

## OBITUARY

Dr. Louis Dienes died on January 31, 1974, at age 88, after an illness of several months. His scientific career spanned a full and productive period of well over six decades. His first investigations were published in 1909, and his last publication appeared in 1971, 20 years after his formal retirement as Director of the Bacteriology Laboratory at the Massachusetts General Hospital (M.G.H.) After "retiring" he continued to work productively in a small research laboratory at the hospital until 1970.

It would probably be more appropriate to speak of Dr. Dienes as having had three careers, an initial one in immunology, a second in bacteriology, and a third in art. In the former two fields, his provocative observations anticipated by several decades major developments and interest. His work on tuberculin or delayed hypersensitivity afforded a firm foundation for subsequent investigations in the field of cellular immunity; his pioneering studies on the L-forms of bacteria and pleuropneumonia-like organisms were important elements in the evolution of current understand-

ing of bacterial cell walls, aberrant bacterial forms, and mycoplasma.

His career as an artist began in early childhood, and he continued to paint until the very end of his life. Indeed, his fondness and talent for painting were sufficiently strong that he spent some time as a young man in Paris deciding whether art was to be his principal calling. Eventually science won out, but his interest in art and morphology continued and were, in a way, wedded in the laboratory, where photomicrographs of his work with L-forms were all taken, developed, and enlarged by him with meticulous concern for detail.

Dr. Dienes was born in Tokay, Hungary in 1885, and what was to be his lifelong fascination with biological science began at an early age. After he received his medical degree at the University of Budapest in 1908, he undertook his first research in biochemistry and microchemical analysis. However, his initial experience with bacteriologic research in 1913 (in the laboratory of F. Neufeld at the Robert Koch Institute in Berlin), coupled with service as an army field bacteriologist



Louis Dienes, 1885-1974

in Poland and the Ukraine during World War I, turned his attention permanently to bacteriology and immunology. His military experience with vast outbreaks of cholera and typhoid, and particularly a valuable collaboration with E. Weil in the study of epidemic typhus fever and the Weil-Felix reaction, clearly focused his interest on the immune response in infectious disease. Thus began his first career.

In 1922, Dr. Dienes came to this country to work at the von Ruck Research Laboratory at a tuberculosis sanatorium in Ashville, North Carolina. Because of his prior experience it was only natural for him to study the immune response in tuberculosis. These classic investigations, begun when immunology was only an embryonic field concerned primarily with humoral immunity, extended over a period of 15 years both in Ashville and later at the M.G.H.

Three major concepts emerged directly from this work: (1) The histologic features of the tuberculin reaction (and of similar types of delayed hypersensitivity), characterized by perivascular mononuclear cell infiltrates throughout the zone of the skin test, are distinctive and set delayed hypersensitivity reactions apart from other types of inflammation and from immunologic reactions mediated by circulating antibodies. (2) Delayed-type hypersensitivity can be elicited with purified nonbacterial antigens such as egg albumen. Thus, the distinctive characteristics of tuberculin sensitivity do not depend, as had been thought, on some unique attribute of bacterial components, but rather are the results of a specific type of sensitivity to any of a variety of antigens. He observed that when a protein antigen is injected into a guinea pig, development of skin reactivity of the delayed type precedes anaphylactic sensitivity. Although his original demonstration of delayed hypersensitivity with purified nonbacterial antigens was carried out in tuberculous animals, he also showed that a similar response could be elicited in the absence of tuberculous infection. Several years later Jones and Mote confirmed Dienes' original observations of this important form of cell-mediated immunity when they observed the development of delayed-type skin reactions in patients accidentally sensitized to small amounts of rabbit serum. (3) The delayed-type hypersensitivity response to a purified protein is exaggerated

when the antigen is injected into an active tuberculous focus such as a caseating lymph node. This important observation of the adjuvant effect was later extended by Freund, who enhanced the development of delayed hypersensitivity by injecting antigen with killed tubercle bacilli in the form of a water-in-oil emulsion.

In 1930, Dr. Dienes moved to Boston to become the bacteriologist at the M.G.H. Prof. Hans Zinsser of the Department of Bacteriology and Immunology at Harvard Medical School, who had also been interested for some time in the tuberculin type of cutaneous reactions, was instrumental in recruiting Dr. Dienes for this position. In addition to running the Diagnostic Bacteriology Laboratory, Dr. Dienes continued his studies of delayed hypersensitivity, in close collaboration with Tracy B. Mallory, until the mid-1930s. Then, during the depression, because of lack of available funds for research, he was forced to make a striking change in the direction of his research. He turned to problems of bacterial growth and morphology, where investigations could be carried out with simple, inexpensive equipment, and he made use of cultures available in the Diagnostic Laboratory. Thus, inauspiciously propelled by external events, he launched into his second career. Fortunately, he lost little momentum in the transition.

The isolation in 1935, by E. Klieneberger, of an unusual organism (designated  $L_1$ ) from cultures of *Streptobacillus moniliformis* aroused his interest. These organisms had many characteristics not observed in bacteria and were similar in many respects to the bovine pleuropneumonia agent. Klieneberger believed that the pinpoint  $L_1$  colonies and the typical bacterial colonies obtained from infections or cultures of *S. moniliformis* represented two unrelated organisms living symbiotically. Using his newly developed in situ staining techniques and patiently following early changes in colonial growth, Dienes observed the sequential transformation of the bacilli to the large bodies of the L-form and the return of bacilli in broth cultures of  $L_1$ . As was the case with his work on delayed hypersensitivity, these observations and interpretations were not immediately accepted. Nonetheless, he persevered, marshalling in his photomicrographs morphologic support for his concepts, which were finally accepted about a decade later. During succeeding years he demon-

strated the spontaneous development of L-type colonies from other pleomorphic organisms (*Bacteroides*, *Flavobacterium*, *Haemophilus influenzae*) and emphasized that the transition from bacillus to L-form seemed to occur through the formation of large bodies similar to those observed in *S. moniliformis*. He showed that penicillin and glycine could be used to induce the development of L-forms in species in which they did not occur spontaneously at a detectable frequency. Most of his morphologic observations were made under light microscopy. However, during his last few years of active laboratory investigation, in collaboration with S. Bullivant and with use of the electron microscope, he confirmed that many (but not all) L-forms were surrounded by a single-unit membrane, lacked a cell wall, and had fine structure.

In studying the growth of proteus strains, Dienes noted an apparent antagonism between different strains when they were cocultivated on an agar plate. Spreading cultures of most strains would not grow into each other, and a clear line of demarcation was evident between them. In contrast, when colonies of the same strain were used, spreading growth readily covered the medium uniformly. The line of demarcation was shown to contain many "large bodies." This incompatibility between different strains of *Proteus* has come to be known as the "Dienes Phenomenon."

Inevitably his initial interest in L-forms led to the study of the properties of the morphologically similar pleuropneumonia-like organisms, which he isolated from the genital tract of patients. In 1937, the first isolation from man of a mycoplasma (at that time designated a pleuropneumonia-like or-

ganism) had been reported by Dienes, who obtained it in pure culture from an abscess of a Bartholin's gland.

His third career, that as an artist, lasted a lifetime. He delighted in painting, and his favorite scenes were those of the outdoors. The fine detail in his painting of a mushroom or of a wooded scene showed his keen perception and appreciation of nature. Thus, it is easy to understand the morphologic approach that characterized his bacteriologic studies.

Dr. Dienes was guided in the laboratory by an unwavering curiosity and a firm belief in strict scientific discipline. He opened paths to two new and important areas of investigation. His observations were overlooked initially; others did not follow the direction in which he pointed. It is gratifying that, in his later years, he could see the significance of his work widely accepted.

He was by nature a warm, kind, and gentle man. Those who knew him best will always remember the twinkle in his eye, his obvious delight in a new observation regardless of who had made it, his deep personal involvement in the work in his often cluttered laboratory, and the devotion he engendered in his students and associates. He was dedicated to the increase of knowledge and to science. When elected an honorary member of the American Society for Microbiology, his response was simply that he considered it as the greatest privilege that he had been able to give the larger part of his life to scientific work and studies.

MORTON N. SWARTZ

HAROLD F. DVORAK

Massachusetts General Hospital  
Boston, Massachusetts