# MYCOPLASMA and the L FORMS of BACTERIA

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# Mycoplasma and the L Forms of Bacteria

A Symposium in Honor of
Louis Dienes

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*Edited by*
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Mycoplasma and the L Forms of Bacteria

Louis Dienes, M. D.

# ACKNOWLEDGMENTS

The Symposium on Mycoplasma and L Forms of Bacteria honoring Dr. Louis Dienes was sponsored by the Massachusetts Chapter of the Arthritis Foundation. The symposium was held in Boston, Massachusetts, on June 18, 1969, at the time of the 20th Anniversary of the Massachusetts Chapter of the Arthritis Foundation. Additional travel funds were kindly supplied by Hyland, Division of Travenol Laboratories, Inc. and Pfizer Diagnostics. The auditorium was made available by the Shriners Burn Institute. Sarabelle Madoff and Willy N. Pachas organized the scientific program, and in addition Marguerite E. Burke, Lawrence J. Kunz, Marian W. Ropes, and Edgar B. Taft were members of the committee responsible for the symposium. Anne M. Sullivan and Helen M. McGrath provided secretarial assistance.

# PREFACE

The product of an intellectual Hungarian family, Louis Dienes at age nine had no difficulty in finding his way into science. After graduating from medical school, he worked on the role of certain carbohydrates and proteins in nutrition, developed several methods for microchemical analysis, and published his first scientific paper in 1909. In 1913 he had his first contact with research in bacteriology in F. Neufeld's laboratory at the Robert Koch Institute in Berlin. As a member of the Institute of Hygiene of the University of Budapest, he worked as an army field bacteriologist in the Ukraine and Poland throughout the duration of World War I. He had experience with the epidemics of typhoid fever, cholera, and dysentery. Most fruitful was the study of typhus fever in collaboration with E. Weil. The influence of an acute infection on the immune response was apparent in typhus fever. He came to the United States in 1922 to do research in tuberculosis. The main result of these studies was the recognition that the characteristic manifestation of the immune response in tuberculosis, tuberculin sensitivity, is the result of a greatly increased intensity of the immune response. This increase, which had been apparent in typhus fever, was investigated by P. A. Lewis in animal experiments. After Louis Dienes came to the Massachusetts General Hospital in 1930, a most fruitful collaboration developed with Tracy B. Mallory in the study of the delayed type of hypersensitivity. Shortage of funds for immunological research as well as his increasing involvement in studies with the Arthritis Group were responsible for his turning entirely to bacteriology. An amateur painter in his youth, he was always fascinated by the form variations of bacteria which he felt must have a meaningful role in their life processes. When Emmy Klieneberger made the observation of the $L_1$ in *Streptobacillus moniliformis*, the technics devised by Louis Dienes enabled him to follow the morphological sequence of the transformation of the bacillus to the L form and the recovery of the bacterium from the large bodies of the L form. Today it seems paradoxical that twelve years elapsed before this discovery was finally accepted. He later extended his observations on the infinite number of possibilities of bacterial variation under the effect of various stimuli. This has opened new questions in regard

to bacterial physiology and taxonomy, which when answered will bring us to a closer understanding of the microbial world.

Dr. Dienes was one of the pioneers in the field of mycoplasma and a champion of the possible role of these organisms in human disease. He was the first investigator to identify mycoplasma in human pathologic material. Together with Walter Bauer and the Arthritis Group at the Massachusetts General Hospital, he devoted his attention to the rheumatic diseases. With the ability to apply his knowledge to the diagnosis of disease, his presence at clinical rounds has been of great value. He recognized the first case of Reiter's syndrome diagnosed at the Massachusetts General Hospital. Attempts to isolate mycoplasma from this clinical entity and other rheumatic diseases provided the stimulus to the design of new media and cultural technics and stimulated the search for these organisms in other clinical conditions. The recent progress in the area of primary atypical pneumonia has demonstrated the significance of mycoplasma in this respect.

For many years Dr. Dienes has studied the morphology and reproductive processes of mycoplasma and the L forms of bacteria and has come to believe that there may exist a connection between these two forms of microorganisms. All the morphologic variations described in the mycoplasma have also been seen in the L forms. His recent work in collaboration with S. Bullivant with the electron microscope has confirmed his findings with the light microscope. Moreover, the similarity between the reproductive processes of the mycoplasma and those of the L forms of bacteria has been clearly established. Louis Dienes has always regarded the role of the large bodies to be of great significance in the reproductive processes of both groups of organisms. That the large bodies play a vital role during the early stages of the development of the L forms he has shown very elegantly in the Group A streptococcus L form (Figures 1 and 2). Further evidence for the biologic importance of the large bodies was provided by his demonstration of the development of bacteria within the large bodies derived from L forms of various bacterial species (Figures 3 and 4). The study of the large bodies requires painstaking step-by-step observation of the cultures throughout their development, and perhaps for this reason these observations of Louis Dienes have remained largely confined to his laboratory. Without doubt, the large bodies hold important information as to the redistribution of genetic material and ribosomes in the transformation of bacteria to L forms and in their return to bacteria. Louis Dienes at age 84 continues to make advances which will provide a great stimulus for future investigators. His most recent work on the influence of certain bacterial species on the rever-

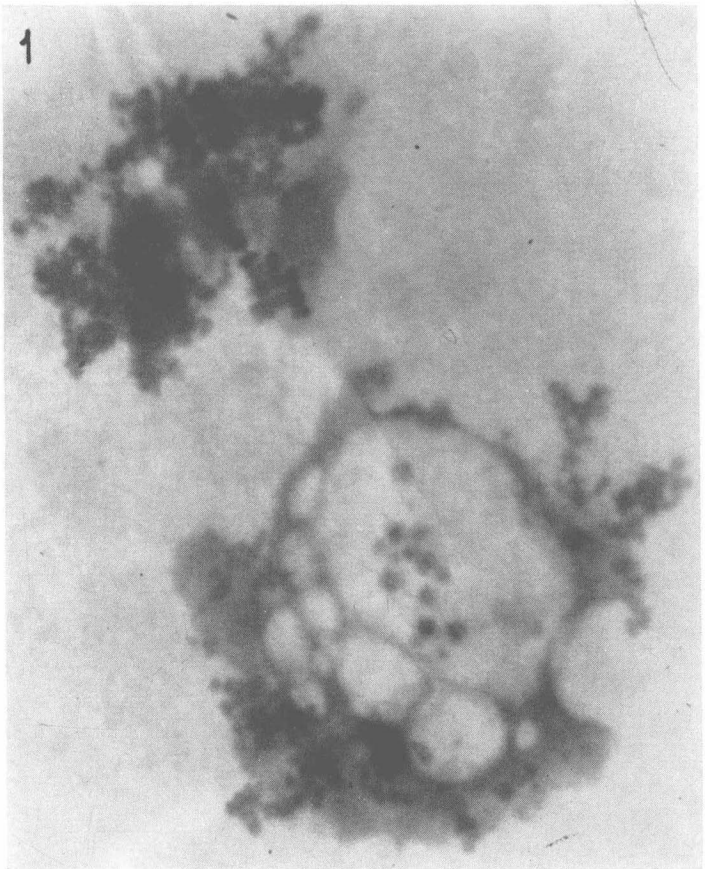


FIGURE 1

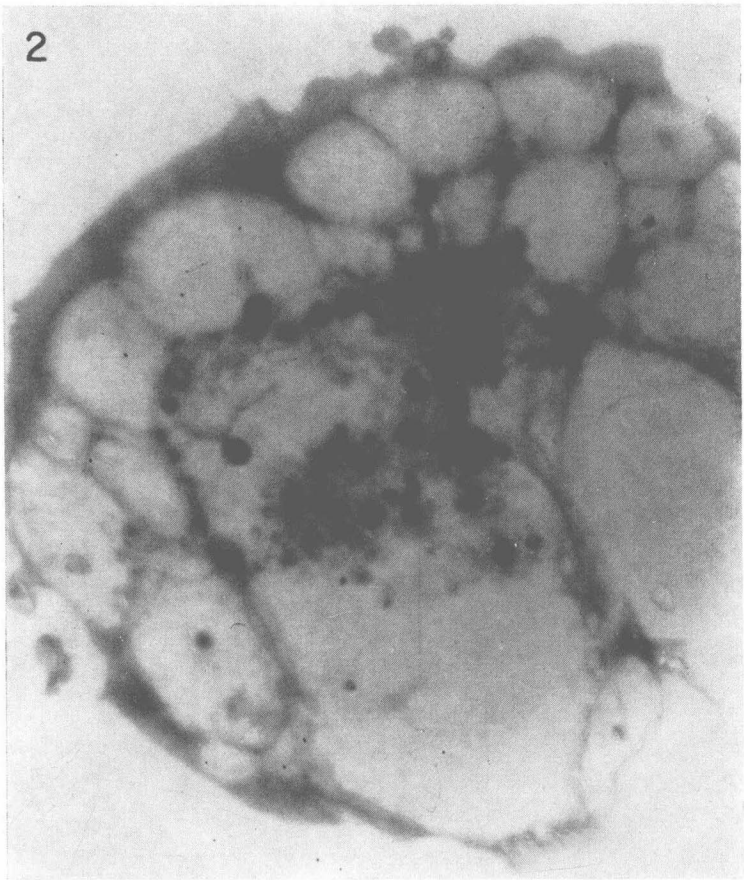


FIGURE 2

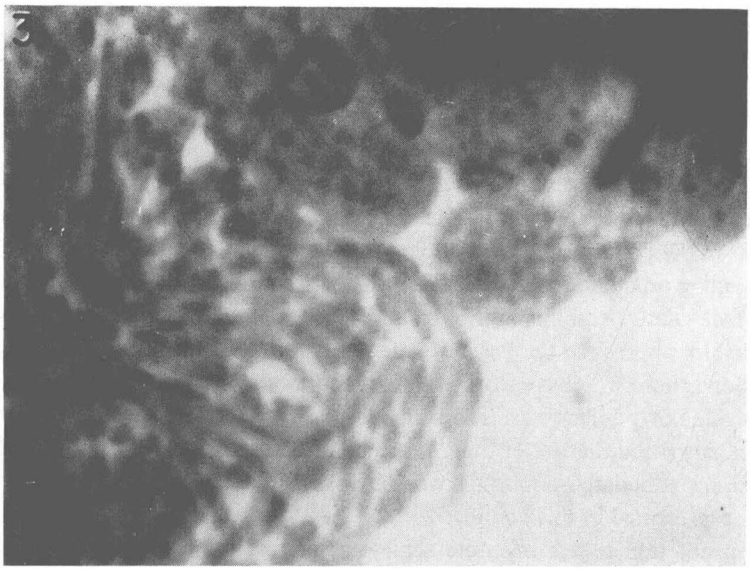


FIGURE 3

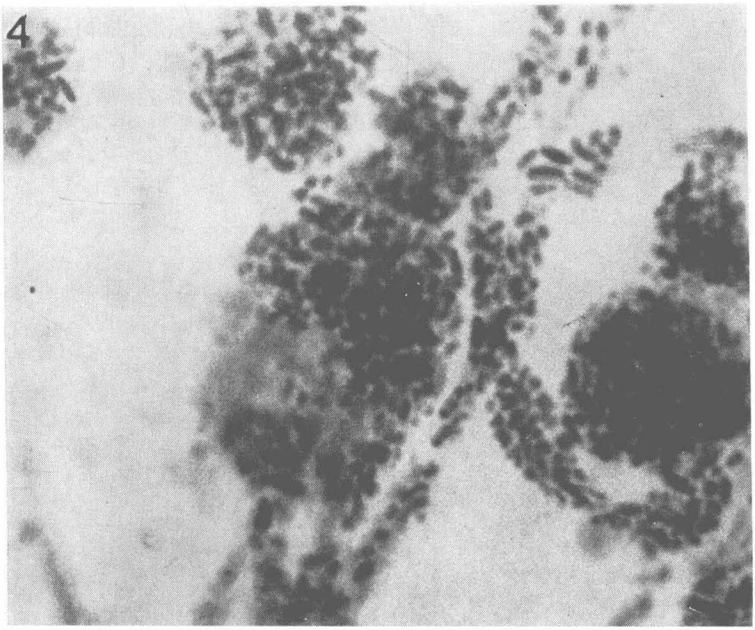


FIGURE 4

sion of *Proteus* and *Salmonella* L forms considered to be in the "stable" state raises new questions regarding possible derepression of enzyme mechanisms involved in *de novo* cell wall synthesis.

Despite the common tendency to consider morphology an outdated scientific tool, the work of Louis Dienes is the best testimony to the fact that in the proper hands morphology can be a most inquisitive and a most revealing scientific method. Dr. Dienes' work has always been characterized by imaginative thinking, tenacity, and strict scientific discipline. All of his disciples appreciate the legacy of these qualities. His influence has spread beyond those who have worked in the laboratory with him or learned from his talks or publications. The soundness of his work in this controversial area has stood the test of time. It has come to be recognized of great value and has stimulated important work by other investigators, some of whom are represented in this volume.

In addition to his scientific achievements and major contributions are Dr. Dienes' remarkable personal attributes—his sincere interest in increased knowledge, no matter by whom attained, his ability to stimulate both colleagues and friends, his sound philosophy and his true friendship.

**Sarabelle Madoff**
**Willy N. Pachas**
**Marian W. Ropes**

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* Invited Speaker

# INTRODUCTORY REMARKS

**Harry E. Morton**

*Hospital of the University of Pennsylvania*
*Philadelphia, Pennsylvania*

WELCOME TO THE Symposium on Mycoplasma and L Forms of Bacteria. We wish to thank the Massachusetts Chapter of the Arthritis Foundation for organizing this Symposium to honor Dr. Louis Dienes, the "Father of Mycoplasma." We were sorry to hear yesterday that Dr. Dienes will be unable to attend. He is here in the hospital and he has prepared a message for me to read to you. This is from Dr. Dienes:

"I am sorry that I cannot be at the Symposium. I am feeling as usual quite well, but the Doctor's order—I hope it is only for a few days—is not to leave the bed. I would have liked to take part in the discussion and to get acquainted by personal contact with the impressions of others who are approaching the problems from different directions. The discovery of mycoplasma and of their importance in animal diseases, and the discovery of L forms by Klieneberger, like other basic discoveries in bacteriology, such as transduction, sexuality, and the fascinating studies on DNA, open new ways for the study of bacteria and the diseases produced by them. It is apparent now that the increase of knowledge in every field of human activity creates new serious problems by eliminating some of the old. I grew up with faith in the beneficial influence of knowledge and I believe that the difficulties created by it can be eliminated by more knowledge and imagination. I hope that the discussions at this meeting will be profitable to everyone attending."

This is the 20th anniversary of the Massachusetts Chapter of the Arthritis Foundation. It is also an older anniversary. Today is the 21st anniversary of a workshop which Dr. Dienes conducted here at Harvard back in 1948. For the newcomers in the field of mycoplasma, I should like to take a few minutes to recount some of those early days. At the meeting of the Society

of American Bacteriologists in Minneapolis in the spring of 1948, Dr. J. Howard Mueller announced to the bacteriologists (we were bacteriologists in those days, not microbiologists) that since Dr. Dienes was soon retiring from the staff of Harvard Medical School and since many of the younger people on the staff had never heard the details about the new organisms, they were going to set aside a week for Dr. Dienes to tell the complete story as he wanted, and they thought some bacteriologists around the country would be interested in coming here for a week and participating. I was one of those who attended. Every morning and afternoon session was opened with a lecture by Dr. Dienes, and then we carried out some of the procedures in the laboratory. Twenty-one years ago today we broke up that workshop to return to various parts of the country, some of us to continue working in the field of mycoplasma, while others digressed into biology, clinical medicine, or administrative work in other fields.

Many of us remember the bacteriology meetings years ago where Dr. Dienes presented the only paper on the program dealing with mycoplasma, or we heard him speak of streptococcus and *Bacterioides* in the L form. Now, 25 years later, at the A.S.M. meeting in Miami last month, there were 32 papers—two full sessions—dealing with mycoplasma. There were eleven papers devoted to L forms. So the 43 papers devoted to mycoplasma and L forms constituted 3.3% of the papers given, and they touched just about every phase of microbiology: medical, general, agricultural, and industrial, as well as microbial physiology and biology. Now people are finding mycoplasma in plant tissues and insects so that perhaps mycoplasma will no longer be confined to the animal kingdom.

# BIOLOGY OF THE MYCOPLASMATALES*

**Leonard Hayflick†**

*Department of Medical Microbiology*
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IT IS INDEED a distinct pleasure and a great honor for me to participate in this Symposium in honor of Dr. Louis Dienes. The word symposium comes from the Greek "to drink together" and although I do not believe that the convenors of this Symposium intended a literal interpretation of this word—at least at this time—I know that you will all join me in an imaginary toast to our absent but honored guest who in addition to his monumental scientific contributions to microbiology has been chiefly responsible for attracting so many other workers into this field. I believe that there can be no greater honor to be paid to Dr. Dienes than to recognize the guidance and inspiration that he has given to so many of us here today, students and colleagues alike.

I have been asked to introduce one of Dr. Dienes' favorite subjects, the mycoplasmas, by presenting to you a brief overview of their basic biology and to consider some aspects of historical developments in this field.

## Definition

Mycoplasmas, the smallest free-living microorganisms, are composed of minimal reproductive units as small as 125 mμ in diameter which enlarge to spherical or branching forms up to 500 mμ in diameter. The organisms

* This study was conducted under contract PH 43-68-1256 within the Special Virus Cancer Program of the National Cancer Institute, NIH, USPHS.

This presentation was based on the chapter "Fundamental Biology of the Class Mollicutes, Order Mycoplasmatales," in *The Mycoplasmatales and the L-Phase of Bacteria*, L. Hayflick, Editor, Appleton-Century-Crofts, 1969.

† Supported by USPHS Career Development Award 5-K3-CA-05938-08.

lack a cell wall and are, in consequence of the effects of external physical forces, highly pleomorphic. They grow on lifeless media, and the center of agar-grown colonies (10 to 600 μ in diameter) is often deeply embedded in the agar. Mycoplasmas are highly susceptible to lysis by osmotic shock, are resistant to penicillin, inhibited by specific antibody, and will tolerate low concentrations of thallium acetate. With the exception of *Acholeplasma laidlawii*, all require sterol and protein for growth and multiplication. Despite a superficial resemblance, they differ from the L-phase of bacteria in that mycoplasmas do not derive from a bacterial parent, a fact that has been substantiated by immunological and nucleic acid homology studies. Furthermore, the bacterial L-phase is not dependent upon sterol and protein for growth, and the minimal reproductive units are generally larger than those of the mycoplasmas.

## History

It is generally believed that Louis Pasteur first recognized that an important disease of cattle called contagious bovine pleuropneumonia was caused by a specific microorganism, although he was unable to see or grow it. However, it was not until 1898 that Nocard and Roux (81) adequately described the disease and, failing to implicate ordinary bacteria, succeeded in growing the etiological agent in broth-filled collodion sacs inserted into the peritoneal cavity of rabbits. Soon they were able to grow the organism in serum-enriched broth *in vitro*, but it was not until 1900 that Dujardin-Beaumetz (33) described colonial growth on solid medium. His description included the light periphery and dark center characteristic of most mycoplasma colonies, and he showed that the darker centers were the result of penetration of the organisms into the medium. The first detailed morphological description of these pleomorphic organisms was made ten years later by Bordet (6) and Borrel et al. (7), and in 1929 Elford (45), using gradacol filters, showed the existence of viable forms 125 to 150 mμ in size. Although capable of growth in cell-free media, the organism was thought by many to be a virus and for 25 years occupied this anomalous position. In subsequent years considerable knowledge of the biology of *Mycoplasma mycoides* var. *mycoides*, the etiological agent of contagious bovine pleuropneumonia, has been obtained, and it has been recently dealt with (25). *M. mycoides* var. *mycoides* is the type species for the Genus Mycoplasma.

Contagious bovine pleuropneumonia is a rapidly spreading lung disease, first reported to have appeared in Germany and Switzerland in 1713 and