease even from those previously aggressively treated.** Infection, 26(6):364-7

"There has been a considerable spectrum of cell wall deficiency demonstrated in our laboratory. B. burgdorferi may exist in various forms depending on its environment. In addition to the spirochetal form, we have demonstrated its growth both as amorphous L-forms and rounded giant L-bodies which have been previously described as cystic forms (11,18). As B. burgdoferi reverts from cell wall deficiency with the rebuilding of its cell wall, classic spirochetal forms can be seen. Most often, in our cultures, B. burgdorferi can be seen in varying stages of reversion, i.e. some L-dependent spirochetal forms within an L-form colony. The L-form variants, osmotically fragile by nature, require precise conditions to grow in culture."

32. Brorson O; Brorson SH. 1997 **Transformation of cystic forms of Borrelia burgdorferi to normal mobile spirochetes.** Infection, 25:240-6

[From the abstract:] "The occurrence of cystic forms of Borrelia burgdorferi in vitro was noted, and these cysts were able to be transformed to normal, mobile spirochetes.

[From the article:] "Ultrastructurally we observed cystic structures with coiled spirochetes inside... The spirochetes inside the cysts were not surrounded by a trilaminar membrane as they are when not inside a cyst; they seemed to have lost one membrane layer. Transverse fissions of bacteria were detected inside some cysts (Figure 6), and several cysts seemed to contain more than one spirochete. ...We also observed fission of the cyst itself (Figure 7). ...

Discussion: Our in vitro experiments with B. burgdorferi demonstrated the transformation of normal, mobile spirochetes to encysted forms. These cystic forms (Figures 3,5,7) seem to be an alternate morphologic state to which B. burgdorferi resorts when the environment becomes too unfavorable. ...

Low biological activity was demonstrated by the absence of change in pH in the culture medium, suggesting a torpor state. When BSK-H medium with serum was added to cystic forms only (as shown in Figure 3), they seemed to wake from this torpor state, and once again became metabolically active (Figure 4). ...The effectiveness of antibiotics requires active metabolism by the bacteria, and therefore it is likely that cystic forms of B. burgdorferi may be resistant to antibiotic treatment. This may explain why Lyme borreliosis can be difficult to treat in some patients (15,19). It is also possible that the membrane surrounding the encysted forms will protect the bacteria against external stress. DNA has been demonstrated in blebs (21), and it is therefore possible that these structures may participate in the protection and transfer of genetic markers. The observation of transverse fission of spirochetes inside the cysts indicates a more complex regeneration of B. burgdorferi than assumed earlier, and may give the bacteria quantitative advantages when they finally escape from the encysted forms."

33. Aberer E; Koszik F; Silberer M. *In Vivo* 1997 **Why is chronic Lyme borreliosis chronic?** Clinical Infectious Diseases, 25 (Suppl 1), S64-S70

"Immunohistochemical staining of ACA skin biopsy specimens with a monoclonal antibody to flagellin has shown that ACA-affected skin harbors several forms of borreliae. Heavily stained, clumped, intertwined forms and granular Borrelia structures among collagen fibers (figure 2) are also seen to form after incubation with antibodies to B. burgdorferi in vitro, and delicate dispersed forms are found lying in degenerating collagen fibers (31). The existence of these forms has been confirmed ultrastructurally (27)."

34. Escudero R; Halluska ML; Backenson PB; Coleman JL; Benach JL. 1997 Characterization of the physiological requirements for the bactericidal effects of a monoclonal antibody to OspB of Borrelia burgdorferi by confocal microscopy. Infection & Immunity, May;65(5):1908-15

The spheroplasts contain both OspA and OspB and are a terminal stage in the bactericidal process induced by Fab-CB2."

[From the article:] "The formation of spheroplasts ultimately leads to cell death; in our studies, only mutants grow after treatment of spirochetes with CB2 (17,18). ...The similarities between the morphological changes that spirochetes undergo in response to the anti-OspB MAb by H6831 and to antibiotics have been noted (35). This may reflect a common pathway for spirochetal death or simply that different killing pathways result in similar morphological characteristics. While antibiotic-induced changes of spirochetes indeed resemble the changes induced by CB2 and H6831, including the formation of spherical structures (4,20,37,43), the changes with the MAbs occur more rapidly. Morphological changes in B. hermsii as a result of treatment with benzylpenicillin required 10 h of incubation (4); 24 h was required for B. burgdorferi with penicillin and vancomycin (20), and 5 days was required with benzylpenicillin (37). ...

From these studies we conclude that Fab-CB2 destabilizes the OM [outer membrane] of B. burgdorferi, with subsequent formation of spheroplasts, through an epitope-specific, bivalent cation-dependent mechanism."

35. Aberer E; Kersten A; Klade H; Poitschek C; Jurecka W. *In Vivo* 1996 **Heterogeneity of Borrelia burgdorferi in the skin.** Am J Dermatopathology, 18(6):571-9

B. burgdorferi granules were documented in skin biopsies using videomicroscopy.

[In cultures] "After incubation with hyperimmune serum,... Rings formed where ends of borreliae fused with the center of organisms. Less mobile borreliae developed granules at their centers or at their ends. These granules were initially connected by a fine stalk and then seemed to be detached from the immobile organisms. ...Studies with antibiotics revealed similar morphologic changes, although the formation of granules of a much larger size (spheroplast-like structures) was obvious."

[In skin biopsies] "Large granules or spherical bodies ("gemmae") 1 to 3 µm were detected among collagen fibers comparable to cysts arising after culture experiments. ...Heavily stained, clumped, and aggregated borreliae and granules, formed by action of hyperimmune sera, were evident as were degenerative changes in the connective tissue."

[Persistence:] "Neuralgias arising 6 months after ECM in spite of antibiotic therapy were evident in a seronegative patient who showed perineural rod-like borrelia structures."

[Intracellular:] "The presence of borreliae in macrophages and keratinocytes, as shown in our studies and also in Berger's silver staining studies, supports the hitherto unproven concept that borreliae may survive intracellularly (33)."

[Seronegativity:] "The morphological forms of borreliae seen in biopsies were correlated with clinical findings. Seropositive patients showed clumped and agglutinated borreliae in tissue (Fig. 4b), whereas seronegative patients exhibited borreliae colony formation (n=2) (Figs. 7b, 8b). ...the behavior of borreliae within collagen fibers is strongly influenced by immune recognition by the patient. Borrelia may escape immune surveillance by colony formation and masking within collagen, resulting in seronegativity."

36. Cluss RG; Goel AS; Rehm HL; Schoenecker JG; Boothby JT. 1996 **Coordinate synthesis and turnover of heat shock proteins in Borrelia burgdorferi: degradation of DnaK during recovery from heat shock.** Infection & Immunity, 64(5):1736-43

[From the abstract:] "Spheroplasts of B. burgdorferi produced by treatment with EDTA and lysozyme were radiolabeled, and specific Hsps were localized to either the cytoplasm or membrane fraction."

37. Mursic VP; Wanner G; Reinhardt S; Wilske B; Busch U; Marget W. 1996 **Formation and cultivation of Borrelia burgdorferi spheroplast L-form variants.** Infection, 24(3):218-26

[Persistence:] "...clinical persistence of Borrelia burgdorferi in patients with active Lyme borreliosis occurs despite obviously adequate antibiotic therapy..." "The persistence of Bb even after therapy with antibiotics has been demonstrated in cerebrospinal fluid (CSF), in skin, iris,heart and joint biopsies."

[Cysts:] In vitro investigation of morphological variants of B. burdorferi, in an effort to explain the clinical persistence of active Lyme borreliosis despite antibiotic therapy. The authors suggest that these atypical forms may allow Borrelia to survive antibiotic treatment.

"Penicillin G was the most effective inducer of SL-forms [spheroplast-L-forms). The reversion of this form to the helical parental forms was mostly achieved by cultivation of isolated SL-colonies in penicillin G-free medium. The atypical forms isolated from patients treated with antibiotics show similar features. The same effect is probably obtained with all other ß-lactam antibiotics."

[Seronegativity:] "With regard to the polyphasic course of Lyme borreliosis, these forms without cell walls can be a possible reason why Borrelia survive in the organism for a long time (probably with all beta-lactam antibiotics) [corrected] and the cell-wall-dependent antibody titers disappear and emerge after reversion."

[Diagnosis/PCR:] "Very interesting are the studies by Hoyer and King who demonstrated the loss of a portion of the chromosomal DNA in an L-form of Enterococcus (43)."

38. Angelov L; Dimova P; Berbencova W. 1996 **Clinical and laboratory evidence of the importance of the tick D. marginatus as a vector of B. burgdorferi in some areas of sporadic Lyme disease in Bulgaria.** European J Epidemiology, 12(5):499-502

[In vivo cystic and granular forms:] "In the sections from the deeper strata of the dermis (str. reticulare) Bb was observed in two extracellulary in deep synovial different structural forms: (a) cylindrical bodies (protoplasm cylinder) with circular ends, covered with a three-layered membrane which undulated in places (Figure 2); (b) in most of the sections another structural form of the spirochete was found; granules, situated among the collagenous fibres in places closely adhered to them, sometimes covered with a membrane (Figure 2). No intracellular Borreliae were observed." [These observations were based on an electron microscopy examination of skin biopsy material from a patient with erythema migrans, a documented tick bite, and positive serologic confirmation of Borrelia burgdoferi infection.]

39. Nanagara R; Duray PH; Schumacher HR Jr. 1996 **Ultrastructural demonstration of spirochetal antigens in synovial fluid and synovial membrane in chronic Lyme disease: possible factors contributing to persistence of organisms.** Human Pathology, 27(10):1025-34

In Vivo

Intracellular Borrelia-like structures were found in Lyme synovium. [From the abstract:] "Electron microscopy [both EM and IEM were used] adds further evidence for persistence of spirochetal antigens in the joint in chronic Lyme disease. Locations of spirochetes or spirochetal antigens both intracellulary and extracellulary in deep synovial connective tissue as reported here suggest sites at which spirochaetes may elude host immune response and antibiotic treatment."

[From the article:] "If spirochetes are already sequestered in tissue that is inaccessible to antibiotics such as in the fibrinous and collagen tissue or within fibroblasts, high-dose parenteral antibiotics, or combination therapies with long duration may be needed to kill the living spirochetes." (p.1032)

Round bodies were also found in synovial fluid and synovium samples from patients with chronic Lyme disease.

40. Stein SL; Solvason HB; Biggart E; Spiegel D. 1996 **A 25-year-old woman with hallucinations, hypersexuality, nightmares, and a rash.** Am J Psychiatry, 153(4):545-51

"The spirochete gradually sequesters itself in cysts or blebs by inducing a surrounding fibroblast reaction, effectively creating an impenetrable barrier to immune cells and many antibiotics. In addition, the organism appears to have a direct immunosuppressant efffect on proliferation of B lymphocytes and on production of interleukin-2. This ability of the B. burgdorferi organism to ensure its survival can result in recurrent relapses, which may be difficult to detect because of seronegativity or may be resistant to standard antibiotic therapy."

41. Kersten A; Poitschek C; Rauch S; Aberer E. 1995 **Effects of penicillin, ceftriaxone, and doxycycline on the morphology of Borrelia burgdorferi.** Antimicro Agents & Chemother, 39(5):1127-33.

B. burgdorferi cultures gradually developed granules when incubated in antibiotics. The degree of alteration was strongly correlated with dose and duration. It is suggested that these morphologic changes may shed light on the ability of B. burgdorferi to survive antibiotic treatment.

"After exposure to penicillin a few individual motile B. burgdorferi organisms could be detected at any time of the 4-day observation period. The morphological alternation developed gradually; initially, after 17 h of incubation, granules of up to 0.8 µm adhering to the end and/or middle regions of the spirochetes developed in cultures incubated with concentrations at the MIC90 or greater. Their numbers increased with the time of incubation, and they formed paired as well as multiple granules after 24 h of incubation. ...After 48 h of incubation with 1.0 or 2.0 times the MIC90, these granules were transformed into up to 1.8-µm vesicle like structures. ...Formation of small colonies undergoing degeneration was observed after 48 to 72 h of incubation. ...

The alterations in the B. burgdorferi organisms incubated with ceftriaxone were identical to those in organisms incubated with penicillin. However, the onset of the alterations was already observed after 8 h of incubation. ...After 48 h no motile borreliae were present even in the presence of concentrations as low as 1/10 the MIC90, but self-propelled rods or granules were evident...

In contrast, doxycycline-treated cultures revealed single organisms with gradually decreasing motilities after 18 h of incubation at concentrations greater than MIC90; after 24 h there was a loss of motility without marked morphological alternations. After 4 days of incubation 90% of the bacteria were immotile. In cultures grown in the presence of concentrations less than the MIC90, the

proportion of motile spirochetes was 25%. Morphological alterations similar to those induced by penicillin or ceftriaxone developed only occasionally after 4 days of incubation. ...

In the present study it could not be evaluated whether the immotile B. burgdorferi organisms are only paralyzed after exposure to doxycycline, similar to T. pallidum in immobilization tests (15), or whether they are killed."

42. Coyle PK; Schutzer SE; Deng Z; Krupp LB; Belman MD; Benach JL; Luft BJ. 1995 **Detection of Borrelia burgdorferi-specific antigen in antibody-negative cerebrospinal fluid in neurologic Lyme disease.** Neurology, 45:2010-14

In Vivo

"There are data to suggest that the spirochete sheds outer surface membrane "blebs" which contain OspA antigen, into surrounding fluids. In earlier work, we were able to detect antigen-like material consistent with OspA in the CSF of patients with neurologic Lyme disease."

43. Bruck DK; Talbot ML; Cluss RG; Boothby JT. 1995 **Ultrastructural characterization of the stages of spheroplast preparation of Borrelia burgdorferi.** J Microbio Methods, (23):219-228

"...we prepared spheroplasts, bacteria stripped of their cell walls, and characterized them ultrastructurally during the preparation process. Their morphological appearance at 38ºC was also observed. ...Approximately 95% of the spirochetes of B. burgdorferi were readily converted to stable spheroplasts by the method used in this investigation [addition of the Tris buffer and lysozyme]. Of the spirochetes converted into spheroplasts, approximately 25% were transformed only partially. ...The success of the conversion from spirochetes to spheroplasts was influenced by the pH of the bathing media. At a pH above 8.0, conversion rates increased... spirochetes cultured in vitro at the relatively high temperatures encountered within their warm-blooded hosts (38ºC) formed protrusions similar to the blebs formed in spheroplasts, although somewhat more irregular in appearance."

44. Hulinska D; Bartak P; Hercogova J; Hancil J; Basta J; Schramlova J. 1994 **Electron microscopy of Langerhans cells and Borrelia burgdorferi in Lyme disease patients.** Zbl Bakt, 280:348-49

In Vivo

Cystic forms of Bb were found in skin biopsy specimens, in CSF, and in blood samples. Surface antigens of the cysts were found to be different from the antigens of coiled spirochetes.

"In the central part of ECM, mainly in the dermis, we found cyst-like forms of Bb, being antigenically different from other coiled spirochetes found in the peripheral part. These cyst-like or granular forms have been reported from culture medium (2) and we found them in the tissue. Some authors believe that cyst-like forms are caused by an inadequate environment. We suggest that these forms may be spores because of their surface envelope which shows a positive reaction with lectin WGA. At the time of the appearance of the cyst-like forms, there were a focal necrosis and edema in the central part of the ECM and a lack of nutrients in the medium. Along the periphery of ECM, Bb were found in the dermis along collagen fibres and their presence is indicated by LCs in the basal epidermis where they multiply. Mitosis of LC's was observed also in AIDS. The observation of tightly packed vesicles attached to the surface of Bb or located freely among collagen fibrils suggested that these vesicles may play a role in the protection of Bb cells aganst detection by the immuno-cell system. Lyme disease spirochetes produce membrane vesicles, which bud from the membrane of the cell to become free-floating packages of spirochetal surface proteins. We found these vesicles also in CSF and blood samples. Garon (7) has suggested that these vesicles transfer intact DNA and thus genetic information."

45. Radolf JD; Bourell KW;Akins DR; Brusca JS; Norgard MV. 1994 **Analysis of Borrelia burgdorferi membrane architecture by freeze-fracture electron microscopy.** J Bacteriology, 176(1):21-31

[Blebs:] "The propensity for B. burgdorferi to shed membrane vesicles (blebs) is a poorly understood property of the Lyme disease spirochete (4,26)." (p.23) ...Limited evidence supports a role for these structures in Lyme disease pathogenesis. Garon and coworkers (20,54) detected B. burgdorferi blebs in specimens from Lyme disease patients and demonstrated that purified blebs stimulate nonspecific proliferation of murine B cells in vitro. ...We reasoned that freeze-fracture analysis might...help to explain the intriguing observation that B. burgdorferi blebs contain extrachromosomal DNA elements (26). Virtually all large blebs were bounded by a membrane identical to the OM [outer membrane] of the parental bacterial cells." (p.28)

"Thus, our findings support the hypothesis of Garon and coworkers that blebs are pinched-off sections of cell wall which contain trapped cytoplasmic material, including plasmids (11,26)." (p.29)

46. Sadziene A; Jonsson M; Bergstrom S; Bright RK; Kennedy RC; Barbour AG. 1994 **A bactericidal antibody to Borrelia burgdorferi is directed against a variable region of the OspB protein.** Infection & Immunity, 62(5):2037-45

"The morphologic effects of bactericidal Fab fragments on cells of B. burgdorferi B311 and B. hermsii were examined by transmission electron microscopy. ...In both situations in which the bactericidal Fab fragment was incubated with its target cells, there was cell disruption and the formation of numerous membrane blebs. ...

In our study, a characteristic morphologic change of susceptible borrelias was the production of large numbers of small membrane blebs. These effects on borrelias were similar to what we had previously observed with penicillin and vancomycin, two cell wall-active antibiotics (10,19)."

47. Schaller M; Neubert U. 1994 **Ultrastructure of Borrelia burgdorferi after exposure to benzylpenicillin.** Infection, 22(6):401-6

B. burgdorferi were observed to form cysts and blebs when treated with penicillin G. "These structures were not found under optimal culture conditions. One may speculate that the borreliae could escape the action of the antibiotic by developing such spherical bodies." (p. 404)

48. Sigal LH. 1994 **The polymerase chain reaction assay for Borrelia burgdorferi in the diagnosis of Lyme borreliosis.** Annals of Internal Med, 120(6):520-21

"Borrelia burgdorferi produces large numbers of blebs, which are small membrane-bound bodies derived from outpouchings of the organism. Many of these contain B. burgdorferi DNA [13] and may persist in the synovium long after the organism [referring to the spirochete form of Bb] has been killed and eliminated. "

49. Dever LL; Jorgensen JH; Barbour AG. 1993 **In vitro activity of vancomycin against the spirochete Borrelia burgdorferi.** Antimicrob Agents & Chemother, 37:1115-21

"Approximately 75% of cells exposed to either penicillin or vancomycin had one or more large membrane blebs, designated gemmas (6), whereas untreated B31 cells in log-phase growth had only occasional (<20% of cells) small blebs that were smaller than those seen in treated cells... Thin sections of B31 cells treated with penicillin or vancomycin were indistinguishable from one another. Both demonstrated numerous gemmas... Numerous smaller spherical blebs were associated with the outer membrane of treated cells and were also found separate from the cell membranes. Untreated cells demonstrated only occasional smaller spherical blebs, found in association with and separate from the outer membrane. Rod-shaped forms or extremely long spirochetes were not observed in treated cultures."

50. Whitmire WM; Garon CF. 1993 **Specific and nonspecific responses of murine B cells to membrane blebs of Borrelia burgdorferi.** Infection & Immunity, 61:1460-7

"Extracellular membrane-bound vesicles, or blebs, are spirochetal structures which are shed from the surface of the spirochete (19). ...In the present study, we compare specific and nonspecific B-cell responses to blebs and whole-spirochete sonicates of B. burgdorferi in the murine model, demonstrate that bleb-induced mitogenesis is significantly greater than that caused by whole spirochetes, and suggest that B-cell mitogenesis is associated with spirochetal membranes with little typical LPS [lipopolysaccharide]."

51. Coleman JL; Rogers RC; Benach JL. 1992 **Selection of an escape variant of Borrelia burgdorferi by use of bactericidal monoclonal antibodies to OspB.** Infection & Immunity, 60(8):3098-3104

Spherical bodies were photographed after exposure to CB2. and in a control exposed to normal mouse IgG. [The formation of these structures is not discussed in the article.]

52. Aberer E; Duray PH. 1991 **Morphology of Borrelia burgdorferi: structural patterns of cultured borrelia in relation to staining methods.** J Clinical Microbio, 29:764-72

"Occasionally, small intensely stained granules were seen around spirochetes (Fig. 7a). ... outer membrane blebs were also seen very distinctly... The cytomorphologic features of B. burgdorferi show marked polymorphism, a fact that makes its detection in tissue or biologic fluid samples challenging to the inexperienced microscopist (Fig.1). ...The significance of membrane blebs in some B. burgdorferi cells awaits further study, but their presence was detected in some of our preparations." Also found in vitro evidence of colonies.

53. Dorward DW; Schwan TG; Garon CF. 1991 **Immune capture and detection of Borrelia burgdorferi antigens in urine, blood, or tissues from infected ticks, mice, dogs, and humans.** J Clinical Microbio, 29:1162-70

"Vesicles were resolved on the surfaces of spirochetes recovered from infected ticks and mouse tissues, indicating that these vesicles are formed by B. burgdorferi in vivo. Gold-labeled, membranous vesicles were also observed in urine and blood."

54. Barthold SW; Persing DH; Armstrong AL; Peeples RA. 1991 **Kinetics of Borrelia burgdorferi dissemination and evolution of disease following intradermal inoculation of mice.** Am J Pathology, 139:263-73

[Early, multisystemic dissemination:] "Microscopy showed early inflammatory lesions around joints in three of five mice as early as day 4, and all mice had arthritis after day 10 (Table 1). ...Inflammation of cardiac tissues was present in all mice examined at day 10 and beyond...These studies show that B. burgdorferi spirochetes disseminate to cause multisystemic infection within a few days after initial infection of the skin.(p.267-71) ...It is curious that intense inflammation occurs only in target tissues such as the heart and joints, despite the presence of spirochetes in other sites, such as skin, kidney, and spleen, with virtually no evidence of host reaction." (p.272)

[Morphology: spirochete forms decrease as the infection ages:] "Leg tissue (knee and tibiotarsus) demonstrated small numbers of spirochetes in areas of inflammation on days 4 and 7, with more organisms present on day 10 and the greatest number of spirochetes on day 15. The number of spirochetes diminished significantly thereafter." (p.269) "...the number of visible spirochetes in infected tissues drops considerably as infection progresses." (p.272)

[Intracellular:] "Spirochetes were usually extracellular, although small numbers were found in intracellular locations in these mice..." (p.272)

[Symptom Causality:] "The onset of inflammation in distant target tissues such as joints and heart coincides with the appearance of spirochetes in these sites. The early onset of inflammation and its direct correlation with spirochetes provides strong evidence that the arthritis and carditis of acute Lyme disease are due to direct effects of the spirochete, rather than an immunopathic mechanism." (p.271)

55. Preac-Mursic V; Wilske B; Reinhardt S. 1991 **Culture of Borrelia burgdorferi on six solid media.** Eur J Clin Microbiol Infect Dis, Dec;10(12):1076-9

[Abstract:] "After incubation in a candle jar and a GasPak for two to four weeks, Borrelia colonies were counted and characterized. Colony morphology was related more to the growth substrate than to the characteristics of the various Borrelia burgdorferi isolates. Culture on PMR agar resulted in the highest recovery rate and the best colony formation, with a size variation of 0.3-3.0 mm."

56. Burgdorfer W; Hayes SF. 1989 **Vector-spirochete relationship in louse-borne and tick-borne borreliosis with emphasis on Lyme disease.** In: Harris, K.F. (ed): Advances in disease vector research. Springer Verlag, NY, Vol 6:127-150

57. Garon CF; Dorward DW; Corwin MD. 1989 **Structural features of Borrelia burgdorferi - the Lyme disease spirochete: silver staining for nucleic acids.** Scanning Electron Microscopy, 3:109-115

[From the abstract:] "Intact DNA was demonstrated both by lysing blebs directly on the surface of microscope grids and by extracting molecules from purified bleb preparation with detergents and solvents. Both linear and circular DNA molecules could be identified in purified membrane blebs."

58. Hulinska D; Jirous J; Valesova M; Hercogova J. 1989 **Ultrastructure of Borrelia burgdorferi in tissues of patients with Lyme disease.** J Basic Microbiol, 29:73-83

In Vivo

Borrelia burgdorferi granules and vesicles were photographed in tissue specimens (skin samples and synovial membrane samples) of Lyme patients.

59. MacDonald AB. 1988 **Concurrent neocortical borreliosis and Alzheimer's disease: Demonstration of a spirochetal cyst form.** Annals NY Academy of Sciences, 539:468-70

In Vivo (human brain)

In vivo finding of Borrelia burgdorferi cysts in an autopsy of a human brain. "An unexpected observation was the identification *of cystic forms of the Borrelia spirochete in dark-field preparations of cultured hippocampus, and in imprints of hippocampus... A cystic form of the Borrelia spirochete would explain the ability of the microbe to persist in the host during a prolonged period of asymptomatic clinical latency, which spans the period between primary infection and the expression of tertiary manifestations of neuroborreliosis."*

60. Kurtti TJ; Munderloh UG; Johnson RC; Ahlstrand GG. 1987 **Colony formation and morphology in Borrelia burgdorferi.** J Clinical Microbio, 25:2054-8

"The small surface colonies were composed of tangles of coiled spirochetes at the periphery and numerous spherical cells.. In contrast, diffuse colonies contained fewer spherical bodies..."

61. Barbour AG; Hayes SF. 1986 **Biology of Borrelia species.** Microbiol Rev, 50:381-400

"Outer envelope blebs are also seen when specific antibody and a complement source are added to borreliae (156), when cells are frozen and thawed (175), when cells are exposed to penicillin (34), and in aged cultures (9). These findings indicate that disturbances to the cell can lead to large bleb formation. ...The nature and function of such structures are unknown; they do not appear to be an artifact of block sectioning. ...

The relapsing borreliae circulate and multiply in the blood until specific antibody appears. Once the concentration of antibody is high enough, the organisms rapidly disappear from the blood. ...When relapsing fever borreliae are no longer detectable in the blood, they may still be found in organs (120)."

[Classification:] "Robosomal ribonucleic acid (RNA) cataloging has, in fact, shown that spirochetes represent an ancient grouping and that a formal rank of class or division (phylum) would be more appropriate than order for this unique collection of microorganisms (96,198)."

62. Hayes SF; Burgdorfer W; Barbour AG. 1983 **Bacteriophage in the Ixodes dammini spirochete, etiological agent of Lyme disease.** J Bacteriology, 154:1436-9

Bacteriophage were detected in Borrelia burgdorferi isolated from a tick. The phage attached to many spirochetal surfaces, including "blebs, gemmae, or spherical bodies." Includes photographs.

63. Barbour AG; Todd WJ; Stoenner HG. 1982 **Action of penicillin on Borrelia hermsii.** Antimicro Agents & Chemother, 21:823-9

"Benzylpenicillin at its minimum bactericidal concentration induced formation of large spherical structures. These structures were bounded by one or both cellular membranes and, in some thin sections, appeared to contain material from disrupted protoplasmic cylinders... they are consistent in appearance with spheroplasts (20,29). ...A prominent electron microscopic finding was the abundance of small membranous blebs or vesicles in the penicillin-treated culture. Blebbing of the outer membrane is said to occur when spirochetes are under 'adverse conditions.' ...

A possible consequence of penicillin-induced membrane vesicle formation is the Jarisch-Herxheimer reaction...
A release of numerous blebs containing such material conceivably could precipitate the Jarisch-Herxheimer reaction."

Other Spirochetes

64. Domingue Sr, GJ; Woody HB. 1997 **Bacterial persistence and expression of disease.** Clinical Microbiology Reviews, Apr, 320-344

"We speculate that the persistence of T. pallidum DNA (despite the absence of symptoms) may also represent nucleic acid derived from dormant, viable persistent forms of the organism...that may or may not elicit clinical symptoms yet maintain the dominant presence of the microbe in tissues and contribute to spirochetal persistence and relapse. Microorganisms within the genera Treponema, Borrelia, and Leptospira are often characterized by large, cyst-like bodies that are present in their developmental cycles. These cyst-like structures have been well documented at the EM [electron microscope] level. These cyst-like bodies resemble L-form large bodies (57,58)."

65. Wolf V; Wecke J. 1994 **Formation of multiple treponemes.** Zbl Bakt, 280:297-303

"The existence of such spherical bodies as pseudomulticellular bacteria seems to be a widespread phenomenon in the tribe of spirochetes."

"It was calculated that the formation of spherical bodies may reduce their surface by up to 75% as compared to the single form. Thus, the reaction surface for antibodies or other compounds produced by the host is considerably diminished. Therefore, such spherical structures being at resting states may represent a survival strategy of spirochetes. ...the spherical bodies may be the starting point of the new inflammatory episode. This wavelike process is typical of many spirochetal diseases."

66. Mattman LH. 1993 **Cell wall deficient forms: stealth pathogens.** CRC Press, Inc., Boca Raton, Fla., 2nd ed.

"The genera Borrelia, Leptospira, and Treponema are characterized by developing large cyst-like bodies. The structure and function of the "cysts" have been documented with countless electron micrographs. They resemble the characteristic L-body of the L-cycle in many respects. Most notably, the classic spirochete may appear in the interior of such cysts. Secondly, an alternate type of reproduction from these bodies is a sprouting filament which may become the spirochete. ...The spirochetal cysts differ from bacterial L-bodies in usually forming only a few spirochetae rather than the numerous parent forms which may pack a reverting L-body of most species. Secondly, a sprouting cyst usually thrusts out a spirochetal form rather than the infinite varieties of rhizoid growth which can emerge from an L-body of most bacteria.

The formation of tiny refractile granules is also well documented for many species of all genera in the Spirochaetae. Whether these are pathogenic per se remains at this date a controversial point. There is little doubt that even for T. pallidum these granules are infective. The multiplication of the granules has been described by careful investigators, and their development into spiral organisms has been described for almost every species."

67. Bergstrom S; Garon CF; Barbour AG; MacDougall J. 1992 **Extrachromosomal elements of spirochetes. [Review]** Research in Microbiology. 143(6):623-8

[From the abstract:] "The presence of nucleic-acid-containing vesicles and its possible role in mediating DNA transfer between borreliae is an additional, very interesting feature of these organisms."

68. Delektorskii VV; Romanenko VN; Gupalo LA; Balakishieva FI. 1990 **The cytoarchitectonics of hard chancre in rabbits with experimental syphilis exposed to soliusulfon and cefamezine.** [In Russian; English abstract available] Vestn Dermatol Venerol, 4:32-6

Describes T. pallidum ultrastructure, and the process of formation of a granule. Cefamezin did not effect spirochetal cysts in the treatment of rabbits.

69. Gebbers JO; Marder HP. 1989 **Unusual in vitro formation of cyst-like structures associated with human intestinal spirochaetosis.** Eur J Clin Microbiol Infect Dis, 8:302-6

In vitro findings suggested that spirochetes may develop in cysts, contrary to the traditional view that transverse fission is their main mode of reproduction. As cysts were found in centrifugates of cultures but not in biopsy specimens, the authors speculate that this mode of reproduction may occur only when in sub-optimal environments outside the host. Includes electron micrographs of maturation of spirochetes within the cyst-like structures as supporting evidence.

[Cysts:] "Examination of ultrathin section of centrifugates of cultured spirochaetes yielded unusual cyst-like structures with an outer double membrane containing spirochaetes in different developmental stages. ...The encystment of the spirochaetes could be related to their protection, multiplication, spread and transmission."

[Intracellular:] "...the spirochaetes were not only attached to the surface but were also found within epithelial cells and in mucosal macrophages."

70. Umemoto T; Namikawa, I; Yamamoto M. 1984 **Colonial morphology of treponemes observed by electron microscopy.** Microbiology & Immunology, 28:11-22

[From the abstract:] "Scanning and transmission electron microscopy revealed that the colonies of Reiter treponemes contained spherical forms almost up to 5 μm in diameter, each consisting of an outer membrane and a treponemal main body."

71. Al-Qudah AA; Mostratos A; Quesnel LB. 1983 **A proposed life cycle for the Reiter treponeme.** J Applied Bacteriology, 55:417-28

This study provides evidence for the viability of cysts and the existence of a complex manner of reproduction. "Although transverse fission may be the main mode of reproduction of Reiter treponemes in optimal growth conditions, the spontaneous formation of cysts increases in aging cultures to the extent that it is rare to find a typical treponeme in old cultures. We conclude that such cysts... [serve to] by-pass adverse environmental conditions and to ensure the propagation of the organism. ... the existence of the causative agent of syphilis in a nonspirochetal form has long been hypothesized to explain the latency of syphilis and the infectivity of tissues devoid of demonstrable treponemes...electron micrographs showed that whole treponemes were packed tightly within the outer sheath and the size of such cysts depends on the number of treponemes packed inside. This agrees with what usually happens in protozoa in nature; ...the majority of cysts in protozoa are a means of protecting their contents against unfavorable conditions... Later, depending on conditions when the harmful exposure is past, protective cysts may become multiplication cysts. They are not merely protective but also serve for reproduction."

"...electron micrographs showed that whole treponemes were packed tightly within the outer sheath and the size of such cysts depends on the number of treponemes packed inside ..."

72. Ivlieva MS; Masiukova SA; 1982 **Detection of atypical Treponema pallidum in the chancre of a white mouse.** Vestnik Dermatologii i Venerlogii, (3)21-4

73. Umemoto T; Namikawa I; Yoshii Z; Konishi H. 1982 **An internal view of the spherical body of Treponema macrodentium as revealed by scanning electron microscopy.** Microbiology & Immunology, 26(3):191-8

"Spirochetes are well known to be microorganisms forming morphologically abnormal structures both in vitro and in vivo. ...External observation of a spherical body by scanning electron microscopy clearly revealed the main bodies [spirochetes] running beneath the inner surface of the spherical body membrane [cyst]. "

74. Ovcinnikov NM. 1981 **Important problems in the serodiagnosis of syphilis.** Vestn Dermatol Venerol, 8:22-6

[According to Mattman, 1993: "It is thought [by Ovcinnikov] that false negative serological tests for syphilis may be explained because cystic and granule stages of the treponeme have not stimulated antibody reactive with the spirochetal stage."]

75. Ovcinnikov NM; Delektorskii VV. 1981 **Treponema pallidum ultrastructure and the mechanisms of cellular protection before and during syphilis therapy.** Vestnik Dermatologii i Venerlogii, (12):37-40

76. Umemoto T; Namikawa I. 1980 **Electron microscopy of the spherical bodies of oral spirochetes in vitro.** Microbiology & Immunology, 24:321-34

[From the abstract:] "...in the presence of a high concentration of sucrose, the outer envelope of one or both terminal ends of this oral spirochete changed into a swollen structure, the SB [spherical body]."

[From the article:] "Spirochetes such as Treponema, Leptospira, and Borrelia form, in vitro or in vivo, spherical structures which have been designated as granules (3,4,7,13), cysts (16,20), and spherical forms (2)."

77. Hovind-Hougen K; Birch-Andersen A; Nielsen H. 1979 **Electron microscopy of Treponemes subjected to the Treponema pallidum immobilization (TPI) test. II: immunoelectron microscopy.** Acta Pathol Microbiol Scand, [C] 87:263-8

In vitro finding of spherical T. pallidum cells that did not react with human IgG antibodies. "...an occasional spheroid cell (6) was present in the cell suspensions studied. Spheroid cells are non-motile and no human IgG globulin could be demonstrated on the outer membrane of these cells (Fig. 6)... We are tempted to identify the non-motile cells with those that do not adsorb human IgG, and our observations would then indicate that only motile cells of T. pallidum are able to react with human IgG antibodies present in serum from syphilitic patients."

78. Blom J; Hovind-Hougen K; Jensen HJ; Birch-Anderson A. 1977 **Electron microscopy of lymph nodes of hamsters experimentally infected with Treponema pertenue.** Acta Pathol Microbiol Scand, [A] Jan; 85A(1):89-98

Treponemes were found intracellularly in macrophages. These treponemes did not show their typically helical shape, but were present as spherical forms.

79. Asai Y. 1977 **Fine structure of oral spirochetes, especially axial fibrils and cyst like structure.** Shigaku, 64(5):919-40

80. Umemoto T; Namikawa I; Nitta H. 1976 **Scanning electron microscopical observation on the spherical body of oral spirochetes.** Japan. J. Ora. Biol., 18:435-41

81. Furukawa K. 1975 **Electron microscopic studies of Treponema.** J. Kyoto Pref. Univ. Med., 84:151-165

[According to Umemoto and Namikawa, 1980: "Furukawa reported that a largely expanded protoplasmic cylinder of T. pallidum was induced by treatment with an antibiotic..."]

82. Ovcinnikov NM; Delektorskij VV. 1975 **Treponema pallidum in nerve fibres.** British Journal of Venereal Diseases, Feb;51(1):10

[Abstract:] "Ultrathin sections of a rabbit scrotal syphiloma were examined by electron microscopy. Treponemes were observed in the endo-, peri-, and epineurium of the nerve fibre. The significance of these findings, in that infection may be transmitted via the nerve fibres and pain reduced by damage to the afferent fibres, are discussed."

[From the article:] "Firstly, in our opinion, this indicates that as well as passing along the blood stream infection may be transmitted directly along the nerves to the spinal canal, meninges, and cerebrospinal fluid." Electron micrographs are provided, with T. pallidum shown in a coccoid form.

83. Ustimenko LM. 1975 **Characteristics of the morphogenesis of Treponema pallidum L forms and the stages of their reversion.** Vestnik Dermatologii i Venerlogii, (2)36-40

84. Umemoto T. 1974 **Spherical body formation of oral spirochetes following addition of sucrose.** Journal of Gifu Dent. Soc. 2:1-15

85. Ustimenko LM. 1974 **Serum factor and the induction of L forms of Treponema pallidum under the action of penicillin during prolonged cultivation of the microorganism.** Antibiotiki, 19(11):998-1003

86. Joseph R; Holt SC; Canale-Parola E. 1973 **Peptidoglycan of free-living anaerobic spirochetes.** Journal of Bacteriology, 115:426-35

"Addition of penicillin G to exponential phase cultures of S. stenostretpa resulted in conversion of the helically shaped organisms into round or distorted cells, as observed by phase microscopy."

87. Dunlop EM. 1972 **Persistence of treponemes after treatment.** British Medical Journal, 2:577-80

Discussion of findings by multiple research teams of morphologically variant T. pallidum forms after antibiotic treatment. "The fundamental question is whether treponeme-like forms found after the treatment of syphilis are Treponema pallidum. ...Morphologically some persisting treponeme-like forms in material from patients are identical with T. pallidum. Animals have been infected with such material by four groups of workers. ...Treponemes have been found after dosages of penicillin sufficient to maintain much higher concentrations of penicillin than the 0.03 U/ml regarded as fully treponemacidal. Nevertheless, a strain of T. pallidum resistant to penicillin has yet to be described."

88. Lauderdale V; Goldman JN. 1972 **Serial ultrathin sectioning demonstrating the intracellularity of T. pallidum.** British Journal of Venereal Diseases, 48:87

Cystic forms of T. pallidum, both intracellular and extracellular, were found in rabbit tissues. "This report of intracellular treponemes should stimulate consideration of the possibility that T. pallidum may be 'stored' intracellularly, with retention of its antigenicity, viability, or even its pathogenicity, in some host cells. ..The speculation of Goldman (1969, 1971) that an intracellular habitat may provide another protective device for the treponemal invader against the action of drugs or the immunological reactions of the host is raised once more."

"Cyst-like forms, as described by Ovcinnikov and Delektorskij (1968, 1969a), were seen in our preparations."

89. Ustimenko LM. 1972 **Effect of the serum factor on the sensitivity of cultural Treponema pallidum to penicillin and on its capacity to L-transformation.** Zhurnal Mikrobiologii, Epidemiologii i Immunuobiologii, 49(5):116-9

90. Ovcinnikov NM; Delectorsku VV. 1971 **Current concepts of the morphology and biology of Treponema pallidum based on electron microscopy.** British J Venereal Diseases, 47:315-28

[Granules:] "Another mode of reproduction resorted to in adverse circumstances consists in the formation of spores which subsequently develop into new treponemes. The breakdown into granules is especially pronounced under the action of penicillin and immune sera."

[Cysts:] "By means of electron microscopy, we have succeeded in demonstrating the presence of cysts in a rabbit chancre... When examining the cysts, we could distinctly see multi-layered membranes and treponemes cut in various places." (p.317)

"Under stressful conditions, the treponeme 'packs' itself into a compact roll (Fig. 8) and becomes covered with a transparent mucoid capsule, which resists the penetration of drugs and antibodies. The organisms may persist in this form for a prolonged period without any reaction from the host. The encysted treponemes and the host coexist more or less peacefully, but under propitious circumstances the cysts may be transformed again into the usual spiral, which damages the cells of the host and elicits a response." (p.316)

"If the stress is not lethal, accessory envelopes are formed and the treponemes become well encapsulated and may survive new stresses many times stronger than the initial one. Encystment as a mechanism of survival and mode of reproduction is widespread in nature, especially among protozoa." (p.316)

"When L-forms are transferred to the usual media they soon reverse to the original forms... Some of them are seen to divide..." (p.327)

[Intracellular:] T. pallidum were found inside a cell taken from the site of a chancre; and L-forms were found inside plasma cells. [Includes photos of intracellular T. pallidum]

91. Ovcinnikov NM; Delektorskii VV; Ustimenko LM. 1970 **L-forms of Treponema pallidum (electron microscopic studies).** Vestnik Dermatologii i Venerlogii, 44(8):53-7

92. Hoyer BH; King JR. 1969 **Desoxyribonucleic acid sequence losses in a stable streptococcal L-form.** Journal of Bacteriology, 97:1516-17

[Note: this study is not about spirochetes, but is included because of its interesting findings concerning DNA sequences and L-forms of bacteria.]

Demonstrated the loss of a portion of the chromosomal DNA in an L-form of Streptococcus.

[From the abstract:] "A portion of the deoxyribonucleic acid sequences present in Streptococcus faecalis were absent in its stable L form. The remaining sequences were common to both forms."

[From the article:] "In the L form, 4 to 6% of the sequences present in the parent (as estimated from Fig. 1a) were lacking. ...A similar, naturally occurring deletion has been described in the genus Brucella (3)."

93. Ovcinnikov NM, Delektorskij VV. 1969 **Further studies of the morphology of Treponema pallidum under the electron microscope.** British J Venereal Diseases, Jun;45(2):87-116

"With lengthy exposure to unfavourable factors at a relatively low level of intensity, cysts are formed and resistance to the particular factor concerned increases. If the treponeme is exposed to very intense unfavourable factors, the cysts which have been formed die and disintegrate. ...

The motility of the spheroids suggests that they are viable... these are formed for defence and long-term survival. ...

Some cysts contain round lamellar structures or formations filled with a granular mass. We suggest that this mass is a store of nutrient material. ...In lengthy periods of observation treponemes can be seen issuing from the cysts. Finally, the seeding of material containing large numbers of cysts and almost no spiral treponemes on to fresh nutrient media with favourable conditions for growth leads to abundant growth of spiral forms.

Cysts are also found in cultivated treponemes, in pathogenic treponemes, in material from rabbits, and in leptospirae (Fig. 85)."

94. Ovcinnikov NM; Delectorsku VV. 1968 **Further study of ultrathin sections of Treponema pallidum under the electron microscope.** British J Venereal Diseases, 44:1-34

Observations of T.pallidum cystic and granular formations under the electron microscope. "...under unfavourable conditions of existence, treponemes form real cysts as a method of persistent survival and multiplication, as occurs not infrequently among protozoa."

"As the treponeme moves, the thickness changes. This indicates that the body possesses a capacity for contraction... The sharply-marked structural elements of the treponeme and its complex and characteristic structure indicate that cysts are not a product of degeneration. In addition, in cultures where there are many cysts, they are very mobile, which is another argument against degeneration... When transfers are made from cultures containing cysts and almost no ordinary spiral forms, growth of ordinary spiral forms occurs."

Includes photo of a treponeme packed into a cyst surrounded by a mucus-like mass.

95. Yobs AR; Clark Jr. JW; Mothershed SE; Bullard JC; Artley CW. 1968 **Further observations on the persistence of Treponema pallidum after treatment in rabbits and humans.** British J Venereal Diseases, 44:116-30

Results of a 4-year study of rabbits treated with penicillin for late latent syphilis. The persistence of syphilis in numerous subjects after antibiotic treatment was confirmed. Cortisone treatment was found to reactivate clinical disease. Various theories are offered to explain the persistence of T. pallidum despite antibiotic therapy, including morphologic changes in the organism. "One may also speculate that T. pallidum has a life cycle in only one stage of which the recognized treponemal morphology is found and in only one stage of which the organism is sensitive to antibiotics."

96. Kats LN; Konstantinova ND; Anan'in VV. 1967 **[Electron microscopic studies of the Leptospira cyst].** Dokl Akad Nauk SSSR, 176(3):710-1

97. Ovcinnikov NM; Delectorsku VV. 1966 **Morphology of Treponema pallidum.** British Journal of Venereal Diseases, 35:223-9

[Cysts:] "...the impression is gained that these round structures separate by constriction into independent granular forms of T. pallidum. In cultures, side by side with spiral treponemes, spherical bodies of various sizes and structures are encountered. Some of these, found in cultures four to six days old, are small and highly motile, with brilliant granules... In older cultures (14-30 days old) the cysts reach a great size and have a thick envelope, which is apparently formed from the outer envelope of the treponeme. ...Inside is the treponeme, and this looks either elongated or round, consisting of separate segments seemingly not connected with each other... These are cysts."

Discussion: The results of this examination of ultra-thin sections under the electron microscope make it possible to affirm that T. pallidum is not a long, solid cylinder of spiral form, but consists of individual segments, whose size differs with the age of the culture. ...The number of particular forms depends on the conditions of existence. Under favourable conditions elongated forms predominate, and under unfavourable conditions the rounded forms."

"...we are inclined to consider the granular forms to be one of the stages of resistant survival, occurring under unfavourable conditions. In our earlier papers (Ovcinnikov, 1955) we have given some evidence on this matter, but we do not yet consider the conclusion completely beyond dispute."

[Reproduction:] "A treponeme may divide not only in two but also into several segments."

98. Pillot J; Ryter A. 1965 **Structure des spirochetes. I. Etude des genres treponema, borrelia et leptospira au microscope electronique.** Ann l'Inst Pasteur, 108:791-804

[According to Aberer, 1996: granules were found to form in old cultures of Borrelia.]

99. Ustimenko LM. 1965 **L Forms of Treponema pertenue.** Vestn Akad Med Nauk SSSR, (20):46-50

100. Bladen HA; Hampp EG. 1964 **Ultrastructure of Treponema microdentium and Borrelia vincentii.** Journal of Bacteriology, 87:1180-91

"Spirochetal granules were frequently observed in thin-sectioned material of both strains FM [Treponema microdentium] and N9 [Borrelia vincentii]. They varied from 0.7 to 25 µ in diameter, and contained 2 to more than 50 protoplasmic cylinders. ...End knobs were usually evident on both ends of the organism and served as attachment sites for fibers of the axial and terminal filaments. ...The end knobs were possibly analagous to the basal granules or blepharoplasts seen in flagellated bacteria, but this could not be determined from our results."

101. Collart P; Borel L; Durel P. 1964 **Significance of spiral organisms found after treatment in late human and experimental syphilis.** British J Venereal Diseases, 40:81-9

[Persistence:] "Pencillin treatment, if given late in the disease, of whatever dosage or duration, is unable to destroy all the treponemes which have been present in the organism for a long time. ...Is the persistence of T. pallida after treatment unique to this species? Probably not; and what we call cure, in a clinical sense, probably does not correspond to total bacteriological destruction. ...The condition of bacteriological quiescence is perhaps what we call clinical cure..."

[Variant Forms:] " As the infection ages, less typical organisms are found... Are the organisms really Treponema pallida? We found spiral organisms in the lymph nodes and the cerebrospinal fluid of rabbits and of treated patients, which do not always show the typical morphological appearance of T. pallida as seen in a chancre or in an acute orchitis. These organisms are the same as those seen in late untreated experimental syphilis and are called T. pallida by numerous authors whose scattered publications do not seem to have attracted much attention."

[Diagnosis/Testing:] "Persistence of treponemes in the tissues provides a satisfactory explanation for the continued presence of immobilizing antibodies after treatment."

[Treatment:] "Cortisone can sometimes reactivate latent syphilis in rabbits. Two rabbits out of twelve which had been treated and then given cortisone presented the classical lesions of late syphilis. These observations appear to be evidence of persistence of the vitality of the T. pallida."

[Methodology:] "Levaditi and Vaisman (1945) has already shown that T. pallidum can be demonstrated by staining even after treatment with arsenic and penicillin, in the syphiloma of a rabbit, when examination by dark-ground microscopy was negative." "This may surprise those who rely on darkground microscopy; in fact, it has already been described but has been forgotten because the work was reported some years ago."

102. Pillot J; Dupouey P; Ryter A. 1964 **La signification des formes atypiques et la notion de cycle évolutif chez les spirochètes.** Ann Inst Pasteur (Paris), 107:484-502, 663-77

[According to Hayes & Burgdorfer, 1993: Pillot et al found that "Gemmae become more numerous in acidic older cultures and can be induced by rapid decreases of pH."]

103. Listgarten MA; Loesche WJ; Socransky SS. 1963 **Morphology of Treponema microdentium as revealed by electron microscopy of ultrathin sections.** Journal of Bacteriology, 85:932-39

" 'Granules' were seen more frequently in older cultures [of T. microdentium]." (p.934)

[Observations concerning cell wall:] "The [cell] envelope had an irregular contour, was easily disrupted during processing, and did not appear essential in maintaining the shape of the protoplasmic cylinder. It is therefore probable that this envelope is quite distinct from bacterial cells walls, which in ultrathin sections appear as regular, well-defined, electron-dense structures." (p.938)

104. Hardy PH; Nell EE. 1961 **Influence of osmotic pressure on the morphology of the Reiter treponeme.** Journal of Bacteriology, 82:967-78

"[Reiter] Treponemes in saline solution were observed while distilled water was pulled into the preparation by capillary action, and it was found that although all treponemes in a field were not changed to spheres simultaneously, the conversion of any single one took place instantaneously." (p.973)

105. Gürün H. 1957 **A new culture method for the organisms of leprosy, tuberculosis, and syphilis.** Ruzarli Matbaa (Ankara), pp.1-42

[According to Mattman, 1993: "Gürün grew T. pallidum in a beeswax-honey medium. In his experience, every isolate grew first as multitudinous granules."]

106. Gängel G; Themann H. 1956 **[Title unknown]** Arch Hyg Bakteriol, 140:559-68

[According to Mattman, 1993: "Nonbinary fission propagation of L. icterohemorrhagiae is beautifully demonstrated... From one active center many Leptospira extend (Figure 8). The authors comment on how greatly their findings with Leptospira resemble the cycles observed in T. pallidum by DeLamater and associates. Again, spirochetes form within cysts."]

107. Swain RH. 1955 **Electron microscopic studies of the morphology of pathogenic spirochetes.** Journal of Pathol. Bacteriol., 69:117-28

[According to Mattman, 1993: "The slender Leptospira with a diameter of only 0.12 µm are sometimes widened by a "bubble," within which a coiled spirochete is seen by fine structure studies. The bubbles appear as early as 5 d."]

108. Czekalowki JW; Eaves G. 1954 **Formation of granular structures by Leptospirae as revealed by the electron microscope.** J Bacteriology, 67:619-27

Leptospira began to show granulation after 2 weeks in a culture. The granules were spaced regularly within the bodies of the spirochetes. After four weeks a larger type of granule appeared which was broader than the body of the spirochetes. These were later "shed free." By the 5th to 7th month, there were no spirochetes observed; the culture contained only granules. The granules consisted of "what appears to be short segments of leptospiral body embedded in homogeneous substance." The authors conclude that the "formation of granules represents a rhythmic and constant process and hence these granules must play a role in the life-cycle of leptospirae."

109. Steiner G. 1954 **Morphology of spirochaeta myelophthora in multiple sclerosis.** Journal of Neuropathology, 13:221-9

"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.

...Morphology and Polymorphism of Spirochaeta Myelophthora: Loops, incomplete, nearly complete or totally complete rings are occasionally seen... 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.

...Classification: ...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.

...Reproduction: ...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.

...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 spirochetal diseases, such as relapsing fever and syphilis... 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.

...Transformation: There is a definite sequence of events in the disintegration of the spirochaeta myelophthora. Breaking-up starts with the appearance of loops, rings (fig. 2d), knobs, (fig. 1r, s, t), partial thickening and the formation of granules of different sizes ....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. ..." [Includes photographs as supporting evidence.]

110. Coutts WE; Coutts WR. 1953 **Treponema pallidum buds, granules and cysts as found in human syphilitic chancres and seen in fixed unstained smears under darkground illumination.** American Journal of Syphilis, 37:29-36

"McDonagh classified the spirochete with the Protozoa and paralleled its development with that of the malaria parasite. Many investigators have observed the small intracellular granules not only in endothelial cells, but in red corpuscles, lymphocytes, fibroblasts, and giant cells (Ross, 1913); Lundie, 1919; Coutts). ...we are firmly convinced of the existence of a T. pallidum life cycle. This cycle is apparently as complex as that of the malaria parasite and is multiphasic. However, up to the moment it is practically impossible to establish an exact correlation between its different phases.

Among these cycle forms we find definite and characteristic dense or vesicular spheroid bodies closely in contact with or attached by short stalks to the cell body and which originate from the treponemal cell wall. As pointed out by several authors who have studied animal strains of T. pallidum, these recall conidia and chlamydospores of higher fungi. Some of them contain a denser granule in the interior. We also find free spheroid or ovoid bodies containing a denser granule in their interiors, which develop into a commalike body. This commalike body is liberated as such and eventually grows and spirals into a typical treponeme.

Another type of free structure may contain numerous dense rounded bodies, commalike bodies, or thin spiral organisms (spirochetal cysts). These spirals are liberated by rupture of the cyst owing to overdistention. ....Spirochetogenic granules are by far more numerous than the cysts."

111. Morton HE; Ford WT. 1953 **Preliminary observations of the action of penicillin on Treponema pallidum in vivo.** American Journal of Syphilis, 37:529-35

"When bacteria [T. pallidum] are brought into contact with sublethal concentrations of penicillin, the morphology is altered markedly."

112. Bryant MP. 1952 **The isolation and characteristics of a spirochete from the bovine rumen.** Journal of Bacteriology, 64:325-35

"In the present study cultures of spirochetes up to two months in age have always shown a few typical spiral forms, but the round bodies have been the predominating type. On transfer to agar dilution series these old cultures gave rise to large numbers of spirochete colonies. Also, young cultures four to five days old have shown actively motile spirochetes with end bodies attached. These observations suggest that the round bodies might be viable."

113. Steiner G. 1952 **Acute plaques in multiple sclerosis, their pathogenic significance and the role of spirochaetes as etiological factor.** Journal of Neuropathology, 11:343-72

Spirochetes, spirochetal cysts, and spirochetal granules were found in autopsies of MS patients. Includes photographs as supporting evidence.

"Extracellular Granular Bodies: These granules were of varying sizes and shapes. Round, ovoid, or irregularly contoured shapes were common. ...Two or more granules in close proximity were also seen. ...

Intracellular Granular Bodies: ...The granules differed in shape and size from the extracellular granules. They were more massive, and of a very irregular shape. ...

The Relationship of Granular Bodies and Spirochetes: There are all intermediate stages between well preserved regularly coiled spirochetes and granular bodies. There are terminal granules with adherent spirochetal threads (fig. 9c); there are granules already freed from the still persisting spirochetal thread, but at a very short distance from it, so that the breaking off of the granule from the spirochetal thread seems very probable. ...There are spirochetes...still showing the structural continuity between the granule and the spirochete. The knobs and loops represent probably the earliest transitional phases from the spirochetal form to granule formation. There is no doubt that the granular bodies, the haptocytes and the spirochetes are in intimate pathogenetic relationship. ...

The biological significance of these bodies in multiple sclerosis is still obscure. One aspect, however, is certain: These granular bodies are definitely related to the presence of well preserved spirochetes and their disintegrating forms.

Granular bodies in general may represent 1) involutional forms (a) with possibility of redevelopment into typical spirochetal forms, (b) representing beginning disintegration and final death of the spirochetes, (c) possibility of (a) and (b), that is, redevelopment into spirochetal forms as well as irreversible disintegration; 2) specific evolutional forms in the life-cycle of the spirochete. At present no decision between 1) or 2) is possible.

114. Angulo JJ; Watson JHL; Wedderburn CC; Leon-Blanco F; Varela G. 1951 **Electromicroscopy of Treponemas from cases of Yaws, Pinta, and so-called cuban form of Pinta.** Am J of Tropical Med, 31:458

115. Delamater ED; Haanes M; Wiggall RH. 1951 **Studies on the life cycles of spirochaetes: VII. The life cycle of the Kazan non-pathogenic Treponema pallidum in culture.** American Journal of Syphilis, 35:216-24

116. Delamater ED; Haanes M; Wiggall RH. 1951 **Studies on the life cycles of spirochaetes: V. The life cycle of the Nichols non-pathogenic Treponema in culture.** American Journal of Syphilis, 35:164-79

Formation of reproductive cysts.

117. Delamater ED; Haanes M; Wiggall RH; Pillsbury DM. 1951 **Studies on the life cycle of spirochetes. VIII. Summary and comparison of observations on various organisms.** J Invest Dermatol, 16:231-56

"Production of gemmae as a means of vegetative reproduction:
The production of gemmae has been observed in all of the organisms cited above except Borrelia novyi. This organism has not as yet been adequately studied. ...It appears in the present stage of our observations that the granule that becomes visible within these minute cysts is the primordium of the daughter spirochete and that the spirochete is produced by elongation and development of this granule. ...

An additional method for the reproduction of spirochetes appears to be by the formation of structures designated here as multispirochetal cysts. ...At the present time it can be said that dense granules, usually lying at one side or at the periphery of the cysts, appear to reduplicate, forming dense aggregates. From these recognizable spirochetal filaments develop... In Figure 7 the central body suggests the possibility that the granules or inclusions, each of which forms a new spirochete, may reduplicate by a process of budding. It will be readily seen that these multispirochetal cysts may obtain tremendous size and may include very large numbers of organisms. ...Emergence of adult forms from these large cysts will be described presently. ...

Current studies...suggest that so far as these observations have been taken, we are dealing with processes of reproduction which apply at least in some degree in most spirochetes.

[Classification:] "It seems likely that the spirochetes should be considered as a separate group of micro-organisms distinct from the bacteria and also distinct from the protozoa."

118. Hampp EG. 1951 **Further studies on the significance of spirochetal granules.** Journal of Bacteriology, 62:347-49

74-month old cultures "consisted of nothing but spirochetal granules and no vegetative forms of the organisms were in evidence. ...subcultures were placed in anaerobic jars and incubated at 37 C. The spirochetal cultures were examined after 48 hours and thereafter at 24-hour periods until growth became apparent... It is a possibility that these granules may be resting bodies formed in response to adverse environmental conditions with reduction of their metabolic activities to a minimum with retention of reproductive capacities."

119. Klieneberger-Nobel 1951 **The filterable forms of bacteria.** Bacteriol Rev, 15:77-103

"FILTERABLE FORMS IN SPIROCHETES: ...It is most interesting that in the cycle of spirochetal evolution a phase seems to occur in which the organism persists in the form of small granules. This form is apparently resistant and latent and becomes infective when it regenerates spirochetes. ...

It is known that growth in immune serum causes organisms to go into the L phase. Through the process of regeneration they may emerge as organisms resistant to the inhibitory serum factor. It is therefore feasible that in spirochetes an antigenic as well as a morphological transformation occur at the same time. ...

There seems to exist an alternation between the actual spirochetal phase and a granular phase which, it is assumed, may represent the regenerative or L phase. This latter phase is at the same time resistant and responsible for the periods of latency. It is able to reproduce young spirochetes which may in various ways differ from the preceding generation. The existing information indicates that the spirochetal L phase consists of particles which are almost submicroscopic."

120. Levaditi C; Vaisman A; Chaigneau H. 1951 **Culture du Spirochaeta Duttoni dans l'oeuf fécondé de poule.** Ann Inst Pasteur, 80:9-20

[According to Klieneberger-Nobel, 1951: "Balls, loops and argentophilic almost submicroscopic granules were observed in abundance. they occurred in the interstices between the cells as well as inside the cytoplasm of the cells themselves. The authors expressed the opinion that the argentophilic granules are able to regenerate the typical wavy spirochetes."]

121. Campbell RE; Rosahn PD. 1950 **The morphology and staining characteristics of Treponema pallidum. literature and description of a new technique for staining the organisms in tissues.** Review of the Yale Journal of Biology and Med, 22:527-43

Demonstrated (via a new staining technique) the following spirochetal forms in an active syphiloma of a rabbit: "filamentous forms, short forms, irregular forms, thick long forms, circular forms, forms with terminal ovoid body, free ovoid bodies, incomplete serrated circular forms, comma forms, intracellular circular smooth and serrated forms, extracellular granular circular forms, and granular forms." Includes photographs.

Also includes an interesting historical account of the discovery of atypical spirochetal forms in the early 1900's, and the attempts of various researchers to establish the function of these forms.

122. Delamater ED; Newcomer VD; Haanes M; Wiggall RH. 1950 **Studies on the life cycles of spirochaetes: I. The use of phase contrast microscopy.** Am J Syphilis, 34:122-5

Includes several small photos of spirochetes emerging from "gemma," which the authors interpret as reproductive forms. "The first [photograph] shows the origination of three dense gemmae from two entwined spiral forms (X3,700). The second shows a very early emergence of a delicate spiral from a gemma (X3,460). Fig. 4 demonstrates further emergence of a spiral form from its gemma (X3,460)."

123. Delamater ED; Wiggall RH; Haanes M. 1950 **Studies on the life cycles of spirochaetes: III. The life cycle of the Nichols pathogenic Treponema pallidum in the rabbit testis as seen by phase contrast microscopy.** J Experimental Med, 92:239-46

"...it seems likely from these observations that there are two means of vegetative reproduction, consisting of (1) transverse division (the most important under usual conditions); and (2) the production of gemmae or buds which eventuate into unispirochetal cysts comparable to those described for saprophytic forms, within each of which single spirochetes develop and differentiate, and from which they subsequently emerge." (p.244)

"..it is suspected on the basis of these studies that the presence of this life cycle may form a part of the basis of the latency problem as it occurs in syphilis."

124. Delamater ED; Wiggall RH; Haanes M. 1950 **Studies on the life cycles of spirochetes: IV. The life-cycle of the Nichols pathogenic Treponema pallidum in the rabbit testis as visualized by means of stained smears.** J Experimental Med, 92:247-50

Studies demonstrating the development of T. pallidum spirochetes from gemmae, using material from rabbit testis.

125. Hampp EG; Bethesda MS. 1950 **Morphologic characteristics of the smaller oral treponemes and Borrelia vincentii as revealed by stained smear, darkfield and electron microscopic technics.** J Am Dental Assoc, 40:1-11

126. Babudieri B. 1949 **The morphology of the genus Leptospira as shown by the electron microscopy.** Journal of Hygiene, 47:390-392.

127. Gelperin, A. 1949 **Morphology, cultural characteristics and a method for mass cultivation of the Reiter spirochaetes.** Am J Syphilis, 33:101-13

128. Jakob A. 1949 **Ein Beitrag zur Frage der Dauerformen (Kornchenstadium) bei den Leptospiren.** Klin Woehsehr, 27:364-6

129. Hampp EG; Scott D; Wykoff RWG. 1948 **Morphologic characteristics of certain cultured strains of oral spirochetes and Treponema pallidum as revealed by the electron microscope.** Journal of Bacteriology, 56:755-69

"Typical free granules, the end products of granule "shedding," ... consist for the most part of what appear to be short sections of spirochetes closely packed together...Although it is not possible to determine from these micrographs that the granules are germinative units, their constant rhythmic occurrence in living cultures suggests this possibility. Further support of this hypothesis is provided by the fact that cultures up to 31 months old, showing only refractile granules by dark-field examination, have invariably given normal growths on transfer to fresh medium (Hampp, 1946)." (p.768).

130. Lennhoff C. 1948 **Spirochaetes in aetiologically obscure diseases.** Acta Dermato-Venereologica, Vol 28 Fasc 3:295-324

"If arsphenamine is injected intravenously into a syphilitic rabbit and the serum taken from the chancre at short intervals is smeared on glass slides, ...the spirochaetes will be found stained, and progressive morphological changes will be noted during their gradual disappearance."

131. Bessemans A; Wittebolie P; Baert H. 1947 **Study by means of micromanipulation of the virulence of one or several spirochaetes as well as viability of spirochaetes or granular forms of culture of supposed Treponema pallidum.** Bulletin of Hygiene, 23:548

132. Wile UJ. 1947 **Transmission of experimental syphilis from mouse to mouse in absence of S. pallida and pathologic changes in presence of successful innoculation.** Am J Syphilis, 31:109-14

Showed that syphilis can be transmitted by tissues from infected hosts in the absence of spirochetes, suggesting that the infectious agent is present in another form. [Note: this study does not specifically mention cysts or granules.]

133. Hampp EG. 1946 **Morphologic alteration of smaller oral treponemas during aging of cultures; Effect of age on viability of spirochetal cultures.** J Am Dental Assoc, 33:201-6

[As described by Hampp, 1951: "...pure cultures of the smaller oral treponemes maintained in anaerobic jars at 37 C up to 31 months and exhibiting only refractile spirochetal granules by dark-field examination have given rise to normal growth in a limited period of time when transferred to fresh medium."]

134. Wile UJ; Johnson SAM. 1944 **Further study of the chick embryo as a culture medium for the Spirochaeta pallida.** Am J Syphilis, 28:187-91

[According to Mattman, 1993: "...chorioallantoic membrane from chick embryo inoculated with T. pallidum might be free of spirochetes by dark-field examination yet produce syphilis when inoculated intratesticularly in rabbits." --suggesting the presence of another form of the organism.]

135. Bessemans A; Wittebolie P; Niemegeers L. 1943 **Modification experiméntale durable de la structure antigenique des leptospires.** Bull acad roy med Belg, 442-5

136. Herreweghe E. 1943 **Coloration des granules leptospiriens.** Acta Biologica Belge, 3-4:245

137. Mudd S; Polevitsky K; Anderson TF. 1943 **Bacterial morphology as shown by the electron microscope; V. Treponema pallidum, Treponema macrodentium and Treponema microdentium.** Journal of Bacteriology, 46:15-24

"The spheroidal bodies shown in the electron micrographs cited we certainly do not believe can reasonably be interpreted as degeneration products. They are definite and characteristic bodies originating from the spirochetal cell. ...Irregularly spheroidal, dense bodies... are often found attached to the spirochetal cell, frequently near the end; such a dense body may be in close apposition to the outside of the spirochetal cell-wall or may be connected to it by a short stalk. The evidence concerning these bodies seems to support the interpretation that they are asexual reproductive bodies."

138. Bessemans A; Wittebolie P; Baert H. 1942 **Le micro-manipulateur et les granules d'une souche de Leptospire aquicole nonpathogene.** Bull ass. diplomés microbiol. fac. pharm., Nancy, 61:72-80

[According to Czekalowski, 1954: Granules from the culture of a leptospira were isolated using a micromanipulator and grown from single cell cultures.]

139. Gastinel P. 1942 **A propos de la présence du granule spirochétogéne chez la souris experimentalement syphilisée.** C rend Soc biol, 136:184

140. Gastinel P; Mollindeo R. 1942 **Sur l'evolution du L. ieterohaemorrhagiae, granule leptospirogene.** Compt. rend soc biol, 136:141-4

141. Levaditi C; Noury H. 1942 **Syphilis inapparent de la souris et granules spirochétogènes.** C. rend Soc. biol., 136:418

142. Morton HE; Andersen TF. 1942 **Some morphologic features of the Nichols strain of Treponema pallidum as revealed by the electron microscope.** Am J Syphilis, 26:565-73

143. Morton HE; Anderson TF. 1942 **Observations on the morphology of Leptospirae and the Nichol's strain of Treponema pallidum with the aid of the RCA electron microscope.** Journal of Bacteriology, 43:64-5

"Granules, lateral buds, and constrictions of the treponemata as described by numerous workers have been observed."

144. Wile UJ; Picard RG; Kearny EB. 1942 **The morphology of spirochaeta pallida in the electron microscope.** JAMA, 199:880-1

"...in many specimens a curious knoblike structure was seen at the end of many organisms. Their almost uniform shape and density suggest that these are not extraneous particles of the preparation but a part of the organism itself."

145. Polevitzky KA; Anderson TF. 1942 **The morphology of various bacterial forms, some of pathogenic significance in oral infections, as shown by the electron microscope.** Journal of Bacteriology, 43:64-5

"The morphologic characteristics of these organisms [Fusiformis dentium and Borrelia vincentii] appear to change with the age of the culture."

"Another series of electron micrographs demonstrates two forms of oral spirochetes: Treponema microdentium and Treponema macrodentium. They were also prepared from pure cultures. These pictures are unusual because in them we can see clearly the end filaments described by Noguchi in 1912."

146. Levaditi C. 1941 **Phases involutives der Treponema pallidum, et granules spirochetiens argentophiles chez les souris atteintes de syphilis experimentale cliniquement inapparente.** C rend Soc biol, 135:467

147. Levaditi C. 1941 **L'involution du Treponema pallidum est-elle un phénomène interessant l'ensemble de l'organisme contaminé?** C rend Soc biol, 135:1105

148. Mollinedo R. 1941 **Essia sur le cycle évolutif des spirochètes.** I.P.P., 6, Pl. du Louvre, Paris

149. Seguin P. 1941 **A propos du granule spirochétogène.** C. rend Soc. biol., 135:1159

150. Wile UJ; Snow JS. 1941 **The chick embryo as a culture medium for Spirocheta pallida.** J Invest Dermatol, 4:103-9

[According to Mattman, 1993: "...chorioallantoic membrane from chick embryo inoculated with T. pallidum might be free of spirochetes by dark-field examination yet produce syphilis when inoculated intratesticularly in rabbits."]

151. Manouélian Y. 1940 **Etude morphologique du Spirochaeta pallida. Modes de devision. Spirochétogène syphilitique.** Annales de l'Institut Pasteur, 64:439-55

153. Seguin P. 1940 **Le granule spirochétogène; étude morphologique et biologique.** Ann Derm Syph, Par., 10:833

152. Manson-Bahr. 1940 **Relapsing fevers.** Manson's Tropical Diseases, 11th edition

"There are those who think that the spirochaete has an invisible stage in the blood. It is said that if a drop of blood containing few spirochaetes is placed in sterile vaseline and incubated, it will in a few hours be found swarming with large numbers arising from forms previously invisible." (p.214)

"There has been controversy as to the meaning of the chromatic granules seen within the body of the tick, but it is probable that these are of two kinds, some representing a degeneration of the defunct spirochaetes, while others are to be regarded as active stages in the developmental cycle of the organism. The
fact remains that for a considerable time after ingestion, fully formed spirochaetes cannot be demonstrated within the body-cavity of the infected tick. " (p.217)

154. Simon C; Mollinedo 1940 **Diagnostic de la syphilis par la recherche du granule spirochétogène.** Presse Médicale, 48:513-6

[According to Klieneberger-Nobel, 1951: "Simon and Mollindeo (124) investigated the cycle of T. pallidum by serial punctures of the lymphatic glands in cases of syphilis during the disease and treatment. They found that T. pallidum underwent a transformation and that one of the stages of the cycle was granules, ("granule spirochetogene"). This granular stage persisted in the glands for a long time during chemotherapeutic treatment. According to the drug applied the adult spirochetes decreased more or less rapidly. The authors believe, that a cure is not achieved unless the granular stage disappears from the human organism as well."]

155. Steiner G. 1940 **Morphologic appearances of spirochetal reproduction in tissues.** Archives of Pathology: 189-99

"In the tissues the organisms are distributed in two ways: (1) they are diffusely scattered; (2) they are accumulated in dense ball-like masses. It is to the latter appearance that I wish to draw special attention. Morphologically these ball-like masses are round or oval accumulations, made up of spirochetes closely packed together. ...

Reproductive colonies are found only in very acute stages of syphilitic diseases. The structure of these colonies in the tissues has an appearance which is almost identical with that of colonies growing in solid mediums. Furthermore, in the final stages of some spirochetal diseases characterized by the agglomerative phase of spirochetal reproduction (relapsing fever and spirochetosis gallinarum) numerous single degenerating spirochetes are almost always found. Degenerating spirochetes are recognized by the presence of spherical granules on one or both ends of the individual organism, by deformed spirals, by rings or loops, by parts fused together or even by isolated granules. Such degenerated forms are not seen in recent conglomerations or in their vicinity, where spirochetes are always rich in number. ...

At present no explanation for this specific conglomerative type of reproduction can be offered."

156. Hassin GB; Diamond IB. 1939 **Silver cells and spirochete-like formations in MS and other diseases of the central nervous system.** Arch Neurology & Psych, 41:471-83

Reviews and confirms the findings of G. Steiner and other researchers who found "silver cells" [spirochetal granules that take a silver stain] in brain autopsies of MS cases. (G. Steiner contended that MS is an infectious disease caused by a spirochete that extrudes granules, and which destroys myelin.) While the authors found granules in the CNS of all 8 MS patients they studied, they dispute Steiner's contention that spirochetes are the causative agent of MS.

"Silver granules were present in all the 8 cases of multiple sclerosis studied. They were numerous in the areas bordering on the plaques... In the areas in which the degeneration is, as it were, in full swing...the granules are exceptionally numerous, while in apparently normal areas they are rare, as here the myelin is merely swollen and not yet broken up.... they are not artefacts due to the various procedures used in their staining as in a normal brain studied by the same method the granules were not seen."

157. Bessemans A. 1938 **Morphologic variations of the syphilitic germ.** Am J Syphilis, 22:294

Discusses pleomorphism in T. pallidum.

158. Levaditi C; Vaisman 1938 **Cycle évolutif du Treponema palldium.** C rend Soc biol, 127:194

159. Nyka W. 1938 **Nouvelles recherches sur le polymorphisme du virus syphilitique dans les ganglions lymphatiques du lapin.** Annales de l'Institut Pasteur, Par., 60:316

160. Blackman N; Putnam **TJ.** 1936 **Nature of the "silver cells" occurring in multiple sclerosis and other diseases.** Arch Neurology & Psych, 54-61

"In 1928 Steiner (1) announced that he had demonstrated spirochetes in the brain of a patient with multiple sclerosis by means of an improved silver impregnation method. He has since described spirochetes in other brains showing typical lesions and free from suspicion of syphilis. ...Far more common than the complete rodlike structures were certain characteristic elements which Steiner named "silver cells (Silberzellen). ...Steiner's work has been repeated by Rogers (3), Kopeloff and Blackman (4) and others...These investigators all agreed that the silver stain is beautifully sharp and specific for spirochetes and that the 'silver cells' occur in cases of multiple sclerosis, with occasional exceptions, and in cases of dementia paralytica but in none of a considerable number of cases used as controls.. ..

A further repetition of Steiner's work was undertaken, first, to determine more closely the nature of the "silver cells"... In both cases of multiple sclerosis "silver cells" were easily seen, and in one they were so plentiful as to constitute the majority of infiltrating elements in the adventitia of blood vessels situated toward the periphery of the plaque, as if they represented an early stage in its evolution. In the center of the plaque, where the lesions are older, the "silver cells" are much rarer and in certain lesions are absent. Only in recent, fresh plaques or in older ones which are apparently enlarging does one see the "silver cells" in their most typical aspect.

...Summary and Conclusions: "Silver cells" are characteristic of multiple sclerosis. ...They are not confined to multiple sclerosis and syphilis, however. They may occur also in vascular lesions under conditions which appear substantially to exclude the possibility of local phagocytosis of microorganisms. They have not been observed by previous investigators in cases of a great variety of other conditions used as controls.

Small though this material is, it appears sufficient to justify the conclusion that the argentophilic particles are not necessarily of spirochetal or bacterial origin. Their occurence in vascular lesions, the fact that similar cells contain yellow pigment and the demonstration in them of what is presumably iron by means of micro-incineration suggest that the silver-staining material may be of hematogenous origin."

161. Nyka W. 1936 **A propos de la multiplication du spirochéte syphilitique.** C rend Soc biol, 121:97

162. Kopeloff N; Blackman N. 1935 **Silver cells (Steiner's method) in multiple sclerosis compared with their presence in other diseases.** Arch Neurology & Psych, 34:1297

[From the article:] "...we examined tissue from the brains of eleven patients with multiple sclerosis (and one other with a borderline case), of two patients with dementia paralytica and of fifty-one patients with various disease conditions.

...Silver cells were seen in the brain tissue of ten of the eleven persons with undoubted multiple sclerosis. The cells occurred in greatest number in or around the walls of blood vessels. In the tissue of the patient with the borderline case, in which the pathologic diagnosis lay between diffuse sclerosis and acute multiple sclerosis, a single silver cell was noted. Silver cells were not observed in the spinal cord (the only tissue examined) of a patients who supposedly had multiple sclerosis. Silver cells and spirochetes were noted in the brain tissue of the two patients with dementia paralytica used as controls.

In the brain tissue of 5 of the patients with multiple sclerosis a few silver-stained bodies appeared which might be interpreted as being degenerated forms of spirochetes, but clearly defined spirochetes could not be found. We prefer to leave open the question of the incidence of spirochetes in cases of multiple sclerosis until we have had an opportunity to examine fresher material.

The search for silver cells in the tissue of patients with other diseases was conducted in the same manner as that in the tissue of patients with multiple sclerosis, except that the diagnosis remained unknown to the observer until the examination was completed.

In the tissues of only one brain in the control series were silver cells seen, viz., in that of a patient with congenital syphilis. Numerous spirochetes were likewise demonstrated in this specimen. It will be noted that in the control series the brain of a patient with dementia paralytica and one of a patient with multiple sclerosis were included. The latter happened to be the only one in which silver cells were not noted among the brains of the 11 patients with multiple sclerosis originally examined.

Steiner's conclusions concerning the presence of silver cells in the tissue of patients with spirochetal diseases and their absence in the brain tissue of other persons are therefore confirmed."

163. Manouélian Y. 1935 **Syphilis tardive. Forms minuscules du Spirochaeta pallida. Spirochetogene syphilitique.** Annales de l'Institut Pasteur, 55:698-708

164. Manouélian Y. 1935 **Placentas syphilitiques, formes minuscules du tréponème et ultravirus syphilitique.** C rend Acad sc, 200:1439

165. Guiraud P. 1934 **Inclusions intramacrogliques dans la sclerose en plaques.** L'Encephale, 29:676

[According to G. Steiner, 1952:] Guiraud believed that granules found in the brains of MS patients are a form of the spirochetal organism itself.

166. Nyka W. 1934 **Le virus syphilitique: ses variations morphologiques, sa multiplication et son action pathogène.** Annales de l'Institut Pasteur, Par., 53:243

167. Kon Y. 1933 **Über die Silberreaktion der Zellen.** Jena, Gustav Fischer.

[According to G.B. Hassin, 1939, who wrote that: "Kon observed silver granules in practically every tissue and organ of the body, including the brain. Fine and coarse black or brown granules were present also in the cytoplasm of the ganglion cells, but not in their nuclei. ...In the nuclei of the bagus nerve granules of the foregoing type were so numerous that they covered the cell nuclei... As the granules disappear after a fresh piece of brain tissue has been in running water for twenty-four hours, it is to be assumed that the substance of the granules, stainable with silver, is not stable. Nor can granules be demonstrated when pieces of brain tissue have been kept in alcohol, solutions of formaldehyde or osmic acid."

168. Földvari F. 1932 **Conduct of Spirocheta pallida in tissue explantations.** Am J Syphilis, 16:145-54

"In this study free buds have often been seen too, further or nearer to the spirochete body, as well as short budding forms."

169. Ingraham NR, Jr. 1932 **The life history of Treponema pallidum. A Critical review of literature.** Am J Syphilis, 16:155-90

Reviews perplexing phenomena in spirochetal infections, such as latency in syphilis, the evidence for alternate forms of the organisms, and emphasizes the -- on a theoretical basis at least -- the existence of a minute granule form of T. pallidum offers a cogent explanation for these phenomena. "...it is worth while to consider that they all may be explained by a single assumption: that the Treponema pallidum may produce, in one stage of its life cycle a minute, resistant, infective granule. ...If a minute, resistant body is the cause of syphilitic infection, the changes that would be wrought in our ideas concerning the etiology, pathogenesis, diagnosis, therapy, and prognosis in this disease need scarcely be pointed out."

The author states that there have been 18 separate experiments in which tissues from infected hosts transmitted infection in the absence of spirochetes.

170. Levaditi C; Schoen R. 1932 **Présence du treponema pallidum chez les souris atteintes de syphilis expérimentale, Inapparente.** C rend Soc biol, 109:811

171. Guiraud P. 1931 **Figures parasitaires intracellulaires dans la sclerose en plaques.** L'Encephale, 26:349

[According to G. Steiner, 1952: Guiraud believed that granules found in the brains of MS patients are a form of the spirochetal organism itself.]

172. Lepine P. 1931 **Forme visible et forme invisible du virus syphilitique.** Rev. méd., Par., 48:721

[According to Campbell, 1950: Hypothesized the existence of a virulent virus or ultramicroscopic organism as the actual cause of syphilis.]

173. Lepine P. 1931 **A propos du cycle évolutif du virus syphilitique: le tréponème pâle est-il virulent?** Presse méd, 39:1233

[According to Campbell, 1950: Hypothesized the existence of a virulent virus or ultramicroscopic organism as the actual cause of syphilis.]

174. Saleeby E; Greenbaum SS. 1931 **Comparative biologic and histologic study of lymph glands from syphilitic patients.** JAMA, 96:98

[As quoted in Ingraham, 1932: "The Spirocheta pallida was demonstrated in five of the twenty-one human inguinal glands studied with the Levaditi method. In two sections the organisms were numerous, and in the other three only an occasional one was noted. But in most of the sections there were small, black mostly intracellular granules, which were suggestive of being spirochetal granules."]

175. Steiner G. 1931 **Krankheitserreger und Gewebsbefund bei multipler Sklerose: Vergleichend-histologisch-parasitologische Untersuchungen bei multipler Sklerose und anderen Spirochatosen. (Comparative studies between MS and other spirochetoses)** Ergebn. d. Hyg., Bakt., Immunitatsforsch. u. exper. Therap., 12:269-464

[Blackman, 1936, wrote that: "In all of seven of twenty-eight cases of multiple sclerosis examination of the brain gave positive results. ...Far more common than the complete rodlike structures were certain characteristic elements which Steiner named "silver cells" (Silberzellen). They consist of spherical bodies, about the size of the nucleus of a lymphocyte... Steiner observed these structures to be present in practically all cases of multiple sclerosis..."]

[Hassin, 1939, wrote that: "Steiner maintained that multiple sclerosis is an infectious disease caused by a specific spirochete, different from any other spirochete--for instance, that of syphilis, of Weil's disease, or of relapsing fever. It is a destroyer of myelin and therefore was termed by him Spirochaeta myelophthora. It is short lived, for it rapidly breaks up into small fragments or granules... Steiner was able to observe within some silver cells fragments of spirochetes, their extracellular portion undergoing degeneration and presenting transitional stages from spirochete to silver cell. Silver cells are thus to be looked on as degenerated spirochetes, representing an advanced stage of a spirochetal infection. Silver cells containing fine granular substance are the older; the fresh, younger cells containing formations in the form of ringlets, commas, loops and rods represent an early stage of spirochetal infection. ...In cases in which the course has been rapid and cases of young persons silver cells are numerous; in cases in which the disease is of long duration silver cells are harder to demonstrate; only the fine granules are present, without the clear threadlike argyrophilic content."]

176. Warthin AS; Olsen RE. 1931 **The apparent sequence of spirochetes and granular forms in syphilitic buboes.** Am J Syphilis, 15:145

"In addition to their presence in aortic necroses, we find similar ring-shaped forms in chancres, buboes, secondary skin lesions, and in late lesions in the heart, aorta, and skin. ...They are both extra- and intracellular. The demonstration of ring forms in latent syphilitic perivascular lesions, in which no typical spirochetes can be found, was the first important link of the chain to be demonstrated by us. ...In only about 50 to 60 per cent of cases showing identical tissue lesions could these typical large forms be demonstrated, and in some cases their number was so small as to be wholly out of proportion to the magnitude of the lesions present in the tissues. ... We are now able to demonstrate hundreds of small spirochetes in tissue-lesions in which the usual technical methods of demonstrating spirochetes show nothing at all."

177. Jahnel F. 1930 **Pathologische Anatomie der progressiven Paralyse, in Bumke, O.: Handbuch der Geisteskrankheiten.** Berlin, Julius Springer, vol. 11, p.513

[According to G.B. Hassin, 1939: Jahnel, in 1919, identified ultramicroscopic granules in tissues -- singly, or in connection with fragments of Spirochaeta pallida. The granules were seen only in areas densely invaded by spirochetes, but never in areas free of them.]

178. Levaditi C. 1930 **Gommes syphilitiques et formes anormales du tréponèmes, ultravirus syphilitiques.** Compt. rend soc biol, 104:477-80

[According to Warthin, 1931: "Levaditi confirmed the work and conclusions of Manouelian. He describes the stages leading from the spirochete to the granules, the ultimate granules being from 0.1 to 0.3 microns in diameter. He believes that these findings might explain late syphilis without spirochetes, paresis without spirochetes, and finally malignant syphilis. The resistant forms are not sensitive to the chemicals that kill the vegetative (spirochete types)."]

179. Levaditi C; Lepine P; Schoen R. 1930 **Relation entre le cycle évolutif du "Treponema pallidum" et la genèse des lésions syphilitiques.** Compt. rend soc biol, 104:72-5

[According to Ingraham, 1932: "Levaditi, Lépine, and Schoen have similarly demonstrated the infectiousness of skin grafts in mice which contain no microscopically visible Treponemata."]

180. Levaditi C; Po LY. 1930 **Cycle évolutif du Treponema pallidum du Spirochaeta pertenuis et du Spirchaeta cunicola.** Compt. rend soc biol, 104:736-40

[Mattman, 1993, wrote that: "...Levaditi and Po...concluded that that granules and serrated forms finally evolve to the almost invisible stage of T. pallidum and that the transition forms and tiny granules are often the only forms in the brain in paresis."]

181. Manouélian Y. 1930 **Syphilis héréditaire et formes évolutives du tréponéme.** C rend Acad sc, 190:332

182. Manouélian Y. 1930 **Gommes syphilitiques et formes anormales du treponemes; Ultra-virus syphilitiques.** Compt. rend soc biol, 104:249-51

[According to Warthin, 1931: Manouelian described granular forms in old gummas and other late lesions. "...he regarded these forms as representing a transmutation series from the typical spirochete form to a minute corpuscle which can pass through a filter. These atypical granules are much more numerous than the typical spirochetes, and are very abundant where the latter are rare or cannot be demonstrated at all. He regards the presence of these granules as confirmatory of the syphilitic nature of a late lesion, even in the absence of typical spirochetes."]

183. Marchoux E; Chorine. 1930 **Le Sang des Poules piquées par les Argas est virulent en l'Absence de Spirochètes apparents.** Compt rend Soc de biol, 104:259

184. Roukavischnikoff EJ. 1930 **Zur Frage der Entwicklungsstadien des Syphiliserregers, die im Blute des infizierten Menschen und der Versuchstiere zirkulieren.** Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig, 115:66-71.

[According to Mattman, 1993: "Roukavischnikoff found that blood in the primary stage of syphilis contains tiny granules which, if properly nutured, develop into the typical spirochete or more often show stages of growth which he recognized as transitional."]

[According to Ingraham, 1932: Roukavischnikoff performed experiments on human blood from untreated cases of latent syphilis. He concluded "that the cause of syphilis circulates in the blood of the infected animal in an avisual stage of its development. If a large portion of blood is brought into artificial cultural conditions, this sets in operation the stimulus for the transformation of the microorganism from the invisible to the microscopically perceptible stage of its development, in which are present spheroidal granules, in size, staining properties, and character of contents, a distinctive picture. Under favorable artificial living conditions, the further development of the spheroidal form into aggregations of spirochetes can be demonstrated."]

185. Seguin P. 1930 **Treponema calligyrum et ultra-virus spirochétique.** C rend Soc biol, 104:247

186. Seguin P. 1930 **Spirochaeta gallinarum et formes dites "ultra-virus."** C rend Soc biol, 104:836

187. Sézary A. 1930 **Les Formes atypiques et la Forme granuleuse du Tréponème pale.** Compt rend Soc de biol, 105:444

188. Warthin AS; Olson RE. 1930 **The granular transformation of Spirochaeta pallida in aortic focal lesions.** Am J Syphilis, 14:433-3

Atypical forms of T. pallidum were found in aortic focal lesions. The progressively smaller shapes suggested that the spirochete transforms itself into a minute granule by a series of contractions. Includes an interesting drawing of the transitional stages observed as a spirochete transforms itself into a minute granular form. The authors raise the question as to whether this progression represents evolution or involution, but seem to emphasize the possibility of involution. Atypical forms were found even when typical spirochetes were absent.

"...What is of the greatest interest is that we can always demonstrate typical spirochetes about the borders of these focal necroses, even when we can find none in the perivascular infiltrations. ...In the interior of the necrotic foci typical spirochetes are found but rarely. They are replaced by atypical forms of various sizes and shapes showing all possible transition stages from a typical spirochete to fine single granules almost submicroscopic in size. A definite cycle of transformation is apparent. The typical forms do not break up into multiple granules or beaded forms. The first stage is apparently the development of a knob, usually at one or both ends, but occasionally in the middle of the organism; the ends then bend together, forming a horse-shoe loop, this in turn becomes an irregular circle, which contracts into a solid irregular granule, finally becoming a single, small, rounded granule. The loop stage does not invariably appear, as some organisms, after the appearance of the knob-like extremities, change into elongated amorphous masses without any central opening, and contract as do the loops until the minute granule is all the remains. A submicroscopic form following the minute granule is inferred, but we are not ready to offer positive demonstration of it at the present moment. ...The degenerative forms are invariably present, even when typical spirochetes may be apparently absent."

189. **Hauduroy P.** 1929 **Les Ultravirus et les formes filtrantes des Microbes.** Mass et Cie., Editeurs, Paris. Deuxième partie: Les Microbes filtrants visibles.

[According to Klieneberger-Nobel, 1951: "Hauduroy (65) reviews Leishman's investigations of a tick-borne infection of monkeys. The spirochetes went through a cycle in the ticks. The day after the intake of infected blood they were found agglutinated inside the digestive tube of the tick. Gradually they underwent fragmentation, and granules of different sizes were liberated into the intestinal tract. The granules became dispersed in the tick. Leishman observed heaps of granules as well as small, very young spirochetal forms in the ovarium of a tick. He found that emulsions of ticks in which spirochetes had not been found by microscopical examination, caused infection in the monkeys. Prowazek, Blanc, Brumpt, Wolbach, Marcloux (65) confirmed Leishman's observations, and all these authors stated that the spirochetal cycle includes an "invisible stage". According to Hauduroy, Borrelia recurrentis, B. duttoni (African tick fever) and B. venezuelensis (American tick fever) have been shown to pass china filters which retain ordinary bacteria."]

190. Hoffman E. 1929 **Zur granulären Form der Syphilissporchäte.** Derm. Wschr, 89:2041

191. Meirowsky E. 1929 **Zur granulären Form der Syphilisspirochäte-Schlusswort.** Derm. Wschr, 89:2042

192. Meirowsky E. 1929 **Der gegenwärtige Stand der Frage eines Entwicklungskreises der Spirochaeta pallida.** Derm. Wschr, 88:765

193. Levaditi C; Sanchis-Bayarri V; 1928 **Le virus syphilitique compor-t-il un cycle évolutif dont le Treponema pallidum n'est qu'une des phases connues?** Annales de l'Institut Pasteur, Par., 42:475

194. Steiner G. 1928 **Spirochaten im menschlichen Gehirn bei multipler Sklerose.** Nervenarzt, 1:457
(Spirochetes in the brain of persons with multiple sclerosis)

[According to Blackman, 1936: Steiner explained the rarity of spirochetes in the brain in multiple sclerosis cases by their extreme lability, which causes their rapid disappearance directly after the onset of the attack.]

195. Levaditi C; Schoen R; Sanchis-Bayarri V. 1927 **Le cycle évolutif du "Treponema pallidum."** Bull acad méd (Paris), 98:149-152

[According to Klieneberger-Nobel, 1951: "An evolutionary cycle for Treponema pallidum has been suggested by Levaditi, Schoen and Sanchis-Bayarri (93) who studied the morphology of the organism quite extensively. They observed that in lymphatic glands of rabbits, infected by the scrotal route, spirochetes were very rarely found microscopically, although the glands were infective for new animals. ...

According to Levaditi the granular form represents the pre-spirochetal phase of the syphilitic agent. The granules are able to retransform themselves into young spirochetes and then into the long, spiral adult form. The granular form persists in the tissues during periods of latency and withstands specific treatment. ...

Levaditi's conception would be in agreement with the fact that spirochetes are not found in certain diseased tissues, that they are not demonstrated in nerve fibres from cases of paralysis of the insane and of tabes and that latent stages of the disease resist chemotherapeutic treatment."]

196. Nicolle C. 1927 **L'evolution des spirochetes et le mecanisme de la crise dans les spirochetoses.** Arch Inst pasteur de Tunis, 16:207-17

[According to Klieneberger-Nobel, 1951: "According to Nicolle (112) and Nicolle and Anderson (114) the relapsing fever spirochetes occur in the louse in two alternating forms, one avirulent and visible, the other virulent and invisible. ...Nicolle's interpretation of the characteristic evolution of the disease is that the parasites go into a granular stage produced by fragmentation of the adult forms. The granular stage is resistant and persists in the tissues. The repetition of the fever is brought about by an invasion of the blood by "previsibles" spirochetes which are fully virulent and which develop into the large adult form."]

197. Nicolle C; Anderson 1927 **Étude comparative de quelques virus recurrents, pathogènes pour l'homme.** Arch. Inst. Pasteur de Tunis, 16:125-206

[According to Klieneberger-Nobel, 1951: "According to Nicolle (112) and Nicolle and Anderson (114) the relapsing fever spirochetes occur in the louse in two alternating forms, one avirulent and visible, the other virulent and invisible. ...Nicolle's interpretation of the characteristic evolution of the disease is that the parasites go into a granular stage produced by fragmentation of the adult forms. The granular stage is resistant and persists in the tissues. The repetition of the fever is brought about by an invasion of the blood by "previsibles" spirochetes which are fully virulent and which develop into the large adult form."]

198. Sanarelli. 1927 **Identité entre Spirochètes et Bacillus Fusiformes--Les Heliconemes, "vincenti."** Ann de l'Inst Pasteur, 41:673

[According to Ingraham, 1932: "Sanarelli has reundertaken the problem of establishing the identity of these two forms and, feeling that he has succeeded in showing the fusiform bacillus to be an anaerobic spirochete very much altered by an aerobic environment and by the toxicity of the end-products of metabolism of coexisting bacteria, suggests the name "Heliconema Vincenti" for it. In his exhaustive study he states that disease in animals can be produced by either form of the organism."]

199. Timmerman H. 1927 **Quoted by Van Thiel, P.H., 1948. The leptospiroses.** Universitaire Pers, Leiden.

Granules develop in response to physical and chemical changes.

200. Kermorgant Y. 1926 **Les formes "invisibles" des spirochètes.** Progr. mèd., Par., 54:599

[According to Ingraham, 1932: "...the dramatic experiments of Kermorgant indicat[ed] the necessity of a symbiotic relationship for the development of a spirochete of the parotid gland..."]

201. Nicolle C. 1925 **Sur la nature des virus invisibles. Origine microbienne des Inframicrobes.** Arch. Inst. Pasteur de Tunis, 14:105-20

[According to Klieneberger-Nobel, 1951: "Nicolle and Blan (113) and Nicolle (111) during their work on relapsing fever conceived the idea that spirochetes must exist in a visible and an invisible stage. They observed that after a louse had fed upon a patient, infected with spirochetes, the parasites transversed the cells of the intestine of the louse in the first few hours, but then the parasites remained undemonstrable until the sixth or seventh day when they reappeared in the insect, but were extremely small. they gradually increased in size until finally they reached the size of the adult spirochetes. The virulence of the louse spirochetes was at its highest on the sixth day after the blood meal when the actual parasites were either still invisible or very minute. After the eighth or ninth day the louse-spirochetes lost their virulence completely."]

202. Szilvási J; Fehér D. 1925 **Beiträge zur Morphologie der Spirochaeta pallida.** Zbl Bakt, 1. Abt., 95:436

203. Aristowsky W; Holtzer R. 1924 **BemerKungen zur Morphologie der Spirochaeta obermeieri.** Zbl Bakt, 91:175-8

204. Bushke; Kroó. 1924 **Experimentelle Analogieversuche zwischen Recurrens und Syphilis.** Arch. f. Dermat. u. Syph., 145:236

[According to Ingraham, 1932: Observed bud formation in spirochetes. Ingraham also quotes the authors as saying that "spirochetes can no longer be pointed out microscopically in the brains of immune mice, in spite of the fact that these brains can none the less cause infection."]

205. McDonagh JER. 1924 **The nature of disease.** Heinemann, London

[As quoted in Ingraham, 1932: "The knob of the Spirocheta pallida is made up of the same constituents as the head of the spermatozoon. Not all spirochetes have knobs thought they appear able to develop them in any part of their length. From this knob, or granule, as it is frequently called, another spirochete may develop. In this way the spirochete multiplies in the cuture tube. Multiplication by granule formation may take place in the body sometimes, for instance in condylomata and in the grey matter of the brain in general paresis. Moisture appears to favor this method of development. That the adult male phase is capable of developing in this way, has led many to think that it is the only way in which it can multiply. These observers forget, however, that a culture tube is a very different thing from the human body."]

206. Antoni. 1921 **Studien über die Morphologie der Spirochaeta pallida nach Beobachtungen im Dunkelfeld.** Arch f Dermat u Syph, 129:70

207. Marchand. 1921 **Considérations pathogeniques sur la Paralysie Générale.** Presse méd, 29:695

[According to Ingraham, 1932: "It is such facts as these [the difficulty of discovering T. pallidum organisms in primary and secondary syphilis] that caused Marchand as late as 1921, to express the belief that paresis is caused by a filterable virus which grows in the ground prepared by the antecedent syphilitic infection."]

208. McDonagh JER. 1921 **The development of the female phase of the leucocytozoon syphilidis.** J Path Bact, Lond, 24:272

209. Saphier J. 1921 **Zur Morphologie der Spirochaeta pallida.** Arch Derm Syph, Wien, 136:59

210. Leishman WB. 1920 **The Horace Dobell lecture on an experimental investigation of Spirochaeta duttoni, the parasite of tick fever.** Lancet, 2:1237-44

S. duttoni, when inside a tick, was found to reproduce by a process of budding and extrusion of granules; the granules grow into young spirochetes. The granules are also themselves capable of multiplication. Their development into spirochetal form within a vertebrate host is an exceptional occurence, brought about by certain environmental circumstances, of which temperature is a very important factor. This interpretation rests on an accumulation of observations by the author and other researchers cited, including the correlation of the temporary disappearance of spirochetes from the tick's stomach with the appearance of large numbers of granules. The discrepancy between the authors results and that of some others is explained as the result of differences in temperature, as the other researchers had performed their studies in the tropics.

"Occurrence and Significance of Granules and Buds: ...I think it may now be accepted as a generalisation that spirochaetes as a class tend at one stage of their life to form small granules which are subsequently liberated from the periplastic sheath. A similar statement may also, I think, be made in connexion with the curious buds or swellings which form upon spirochaetes either terminally, subterminally, or laterally. These, too, have been observed by so many workers and in connexion with so many different spirochaetes that their existence must also be taken as proved, whatever view be held as to their nature. ...

As the numbers of granule clumps found in the intra-ovarian eggs is never large it is obvious that the granules can increase in numbers without any fresh spirochaetal infection. Assuming for a moment that the vital theory is correct it seems certain that they are therefore capable of multiplication in the granular form, and probable that their development into spirillar shape is an exceptional occurrence brought about by influences not as yet fully determined. ...the granules are derived from Sp. duttoni, represent a vital process in the life of the spirochaete, and are neither degeneration products of these organisms nor granules derived from the cells of the host. ...

Influence of Temperature: At temperatures below 25ºC, the spirochaetes maintain their motility, characteristic shape, and staining reactions for three or four days; after this they rapidly become motionless, distorted in shape, tend to aggregate in tangles, and show very irregular staining. In the days following these changes become more pronounced, and it is increasingly difficult to find an unaltered spirochaete, until, on or about the tenth day after the feed, they are found to have disappeared entirely from the gut.

...Turning now to ticks kept after feeding at temperatures above 25ºC. ...By the eighth to the tenth day after the meal active unaltered spirochaetes had either vanished completely from the tick's body or were extremely hard to find. But--and this is the interesting point--at or about this same period there was a sudden reappearance of spirochaetes in various tissues, but spirochaetes of an altogether different type--small, delicate, faintly staining, and less regularly curved than those found in the blood. When first seen they were usually present in enormous numbers and showed no increase in the days following, rather a slow decline. It gave a strong impression of a simultaneous development or origin rather than of a rapid process of multiplication from a few individuals. ...

Another interesting point which was noticed in several of these experiments was that the young spirochaetes appeared in successive waves at intervals, roughly, of 7-10 days, as long as the ticks were kept at the higher temperature. The suggestive bearing of this observation upon the successive crops of organisms which synchronise with the febrile relapses in man and animals will be obvious. ...I am convinced that they [the "young" spirochetes] are formed within the tissues--probably from the granule clumps--and that it is only at a later stage and under certain conditions that they grow to full size...

Again, spirochaetes kept in vitro for many days at temperatures approaching the freezing-point may show no trace of motility on examination, but on placing them on the warm stage I have seen great numbers become once again actively motile.

211. Lundie C; Goss FH. 1919 **Observations on the sporulation of syphilis organism as seen on the dark ground.** Lancet, 2:1025-6

Large numbers of "coccal bodies" were found in scrapings from syphilitic sores. A "leucocyte" was seen to burst and release hundreds of "spores". "All these phenomena were noted only in slides taken from sores that were clinically syphilitic, and from no others."

212. Leishman WB. 1918 **A note on the "granule clumps" found in Ornithodorus moubata and their relation to the spirochaetes of African relapsing fever (tick fever).** Annales de l'Institut Pasteur, 32:49-59

Innoculation of tissues containing only granules produced spirochaetosis in mice.

Some granules were observed to develop into spirochetes. Periods of several days were noted where few, if any, spirochetes could be found inside a tick, followed by sudden re-invasion of tissues with mostly young and vigorously motile spirochetes, particularly in ticks kept at higher temperatures. This sequence was found to repeat in a regular pattern. The author concludes that this phenomenon is related to the reproductive habits of the organism. "...regular relapses appear to take place in the body of the tick, as regards the appearance and disappearance of the spirochaetes, just as in the case of the warm-blooded host."

Granules were also associated with the transmission of infection from mother to baby ticks. "The occurence of similar granules in the eggs of the fecundated female tick and my almost invariable failure to find spirochaetes in such eggs, even when the mother tick had been heavily infected shortly before, further suggested to me that it might be in this form that the virus passed to the next generation of ticks."

213. **Noguchi H.** 1917 **Spirochaetes.** Am J Syphilis, 1:261-346

[As quoted by Ingraham, 1932: "The body [a coccus-like body] is more frequently present in old cultures in which innumerable granules are also found. By making a transplant of such a culture into a new medium, it was found, when examined several days later, the new culture contained many short spiral forms, which were in one manner or another intimately connected with the granules. This phenomenon suggests the possibility of representing the sprouting of spiral forms from granules."]

214. Fantham HB; Cantab MA. 1916 **Spirochaetes and their granule phase.** British Medical Journal, 1:409-11

"It must also be borne in mind that coccoid bodies may be present when spirochaetes as such cannot be detected. ...There is no doubt that spirochaetes produce such granules; it is only their significance, whether cyclical or degenerative, that is in question. ...

Within the Malpighian or genital cells of a transmitting tick, ...the coccoid bodies often seem to be liberated by the disintegration of the periplast. Groups of coccoid bodies still retaining the outline of the spirochaete from which they originated are of fairly frequent occurence. ...

Further, it should be noted that in experiments with the invertebrate transmitters of such spirochaetes as S. duttoni, S. recurrentis, and S. gallinarum, careful attention should be paid to the temperature, humidity, and other climatic conditions under which the investigations are conducted, since these factors undoubtedly influence the development of spirochaetes therein."

215. Inada R; Ido Y; Hoki R; Kanedo; Ito H. 1916 **The etiology and mode of infection and specific therapy of Weil's disease. [Spirochaeta icterohaemorrhagica]** J Experimental Med, 23:377-402

"At the period at which fatal cases come to autopsy the liver is either devoid of spirochaetae or they are so few or modified in form as to be difficult of discovery or recognition. ...The forms present in the liver are as variable as are the differences in length. One sees round or oblong granules, sometimes three or four in number... In addition still larger granules sometimes project from the body of the organism forming the so called bud of the spirochaeta."

"The lymph glands and spleen contained a small number of spirochaetae, mostly in a degenerated condition. ...in autopsied cases typical organisms rarely were met with. ...Thus the distribution of the spirochaetae in the human body differs from that of the guinea pig in that the number present is smaller, the degenerative forms are more abundant, they are more within cells...

The greater occurrence of intracellular organisms is probably due to the fact that the spirochaetae invade cells in order to escape from the action of the immune body..."

216. Warthin AS. 1916 **The persistence of active lesions in the tissues of clinically inactive or "cured" syphilis.** Am J Med Sc., 152:508

217. Fantham HB. 1914 **The granule phase of Spirochaetes.** Annals of Tropical Medicine, 8:471-84

"That spirochetes divide by multiple transverse fission into small portions--the granules, coccoid bodies, or spores of various authors--really is not open to controversy. ...

I believe, however, that it is highly probable that spirochaetal granules are connected with relapses when such occur in spirochaetosis in the vertebrate host. ...Also I think that the granules are more resistant to drugs than the spirochaete forms, and in this way are responsible for relapses, sometimes long deferred.

...As regards the failure to infect vertebrates by the injection of coccoid bodies--on which some stress has been laid--that, unfortunately, is sometimes, though not always, the case. Perhaps, as Hindle (1912, p. 474) remarks, there is some undermined factor (? coxal fluid) connected with the development of coccoid bodies in such cases. ...

Personally, I have frequently found spirochaetes in every organ of the body of the tick, especially if the tick had previously been kept for a short time at 30ºC to 35ºC. Many investigators seem to overlook the importance of recording the temperature or other climatic conditions under which the ticks, dissected or otherwise used by them, were previously kept."

218. Meirowsky E. 1914 **Untersuchungen über die Stellung der Spirochäten im System.** München med. Wochschr, 61:592

219. Meirowsky E. 1914 **Protozoischer oder pflanzlicher Entwicklungskreiss der Spirochaeten?** Dermat. Wschr. 58:225

220. Meirowsky E. 1914 **Beobachtungen an lebenden Spirochaeten.** Arch Derm Syph, Wien, 199: pt.1, 200

221. Meirowsky E. 1914 **On the biological position of the Spirochaeta pallida and its development.** British Journal of Dermatology, 26:185
(Abstract by Dr. H. C. Semon)

"Dr. Meirowsky observed the aggregation of apparent chromatin granules into small globules, or expansion which might assume a lateral or end-on position to the spirochaetal body. Extrusion of these followed, and the buds thus formed remained attached by a fine pedicle or stalk at the point of extrusion.... spirochaetal buds have the property of dividing."

Meirowsky believed spirochetes reproduced "by transverse division, budding and sporulation." He opposed McDonagh's contention that spirochetes were protozoa. "Summarising his views, Meirowsky states that the absence of a nucleus, an undulating membrane, and a blepharoblast are very cogent arguments against the protozoal nature of Spirochaeta pallida... the spirochaete can reproduce its species by budding like other members of the true vegetable order. On this basis the author reiterates his conviction that Spirochaeta pallida are true vegetable parasites..."

222. Meirowsky SE. 1914 **Studien über die Fortflanzung von Bakterien, Spirillen and Spirochaeten.** Julius Springer, Berlin

223. Nicolle C; Blanc G. 1914 **Les spirilles de la fièvre récurrente, sont-ils virulants aux phases successives de leur évolution chez le pou? Démonstration de leur virulence à un stade invisible.** Compt rend Acad d sci, 158:1815-17

[Fantham (1916) wrote that: "Nicolle and Blanc (1914) find that the causal agents of relapsing fever are virulent or infective in the louse just before they reappear as spirochaetes. They think there is an invisible stage in the life-cycle, though they do not appear to have examined for a granule stage, which might be easily overlooked."]

224. Nicolle C; Blanc G. 1914 **Fièvre recurrente et spirillose.** Arch Inst Pasteur de Tunis, 9:63-9

[According to Klieneberger-Nobel, 1951: "Nicolle and Blan (113) and Nicolle (111) during their work on relapsing fever conceived the idea that spirochetes must exist in a visible and an invisible stage. They observed that after a louse had fed upon a patient, infected with spirochetes, the parasites transversed the cells of the intestine of the louse in the first few hours, but then the parasites remained undemonstrable until the sixth or seventh day when they reappeared in the insect, but were extremely small. they gradually increased in size until finally they reached the size of the adult spirochetes. The virulence of the louse spirochetes was at its highest on the sixth day after the blood meal when the actual parasites were either still invisible or very minute. After the eighth or ninth day the louse-spirochetes lost their virulence completely."]

225. Sergent E; Foley H. 1914 **De la periode de latence du spirille chez le pou infecte de fievre recurrent.** Compt rend acad sci., clix, pp. 119-22

[As described by Leishman, 1920: After ingestion into ticks, the spirochetes studied disappeared after 24 hours. After the 6th day, new, actively mobile spirochetes reappeared suddenly in great numbers. Infectivity was highest on the 6th day prior to this reappearance, despite of the absence of demonstrable spirochetes. Transverse fission of the spirochetes in the louse was only rarely observed. When classic-shaped spirochetes were present, their numbers did not increase.]

[According to Mattman, 1993: "After the flea ingests blood from an infected animal, no borrelia are found in the insect for 8 d, although the entire flea is examined by dark-field microscopy. However, during these 8 d, the flea can infect monkeys."]

226. Sergent E; Foley H. 1914 **Des periodes de latence du spirille chez le malade atteint de fievre recurrent.** Compt rend acad sci, clviii, pp. 1926-8

[According to Fantham, 1916: states "that the spirochaete in the louse assumes a very small form which is as virulent as the spirochaeti-form stage. During eight days following a meal of infected blood the body of the louse does not contain any spirochaetes as such, though the spiral organisms reappear later..."]

227. Todd; Wolbach. 1914 **Concerning the filterability of Spirochaeta duttoni.** J Med Research, 30:27

228. Balfour A. 1913 **Notes on the life-cycle of the Sudan fowl spirochaete.** Trans. XVII Internat. Congress of Med, London, pt.ii, sect. xxi, pp.275-8

[According to Fatham, 1916: "Balfour (1913) thinks that he seems to have succeeded in growing spirochaetes in vitro from infected tick eggs in which granules only could be demonstrated."]

229. Leishman WB. 1913 **Relapsing Fevers.** Trans. XVII Internat. Congress of Med., London, pt ii. sect. xxi, p. 282

230. McDonagh JER. 1913 **The complete life history of the organism of syphilis.** British Med J Dermatology & Syphilis, 25:1-14

A detailed description of a complex life cycle of Treponema pallidum, which the author believed to include a spore stage, and both asexual and male/female stages.

"In most specimens the female gametocytes and zygotes are to be found in greatest abundance; it seems that neither salvarsan nor mercury has any influence upon them..."

231. Meirowsky E. 1913 **Beobachtungen an lebenden Spirochäten.** München med. Wochschr, 60:1870-3

232. Ross EH. 1913 **The intracellular parasites in syphilis.** British Medical Journal, 1:195

"Two hundred consecutive cases of human syphilis have now been examined, and Lymphocytozoon pallidum [bodies Ross believed were parasitic forms of syphilis] found in every case. ...Therefore I think we are now justified in naming these 'bodies' parasites, and regarding their presence as diagnostic of disease in the various animals concerned, including human syphilis."

Ross also reports that "the 'bodies' [are] seen outside and within the cells of the blood and lesions in primary and secondary syphilis".

233. Balfour A. 1912 **The life cycle of Spirochaeta gallinarum: an appreciation and criticism of E. Hindle's recent paper.** Parasitology, 5:122-6

234. Hindle E. 1912 **On the life-cycle of spirochaeta gallinarum.** Parasitology, Vol IV, pp. 463-7

"By means of examination with the dark-ground illumination, I have frequently observed the breaking up of the spirochaete into a number of coccoid forms (? spores), in the manner described by Balfour (1911) for this species, and also by Bosanquet (1911) for S. anodontae. I can entirely confirm Balfour's description of this interesting process, which takes place at the crisis of the disease or after drug treatment. ...The spirochaete gradually assumes the appearance of a chain of beads (Fig. 2 a-d) contained within the transparent cell-wall.

After swimming about for some time in this form, the spirochaete appears to rupture at one end and the coccoid bodies escape into the surrounding medium, leaving an empty sheath behind them (e). In some cases the whole cell-wall seems to disintegrate before the coccoid bodies escape, but the final result is the same, viz. the liberation of a varying number of minute round or ovoid bodies (f). ...The true nature of these bodies is problematical, for although in some respects they resemble the spores of bacteria--especially the Disporea--in their formation, yet the fact that they stain deeply and also multiply...at once differentiates them from true spores. ...

The development of intracellular coccoid forms into normal spirochaetes and also into fusiform bacilli has been repeatedly observed in the tick. ...In order to develop into spirochaetes it is necessary for them to escape from the cell into a fluid medium...

It is possible that when the coccoid bodies mixed with the coxal fluid enter the wound caused by the tick's bite, the spirochaetes multiply at the site of infection before entering the general circulation....

Therefore, it is possible that one of the stages of the spirochaete may be cultured without the spirochaete form being developed."

235. Jennings E. 1912 **The parasites recently found in syphilis.** British Medical Journal, 2:1655

Found a coccoid, "protozoal parasite" in syphilitic chancres and blood using the jelly method. "The parasites appear as small, round, brown-coloured bodies lying free in the plasma. Each one contains some deeply staining granules and a vacuole (Fig.1)." These findings confirmed those reported by E.H. Ross in 1912.

[Diagnosis:] "The jelly method is so very simple that I have written this note with the view of its general adoption as a means of diagnosis in syphilis. ...These parasites can also be seen in the peripheral blood in syphilitics, but here they are more scarce, and for diagnostic purposes I advise the examination...

236. McDonagh JER. 1912 **The life-cycle of the organism of syphilis.** British Journal of Dermatology, 24:381

237. McDonagh JER. 1912 **The life cycle of the organism of syphilis.** Lancet, 2:1011

The author argues that the Treponema pallidum is the adult male phase of a coccidial protozoan, and that the spores that result from the conjugation of the two sexual phases are the actual infectious agent of syphilis. The spores were observed to develop inside of cells. These atypical forms seen are said to have diagnostic value. Swellings at the end or middle of the spirochaetes were observed.

"...So firm has been the belief in the spirochaeta pallida, that that organism is taken for granted as being the sole agent of everything syphilitic. Now let us, for a moment, ask ourselves two questions: 1. Why is the incubation period of syphilis so long? 2. Why do not one or two injections of salvarsan cure every case? If syphilis is conveyed by the passage of spirochaetae from one person to another, ought not the initial lesion to begin to show itself two or three days after intercourse, as is more or less the rule with bacterial infections--viz., ulcus molle, gonorrhoea, diphtheria, &c.?

The diseases which have a long incubation period are nearly all due to protozoa; the incubation period is long because the infective organism has to go through a cycle of changes before it can give rise to symptoms. Since the spirochaeta is a protozoon--as assumption which one may safely make, owing to its rapid destruction under salvarsan--is it not possible that it is only one of the phases in the life cycle of the syphilitic parasite? The action salvarsan has on spirochaetae in general is phenomenal. No spirochaetae are found in films made from the blood or discharge from a chancre after 48 hours following a single injection. ...In spite of this recurrences occur again and again. ...

Another little point! All are agreed that it is fearfully difficult -- is it possible at all? -- to find the spirochaeta pallida in a gumma. In the tertiary stage of syphilis, then, the number of spirochaetae must be considerably less than in the secondary; but which stage of the disease is the harder to cure?..."

238. Moolgavkar SR. 1912 **On certain bodies found in syphilitic lesions demonstrated by the jelly method.** British Medical Journal, 2:1655

Round "bodies" were found in samples from syphilitic chancres and glands using the jelly method, confirming the findings of EH Ross, 1912. "I have examined 25 chancres and 22 glands by this method, and have found the bodies in every syphilitic case." The bodies were both intracellular and extracelluar.

239. Noguchi H. 1912 **Pure cultivation of Spirochaeta phagedenis.** J Exper Med, 16:261

[As quoted by Ingraham, 1932: "All the various spirochetes that I have studied have shown features which are more highly differentiated than those seen in bacteria. For example, in most of the spirochetes we observe during certain periods of their life the secretion of a small round body that stains like chromatin. The organisms often concentrate the chromatin material at one part of their body and then udnergo a peculiar segmentation. The granules thus liberated seem to remain alive and at certain periods develop into spiral forms."]

240. Noguchi H. 1912 **Treponema mucosum (new species) a mucin producing spirochaeta from pyorrhea.** J Experimental Med, 16:194-8

"When cultivated under unfavorable conditions a large number of irregular forms appear. ...There are also many granular particles. These particles may be merely degenerative products or they may be segments which under favorable conditions are capable of reproducing the spirochaetae. These segments or granules take the chromatin stain and vary in size. Not infrequently a long spirochaeta is found undergoing a granular segmentation (degeneration?), or a small spirochaeta is seen attached to a round body as if it had just sprouted out of the latter."

241. Ross EH. 1912 **An intracellular parasite developing into spirochetes.** British Medical Journal, 2:1651

The jelly method "enabled the development of this parasite, which was named Lymphocytozoon cobayae, to be demonstrated. It showed how the chromatin within the inclusion becomes formed into spirochaete-like bodies, and how, after the inclusion has burst, the spirochaetes swim freely in the blood. ...Since then 143 cases of primary and secondary syphilis have been examined by this method, and the intracellular and extracellular bodies have been found in every case. ...They have not been seen apart from syphilis, although a great many controls of blood and tissues have been examined on the jellies."

242. Balfour A. 1911 **The infective granule in certain protozoal infections, as illustrated by the spirochaetosis of Sudanese fowl.** British Medical Journal, 1:752

Spirochetes were observed to discharge large numbers of granules. "...the spirochaetes undergo an astonishing change. They discharge from their periplastic sheaths spherical granules, and it is apparently these granules which enter the red cells, develop in them and complete a cycle of schizogony...In process of time the spirochaete loses its activity, becomes difficult to see, and eventually all that is left of it is the limp and lifeless... [that the granules] do not appear to take on the Romanowsky stain may explain why they have not previously been noticed... I have found these granules to be resistant forms and their presence in countless numbers in the tissues might explain part of the mechanism of relapse and the difficulty of curing completely some of the more chronic spirochaetal infections, as, for example, syphilis and yaws."

243. Balfour A. 1911 **The infective granule in certain protozoal infections, as illustrated by the spirochaetosis of Sudanese fowls.** British Medical Journal, 1:870

244. Fantham HB. 1911 **Some researches on the life-cycle of spirochaetes.** Annals of Tropical Med & Parasitology, 5:479-96

Minute, ovoid bodies from spirochetes caused spirochaetosis and death in animals when the ovoid bodies were first incubated at 34º to 37ºC. "...the ovoid bodies reach the ovary [inside the tick], where they intermingle with the developing ova, and become incorporated with some of them. The eggs when laid may contain these minute bodies. ...when the eggs were kept in an incubator at 34º to 37ºC. for four to six days before being injected, the experimental animals developed spirochaetosis and died in a short time (3 to 6 days). ...

The spores or coccoid bodies are probably able to withstand conditions unfavourable to the spirochaetiform stage of the parasite."

245. Leishman WB. 1911 **An address on the mechanism of infection in tick fever, and on hereditary transmission of Spirochaeta duttoni in the tick.** Lancet, 133:11-4

[According to Fantham, 1912: "Leishman's results essentially were that spirochaetes gave rise by multiple fission to granules or coccoid bodies inside the invertebrate host, and that these granules or coccoid bodies found their way more especially to the Malpighian tubules, gonads, and other organs of the tick. The granules themselves multiplied. The eggs of the female tick became infected with granules, and the progeny of infected females might be born infected. The observations of Leishman have been confirmed and extended by Balfour (1911), Fantham (1911), and Hindle (1911)."]

[From the article:] "The result may be stated briefly: no recognisable spirochaetae could be detected [in ticks] later than the tenth day. ...The principal features were the extrusion of lateral, more rarely terminal, swellings, which contained one or more particles of chromatin, and the breaking up of the chromatin core of the spirochaeta into numerous fragments of coccoid or bacillary shape. ...The subsequent fate of these granules was studied from day to day after the first recognisable embryonal cells made their appearance, the granular clumps were found in the protoplasm of some of these cells. ...From this point all the granule clumps seen were intracellular, never free unless such cells had been ruptured. ...

Assuming their spirochaete origin, it is obvious that these granules are not mere "resting forms," as they multiply in the egg and in the young tick, the few clumps seen in the most minute egg giving rise to the thousands found at a later stage in the Malpighian tubes."

246. Noguchi H. 1911 **A method for the pure cultivation of pathogenic Treponema pallidum.** Journal of Experimental Medicine, XIV:99-112

[Morphology:] "Another interesting feature shown in this figure [Plate 12] is the presence of peculiar spore-like round bodies in some pallida. ...It is not rare to find a round body connected with one or two young pallida as though the latter were just sprouting from the former. The pallida with these round bodies are motile."

Also observed T. pallidum colonies. "Isolated colonies are seldom formed apart from the tissue."

247. O'Farrel WR; Balfour A. 1911 **Granule-shedding in Treponema pallidum and associated Spirochaetae.** J Royal Army Medical Corps, Vol XVil, p.225

248. Leishman WB. 1910 **Observations on the mechanism of infection in tick fever and the hereditary transmission of Spirochaeta duttoni in the tick.** Journal of Trop. Med. Hyg., 13:42-5

249. Sézary A. 1910 **Sur une forme annulaire du tréponeme pâle.** C rend Soc biol, 69:339

250. Balfour A. 1907 **A peculiar blood condition, probably parasitic, in Sudanese fowls.** British Medical Journal, Nov. 9th:1330-3

"...though coccoid bodies have been found in the blood after relapsing fever in man, very little is known about them, and they occur free in the plasma."

251. Breinl A. 1907 **The morphology and life-history of Spirochaeta Duttoni, No. 3.** Annals of Tropical Medicine & Parasitology.

[According to Dutton (1907) and Fantham (1916): Observed encysted forms of S. duttoni in the spleen. The cysts broke into granular bodies from which new generations of spirochetes emerged.]

[Dutton (1907) wrote: "Breinl recalls the fact that blood which contained spirochaetae is still infective after it has pass through a Berkefeld filter. He surmises that this may be explained by the presence of the granules described above and he suggests that the above cycle of development, which we think occurs in the tick, may also take place in the mammalian host."]

252. Dutton JS; Todd JL. 1907 **A note on the morphology of Spirochaeta Duttoni.** Lancet, 2:1523

Spirochetes within sporocyst-like bodies were found in the blood even when other forms had disappeared. It is suggested that spirochetes may have more than one method of reproduction, perhaps including a process involving extrusion of granules which subsequently develop into new spirochetes.

"Spirochaetae frequently occur which possess either median or terminal knob-like swellings (7). ...A swelling in either situation is sometimes placed laterally and definitely outside the parasite, though still attached to it by a pink-staining band.

Striking changes occur in the parasites contained in the organs, particularly the spleen, bone marrow, and liver. ...[Just before a crisis] A very few similary coiled parasites undergo a remarkable change. ...They lie, placed in a bluish-staining ground substance, within a definite cyst wall and so form a sporocyst-like body of about the same size as a red blood cell. These forms may be seen in the blood after all other forms have disappeared. ...In the blood and organs of infected animals, and also in the blood contained in the alimentary canal of ticks, blue bodies, about 3 μ in diameter (in the tick as small as 1 μ) with red central granules constantly occur."

253. Ewing J. 1907 **Note on involution forms of Spirochaete pallida in gummata.** Proceedings of the New York Path. Soc., 1907-8, n.s. 7:166-71

In vivo findings of "abundant transitional forms between intact spirochaetae and their granular detritus" are interpreted as the progressive destruction of spirochetes by intracellular digestion. The author points out that tertiary lesions "have usually been found free from readily recognizable parasites." He suggests that the presence of these transitional spirochetal forms may be useful as an alternative means to diagnosis syphilis, since "their appearance is characteristic, and I have not been able to find such cells in a considerable series of tumors of necrotic lesions other than syphilis."

The description of transitional spirochetal forms includes the following comments: "The organism may appear as a chain of granules which outline a complete spirochaete." "Finally, the cell may contain several foci of compact granules of the above type, and eventually the granules may lose their capacity to take up the silver and appear as yellow-ish granules, in which condition they are no longer recognizable as derivatives of spirochaetae."

254. Jacquet L; Sézary A. 1907 **Des formes atypiques et dégénératives du tréponéme pâle.** Bull mem Soc Med Hop Par., 3.s., 24:114

255. Ehrmann S. 1906 **Die Phagozytose und die Degenerationsformen der Spirochaete pallida in Primäraffekt und Lymphstrang.** Wiener Klinische Wochenschrift, 19:828

256. Herxheimer K. 1906 **Weitere Mitteilungen über die Spirochaeta Pallida.** München med Wochschr, 53:310-2

[According to Czekalowki, 1954: Found that the classic spiral form is not the only form that spirochetes may assume.]

257. Leuriaux C; Geets V. 1906 **Culture de Treponema pallidum de Schaudinn.** Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt 1 Orig, 41:684-8

[According to Mattman, 1993: "A very early attempt to culture T. pallidum with simple medium demonstrated the atypical stages. Two parts of spinal fluid from an individual with central nervous system syphilis were added to one part of peptone broth. By Day 4 of incubation, many motile ovoid bodies...were seen which gradually went through a multiplicity of morphologies, only one of which was the typical tightly coiled treponeme."]

258. Novy FG; Knapp RS. 1906 **Studies on the Spirillum obermeieri and related organisms.** Journal of Infectious Diseases, 3:291-3

According to Czekalowski JW, 1954: Found that the classic spiral form is not the only form that spirochetes may assume.
According to Delameter ED, 1951: produced infective filtrates of B. recurrentis which contained no spirochetes.

259. Herxheimer K. 1905 **Zur Kenntnis der Spirochaeta Pallida.** München med Wochschr, 53:310-2

[According to Czekalowki, 1954: Found that the classic spiral form is not the only form that spirochetes may assume.]

[According to Mudd et al, 1943: "Granules within the protoplasm were shown in a drawing of a stained spirochete by Herxheimer (1905)..."]

[According to Földvari, 1932: "In 1905, Herxheimer found minute corpuscles inside and outside of the body of the Spirocheta pallida as well as similar ones nearer or further from the spirochete body, but quite independent of it and freely situated."]

260. Krzystalowicz F; Siedlicki M. 1905 **Contribution à l'étude de la structure et du cycle évolutif du Spirochaete pallida de Schaudinn.** Bull Acad Sc Cracovie, 9:713 Rev prat Mal cutan, 1906, 5:43.

[According to Campbell, 1950: Described most of the forms ascribed to the evolution or involution of the spirochete of syphilis.]

261. Krzystalowicz F; Siedlicki M. 1905 **Spostrzezenia nad budowa i rozwajem Spirochaeta pallida Schaudinn.** Rozpr. wydz. mat, przyrold. Polska Akad., 5:414

[According to Campbell, 1950: Described most of the forms ascribed to the evolution or involution of the spirochete of syphilis.]

262. Schaudinn F; Hoffman S. 1905 **Über Spirochaeta pallida bei Syphilis und die Unterschiede dieser Form gegenuber anderen Arten dieser Gattung.** Berlin Klin Wochschr, 42:673-5

Found that the classic spiral form is not the only form that spirochetes may assume.
[According to Novy & Knapp, Schaudinn believed that spirochetes were protozoa, not bacteria.]

~JR~