

Interest in Mycoplasmas Centering on Five Areas

Microorganisms are found important to genetic coding, for 'nuisance value,' and as agents in many diseases

MEDICINE IN THE MAKING

The mycoplasmas, a group of microorganisms intermediate in size between bacteria and viruses, have been implicated as the cause of certain specific diseases in man, such as primary atypical pneumonia, and in all of the common domestic animals raised for food.

These ubiquitous agents have been recovered by investigators from sites other than the respiratory tract of man and animals and are now thought to be causative factors of disease in the genitourinary tract, mammary glands, joints, heart, and brain of one or more species of animals, including man. Recently they have been strongly implicated in the pathogenesis of cancer in man.

Although the first mycoplasma was isolated and grown in vitro from a case of pleuropneumonia in cattle in 1898, it has been only with the development of ultra-sophisticated laboratory and electron microscope techniques that the microorganisms have come under intensive investigation.

The scope of this work and some of the problems were outlined recently by Leon-

ard Hayflick, Ph.D., a medical microbiologist at the Wistar Institute, Philadelphia, when he presided as chairman of the second conference on the Biology of the Mycoplasmas sponsored by the New York Academy of Sciences. This meeting drew several hundred investigators from 14 countries.

"Essentially five broad areas of inquiry in the past seven years, since our first conference was held, have yielded results that have intensified interest in the mycoplasmas," he said. "First is the realization by molecular biologists that the mycoplasmas, as the smallest free-living microorganisms, are important materials for the elucidation of genetic coding and packaging.

"Second, the mycoplasmas have been of very great interest for their nuisance value to the army of investigators working with cell cultures. They have been shown to turn up with great regularity in cells maintained for any time in culture.

"Third, the demonstration five years ago that for the first time a particular

mycoplasma species was shown to be the etiological agent of a human disease known as primary atypical pneumonia.

"Fourth, their increasing importance as causes of animal diseases throughout the world. In particular, the observation that, as in other domestic and laboratory animals, mycoplasmas are etiologically responsible for economically important diseases of poultry and swine.

"Fifth, many people have become interested in mycoplasmas by having encountered them in what were first thought to be virus preparations obtained from human leukemia or cancerous tissues."

Dr. E. A. Freundt, of the Institute of General Pathology and Bacteriology at the University of Aarhus, Denmark, suggested that the investigators concerned with understanding the mycoplasmas are confronted with a situation that "parallels the problems we are facing with the viruses."

"During the past five years," he said, "there have been remarkable improvements in electron microscopy which in turn have led investigators into an increasing appreciation of the fundamental significance of morphology and ultrastructure for the definition of the mycoplasmas."



DR. HAYFLICK

An English medical scientist, Dr. D. G. ffarington Edward, of the Wellcome Research Laboratories in Beckenham, Kent, suggested that the order of Mycoplasmales, excluding *M. laidlawii*, may be defined as a group of microorganisms whose minimal viable units (elementary bodies) measure about 200 millimicrons.

"These cells," he said, "enlarge during growth and have a potential for growing in branching filaments. Reproduction appears to take place by the development within the filaments and possibly also in the cytoplasm of the larger cells of further elementary bodies and their subsequent release by fragmentation and disintegration of the filaments.

"The organisms lack a cell wall and moreover are incapable of synthesizing cell wall components, such as muramic acid and diaminopimelic acids. By reason of the lack of a cell wall they are plastic and assume their characteristic pleomorphic morphology. Also associated with a lack of a cell wall are the property of growing into a solid agar medium with the formation of characteristic colonies, comparative easy lysis by osmotic shock, absolute resistance to inhibition by penicillin and inhibition of growth by antibody. They require sterol (for example, cholesterol) for growth, presumably to maintain the integrity and function of the cell membrane."

Dr. Edward noted that L-phase organisms resemble mycoplasma in certain respects because they also lack a cell wall and are "pleuropneumonia-like." The L-phase organisms differ from Mycoplasmales because their minimal viable units (and genomes) are larger (600 millimicrons) and they do not require sterol for growth.

Unlike bacteria, mycoplasmas have no thick, rigid wall to hold them in shape, and in this respect they do resemble the L-forms into which certain bacteria can be transformed. However, microbiologists have been unable to transform mycoplasmas into bacterial forms, something they are always able to do with the L-forms. And they have also been unable to produce disease in animals with L-forms as they can with the mycoplasmas.

According to Mark E. Tourtellotte, Ph.D., a microbiologist at the University of Connecticut, the mycoplasmas do seem to be closely related to bacteria because both build proteins from amino acid. Also, they are especially like bacteria because their messenger RNA is short-lived and because a chemical—cycloheximide—interferes with the protein-making process in both the bacteria and the mycoplasmas.

However, a group of investigators at the National Cancer Institute said that

their studies indicate that "the mycoplasmas are distinct from bacteria."

Reporting for this group, Norman L. Somerson, Ph.D., said they matched the DNA of three species of mycoplasmas with three species of bacteria deemed most likely to be their parents. The DNA of one stable L-form was found to be identical with that of its known bacterial parent.

"Species within the genus *Mycoplasma* are sufficiently unrelated in some cases to warrant their inclusion in separate genera within the family Mycoplasmales," said Dr. Somerson. He noted these observations were made after DNA-matching tests on 20 species of mycoplasma.

It was the opinion of Drs. Louis Dienes and Stanley Bullivant, of Massachusetts General Hospital, Boston, that the mycoplasmas may be derived from bacteria. Like others attending the conference, they found the mycoplasmas to be very similar to the L-forms.

Both of these investigators found the similarities so great that they referred to mycoplasmas as pleuropneumonia-like organisms (PPLO), rather than as mycoplasmas. They pointed out that PPLO and L-forms are similar in size, shape, and internal structure.

"Of special interest," they said, "is the presence of the so-called elementary corpuscles and very thin regular filaments. It is impressive that there is so much similarity between PPLO and L-forms when they are grown under variable culture conditions."

Drs. Dienes and Bullivant pointed out that the main difference between L forms and PPLO seems to be that strains of PPLO are better adapted to grow in bacteriologic media and especially to grow as small granules. They said that the similarities between the two groups suggest that PPLO, like L forms, may be derived from bacteria. While there is no direct evidence for this, they said it is one of the important problems in studying PPLO.

Dr. Edward posed several as yet unanswered questions and problems for his colleagues to consider.

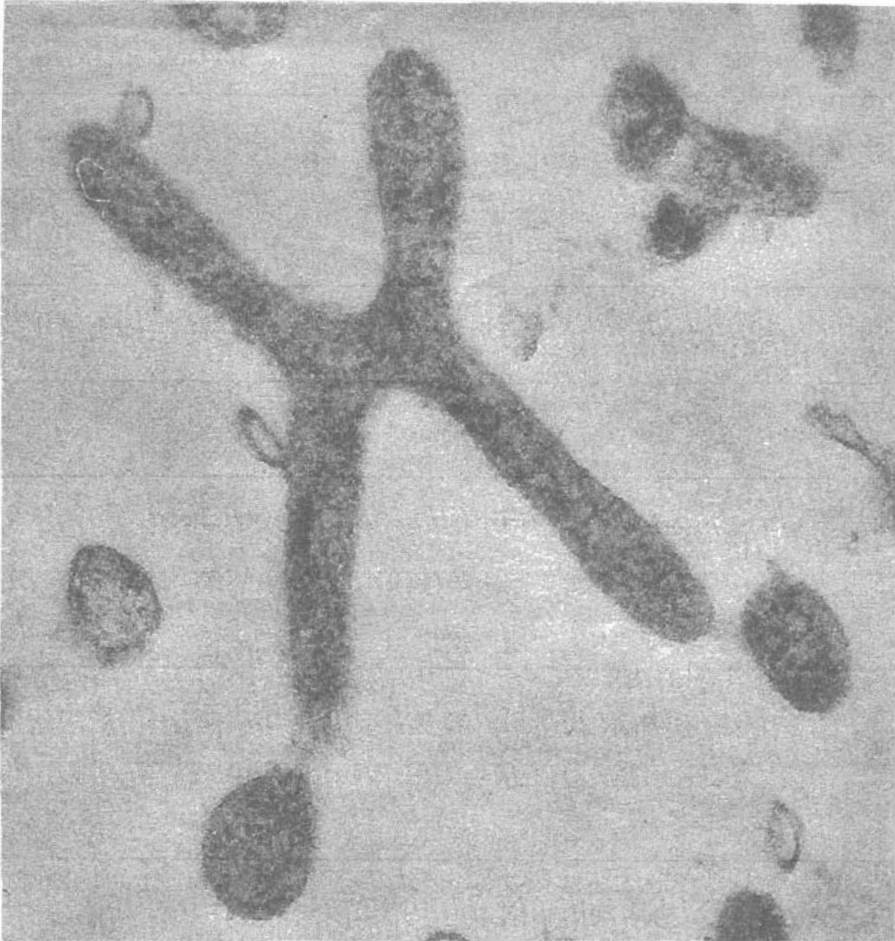
"What happens in practice when a new isolate is included in the order of Mycoplasmales? Identification is usually based on colonial appearances and on a rough look at its morphology, together with the knowledge that it has not knowingly been derived from a bacterium and shows no tendency to revert to one.

"I think we should bear in mind the need for a simple and practical definition of Mycoplasmales. My definition is more of a general description rather than a useful and practical one. Moreover, it is one that in detail is based only on examination of a minority of recognized species.

"Another problem to be borne in mind is the definition of a species. In examining a number of isolates of mycoplasma, what criteria should be used to classify them into species? The antigenic similarities revealed by what serological tests distinguish a species? How can biological properties be extended and tests standardized? What should be regarded as a variant or serotype, rather than a distinct species?

"We can do no more than raise these as questions that urgently need an answer."





Ultrathin section through filaments in branching stage of *Mycoplasma mycoides* is shown in this micrograph, magnification x 94,000, made by Dr. E. A. Freundt.