MICROBIOLOGY

Association of Bacterial *L*-Phase Organisms in Chronic Infections

The pathogenicity, in the classic sense, of bacterial *L*-phase cells has yet to be conclusively demonstrated. The association of the *L*-phase of certain bacteria with diverse clinical states has been noted in the past, and this association is being reported more frequently as knowledge and interest in these forms have increased[1–9]. Such reported investigations lead to the assumption that host resistance mechanisms tend to create the *L*-phase of a bacterium. Experiments designed to determine the possible pathogenicity of the bacterial *L*-phase have usually ignored the host. It is not unreasonable to assume that the *L*-phase is the bacterial form most readily handled by normal host resistance mechanisms.

In regard to these assumptive possibilities, it was thought that chronic staphylococcal infections may be associated with *L*-phase bacteria. Patients were chosen for their history of chronic infection, usually associated with staphylococci. Blood samples, 10 ml., were drawn after the remission of clinical symptoms of disease brought about by antibiotic therapy. 5·0 ml. of each sample was injected immediately into 5·0 ml. double-strength *L*-phase minimal medium prepared according to the Mattman modification of the method of Medill and O'Kane[10]. The remaining 5·0 ml. was inoculated into 50 ml. 'sloppy' brain heart infusion medium (BHI plus 0·1 per cent agar). The inoculated *L*-phase medium was then incubated anaerobically until a change was obvious. Sometimes the only change was a slight haemolysis of the blood layer. Growth generally appeared by the end of the 5th–7th day of incubation. The 'sloppy' BHI was incubated aerobically for a minimum of two weeks.

When *L*-phase medium cultures were 5–7 days old, they were removed from the anaerobic chambers (95 per cent nitrogen; 5 per cent carbon dioxide) and two 0·5 ml. samples were inoculated into 4·5 ml. fresh medium. They were incubated either aerobically or anaerobically. Growth appeared in both tubes, but generally was greater in the tube incubated in the absence of air. At the time of maximal development (3–5 days) the organisms were again transferred to fresh medium and incubated aerobically. Adequate growth of the organism occurred in 3 days. These *L*-phase cells were then reverted to classic bacteria by daily transfers in BHI and on to 'Difco 110' medium slants. The cultures on the 'Difco 110' medium usually underwent a colour change from white to bright yellow during the successive transfers. The 'sloppy' BHI medium remained negative for any culture, even though the reverted *L*-phase cells were able to grow in this medium. Medium controls remained aseptic.

From a total of 11 patients selected for their history of recurring boils and staphylococcal problems, positive *L*-phase cells were obtained without the appearance of the classic bacterial forms. The *L*-phase cells all reverted to staphylococci after continued sub-culture.

As a control of the methodology, blood samples were taken from 22 apparently uninfected persons from the hospital staff. These blood samples were cultured in the routine process, and of these, 20 were negative. On re-testing samples of blood from the two whose blood had given positive cultures, no culture was obtained. The possibility of sub-acute infection in these two persons may be a reasonable explanation.

When large numbers of *L*-phase bacterial cells are injected into a healthy animal, no disease occurs and the *L*-phase cells are rapidly cleared. This may indicate merely that the animal is normal and therefore capable of efficiently handling the intruding cells. The classic bacterial growth form is not handled as well, if at all, and thus causes the symptomatic onset of disease. However, if the animal host resistance is interfered with by mucin injection, then the *L*-phase cells survive, revert to the classic form and cause overt disease (unpublished results). Unfortunately, mucin is so non-specific in its interference with resistance that many factors only indirectly involved in host resistance are undoubtedly affected. The usual interpretation of this sequence of events is that the *L*-phase cells are non-pathogenic and are therefore not involved in the infectious process or the disease state. This interpretation may not be correct. We speculate that the inability to handle *L*-phase bacteria may be related to an incomplete host resistance system caused by stress or inherited deficiencies. The consequence of survival of the *L*-phase in tissues not amenable to therapy would, therefore, be chronic disease. During therapy no overt disease state may exist. If therapy should be discontinued before the host resistance system is functioning efficiently, the surviving *L*-cells may slowly revert to classic growth forms and eventually give rise to overt disease. It would be expected that during therapy, however, the host defence mechanisms would become more active or that the physical or biochemical stress would have disappeared. This would allow complete recovery. Complete recovery may be primarily dependent on the reacquisition of host resistance factors rather than on the course of chemotherapeutic treatment.

A more extensive investigation of chronic staphylococcal *L*-phase infection should be initiated. Other chronic disease states should be examined during various phases of treatment and non-treatment for possible *L*-phase association. Rheumatic fever comes to mind as a most logical chronic disease that could be associated with streptococcal *L*-phase survival, perhaps localized in heart valve tissue.

The association of *L*-phase cells in cases of chronic infections of humans which have been treated with antibiotics requires greater consideration than has been outlined in this report. The possible involvement of the bacterial *L*-phase in this type of infection should no longer be ignored.

C. W. Godzeski
Gordon Brier
R. S. Griffith
H. R. Black

Lilly Research Laboratories,
Eli Lilly and Co.,
The Lilly Clinic,
General Hospital,
Indianapolis, Indiana.

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CYTOLOGY

A New Basic Chromosome Number in the Family Fagaceae

Chromosome numbers have been determined previously for some 58 species in the family Fagaceae, covering the three genera of the northern temperate zone, *Quercus*, *Castanea* and *Fagus*[1–3]. These determinations are uniformly $2n = 24$, the Fagaceae resembling many other families of woody angiosperms in this stability of chro-