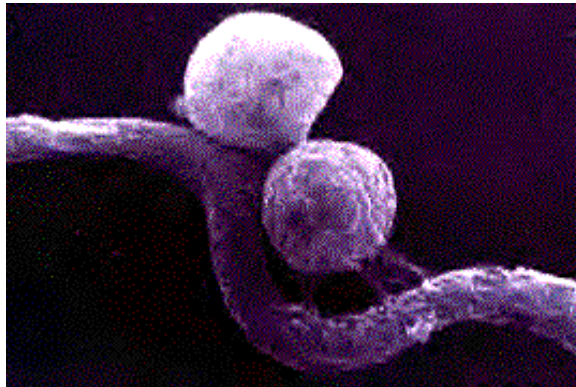


Lyme Disease

Studies on the Cystic Form of *Borrelia burgdorferi*

Mechanisms of Persistence



V. Preac Mursic et al. Infection Vol. 24 No. 3, p.222. 1996.

June, 2001

Another aspect [of spirochetal infections]... is the apparent continuation of the disease process after the organisms are no longer detectable. The “sequestration” or disappearance from the blood of the *Borrelia* in the afebrile periods between relapses in relapsing fever may be due to the action of specific antibodies, but it can also represent the simplest example of “hiding.” Why is it so difficult to culture or even visualize spirochetes in the synovium and synovial fluid of patients with Lyme arthritis of long duration? What is tertiary syphilis in the absence of *Treponema pallidum*?

Jorge L. Benach, Ph.D. and James L. Coleman, Ph.D. 1993.
Overview of Spirochetal Infections.
In Lyme Disease, ed. Patricia K. Coyle, M.D.
St. Louis: Mosby-Year Book, Inc., p. 61.

Formation of Cysts: A Survival Mechanism

Descriptions of spirochetal encystment — from the literature on Treponema

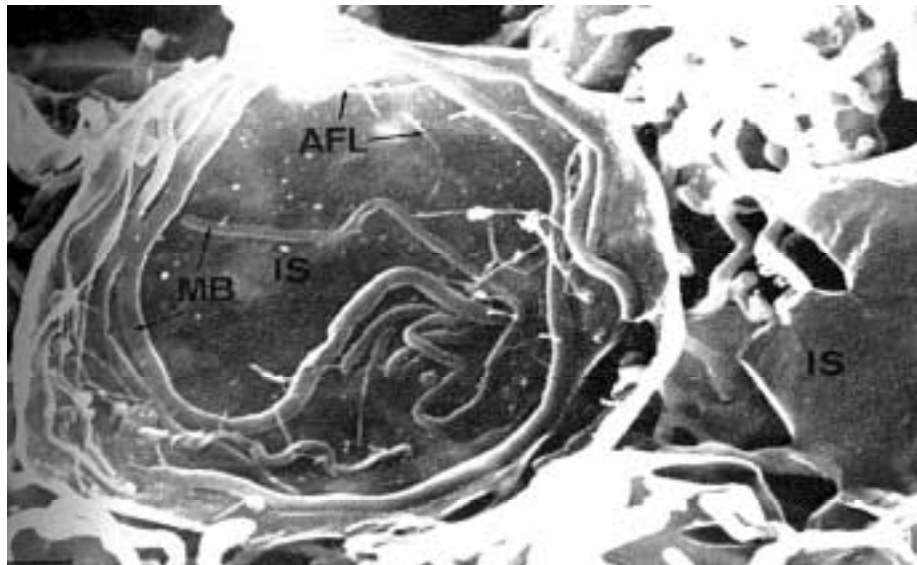
Under stressful conditions, the treponeme ‘packs’ itself into a compact roll (Fig. 8) and becomes covered with a transparent mucoid capsule, which resists the penetration of drugs and antibodies. The organisms may persist in this form for a prolonged period without any reaction from the host. The encysted 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.

Ovcinnikov NM; Delectorsku VV. 1971.
Current Concepts of the Morphology and Biology of Treponema
Pallidum Based on Electron Microscopy.
British Journal of Venereal Diseases, 47:316.

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... 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 but some of them are designed rather to ensure a long period of rest. 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.

Al-Qudah AA; Mostratos A; Quesnel LB. 1983.
A Proposed Life Cycle for the Reiter Treponeme.
Journal of Applied Bacteriology, 55:426-427.

T. macrodentium cyst showing spirochetes coiled inside



Umemoto T; Namikawa I. et al. 1982.
In “An internal view of the spherical body of Treponema macrodentium as revealed by scanning electron microscopy.” Microbiology & Immunology, 26(3):195.

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Conversion of *Borrelia garinii* Cystic Forms to Motile Spirochetes In Vivo.

Date of Publication: May, 2001
Source: APMIS, 109(5):383-8.
Authors: Gruntar I; Malovrh T; Murgia R; Cinco M.
Institution: Institute of Microbiology and Parasitology, Veterinary Faculty,
Ljubljana, Slovenia.

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.

Quotations From The Full-Text Article

“Interesting conclusions can be drawn from the results of cyst inoculation experiments on mice:
***B. garinii* cystic forms maintain their capability to reconvert into normal spirochetes not only in vitro but also in vivo and can therefore be considered infective, at least in BALB/c mice.**”

“Dormant borreliae evacuated in cysts might be resistant to antibiotics (due to low metabolic activity), and stage under non-optimal environmental conditions. **Borrelial cystic forms could therefore be responsible for the frequent failures of antibiotic therapy and for the commonly reported relapses of Lyme disease.**”

An In Vitro Study of the Susceptibility of Mobile and Cystic Forms of *Borrelia burgdorferi* to Metronidazole

Date of Publication: June, 1999
Source: APMIS, 107(6):566-576
Authors: (1) Brorson O; (2) Brorson S.
Institution: (1) Department of Microbiology, Vestfold Sentralsykehus, Tonsberg
(2) Department of Pathology, Ulleval Hospital, Oslo, Norway

Abstract

The aim of this study was to examine the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to metronidazole. Because *B. burgdorferi* is a microaerobic bacterium like *Helicobacter pylori*, metronidazole (MZ) was chosen in the susceptibility test. For both microaerobic and aerobic incubation the normal mobile spirochetes were resistant to this antibiotic with an MBC ≥ 512 [micro]g/ml. Conversion of mobile spirochetes to cystic forms was not observed when they were incubated with MZ. When they were incubated under microaerobic conditions, the biologically active cystic forms had an MBC ≥ 4 [micro]g/ml, but the MBC was ≥ 32 [micro]g/ml with aerobic incubation at 37[degrees]C. Staining with acridine orange (AO), dark field microscopy (DFM), and transmission electron microscopy (TEM) revealed that the contents of the cysts were degraded when the concentration of MZ was $\geq$ MBC. Some cysts were also ruptured. When incubated with a sufficient concentration of MZ, core structures did not develop inside the cysts, and AO revealed less RNA in the cysts. **Our observations may help efforts to treat resistant infections caused by *B. burgdorferi* with a combination of MZ and other antibiotics in order to eradicate both cystic and mobile forms of *B. burgdorferi*.**

Quotations From The Full-Text Article

“Serology, PCR, and cultivation are important for the conclusive diagnosis of Lyme borreliosis, but all these techniques have shortcomings, and false-positive and false-negative results are frequent. Many reports claim that all known antibiotics have shortcomings in the treatment of Lyme borreliosis.

***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. This phenomenon, combined with the ability of the cysts to revert to normal mobile spirochetes may explain a reactivation of the disease after an illusory cure – and not a “post Lyme syndrome” as postulated by other researchers.”** (p.566)

“*Helicobacter pylori* is also [like *B. burgdorferi*] capable of transforming to coccoid (cystoid) forms and reversing to normal mobile forms, and for this bacterium treatment with three or more antibiotics has been established. Therefore dual medication with MZ [metronidazole] as one of the antibiotics could be of value, also for curing infections caused by mobile and cystic forms of *B. burgdorferi*. (p.574)

A Rapid Method for Generating Cystic Forms of *Borrelia burgdorferi*, and Their Reversal to Mobile Spirochetes

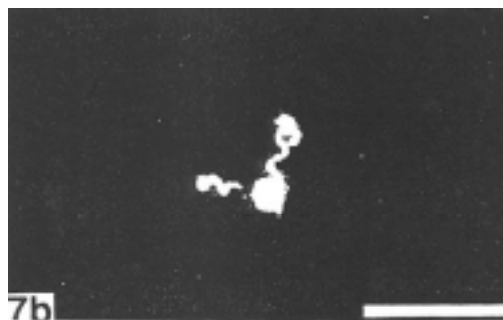
Date of Publication: December, 1998
Source: APMIS, 106(12):1131-1141
Authors: (1) Brorson O; (2) Brorson S.
Institution: (1) Department of Microbiology, Vestfold Sentralsykehus, Tonsberg
(2) Department of Pathology, Ulleval Hospital, Oslo, Norway

Abstract

Mobile *Borrelia burgdorferi* were transferred to distilled water (106 per ml). The cultures were observed by dark field microscopy (DFM), interference contrast microscopy (ICM) and transmission electron microscopy (TEM). **95% of the spirochetes were converted to cysts after 1 min, and after 4 h no normal mobile borreliae were observed.** When transferred to growth medium (BSK-H), the cysts became smaller and more irregular, and were filled with organic substances. After 1 day, 1-5 thin structures sprouted from the cysts. They continued to grow in both length and thickness until they attained a normal spirochetal structure. Finally, these **new-born spirochetes detached from the cysts**, by which time their mobility had become normal. The present method for producing large amounts of cystic forms of *B. burgdorferi* is well suited for further studies of this unique microbe.

Quotation From The Full-Text Article

“...cystic forms may occur in the human organism (11,14,15), and they may explain the long periods of latency, resistance to antibiotics, negative serological results (3-7,10,12,13,28), and low PCR sensitivity (5,8,10).” (p.1139)



B. burgdorferi. Developing spirochetes seen protruding from a cyst.

Brorson O; Brorson SH. 1999.

In Vitro Conversion of *Borrelia burgdorferi* to Cystic Forms in Spinal Fluid, and Transformation to Mobile Spirochetes by Incubation in BSK-H Medium

Date of Publication: May-Jun, 1998
Source: Infection, 26(3):144-50
Authors: Brorson O; Brorson S.
Institution: Dept. of Microbiology, Vestfold Sentralsykehus, Tonsberg

Abstract

The purpose of this study was to examine the structural alterations of *Borrelia burgdorferi* when exposed to spinal fluid. **Normal, mobile spirochetes were inoculated into spinal fluid, and the spirochetes were converted to cysts (spheroplast L-forms) after 1-24 h. When these cystic forms were transferred to a rich BSK-H medium, the cysts were converted back to normal, mobile spirochetes** after incubation for 9 to 17 days. The cultures were examined by dark field microscopy (DFM), interference contrast microscopy (ICM) and transmission electron microscopy (TEM). **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*.**

Quotation From The Full-Text Article

“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.”

Transformation of Cystic Forms of *Borrelia burgdorferi* to Normal, Mobile Spirochetes

Date of Publication: 1997
Source: Infection, 25(4):240-246
Authors: (1) Brorson O; (2) Brorson S.
Institution: (1) Department of Microbiology, Vestfold Sentralsykehus, Tonsberg
(2) Department of Pathology, Ulleval Hospital, Oslo, Norway

Abstract

Summary: The purpose of this study was to evaluate the behaviour of *Borrelia burgdorferi* under controlled conditions. The occurrence of cystic forms of *Borrelia burgdorferi* in vitro was noted, and these cysts were able to be transformed to normal, mobile spirochetes. *B. burgdorferi* was cultivated in a commercial culture medium without serum. The spirochetes multiplied only slowly in this medium, and transformation to encysted forms was observed after 1 week. When these cysts were transferred to the same culture medium with rabbit serum, **the encysted forms developed into regular, mobile spirochetes after 6 weeks**, and their regeneration time was normal. Examination of these cysts in the transmission electron microscope revealed transverse fission inside the cysts. It is probable that similar phenomena may occur *in vivo* under conditions unfavourable for spirochetes. **These observations may help to explain why diagnosis and treatment of *B. burgdorferi* infections in humans can be difficult.**

Quotations From The Full-Text Article

“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. ...In vivo these encysted forms may explain why *Borrelia* infection can be temporarily dormant, why a reactivation of the disease may occur when the conditions suit *B. burgdorferi*, and why the infection may relapse after treatment with antibiotics.” (p.245)

“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).” (p.244)

Serum Starvation-Induced Cyst Formation in *Borrelia burgdorferi* Under Defined Conditions

Paper confirming the Brorsons' work on cystic forms of Borrelia burgdorferi.

Alban PS; Nelson DR. University of Rhode Island, Kingston, RI 02181

Presented at the 1999 International Conference on Lyme Disease in Munich, Germany.

Subsequently published in Microbiology, Jan 2000;146 (Pt 1):119-27.

It has recently been demonstrated that cells of *Borrelia burgdorferi* transform from mobile spirochetes into nonmotile cysts when cultured for several weeks in BSK medium lacking rabbit serum (BSKrs-) or within 24 h in cerebrospinal fluid. Additionally, cyst forms of *B. burgdorferi* can successfully convert back to the motile vegetative form when incubated for 6 wk in BSK medium containing 6% rabbit serum (BSKrs+). The aim of this study was to investigate cyst formation by *B. burgdorferi* cells under defined conditions in order to understand the physiological basis of this transformation and to identify proteins involved in cyst formation. **We confirmed that motile *B. burgdorferi* cells transform into cysts after a 30 d incubation in BSKrs-. When *B. burgdorferi* cells were incubated in RPMI, a lipid-free defined medium rich in glucose, vitamins, and amino acids, >90% of the cells formed cysts within 48h. Cyst opening and recovery of spiral-shaped non-motile organisms was induced within 1 min by the addition of either BSKrs+ or rabbit serum (6%v/v, final concentration). Cells regained motility with additional incubation in BSKrs+.** The percentage of cells recovered (70 – 10%) and the recovery time (2 h - 8d) were inversely proportional to the cyst culture age. Motile cells were not recovered from cyst cultures older than 9 days. Cysts were not formed in HEPES buffer, pH 7.6, nor could motile calls be recovered after 24h in HEPES buffer. Cyst formation was completely inhibited by the addition of tetracycline (150 ~mcg/ml) to RPMI. Protein expression during cyst formation was analyzed by one- and two-dimensional gel electrophoresis of unlabeled and [35S]methionine-labeled cells. At least five proteins (13, 20.5, 26.5, 27, and 97 kDa) were found to be induced during cyst formation and numerous proteins were repressed. Several of the serum starvation-induced cyst proteins were found to be antigenic when western blots were probed with human Lyme disease patient sera. **These data suggest that cells of *B. burgdorferi* although possessing a small genome and extremely limited biosynthetic capabilities have evolved a stress response to serum starvation. Cells rapidly respond to conditions of serum starvation by inducing changes in protein synthesis and cell morphology. This may help explain how cells of *B. burgdorferi* can survive in different hosts and host tissues.**

The cyst forms seem resistant to conventional antibiotics. Note the enormous concentration of tetracycline needed to inhibit cysts, much greater than that achievable in humans. Flagyl has activity against cysts.

Quotations From The Full-Text Article (Microbiology, Jan 2000)

“...Western blots displayed consistent differences between the protein antigens recognized in vegetative cells and cysts. ...both the 46 kDa and 41 kDa (flagellin) protein bands exhibited less reactivity to sera from humans or monkeys in blots prepared from cysts, suggesting that both proteins were present in decreased amounts in cysts.” (pp.123-4)

“By forming cysts, it is also conceivable that *B. burgdorferi* cells evade detection by the immune system.” (p. 125)

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*Boldface has been added and is not part of the original text.***

Formation and Cultivation of *Borrelia burgdorferi* Spheroplast-L-form Variants

Date of Publication: May-June, 1996
Source: Infection, 24(3):218-26
Authors: Mursic VP; Wanner G; Reinhardt S; Wilske B; Busch U; Marget W.
Institution: Max von Pettenkofer-Institut, Ludwig-Maximilians-Universitat
Munchen, Germany

Abstract

As clinical persistence of *Borrelia burgdorferi* in patients with active Lyme borreliosis occurs despite obviously adequate antibiotic therapy, in vitro investigations of morphological variants and atypical forms of *B. burgdorferi* were undertaken. In an attempt to learn more about the variation of *B. burgdorferi* and the role of atypical forms in Lyme borreliosis, borreliae isolated from antibiotically treated and untreated patients with the clinical diagnosis of definite and probable Lyme borreliosis and from patient specimens contaminated with bacteria were investigated. Furthermore, the degeneration of the isolates during exposure to penicillin G in vitro was analysed. Morphological analysis by darkfield microscopy and scanning electron microscopy revealed diverse alterations. **Persisters isolated from a great number of patients (60-80%) after treatment with antibiotics had an atypical form.** The morphological alterations in culture with penicillin G developed gradually and increased with duration of incubation. Pleomorphism, the presence of elongated forms and spherical structures, the inability of cells to replicate, the long period of adaptation to growth in MKP-medium and the mycoplasma-like colonies after growth in solid medium (PMR agar) suggest that ***B. burgdorferi* produce spheroplast-L-form variants.** **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.**

Quotations From The Full-Text Article

“However, some patients develop late symptoms despite apparently adequate antibiotic treatment (11-15). **The persistence of Bb even after therapy with antibiotics has been demonstrated in cerebrospinal fluid (CSF), in skin, iris, heart and joint biopsies.**”

“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).”

Heterogeneity of *Borrelia burgdorferi* in the Skin

Date of Publication: December, 1996
Source: American Journal of Dermatopathology, 18(6):571-9.
Authors: Aberer E; Kersten A; Klade H; Poitschek C; Jurecka W.
Institution: Department of Dermatology, University of Vienna, Austria.

Abstract

The reliability of various in vitro techniques to identify *Borrelia burgdorferi* infection is still unsatisfactory. Using a high-power resolution videomicroscope and staining with the borrelia genus-specific monoclonal flagellar antibody H9724, we identified borrelial structures in skin biopsies of erythema chronicum migrans (from which borrelia later was cultured), of acrodermatitis chronica atrophicans, and of morphea. In addition to typical borreliae, we noted stained structures of varying shapes identical to borreliae found in a "borrelia-injected skin" model; identical to agar-embedded borreliae; and identical to cultured borreliae following exposure to hyperimmune sera and/or antibiotics. **We conclude that the H9724-reactive structures [atypical shapes of borreliae] represent various forms of *B. burgdorferi* rather than staining artifacts. These "atypical" forms of *B. burgdorferi* may represent in vivo morphologic variants of this bacterium.**

Quotation From The Full-Text Article

"After incubation with hyperimmune serum, some of the bacteria were immobilized while others paired off, intertwined, and showed intense movement resulting in rope ladder-like formations... Rings formed where ends of borreliae fused with the center of organisms. Less mobile borreliae developed granules at their centers or at their ends (Fig. 3a). 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** (Fig. 5a)."

"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). Neuralgias arising 6 months after ECM in spite of antibiotic therapy were evident in a seronegative patient who showed perineural rod-like borrelia structures. ...**Also, small granular structures were evident among collagen fibers** (Fib. 6b)."

"...simply knowing that *B. burgdorferi* are morphologically diverse may explain the large spectrum of Bb-associated diseases, [and] may indicate a **heterogenous immune responses** [sic] in individuals..." (p. 578)

Effects of Penicillin, Ceftriaxone, and Doxycycline on Morphology of *Borrelia burgdorferi*

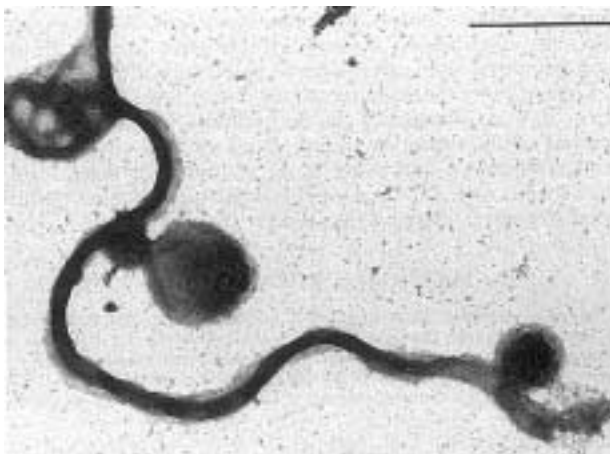
Date of Publication: May, 1995
Source: Antimicrobial Agents & Chemotherapy, 39(5):1127-33
Authors: Kersten A; Poitschek C; Rauch S; Aberer E.
Institution: Department of Dermatology, University of Vienna, Austria

Abstract

Antibiotic therapy with penicillin, doxycycline, and ceftriaxone has proven to be effective for the treatment of Lyme borreliosis. **In some patients, however, it was noticed that borreliae can survive in the tissues in spite of seemingly adequate therapy.** For a better understanding of this phenomenon, we investigated the different modes of degeneration of *Borrelia burgdorferi* suspensions during a 96-h exposure to various antibiotics. **By dark-field microscopy and ultrastructural investigations, increasing blebbing and the gradual formation of granular and cystic structures could be followed during the exposure time.** Although antibiotic concentrations at the MIC at which 90% of organisms are inhibited after 72 h were 80% or even greater, motile organisms were still present after incubation with penicillin and doxycycline but not after incubation with ceftriaxone. **By transmission electron microscopy, intact spirochetal parts, mostly situated in cysts, were seen up to 96 h after exposure with all three antibiotics tested. According to experiences from studies with other spirochetes it is suggested that encysted borreliae, granules, and the remaining blebs might be responsible for the ongoing antigenic stimulus leading to complaints of chronic Lyme borreliosis.**

Quotation From The Full-Text Article

“Morphologically intact borrelia parts seen after 4 days of incubation with antibiotics, however, may also persist in humans during antibiotic treatment. ...granules and encysted *B. burgdorferi* should be investigated further in view of their long-term persistence in infected tissues and their contribution to the pathogenesis of Lyme borreliosis.” (p.1132)



B. burgdorferi: After incubation with ceftriaxone

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*Boldface has been added and is not part of the original text.***

Electron Microscopy of Langerhans Cells and *Borrelia burgdorferi* in Lyme Disease Patients

Date of Publication: 1994
Source: Zbl Bakt, 280:348-349.
Authors: Hulinska D; Bartak P; Hercogova J; Hancil J; Basta J; Schramlova J.
Institution: Institute of Public Health, Prague.

Quotations From The Full-Text Article

“The form of spirochetes was unusual, i.e. cyst-like (Fig.5). **The surface membrane of cyst of Bb was antigenically different**, negative with McAb H₅₃₃₂, positive with lectin WGA in the IEM method (Fig.7) in contrast to the material of the spirochetes inside the cyst, which was positive with McAbH₅₃₃₂ in the outer envelope on cross section (Fig.6).”(p.353)

“The structure of Bb in tissue was more delicate and characterized with lower numbers of flagellae than those Bb commonly have in culture but **cyst-like forms were found in both tissue and culture.**”

“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 [Langerhans cells] 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 against 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. Geron (7) has suggested that these vesicles transfer intact DNA and thus genetic information.**”(p.357)

Concurrent Neocortical Borreliosis and Alzheimer's Disease Demonstration of a Spirochetal Cyst Form

Date of Publication: 1988
Source: Annals of the New York Academy of Sciences, 468-470
Author: Alan B. MacDonald
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A 71 year old man died in Arizona 3 years after the onset of progressive dementia. A diagnosis of probable Alzheimer's disease was based on clinical criteria. The brain was removed at autopsy, frozen (unfixed), and transported to the Department of Pathology, University of California, San Diego, School of Medicine where it was stored at -70 degrees C for further study. The author received the frozen brain and utilized methods previously described (1) for in vitro culture, cytologic, immunohistochemical, and silver impregnation studies. Argyrophilic plaques and neurofibrillary tangles were found in the frontal lobe and hippocampal formation in sufficient number to establish the neuropathologic diagnosis of Alzheimer's (fig 1 A). Spirochetes were visualized in imprint preparations of freshly thawed frontal lobe cortex with monoclonal antibody H5332, which specifically binds to the outer surface membrane of *Borrelia burgdorferi* (fig.2). *Borrelia* spirochetes were recovered from cultures of freshly thawed cerebral cortex and hippocampus in Barbour-Stoenner-Kelly medium. **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** using the monoclonal antibody H9724, which binds to class-specific axial filament proteins of *Borrelia* spirochetes. Oil immersion examination of sections from the hippocampus impregnated with silver disclosed a rare cystic structure (fig. 1B). **Previous workers have identified spirochetal cyst forms in cultures of non-pathogenic treponemal spirochetes** and have suggested that spirochetes have a complex life cycle. (2-5) **Dark-field examination of aged cultures of the B31 reference strain of *Borrelia burgdorferi* disclosed cystic structures similar to the cysts found in the autopsy brain culture.**

The following hypothesis is offered based on these observations. *Borrelia* spirochetes have a complex life cycle which includes corkscrew shaped forms, uncoiled filamentous forms, L-forms lacking a cell wall, cystic and ameboid forms, and granular forms. These forms may exist as either extracellular or intracellular pathogens. The cystic form of *Borrelia burgdorferi* may explain the Pick body, which is found in Pick's disease, and the granular form of *Borrelia* may explain granulovascular degeneration of nerve cells in the hippocampal formation in Alzheimer's disease. **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.**

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*Boldface has been added and is not part of the original text.***

The Infective Granule In Certain Protozoal Infections, As Illustrated By The Spirochaetosis of Sudanese Fowl

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Author: Andrew Balfour, M.D.

Preliminary note.

At the first meeting of the Tropical Medicine Section of the British Medical Association in London last year **I advanced the view that, in all probability, what might be called the “infective granule” would yet be found to play an important part in certain protozoal infections, and more especially in spirochaetosis and trypanosomiasis.** I based this belief on the work of Leishman as regards the changes undergone by *Spirochaeta duttoni* [a strain of relapsing fever] in *Ornithodoros moubata*, and on the allied changes which I had found to occur in the Sudan fowl spirochaete when ingested by *Arga pericus*. I have been continuing the work on fowl spirochaetosis and have recently arrived at some most interesting and significant results, which may yet have considerable bearing on the view we must take of the pathology of this and other spirochaetal diseases, and possibly also on their treatment. The full account of these later researches will be presented in the fourth report of these laboratories, which is now in press, and is due to appear in the autumn of the present year; here I wish merely to place on record a few of the more salient features of the work.

It will perhaps be remembered that one found intracorpuseular forms in this fowl spirochaetosis, and that following Sambon, one had to come to the conclusion that these endoglobular bodies represented a stage in the lifecycle of the spirochaete -- constituted, in short, its stage of schizogony [reproduction by multiple asexual fission] in the fowl. Sambon, however, who expressed this view from the study of a few slides I gave him, did not indicate how this red cell invasion occurred. For a long time I believed the spirochaetes themselves entered the red cells and broke up, or coiled up, within them to form these remarkable bodies. As the parasites can and do enter and leave the erythroblasts of the fowl, there was good ground for this supposition. Now however, I know better.

By the use of the dark-field method, and more especially by practising liver puncture on chicks at the crisis or on chicks which have been given a sufficiently large dose of salvarsan, I have found that in the liver in particular, also in the spleen and lung, **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.** The appearance is very remarkable. If a well-infected chick be given a dose of salvarsan, the peripheral blood is soon cleared, or nearly cleared of spirochaetes. If then a drop of liver juice be examined by the dark-field method, it will be found swarming with spirochaetes and with highly refractile granules. The source of the latter is soon apparent, for attention will be directed to spirochaetes which are not moving in the usual way, but are in a state of violent contortion, or are, so to speak, shaking themselves to and fro. Indeed, I cannot give a more apt comparison than by likening their movements to those of dogs which have been in water and are shaking themselves vigorously to dry their coats. **The object of the spirochaetes, however, is to rid themselves of the bright spherical granules which can be seen within them and which may or may not be aggregations of the so-called chromatin core.** They are forced along the periplastic sheath and suddenly discharged, so that they become free in the medium and dance hither and thither as tiny, solid, spherical, brilliant white

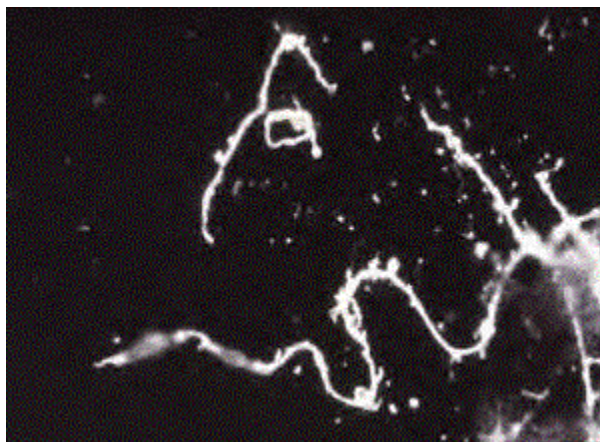
particles. **In process of time the spirochaete loses its activity, becomes difficult to see, and eventually all that is left of it is the limp and lifeless sheath** drifting aimlessly in the fluid and liable to be caught up and swept away by some still vigorous parasite. Such a sheath may still retain one or two of the granules which it has been unable to discharge. As may be imagined, the process is most fascinating to watch, and my observations have been confirmed by Captain Fry and Mr. Buchanan, of these laboratories and Captain O'Farrell, R.A.M.C. I may also say that the first-named had previously seen a shedding off of granules by trypanosomes in the peripheral blood of experimental animals, a phenomenon which he is now studying.

It is these spirochaete granules in the liver, spleen and lung, and possibly also in other internal organs, which I believe, invade the red cells. I think I have seen the penetration occur, but require to make further observations in order to be certain as to the mode of entry. **Such a chain of events fully explains all the puzzling features which this intracorporeal infection has hitherto presented, and moreover, brings it into line with the infective granules found in the ticks,** for these very closely resemble those seen in liver-juice films both when examined by dark-field method and when stained by the Levaditi process. There are various other points more especially as regards the peculiar staining reactions of these granules, into which I need not enter beyond saying that the fact that, when free, **they do not appear to take on the Romanowsky stain may explain why they have not previously been noticed.** The work is also not yet complete as it is necessary to find out if the spirochaetes ingested by ticks behave in a similar manner and thereby produce the granules of Leishman.

I see that Jowett in South Africa has recently discovered what appears to be an identical form of fowl spirochaetosis, and I trust he will employ the dark-field method and endeavour by liver puncture and the use of salvarsan, for the purpose of creating an artificial crisis to follow out the curious cycle I have indicated.

From these observations and others which will be fully detailed at a later date I have come to the conclusion that this fowl spirochaete must be classed as a specific entity and I am proposing for it the name *Spirochaeta granulosa penitans*, which, though lengthy, suitably indicates its more important peculiarities. **At the same time it is quite possible — nay, even probable — that other pathogenic spirochaetes behave in a similar manner. 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.**

In conclusion, I must thank Professor Erlich for most kindly placing at my disposal an ample supply of his new and valuable remedy. [Professor Erlich's remedy for syphilis was salvarsan, which contains arsenic.]



Borrelia burgdorferi, "Granule shedding."

Burgdorfer W. 1999.

12th International Conference on Lyme Disease and Other Spirochetal and Tick-Borne Disorders.