**Causative Agent**

WARREN A. ANDIMAN, M.D.

*Moderator*

*Lyme Disease Spirochete: In Vitro Growth Characteristics, Antibiotic Susceptibility, and in Vivo Clinical Responses*

Since stock cultures of organisms that have been passaged many times in the laboratory may undergo changes in their pathogenicity, concern was expressed as to whether antimicrobial sensitivity tests performed *in vitro* accurately reflect the clinical responses one might expect in treating patients. Dr. Feeley believed that various strains of the Lyme disease spirochete are stable *in vitro*, but could offer no assurance that there was a perfect correlation between *in vitro* tests and clinical responses. He stated that because some spirochetes contain plasmids associated with virulence or antimicrobial resistance it might be best, in some circumstances, to use strains that were recently isolated to assure that there is no plasmid loss. It was pointed out that the imperfect correlation between *in vitro* tests of susceptibility of the Lyme disease spirochete to erythromycin and its possible shortcomings in treating patients should encourage physicians to depend first on their cumulative clinical experience in treating patients. *In vitro* susceptibility tests should be used only as a general guide to the clinician as to what antimicrobials might be useful in treating patients.

*Lyme Disease Spirochete: Surface Characteristics and Changes in Morphology*

The composition of the outer envelope varies with the organism. For example, leptospires contain up to 35 percent lipid in the envelope, whereas the concentration in treponemes is lower, perhaps in the range of 15 percent. This aspect of the Lyme disease spirochete has not yet been studied. It appears that the membranes of these organisms are very fluid, but it has not yet been demonstrated whether these organisms have true bacterial pores to allow the transport of essential nutrients, such as glucose or amino acids. Some of this transport may occur directly via lipids in the membrane.

It is conceivable that the surface layer of *Borrelia* changes following each recurrence of disease, thereby evading some critical host defense. There is evidence that such surface changes may occur *in vitro* during continuous passage. Intermittent cloning of the spirochete might result in more dramatic changes in the morphology, such as a fluctuation in the number of internal flagella. Thus far, several examinations of the B31 clone have revealed seven or 11 flagella, but an intermediate number of flagella or axial filaments has not been observed.

The surface layer may also be responsible for some of the clumping observed in cultures of these organisms. Because the clumping tends to recede after several subcultures, clumping may be an active process. The organisms tend to stick at one end to the microscope glass and at the other end to one another, once contact is made.
**Lyme Disease Spirochete: Cultural Requirements for Oxygen**

The spirochete appears to grow best in a tube that is partially filled—perhaps two-thirds full. The optimal quantity for air left in the culture tube may vary with altitude. Experiments are presently being done in which the inoculum size and the amount of air in the tube are varied in a checkerboard pattern. The organism may be very sensitive to radical formation resulting from oxidation.

**Taxonomic Classification of Spirochetes**

There is great difficulty in deciding upon a proper taxonomic niche for members of the spirochete family of organisms. These organisms are classified in two different ways—by phenotype and genotype. Some investigators have reclassified the three Borrelia species—*B. hermsii, B. turicatae*, and *B. parkeri*—into a single species based on the considerable extent of their shared DNA homology. The same appears to be true of the Lyme disease spirochete; what were originally believed to be three different species now seem to be one.

Dr. Kari Hovind-Hougen issued a warning regarding the use of *T. hyodysenteriae* as a laboratory model of treponemal infection. Although workers use it because it can be easily cultivated, it differs from other treponemes in the absence of cytoplasmic tubules and the fact that it divides by septum formation. However, to date, it has been firmly classified as a treponeme and has been the only member of that genus with a G + C content at all similar to that found in the Lyme disease spirochete.

**Clinical Characteristics of Lyme Disease versus Other Diseases Caused by Borrelia; Herxheimer Reactions**

Because Lyme disease manifests clinically in a very different way from relapsing fever, Dr. Steere asked whether there is more information about other diseases caused by the Borrelia group of organisms. It was generally felt that there is a dearth of up-to-date information about this subject. Most of the relapsing fever group of diseases are rarely seen today. *B. anserina* appears to undergo antigenic change, as do other borrelia, but little is known about its clinical behavior in animals.

Herxheimer-like reactions have occurred in association with antibiotic treatment of Lyme disease. They are intermediate in intensity between the almost unavoidable reactions seen in secondary syphilis and the rarer and less dramatic reactions associated with the Borrelia. In many ways, the reaction manifests as a modest intensification of those symptoms which the patient is already experiencing—e.g., the fever may rise one or two degrees, the muscle pain may get worse, and the rash may become more erythematous. Patients with Lyme disease who are treated with erythromycin have less intense reactions than those treated with penicillin. One possible explanation is that erythromycin may kill the organisms more slowly or it may act over a greater number of hours, thereby blunting the reaction.