

## Almost everything you wanted to know about Borrelia

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## The Questions

- Thanks for all the questions, sorry I don't have time to answer them all - please see me afterwards.
- Sorry, I don't know about:
  - cycling (rotating) antibiotics
  - lengthy antibiotic treatment
- Two of the questions were answered during my talk in 2014. I have avoided repeating that material.

## What does Borrelia live on food/nourishment wise?

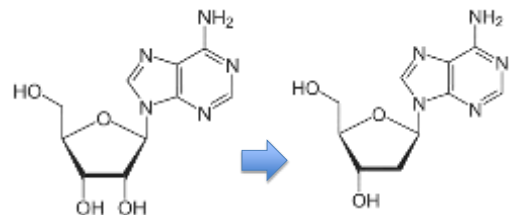
- Borrelia can only utilise a small range of carbohydrates. These include: glucose, mannose, maltose, glycerol, N-Acetylglucosamine (GlcNAc). [1]
- In the tick-stage of the Borrelia life cycle Glycerol appears to be an important source of energy. [2]
- In the human body, Glucose is the primary source of energy for Borrelia.
  - The concentration in human blood is ~95 mg per 100ml [3]
  - This may increase up to 140 mg/100ml after a meal
  - Low glucose levels will kill you before killing the Borrelia
- [1] von Lackum K, Stevenson B (2005) Carbohydrate utilization by the Lyme borreliosis spirochete, *Borrelia burgdorferi*. FEMS Microbiol Lett 243: 173–179.
- [2] Pappas (2011) *Borrelia burgdorferi* requires glycerol for maximum fitness during the tick phase of the enzootic cycle. PLoS Pathog. PLoS Pathog.
- [3] Young, (1971) CLINICAL CHEMISTRY, Vol. 17, No. 5,

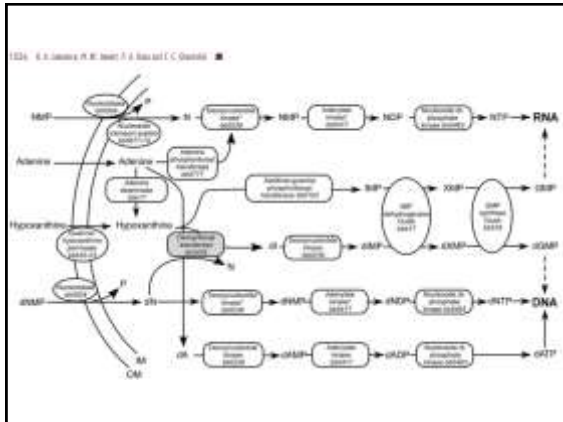
- As an obligate parasite with a minimal genome Borrelia must scavenge nutrients from the host.
- **amino acids** required to make proteins
- **fatty acids** for lipids/lipoproteins
- **nucleosides** for the biosynthesis of DNA/RNA

- For the synthesis of DNA, Borrelia relies on host-derived sources of
  - deoxypurine bases (A & G) and
  - deoxypyrimidine bases (C & T)

See the introduction to: Lawrence, (2009). *Borrelia burgdorferi* bb0426 encodes a 2'-deoxyribosyltransferase that plays a central role in purine salvage. *Molecular Microbiology*, 72(6), 1517–1529.

- **Adenosine**
  - (in RNA)
  - a ribonucleoside
- **Deoxy-adenosine**
  - (in DNA)
  - A deoxy-ribonucleoside





**This highlights an interesting different between Lyme disease and relapsing fever *Borrelia***

- Relapsing fever *Borrelia* have a functional ribonucleotide reductase (RNR).
  - This enzymatically reduces ribonucleotides to their deoxy-analogues
  - Allows greater biochemical flexibility
- Lyme disease *Borrelia* lack a functional RNR and so needs to acquire the deoxynucleotides from the host.

Lawrence et al (2009). *Borrelia burgdorferi* bb0426 encodes a 2'-deoxyribosyltransferase that plays a central role in purine salvage. *Molecular Microbiology*, 72(6), 1517–1529.

**Other Questions about Salt/Sugar/Alcohol/Alkaline diets**

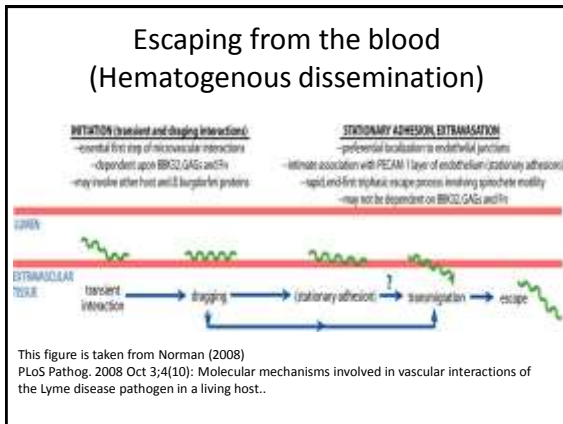
- Your body will maintain homeostasis.
- Attempting to adjust these concentrations by altering diet is futile (and not safe).
- I am not aware of artificial sweeteners influencing *Borrelia* growth.

See this paper for typical values of various salts/sugars in 10 people Young, *CLINICAL CHEMISTRY*, Vol. 17, No. 5, 1971)

**Is it possible that levels of *Borrelia* can be reliably measured in the blood?**

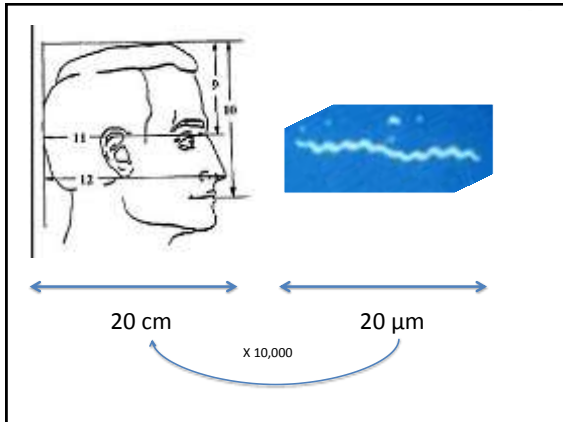
- A problem- The adaptive immune response rapidly clears *Borrelia* from the blood
- B. burgdorferi* rarely achieves more than 100 cells/ml [1]
- However the inflammatory response is out of proportion to the number of cells.
- The intense inflammatory response is due to bacterial surface molecules and their interactions with the innate immune modulators, in particular the Toll-like receptors.
- Will this indicate the effectiveness of therapy?
  - No, because of rapid clearance from the blood
  - Borrelia* invade various tissues

[1] *Borrelia: Molecular Biology, Host Interaction and Pathogenesis* By D. Scott Samuels



**Size?**

- Borrelia* are approximately 0.2 x 20 micrometers.
- Cell widths are reproducible
- Cell lengths vary with culture conditions and source of the *Borrelia*.
- I was going to use an analogy to a human hair but:
  - not really useful.
  - The width of a human hair can range from about 20 to 200 microns



- The human head is about 20 cm (longest diameter)
- Borrelia is 20 μm (length)
  - x1000 = 20 mm
  - x10 = 200 mm or 20 cm
- So the human head is ~10,000x larger than Borrelia



- I guess the lecture theatre might be about 20m
  - 20 metre/10,000 = 0.2 cm or 2 mm

So if we imagine that our head is as large as a 20 metre lecture theatre, then a single Borrelia cell is like a fragment of human hair 2 mm long.

### For comparison

- Bone marrow cell (diameter ~40 μm)
- Red blood cell (diameter ~7-8 μm)
- Borrelia width 0.2 μm, length ~20 μm

### Would raising the body temperature kill Borrelia?

“no growth or motility of any strain was observed after 4 days at 42C.”



- Hubálek Z1, Halouzka J, Heroldová M (1998). Growth temperature ranges of Borrelia burgdorferi sensu lato strains.. J Med Microbiol. Oct;47(10):929-32

- **Both Borrelia and humans are temperature sensitive**
- **So don't try this at home!**

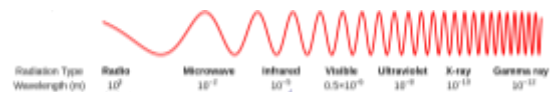
### The Rife Machine

- The Rife Machine is based on the pseudoscience of Radionics.
- The idea that diseases can be diagnosed and treated by “tuning in” or bombardment with “radio-like” frequencies
- There is no evidence that this does anything
- Not to be confused with MRI scanners!!!



### The Rife Machine?

- Electromagnetic Radiation



- An excellent summary can be found here:

<http://www.cancerresearchuk.org/about-cancer/cancers-in-general/cancer-questions/ribe-machine-and-cancer>

## To what extent is *Borrelia* an intracellular pathogen?

- Many papers describe an extracellular niche
  - association with the extracellular matrix
- In previous lectures I have also discussed paracellular invasion (See Norman *et al*, 2008)
- Here I will summarise the evidence that *Borrelia* can invade non-phagocytic cells
  - Synovial cells
  - Neuronal cells

PLoS Pathog. 2008 Oct 3;4(10):e1000169. **Molecular mechanisms involved in vascular interactions of the Lyme disease pathogen in a living host** Norman MU1, Moriarty TJ, Dresser AR, Millen B, Kubus P, Chaconas G.

Rheumatol Int (1996) 16: 125–131  
ORIGINAL ARTICLE  
H. J. Girschick · H. L. Huppertz · H. Rüssmann · S. Krenn · H. Karch

**Intracellular persistence of *Borrelia burgdorferi* in human synovial cells**

- A *Borrelia* infection model using cell cultures of
  - human synovial cells and
  - human macrophages.
- morphologically intact *Borrelia burgdorferi* were found in the cytosol of synovial cells without engulfment by cell membrane folds or phagosomes

## Human synovial cells infected with *Borrelia*

- Ceftriaxone added at a concentration of either 1.0 or 4.0 micrograms/ml for 9 days
- No antibiotics after day 10
- Supernatants were probed for viable spirochetes once a week for 63 days.
  - No viable spirochetes found by either phase contrast microscopy (PCM) or re-cultivation.
- Treatment with ceftriaxone eradicated all extracellular *Borrelia burgdorferi*.

## Intracellular *Borrelia* survived

- After 63 days the synovial cells were lysed.
- Viable spirochetes were reisolated
- “cytosolic *Borrelia burgdorferi* were still detectable by TEM. After lysis of the SC at day 63, viable *Borrelia burgdorferi* were identified 7 days later by PCM and recultivation from the cell lysates; this did not occur in the uninfected controls”

Rheumatol Int. 1996;16(3):125-32. **Intracellular persistence of *Borrelia burgdorferi* in human synovial cells.** Girschick HJ1, Huppertz HJ, Rüssmann H, Krenn V, Karch H.

## Invasion of human neuronal and glial cells

- *B. burgdorferi* were shown to interact with and invade human neuronal and glial cells
  - *Borrelia* remained viable
- Gentamicin was used to kill extracellular *Borrelia*
- “These results suggest that the association of infectious *B. burgdorferi* with these cell lines is a specific and targeted binding mechanism, rather than a non-specific endocytic event”

• Microbes Infect. 2006 Nov-Dec;8(14-15):2832-40. Epub 2006 Sep 22. **Invasion of human neuronal and glial cells by an infectious strain of *Borrelia burgdorferi*.** Livengood JA1, Gilmore RD Jr.

## Human cortical neuronal cells (HCN-2)



Red = Borrelia associated with the cell surface  
White arrows indicate intracellular Borrelia

Figure from: *Microbes Infect.* 2006 Nov-Dec;8(14-15):2832-40. [Invasion of human neuronal and glial cells by an infectious strain of Borrelia burgdorferi.](#) Livengood JA1, Gilmore RD Jr.

## no cellular cytotoxicity

- "...these cells could serve as a site for *B. burgdorferi* to be sequestered from the host's immune defenses, and/or act as a locale for prolonged infection without causing immediate harm or cell death to their host"
- However, there is likely to be some physiological change in the function of the cell.

## There are many unknowns

- The mechanisms involved in cell penetration are unknown
- What factors determine if Borrelial will survive within cells?
- Clinical relevance?

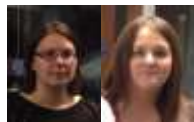
## Summary

- The infected cell is not killed- limited immune response.
- Intracellular Borrelia are shielded from the antibiotics.
- Borrelia remained viable for the duration of the experiment (several weeks)

## Acknowledgments

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