

## CME Infor

[Target Learning](#)
[Auth Disc](#)
[Accr Stat](#)
[Commer](#)
[Hardwa](#)
[Requ](#)
[Priv](#)
[Confi](#)

Return to Medscape coverage of: [12th International Conference on Lyme Disease and Other Spirochetal and Tick-Borne Disorders](#) | [Day 1 - Friday, April 9](#)



Email this Article



Print this Activity for Offline Reference

[Next Article](#)

**CME** in  
indicates  
educator  
profes

## Keynote Address - The Complexity of Vector-borne Spirochetes (*Borrelia* spp) **CME**

### [Disclosures](#)

The keynote speaker at this conference was Willy Burgdorfer, PhD, of the National Institutes of Health. In the late 1970s, Dr. Burgdorfer and I began debating whether the lone star tick, *Amblyomma americanum*, transmits pathogenic spotted fever group rickettsia to humans. In 1981, Dr. Burgdorfer was looking for rickettsia in New York ticks when he detected spirochetes -- the organisms, subsequently named *Borrelia burgdorferi*, that we now know to be the etiologic agents of Lyme disease. In his lecture, Dr. Burgdorfer reviewed the scientific literature past (much of it no longer written in English) and present, dealing with spirochetal cysts, blebs, and spherules.

Because of the seminal nature of his work, Dr. Burgdorfer's presentation is posted here in its entirety. As you read his review of work in this area to date, keep in mind much remains to be done. It is possible that spirochetal bleb formation signals the organism's impending death. The research reviewed here, however, suggests that it is a survival mechanism, which of course, has implications regarding patient management.

-- Julie Rawlings, MPH

## The Complexity of Arthropod-borne Spirochetes (*Borrelia* spp)

**Speaker: Willy Burgdorfer, PhD**

Today's investigators, eager to apply their sophisticated microscopic, immunochemical, molecular and genetic methodologies often are not aware that their research objectives are similar if not identical to those of earlier workers whose publications unfortunately may no longer be available or are published in foreign journals.

My talk today identifies the highly controversial historical findings related to the biology and vector(s)/host relationships of borreliae, and emphasizes their importance to our current investigations of Lyme disease and its spirochetes. Let us briefly recall that the first discovery of spirochetes pathogenic to humans is credited to the German physician Dr. Otto Obermeier (Fig. 1) who as early as 1868 during an epidemic in Berlin detected in the blood of relapsing fever patients highly motile threadlike microorganisms (Fig. 2) that in morphology were similar to the water spirochetes *Spirocheta plicatilis* -- a spirochete detected in 1835 by Dr. Ehrenberg.





Figure 1

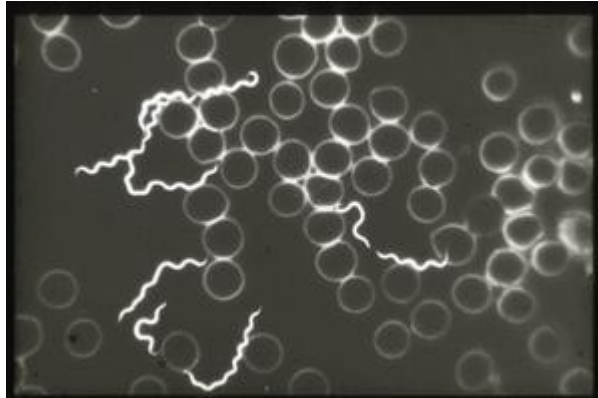


Figure 2

In 1878, the physician Gregor Münch was the first to express the idea that recurrent or relapsing fever may be transmitted by the bite of blood-sucking arthropods such as lice, fleas and bugs. The theory of lice being the vector was confirmed later by the French microbiologists Sergent and Foley in 1910.

At the turn of the century, 1903 through 1905, the British physicians Dutton (Joseph Everett) and Todd (John Lancelot) working in the Congo, and independently Ross (Philip Hedorland) and Milne (Arthur Dawson) active in Uganda, found that the disease referred to as "human tick disease" by Livingston (David) as early as 1857, was caused by a spirochete transmitted by the African soft-shelled or argasid tick, *Ornithodoros moubata* (Fig. 3). Both Dutton and Todd contracted the disease. Dutton, a pathologist, infected himself accidentally during a post mortem and died. He is remembered by having had named the East African relapsing fever spirochete *Borrelia duttonii*.



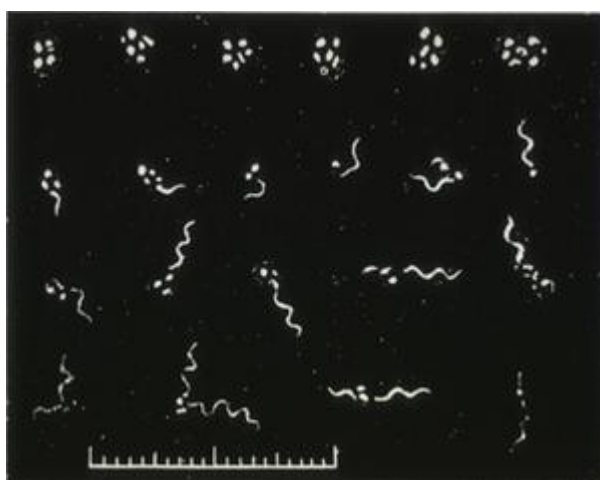
Figure 3

Also playing an important role in relapsing fever research was the German microbiologist Robert Koch. At the end of 1904, he was called to East Africa to investigate the widely distributed East Coast Fever in cattle. He soon learned that most Europeans traveling into the interior regions had been suffering of recurrent fever first thought to be malaria. Although Koch was not aware of the British findings in the Congo and Uganda, he confirmed the vector role of the *Ornithodoros moubata*. He was the first to demonstrate that spirochetes were transmitted via eggs (transovarial transmission) to the progeny of infected female ticks.

Ever since it was demonstrated that the body louse (*Pediculus humanus humanus*) and the African *O moubata* were the vectors of the relapsing fever spirochetes known today as *Borrelia recurrentis*

and *B. duttonii*, respectively, intense studies have been carried out on the development of these microorganisms in their vectors, and on the mode of transmission to humans. Thus, in 1912, the French worker Charles Nicolle and coworkers studied the behavior of *B. recurrentis* in lice and noted that the spirochetes had disappeared from the midgut 24 hours after they had been ingested; they were no longer detectable until days 6 to 8 when they suddenly reappeared in the hemolymph.

A similar "negative phase" had previously been reported for *B. duttonii* in *O. moubata* by Dutton and Todd (1905-1907), Leishman and other investigators (1907-1920), Fantham (1911-1915), Hindle (1911), and later also by Hatt (1929) and Nicolle and associates (1930). According to these investigators, ingested spirochetes invade the gut epithelium where they lose motility and after 3 to 4 days develop into cysts (blebs, vesicles) that contain granules or chromatin bodies (Fig. 4). Dutton and Todd postulated that these spherules are formed by protuberance of the spirochetes periplasmic membranes; they may occur at any point along the spirochete. At some time during their development, these spherules or cysts were said to burst and release their granules. By the 10th day after infectious feeding, Dutton and Todd no longer found morphologically typical spirochetes, but instead large numbers of granules from which eventually new spirochetes developed provided the ticks were maintained at temperatures above 25° C.



**Figure 4**

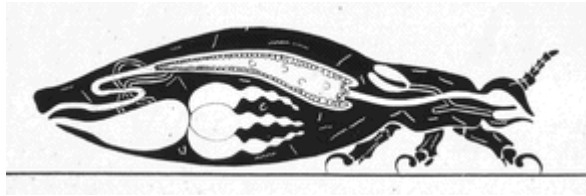
Hindle, in 1911, reported similar observations. In infected ticks held at 21° C, the spirochetes had disappeared from the midgut by the 10th day after infectious feeding. They could no longer be detected either in the gut or in the tissues. However, triturates of such ticks injected into mice regularly proved infective, and an increase in temperature to 35° C resulted in the reappearance of morphologically typical spirochetes.

This "granulation theory" -- as it was referred to -- received a significant boost in 1950 when Edward Hampp of the National Institute of Dental Research in Bethesda showed by stained smears, darkfield and electron microscopy that oral treponemes and *Borrelia vincenti* in cultures produce blebs and granules that were considered "germinative units." His hypothesis was supported by the observation that 31 month-old cultures containing only granules invariably resulted in typical spirochetes upon transfer to fresh medium.

Similar observations were also reported by DeLamater and coworkers from the University of Pennsylvania Medical School. They provided evidence for the occurrence of a complex life cycle in the pathogenic and nonpathogenic strains of *Treponema pallidum*. Accordingly, these spirochetes multiply by (1) transverse or binary fission, and (2) by producing gemmae (cysts in which a single or more granules appeared to be the primordia of daughter spirochetes).

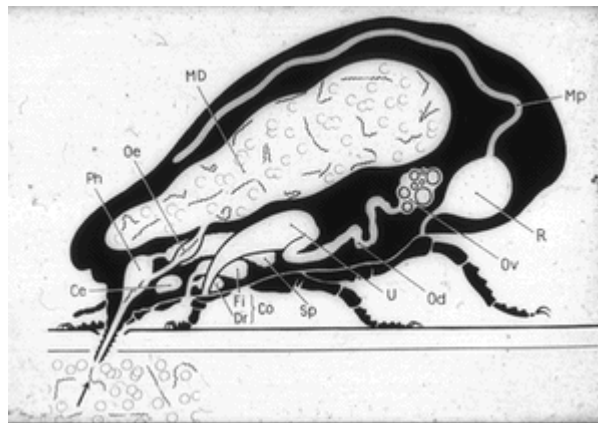
In contrast, there were many investigators, including Wittrock (1913), Kleine and Eckard (1913), Kleine and Krause (1932), Feng and Chung (1936-39), and your speaker (Burgdorfer, 1951), who conducted dynamic investigations on the development of various species of borreliae in lice or ticks and found no evidence of a negative phase or complex life cycle.

It is now generally accepted that *B. recurrentis* spirochetes in the body louse, *P. humanus humanus*, following ingestion with a patient's blood, arrive in the midgut where most are destined to die (Fig. 5). Those that survive, within a few hours after ingestion pass through the gut wall into the hemolymph where they undergo massive multiplication by binary fission. As a result, large concentrations of spirochetes are found in the hemolymph surrounding the various tissues as early as 8 to 10 days after ingestion.



**Figure 5**

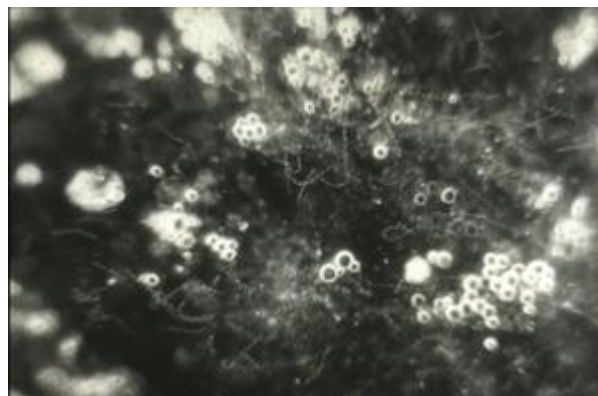
Similarly, *O. moubata* and other relapsing fever ticks, during their short feeding (10 to 30 minutes), ingest spirochetes into the midgut where they can be demonstrated in gradually decreasing numbers for about 14 days but not longer (Fig. 6). Within hours after the ticks had engorged, spirochetes accumulate in the intercellular spaces of the gut epithelium (Fig. 7) from where as early as 24 hours after ingestion, they penetrate the basement membrane to enter the body cavity where they undergo massive multiplication by binary fission (Figs. 8, 9).



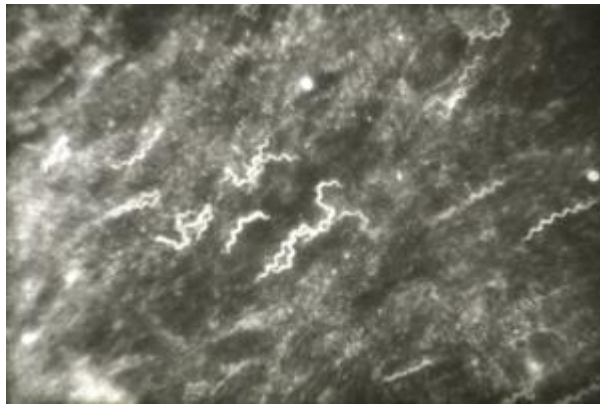
**Figure 6**



**Figure 7**



**Figure 8**



**Figure 9**

Although most of the above mentioned opponents of the "granulation theory" verified the formation and existence of cysts, blebs, spherules associated with spirochetes, they considered them as degeneration products.

Thus the question of "negative phase" and "complex developmental cycle" appeared to be settled until in 1981 your speaker discovered the Lyme disease spirochete -- now known as *Borrelia burgdorferi* -- associated with ticks of the *Ixodes ricinus/persulcatus* complex (Fig. 10). This relatively large *Borrelia* 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.



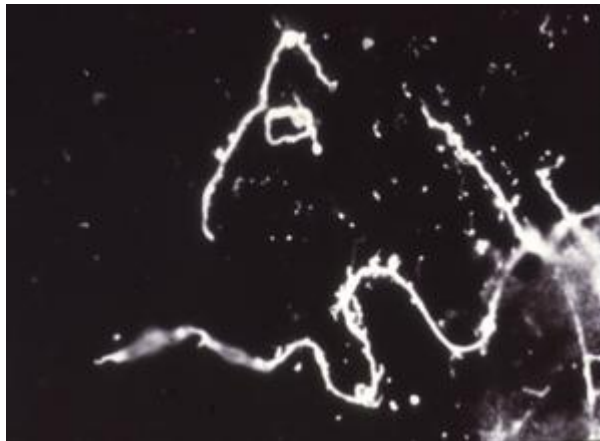
**Figure 10**

Unlike the other louse and tick-borne borreliae that leave the midgut of their vectors shortly after ingestion to cause a hemolymph-limited or systemic infection, *B burgdorferi* in most of its tick vectors remains in the midgut where it aggregates near the microvillar brushborder and in the intercellular spaces of the gut epithelium. From there, it may penetrate the gut wall during and after engorgement and may initiate a systemic infection particularly in the tissues of the ticks' central ganglion, ovary and Malpighian tubules. Regardless of such generalized infections, however, the midgut remains infected throughout the life span of the tick.

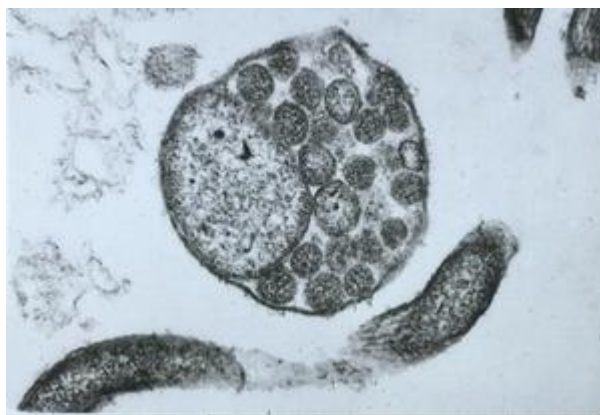
Of particular interest to our discussion is the presence in freshly engorged Lyme disease ticks of



spirochetes with outer membrane-associated cysts, blebs or spherules that often contain numerous granules with surrounding trilaminar membranes (Figs. 11,12). Because the internal material of these granules is similar in appearance and electron density to that of typical spirochetes and because these cysts (blebs, spherules) strongly react when treated with FITC-labeled conjugates, the questions concerning a complex life cycle of borreliae have again been raised and induced investigators to critically examine the nature and function of these formations -- a research problem referred to for the first time almost 100 years ago by Dutton and Todd.



**Figure 11**



**Figure 12**

Thus, RML scientists Dave Dorward and Claude Gron 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). The fact that this material was packaged within the membrane-derived vesicles suggested that it might play a role in the protection of genetic markers. *In vivo* and *in vitro* exposure of *B burgdorferi* to antibiotics (penicillin G, ceftriaxone) were shown by Preac-Mursic and associates to produce cytomorphic atypical but motile spirochetes with numerous membrane-derived vesicles (spheroblast -- L-forms) (Fig. 14).<sup>[1]</sup>

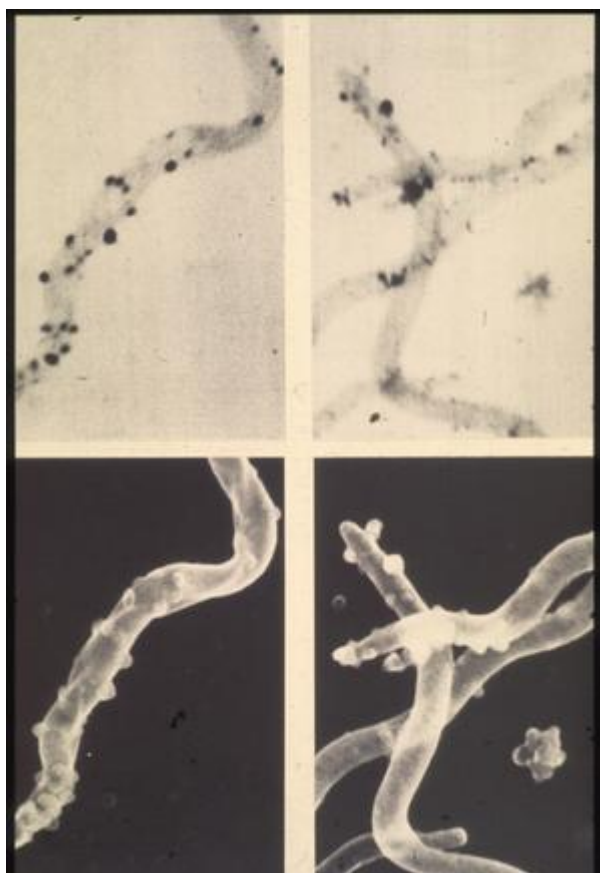


Figure 13



Figure 14

In their recent publication, Brorson and Brorson reported on the "*In vitro* Conversion of *Borrellia burgdorferi* to Cystic Forms in Spinal Fluid, and on the Transformation to Mobile Spirochetes by Incubation in BSK-H Medium."<sup>[2]</sup> Accordingly, *B burgdorferi* converted rapidly to cystic forms when transferred to spinal fluid. No normal spirochetes were left after 24 hours of incubation at 37° C; all were converted to cysts. 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.

These most recent findings do confirm the development of membrane-derived cysts, blebs, spherules, vesicles and the potential transformation to motile, helical spirochetes, not as part of a complex developmental cycle -- as postulated by Dutton and associates -- but rather as a "survival mechanism" of spirochetes to overcome or escape unfavorable conditions. Such conditions prevail during early phases of infection when spirochetes ingested into the midgut of ticks or lice become exposed to the vectors' digestive enzymes and tissue barriers (peritrophic membrane, gut epithelium). As a result, most detectable spirochetes produce numerous cysts often filled with granular material.

Other *in vitro* and *in vivo* factors shown to induce development of cysts include unsatisfactory culturing conditions, presence of antibodies and the effects of antibiotics.

Using silver impregnations and immunochemical staining, cystic material has been demonstrated in

every animal and human tissue infected by *B burgdorferi*. As yet, it is not known whether these forms of *Borrelia* represent products of degenerated spirochetes or of surviving organisms capable of transforming to typical spirochetes once the favorable environmental conditions are restored. It is tempting to speculate, however, that the survival mechanism of spirochetes is responsible for the diverse pathology of these organisms as well as for their ability to survive as cystic forms thereby producing prolonged, chronic and periodically recurrent disease.

## References

1. Preac-Mursic V, Wanner G, Reinhardt S, et al: Formation and Cultivation of *Borrelia burgdorferi* Spheroplast-L-Form Variants. *Infection* 24(3):218-225, 1996.
2. Brnson O, Brnson SH: *In Vitro* Conversion of *Borrelia burgdorferi* to Cystic Forms in Spinal Fluid, and Transformation to Motile Spirochetes by Incubation in BSK-H Medium. *Infection* 26(3):218-225, 1996. *Infection* 26(3):144-150, 1998.

[Next Article](#)

Copyright © 1999 Medscape Portals, Inc.

Search Medscape, MEDLINE and Drug Reference

• [About Medscape](#) • [Privacy & Ethics](#) • [Terms of Use](#) • [WebMD Health](#) • [WebMD Corpor](#)

All material on this website is protected by copyright, [Copyright](#) © 1994-2006 by Medscape. This website also contains material copyrighted Medscape requires Microsoft browsers in versions 6 or higher.