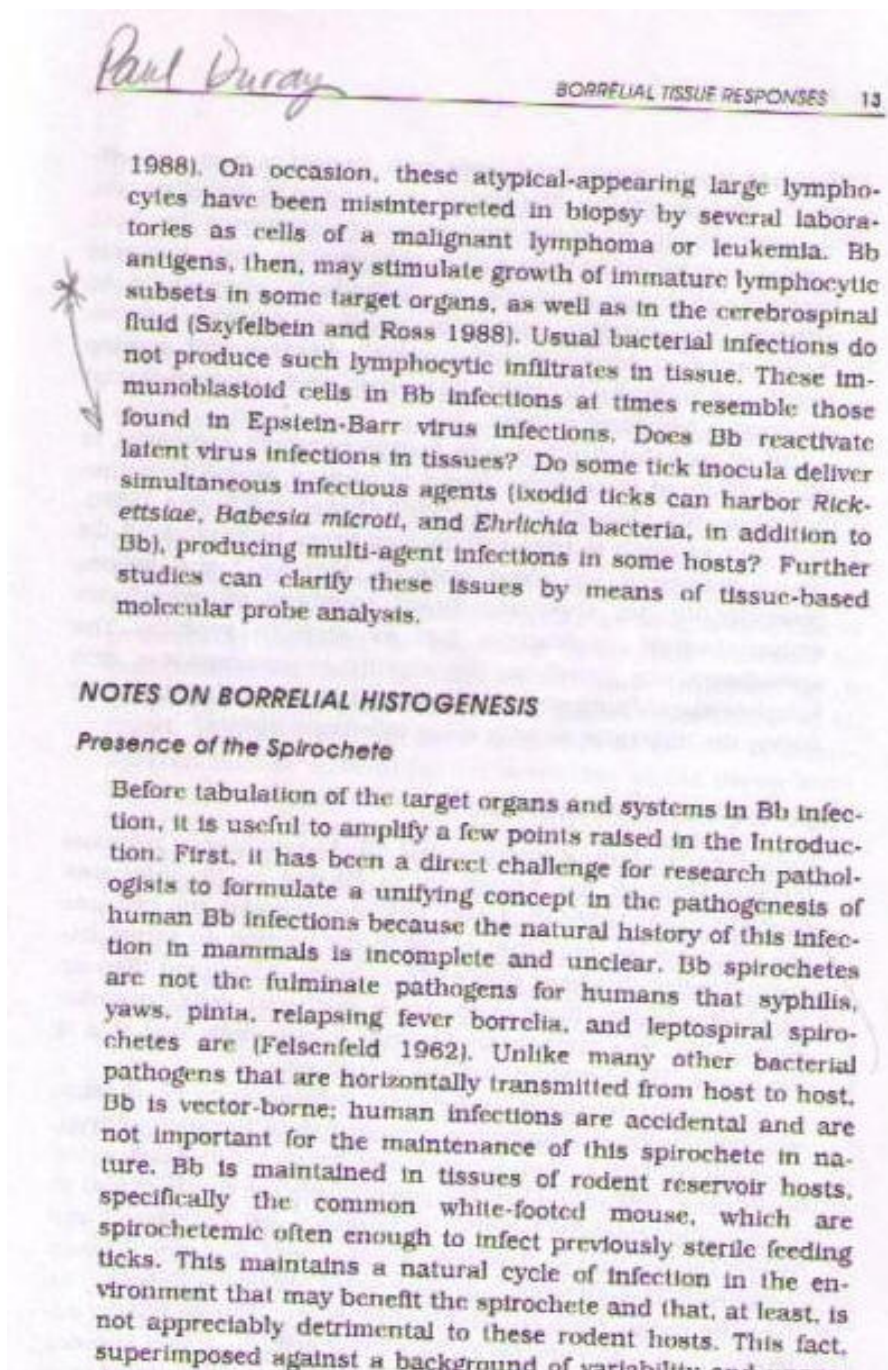


1992: NASA/NIH/NIC/US Army Top Pathologist: "In Chronic Lyme victims, I see what look like Epstein-Barr transformed lymphocytes."



[Paul Duray, NASA rotating bioreactors \(culturing in zero gravity\) and borreliaskbook/micro/mg98/agreement.html](#)

**FY 98 INTER-AGENCY AGREEMENT**

**NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT**

**NATIONAL INSTITUTES OF HEALTH**

and

**OFFICE OF LIFE AND MICROGRAVITY SCIENCES AND APPLICATIONS**

**NATIONAL AERONAUTICS**

**AND SPACE ADMINISTRATION**

**8. Assessment of the RWV as a "universal" pathogen culture system**

Primary Investigators: **Dr. Steven Hatfill**, Laboratory of Cellular and Molecular Biophysics, NICHD, NIH and USAMRIID, **Dr. Paul Duray**, Department of Pathology, NCI, NIH, and **Dr. Michael Bray**, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Ft. Detrick, MD.

Aim of Experiments: To demonstrate the potential ability of the RWV Bioreactor to act as a "universal" pathogen culture system for the primary isolation of previously unrecognized pathogens during outbreaks of emerging disease. By demonstrating the ability of the RWV to culture a variety of known pathogens of different classification, i.e. RNA viruses, retroviruses, DNA viruses, parasites, spirochetes, etc., we hope to demonstrate the applicability of the RWV system for de novo primary pathogen isolation protocols.

#### BACKGROUND:

At present, the de novo isolation of previously unknown or unrecognized emerging disease pathogens requires elucidating the proper culture media or permissive cell line applicable for growth of the pathogenic agent in culture. A few examples serve to illustrate the difficulty inherent in this process using conventional technology. The 1970's outbreak of Legionnaires disease required months to identify the causative agent as a bacterium. The HIV-1 retrovirus required over 2 years to isolate and in the 1993 outbreak of Myuro Canyon disease in the four-corners region of the United States it took 7 weeks to grow the Hantavirus in culture. Clearly these timelines are unacceptable with respect to many public health threat scenarios for emerging disease agents. By utilizing the ability of the RWV to maintain a normal three-dimensional cytoarchitecture and microenvironment for a number of tissues, the possibility of using human tissue explants for primary pathogen isolation, becomes a distinct possibility.

Experiment Design: Attempts will be made to culture a variety of infectious pathogen in the same simple medium (RPMI-1640, 15% FCS) containing human tonsil tissue explants maintained in the RWV Bioreactor. In addition, a human liver and epithelial tissue equivalent will be formulated from established cells lines grown on Cytodex 3 microcarrier beads. These will be co-cultured with the tonsil explants. Known pathogens which have proved to be difficult to isolate by normal protocols, will be introduced into the RWV culture and allowed to incubate with the tissue and tissue equivalents for two weeks.

#### Continuing Results:

1. It has been previously demonstrated that the spirochetal organism *Borrelia burgdorferi*, the agent of Lyme disease, grows and proliferates in this system. The system has been shown to support productive infection with HIV-1 strains. Ebola virus was also shown to be amplified in this culture system.
2. The RWV Bioreactor system has been used to study differences between human Ebola strains and Ebola Reston, a macaque strain. Human exposure to Ebola Reston causes no apparent illness, but has an almost 100% mortality rate in non-human primates (NHP). The system has allowed the demonstration that in human tissues unlike in NHPs, Ebola Reston is unable to infect the intact epithelial lining of human blood vessels and this presumably accounts for the non-pathogenicity of the strain in humans.
3. The RWV Bioreactor has also been shown to support productive infection of Monkeypox orthovirus in human lung tissues. This model will hopefully shed light on the pathogenesis of not only monkeypox but also the related smallpox.

Plans: The system will be used to further explore the tissue tropism of Ebola strains, Reston, Zaire, Sudan, and Gabon. RWV Bioreactor cultures will be used to explore the basic pathogenesis of monkeypox in both human and NHP tissues, with particular reference to postulated cytokine production. If possible, in the scientific and political context, this work will be extended to study smallpox infection in human tissues since this type of experiment has never been done before. The RWV will be used in an attempt to culture *Treponema pallidum*, the causative agent of syphilis. This organism is notorious for its resistance to culture by conventional methods.

## **9. Study of Lyme disease in RWV Bioreactor**

Primary Investigators: Dr. Paul Duray, Department of Pathology, National Cancer Institute, NIH.

Aim of Experiments: To use the RWV Bioreactor to culture *Borrelia burgdorferi*, the etiologic agent of Lyme disease, in tissues. Proliferation of the spirochete in tissues is desired so that the infection process and ensuing disease progression can be studied in an *in vitro* model. The model could be used to study the infection process and understand basic questions about the parasite, such as whether it actually infects cells or whether it replicates in intercellular spaces.

### **BACKGROUND:**

Lyme disease is a multisystem inflammatory disease caused by infection with the tick-borne spirochete *Borrelia burgdorferi* and is the most common vector-borne infection in the United States. *Borrelia burgdorferi*, is able to persistently infect humans and animals for months or years in the presence of an active immune response. It is not known how the organisms survive immune attack in the mammalian host. There is not an appropriate *in vitro* model in which to study the progression of Lyme disease in humans or an animal model that presents all the symptoms of human disease.

Experiment Design: Tissue biopsies from patients with Lyme disease will be cultured in RWV Bioreactors to see if proliferation of spirochetes in primary infected tissue can be seen. The RWV will also be used to culture normal human tissues with *Borrelia*, the Lyme disease parasite, to study the infection process. Media other than BSK will be used to determine whether *Borrelia* is replicating in the medium in cultures or in the tissue itself, since it is known that *Borrelia* does not replicate in media other than BSK.


### **Preliminary Results:**

1. Very good replication of *Borrelia* has been seen tonsils infected with the spirochetes. Infection has been confirmed by silver staining of tissue sections and by PCR.
2. Cultures of tonsils infected with *Borrelia* in media other than BSK show good replication, suggesting that the spirochete replicates in the tissue blocks and not in the medium.


Plans: Methods of quantitation of spirochete load in infected tissues are being researched so that better methods of comparison are available. With this model of infection we hope

to begin studying the differences between infectious and non-infectious *Borrelia*. We plan to look for genetic variation of infectious spirochetes during a long-term culture.


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