# **Round** forms of *Borrelia burgdorferi*
# Survival of the Microbe and Attack Models

University of New Haven
Research Seminar Presentation

**Alan B. MacDonald MD**

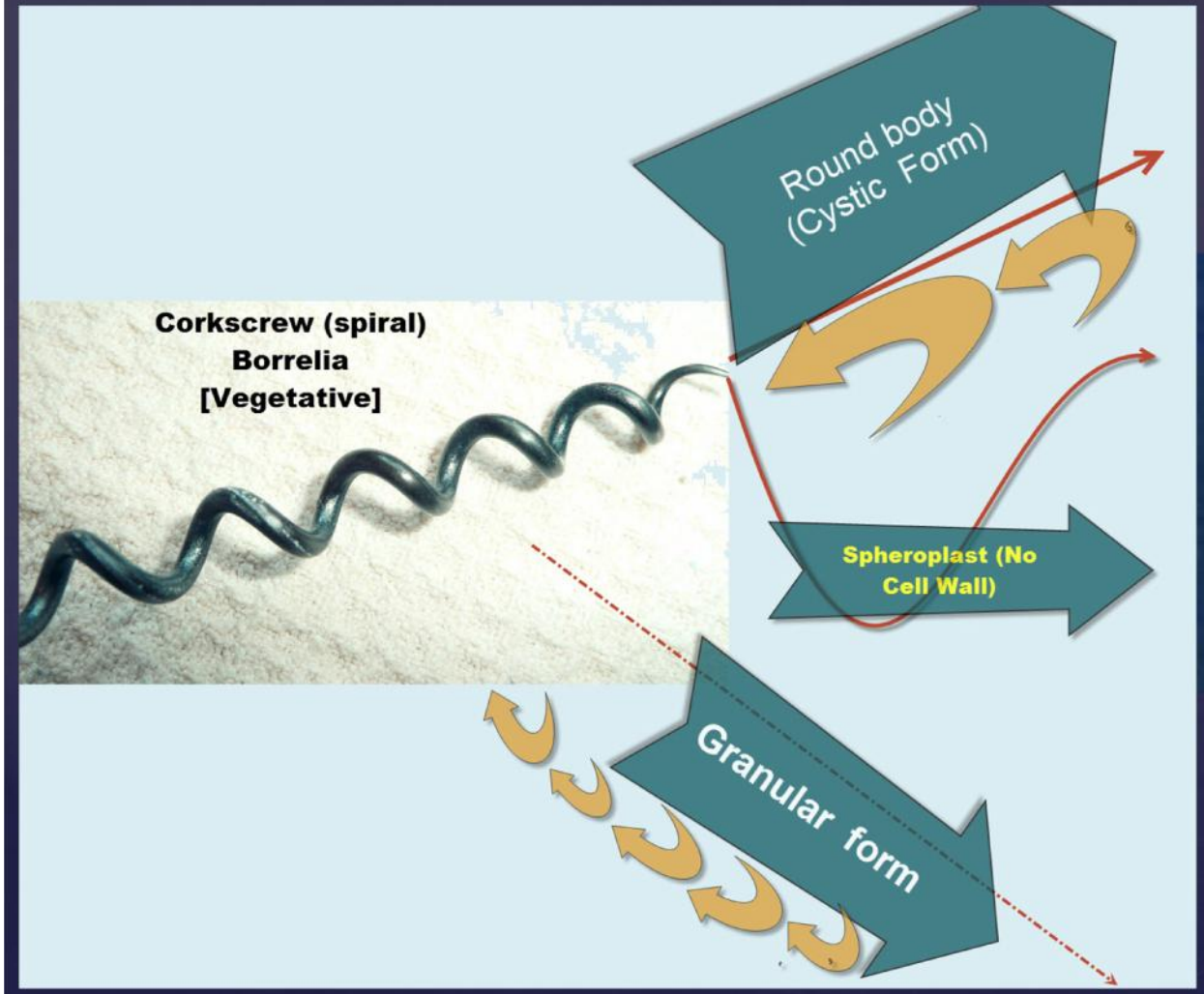
# Borrelia Burgdorferi Survives in adverse environments

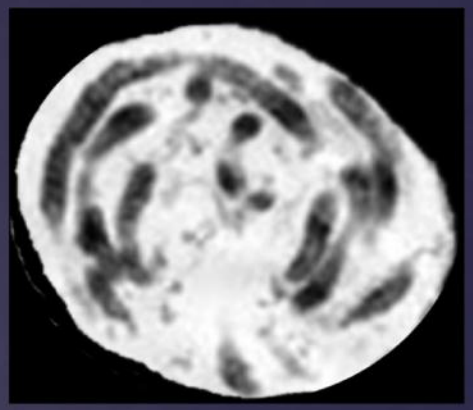
The Shape of Borrelia
Is capable of Change, and these changes proceed in parallel with Changes in its Transcriptome. Upregulation and Down regulation of key Genes …Unfold as the organism senses New environments.
…..
Changes of Shape of the Living ( but not mutant) Borrelia are diverse……
Not all of the possible Transcriptome determined Shape changes for this spirochete are Recognized, even by expert Researchers in the field of Borreliosis

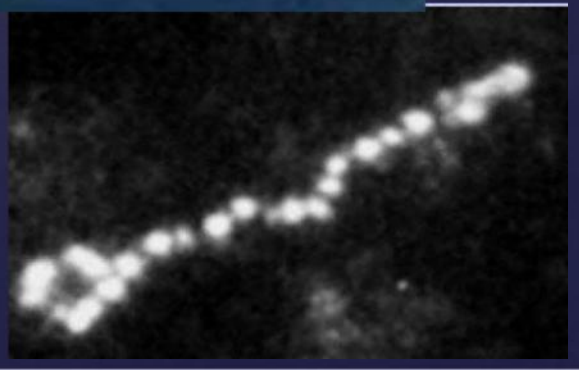
# Educational Objectives:

1. Cystic forms Are Living and Essential to the Borrelia lifestyle
2. Cell Division in the Cystic form is Independent of Cell division in the Spiral (vegetative) form of Borrelia.
3. Unique proteins produced by the Cystic Transcriptome indicate Upregulation and Down Regulation of the Borrelia Genome
4. The Envelope of the Cyst is Devoid of a Slime Layer component , OspA and may present Naked Peptidoglycan cell wall with no overlying glycoproteins.
5. Cell wall Active Antibiotics (combination therapy) can kill Cystic Forms -
   Such antibiotics are only capable of Killing Actively dividing Bacterial forms
6. Cystic forms of Borrelia produce infection (independently of spiral transformations) in Mice
7. Cystic forms of Borrelia are present in Human brain tissue in some patients with Tertiary Neuroborreliosis..
8. Cystic forms of Borrelia are prominently represented in In Vivo biofilms of Borrelia burgdorferi

# Borrelia Shape Changes
# An Introduction



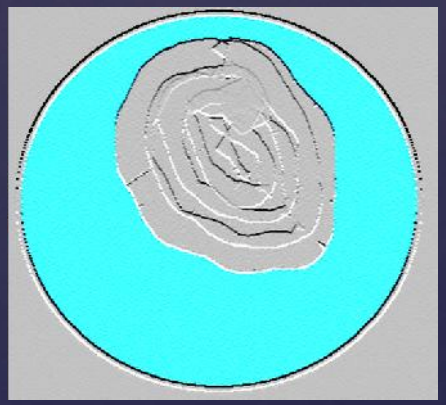



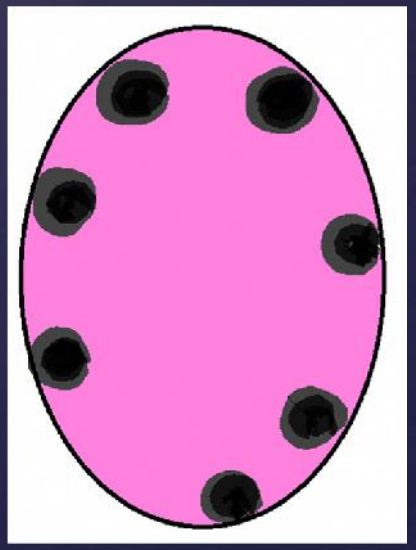


# Spirochetes are expected to be Spiral (corkscrew) in shape according to Textbook teaching

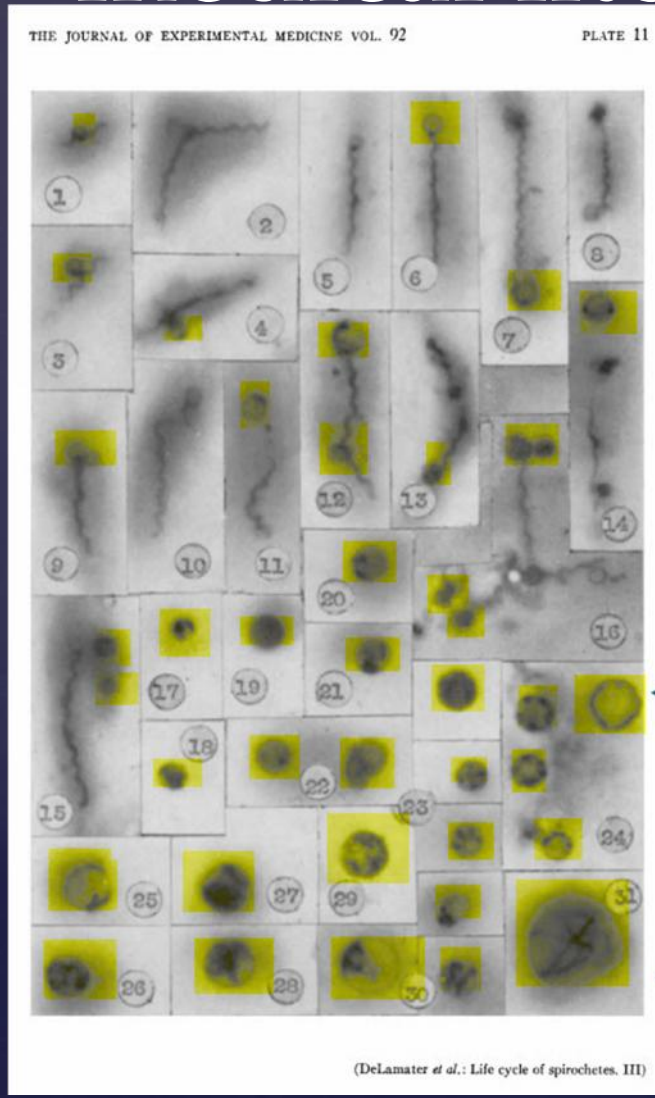
# Round Bodies

are established as part of the repertoire of spirochetes





# …if you are Ignorant of the medical literature …….




***Reference:***
***Journal of Experimental Medicine ,1950***
***Vol 92,***
***pages 239-253***

***Edward Delamater MD et al***

***ROUNDED***
**Spirochetes (Highlighted in Yellow)**

**…….You will be Unaware That *Round* Spirochetal *Forms* were Identified as important hallmarks of spirochetal infection**

**When *Treponema Pallidum* was a public health issue…**

# Look at the Movies

Links to Movie Clips are available on www.molecularalzheimer.org

First Movie (Univ of Connecticut)
Image Credit – PloS Pathogens Feb 2012
Conversion of Round
to Spiral form
Full Motion Video

Second Movie
Image Credit Stan Dembowski – B31 Reference B. Burgdorferi
Conversion of Spiral form
to Round Body
Full Motion Video

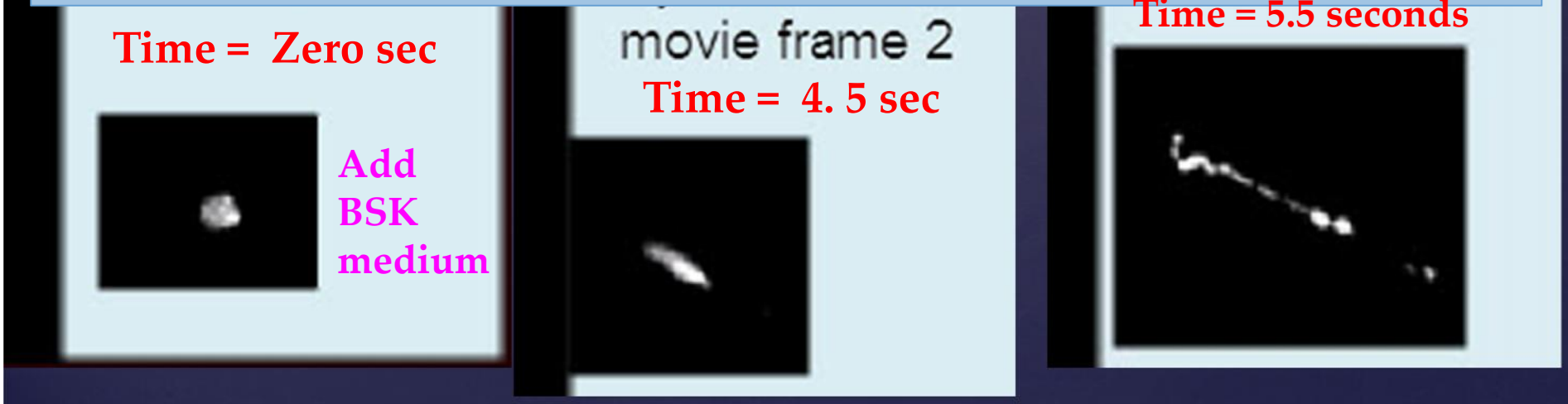
First –
Stop Action Video Clips .....

# Borrelia burgdorferi – Movie frames showing

## Conversion of Round Body to Spiral form

# after adding nutrients to culture medium –

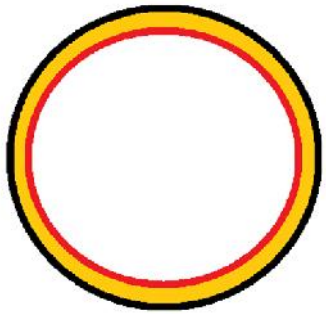
## Elapsed time in seconds

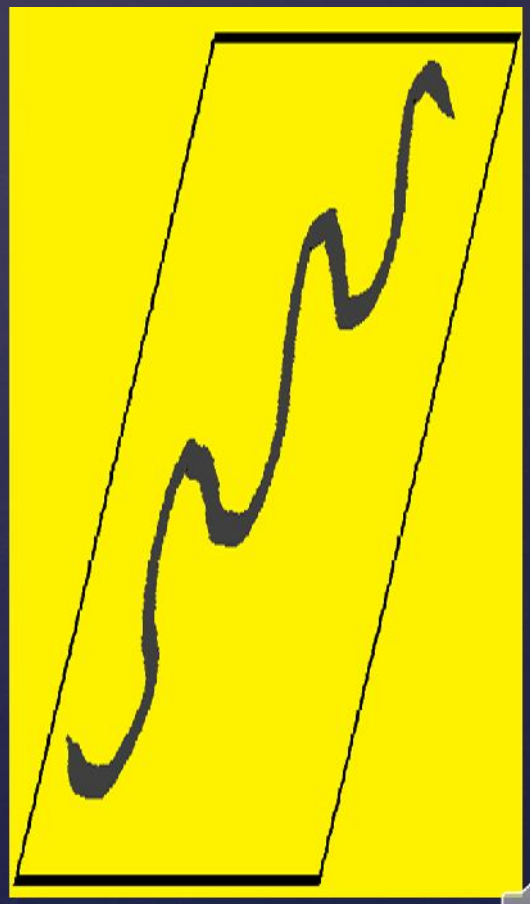



Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al

# What Did You Just See??

Round Body Form
Changes
Into
***Spiral (corkscrew)***





sec 4.66

# FULL ACTION VIDEO

**Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al**

# The Round Body Produces a Spiral Spirochete

**Spiral Form here**

**Round Body Dynamic**

This video must be loaded separately
- See next slide for Web Link

sec 4.56

# FULL ACTION VIDEO

**Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al**

# The Round Body Produces a Spiral Spirochete

**Spiral Form here**


**Round Body Dynamic**

The live video link must be loaded Separately – see www.molecularalzheimer.org for Web link

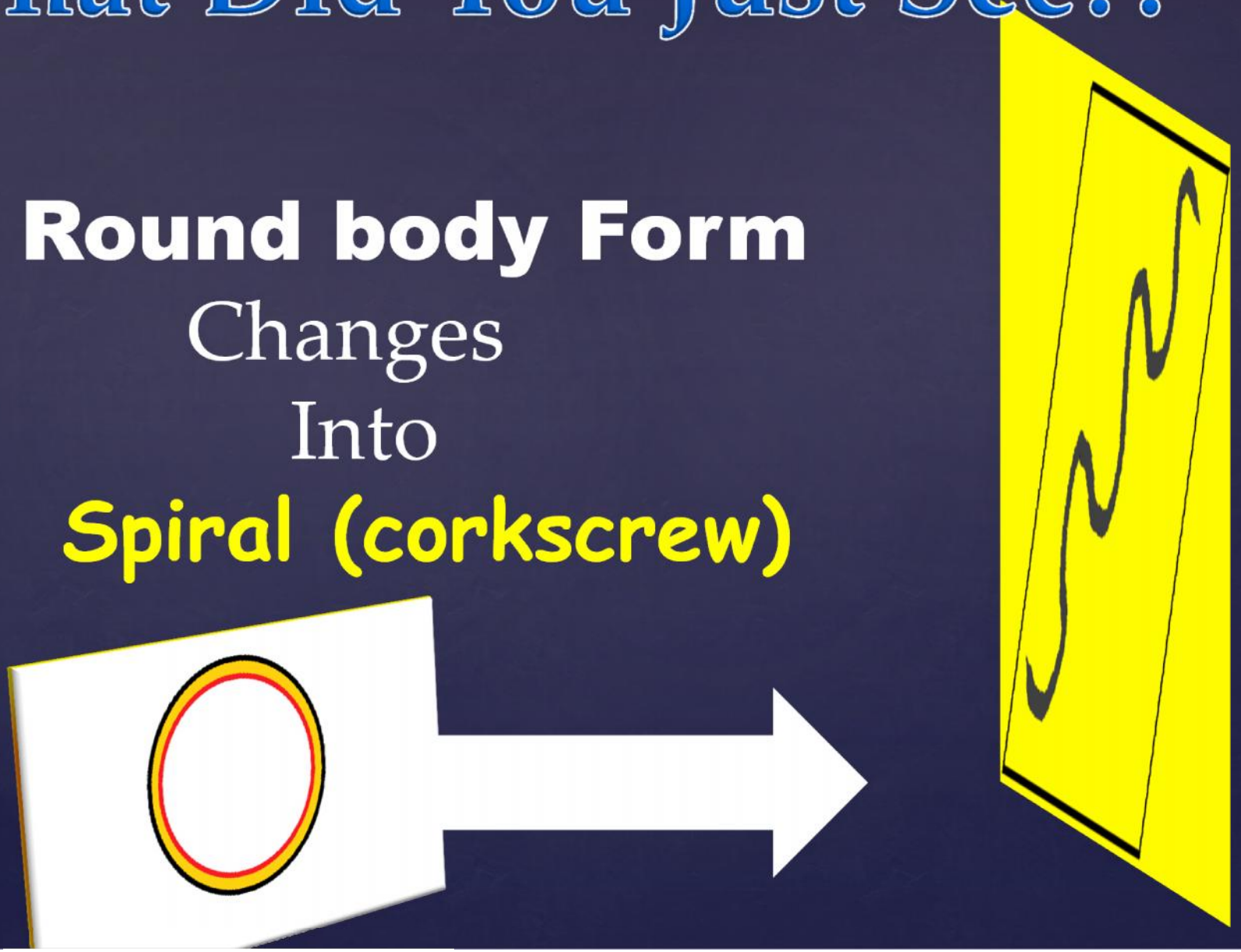
# What Did You Just See??

**Round body Form**

Changes

Into

**Spiral (corkscrew)**



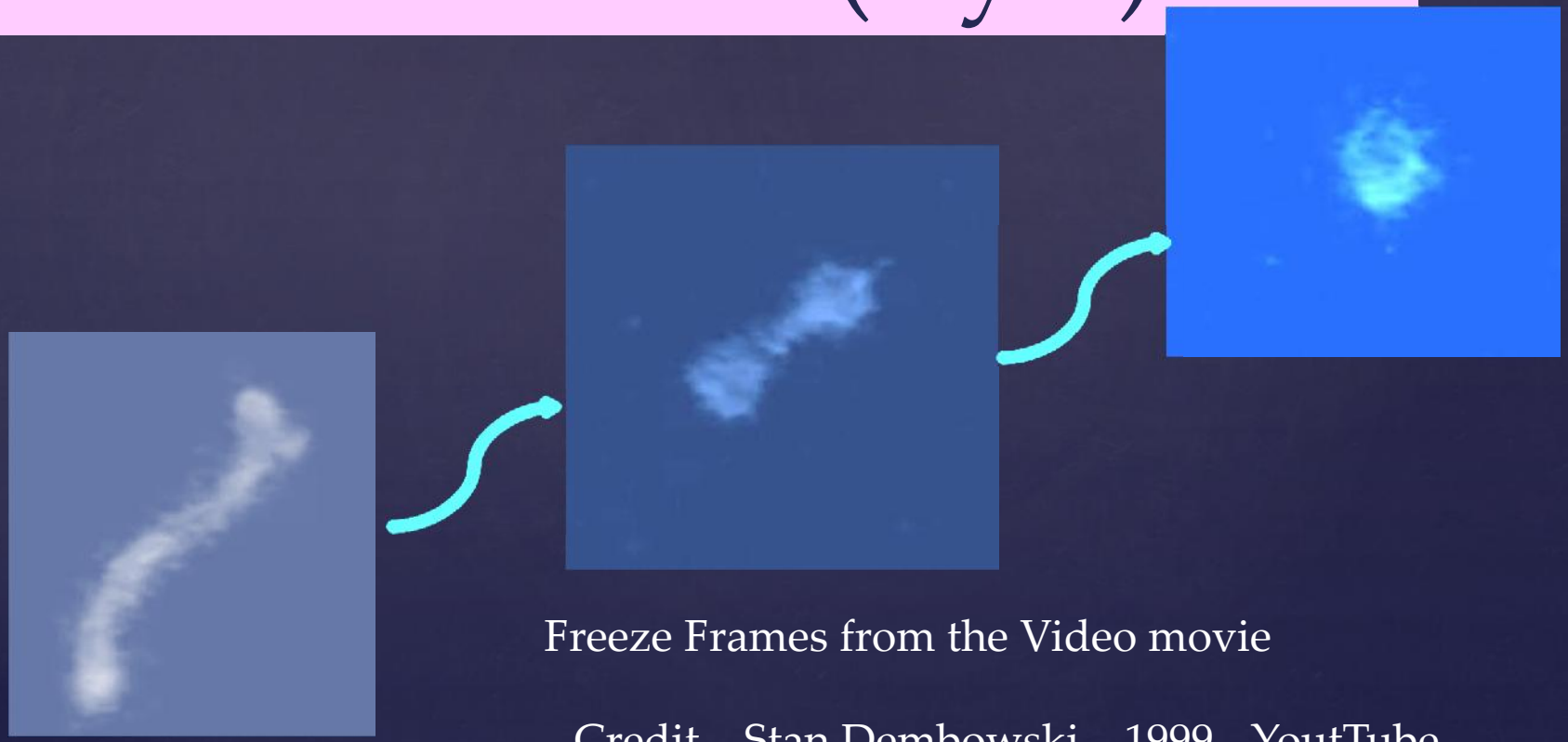
Now
A second movie
Which shows
The
REVERSE of the movie
Which you have Just
viewed

# Spiral to Round Body Transformation of B31 Borrelia burgdorferi

FULL ACTION VIDEO

The Video must be Loaded separately – Web Link available At www.molecularalzheimer.org

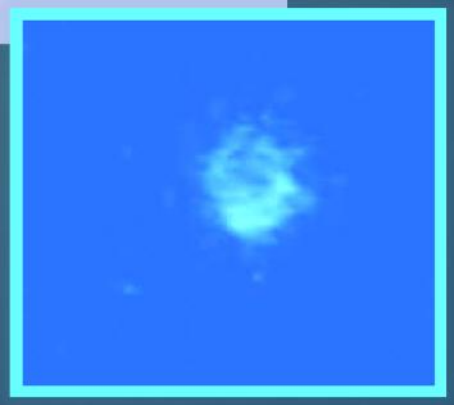
# SPIRAL TRANSFORMS TO ROUND BODY (Cyst)



Freeze Frames from the Video movie

Credit – Stan Dembowski – 1999 - YoutTube

# Shape Changing in Living Borrelia burgdorferi



Freeze Frames – Credit – Stan Dembowski – 1999-YouTube

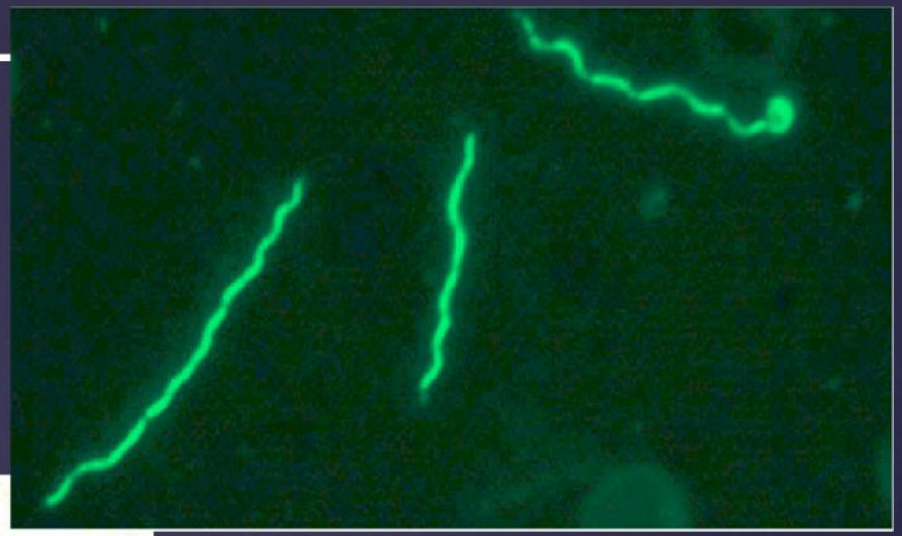
WebLink for Live Video Movie www.molecularalzheiimer.rg

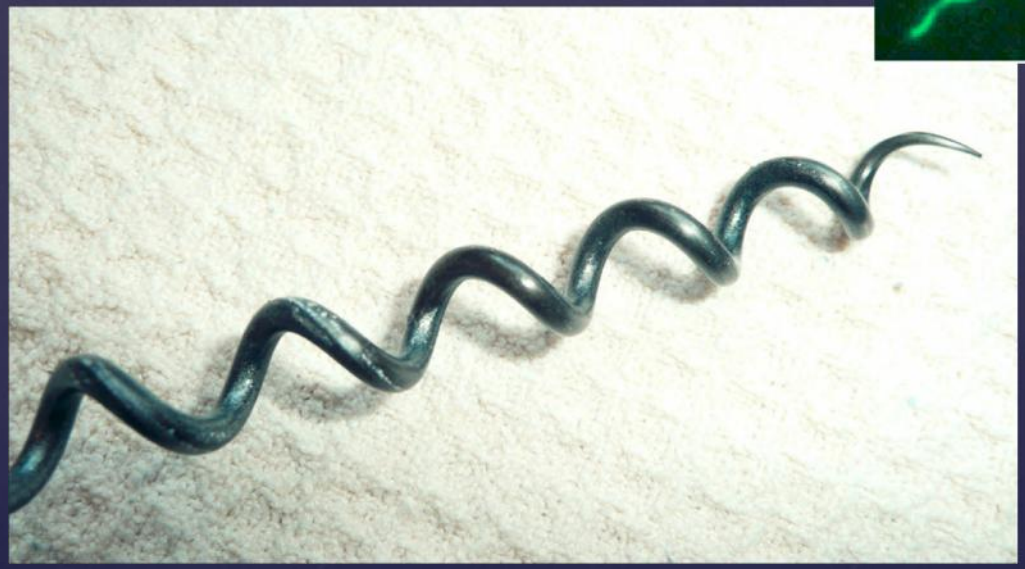
# Two Dimensional Models

for Round Body Transformations in Borrelia Burgdorferi

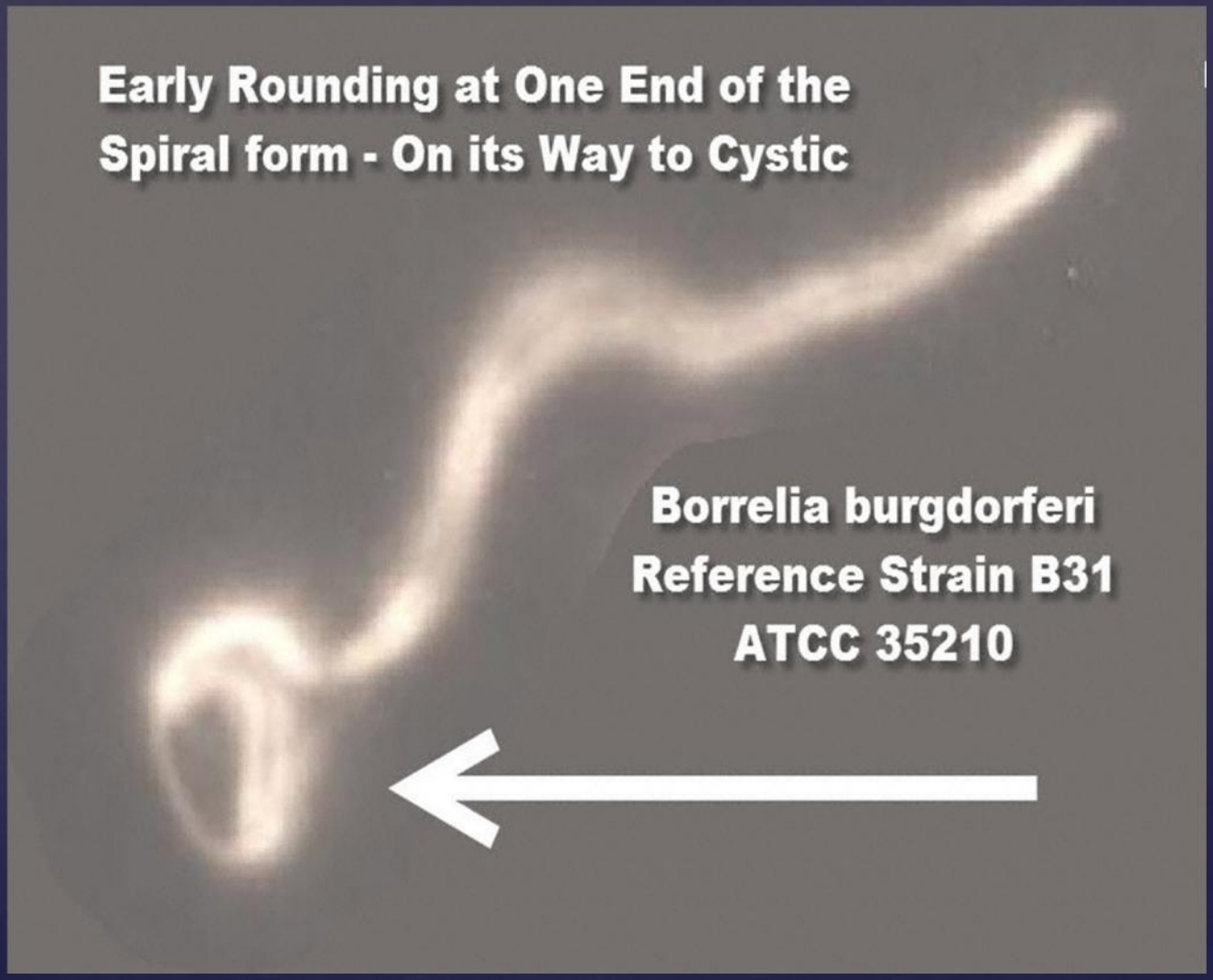
*Borrelia burgdorferi*
national reference strain (B31) obtained from the American Type Culture Collection (ATCC)[35210]

# The Beginning -
# *All Spiral*
## All the time

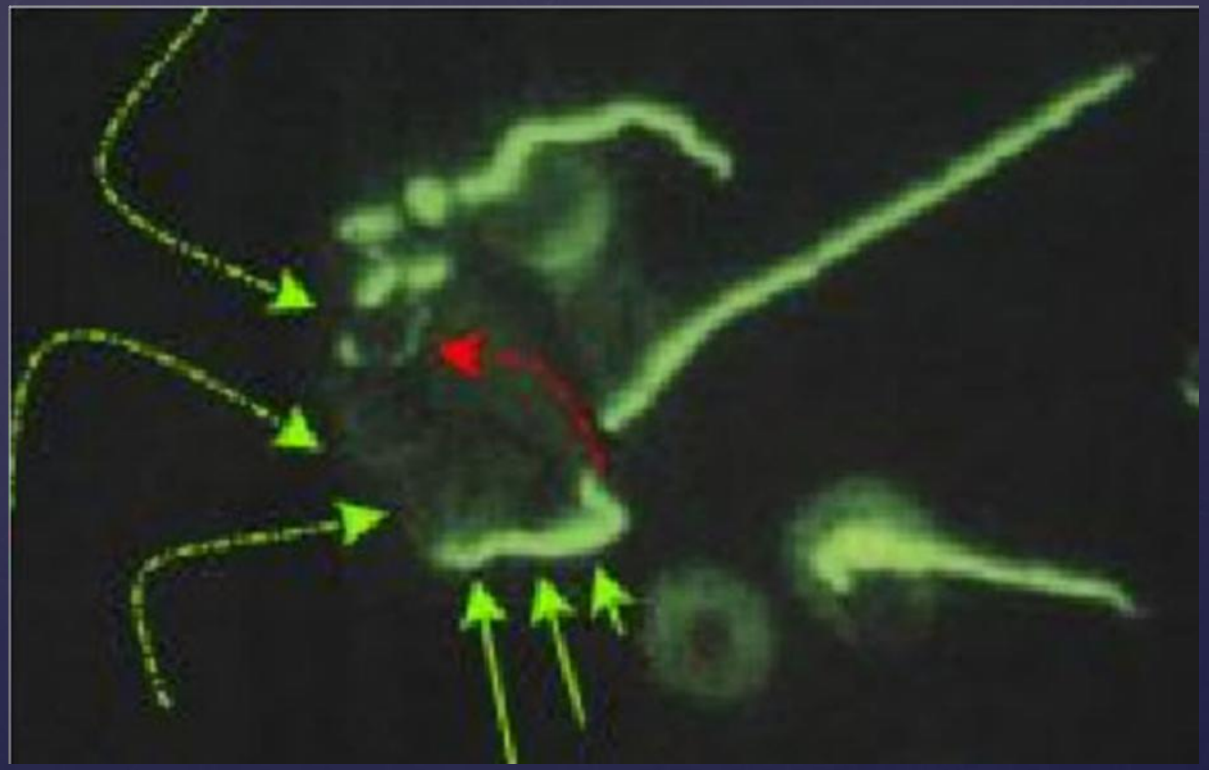




# The Early Shape Change……



# A Rounded area begins to develop at one end of the spiral



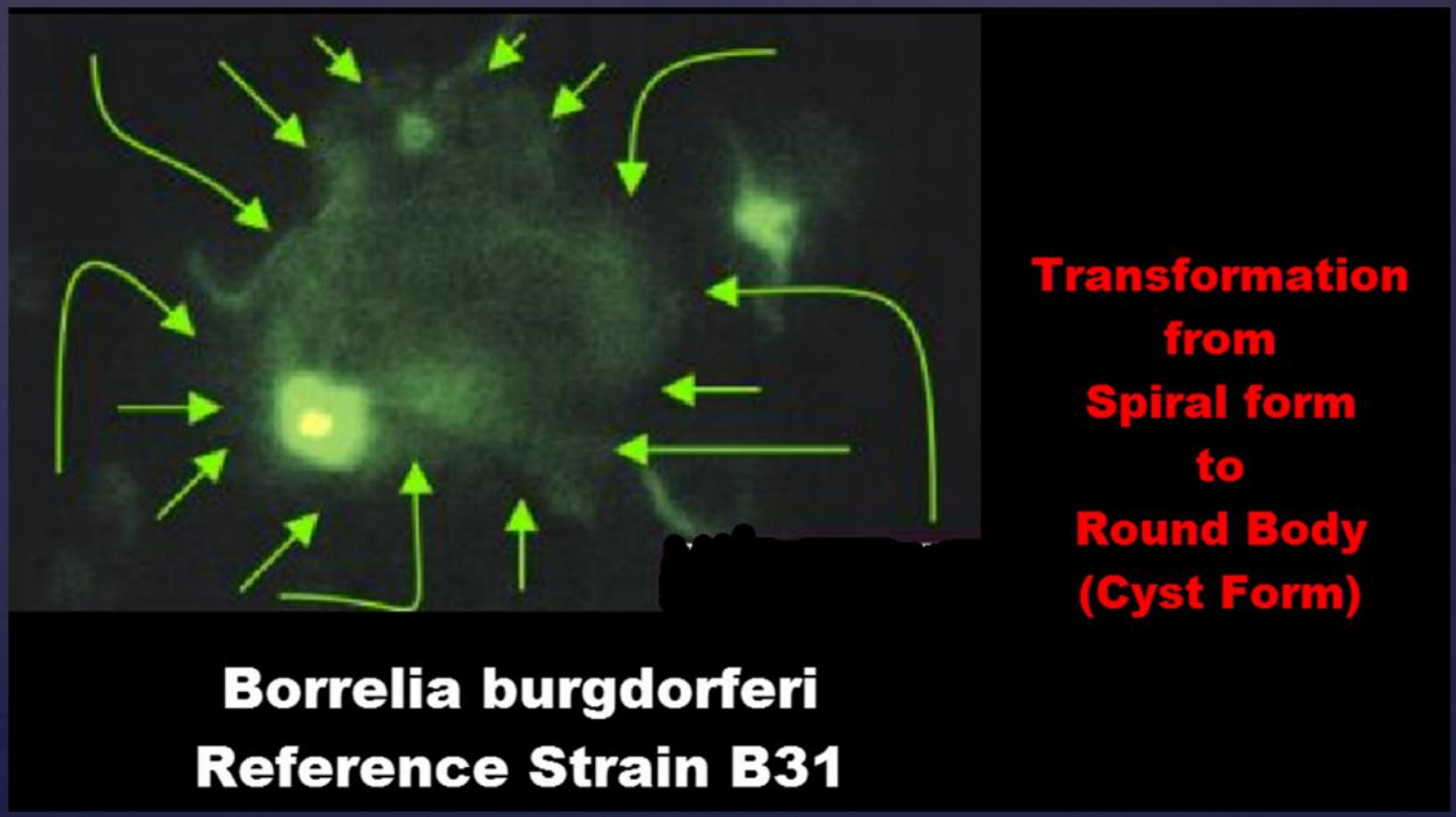
Borrelia burgdorferi strain B31
Darkfield microscopy image
1000x magnification

Transition between Spiral (Vegetative) and Cystic (Round) forms

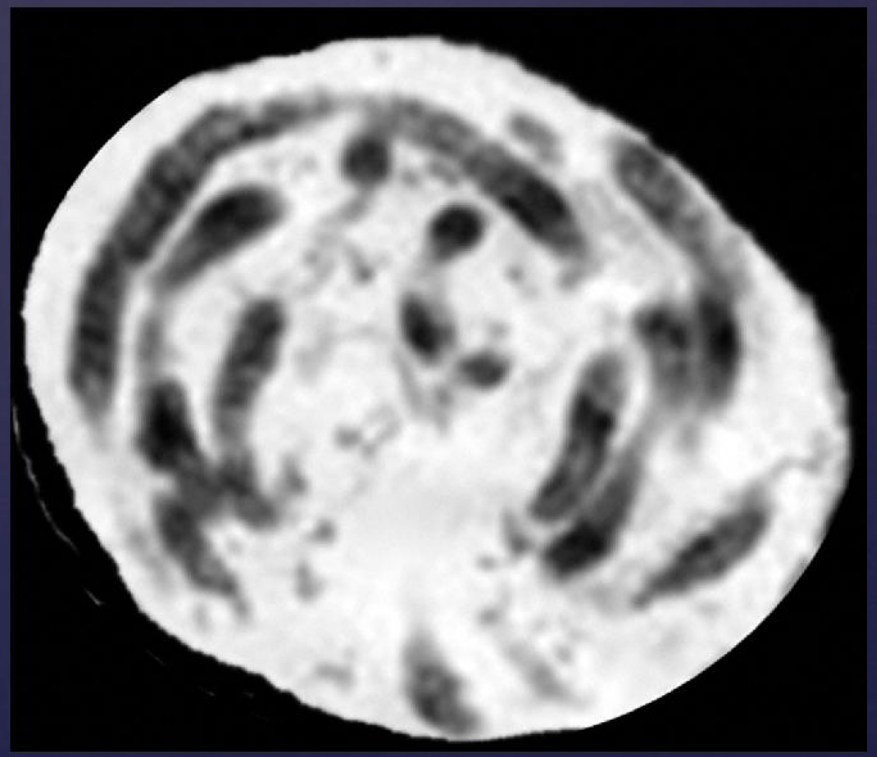


# Transition forms on the the road to Round body Cystic forms

# Intermediate form on the way to Round (Cystic)



# Completion of the Transformation
# All Round (Cystic)
# All the time



# A Peek Inside the Borrelia Round form (Cystic form)

## ATOMIC FORCE microscopy

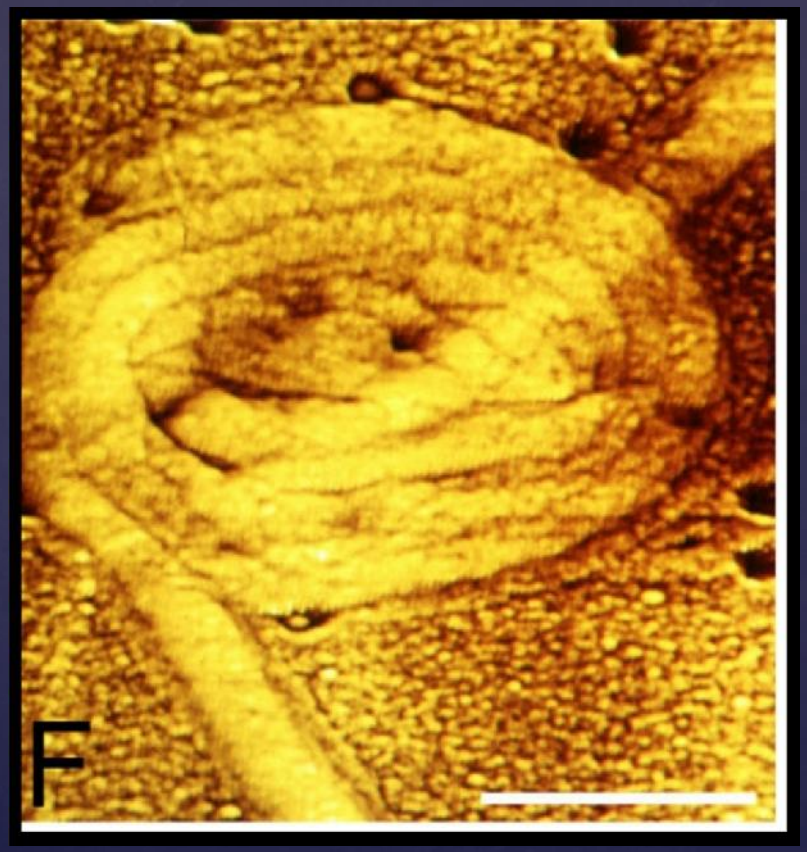



Image Credit - Judith Miklossy MD PhD

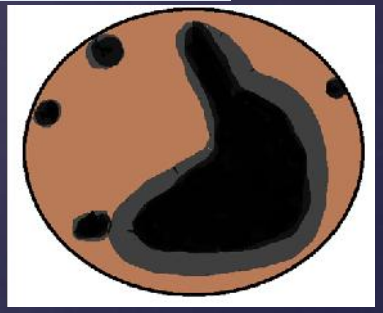
SHORT TERM serum starvation

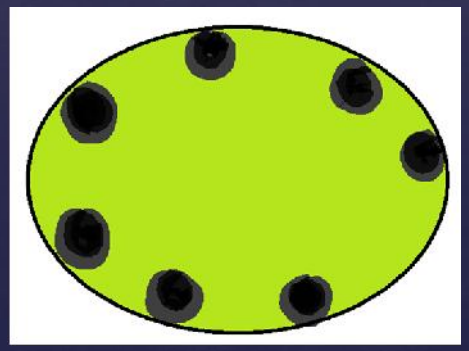
- "Rolled-Up Types"

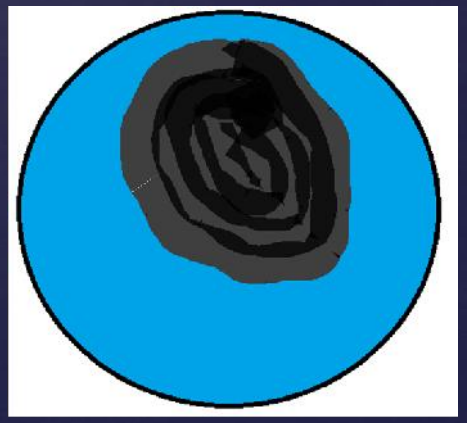
LONG TERM serum starvation

- "Nucleoid /Granular Types"

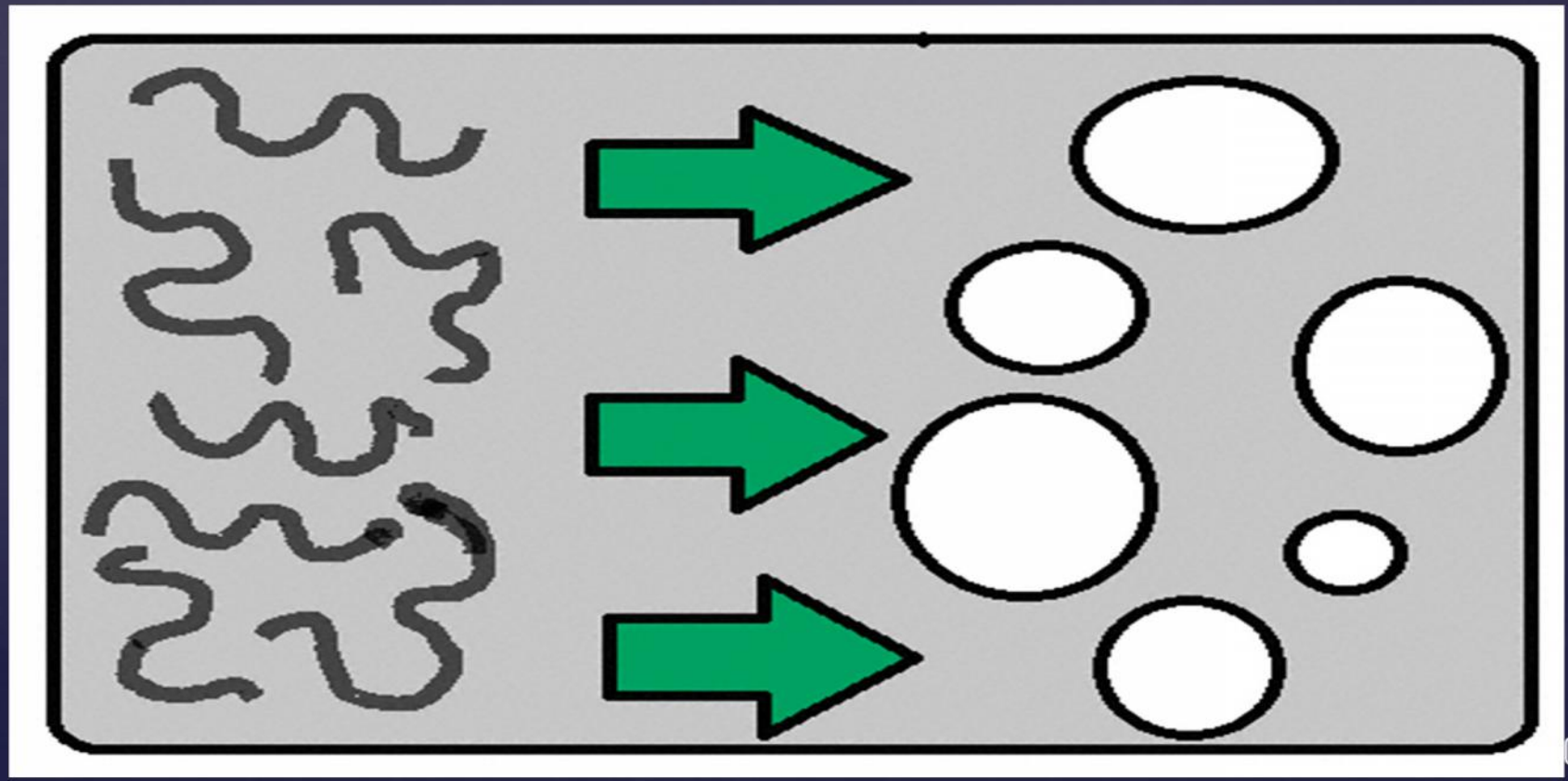
**Round bodies come in different varieties**



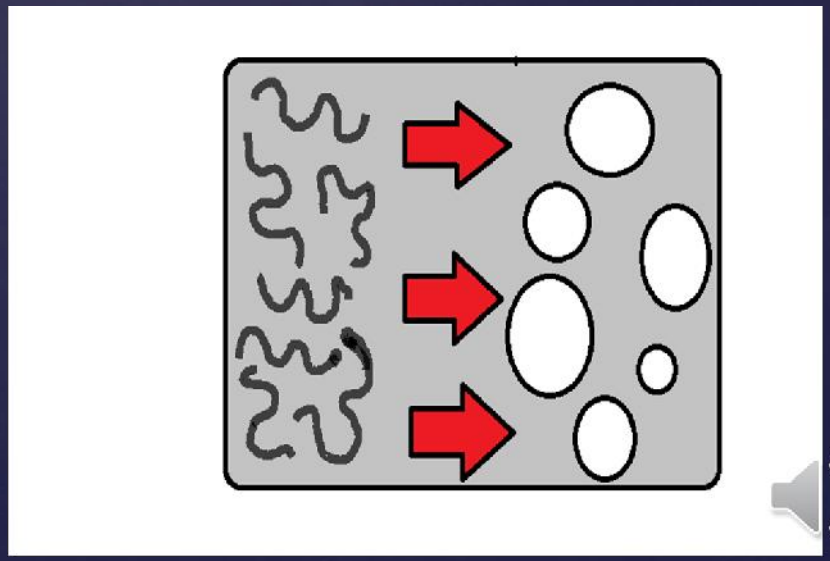


# A Visual Introduction
# Spiral versus Rounded
# Borrelia burgdorferi



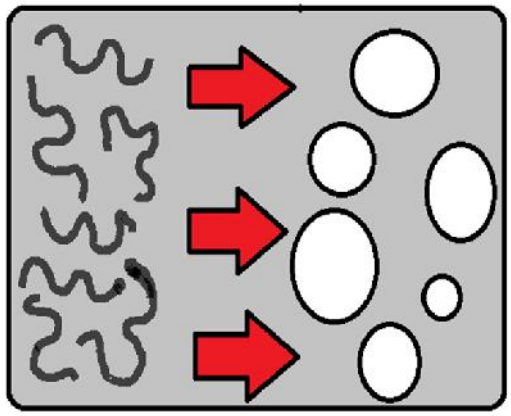
# **Spiral** Borrelia to **Rounded** Borrelia *Interconversions*

- In the **laboratory** – YES
- In the **Ixodid Tick** vector's gut -YES
- In **Human Cerebrospinal fluid** -YES
- In **Human Brain tissue** – YES
- In **laboratory animals** - YES



# Spiral Borrelia burgdorferi lose their corkscrew profile - Laboratory models

*Round Bodies are generated in laboratory conditions*
by placing the spiral forms
**into various liquid media which do not contain serum.**



Spiral Borrelia burgdorferi lose their spiral profile ….

**Alternate Pathways to the State of Round**

- **Aging of the Cultures**
- **Exposure to Antibiotics**
- **Tissue culture medium (RPMI)**
- **Spinal Fluid**
- **Distilled water**
- **Hydrogen peroxide**
- **Changes in pH, Metabolites**

Pupa as the equivalent of a Round Body / Cystic Form

# Shift Happens

# Are Rounded Borrelia Robust or Fragile?

- *Laboratory Observations –*

*Not all laboratories agree …..*
*[ 2 Labs have published In Vitro studies ]*

Durability of Round forms of Spirochetes in the Laboratory provides clues to
the Durability of Round forms of spirochetes in the bodies of living Hosts

( for instance .. Insect Vectors (tick) and mammalian hosts)

# 3 Dimension Model

for the Transformation
Spiral to Round Body
(Cystic form)

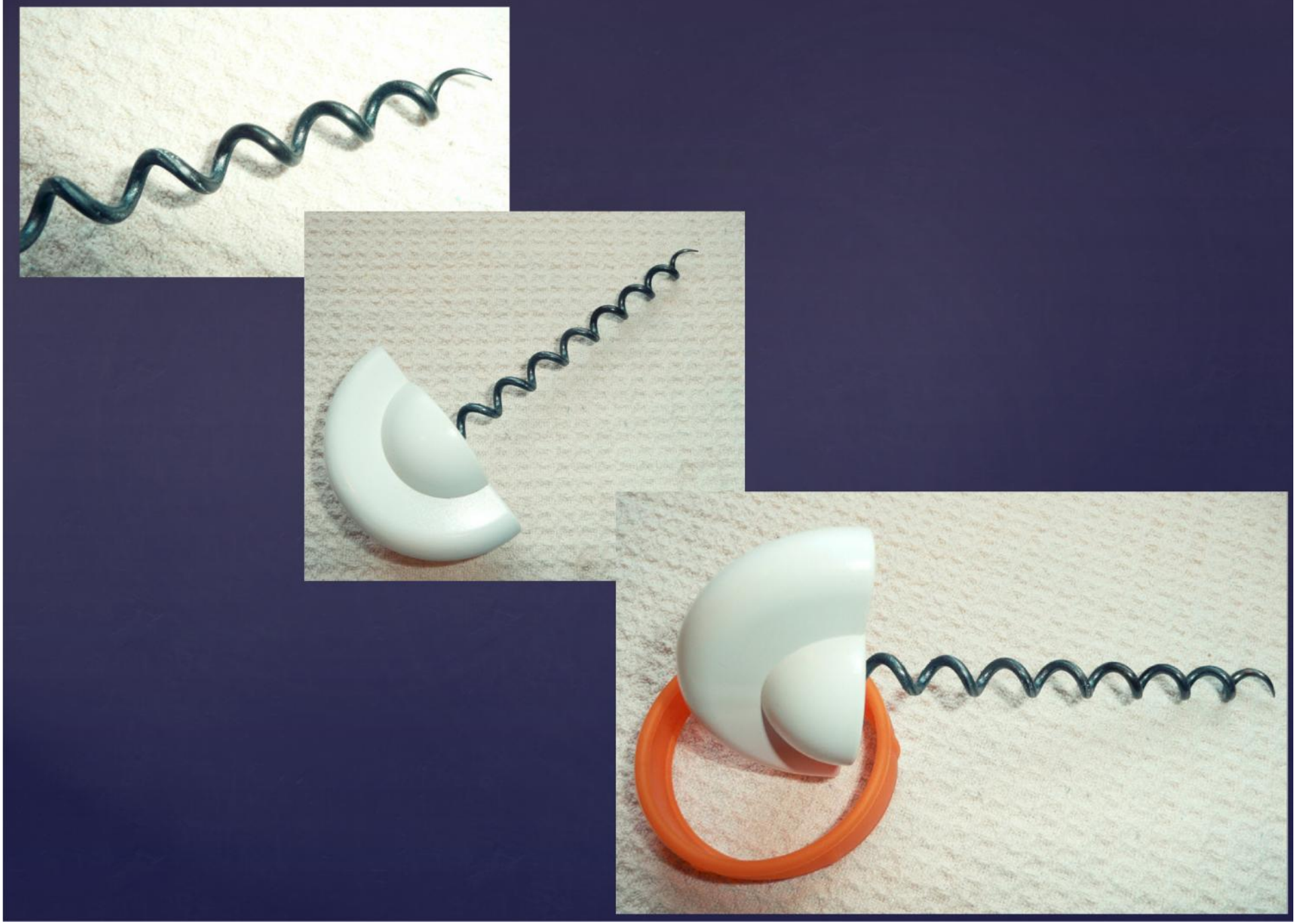
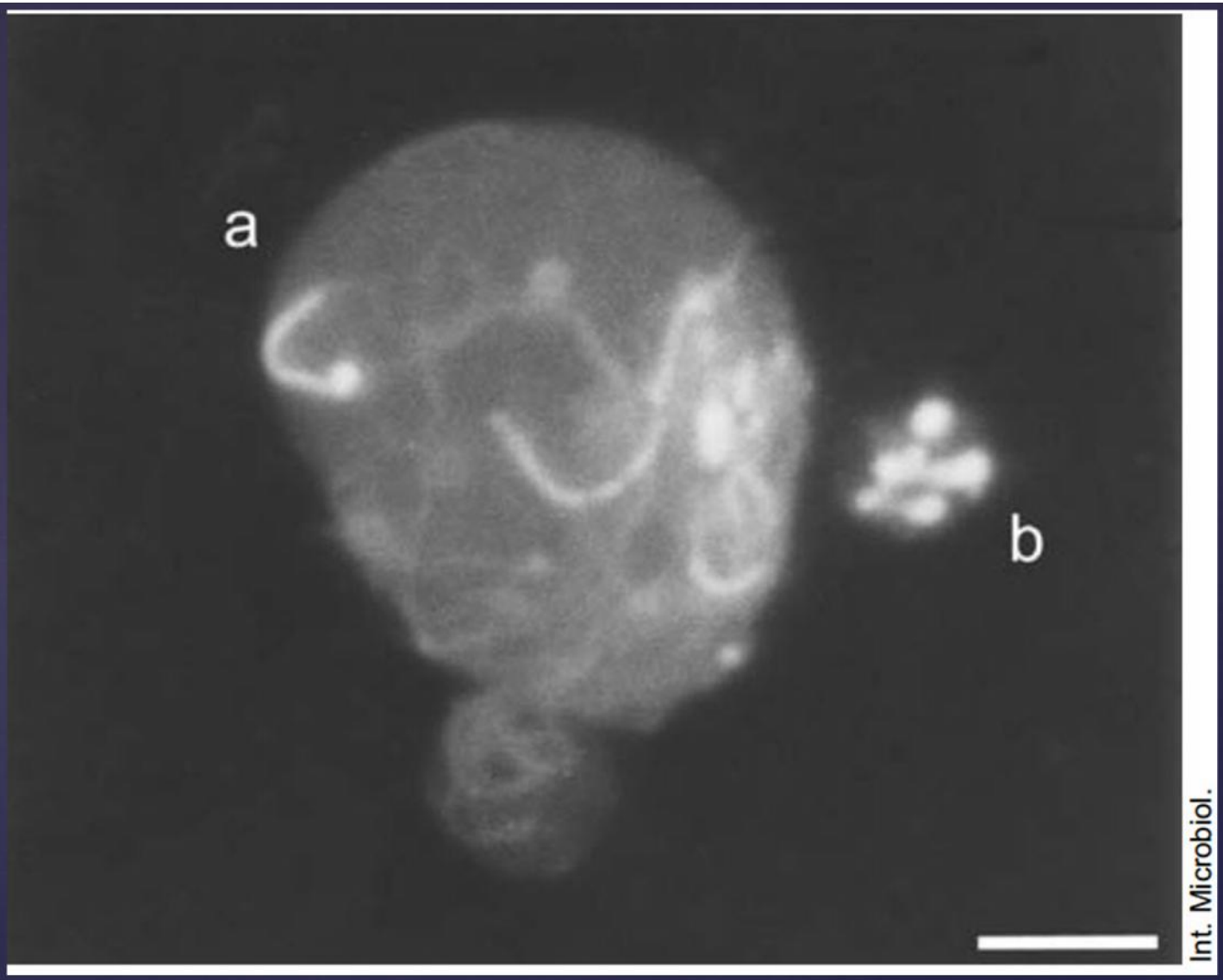


**Photo Credit:**

**Oystein Brorson MD**




Cystic Borrelia burgdorferi (2) with
Internal content of Spirochetal forms [Large "a"}
and Smaller Cystic form with rounded granular bodies.

# Round Body Researchers points of Agreement

# Basic biology of Round Bodies (Cystic Forms) of Borrelia Burgdorferi

# Round Body Manifesto

1. Spiral may become Round, …but the DNA is Constant

2. Round Bodies are Living forms, and undergo Shape changes.

3. Round Bodies are not Motile

4. Round bodies are contained within an
   **Envelope which contains Cell Wall Material**.
   Cell wall in the Envelope of Round bodies removes them
   from the category of Cell Wall deficient forms [L forms or
   Spheroplasts]

5. Electron Microscopy and Atomic Force
   microscopy demonstrate that diverse
   interior structures may be present
   inside Round bodies.

[ Continued – Next page – Points 6 and 7 ]

# Round Body Manifesto

6. **Flagellae are present Inside of the Cell Envelope (Cell wall)** of Round Bodies.

When Spiral forms Emerge from Round bodes, Flagella are immediately identifiable.

Note: Deep versus Superficial positions of Flagellae

Electron Micrographs of **Spiral Bb – Flagellae EXTERNAL to the Cell Wall**

Electron Micrographs **of Cystic (round body) Bb- Flagellae INTERIOR to Cell wall layer of Cyst Envelope**

7. Protein Metabolism of Round Bodies differs from the Protein constituents of spiral forms

# Unique Contributions from Each of the established Round Body Researchers

Drs. Brorson ( Oystein and Sverre Henning) Unique Research findings concerning Round Body biology

# Brorson and Brorson Unique Research Contributions to Round body Cystic form Biology

1. **Round bodies are capable of cell division, independent of a spiral form (Brorson)**
2. **Human Cerebrospinal Fluid may contain Round Bodies (Brorson)**
3. **Multiple Sclerosis spinal fluid –positive Laboratory cultures for Round Bodies (Brorson)**
4. **Antibiotic sensitivity studies on Round Bodies – Metronidazole, Tinidazole, Telithromycin, Hydroxychloroquine, Bismuth Ranitidine Grapefruit seed extract. (Brorson)**
5. **Flagellae (released from their normal position between the Outer surface membrane and the Cell wall) have free range access to the Cyst Interior.**

# Cell division INSIDE of Borrelia Cysts

- Brorson and Brorson ( 1997,1998)
  - Spiral forms of Borrelia burgdorferi were added to human spinal fluid. Cystic forms were noted to replace spiral forms in one week ( Cysts contain rolled up spirochetes)
  - Transverse fission (division of rolled up spirochetes inside cysts) was noted. (**Several cysts contained more than a single spirochete indicating ongoing spirochete cell division after Cysts were formed)**

# Brorson and Brorson – Cyst forms show cell division


**Cell Division in Cystic Form**

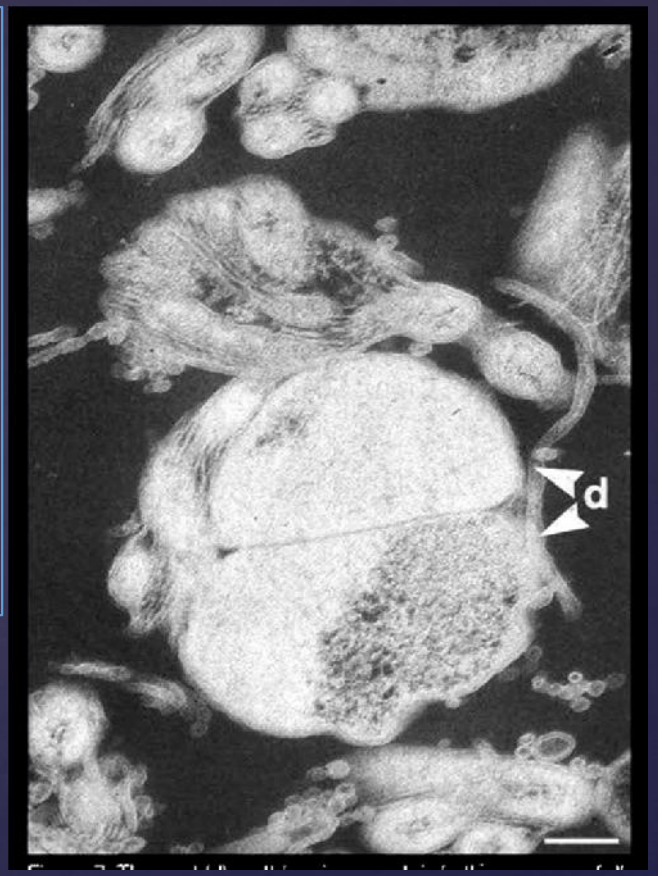
**Photo Credit Dr.Sverre HenningBrorson, Dr. Oystein Brorson**

# Brorson and Brorson -

## Brorson and Brorson:

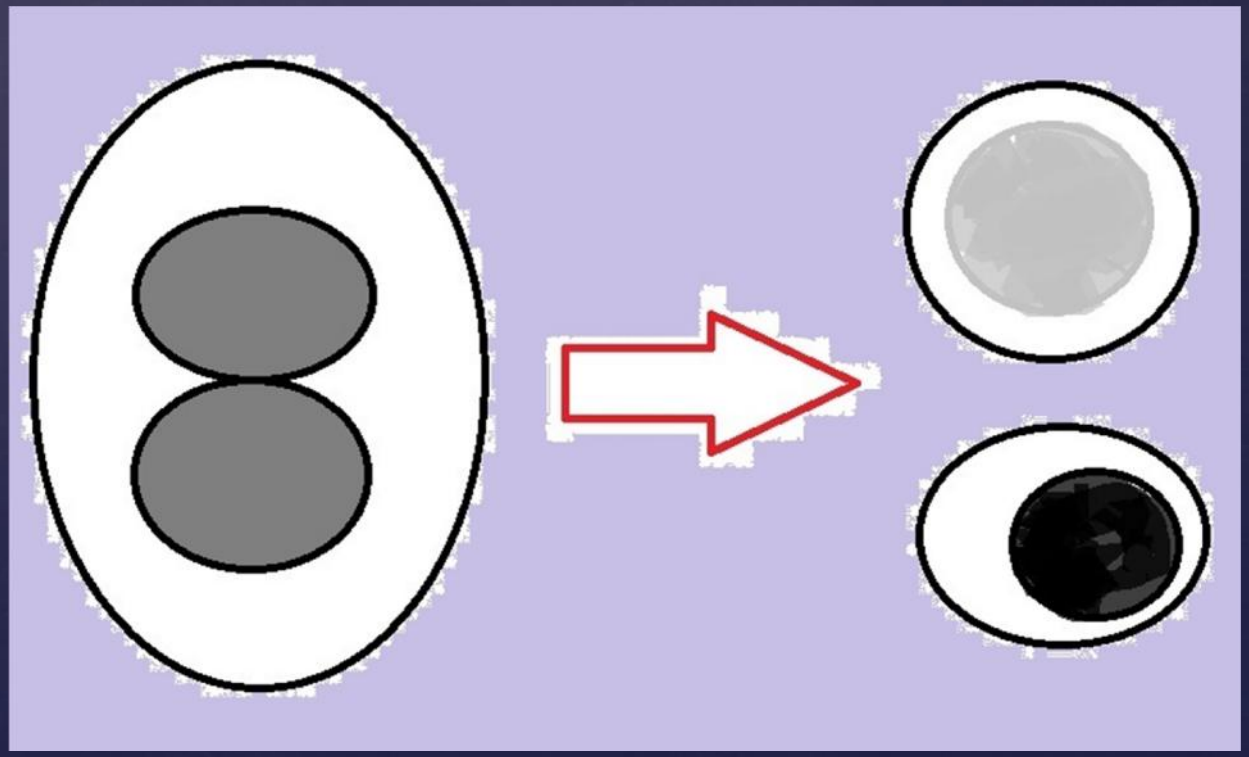
Fission ( cell division) of the actual Borrelia Cyst was identified, Indicating that Cysts have an independent life of their own.

Ref: Brorson and Brorson – 1998 – figure 7.




# Actual Cyst division – Cell division

# Consequences of Cell Division in Borrelia Cysts

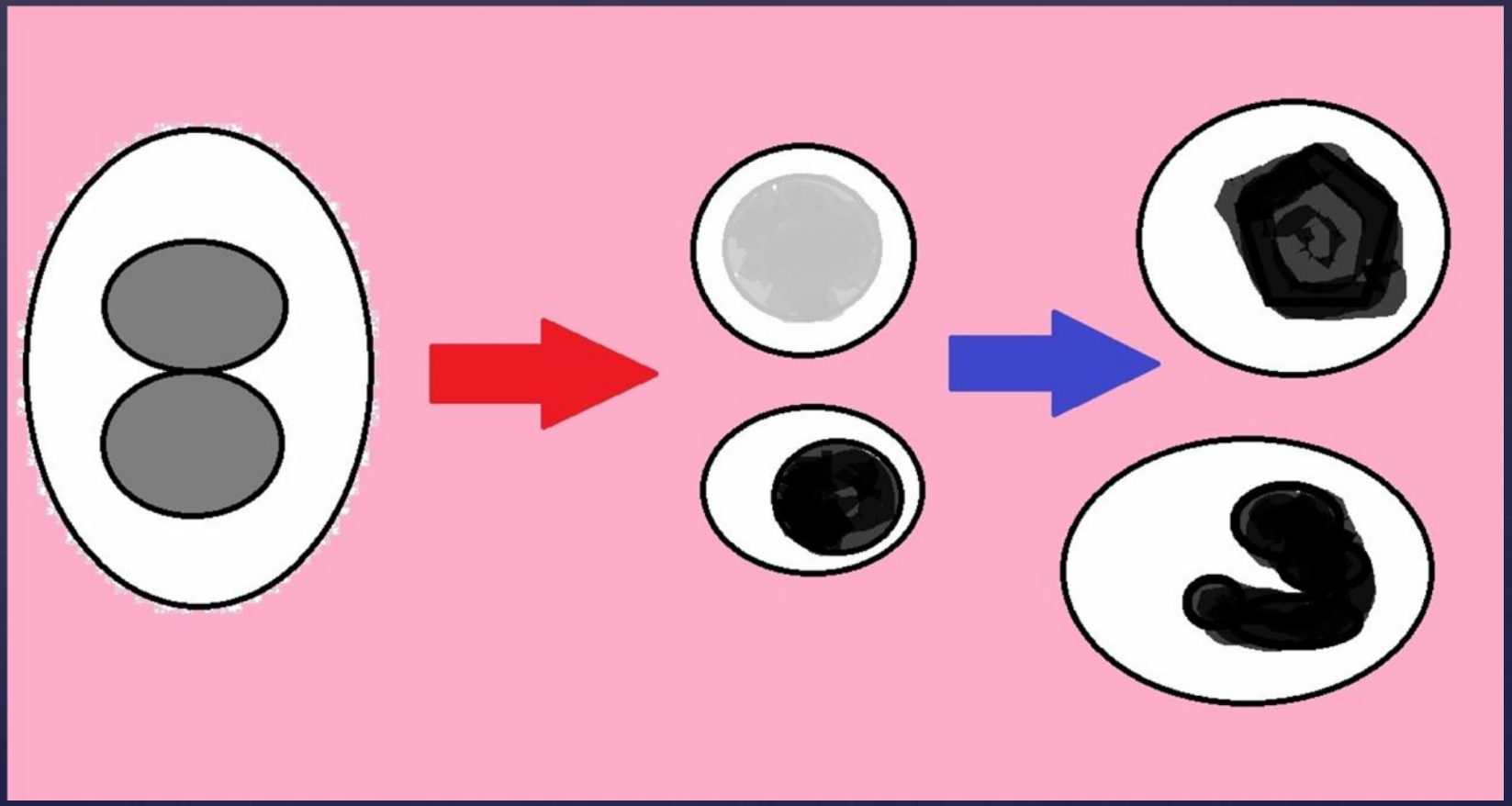


# Brorson and Brorson – Cyst forms show cell division

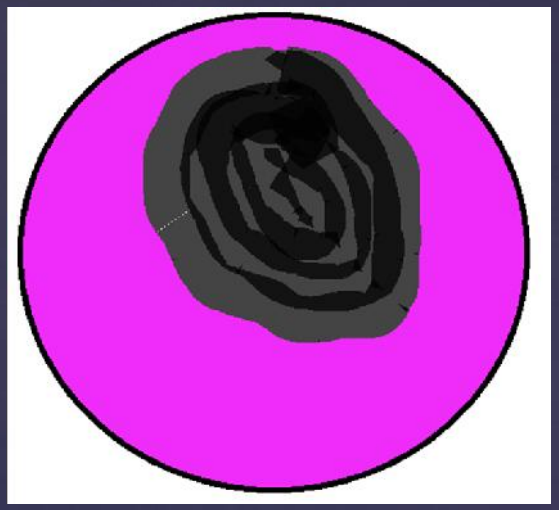

**Cell Division in Cystic Form**

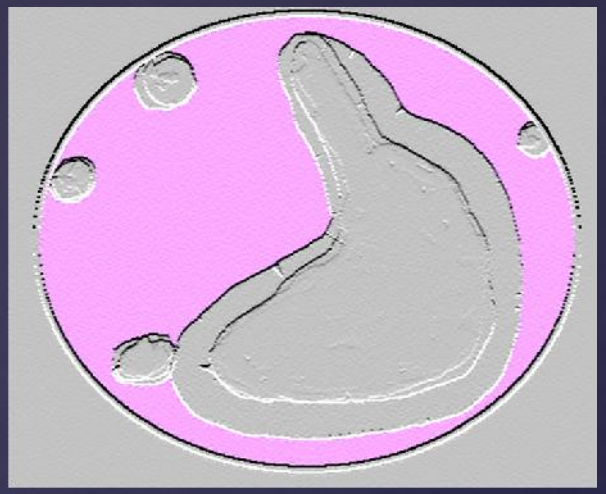
**Photo Credit Dr.Sverre HenningBrorson, Dr. Oystein Brorson**

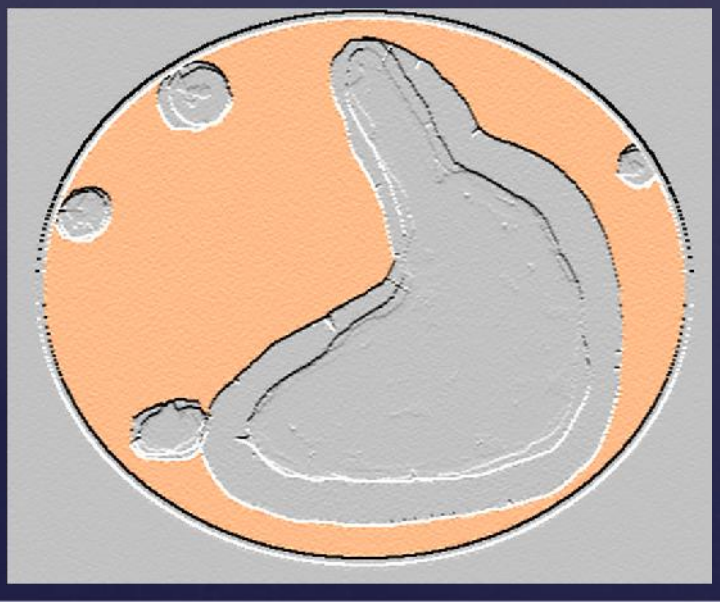
# Consequences of Cell Division in Borrelia  Cysts

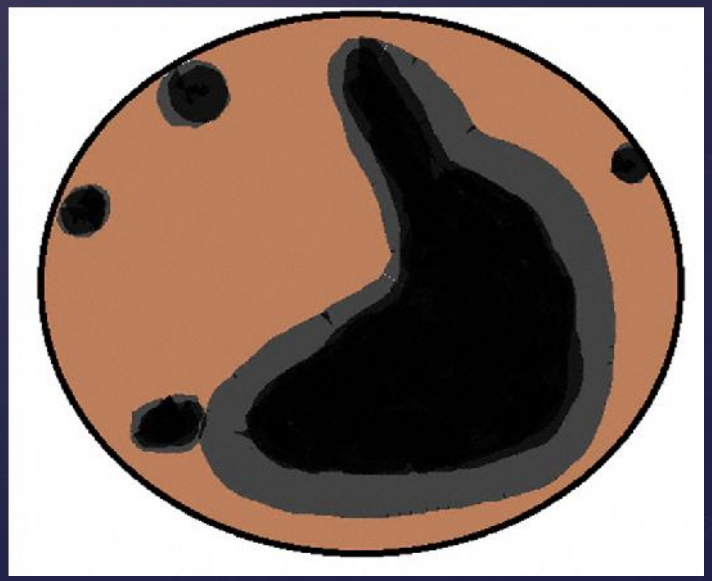


# Consequences of Cell Division in Borrelia Cysts



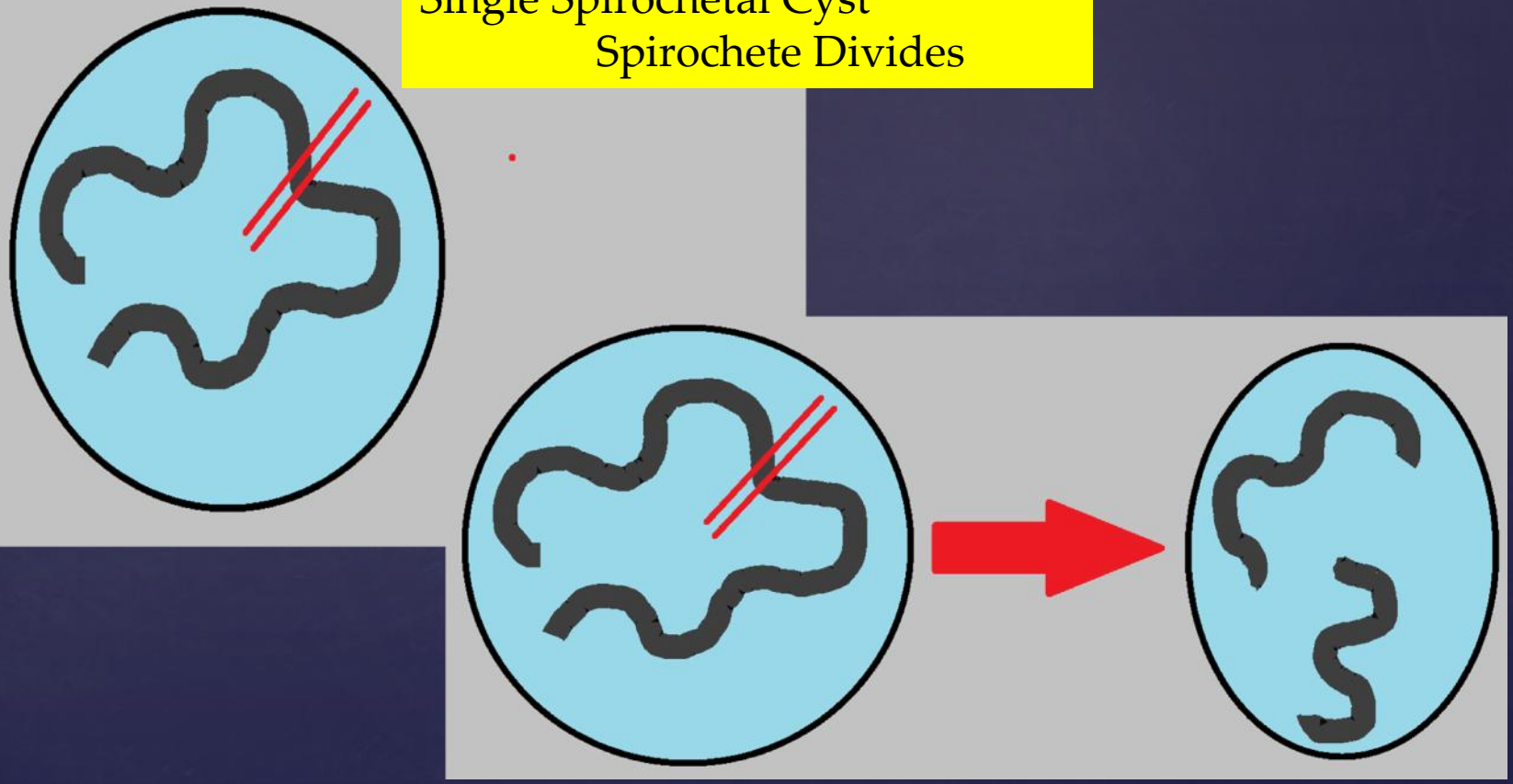






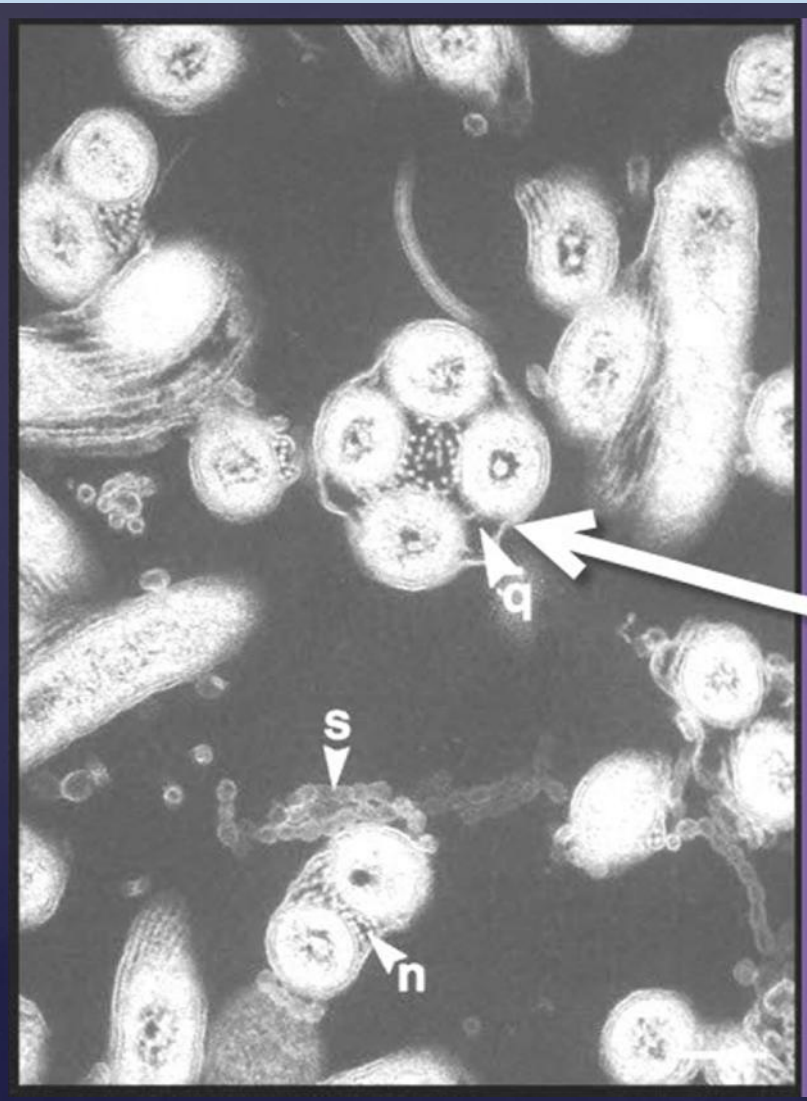
# Consequences of Cell Division in Borrelia Cysts

Single Spirochetal Cyst
Spirochete Divides



Multiple spirochetes within a Cyst = ***Multispirochetal Cyst***

# Brorson and Brorson – Cell division INSIDE of Cystic borrelia

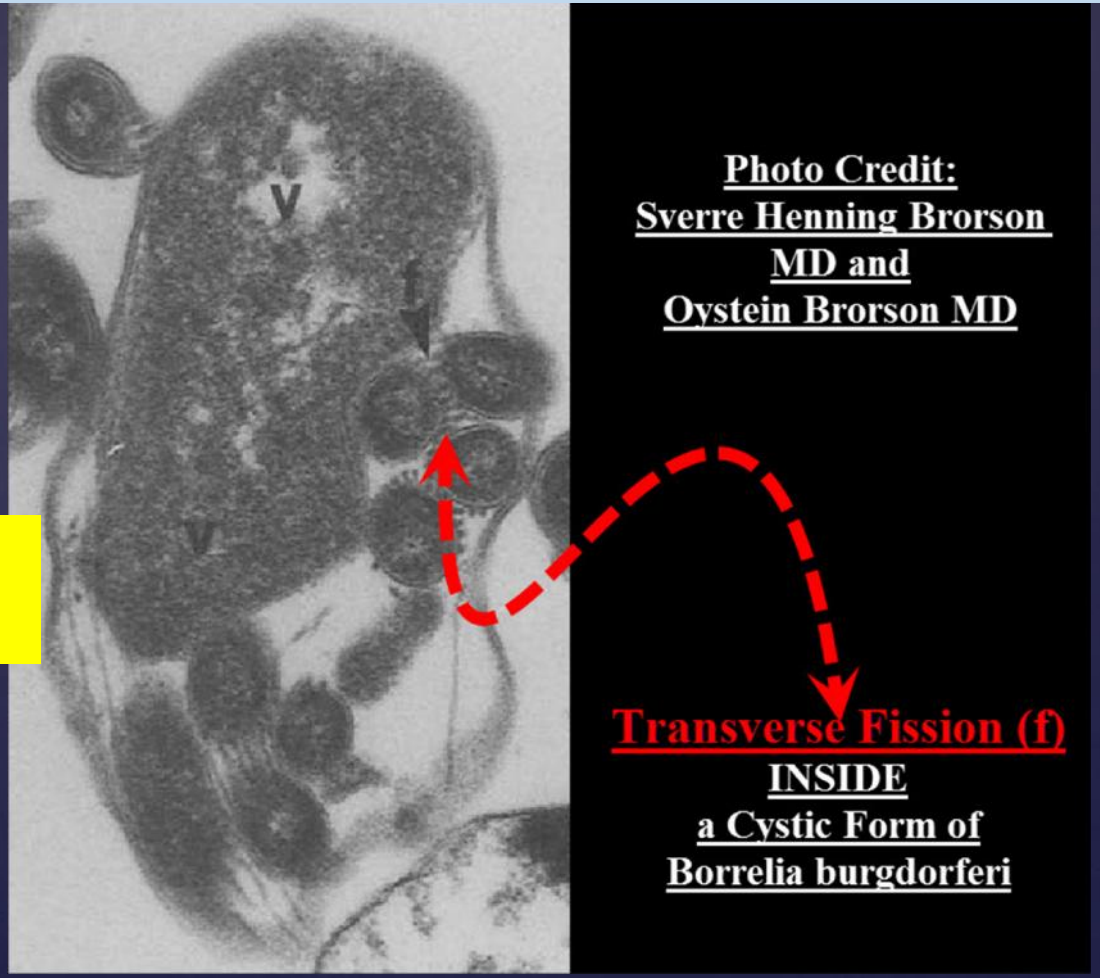



**Cell Divison in Cystic Borrelia**

Multispirochetal Cyst

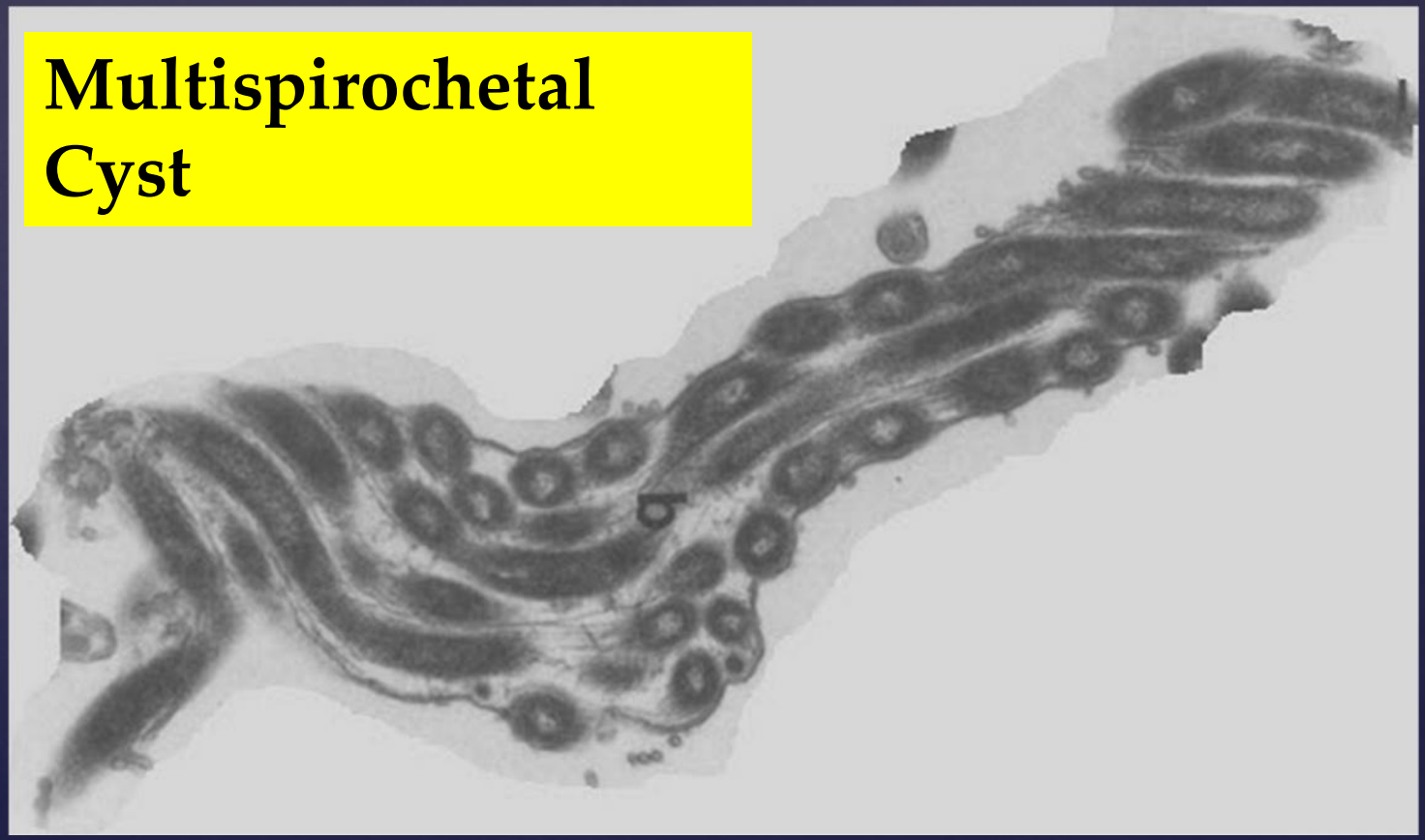
**Photo Credit:**
**Dr Sverre Henning Brorson,**
**Dr. Oystein Brorson**

# Brorson and Brorson Transverse fission occurs inside of the Cyst form

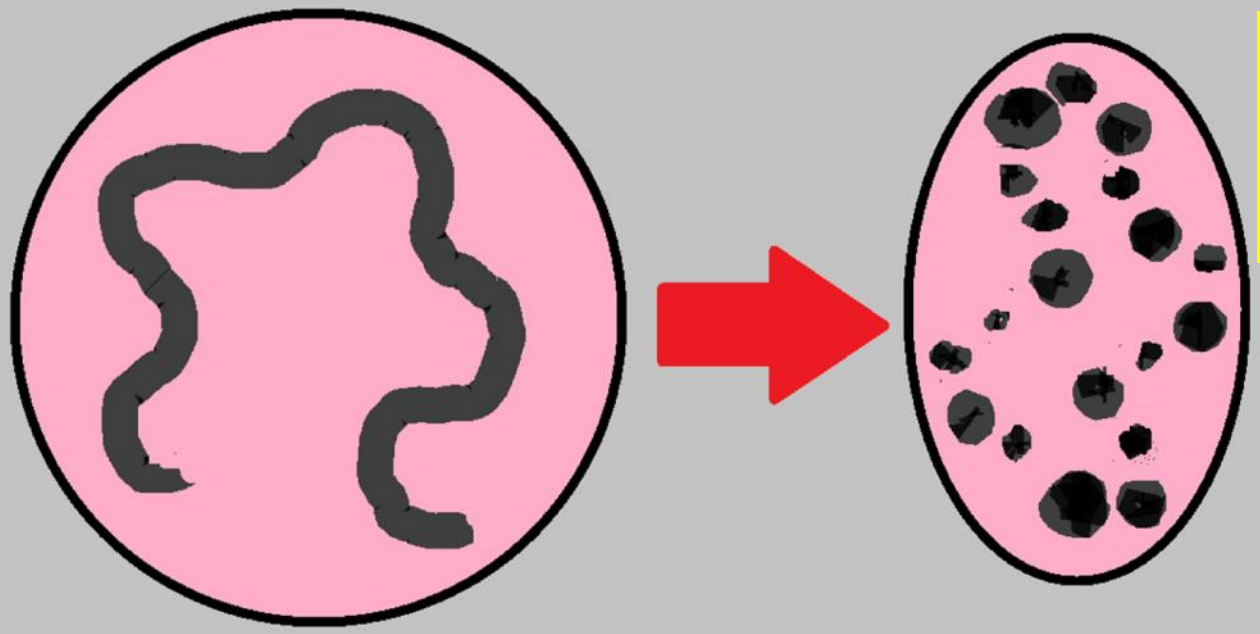



**Multispirochetal Cyst**

# Brorson and Brorson- One Cyst contains 4 spiral forms

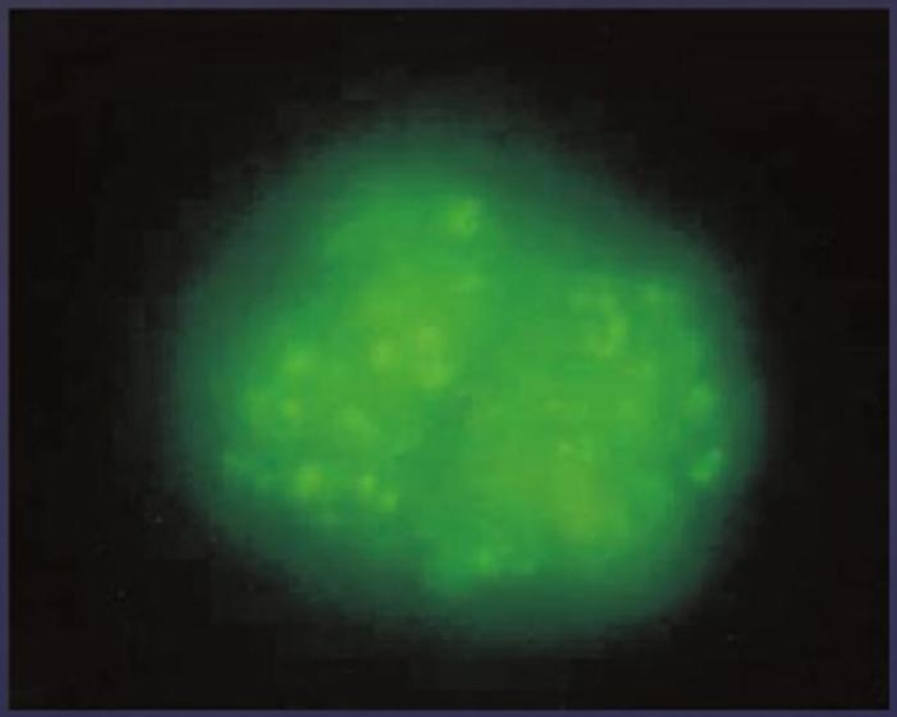


# Consequences of Cell Division in Borrelia Cysts – Granular form Segmentations



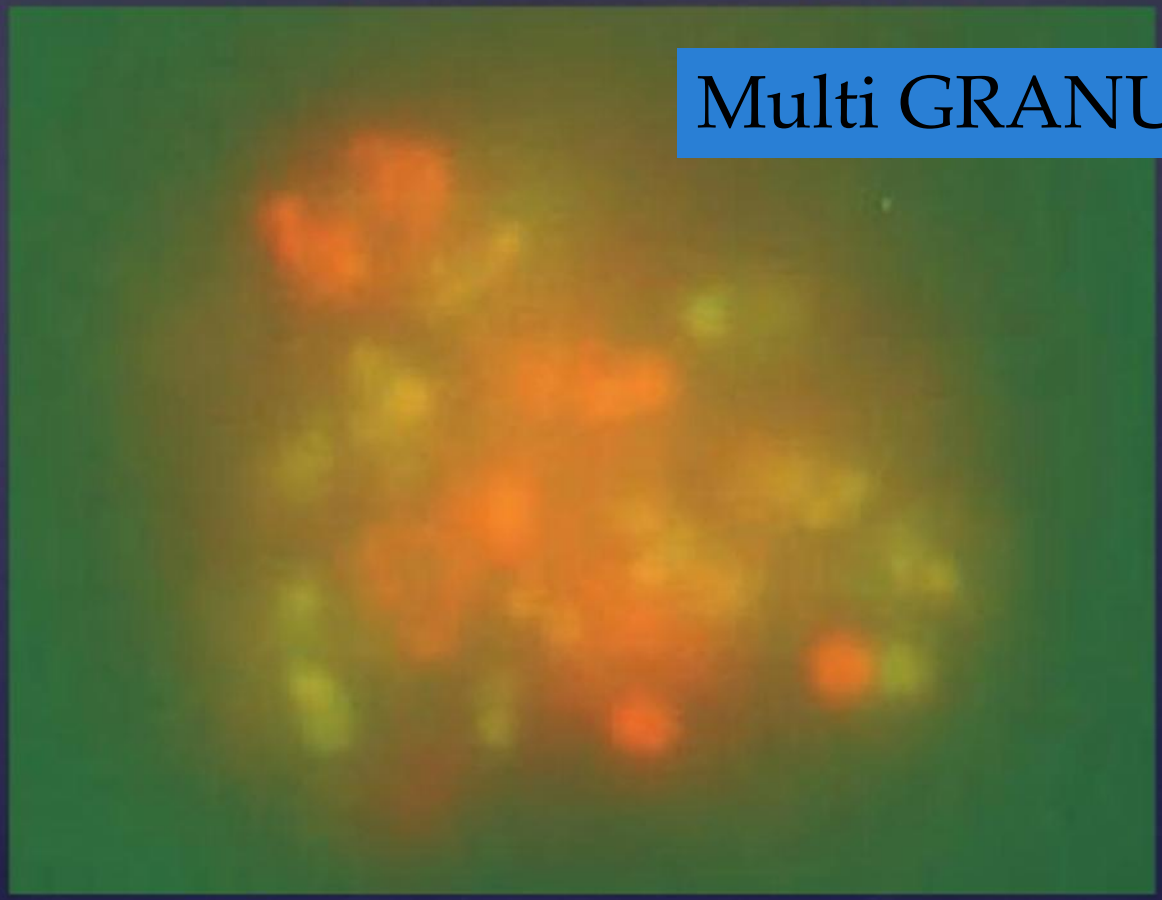
**Multigranular Spirochetal Cyst**

Brorson and Brorson –
Cystic Borrelia with Internal Granules



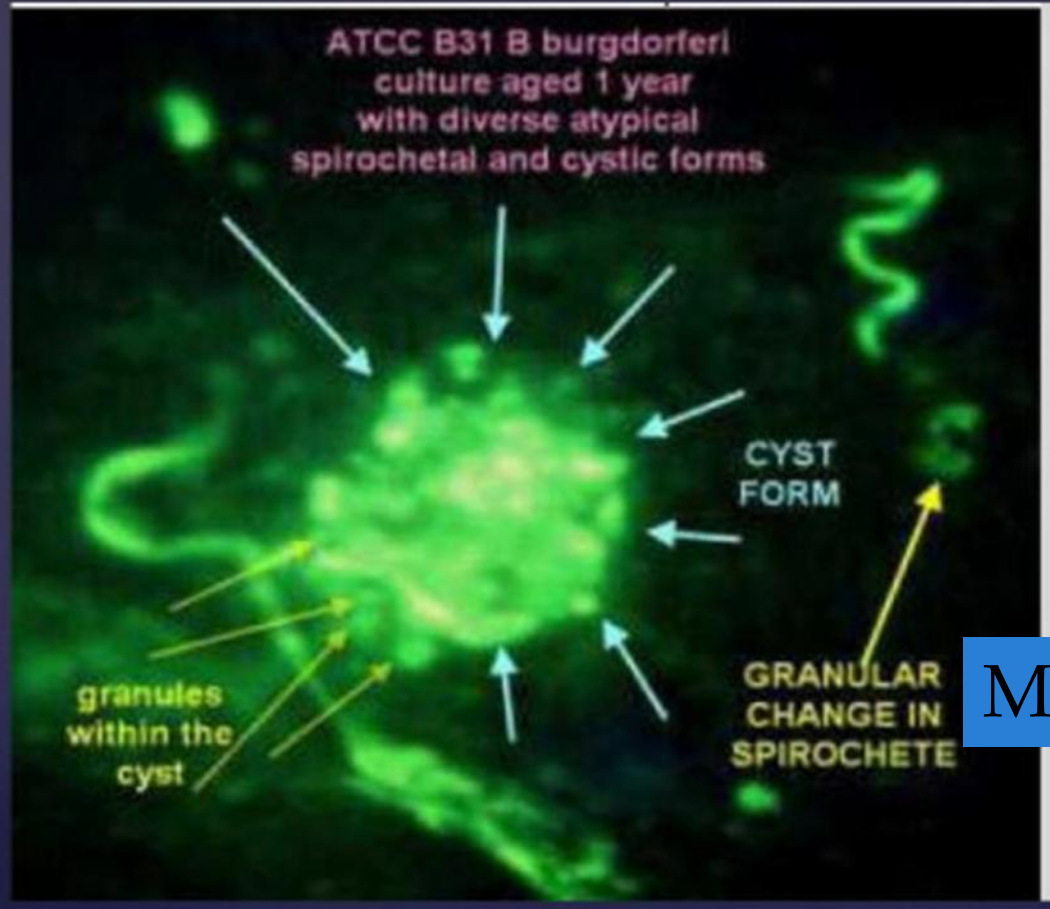
Multi – GRANULAR Cyst

# Brorson and Brorson- Cystic Borrelia with internal granules – treated with antimicrobials



Multi GRANULAR Cyst

# Consequences of Granular elements in Borrelia Cysts




**Cystic Borrelia burgodorferi with Abundant Granular elements**

**Photo Credit: Alan B. MacDonald MD 1988**

Multi –GRANULAR Cyst

# Consequences of Cell Division in Borrelia Cysts

- If you Start with a laboratory adapted
- Spiral form of Borrelia burgdorferi,
- Then *cloning by limiting dilution to a Single*
- *Spirochete* can produce Log phase growth
- In BSK Medium( Alan G. Barbour MD, Rocky Mountain Laboratory NIH, NIAID)

**SPIRAL** FORM

**CYSTIC** FORM

Cystic forms of Borrelia burgdorferi are less demanding

**RPMI ( WITHOUT N acetlyglucosamine) – Works well**

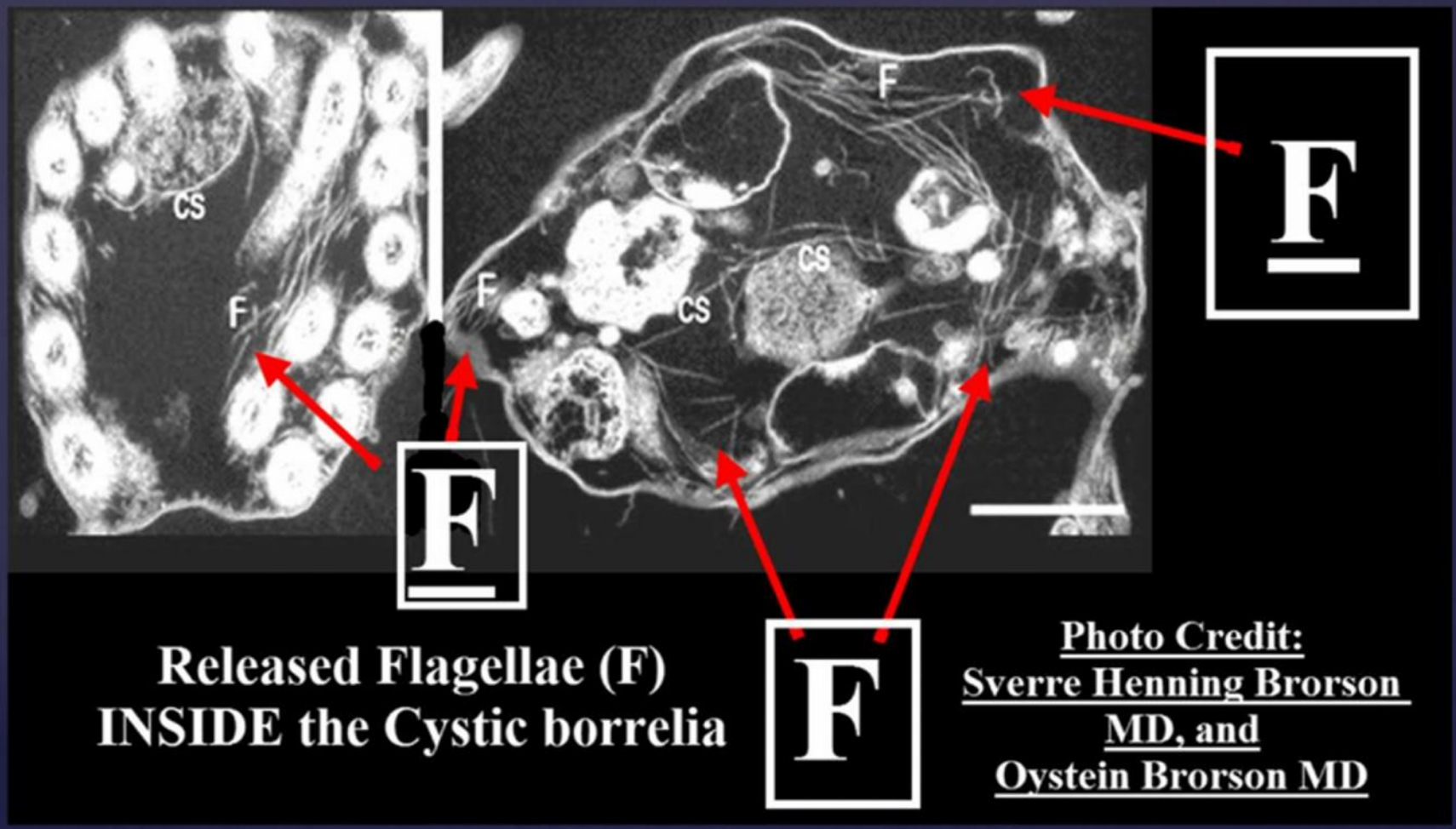
RPMI (50%) with CMRL 1066(50%) - Works well

**MacDonald Editorial comment: Perhaps the focus for culture of Borrelia burgdorferi should focus on growing Cysts!!**

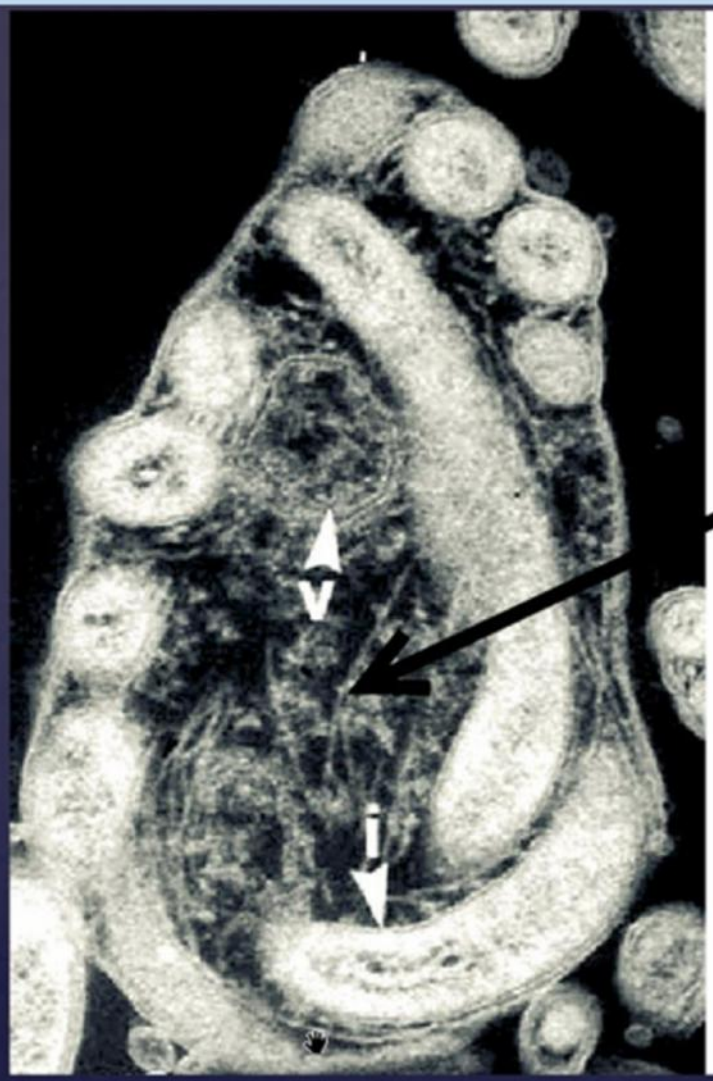
# Consequences of Cell Division in Borrelia Cysts

- **Biological Multiplication = Amplification**
- **of**
- **Pathogen**
- **Number**

# Flagellae inside of Borrelia Cysts – Brorson and Brorson



# Flagellae Inside of Borrelia Cysts – Brorson and Brorson



**Flagellae liberated from Spiral Borrelia cylinders INSIDE a Cystic Borrelia**

**Photo Credit: Sverre Henning Brorson MD and Oystein Brorson MD**

# Borrelia Cysts Containing Liberated Flagellin Units

Alzheimer's Disease – Cystic Borrelia – Reactive with Murine Monoclonal Antibody H9724 ( a Gift from Alan G. Barbour,MD

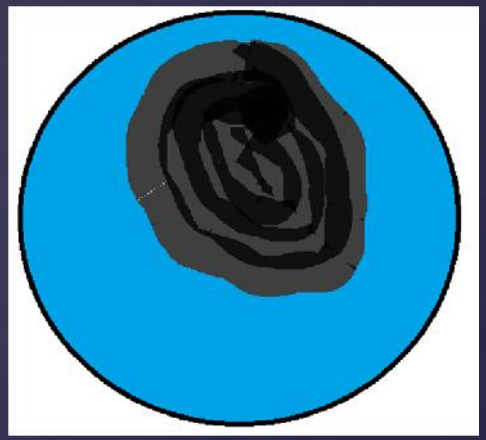
Photo credit : Alan B. MacDonald MD , Photograph date 1987

# Drs. Oystein and Sverre Henning Brorsons' observations on Round Body Biology

- **(In Vitro)** 
- [**Rolled up spirals** visible in cyst]
- **The Round bodies may take 1 week to form in spinal fluid**
- ***"young"*** *Round body"* regenerate spiral forms with 1-2 weeks

- ***"aged"*** ***Round Body*** regenerate spiral forms
- but the regeneration may require longer incubations
- (after 6 weeks)
- [**No longer are rolled up spiral forms visible – but dense nucleoids visible**]

- Dr. O. Brorson and Dr S.H Brorson have elegantly demonstrated cystic forms of Borrelia burgdorferi from cultures of human patients. Ultrastructural studies have evaluated a population of Borrelia cysts which contain densely staining **nucleoid-like** contents. Unlike the Borrelia cysts demonstrated by Drs. Alban and Nelson which contain rolled up spirochetal profiles, and which rapidly revert to spiral motile forms…



# Cystic Borrelia burgdorferi cultured in RPMI from spinal fluid - comments

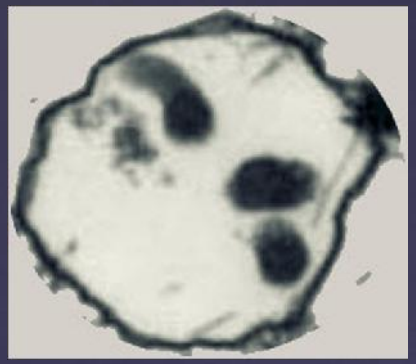
# Images of AGED Cystic Borrelia burgdorferi grown from cerebrospinal fluid
# Dr. Oystein Brorson and S. H. Brorson



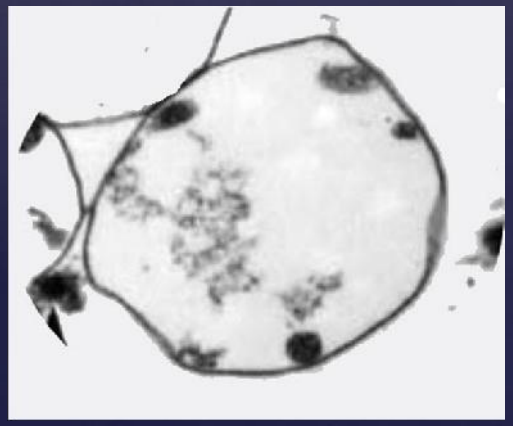

These Images show a dense nucleus-like structure and a surrounding envelope. They closely resemble degenerating human cells

# Side by Side Comparisons
# Images from Dr. O. Brorson Dr S.H Brorson

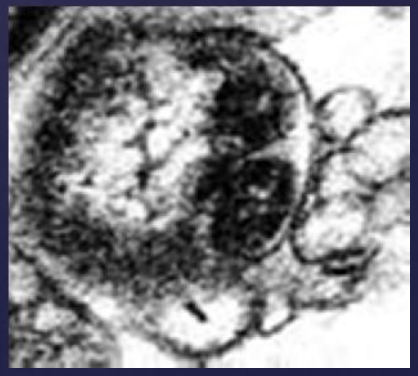
**cysts – recovered from human spinal fluid**




**Young** Cysts –
**small Cylinders inside an Envelope**

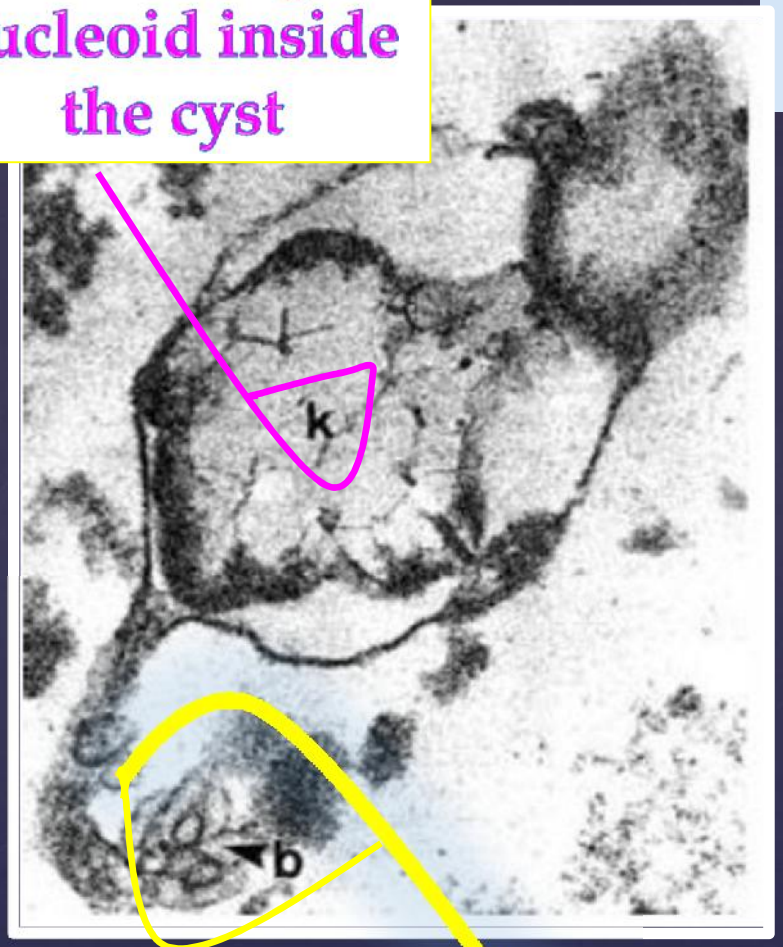


**Aged** Cysts - Large Densely staining Dark Nucleoid- No small cylinders left



# Brorson and Brorson Large Aged Round form of Borrelia Burgdorferi

K is a large nucleoid inside the cyst

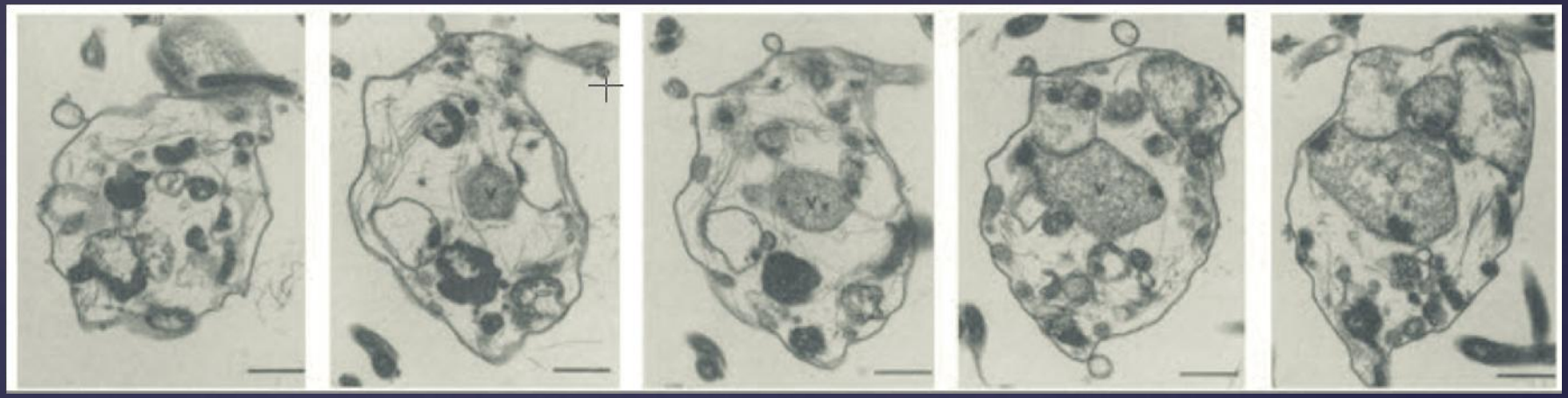



Vesicles (Blebs) are shed from the outer surface membrane of spiral forms of Borrelia burgdorferi. Vesicles contain portions of the organism's DNA. It is noteworthy that Cystic forms of Borrelia burgdorferi also shed Vesicles. Human cells do not shed Vesicles.

Note: Vesicles ("b" small arrow) at 7 o'clock position(Borrelia Cyst)— Brorson and Brorson study

# Brorson and Brorson

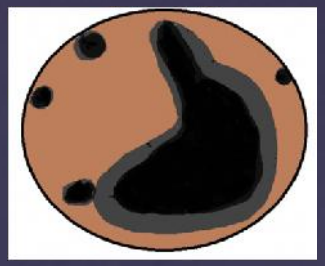
Photo Credit : Sverre Henning Brorson MD, Oystein Brorson MD



## Aging Changes in Cystic Borrelia Burgdorferi

Note the progressive
**Nucleoid** Enlargement
from left panel to right Panel

# Brorson and Brorson -



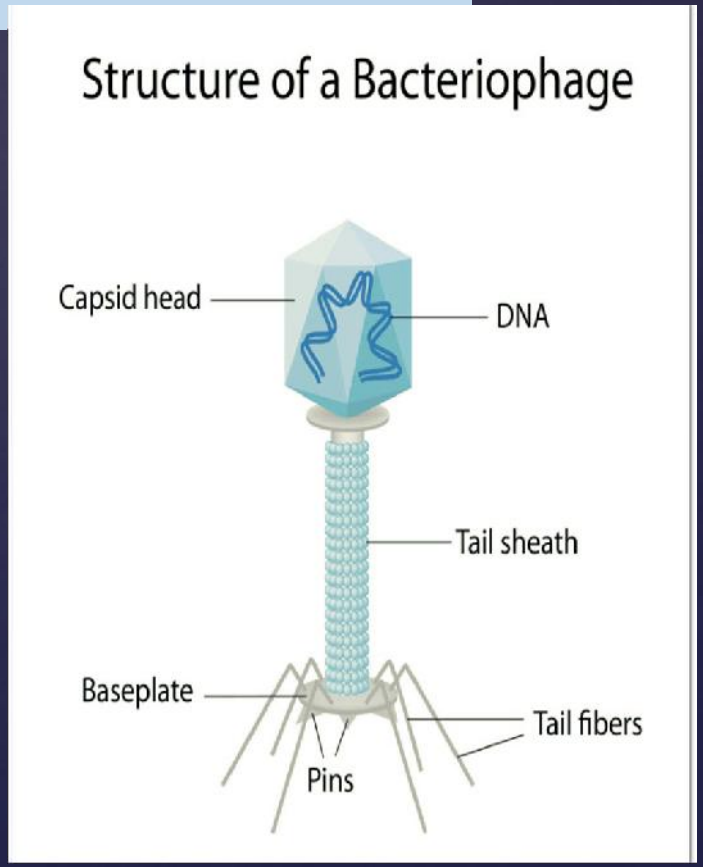
- The Cysts of Drs. Brorson and Brorson which demonstrate dense nucleoid-like content ***do not revert rapidly to vegetative spiral forms when appropriate culture medium (BSK H) is added to them.***
- The pathobiology of the Brorson nucleoid containing cysts has not been elucidated.

# Cystic Borrelia burgdorferi cultured in RPMI from spinal fluid – (continued)

# Willy Burgdorfer PhD, MD (Hon)
# Unique Research Findings Concerning Round bodies

1. Round bodies are named Gemmae

2. Bacteriophages may attack Round Bodies



# Burgdorfer – Unique Research findings Borrelia burgdorferi Bacteriophages and Gemmae



**Lysis of Borrelia burgdorferi Bacteriophages are indicated by Red Arrows**

**Image Credit Barbour and Hayes Micobilolgical Reviews**

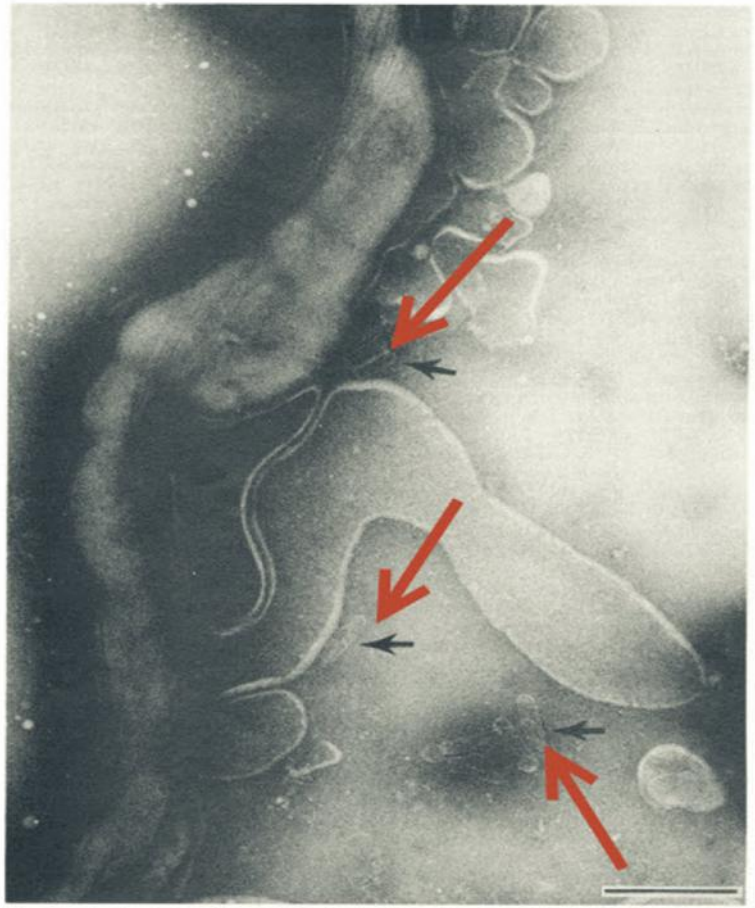


FIG. 6. Electron photmicrograph of a *B. hermsii* cell from a broth culture that was undergoing spontaneous lysis. Evident are numerous bacteriophage heads, some of which are indicated by arrows, and the disruption of the spirochete. The preparation was negatively stained with 2% ammonium molybdate. Bar, 0.2 μm.

# Burgdorfer and Hayes– Unique Research findings Borrelia burgdorferi Gemmae and Bacteriophages

Gemma of Borrelia burgdorferi [Image "a'] showing Internalized Bacteriophages (Phage heads - red arrows)

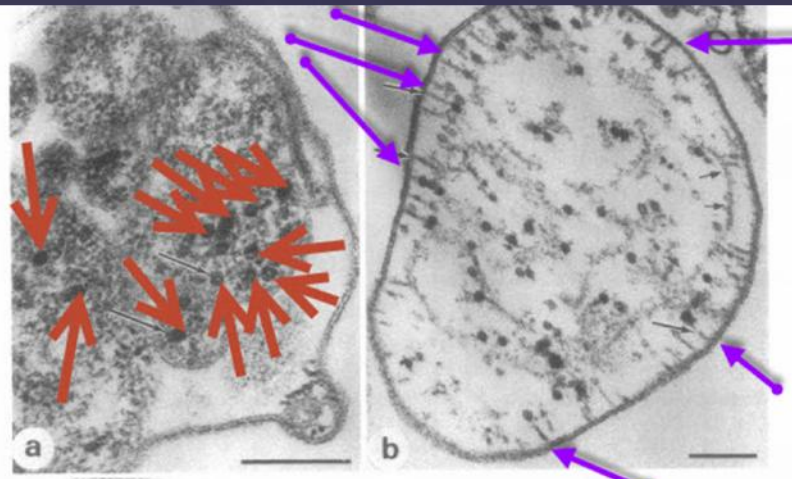


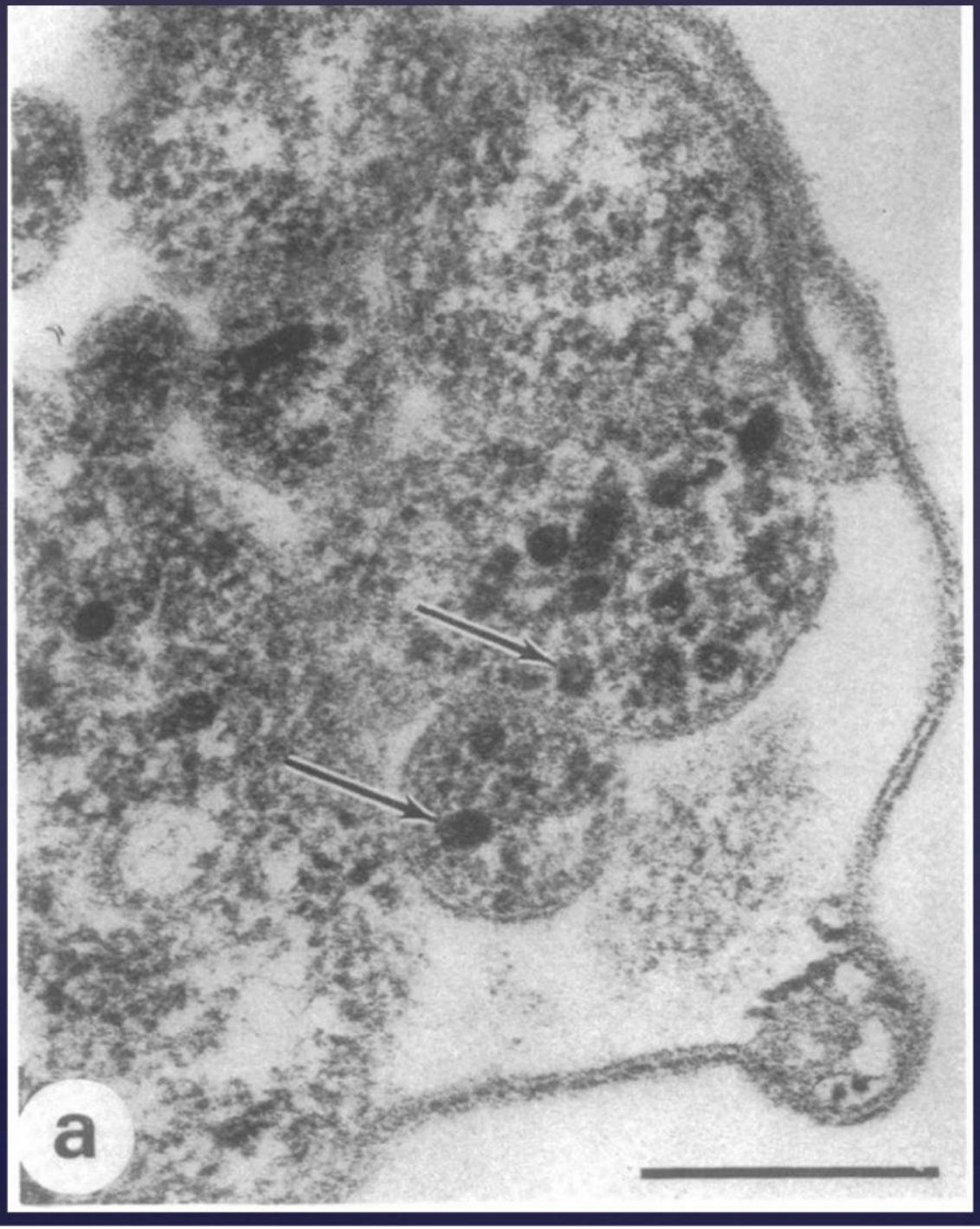

FIG. 2. (a) Section profile of a gemma with its attendant membrane-bound granules or spherical bodies. Arrows denote the cross-section profiles of electron-dense bacteriophage heads. Bar, 0.2 μm. (b) Internal attachment of bacteriophage to outer membrane material after plasmolysis of the spirochete. Arrows indicate remnants of plasma membrane. Bar, 0.2 μm. (c) Phosphomolybdic acid (2%) staining of spirochete (pH 5.0), showing elongated head structures within the spirochete. Bar, 0.2 μm.

determined from the direction of coiling [Fig. 1d, arrows] of the endoflagella [clockwise = right-handed; counterclockwise = left-handed].) Thus far, only those spirochetes showing left-handed coiling have been found to be phage infected.

Remnant of Outer Surface Membrane of B.burgdorferi Showing BACTERIOPHAGES attached- [Purple arrows]

BUT the Phages are atached to the INSIDE of the Osp.

This membrane is turned inside/out




# Gemma

Penetrated by
Bacteriophages

# Hayes and Barbour– Unique Research findings Borrelia burgdorferi Gemmae and Bacteriophages



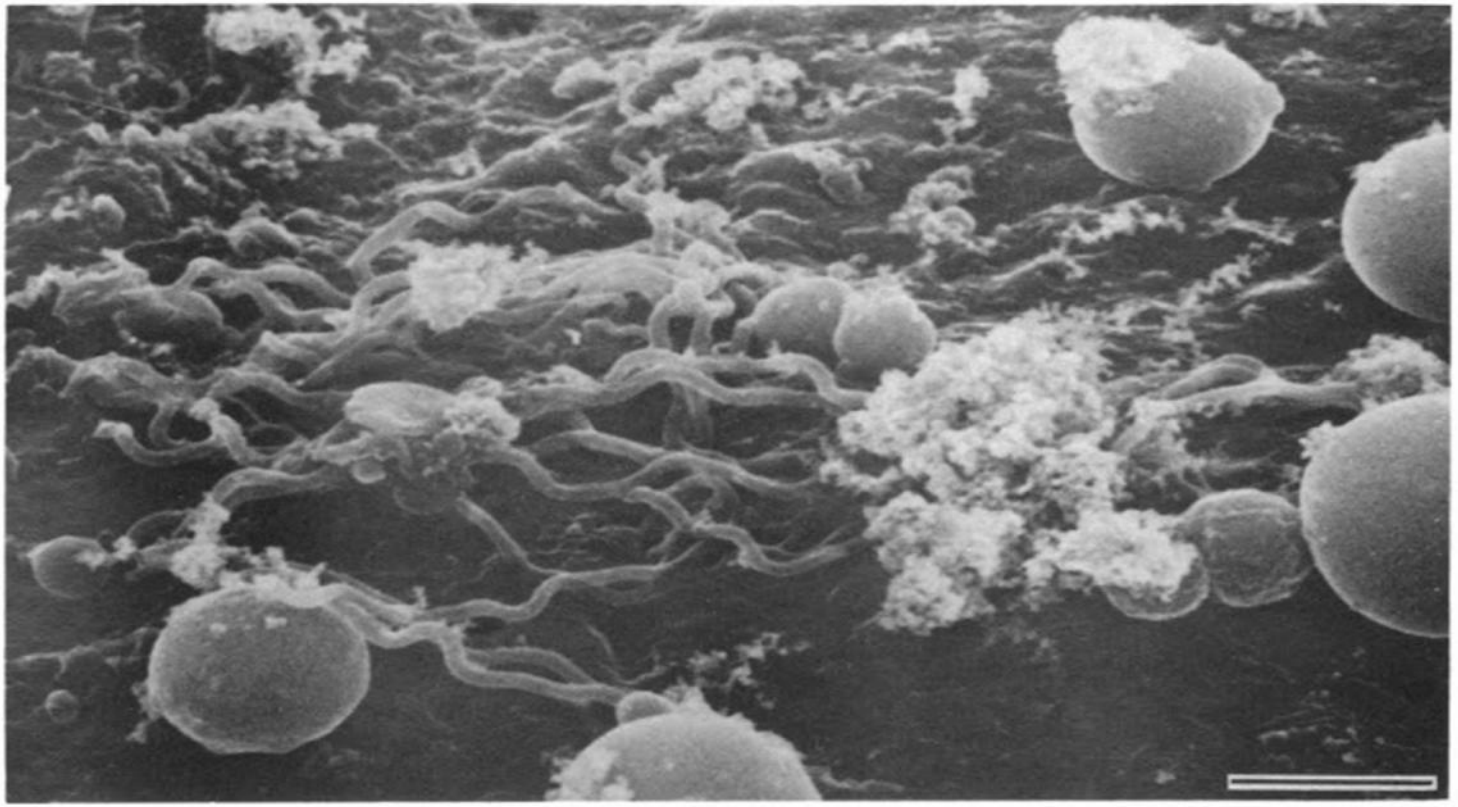


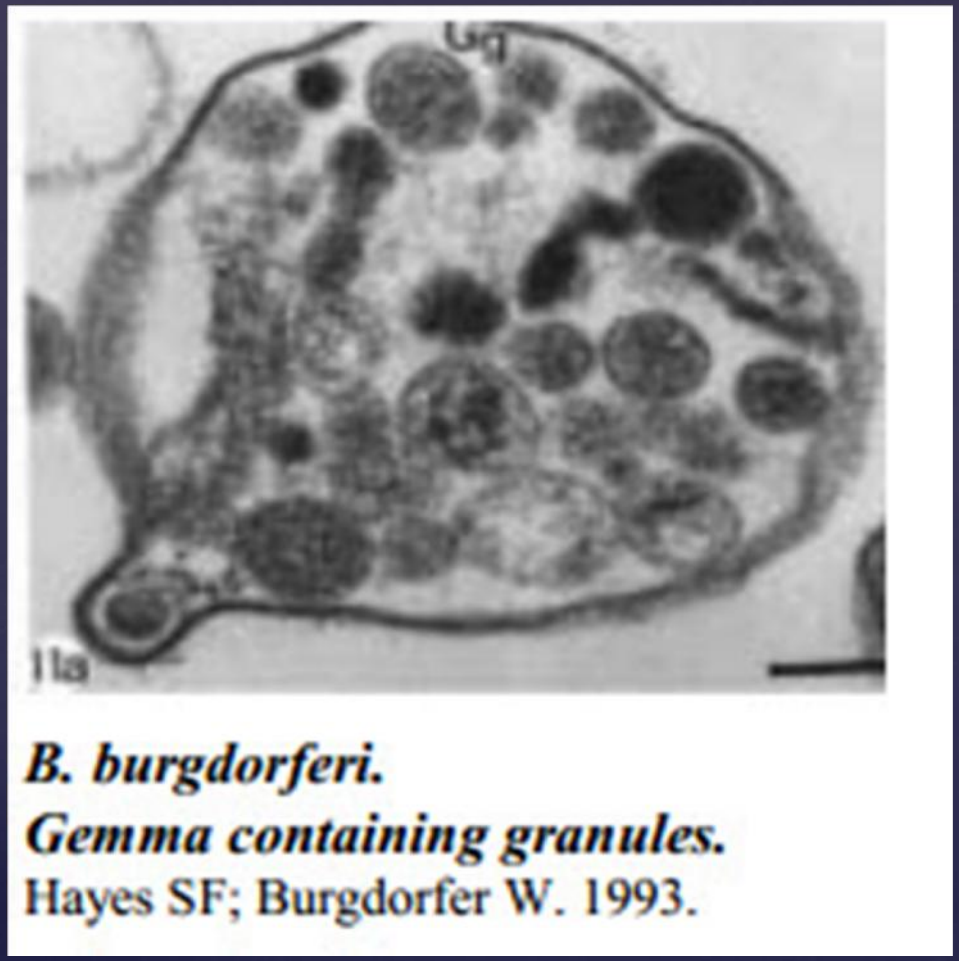
FIG. 8. Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of an *I. dammini* tick. Bar, 2.0 μm. (Photograph courtesy of D. Corwin, Rocky Mountain Laboratories.)

# Burgdorfer and Hayes – Unique Contributions

MacDonald Editorial Comment:
The Image of Hayes and Burgdorfer
Clearly demonstrates "Round things"
in the Electron Micrograph of the Ixodid tick Midgut.
The "Round Things" did not merit a comment from either author, probably because **Attention was directed toward the Net-like "Carpet" of typical Spiral Forms** of Borrelia burgdorferi in the Midgut of the Ixodid tick .

The Motif of "Hiding in Plain Sight" comes to Mind….

# Burgdorfer – Unique Research findings
# Borrelia burgdorferi
# Gemmae

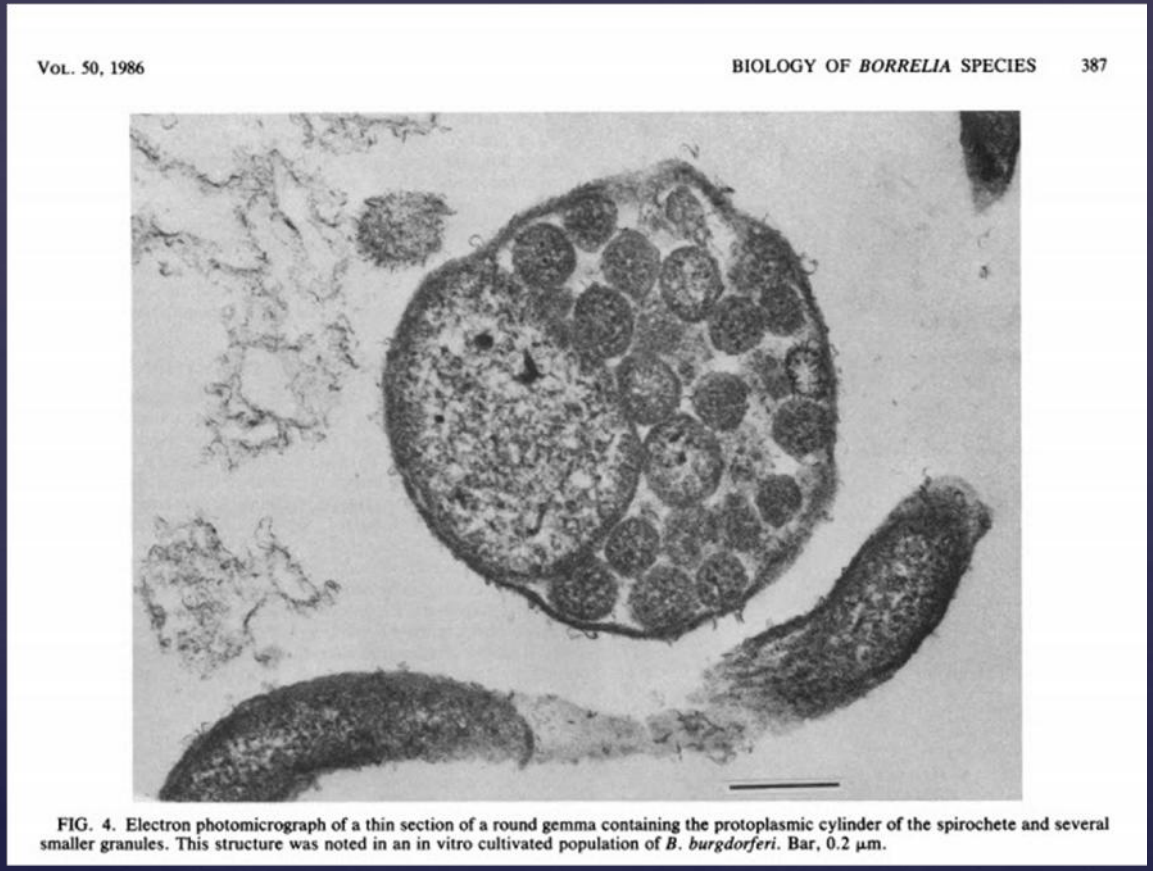



***B. burgdorferi.***
***Gemma containing granules.***
Hayes SF; Burgdorfer W. 1993.

## Photo Credit:

1993
Springer-Verlag
"Aspects of Lyme Borreliosis
K. Weber MD (Editor)
Ultrastructure of Borrelia burgdorferi
S.F.Hayes and W.Burgdorfer

# Burgdorfer – Unique Research findings
# Borrelia burgdorferi
# Gemmae



VOL. 50, 1986 BIOLOGY OF *BORRELIA* SPECIES 387

FIG. 4. Electron photomicrograph of a thin section of a round gemma containing the protoplasmic cylinder of the spirochete and several smaller granules. This structure was noted in an in vitro cultivated population of *B. burgdorferi*. Bar, 0.2 μm.

## Photo Credit:

Barbour, A.G,
and Hayes, S.F.
The Biology of Borrelia Species ,1986

# Burgdorfer and Hayes – Unique research findings Round bodies – and Bacteriophage

University of Rhode Island
Borrelia Research Group
Drs. Alban, Johnson, Nelson
Unique Research
Contributions concerning
Round bodies

# **Dr. Alban's group observations**:

# Are Round Body Forms of Borrelia burgdorferi Robust or Fragile?

1. Living , not Dead , with Diversity of Internal Structure and overall diameter(Brorson)
2. Develop from Spiral forms (Brorson)
3. Envelope Contains :
   - Cell wall - [ Cell wall presence removes these from Spheroplast]
   - Outer Surface membrane
   - Inner Surface membrane
4. Capable of Prolonged Survival (Brorson)
5. May not be eradicated by short term antibiotic therapy
6. Capable of regenerating motile Spiral forms (Brorson)

# University of Rhode Island – Unique contributions To Round body Biology

## Starvation and its Impact on Survival of Borrelia

1. **Round Bodies have limited ability (52% down to 3%) to revert to spiral forms (Alban ,Brorson)**

2. **An Inverse relationship** exists between **Round body Age and Viability (Alban)**

3. **Active protein Synthesis occurs during Round body formation (Alban)**

4. **Tetracycline (which blocks protein synthesis) blocks Round body formation. (Alban)**
5. **During Encystification activities,… Round bodies produce 20 Novel proteins (Alban)**

# University of Rhode Island – Unique contributions To Round body Biology

## Starvation and its Impact on Survival of Borrelia

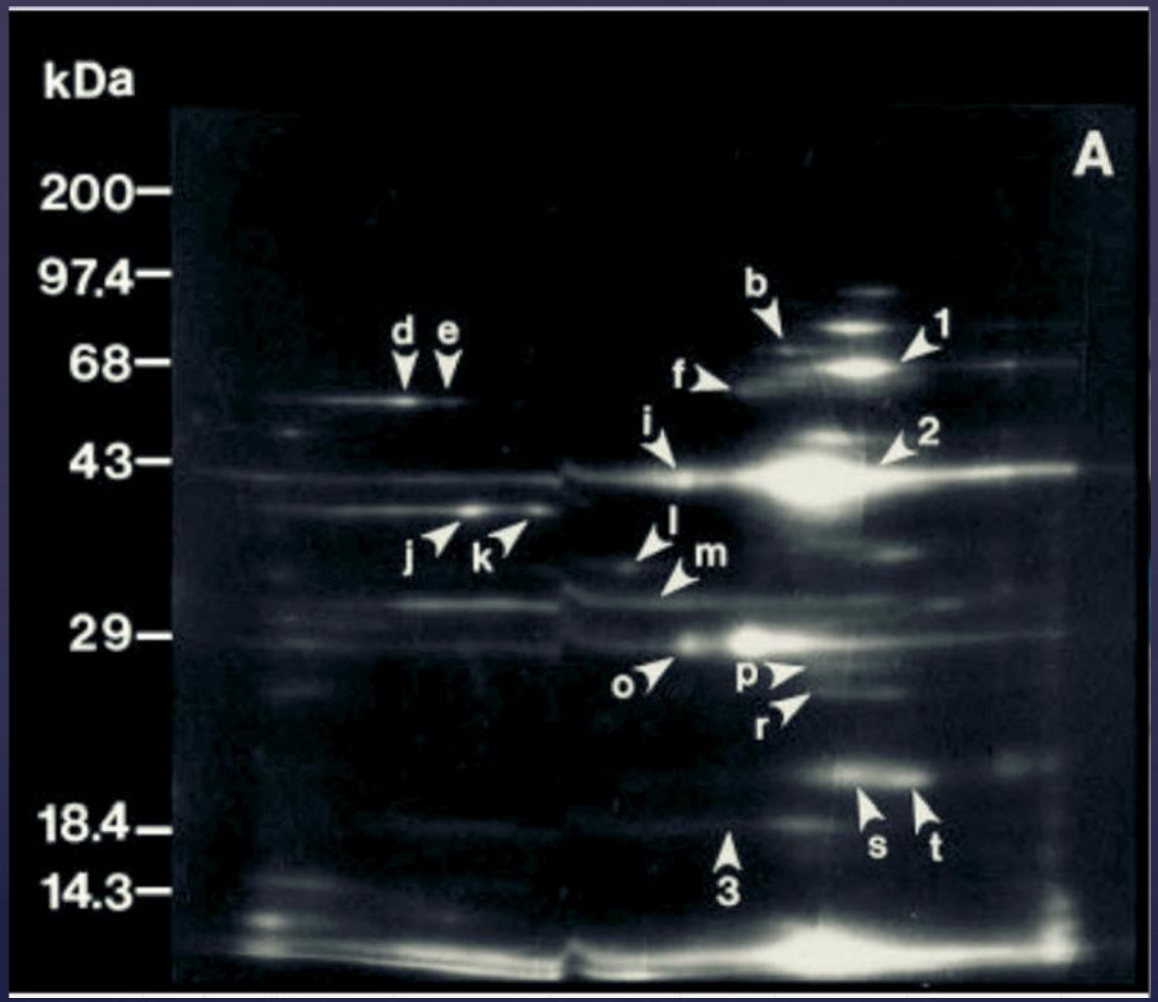
6. One end of the rolled up spiral form often protrudes from the edge of the body (Alban) [ MacDonald calls this the"tail" phenomenon]

7. "It is unclear whether the cyst membrane is part of the vegetative cell or whether it was constructed during Cyst formation" (Alban) [Novel proteins n=20 are synthesized in first 19hrs of starvation]

7. There is an apparent loss of External Envelope OspA during encystification. [ Alban quoting (Dr. Hulinska) ]

8. Gemmae contain DNA but are Not Viable and Gemmae do not revert to Spiral. (Alban) Clumped Spheroplast cell groupings form in Distilled H2O, and these rupture when serum or BSK culture medium is added.

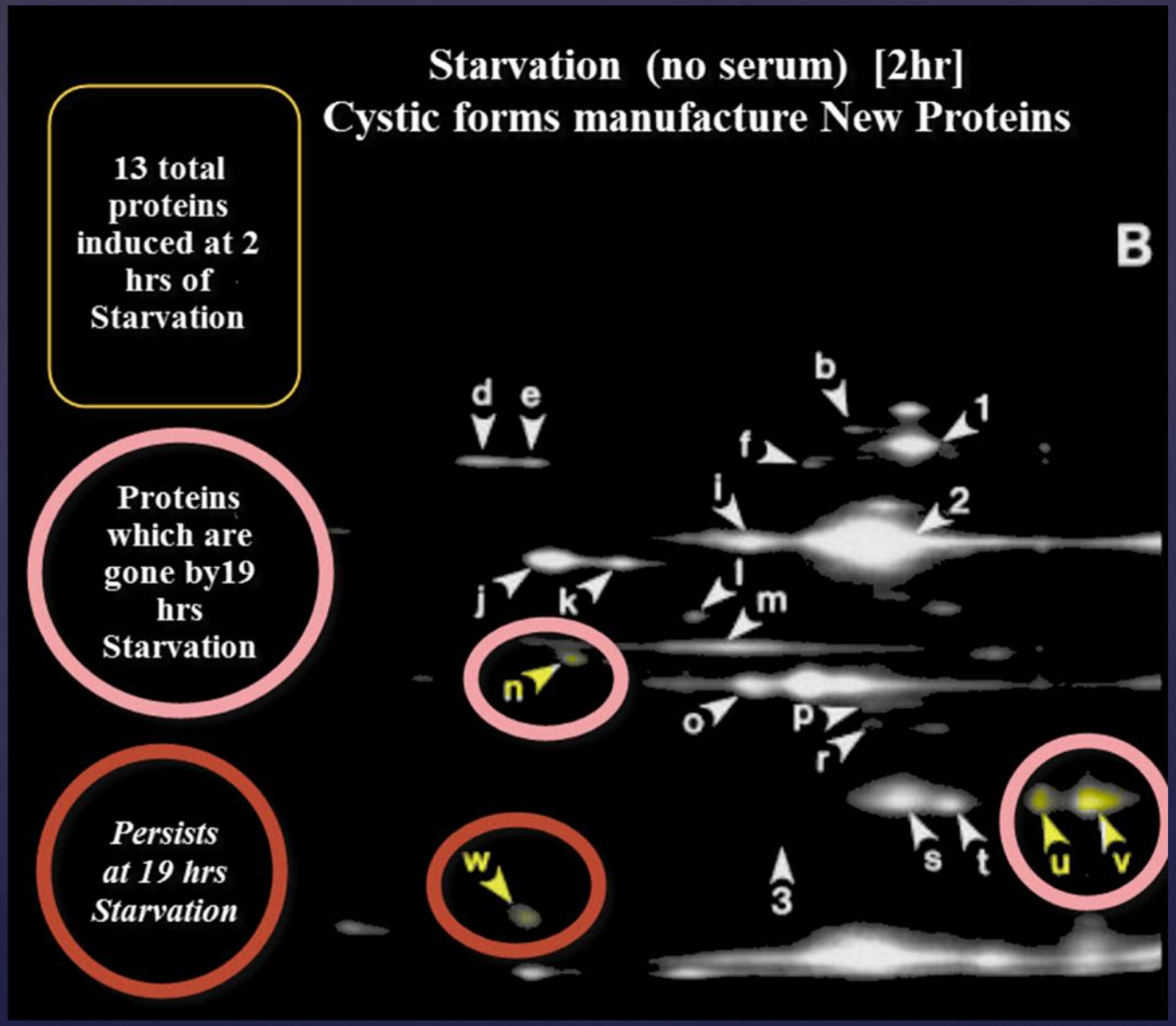
# Alban et al Rhode Island



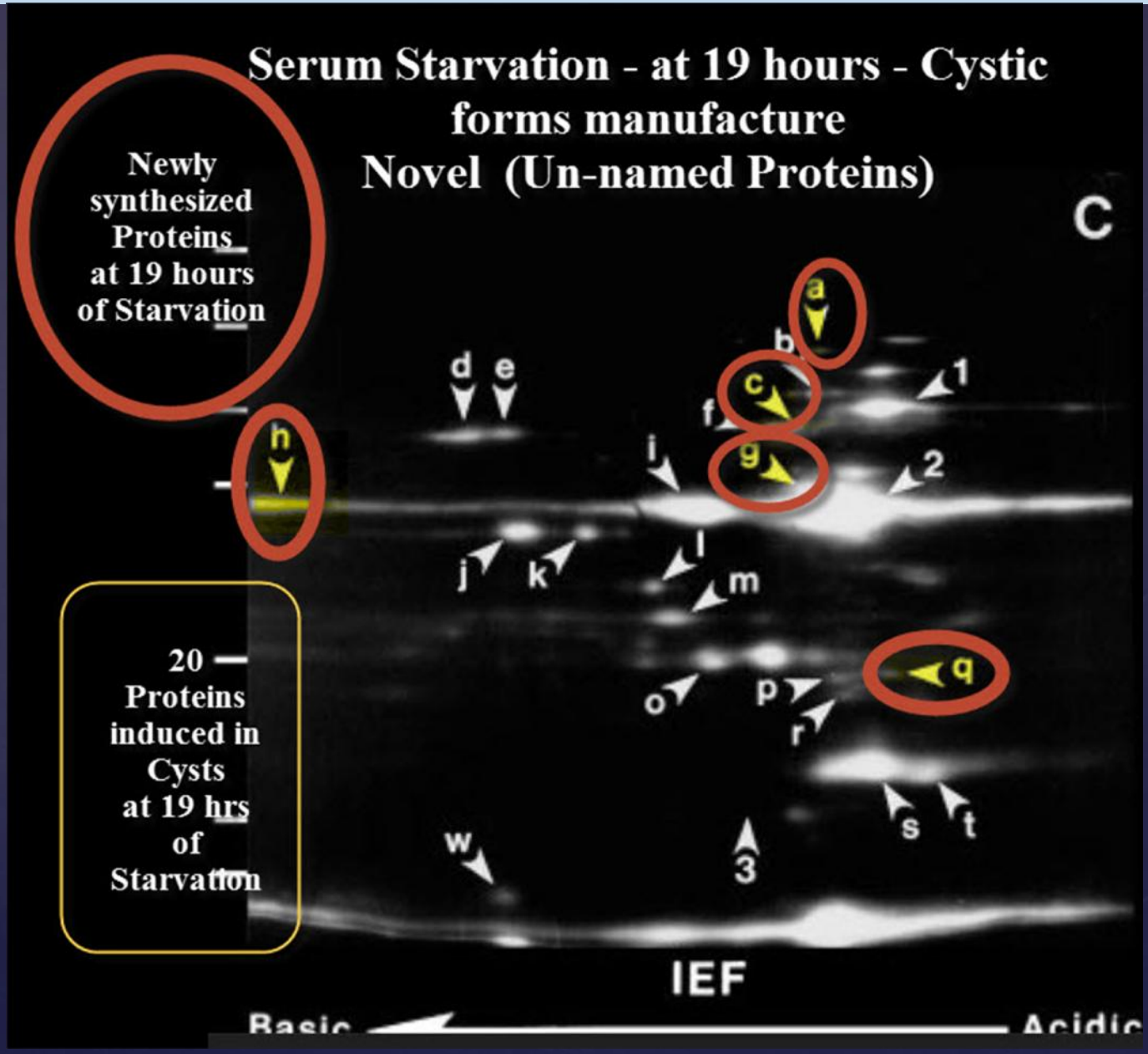

Proteins
Change
As
Cysts
Adapt
To
Adverse
Conditions

2D Electrophoresis
Study
Identifies
17 bands
When
Borrelia grow
In Serum

# Alban et al Rhode Island

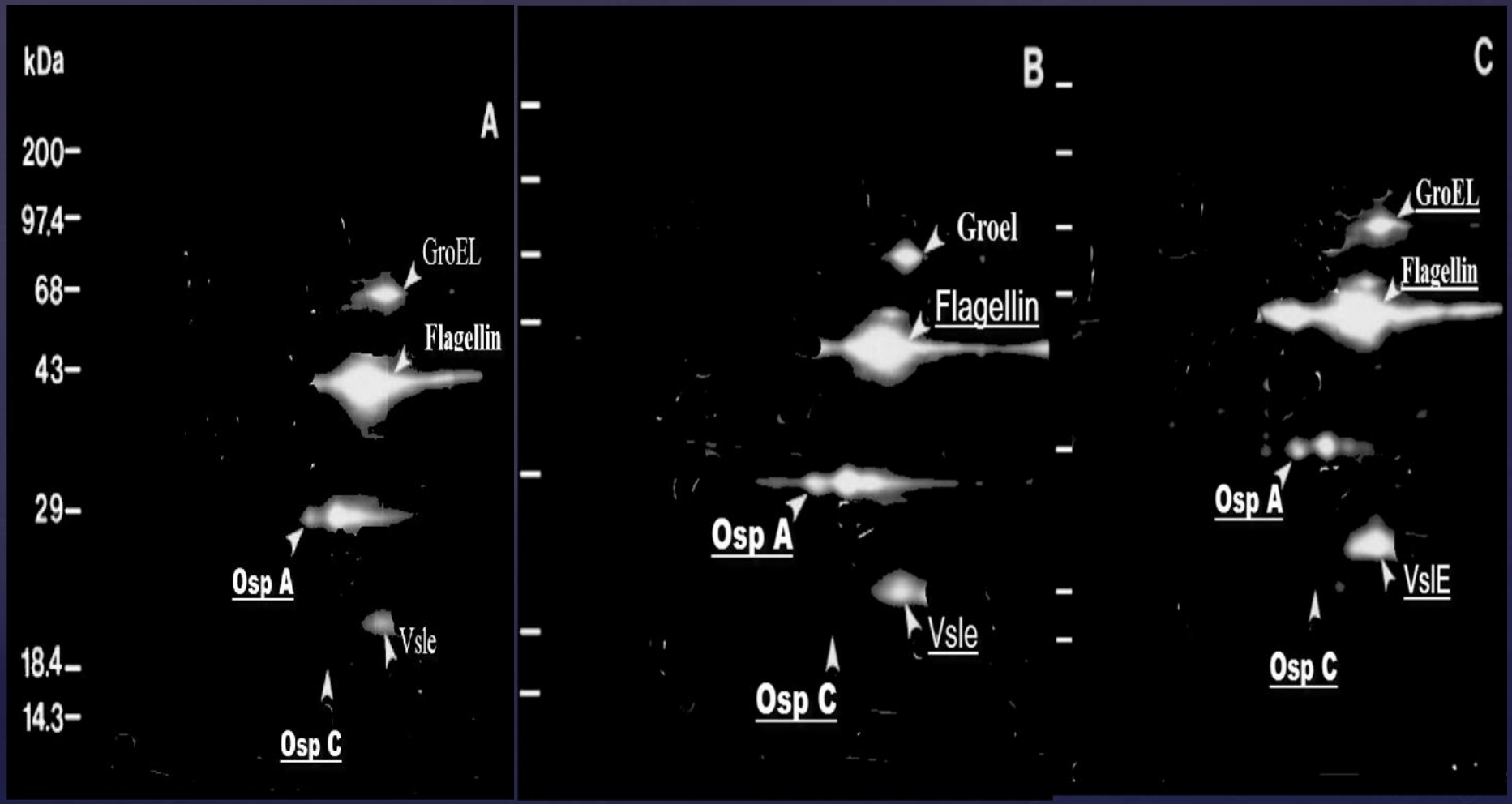


# Alban et al Rhode Island



# Alban et al Rhode Island

Proteins with Known function (N=5) out of a total of 20 Proteins
Found in Borrelia Cysts during starvation>
Note: Old friends …OspA, OspC, Flagellin, VslE, GroEL



# Alban et al Rhode Island Starvation Cystic Borrelia Study

| Medium | Time (d) | Percentage viability ± SD* | Motility† |
|---|---|---|---|
| RPMI | 0 | 100·0 ± 0 | + |
| | 2 | 52·5 ± 13·4 | − |
| | 3 | 18·2 ± 12·5 | − |
| | 5 | 15·7 ± 12·5 | − |
| | 8 | 2·9 ± 1·9 | − |
| RPMI + S | 0 | 100·0 ± 0 | + |
| | 2 | 56 ± 8·5 | + |
| | 5 | 0 ± 0 | − |
| HEPES | 0 | 100·0 ± 0 | + |
| | 1 | 0 ± 0 | − |

* Percentage viability = (viable cells as determined by MPN technique)/(direct microscopic count at $t = 0$). The data shown are averaged from two independent experiments.

# Alban et al Rhode Island – Emergence of New Proteins in Starved Cystic Borrelia

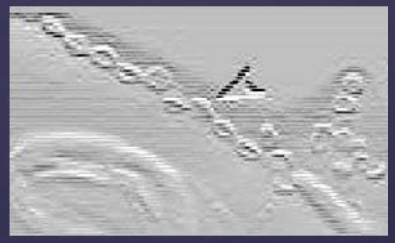
| | | | 0–2 h | 2–19 h |
|---|---|---|---|---|
| a | 89 | 5·8 | 0 | + |
| b | 66 | 5·9 | 0 | + |
| c | 58 | 6·0 | 0 | + |
| d | 57 | 8·4 | 0 | + |
| e | 57 | 8·1 | + | + |
| f | 57 | 6·1 | + | + |
| g | 43 | 5·9 | 0 | + |
| h | 41 | 10 | 0 | + |
| i | 41 | 6·7 | 0 | + |
| j | 37 | 7·9 | + | + |
| k | 37 | 7·4 | + | + |
| l | 34 | 7·0 | + | + |
| m | 32 | 6·9 | + | + |
| n | 31 | 7·7 | + | 0 |
| o‡ | 29 | 6·9 | + | + |
| p | 28 | 5·7 | 0 | + |
| q | 28 | 5·7 | 0 | + |
| r | 27 | 5·7 | 0 | + |
| s‡ | 24·5 | 5·5 | + | + |
| t | 24 | 5·0 | + | + |
| u | 24 | 4·3 | + | 0 |
| v | 24 | 4·0 | + | 0 |
| w | 13·5 | 8·0 | + | + |

# Alban et al Rhode Island

Cystic Borrelia Burgdorferi with protruding segments – **“ tails”**

# Alban et al Rhode Island

*String of Pearls formation* - **a form of "beading" in borrelia**



# University of Rhode Island – Unique contributions To Round body Biology - the "String of Pearls" formation

The "String of Pearls" structure is one of the diverse formations of Borrelia burgdorferi which was first described By Dr Willy Burgdorfer and Mr Fred Hayes at the Rocky Mountain Laboratory, National Institutes of Health, Hamilton, Montana in the early 1980's.

Comment: Alan MacDonald believes, perhaps with some imperfect remembrances, that he first suggested " String of Pearls" based on the title of a Jazz Musical "standard" by the same name. All this notwithstanding, the Phrase is embraced As "standard terminology" today to describe the unique structure illustrated below, which is one of many "signatures" of Borrelia.

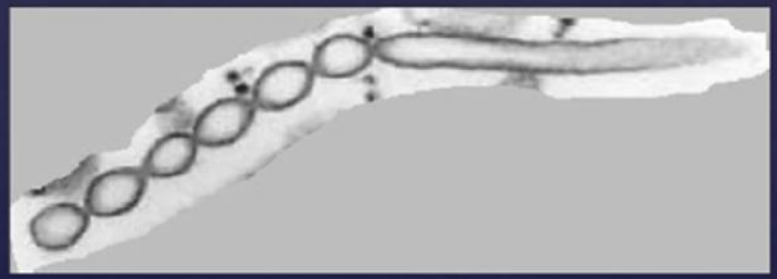
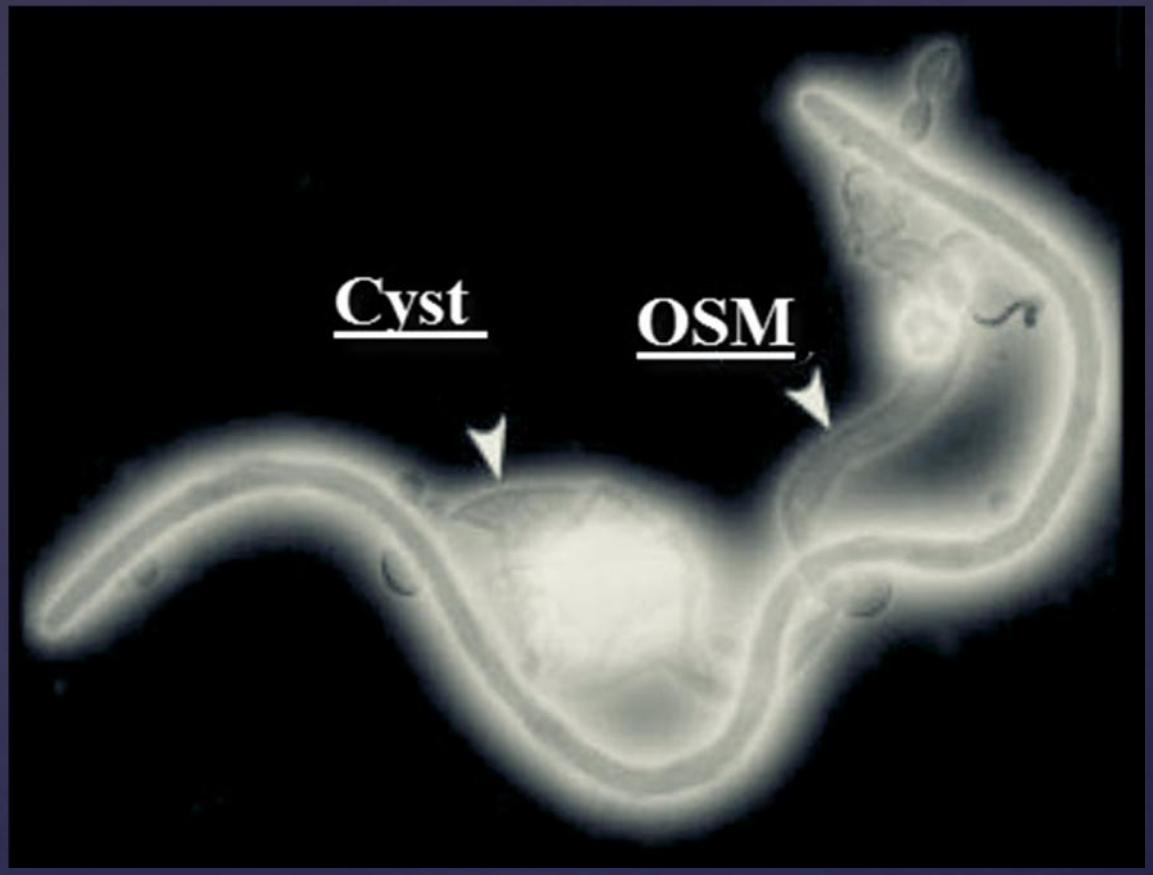


**Image Credit: Brorson,O and SH 2001**

# Alban et al Rhode Island




Carcass of the Envelope of the Cystic Borrelia – Left Behind after
The emergence of the spiral form from the round body

Dr. Eva Sapi and the Borrelia Research Group at the University of New Haven Unique Research findings concerning Round Body biology

# Dr. Eva Sapi's Unique Research findings concerning Round body Biology

1. Antimicrobial Sensitivity Studies of Round Bodies with Determination of MIC and MBC Levels as distinct from Antimicrobial sensitivity Of Spiral forms
1. Identification of Round Bodies as Essential Constituents of Biofilms of Borrelia burgdorferi

# Dr. Eva Sapi University of New Haven – Cystic Borrelia

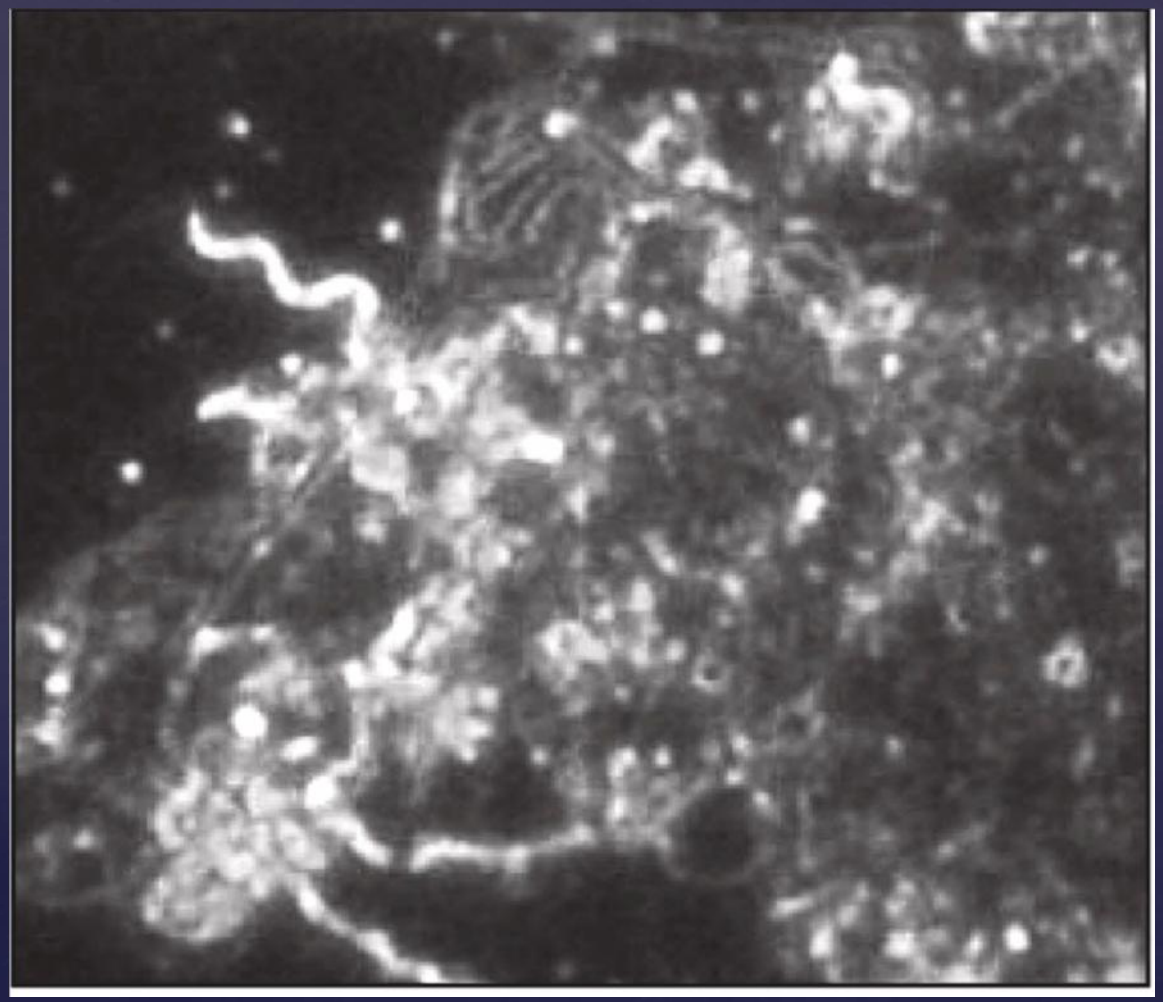


Photo credit: Dr. Eva Sapi

**Biofilm of Borrelia burgdorferi**
**Includes Cystic forms**

American Journal of Clinical Pathology 2008 Vol 129:988-990

# Dr. Eva Sapi –University of New Haven

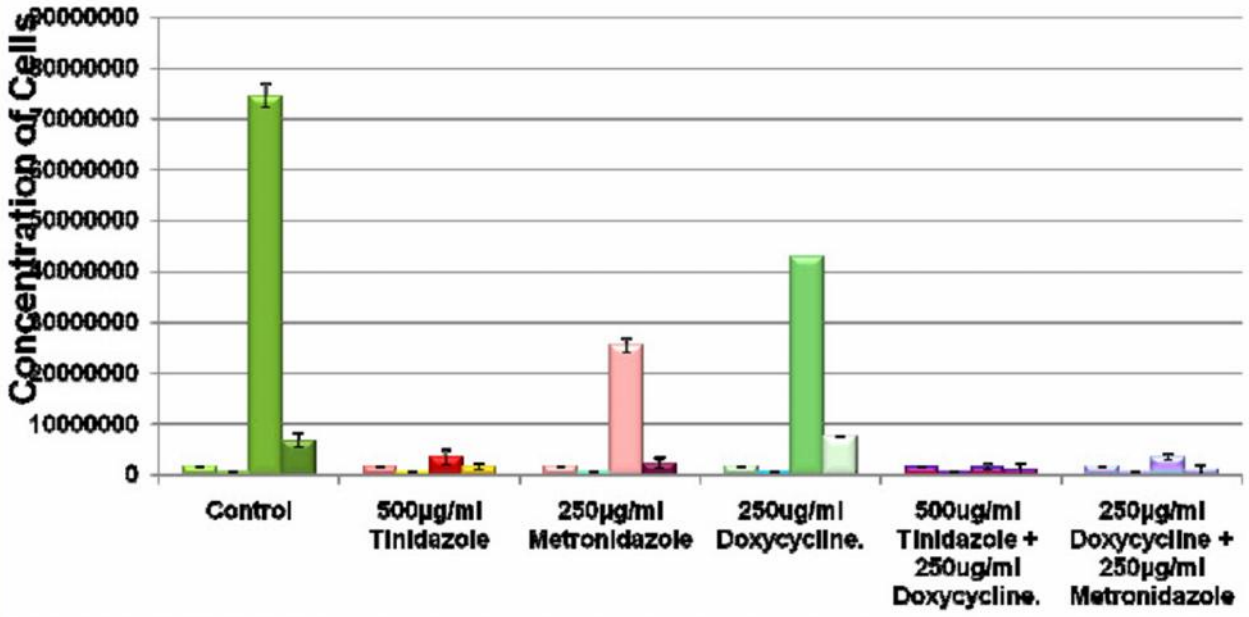
## An *in vitro* evaluation of antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*

- To test the *in vitro* susceptibility of spirochete, cyst and biofilm forms of *Borrelia burgdorferi* to different antibiotics and natural agents
- To test and develop an optimal combination of these tested antibiotics in order to eliminate all of these different forms of *Borrelia burgdorferi* using several microscopic and viability assays
  - The research project supported by *Californian Lyme Disease Association* / April 2010

UNIVERSITY OF NEW HAVEN

# Dr. Eva Sapi –University of New Haven

## Comparison of the effects of doxycyline, tinidazole and metronidazole on the spirochete and cyst formation of *Borrelia burgdorferi*




Kaur N, Datar A and Sapi E unpublished data 2009

# Dr. Eva Sapi –University of New Haven

## In summary

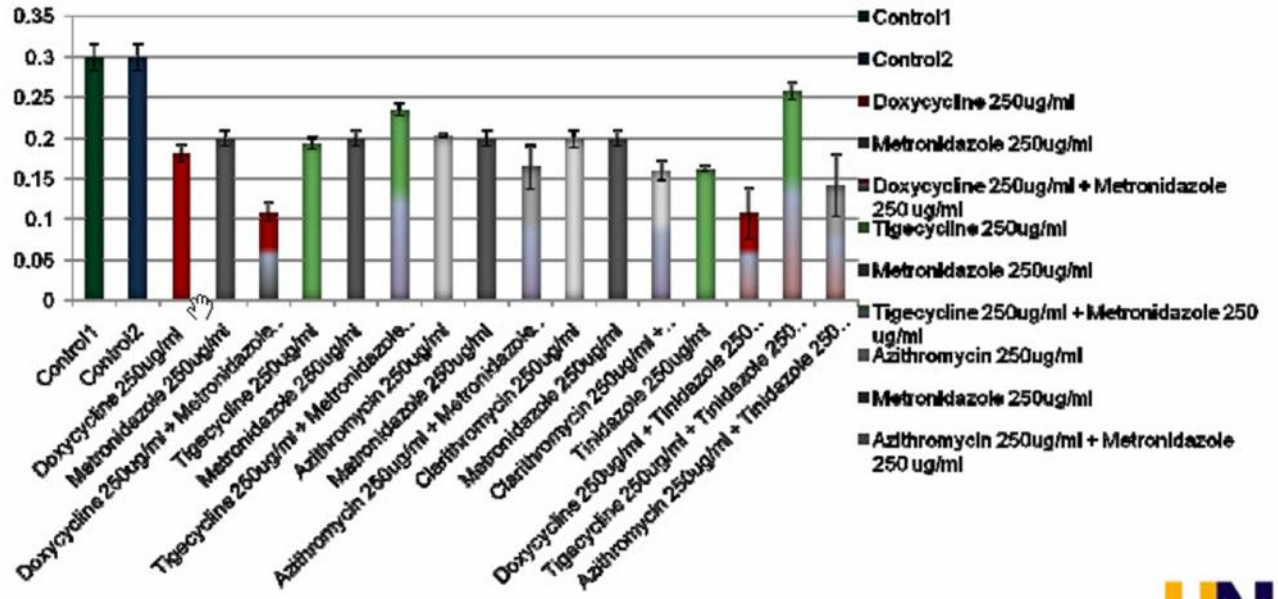
- Our *in vitro* results strongly suggested that different morphological formations of *Borrelia burgdorferi* have different antibiotics sensitivity

- Certain combinations of antibiotics and natural herbal agents can effectively eliminate all these known forms of Borrelia (spirochete, cystic, round bodies and biofilm)

UNIVERSITY OF NEW HAVEN

# Dr. Eva Sapi –University of New Haven

## Treatment of *Borrelia* biofilm with various antibiotics and evaluation by crystal violet staining method




Kaur N, Datar A and Sapi E unpublished data 2010

UNIVERSITY OF NEW HAVEN

# Dagmar Hulinska MD and the Borrelia Research Group , Prague, Czech Republic

1. Round bodies are present in Human skin at the Erythema Migrans site by Electron Microscopy.
2. Envelope of the Round body has NO OUTER SURFACE PROTEIN A ( Osp A is not present on the external surface of the Round Body (Cystic Form)

# Hulinska Borrelia Research Group

1. **Immune Electron Microscopy of Cystic Borrelia in Human EM skin**
   - **Lesion demonstrates ABSENCE OF OspA ON THE SURFACE ENVELOPE of Cystic Borrelia (round bodies)**

# Hulinska Borrelia Research Group

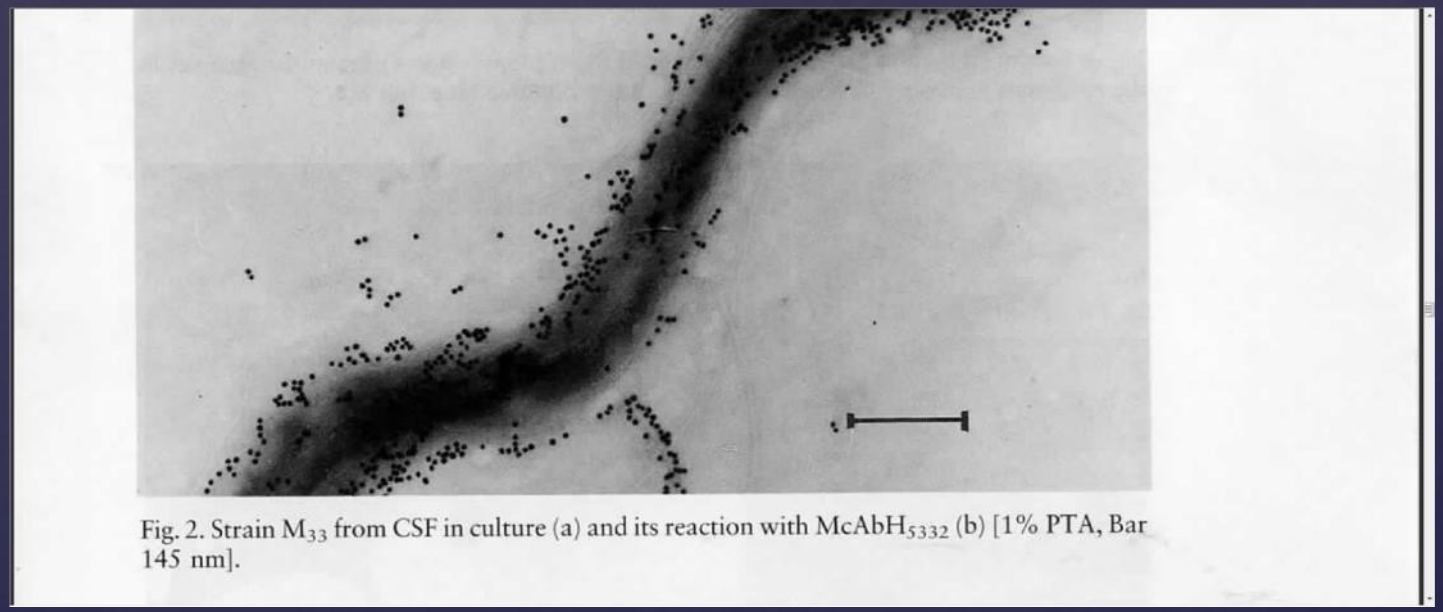


Fig. 2. Strain $M_{33}$ from CSF in culture (a) and its reaction with $McAbH_{5332}$ (b) [1% PTA, Bar 145 nm].

Domain of OspA –Outer Surface Protein A- in a normal spiral form of Borrelia burgdorferi – identified by Monoclonal antibody H5332 ( Murine – from Alan G.Barbour MD – unique Specificity for Osp A of Borrelia burgdorferi- [Note the Antibody binds to the OUTERMOST regions of the envelope]

**MacDonald Editorial Comment……. Here in the Spiral forms of Borrelia Burgdorferi The little black dots MARK the Position OspA, Which is a protein on the OUTER SURFACE…**

# Hulinska Borrelia Research Group

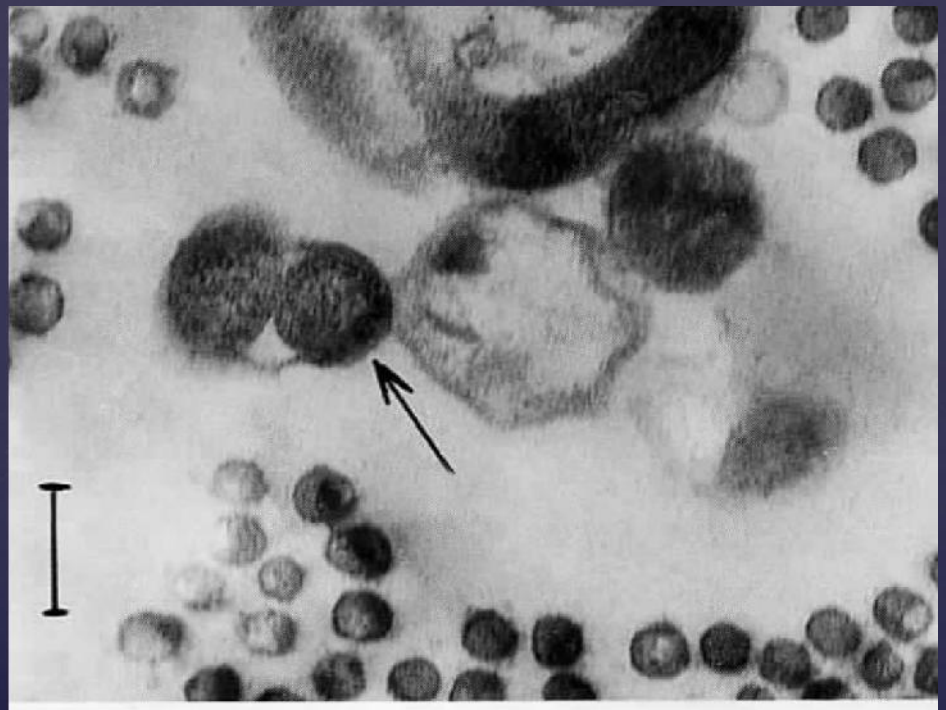


Fig. 5. Cyst-like material of spirochetes in upper dermis

Cyst form of *Borrelia burgdorferi*
Situated in the Dermis of Human skin biopsy
Of Erythema Migrans Lesion

# Hulinska Borrelia Research Group
## Key Observation – Envelope of the Cystic Borrelia in Human Skin

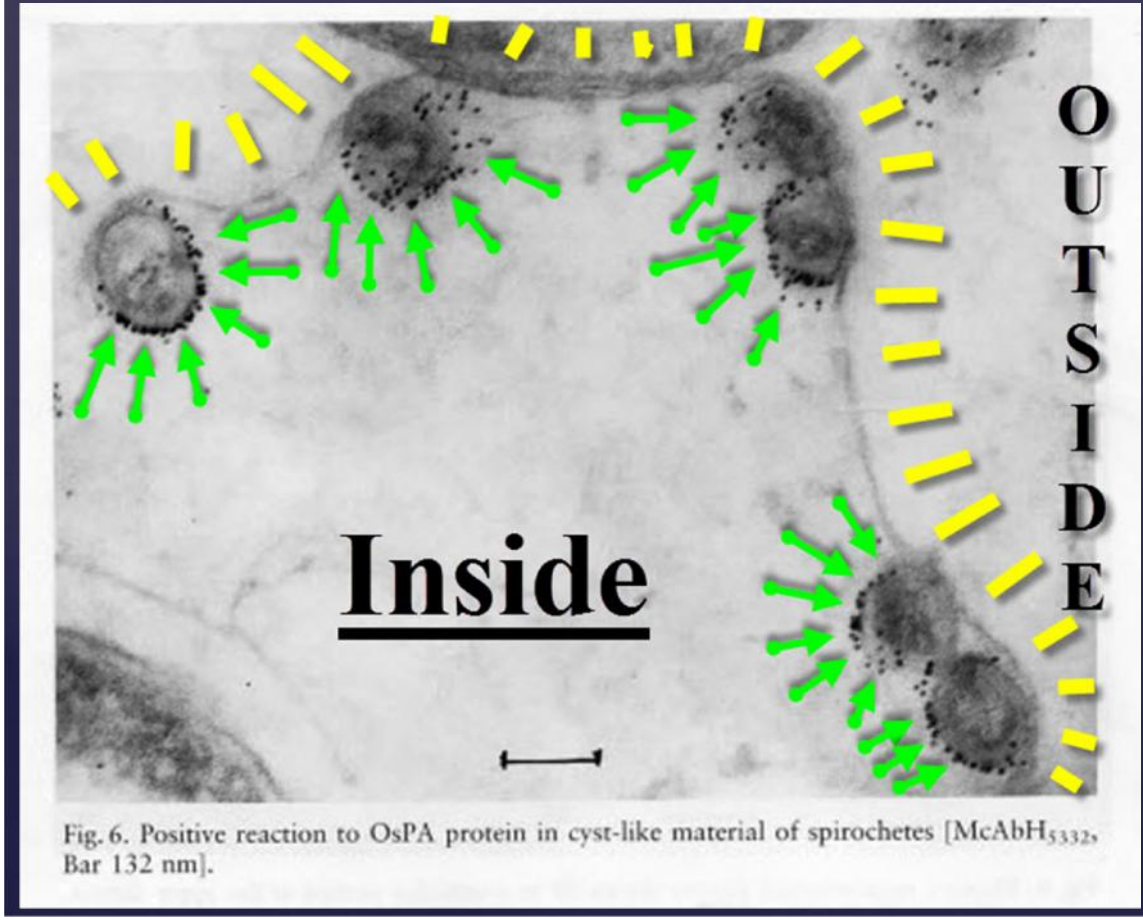



Fig. 6. Positive reaction to OsPA protein in cyst-like material of spirochetes [McAbH$_{5332}$, Bar 132 nm].

Cystic form of Borrelia burgdorferi in biopsy of human Erythema Migrans
Skin lesion –
Note: the Immunogold label in electron microscopy
OspA Binds only to the spiral forms
Of the spirochete
INSIDE
The Cyst.

**The Outer envelope of the Cyst is NEGATIVE FOR OspA protein.**

# Hulinska Borrelia Research Group Key Observations – Envelope Structure – Cystic Borrelia

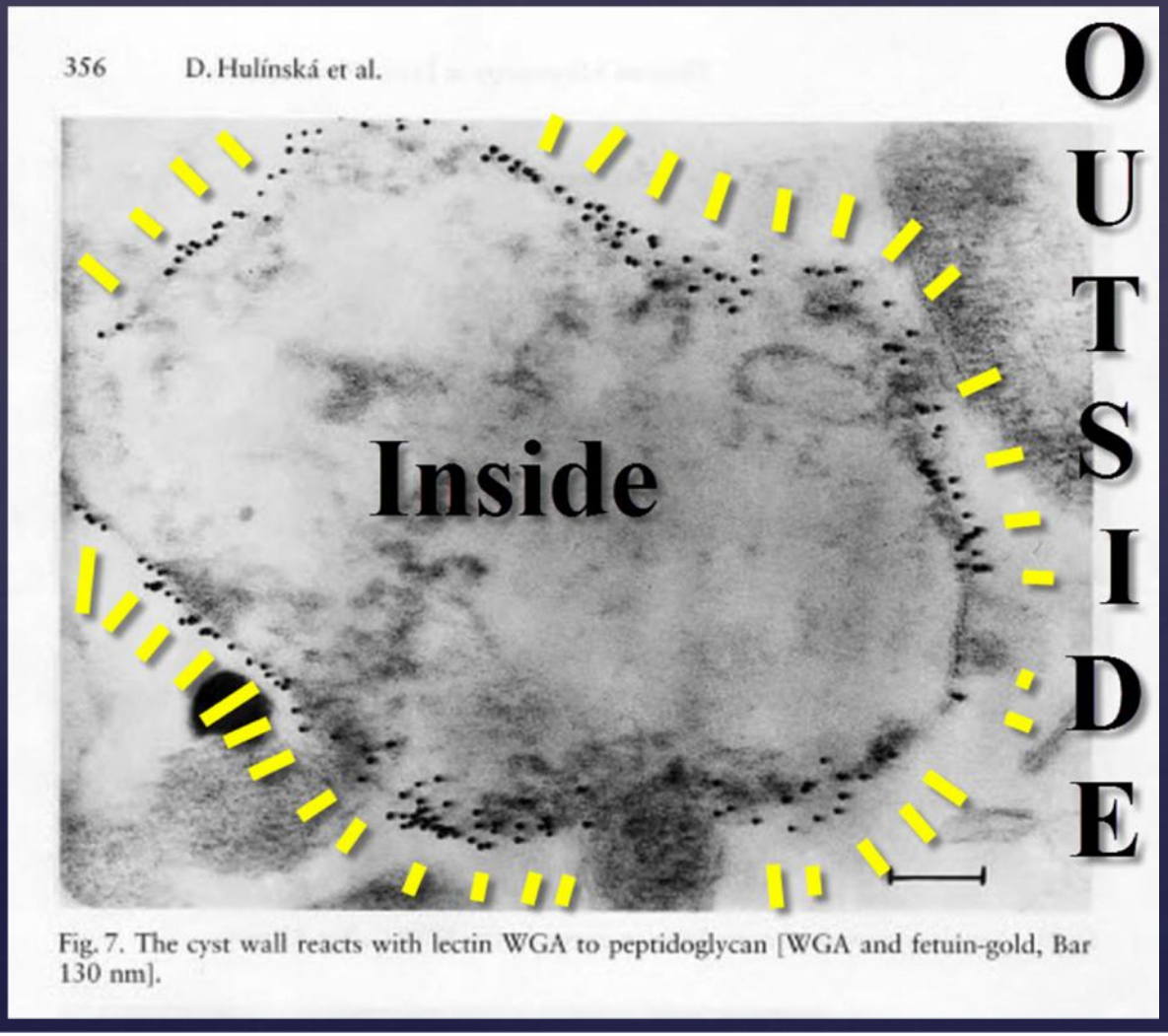


Fig. 7. The cyst wall reacts with lectin WGA to peptidoglycan [WGA and fetuin-gold, Bar 130 nm].

Outer Envelope Regions Of Cystic Borrelia Burgdorferi Binds a Marker for the Naked Cell Wall (Peptidoglycan) Recognized by WGA [Wheat Germ Agglutinin]

---

Note: This is the region where OspA would normally bind.

"Naked Cell Wall material Suggests that the Outer Surface membrane is gone!

# Hulinska Borrelia Research Group

MacDonald - Editorial Comment:
The Hulinska Group Immunoelectron Microscopy Data Point to a Structure of the Envelope of the Borrelia Cyst –

## Is Remarkable!!!

It is possible that the Exposed elements of the Borrelia Cyst Envelope are 100%different from the Exposed proteins of the Spiral (Vegetative) Forms.

**The clinical significance of this possibility is that ALL Antibodies produced by the body against the motile forms , would be useless against the Encysted borreliae.**

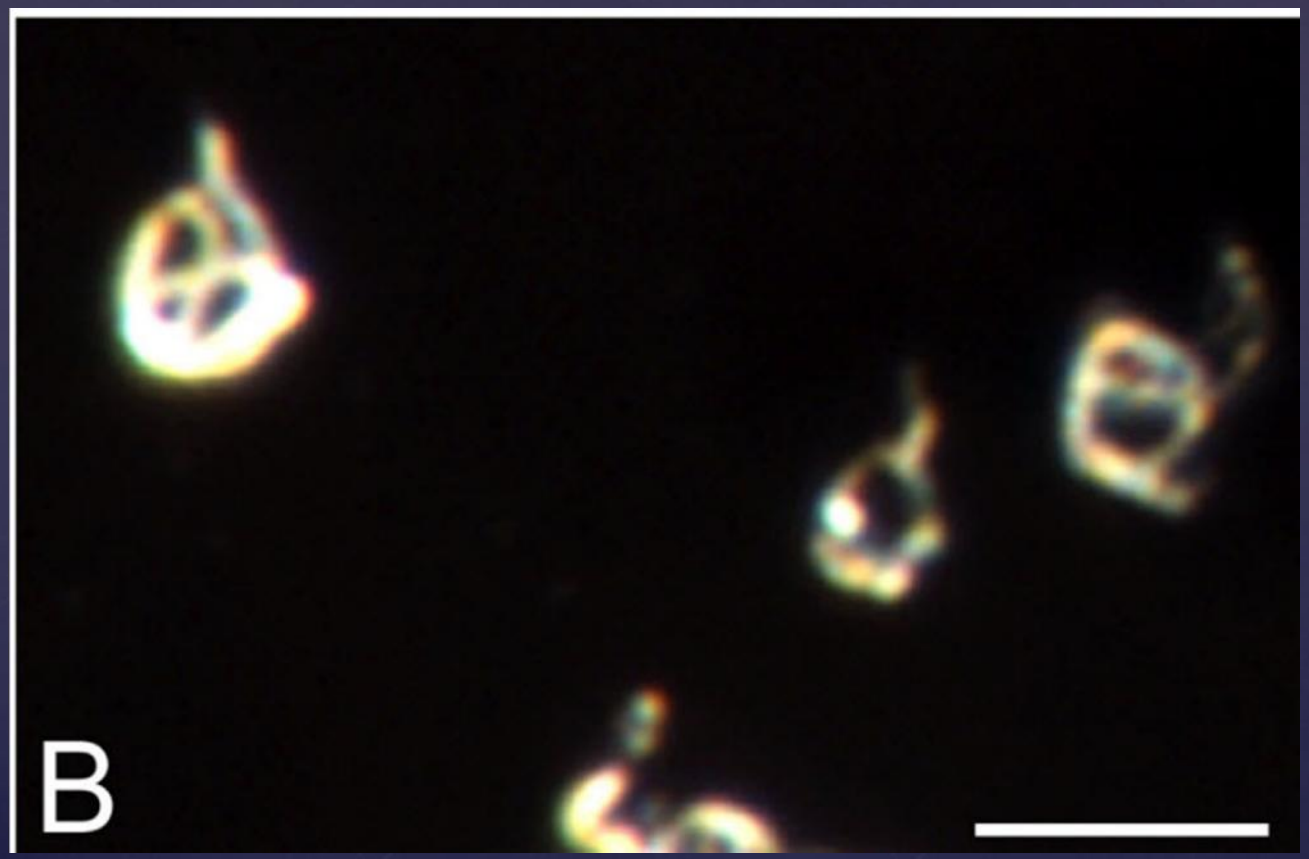
This scenario would be the ideal Example of " Stealth Pathogens"

# Judith Miklossy's Research Contributions to Cystic Borrelia Structure

1. Cystic forms of Borrelia are encountered in Diseased Human Brain Tissues

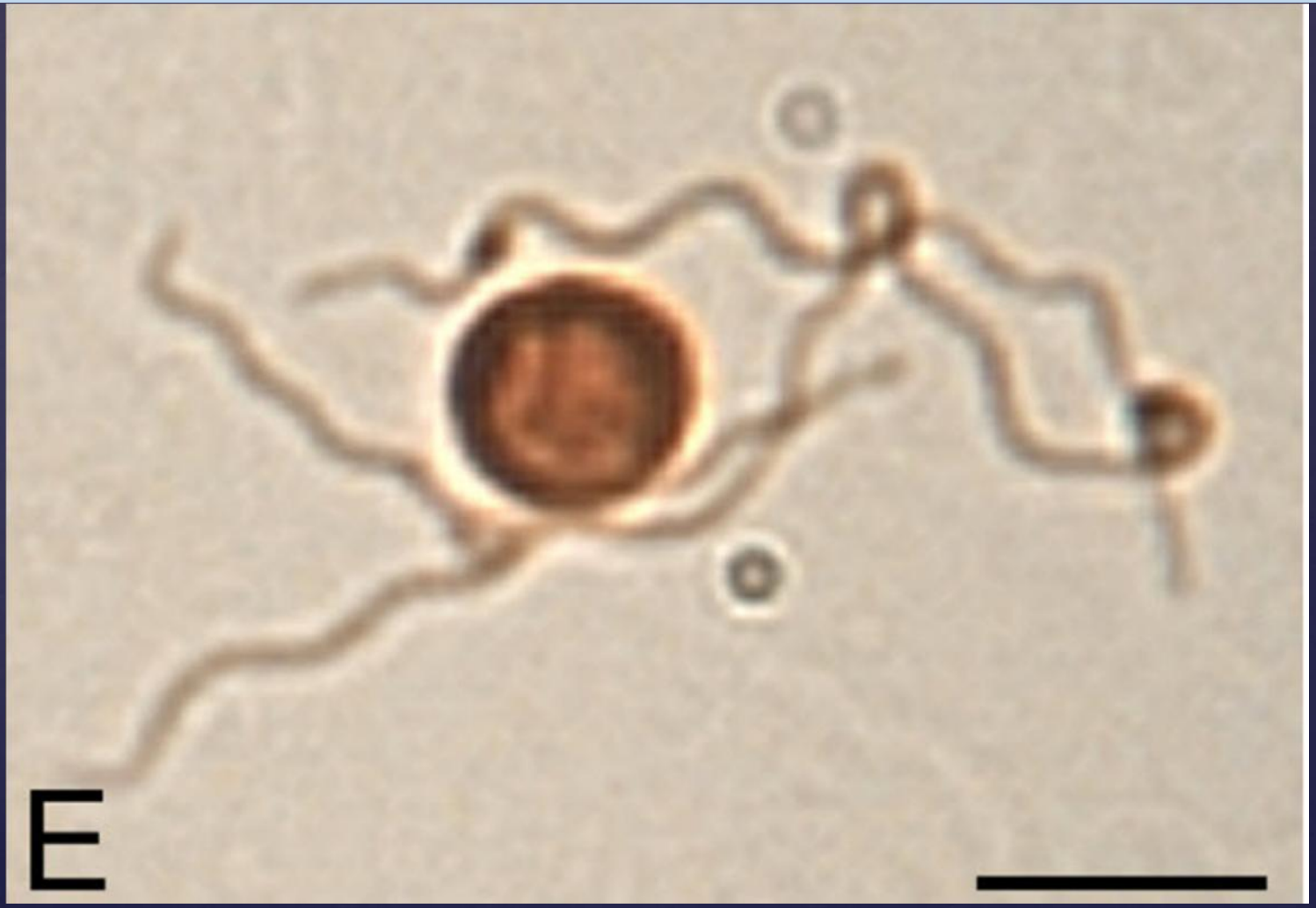
2. Atomic Force Microscopy Study (AFM) – first utilized by Dr. Miklossy to Examine the Inner Structure of Borrelia Cysts

# Miklossy Laboratory
# Unique Contributions –Cystic Borrelia

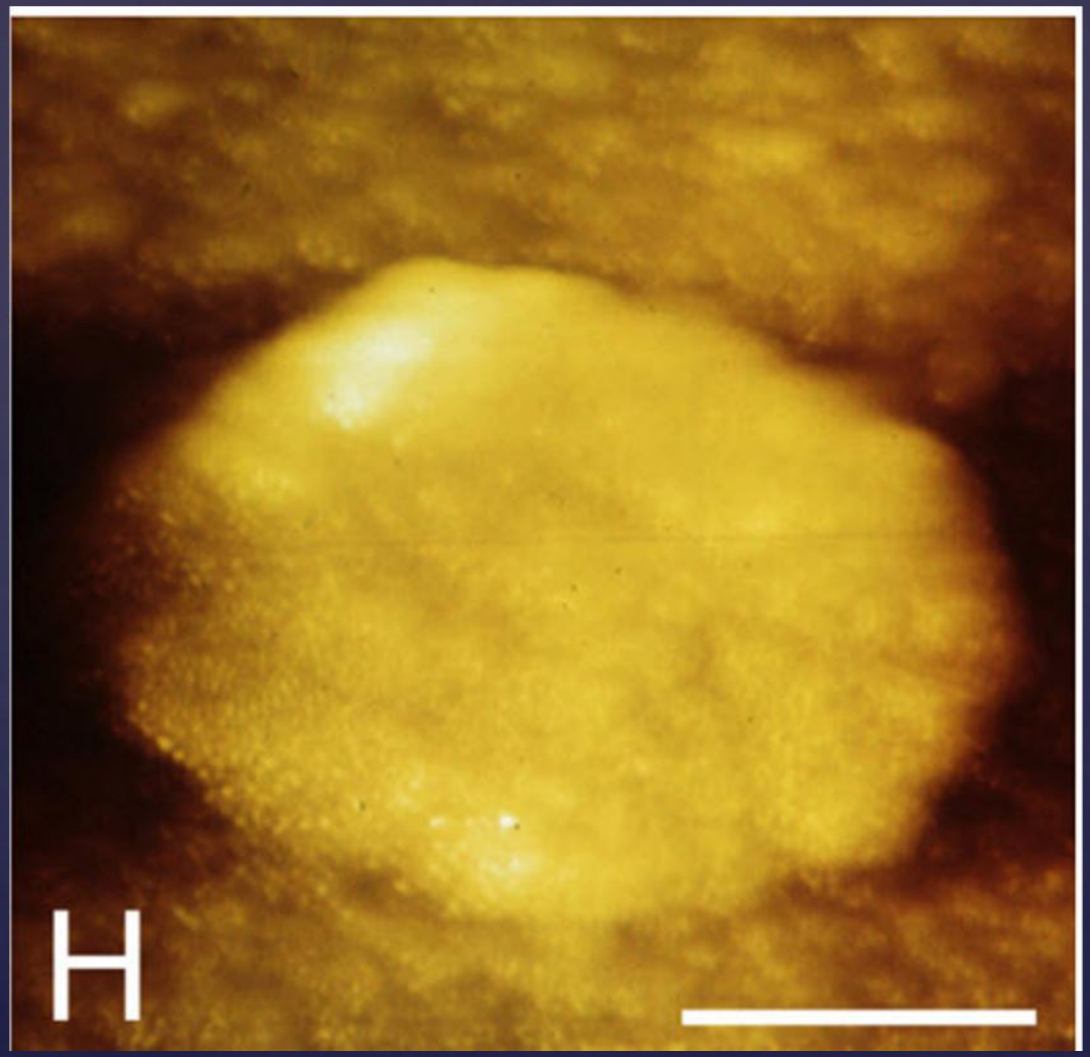



Darkfield Microscopy Image of Borrelia Cyst forms…
Note: some Cysts show "tails" protruding from the Envelope of The Cyst , just Like the Images of the Alban Group At Univ. R.I.

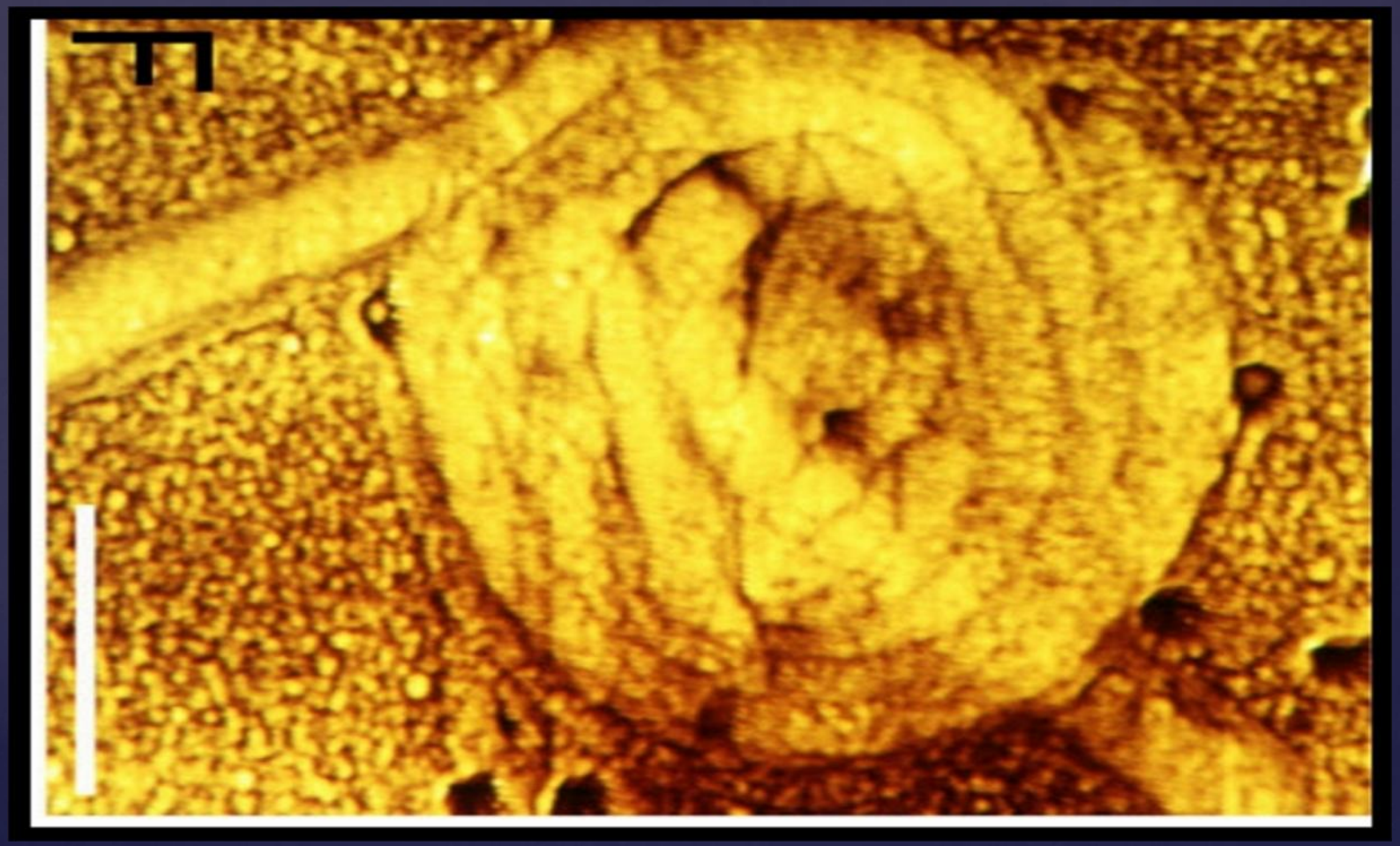
# Miklossy Laboratory
# Unique Contributions –Cystic Borrelia



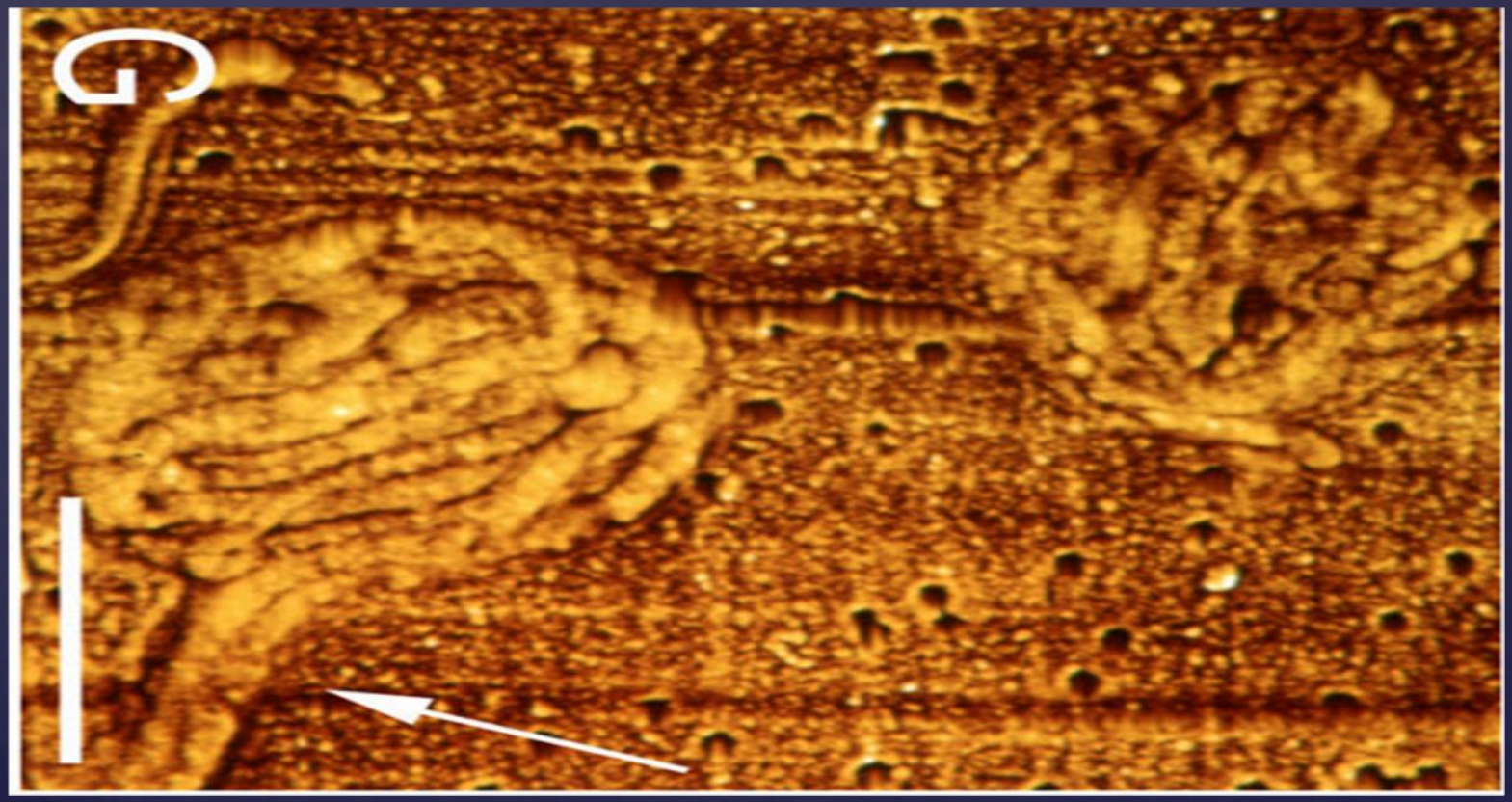
Miklossy Laboratory
Unique Contributions –Cystic Borrelia
Atomic Force Microscopy



Miklossy Laboratory
Unique Contributions –Cystic Borrelia –
Atomic Force Microscopy



Miklossy Laboratory
Unique Contributions –Cystic Borrelia – Atomic Force Microscopy



# MacDonald Editorial Note: Miklossy Cystic Images

The Fascinating Images of Borrelia Cysts from Dr Judith Miklossy are the Atomic Force Microscopic (AFM) Images ever obtained with the AFM microscope.
They have the advantage [ over pre-existing high resolution Microscopes (Electron Microscopes) in that Living organisms Can be examined, without the possible distortions of chemical Fixatives, which are required to produce Electron Micrographs.
All of Dr. Miklossy's Images represent the so called "Young" Cysts of Brorson &Brorson, because a coiled spirochetal structure is contained within the Envelope of
The cyst. No Dense Nucleoids –(typical of "Aged Cysts")
Are seen in this series of excellent Images.

Alan B.MacDonald MD comment

# Dr. Gruntar and the Borrelia Research Group Unique Contributions to the biology of Round Bodies

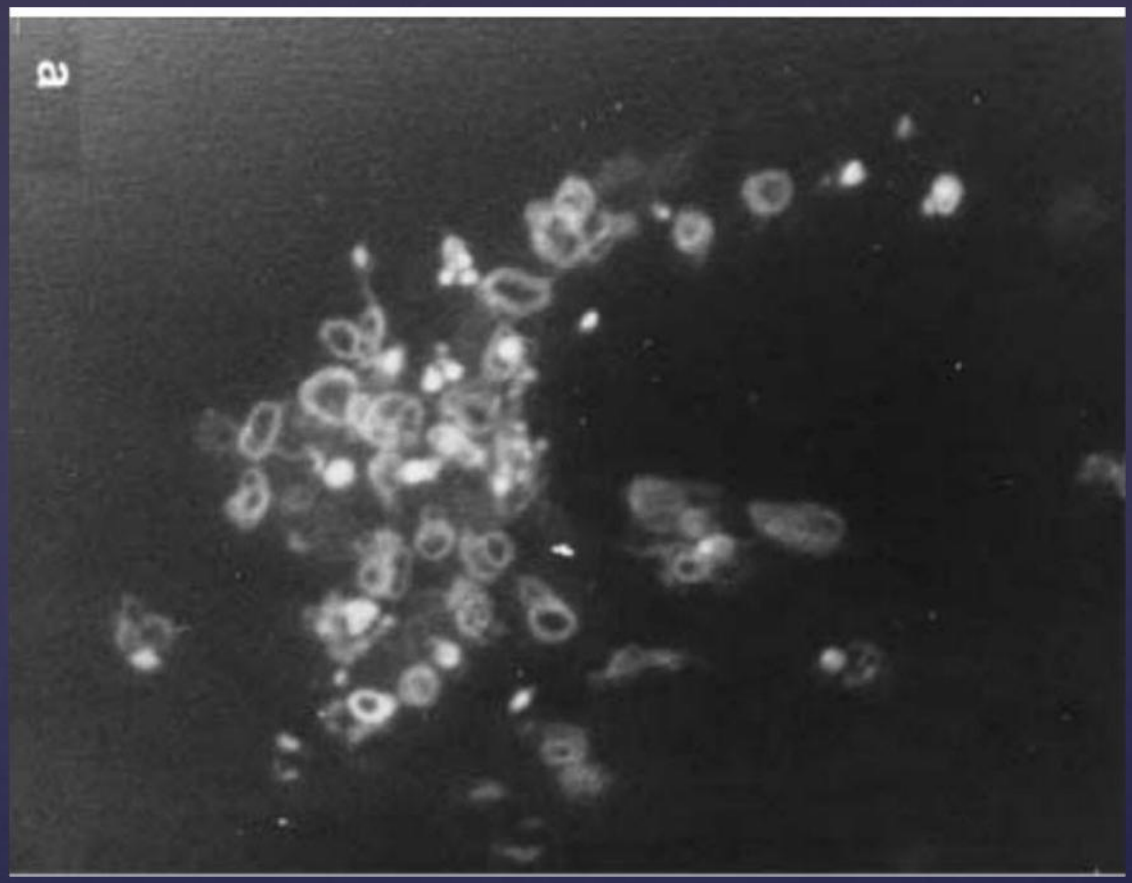
1. Injection of Pure suspensions of Round Bodies into the Peritoneal Cavities of Mice causes Borrelia infection, with Spiral forms recovered from the Laboratory Infected Mice.

# Study of Gruntar and Colleagues 2001

- Borrelia garinii cystic forms created by subjecting spiral (vegetative) forms to Distilled water.
- Mice inoculated( intraperitoneal injection with cysts produced in the laboratory) –
- 2 of 15 mice produced motile spirochetes as evidence of transmissibility of Cysts to cause Infection in a mammalian animal model.

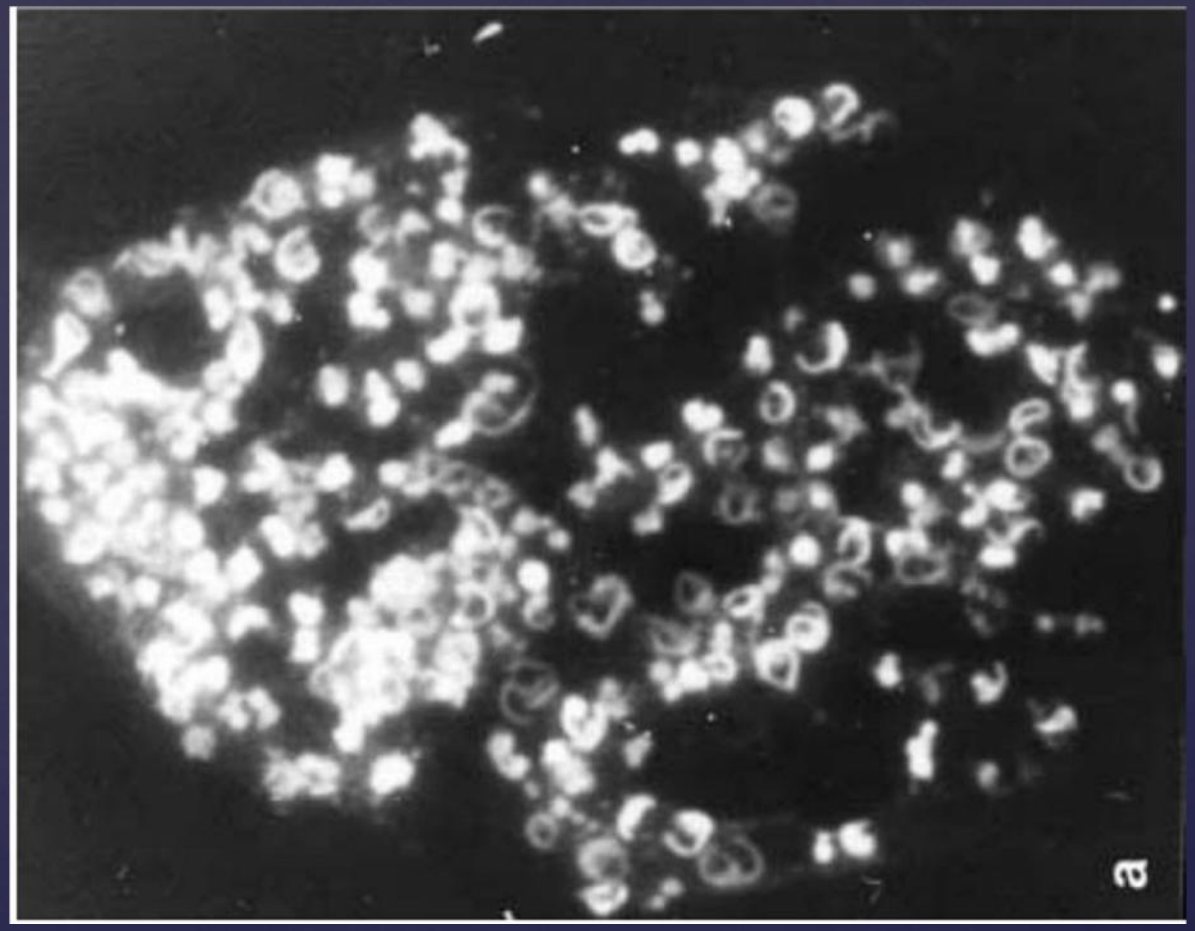
## Infectious nature of Borrelia Cysts

# Study of Gruntar and Colleagues 2001



Infectious nature of Borrelia Cysts

# Study of Gruntar and Colleagues 2001




Infectious nature of Borrelia Cysts

Study of Gruntar and Colleagues 2001

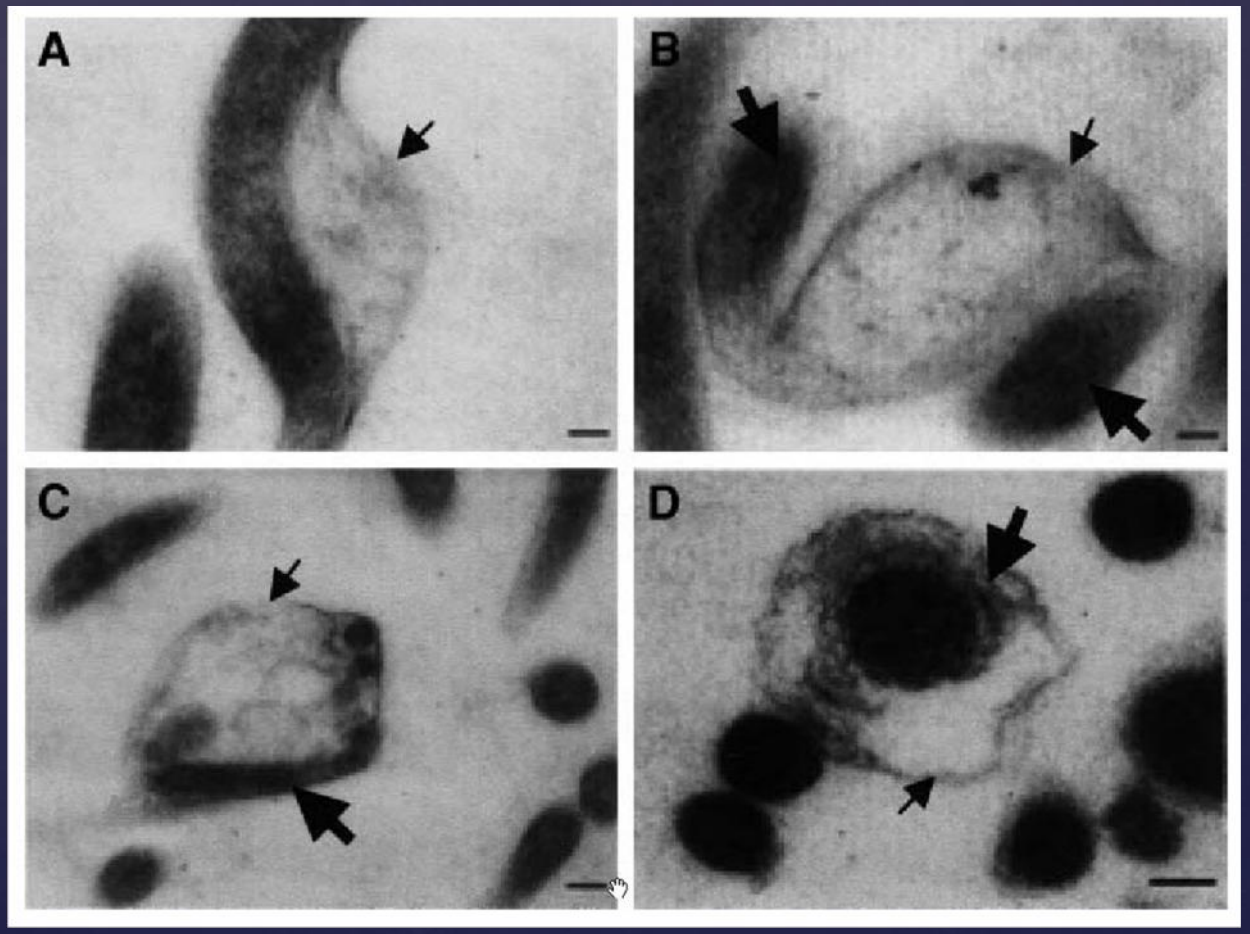
# Infectious nature of Borrelia Cysts in a Mouse model

# Dr. Cinco and the Borrelia Research Group, Italy Unique Research contributions to the biology of Round bodies

# Dr. Murgia Dr Cinco Unique Research findings


1. The borrelia folds itself into its outer surface membrane during Round body formation
2. Limited survival potential is provided by Cyst formation
3. Limited Utilization Amino Acids by Cystic Forms

# Murgia Group contributions
Folding Into Itself to make a Cystic form



Dr. Justin Radolf's Borrelia Research Group, University of Connecticut Unique Research contributions to the Biology of Round bodies

# Radolph Group – University of Connecticut

- A report in the February 2012 issue of Plos Pathogens by the research group at the University of Connecticut School of Medicine headed by Dr Justin Radolf has provided conclusive image data demonstrating
- the formation of **Rounded forms of** ***Borrelia burgdorferi***
- ***during Residence in living Ixodid ticks Which are Triggered by specific genes… (RpoS)***

***Tick midgut contains both Cystic and Spiral forms of Borrelia burgdorferi***

# Witness the Tick gut model… From the Univ.of Connecticut

Spiral Borrelia Burgdorferi change into (Round bodies) in midgut of living Insect Vectors – Ixodes Scapularis

**The Implications of the Spiral to Round transformations……**

**Opinion of Dr Alan MacDonald – Round bodies demonstrate a response to localized Adverse conditions in the Tick Mid Gut, which may be *temporary condition until proper nutrients***

***Are re-established in the tick Mid gut.* Is the gut Round body demonstrating how the Borrelia survives during periods of Adversity**

# Radolph Group –University of Connecticut

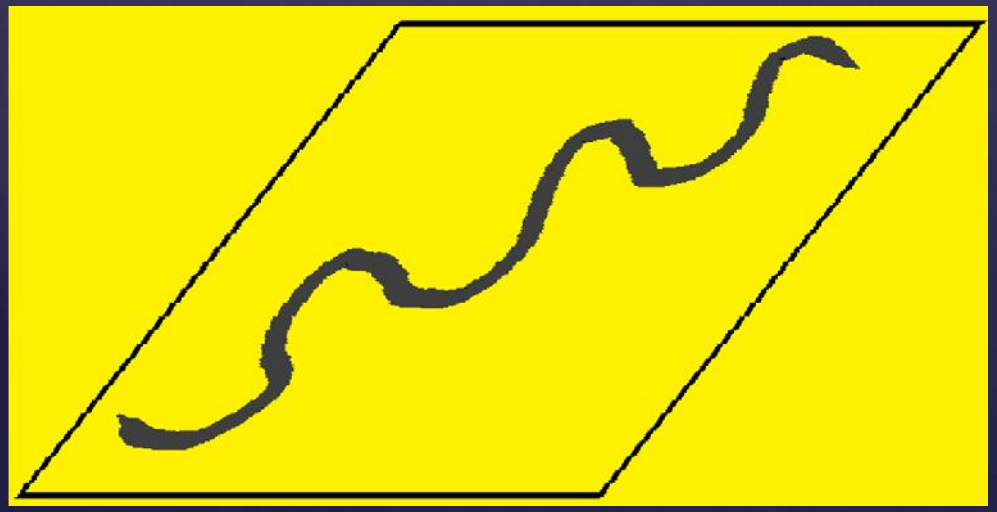
## Live Borrelia survive in Tick body compartments

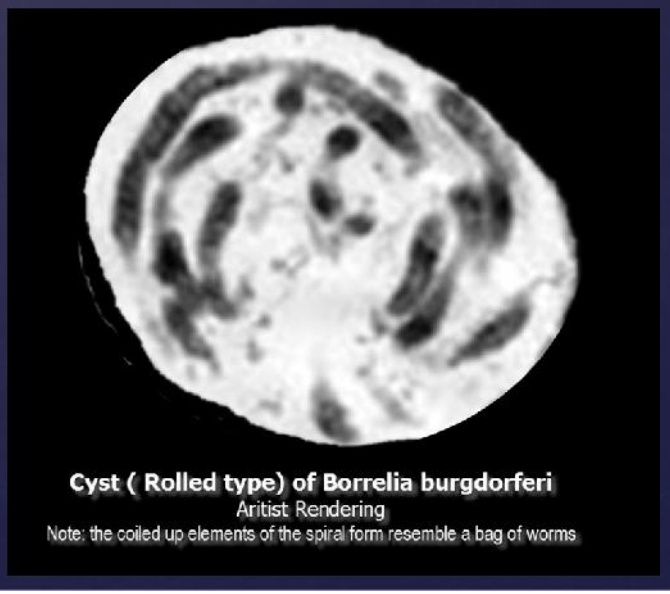
*Mid gut of tick (Only)*

**Tick Mid Gut - Tick Hemolymph**
**Tick Salivary Gland**

**Living Not Motile ???Virulent?**

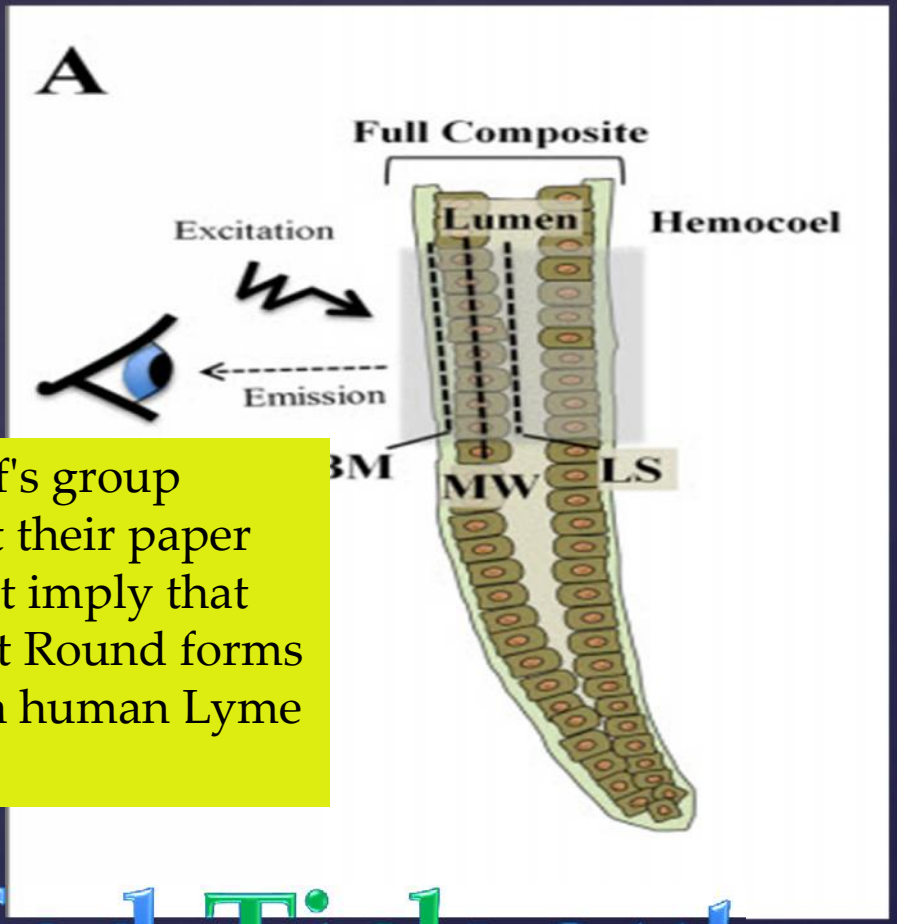
Living Motile Virulent





# Radolph Group – University of Connecticut

The survival of Borrelia burgdorferi In adverse conditions is best exemplified by the presence of living ( Spiral and Rounded) Borrelia burgdorferi in the midgut of Ixodes Scapularis, the North American Vector for Lyme Borreliosis.
…. Comment of Alan MacDonald MD…



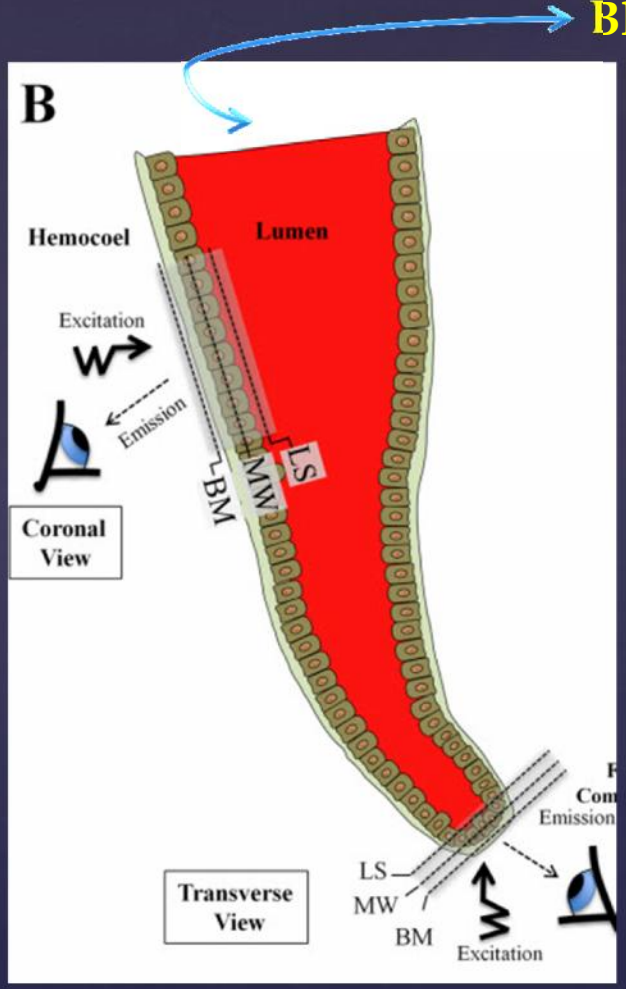

Note: Dr. Radolf's group emphasizes that their paper (below) does not imply that they believe that Round forms are important in human Lyme borreliosis

**Unfed Tick gut**

**Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al**

# Radolf Group – University of Connecticut

*Well Fed tick gut*

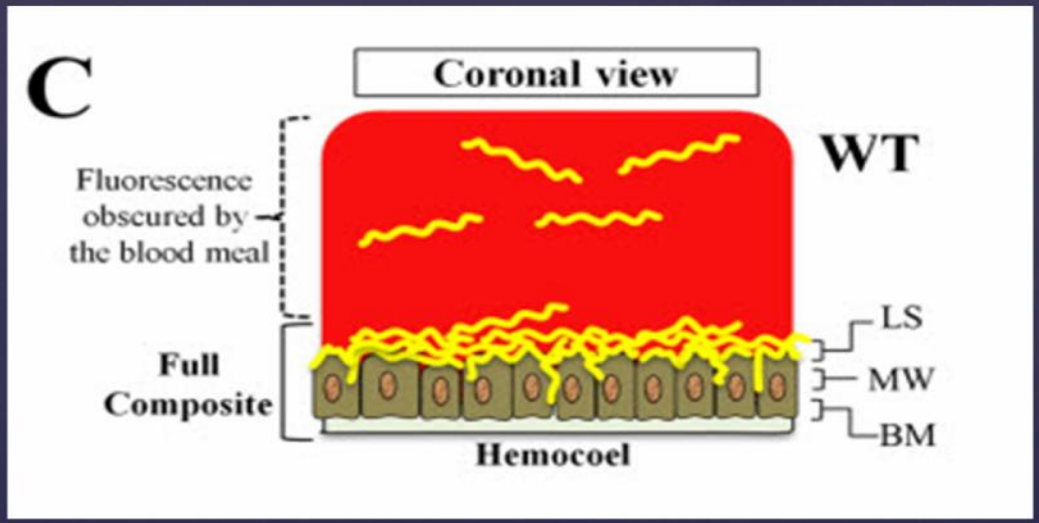



**Spirochetes may be Present in the blood meal**

**Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al**

# Radolf Group – University of Connecticut

## *The Well Fed Tick Gut - Now Containing Borrelia Burgdorferi*




**A Net of spirochetes is attached to the surface of the Tick Gut epithelial cells**

**OR.... Spirochetes may already be present in the tick gut when a blood meal is taken**

**Reference: PLOS Pathogens, Feb 16,2012, Vol 8(2):e1002532 , Dunham-Ems, S. et al**

Hayes and Barbour– Unique Research findings Borrelia burgdorferi
Gemmae and Bacteriophages – Compare with Radolf Group
Net of Borrelia spirochetes in Midgut of tick



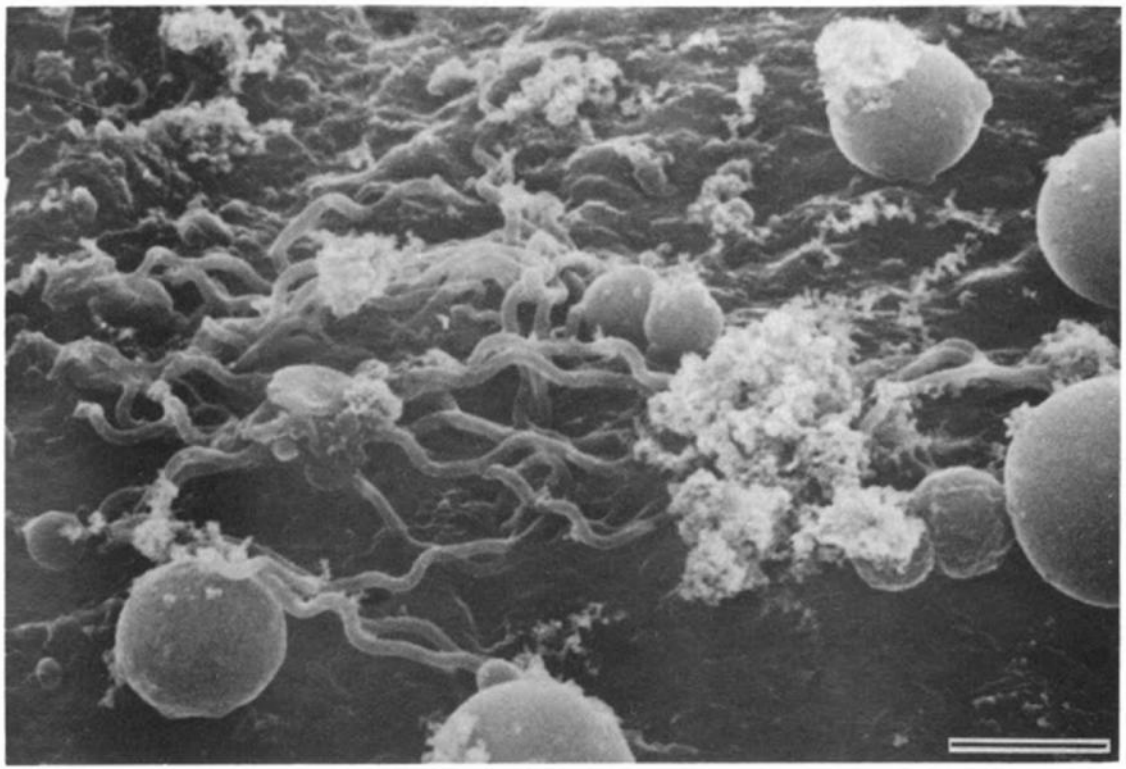
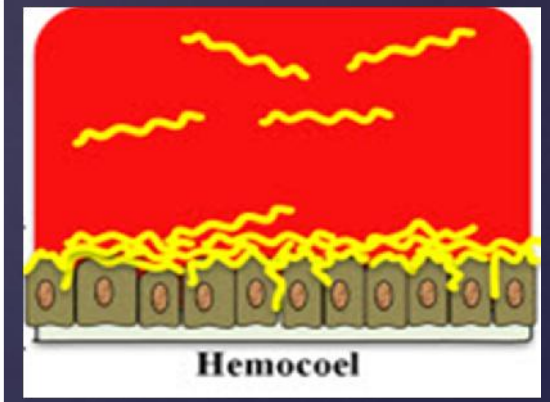


FIG. 8. Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of an *I. dammini* tick. Bar, 2.0 μm. (Photograph courtesy of D. Corwin, Rocky Mountain Laboratories.)



# Radolph Group – University of Connecticut

**Living Ixodid tick midgut Produces**

**of *Borrelia burgdorferi***

**Round Bodies**

**after 72 hours post feeding.**

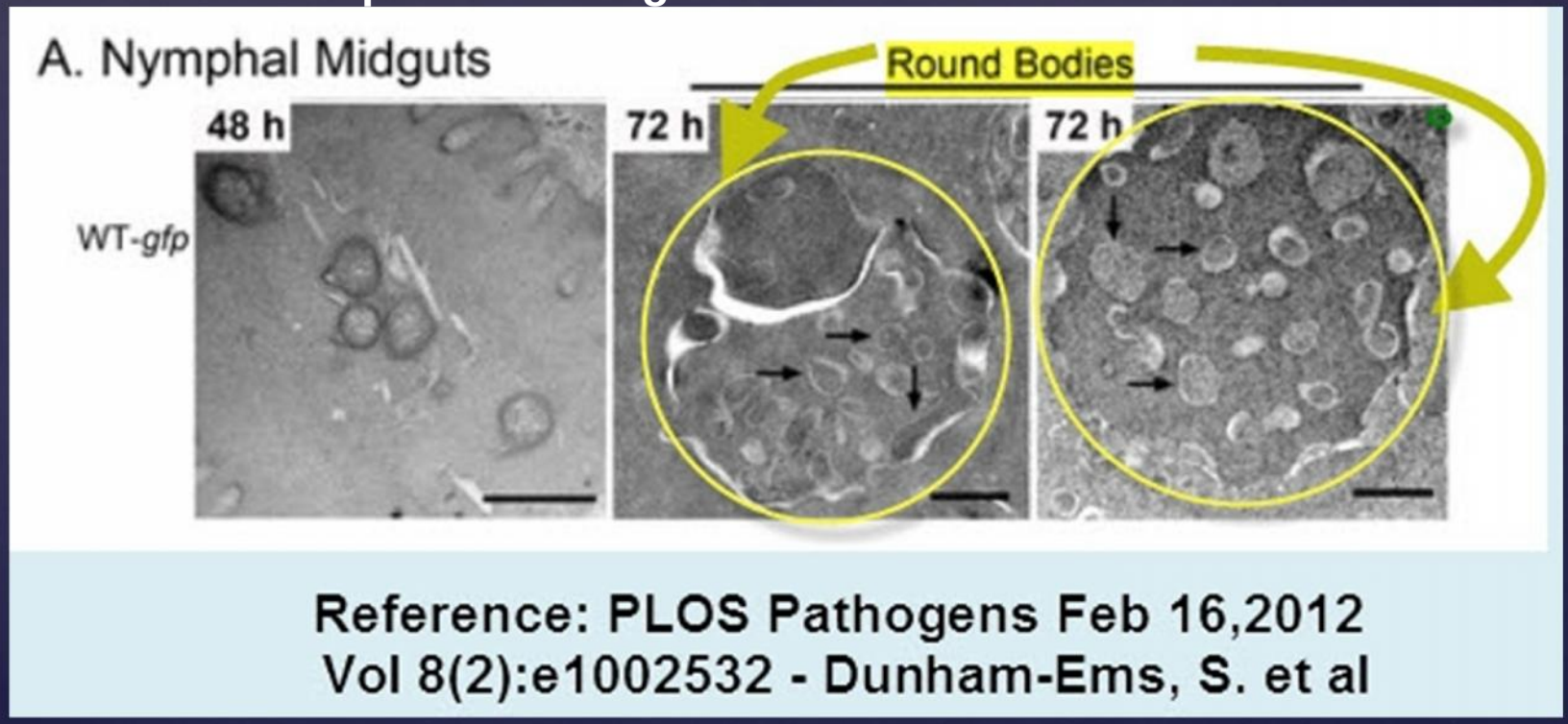



**Figure 6 A – Wild type Borrelia burgdorferi [WT-gfp]**

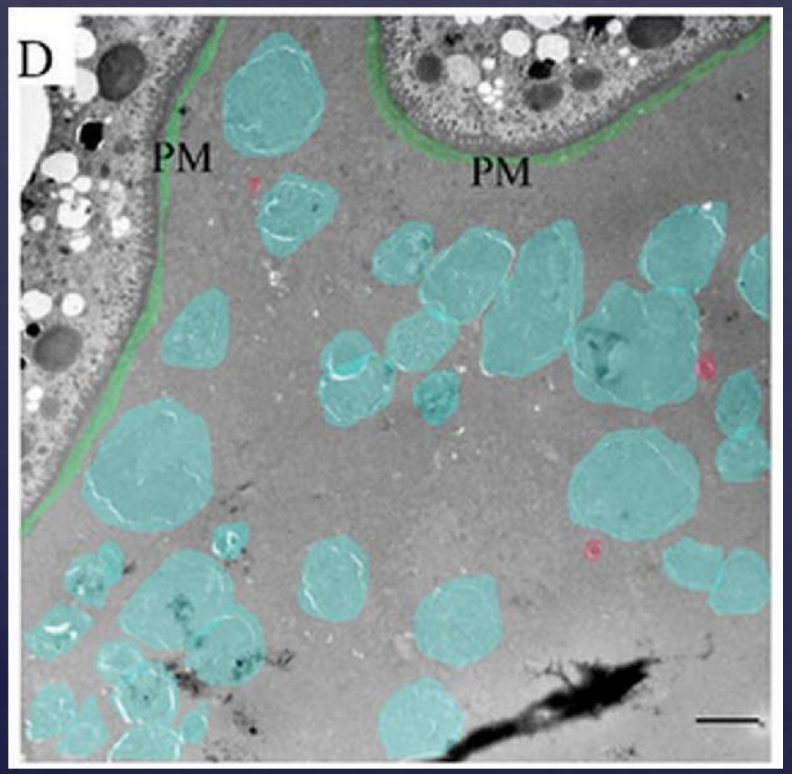
# Radolph Group – University of Connecticut

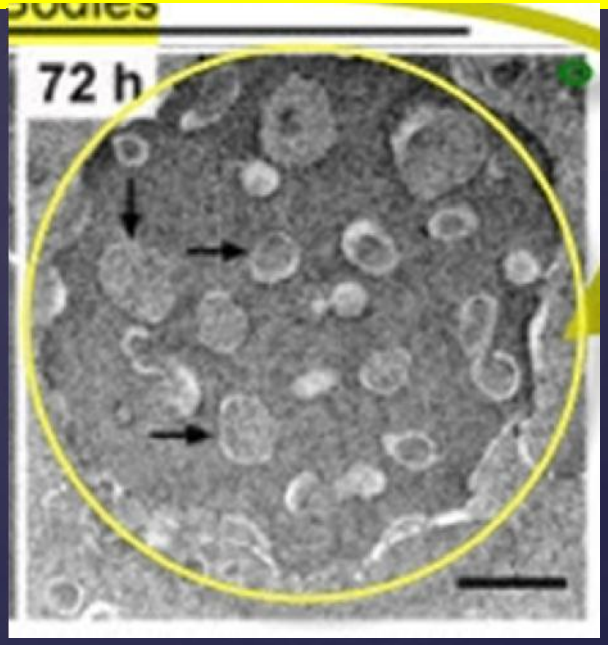
**Living Ixodid tick midgut Produces**

**of *Borrelia burgdorferi***

**after 72 hours post feeding.**

**Round Bodies**



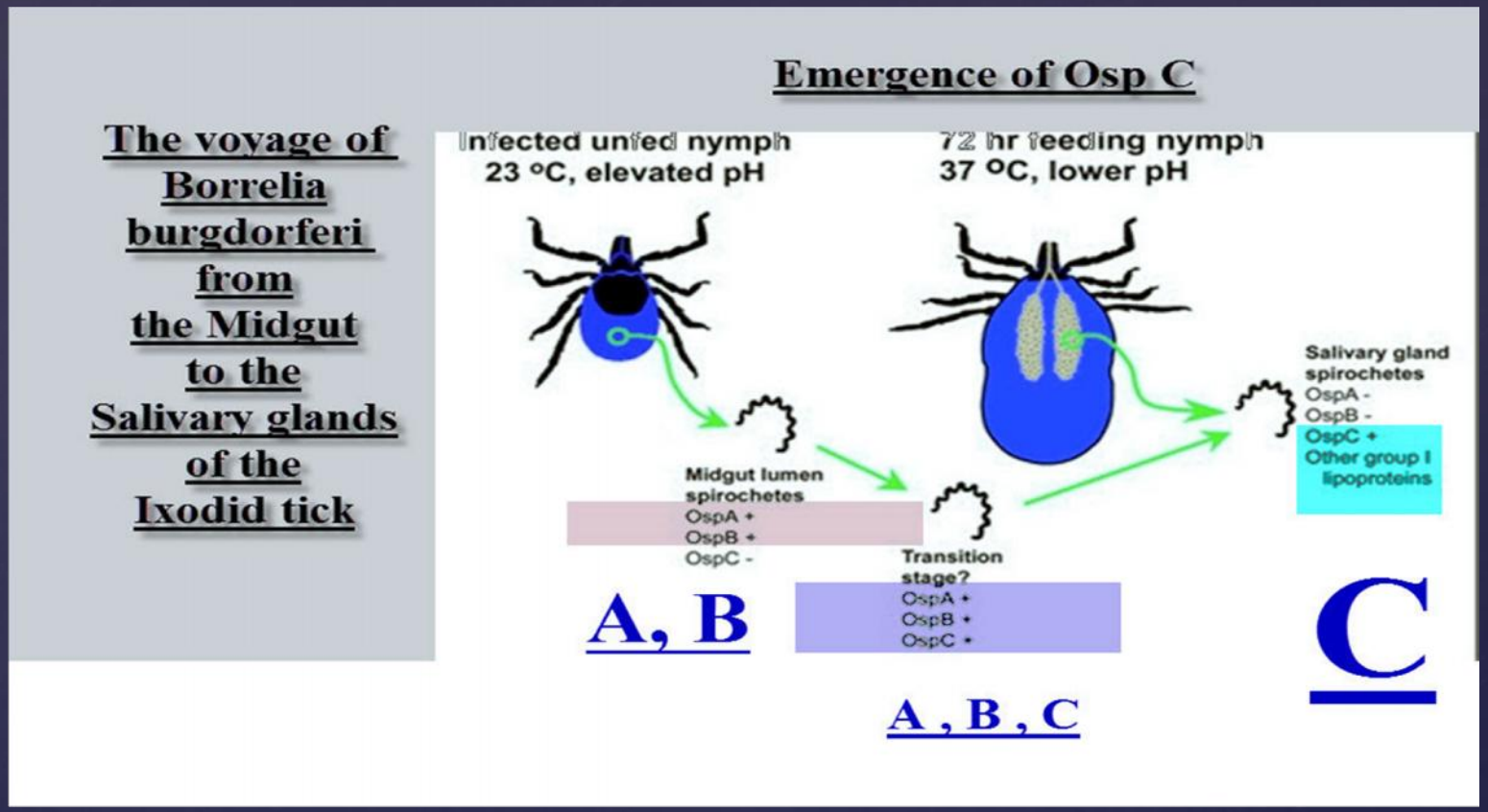




For Comparison with Previous Image (above) – Round bodies in Blue

# Radolph Group – University of Connecticut

**Note: Dr. Radolf's group emphasizes that they did not evaluate the tick midgut for *so called starvation conditions* in the regions where Round Bodies of Borrelia Developed.** The focus of their investigation was to examine the activity of the gene RpoS And the contribution of the RpoS gene to Round Body formation

# Salivary Glands of the Tick

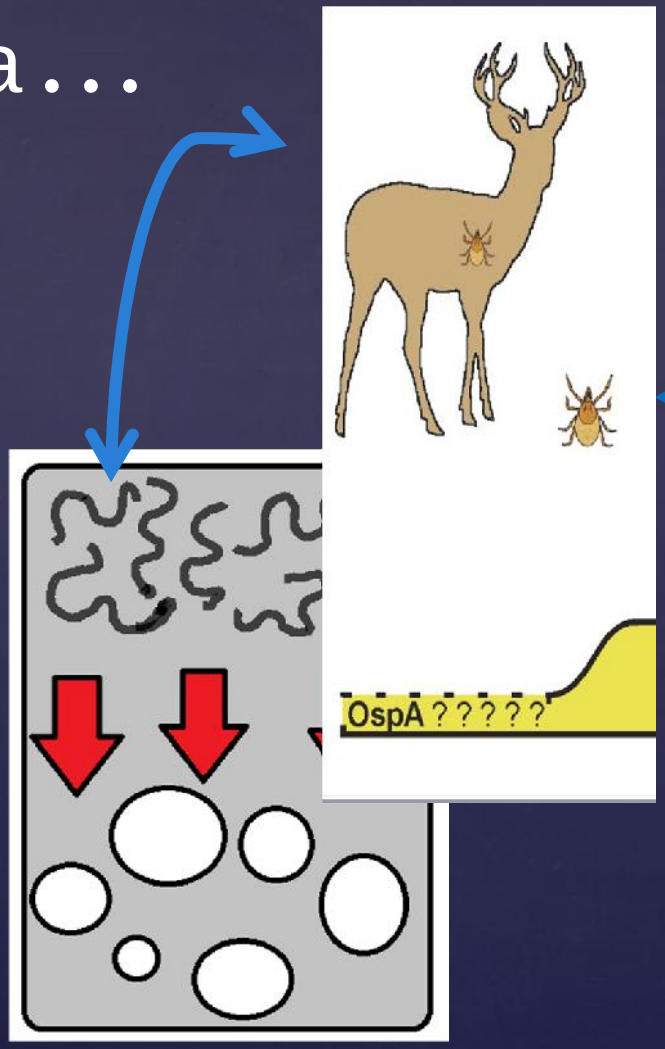



The Spiral form of Borrelia burgdorferi
Passes from the tick's salivary glands to enter the Mammalian bloodstream

Diagram Modified from: Journal of Experimental Medicine, 2004, 199:603-5

# Before the human Bite ...
# There is the Tick bite of the Reservoir mammal containing Spiral borrelia...

Image Credit: Michal Krupka , MD




Reservoir Animal Blood Contains Spiral forms Of Borrelia Burgdorferi

Nutrient conditions in gut of Tick

**Starvation conditions** …MacDonald's personal comments…

the nutrients in the blood meal
are eventually exhausted…

…..The spiral forms run out of nutrients

Genes Sense the lack of nutrients…

Genes trigger changes in the spiral forms of Borrelia burgdorferi

Conflicting data –

-- Brorson & Brorson– No time limit stated for reversion of spiral forms from cystic forms.

-- Gruntar et al – Reversion to spiral is possible at any time – (even after freezing/thaw of cysts)

-- Murgia and Cinco - Reversion to spiral is limited by the conditions of Stress (pH, H2O2, Heating) with time to revert ranging from 10 to 70 days.

-- Alban et al – limited regeneration to spiral motile spirochetes up to 8 days.

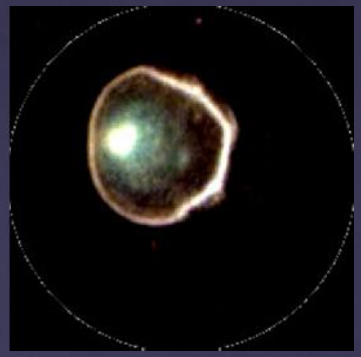
# Are Cystic Borrelia Robust or Fragile?

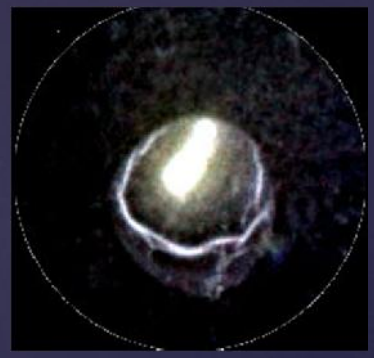
# Norwegian Group –Dr. Morten Laane, Oslo Unique Contributions

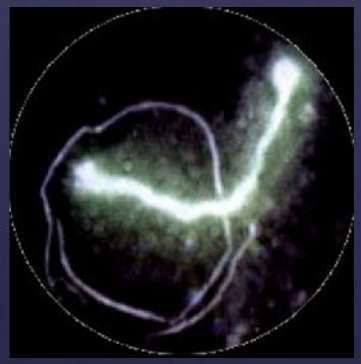
1. Cyst Envelope is rendered in High Definition by Treatment with 45% Acetic Acid -
   A Wrinkled and ***refractile cell envelope*** is visible
2. String of Pearls forms accompany Cystic forms
3. Application of techniques to examine
   Fresh Blood Smears for
   Cysts of borrelia burgdorferi

# Norwegian Group – Dr. Morten Laane, Oslo ,Unique Contributions

**Highly Refractile Envelopes of Cystic forms (round bodies)**
***WRINKLED appearance of Intact Envelopes***
***After Acetic Acid treatment***



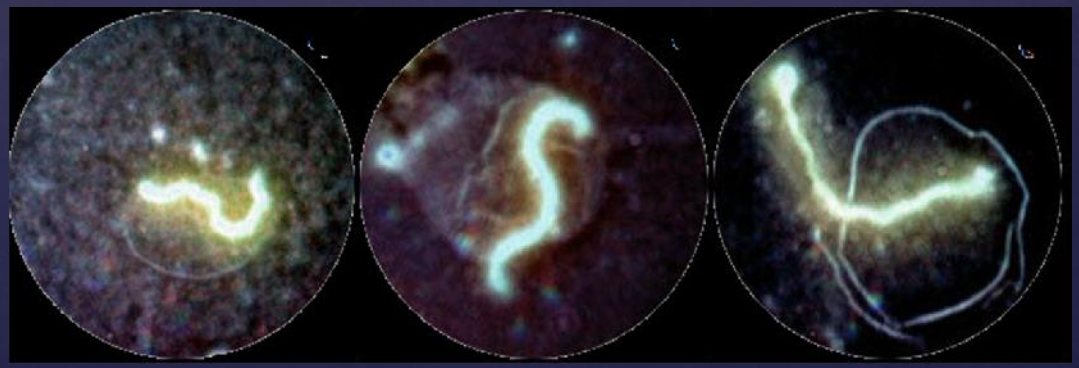




**Rupture of the Envelope –**
**The envelope after rupture is no longer WRINKLED, ….**
**Spirochetes Emerge**

# Norwegian Group, Dr. Morten Laane – Oslo, Norway

ACETIC ACID ENHANCES
VISUALIZATION
OF ENVELOPE
OF THE
ROUND BODY ( CYSTIC FORM )



Norwegian Group – Dr. Morten Laane, Oslo,

**The String of Pearls** – First Identification in Human blood – Patient with Borreliosis

# Alan MacDonald, MD Unique Research Findings on the Biology of Round Bodies

1. Human Autopsy tissue – Identification of Cystic forms of Borrelia burgdorferi
2. Culture of Human Cerebrospinal fluid – Recovery of Cystic forms of Borrelia burgdorferi – Without associated Spiral forms
3. Biofilms of Borrelia burgdorferi - Cystic Borrelia Contribution

# MacDonald's Unique Research Findings concerning the Biology of Round Body

1. **Round Bodies of Borrelia Burgdorferi are present in Autopsy Brain tissue From patients with Alzheimer's Disease.**

2. **Imprint (Fingerprint) technique is highly effective in detection of Round bodies in fresh human tissue.**

3. **Molecular Beacons ( Highly specific DNA probes) maybe utilized to Detect Round Bodies in human Diseased tissue (Borreliosis)**

5. Round bodies are *capable of Intracellular residence in Human Brain Cells.*

# MacDonald's Unique Research Findings concerning the Biology of Round Body

6. *Round Bodies are Immuno Reactive for Murine Monoclonal Antibody H9724 which recognizes Flagellin B epitope.*

7. *Round bodies may demonstrate Inversion ( inside/out ) transformation of their envelope constituents, such that surface antigens of the Spiral borrelia From which they are formed, are hidden on the inner regions of the round Body.*

8. *Copy number ( Chromosome + Plasmids) for Borrelia burgdorferi probably equals the Genome of Borrelia Hermsii ( i.e.. 16 copies of chromosome + plasmids per spiral or Cyst)*

# MacDonald

Before discussing the Alzheimer's studies and the

Biofilms of Borrelia burgdorferi

I will report on the Cerebrospinal Fluid Primary Isolation

Of Borrelia Burgdorferi Cystic forms study.

# MacDonald

- Cystic Borrelia, when present in human
  Cerebrospinal fluid, are viable after long term Freeze/thaw in storage – and Long term "Freezer Desiccation" (freezer burn)

- **The Cystic borrelia in Spinal fluid are capable of replication**, although the growth is very slow and months may pass before organisms are visible under the microscope.

- **Dense Brorson-type nucleoids are present in Borrelia cysts undergoing slow long term growth. NO YOUNG CYSTIC forms appeared in the long term cultures.**

## Conclusions –Spinal fluid culture study

MacDonald – Cystic Forms of Borrelia burgdorferi

# Recovery of Borrelia Burgdorferi from Frozen human spinal fluid

- **This short presentation illustrates the feasibility of in vitro culture for Borrelia burgdorferi from human body fluids obtained from patients with Clinical and Laboratory documented evidence of Lyme Borreliosis.**

# Human Cerebrospinal fluid as an Adverse environment for spiral forms of Borrelia burgdorferi

Spinal fluid is an ultrafiltrate of plasma. Normal spinal fluid contains limited constituents which are required for the spiral form of Borrelia burgdorferi to maintain its shape and motility in serum

# MacDonald

## A study of Frozen Spinal Fluid from a patient with well documented Neuroborreliosis

1. The study which is described below was designed to replicate the research of Dr. Oystein Brorson and Dr Sverre-Henning Brorson.
2. Are frozen Borrelia in stored spinal fluid specimens actually viable ( as in the Gruntar experiment) ???

## MacDonald

# Cystic Borrelia burgdorferi cultured from human cerebrospinal fluid

**60 year old woman presented with Mononeuritis multiplex**

**Lyme disease Western blot studies were positive**

**In blood and cerebrospinal fluid to meet CDC criteria for Intrathecal antibody production ) [IgA, IgG IgM detected on Western Blots of Cerebrospinal fluid]**

**Studies performed at Imugen Labs, Norwood, Mass**

**A full panel of cerebrospinal fluid testing was completed Cell counts showed no Pleocytosis. Chemistry studies were within normal limits. Routine cultures of CSF yielded no growth of pathogens.**

**After all testing was completed, one unopened tube of CSF obtained at the time of lumbar puncture was Frozen at minus 20degrees C. and maintained in the freezer for one year.**

# MacDonald

## Permission to Undertake this study

Application was made and approved by the Institutional Review Board of the Hospital. The patient had been diagnosed and successfully treated for Neuroborreliosis ONE YEAR prior to the beginning of the study.

## Laboratory protocol - Routine

Frozen CSF specimens are by usual protocol retained in the Laboratory Freezer. After one year of frozen storage the specimens are discarded

# MacDonald

## Protocol
## Cerebrospinal fluid
## Borrelia culture study

- RPMI tissue culture medium with CMRL 1066 (equal parts v/v) Was added to the tube of Frozen CSF using strict aseptic technique in a Laminar Air Flow laboratory hood ( to avoid contamination)
- The tube (containing 1cc of frozen CSF) was completely filled with RPMI/CMRL tissue culture medium. The tube was then closed with the provided sterile plastic screw cap. It was incubated at room temperature.

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## Initial Microscopic Examination

- At time zero, one drop of fluid was removed from the cultured Cerebrospinal fluid using aseptic technique,
- Placed on a clean glass slide,
- A glass coverslip was applied.
- Darkfield examination of the fluid under 500x and 1000x oil immersion magnification was completed. No spiral spirochetes were identified. No round cystic forms were noted at time zero

# MacDonald

## Weekly Inspection of the culture

- The tube was inspected weekly by external examination only (without opening the tube).
- No changes in the color of the pH indicator were seen.
- No turbidity was seen.
- No clumps of material within the tube were seen
- The tube remained pink and crystal clear

## Incubation at 24 degrees C. was allowed to proceed

# MacDonald

After 11 months of incubation
the author became aware of the utility of Oligreen ® Invitrogen Inc. for its utility in demonstrating Picogram amounts of DNA, and in particular the use of Oligreen for the detection of viral sized elements in fluorescence microscopy.
Oligreen stains single strand DNA.

# Oligreen staining technique

## MacDonald

One drop of the culture fluid was removed from the tube using strict aseptic conditions, placed on a clean glass slide, covered with a clean glass coverslip, and excess fluid was gently expressed from the slide preparation using a sterile gauze pad and gentle pressure to remove all air bubbles. **Oligreen stain was then applied as a line of liquid along one short axis edge of the coverslip.**

MacDonald

# Oligreen Staining technique (2)

- No pressure was applied to the glass coverslip after the line of Oligreen stain was in place.
- Capillary action was allowed to gradually mix the advancing edge of the Oligreen stain with the coverslipped culture fluid.
- The advancing edge of the Oligreen stain was observed by Epifluorescence microscopy with an excitation/barrier filter cube appropriate for Oligreen.

## MacDonald

# Epifluorescence Microscopy Technique

Oil immersion lenses to achieve 500x magnification ( for scanning) and 1000x magnification (for photography) were used in tandem to photograph fluorescent cystic forms from the culture. No spiral forms of Borrelia burgdorferi were found.

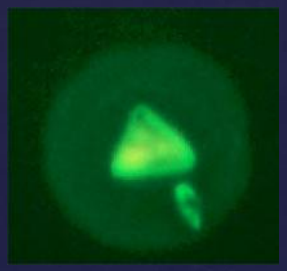
# MacDonald

Morphologies of Cysts of Borrelia burgdorferi in RPMI long term culture

--No Cysts corresponding to the Brorson category of so-called "Young Cysts" with Rolled up spirals of borrelia Within the Cyst, were recovered. All of the Cysts cultivated were Brorson type " Aged Cysts" – with either Dense staining nucleoids or with Granular forms inside the cysts. Some "empty" cysts were found….This is the first report of such "empty" cysts of Borrelia.

--Many of the observed cystic forms contained punctate granular elements. Variability in the number of granules per cyst were evident.

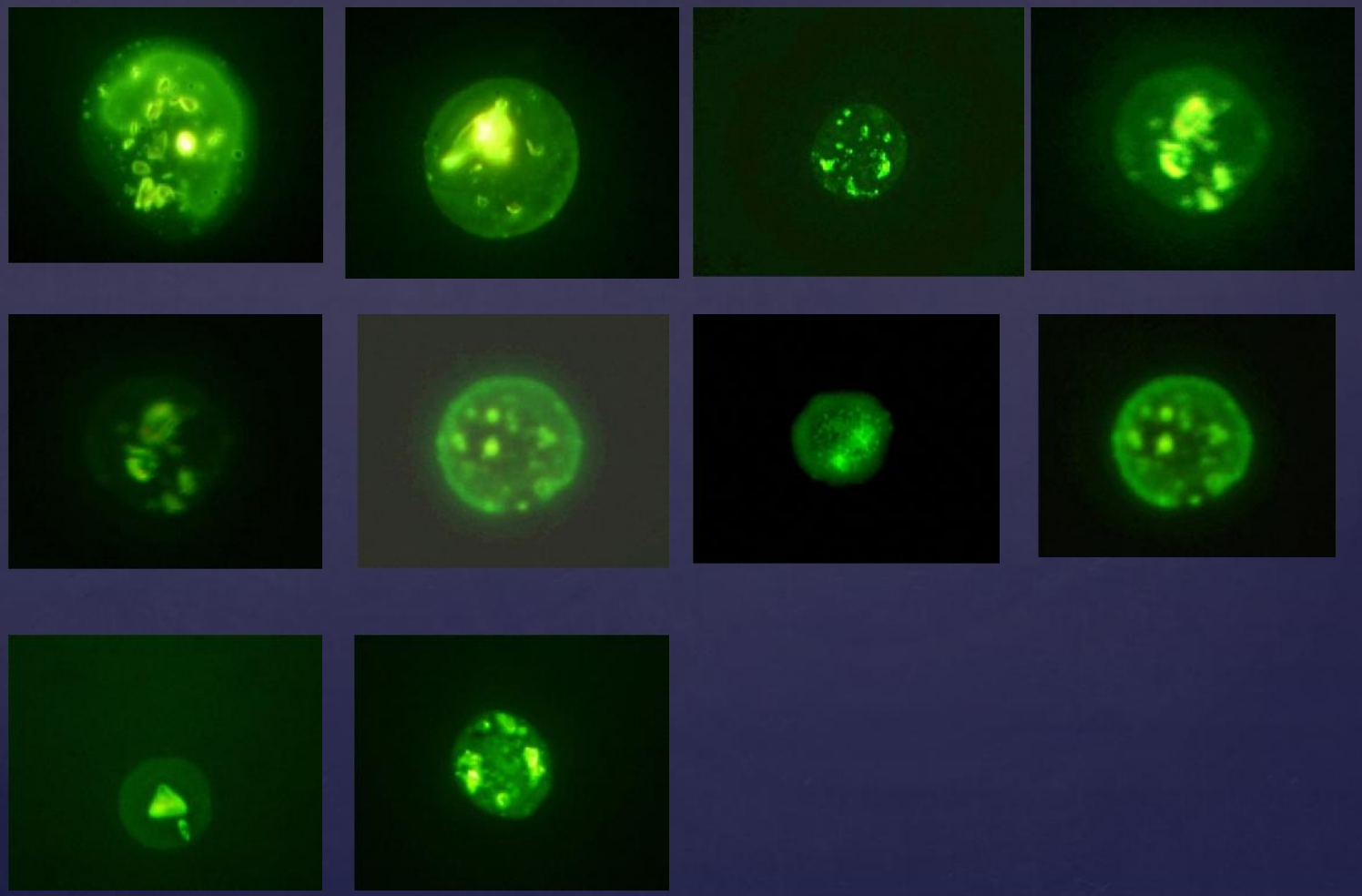
--Some of the cysts contained no granular or solid content, but showed a uniform ground glass appearance.

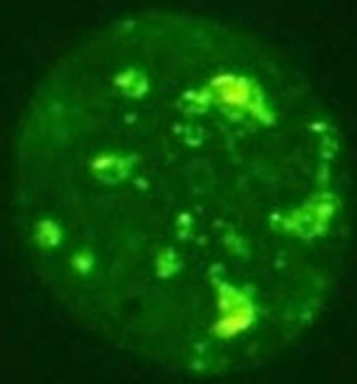


--Some of the cysts contained large nonround nucleoid elements with sharply angular profiles.

-- ( These did not resemble the Gemmae forms of Burgdorfer and Hayes)

# MacDonald – Image Gallery of Spinal Fluid Borrelia Cyst forms





Cystic Borrelia burgdorferi cultured from human Cerebrospinal Fluid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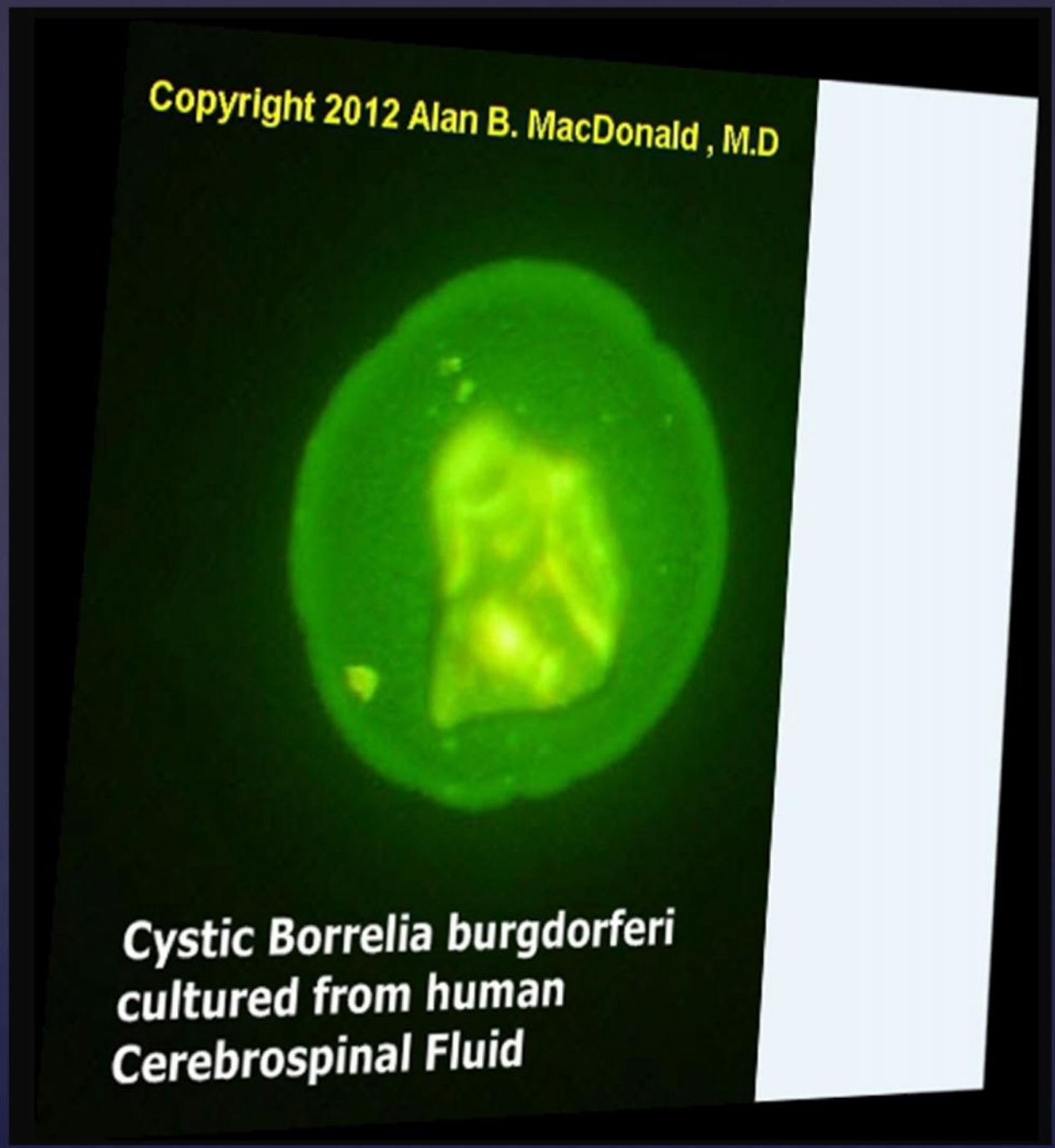
Cystic Borrelia burgdorferi
cultured from human
Cerebrospinal Fluid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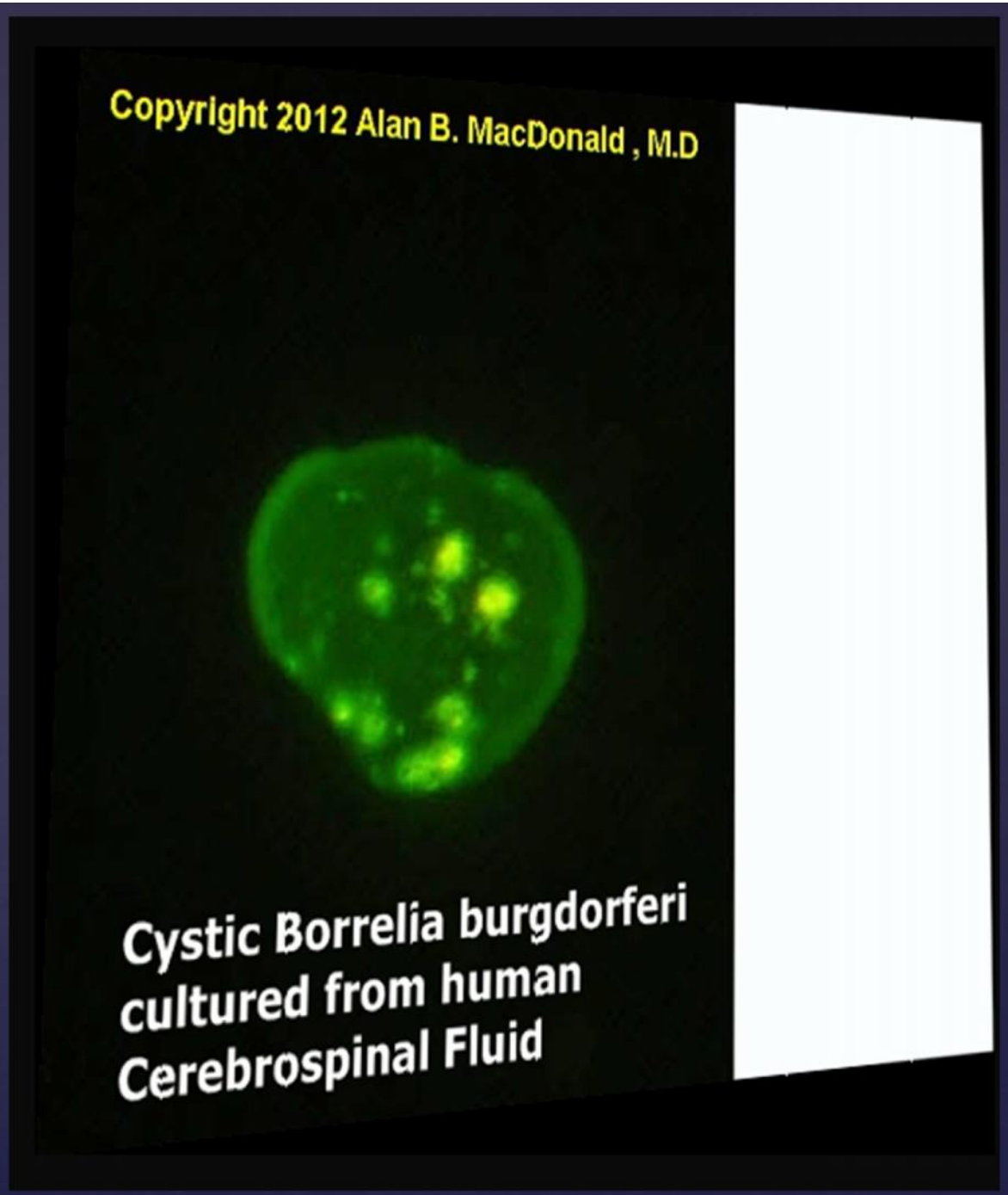
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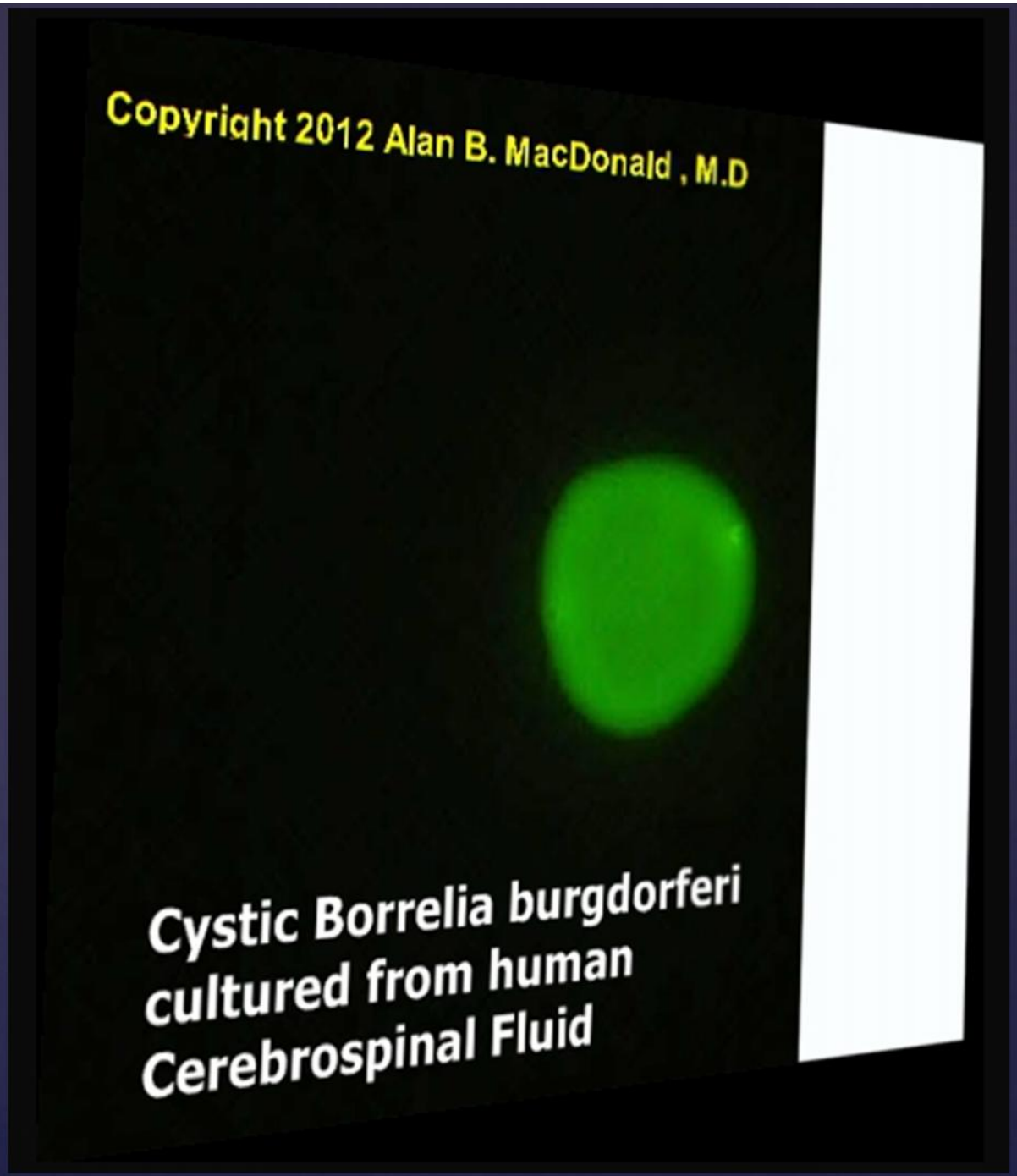
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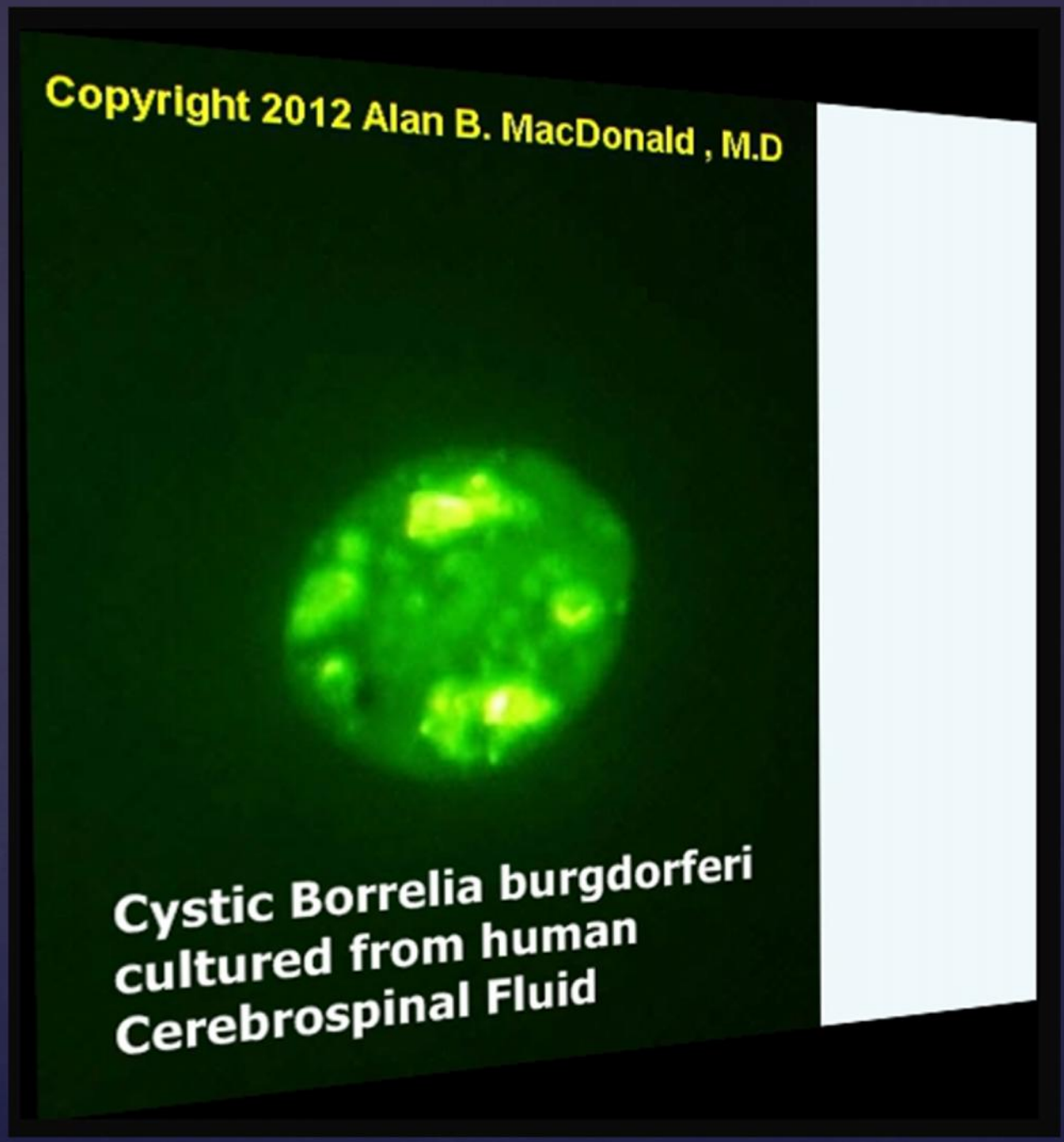
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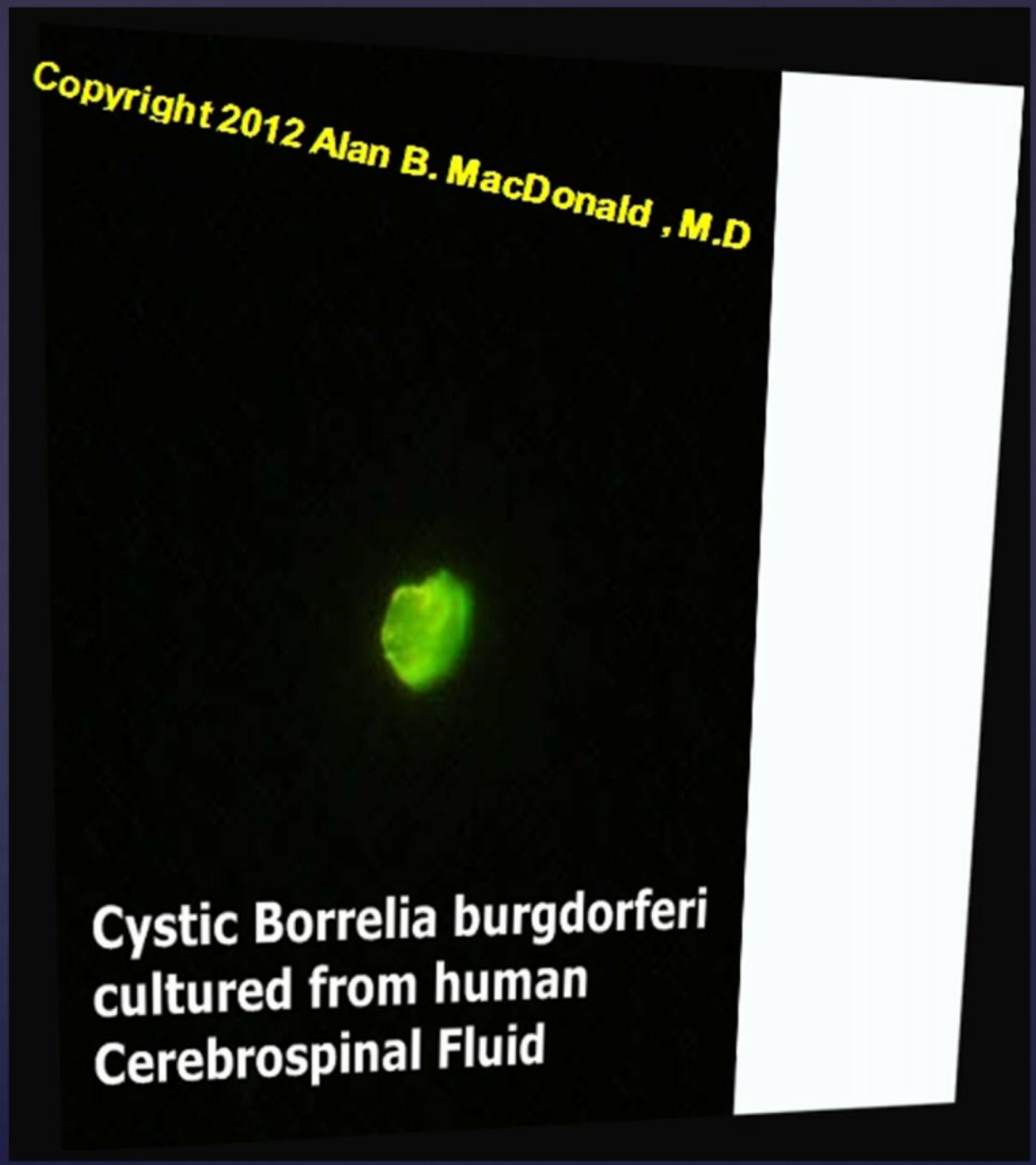
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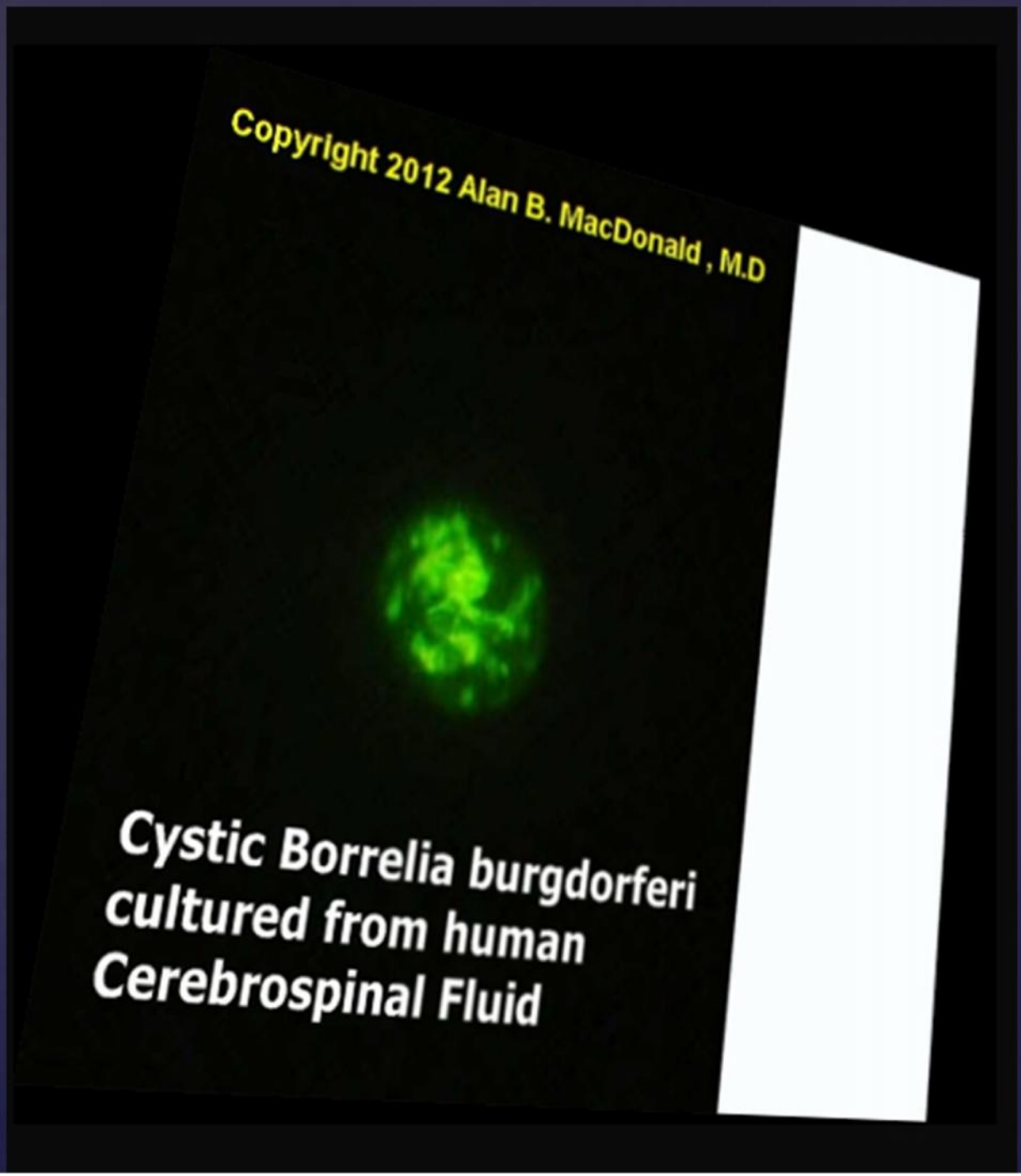
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# Why was RPMI with CMRL1066 used instead of BSK H?

- Drs. Brorson and Drs. Alban and Nelson have demonstrated that when Borrelia burgdorferi is cultured in RPMI, the spirochete survives, but its form becomes Cystic. Cystic forms rapidly revert to spiral and motile in less than 1 minute when nutrients are provided.

- In this experiment, No BSK H was available in the laboratory, but an abundant supply of RPMI/CMRL was available for harvesting tumor tissue for immunohistochemistry studies and for genetic studies.

# MacDonald

## Patient follow-up two years after Intravenous Ceftriaxone Therapy

- Patient reports resolution of the symptoms of painful peripheral neuropathy.
- Patient reports no complaints in joints, skin, or cardiac systems
- Patient reports no cognitive difficulties
- Patient not currently taking antibiotic therapy.

# MacDonald

What is Missing-
From the Images of Borrelia Cystic forms recovered from Long term culture of Human cerebrospinal fluid???

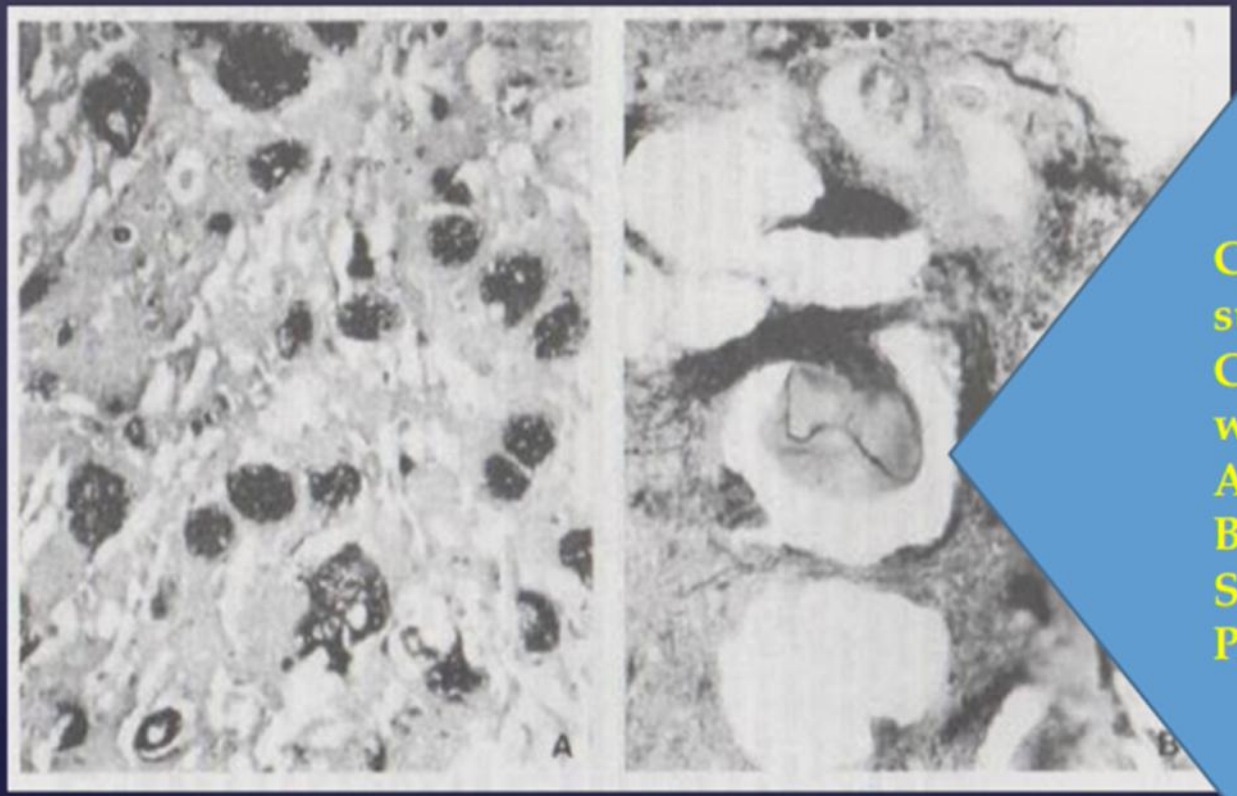

Answer: No "young" Cystic Forms (rolled up type) were recovered from Long term Culture of Human Cerebrospinal fluid .

All of the Cystic forms of Bb, were so called "Aged" Cystic forms of Bb.

# MacDonald – Alzheimer tissue Studies – Contribution of Cystic Borrelia in diseased Brain tissue

Cystic forms of Borrelia – Reactive with Monoclonal Antibody H9724 [for Flagellin of Borrelia burgdorferi] – Recovered from fresh Brain tissue Imprints..

# MacDonald – Alzheimer's Disease tissue - studies for presence of Borrelia burgdorferi




MacDonald comment: Compare "wrinkled Borrelia Cysts from Oslo, Norway Study – Dr. C.M. Laane

**Cyst with "wrinkled" surface**
**Contour – merges with Black staining Alzheimer Plaque – Bielschowsky Silver Stain for Alzheimer Plaques**

# MacDonald – Alzheimer's Disease tissue Studies – Further Immunohistochemical Evidence for Borrelia burgdorferi in Brain tissue

Alzheimer Brain – Fresh tissue Imprints – Spirochetes Reactive to Monoclonal antibody H5332 for OspA of Borrelia burgdorferi
( Mab H5332 – a gift from Alan G. Barbour MD,Rocky Mtn Lab, NIAID

MacDonald – Alzheimer's Disease Brain Fresh tissue in Culture ( BSK) showing Spirochetes by Darkfield Microscopy

# MacDonald- Alzheimer's Brain studies for Borrelia Spirochetes


Note:
The Cyst has "wrinkles" in its surface

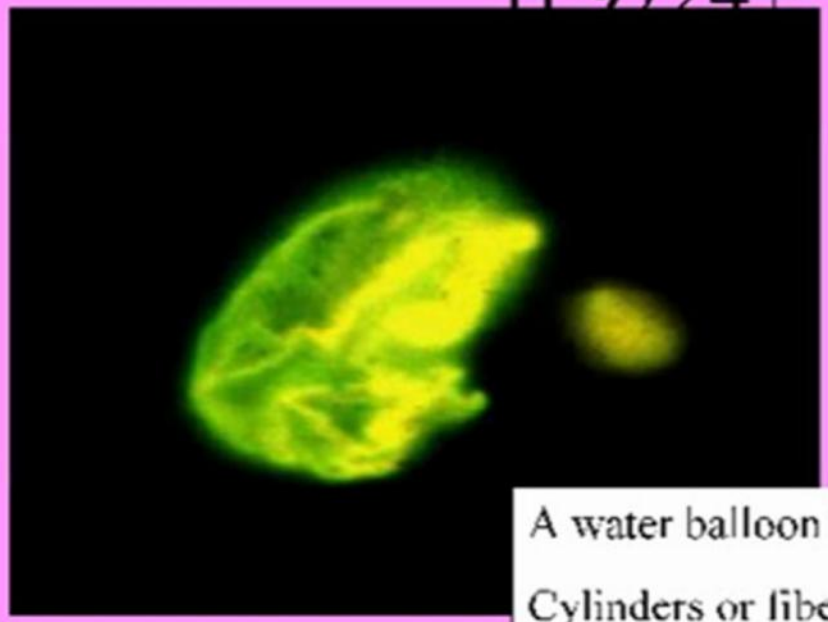
Cystic Borrelia – Control –Oslo Study

Cystic Borrelia in Alzheimer's Brain

MacDonald's Editorial Comment: Acetic Acid is present in fixative used in each of the above preparations; and it Enhances the Cyst Envelope.

# MacDonald – Borrelia Cysts in Alzheimer's Disease

MacDonald – 1987
Cyst form Alzheimer Brain
H 9724+



A water balloon like structure with

Cylinders or fibers inside it

# Borrelia Cysts and Borrelia Biofilm Communities

Established Data points:

1. Leptospires form Biofilm Communities. Some of the Leptospire Biofilms are “Floating Biofilms”, that is To say that these variants of Biofilm do not attach To an Abiotic surface or to a Biotic surface.

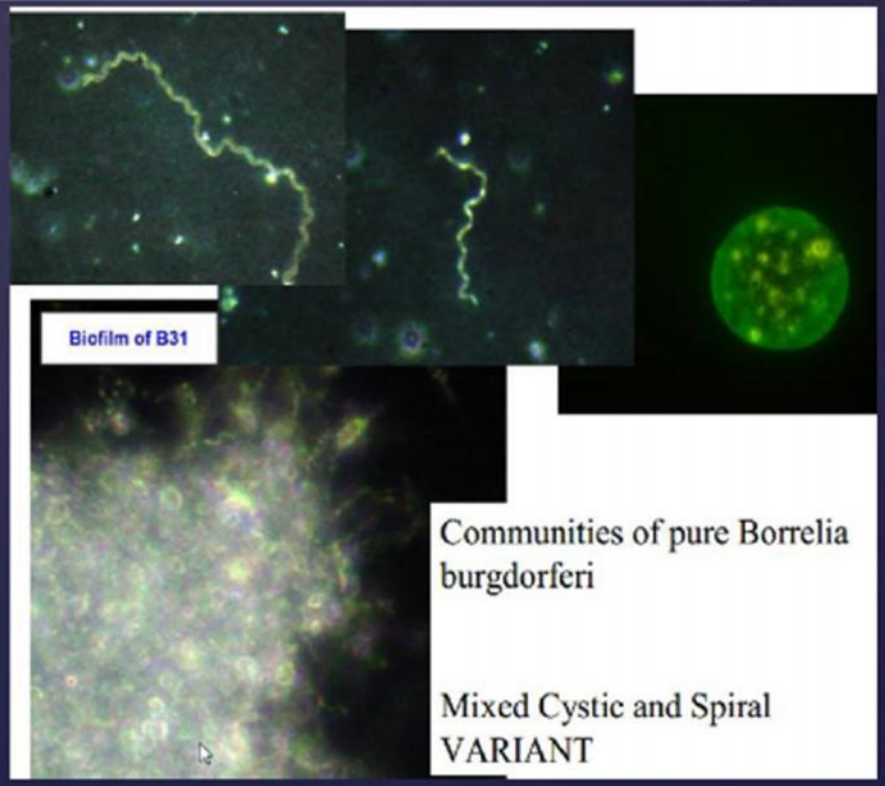
2. Oral Treponema form Biofilm communities in the Oral cavity, which constitute Dental Plaque.

# Borrelia Cysts and Borrelia Biofilm Communities

Definition: BIOFILM ( Dr. W.J.Costerton etal,1995)

"Complex communities of micro-organisms attached to a surface or interface
Enclosed in an exopolysacccharide matrix of microbial and host origin to Produce a spatially organized three dimensional structure."

from: Medical Biofilms, 2003, Jass, J.Surman, S, Walker,J.T.



# MacDonald – Borrelia Cysts and Biofilms of Borrelia

# MacDonald – Borrelia Cysts and Biofilms of Borrelia



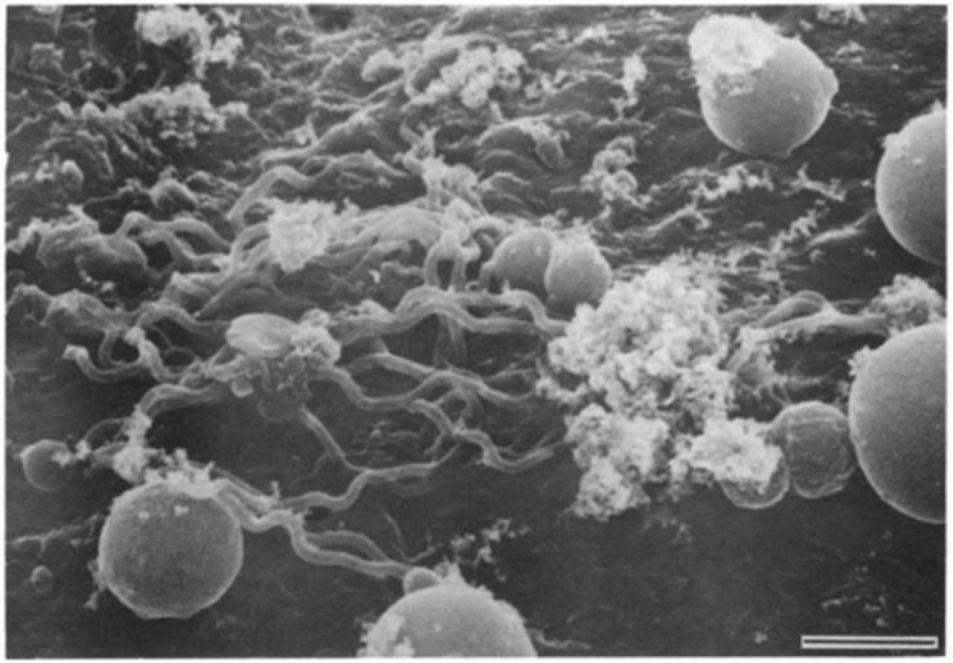
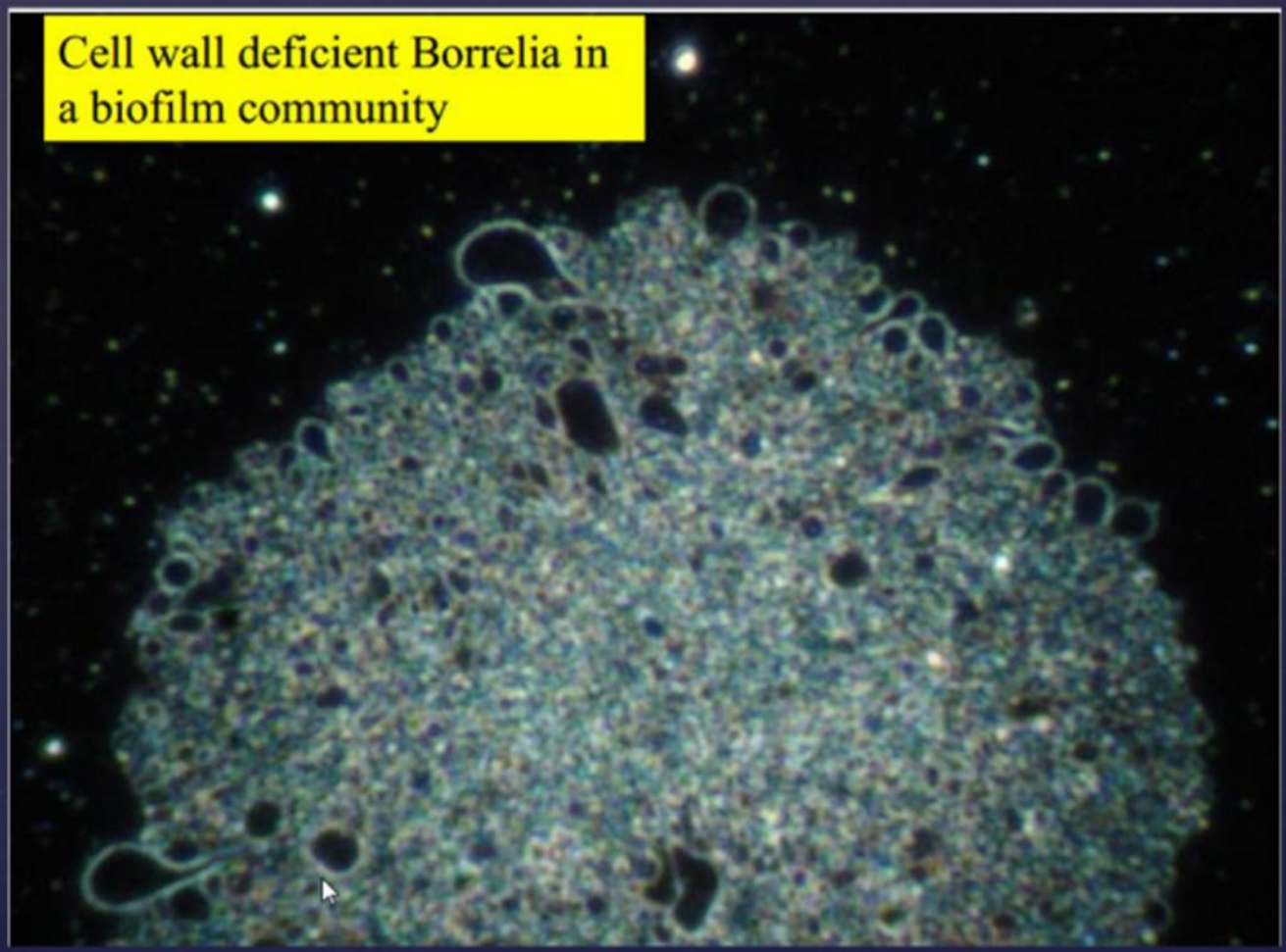


FIG. 8. Scanning electron microscope picture of *B. burgdorferi* spirochetes associated with the epithelium of the midgut of an *I. dammini* tick. Bar, 2.0 μm. (Photograph courtesy of D. Corwin, Rocky Mountain Laboratories.)

**MacDonald's Editorial comment:**
**It is plausible that the NET of Spirochetes described by Burgdorfer and Hayes and again described by Radolf's group in 2012, is indeed a Community of Spirochetes and Cystic forms – Ergo – A Biofilm!!**

# MacDonald – Spheroplast Borrelia Biofilm variant



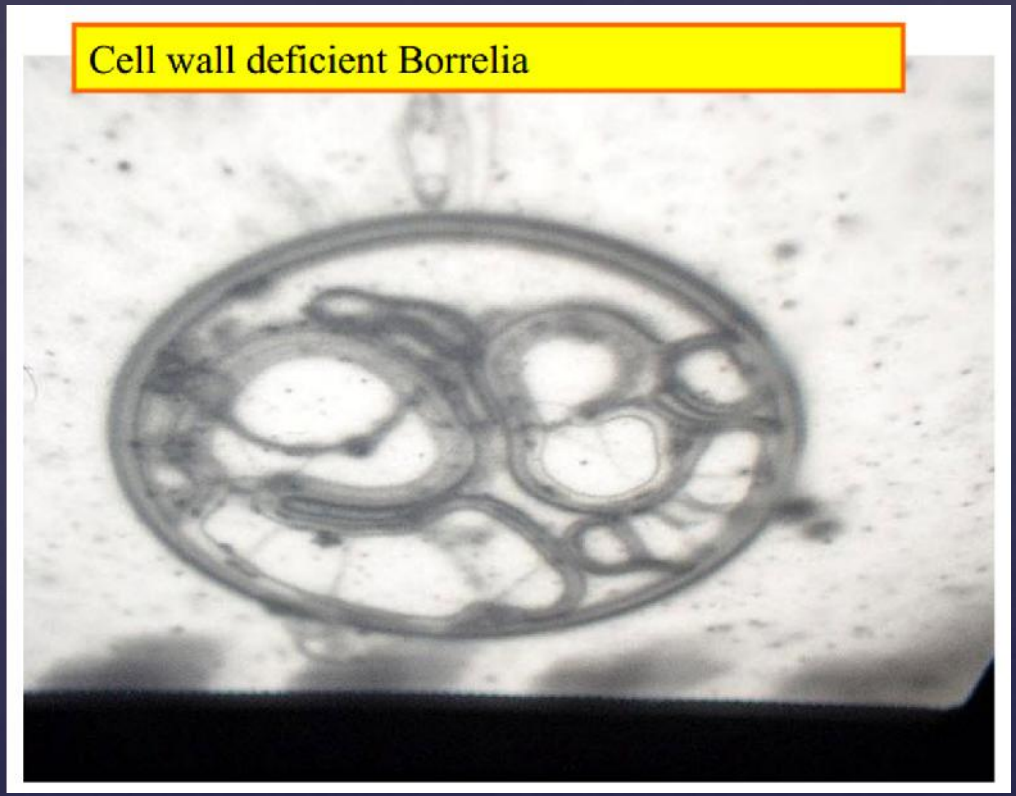
Spheroplasts –
What makes them different? After all, they are Round, Contain DNA, and are derived from Spiral borrelia..

# Spheroplasts
# Do Not Have a Cell Wall

Cell Wall Deficient (CWD)

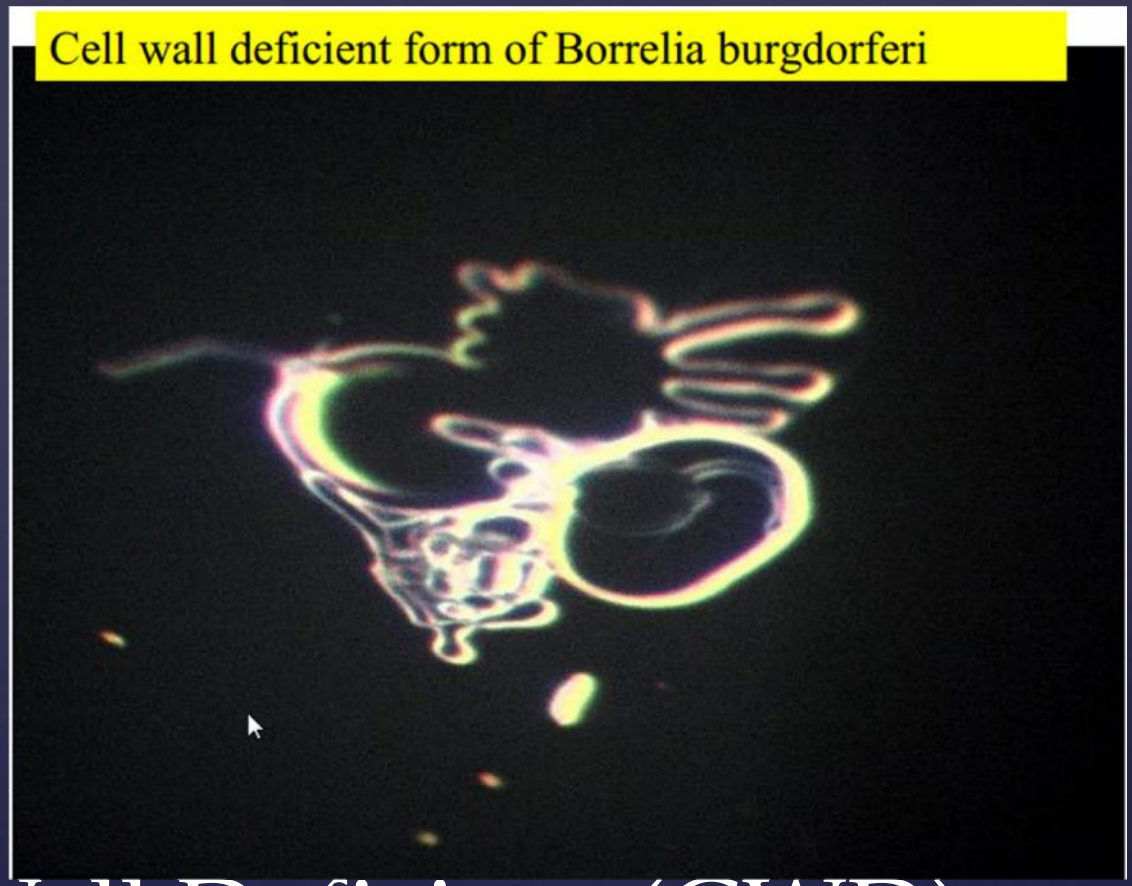
L forms - ( named for the Lister Institute where they were first described

# Spheroplasts
# Do Not Have a Cell Wall




Cell Wall Deficient (CWD)

# Spheroplasts
# Do Not Have a Cell Wall




Cell Wall Deficient (CWD)

# Spheroplasts
# Do Not Have a Cell Wall

Cell Wall Deficient (CWD)

# Spheroplasts of Borrelia burgdorferi
# A Master's Thesis Dissertation

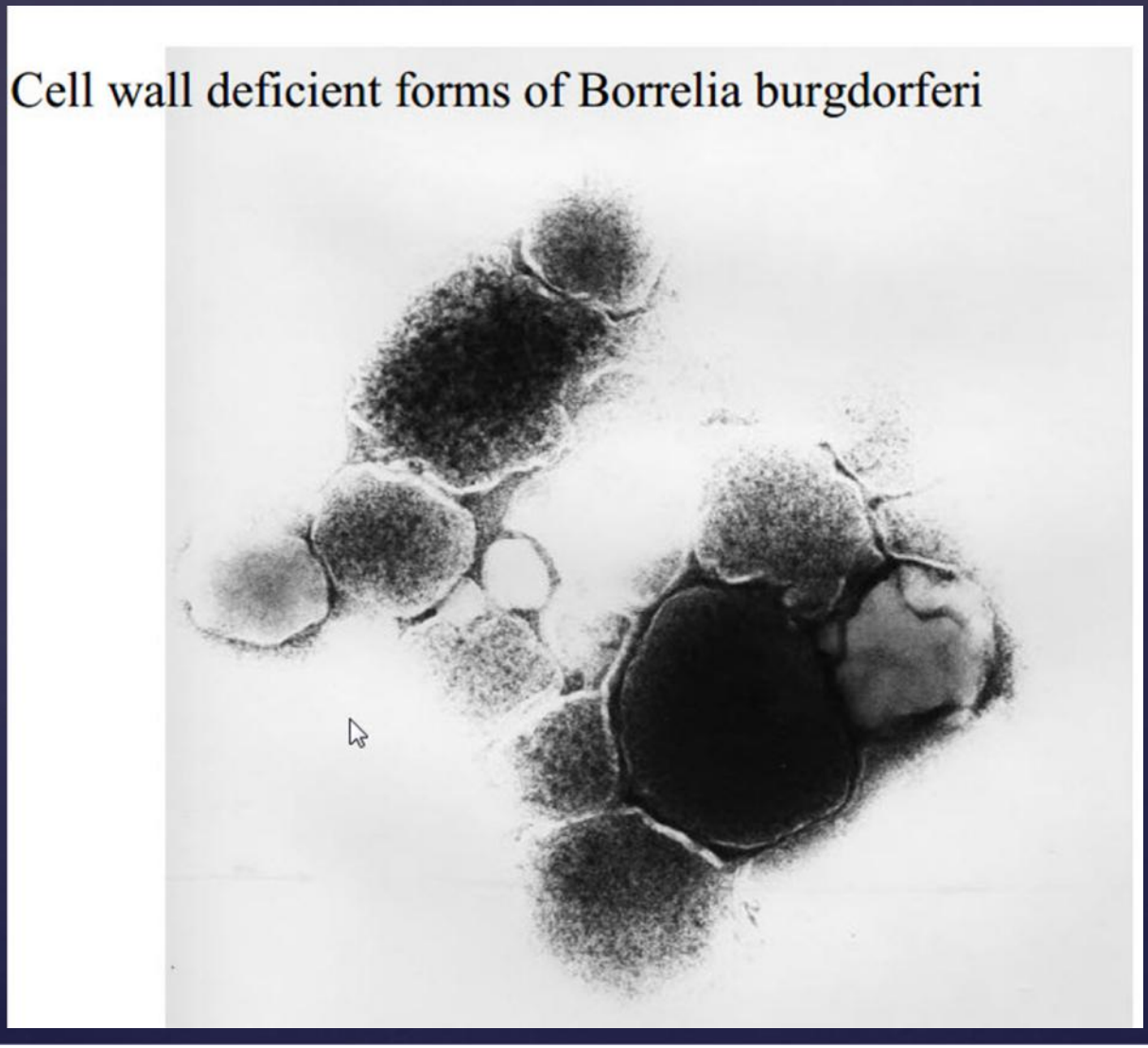


1991

## Spheroplasts of Borrelia burgdorferi

Mary Leigh Talbot
*San Jose State University*

# Spheroplasts of Borrelia burgdorferi - Ultrastructure



# Research Support
# Alan B. MacDonald MD

- Turn the Corner Foundation
  New York, New York
- St. Catherine of Siena Medical Center Smithtown New York 11787
- University of New Haven, Borrelia Research Group

# A Few Ideas which go Beyond

**" Why is there Air ?"**
**Bill Cosby**

*"Why are Round bodies of Spirochetes Important?"*
*Alan MacDonald*

# Questions to Ponder

# Disease production and Cystic Borrelia Two hypotheses for consideration

- **#1 Cystic Borrelia might be capable of producing tissue injury in mammalian cells without the participation of Spiral (vegetative) forms being present.**

- **#2 Cystic Borrelia might only produce tissue injury by reverting to spiral (vegetative) spirochetes**

Borrelia Cystic Forms
(Round bodies)
?? The Ultimate
"persister"
in Chronic Infections

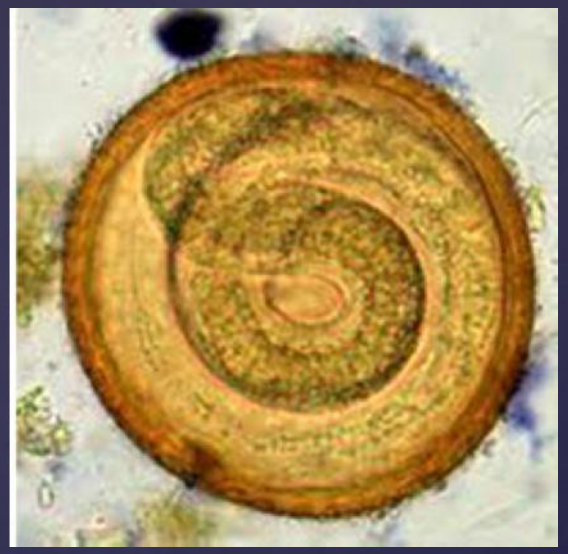
Borrelia Cystic forms (Round Bodies) in various Human Neurodegenerative Conditions

MacDonald

Medical Hypothesis 2006

# Biological Parallels -- Round Bodies in Various Parasitic Diseases

**Trichinella Spiralis**



**Toxocara canis**

# MacDonald – Work in Progress

Can the Brorson Aged type
Round Bodies
[ the types with a Darkly
staining irregular Nucleoid]
actually Masquerade as degenerating
nerve cells
in the Human Central Nervous system ?

Semantics –
Is the choice of the word
“cyst”
less scientific
than the words
“round body” ???

# Copyright Statement



References for this presentation are available on the website
http://www.molecularalzheimer.org -