

A Review of Some Aspects of L-Forms and Gonococci* **

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Systemic manifestations of gonococcal disease, such as arthritis, are often sterile on the usual culture methods used to grow gonococci. Allergic mechanisms have been invoked to explain this but with little evidence to support the concept (1-3). With the report by Holmes et al. (4), that L-forms of gonococci were isolated from joint fluid of a patient with gonococcal arthritis, we decided to investigate the possible role of L-forms in gonococcal disease.

Biology of L-Forms of Bacteria. Kleineberger in 1935 (5) first described L-form formation in *Streptobacillus moniliformis* organisms. L-forms of organisms are also known as protoplasts, variants, or spheroplasts. In protoplast formation, organisms lose their normal rigid cell walls and are able to survive, reproduce, and revert to the normal cell wall possessing parent forms. These features distinguish L-forms of bacteria from mycoplasmas, which never have rigid cell walls. Media, made hypertonic with sucrose, sodium chloride, or other things, are necessary to grow L-forms without lysing the cells and destroying them. Lacking cell walls, L-forms do not show up on the usual Gram's stain.

Numerous species of bacteria, including *E. coli*, staphylococci, enterococci, and certain fungi are capable of L-form formation (6). This phenomenon can be induced by the presence in hypertonic media of certain amino acids, some antibiotics, lysosomal

enzymes, or the combination of complement and antibodies.

The question of pathogenicity of L-forms is far from answered. Work by Gutman et al. (7) clearly demonstrated that protoplast formation is a mechanism whereby organisms can persist in the urinary tract despite antibiotic therapy. Antibiotic therapy with ampicillin caused *Proteus mirabilis* organisms in their patient to revert to L-forms which are quite resistant to ampicillin. With cessation of therapy, the organism can revert to the parent form possessing a cell wall—thus assuring persistence of the infection.

Seven L-form media, the formulae of which have previously been published (8), were simultaneously inoculated with a recently isolated strain of *Neisseria gonorrhoeae*. In order to increase L-form colony recovery, one of the four cell wall antibiotics (ampicillin, benzathine penicillin, methicillin, or potassium penicillin G) was incorporated into the seven media. This comparison was repeated with 21 strains of gonorrhoeae and with each of the four antibiotics to give a total of 588 comparisons. There was a total of 187 cultures which demonstrate the presence of L-form cultures; however, three media accounted for 62% of the positive cultures. These three high recovery media were used, without antibiotics, in conjunction with chocolate and Thayer-Martin media, in two clinical studies designed to isolate L-forms or coccal forms of gonorrhoeae.

In the initial clinical study we cultured on high recovery media material from the urethral exudate of twelve patients with acute urethritis. None of these grew L-forms. In the second clinical study we

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cultured joint fluid from 17 patients with acute arthritis. Twelve of these had clinical features suggestive of gonococcal arthritis; again, no L-forms were grown. Five patients with either gout or osteoarthritis grew no organisms. Thus we were unable to demonstrate a role for L-forms in the pathogenesis of gonococcal disease.

That L-forms are present in some patients and stages of infection cannot be denied on the basis of Holmes' work; neither can it be denied on the basis of the work of Orcinnikov and Delektorskij (9) who have demonstrated, by use of electron microscopy, the presence of wall-less gonococcal cells in prostatic secretions. The possibility also exists, however, that gonococcal L-forms are transitory—that is, they are injured cells on the way to death and do not significantly enter into the pathogenic process. This possibility is reinforced by the present study and also by that of Orcinnikov and Delektorskij, because they were able to demonstrate gonococcal L-forms using electron microscopy; but in no case were they able to grow the organism.

REFERENCES

1. SPINK WW, KEEFER CS: The diagnosis, treatment and end results in gonococcal arthritis. *N Engl J Med* 218: 453, 1938.
2. KEEFER CS, SPINK WW: Gonococcal arthritis: Pathogenesis, mechanism of recovery, and treatment. *JAMA* 109:1448, 1937.
3. WEHRBEIN HL: Gonococcus arthritis: A study of 610 cases. *Surg Gynecol Obstet* 49:105, 1929.
4. HOLMES KK, GUTMAN LT, BELDING ME, TURCK M: Recovery of *Neisseria gonorrhoeae* from "sterile" synovial fluid in gonococcal arthritis. *N Engl J Med* 284:318, 1971.
5. KLEINEBERGER E: The natural occurrence of pleuropneumonia-like organisms in apparent symbiosis with *Streptobacillus moniliformis* and other bacteria. *J Pathol Bact* 40:93, 1935.
6. FEINGOLD DS: Biology and pathogenicity of microbial spheroplasts and L-forms. *N Engl J Med* 281:1159, 1969.
7. GUTMAN LT, SCHALLER J, WEDGEWOOD RJ: Bacterial L-forms in relapsing urinary-tract infection. *Lancet* 1:464, 1967.
8. BALDUCCI YM: Select media for the production of L-forms. Thesis submitted in partial fulfillment of the requirements for Master of Science Degree to the Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia. June, 1972.
9. ORCINNIKOV NM, DELEKTORSKIJ VV: Electron microscope studies of gonococci in the urethral secretions of patients with gonorrhoea. *Br J Venereal Dis* 47:419, 1971.