L. GIVORD

COMPTE - RENDU DU CONGRÈS SUR LES MYCOPLASMES ET AGENTS SIMILAIRES (MLO) DE L'HOMME, DES ANIMAUX ET DES PLANTES

8, 9 et 10 Janvier 1973

Académie des Sciences de New - york

OFFICE DE LA RECHERCHE SCIENTIFIQUE ET TECHNIQUE OUTRE-MER

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Président du Congrès : KARL MARAMOROSCH, Ph. D.
Boyce Thompson Institute Yonkers N.Y.

Organisateur : Rear admiral L.R. NEVILLE, USN (Ret.)

Le Docteur Karl Maramorosh a fait une courte introduction en rappelant qu'il y avait eu déjà deux congrès sur les mycoplasmes organisés par l'Académie des Sciences de New-York mais où il n'était pas question de mycoplasmes des plantes puisque leur découverte est plus récente.

Nous présentons tout d'abord la liste des conférenciers:

Arai, Dr. S.
Banttari, Dr. E.E.
Barile, Dr. M.F.
Belly, Dr. R.T.
Biberfeld, Dr. G.
Black, Dr. F.T.
Boatman, Dr. E.
Bové, Dr. J.M.
Bredt, Dr. W.
Brock, Dr. T.D.
Brunner, Dr. H.
Cassell, Mr. G.H.
Chanock, Dr. R.
Cherry, Dr. J.D.
Clyde, Dr. W.A., Jr.
Collier, Dr. A.M.
DelGiudice, Mr. R.A.
Edward, Dr. D.G.ff.
Fabricant, Dr. J.
Fogh, Dr. J.
Frederick, Mr. R.J.
Freundt, Dr. E.A.
Frey, Dr. M.L.
Gourlay, Dr. R.N.
Grabowski, Mr. M.W.
Hamparian, Dr. V.V.
Hayflick, Dr. L.
Hirumi, Dr. H.
Hopps, Ms. H.E.
Iida, Dr. T.T.
Jensen, Dr. D.D.
Kahane, Dr. I.
Kenny, Dr. G.E.
Klainer, Dr. A.S.
Lemcke, Dr. R.M.
Liss, Mr. A.
Maniloff, Dr. J.
Maramorosch, Dr. K.
Masover, Mr. G.K.
Mclntosh, Dr. A.H.
Meyer, Ms. B.C.
Mogabgab, Dr. W.J.
Morowitz, Dr. H.J.
Neimark, Dr. H.C.
Peterson, Mr. J.F.
Pollack, Dr. D.
Razin, Dr. S.
Riggs, Mr. D.B.
Rodwell, Dr. A.
Rodwell, Dr. E.S.
Ross, Dr. R;F.
Senterfit, Dr. L.
Singer, Dr. S.H.
Smith, Dr. P.F.
Somerson, Dr. N.L.
Stone, Dr. S.S.
Taylor-Robinson, Dr. D.
Tully, Dr. J.G.
Wallace, Mr. D.C.
Wittler, Dr. R.G.
Wolanski, Dr. B.S.
Yedloutschnig, Mr. R.J.

La séance du lundi matin (8 janvier 1973) était présidée par E.A. Freundt M.D. FAO/WHO. Reference Centre, Institute of Medical Microbiology, University of Arhus, Denmark, en remplacement de D.G. ff. Edward M.D., State University of New-York, Downstate Medical Center, Brooklyn. N.Y., malade.

Le résumé des conférences est présenté dans l'ordre chronologique

Titre de la séance :

CLASSIFICATION OF THE MYCOPLASMATALES

1 - PRINCIPLES OF MYCOPLASMA CLASSIFICATION. E.A. Freundt en remplacement de D.G. ff. Edward.

Class : Mollicute
Order : Mycoplasmatale
Family 1 : Mycoplasmataceae
Genus : Mycoplasma (37 species)
(1) - sterol required for growth
(2) - sensitive to digitonin and amphotericin B
(3) - genome size : 4,5 x 10^8 daltons

Family 2 : Acholeplasmataceae
Genus : Acholeplasma
(1) - sterol not required for growth
(2) - resistant to digitonin and amphotericin B
(3) - genome size : 1,0 x 10^9 daltons

Acholeplasma laidlawii Acholeplasma granularum	carotenoid synthesized
Acholeplasma axanthum	carotenoid not synthesized

Example of genome size

Mycoplasma pneumoniae	: 4,8 x 10^8	daltons
" orale	: 4,7 x 10^8	"
" salivarium	: 4,7 x 10^8	"
" fermentans	: 4,8 x 10^8	"
" hominis	: 4,5 x 10^8	"
" gallisepticum	: 4,9 x 10^8	"
" arthritidis	: 4,4 x 10^8	"
" T. Strain n° 27	: 4,7 x 10^8	"
Acholeplasma laidlawii A	: 1,1 x 10^9	"
" laidlawii B	: 1,0 x 10^9	"
" granularum	: 9,5 x 10^9	"
" axanthum	: 1,1 x 10^9	"

2 - MOLECULAR EVOLUTIONARY STUDIES ON MYCOPLASMAS AND ACHOLEPLASMAS by Harold C. Neimark, Ph.D., Dept. of Microbiology and Immunology, College of Medicine, S.U.N.Y., Downstate Medical Center, Brooklyn, N.Y.

Study of enzymes of energy metabolism from mycoplasmas and acholeplasmas has centered on lactate dehydrogenases (LDHs) from fermentative strains. Certain acholeplasma LDHs differ from mycoplasma LDHs by being activated specifically by fructose-1, 6-diphosphate (FDP) ; FDP appears to be an allosteric activator of these enzymes. Molecular and regulatory properties of acholeplasma LDHs have been examined in detail because the FDP activation mechanism is unusual and is known to occur only among the Lactobacillaceae. Individual acholeplasma and streptococcal LDHs were found to share a few to several specific molecular properties ; members of the two groups differ in apparent number of cooperative binding sites for activator. However not all groups of lactic acid bacteria have been examined for this property. It remains to be determined whether occurrence of very similar regulatory enzymes in acholeplasmas and lactic acid bacteria is a result of convergent evolution or a true evolutionary relationship.

3 - LIPIDS OF MYCOPLASMAS by Paul F. Smith, Ph. D., Dept. of Microbiology, Univ. of South Dakota, Vermillion, So. Dak.

Glycolipids and phosphoglycolipids are emerging as possible keys to explain distinctions and evolutionary origins of mycoplasmas. Glycolipids can be considered counterparts in gram-positive bacteria and some mycoplasmas of the lipopolysaccharides of gram-negative bacteria. Identical structures for glycolipids have been found for Acholeplasma laidlawii and Streptococcus, Mycoplasma neurolyticum and Staphylococcus, M. mycoides, M. pneumoniae and the pneumococcus, the Squire strain of mycoplasmas and Lactobacillus, and M. gallinarum and Corynebacterium. Phosphoglycolipids consist of a glycerophosphoryl or a phosphatidyl radical attached to a glycosyl diglyceride. These occur as major lipids in A. laidlawii and Thermoplasma acidophilum. Trace amounts are found in streptococci and staphylococci. The belief exists that phosphoglycolipids are covalently attached to membrane teichoic acids in gram-positive bacteria and are not extractable with lipid solvents. In mycoplasmas these lipids are extractable because these organisms are deficient or devoid of high molecular weight teichoic acids. Possibly the accumulation of phosphoglycolipids in mycoplasmas marks the site of evolutionary progress or degeneration of cell wall biosynthesis.

4 - THE BINDING OF PROTEINS AND LIPIDS TO MYCOPLASMA MEMBRANES by Samuel Razin, Dept. Clinical Microbiology, Hebrew Univ.-Hadassah Medical School, Jerusalem, Israel.

n Acholeplasma laidlawii membranes bound electrostatically large amounts of cytochrome c and lysozyme mostly to the polar groups of membrane lipids causing a decrease in the mobility of the hydrophobic tails. Solubilized membrane proteins and serum albumin were bound to the membranes in smaller amounts mainly by hydrophobic bonds. The bound proteins retained their immunogenicity. The binding of cholesterol by A. laidlawii membranes was tested in buffer containing labeled cholesterol and Tween 80. The data suggest that the major portion of bound cholesterol is incorporated into the lipid bilayer core at a rate dependent on the degree of fluidity of this core which is influenced by the fatty acid composition, interaction with di- or polyvalent cations and by temperature. Complete saturation of the cholesterol binding sites was never achieved, indicating that the mechanism controlling cholesterol uptake by growing cells either does not operate or operates fautily in isolated membranes. Cholesterol concentration depends of species.

5 - THE ORIENTATION OF PROTEINS IN MEMBRANES by Itzhak Kahane, Ph. D. and Vincent T. Marchesi, M.D., Ph. D., Department of Pathology, Yale School of Medicine, New Haven, Connecticut.

Selective enzyme treatments have been used to study the orientation of proteins in plasma membranes of mycoplasma and other cells. Proteins which are located on the external surfaces of membranes can be selectively digested with proteases or differentially iodinated using lactoperoxidase. External proteins or exposed parts of polypeptides are identified either through their removal by the protease or their capacity to incorporate radioactive iodide. Only two proteins (of $\sim$20) are on the external surface of red blood cells, one of which is a glycoprotein. This molecule is oriented at the cell surface so that part of its polypeptide chain (the N-Terminal segment bearing oligosaccharrides) is exposed to the cell exterior while the C-Terminal segment is embedded in the lipid region of the membrane. Similar experiments with mycoplasma membranes also suggest that a relatively small number of proteins ($\sim$ 6 polypeptide chains in M. pneumoniae) are located external to the lipid barrier of the membrane. One of these proteins is a glycoprotein which has been isolated and partially purified. These studies show that membrane proteins of these cell types are distributed asymetrically at the cell surface, with the bulk of the polypeptide chains either within or internal to the lipid framework of the membrane.

6 - SEROLOGICAL REACTIONS OF MYCOPLASMAS by Ruth M. Lemcke, Ph.D., Lister Institute of Preventive Medicine, London, SW1W 8RH.

The serological differentiation of mycoplasmas may not be acceptable to theoretical taxonomists because it fails to provide positive data about the properties of the organisms. In practice, however, serology constitutes the ultimate tool for identifying unknown mycoplasmas by comparison with authentic species and for establishing that new species have been isolated. Two fundamental considerations pertaining to the use of serology in identification are, the mode of preparation of hyperimmune sera and, the type of test chosen. In regard to the former, the inclusion or omission of adjuvant as well as the amount of the immunogen determines the range of antigens against which antibodies are formed. In regard to choice of test, studies with M. hominis showed that antibodies active in growth-inhibition, metabolic-inhibition and indirect haemagglutination are directed against membrane antigens whereas complement-fixing antibodies are directed against both membrane and soluble antigens. Moreover, in strains within the species M. hominis, differences occur in the membrane rather than in the soluble antigens. Thus, tests which involve membrane antigens reveal intraspecies differences whereas the complement-fixation test does not.

7 - SEROLOGICAL IDENTITY OF GLYCOLIPIDS FROM MYCOPLASMA PNEUMONIAE AND SPINACH. George E. Kenny, Department of Pathobiology, University of Washington, Seattle, Washington.

The major complement-fixing antigen of Mycoplasma pneumoniae is found in the glyceroglycolipid fraction. At least four glycolipid components may be resolved on thinlayer chromatography. Two of these components appear to have the composition of digalactosyl diglyceride and trigalactosyl diglyceride respectively. Since it has been reported that digalactosyl diglyceride from spinach cross-reacts with animal antisera to M. pneumoniae, digalactosyl diglyceride was purified from spinach chloroplast lipids. During the fractionation, an additional component with migration characteristics on thin-layer chromatography similar to those of M. pneumoniae trigalactosyl diglyceride was observed. This fraction has been purified and found to contain glycerol, galactose and fatty acids. Crude M. pneumoniae lipids, spinach digalactosyl diglyceride and spinach "trigalactosyl diglyceride" were compared by complement fixation with sera from pneumonia patients. Nearly all sera showed the same antibody response when tested with spinach "trigalactosyl diglyceride" as they showed to crude M. pneumonia lipids, but only about half of the positive specimens showed antibody measureable with spinach digalactosyl diglyceride.

8 - GENOME SIZE AND LIFE CYCLE OF THE MYCOPLASMA by Harold J. Morowitz, Ph. D., Dept. of Molecular Biophysics and Biochemistry, and Douglas C. Wallace, M. Ph., Dept. of Microbiology, Yale Univ., New Haven, Conn.

Examination of genome sizes by renaturation kinetics and the "Kleinschmidt" technique have substantiated the two Mycoplasmataceae genera, the Mycoplasma with about $5x10^8$ dalton (d.) genomes and the Acholeplasma with about $1.0x10^9$ d. genomes. Comparing genome sizes of the mycoplasma with those of other prokaryotic cells except obligate intracellular parasites and with the piece size of yeast chromosomal DNA suggest a possible central role of organisms with genomes of $5x10^8$ d. in the evolution of prokaryotic and eukaryotic cells.

The life cycle of mycoplasma has been assumed to involve elementary bodies. The elementary body hypothesis, however, is weakened because 1) microscopic studies are at the limit of resolution, 2) lack of evidence for the viability of the small objects seen in the microscope, and 3) inadequacy of filtration to measure the size of fluid objects such as mycoplasma. New information on mycoplasma genome size makes it extremely unlikely that any viable unit of less than 0.33 μ in diameter exists. Hence, binary fission with or without filament formation provides an adequate explanation of the reproductive activities of mycoplasma.

H.J. Morowitz presented an electron micrograph of a circular molecule of DNA (Mycoplasma genus).

La séance du lundi après-midi (8 janvier 1973) était présidée par J.G. Tully, Ph. D. Laboratory of Microbiology, National Institutes of Health, Bethesda, Maryland.

Titre de la séance :

MYCOPLASMATALES - NEWER AGENTS

9 - BIOLOGICAL AND SEROLOGICAL CHARACTERISTICS OF THE ACHOLEPLASMAS by Joseph G. Tully, Laboratory of Microbiology, National Institute of Allergy & Infectious Diseases, Bethesda, Maryland.

Acholeplasmas, as sterol-nonrequiring mycoplasmas, are receiving renewed interest since they are frequently isolated from animals and have become the most prevalent mycoplasmas reported as tissue culture contaminants. The present study was directed to some of the problems in identifying Acholeplasma species by conventional techniques. Strains belonging to the three classified species and approximately 15 unclassified strains were examined in several biochemical and serological procedures. A. laidlawii strains varied in their ability to ferment mannose and easculin, although most produced pigmented carotenoids. A. granularum and A. axanthum strains each appeared to be a more homogenous group but both were clearly distinct from each other and from A. laidlawii in serological tests. Most of the unclassified strains possessed biochemical properties similar to A. axanthum, although serological tests indicated that they were not related to each other or to the three classified Acholeplasma species. This information and other data suggest that the genus Acholeplasma represents a much larger number of distinct species than heretofore thought.

10 - THE GENUS THERMOPLASMA by R.T. Belly, Ph. D., B.B. Bohlool, Ph. D. and T.D. Brock, Ph. D., Dept. of Bacteriology, Univ. of Wisconsin, Madison.

Thermoplasma acidophilum is a free-living mycoplasma which grows optimally at 55 C and pH 2.0. The original isolate was obtained from a thermal and acidic region of a coal refuse pile. This organism appears to be absent in naturally occurring thermal, acidic soils and hot springs, but it has been readily isolated from 20 of the 30 thermal coal refuse piles sampled. All isolates were similar morphologically, lacked detectable hexosamine, grew at a pH range of 0.5 to 4.5, and a temperature range of 37 to 65 C. None of the isolates grew autotrophically on S^0 or Fe^{++} or heterotrophically in the absence of yeast extract. Five isolates were studied in detail for possible strain differences. All of these isolates demonstrated reduced cytochrome peaks at 555nm. In the presence of a growth-limiting concentration of yeast extract, growth of all isolates was stimulated by hexoses and some disaccharides, but not by amino acids, polyols, or pentoses.

Sucrose, glucose, mannose and fructose produce growth stimulation but no growth stimulation obtained by ribose, lactose, aspartate, glutamate and glycine. Polyacrylamide gel electrophoresis of cellular proteins demonstrated several common bands. Immunofluorescence is being used to study serological differences, as well as the ecology of this organism. Some serological differences have been detected among the isolates.

Some characteristics of Thermoplasma :

	Mycoplasma	Thermoplasma
Size	0,125 - 0,5 µ	0,3 - 0,7 µ
Filtration	pass trough 0,45 µ filter	pass trough 0,45 µ filter
cell wall	no	no
distilled water	sensitive	resistant
S L S	"	less sensitive

11 - THE DISTRIBUTION OF T-MYCOPLASMAS WITHIN AND AMONG VARIOUS SPECIES by D. Taylor-Robinson, M.D., Clinical Research Centre, Harrow, Middlesex, England.

In newborn infants T-mycoplasmas have been isolated from the genital and oral regions and from conjuctivae, and in adults from semen, urine, urethral, vaginal, rectal and oropharyngeal specimens. In addition, in women they have been found in Fallopian tubes, suprapubic urine samples, as well as in the blood in a case of puerperal fever and in products of conception. The organisms have been recovered from semen, preputial sac, nose, lungs and conjuctivae of cattle, and from the oropharynx of both squirrel monkeys and cats. Semen, preputial and vaginal specimens from canine species also contain these mycoplasmas. The author has not recovered them from rabbits, hamsters, guinea pigs, mice, rats or pigs. Without reinfection the organisms may persist in man for years as shown by studies on subjects isolated in the Antarctic. In organ cultures there is a lack of tissue specificity ; in vivo Gourlay showed that bovine T-mycoplasmas produced a mastitis in cattle but human strains did not. Polyacrylamide electropheresis on a few strains has indicated that those from different animal species are closely related. However, several strains from man are serologically distinct and also distinct from some of those of other species. Nevertheless, cross reactions occur among strains of a species and between strains of different species, although it has not been shown that strains from different animal species are serologically identical.

12a - THE GROWTH OF T-STRAIN MYCOPLASMAS IN MEDIUM WITHOUT ADDED UREA by Gerald K. Masover, M.S. and Leonard Hayflick, Ph.D., Stanford University, Stanford, Calif.

It has been reported that urea must be added to medium formulations for growth of T-strain mycoplasmas. We have shown this not to be so. Several T-strain mycoplasmas were passaged through multiple dilutions in medium without added urea (1) with 0.033 M putrescine, or (2) without added putrescine. Analysis of the medium detected nanomolar amounts of putrescine. No urea could be detected in the medium by a liquid chromatographic method sensitive to 10 µg/ml although analysis with urease detected about 15 µg/ml. The chromatographic method also indicated that putrescine was not depleted. Medium in which all undefined components were dialyzed allowed growth of one of the T-strains only if putrescine, additional magnesium, and urea were also added. The later 3 components could be replaced with agmatine or the urea with allantoin. The type strain (T-960) was passaged in medium containing dialyzed components plus putrescine and allantoin. Thus, properties of T-strains grown in the absence of elevated pH and (NH_4^+) could be evaluated. The results imply a need to reassess the urea requirement for T-strains.

12b - BIOLOGICAL AND PHYSICAL PROPERTIES OF HUMAN T-MYCOPLASMAS by Finn T. Black, M.D., Institute of Medical Microbiology, University of Aarhus, Denmark.

The serotypes I-VIII of human T-mycoplasmas were tested for biological and physical properties. They seem to constitute a very homogenous group, only the hemadsorption test could differentiate within the human T-mycoplasmas. Other properties, however, like ureasplitting - phosphatase activity - haemolysis of guinea pig and rabbit erythrocytes susceptibility to antimicrobial agents and digitonin together with their ability to grow at low temperature are very useful in the characterization of the human T-mycoplasmas as a whole.

13 - THE MOLECULAR BIOLOGY OF MYCOPLASMATALES VIRUSES by Jack Maniloff and Alan Liss, Univ. of Rochester, Rochester, N.Y.

About 50 isolates of mycoplasma viruses have been reported. Morphologically and serologically, these form two groups : (1) most isolates are naked bullet-shaped particles, designated L1-type ; (2) two of the isolates are enveloped particles, designated L2-type. All viruses contain DNA. We will only consider the biology of L1-type viruses.
VIRUS CHARACTERIZATION : The L1-type viruses have different UV inactivation rates, host ranges, one-step growth curves, and rates of inactivation with anti-L1 serum. The protein capsid of the bullet-shaped L1-type particles has helical symmetry.

VIRUS INFECTION : These studies have been done with an L1-type virus, MVL51. At MOI less than 10, virus infection is not lytic : infected cells grow slower than uninfected ones. There is a continual production of virus without cell lysis and virus maturation appears to take place as the viral DNA is packaged and extruded at the cell membrane. The lack of intracellular infectious particles during viral replication was confirmed by a premature lysis experiment.

VIRAL DNA INFECTION : Viral DNA added to cells is taken up and mature viruses are produced. The kinetics and stoichiometry of the DNA-cell interaction have been examined.

14 - EVALUATION OF REFERENCE REAGENTS FOR MYCOPLASMAS by E.A. Freundt, M.D., FAO/WHO International Reference Centre for Animal Mycoplasmas, Institute for Medical Microbiology, University of Aarhus, Denmark.

Seed and serum reagents for 29 Mycoplasma and Acholeplasma species or subspecies were tested. The identity of the seed reagents was confirmed biochemically and serologically. The number of colony forming units/ml varied for frozen samples from $5x10^4$ to 10^8 ; it was lower and often unacceptable for lyophils. Potency and specificity of the serum reagents was determined by cross titrations using disc growth inhibition (DGI), growth precipitation (GP), metabolic inhibition (MI), indirect hemagglutination (IHA), and complement fixation (CF) tests. Homologous titers were usually sufficiently high in all or most tests. All sera were highly specific as determined by DGI, GP and MI, while a rather high degree of cross-reactivity was found in IHA and CF tests. The cross-reactivity in IHA could be considerably reduced when using formalinized erythrocytes instead of fresh cells. With some reservations the reagents were found to fulfil the requirements for international reference reagents.

La séance du Mardi matin (9 janvier 1973) était présidée par Samuel Razin, Ph.D. the Hebrew University, Hadassah Medical School, Jerusalem, Israël.

Titre de la séance :

ULTRASTRUCTURE AND MORPHOLOGY

15 - MORPHOLOGY AND ULTRASTRUCTURE OF MYCOPLASMAS by Edwin Boatman, Ph.D., School of Public Health and Community Medicine, University of Washington, Seattle, Wa.

One outstanding characteristic of Mycoplasma is their heterogeneous morphology or 3-dimensional shape. With more care given to preparative procedures errors in interpretation have been reduced. In early exponential phase the basic cell form is coccoidal which, depending upon the species involved may remain coccoidal or become filamentous. Cellular extensions may be extensive or relatively minor. Consistent differences exist in cell size. The internal ultrastructure of mycoplasmas consists of ribosomes, a fibrillar nuclear component and in some, specialized structures at specific locations, the whole, bounded by a double membrane. With the techniques at hand, it is not enough to describe for any isolate either its morphology or ultrastructure, but both are required and in conjunction with estimates of cell viability throughout growth. Fixation has indeterminable effects on both morphology and structure ; the importance of observing unfixed organisms is obvious. The choice of fixatives remains subjective. Adequate sampling and quantitation are necessary particularly when sectioned material is observed. The aim of the morphologist is to relate structure to function and with the mycoplasmas, the task is a challenging one.
E. Boatman presented a series of figures showing different stages of growth and change in morphology of a Mycoplasma particule.

16 - NATURE OF STRIATED STRUCTURES IN MYCOPLASMAS by Alan Rodwell, Ph.D., J.E. Peterson, B.V.Sc., and E. Shirley Rodwell, Ph.D., C.S.I.R.O., Animal Health Research Laboratory, Parkville, Victoria, Australia.

A form of Mycoplasma, designated as the rho (ρ) form, is characterized as a relatively rigid, filamentous organism, often with discoidal swellings, and containing an intracytoplasmic, axial fibre terminating in a characteristic structure fig. 1. The fibre appears to be composed of parallel fibrils about 3nm diameter with structural features in lateral register producing a cross banding pattern after negative staining with a periodicity of 12 to 14.5nm. The ρ form was commonly found in caprine mycoplasmas, but occurred also in other mycoplasmas. The growth medium is important for selection of the ρ form genotype. For expression of the ρ character, a medium of relatively high tonicity and an unrestricted energy source is required. These conditions are not fulfilled by some of the widely used commercial media. Evidence indicates that the fibre contains protein.

By polyacrylamide gel electrophoresis, cultures rich in ρ forms were shown to contain two proteins of molecular weights approximately 130,000 and 28,000 daltons, which were absent or in low concentration in cultures without ρ forms.

17 - ULTRASTRUCTURE OF PLANT MYCOPLASMALIKE AGENTS by Hiroyuki Hirumi, M.D., Boyce Thompson Inst., Yonkers, N. Y.

The basic morphology of microorganisms observed in the phloem tissues of yellows diseased Nicotiana rustica and Salix rigida plants and of Cajanus cajan plants with a proliferation disease was similar to that described for many mycoplasmata. In a diseased N. rustica plant, spherical, as well as filamentous, bodies bounded by a smooth unit membrane contained ribosomes and deoxyribonucleic acid-like strands and varied considerably in size, shape and electron opacity. Colony-type accumulations, consisting of mushroom- or horseshoe-shaped forms, were seen in the phloem parenchyma cell cytoplasm of the host plant. Ultrastructural abnormalities of the mycoplasmalike organisms (MLO) were also seen, indicating that there is a natural degeneration of the agents in situ. In S. rigida plants, an electron-opaque layer surrounded the unit membrane of the MLO. This layer may be characteristic for certain species of the agents infecting plants, or it may be formed by woody plant hosts. In C. cajan, spherical MLO that resembled those found in N. rustica were observed.

18 - NEGATIVE STAINING OF PLANT AGENTS by Bohdan S. Wolanski, Ph.D., Merck Institute for Therapeutic Research, West Point, Pennsylvania.

The use of phosphotungstic acid (PTA) stain for the identification of the presumptive mycoplasma agents of aster yellows and corn stunt diseases has been found unsatisfactory. Structures generally considered as characteristic mycoplasma-like bodies are found in negatively stained extracts from both healthy and diseased aster and corn plants. These bodies are thought to be preparation and staining artifacts as no similar structures can be found in either healthy or diseased plant material pretreated with glutaraldehyde and osmium tetroxide. Artifacts similar to those seen in plant extracts can be produced also by PTA staining of HeLa cells and of Mycoplasma laidlawii : these artifacts are not present in material treated with fixatives prior to negative staining. A decrease in the amount of deformation produced in the plant material resulted from the use of uranyl acetate and ammonium molybdate instead of PTA. Filamentous bodies different from any structures seen in healthy plants were found in samples of corn stunt infected Zea mays and may be a form of the mycoplasma-like organism associated with this disease.

19 - SCANNING ELECTRON MICROSCOPY OF MYCOPLASMA AND MYCOPLASMA-LIKE AGENTS by Albert S. Klainer, MD, West Virginia University Medical Center, Morgantown, West Virginia and J. Dennis Pollack, Ph.D., College of Medicine, Ohio State University, Columbus, Ohio.

The morphology and the existence of a growth cycle of Mycoplasma pneumoniae has been clearly established by scanning electron microscopic studies which showed an orderly and sequential metamorphosis during its life cycle from spherical to filamentous to large round forms during the period of growth from 8 hours to 10 days. Further studies showed morphologic changes in Acholeplasma laidlawii B, A. granularum, A. axanthum, M. gallisepticum, M. hyorhinis, and T-strain grown in SSR-2 medium without oleic acid or Shepard's U-9 medium for 1/2 to 10 days at 37°C. Scanning electron microscopid studies have provided unique information concerning growth-related morphologic changes of intact fixed organisms not previously obtainable with other techniques. Scanning electron microscopic techniques, therefore, have become of great value in the investigation of the morphology of these organisms.

20 - MOTILITY OF MYCOPLASMAS by W. Bredt, Inst. f. Med. Microbiol., Johannes Gutenberg Univ., Mainz, Germany.

Some Mycoplasma species are able to glide actively along surfaces. Experiments were performed on M. pneumoniae strain FH. The cells were observed on glass surface in liquid medium with 3-5 % gelatine. The cells moved in irregular, mostly circular patterns. Their maximum speed was 0.75 µm/sec. The cells appeared to stick to the glass with a certain area of their front part. They always moved forwards with their front part, never switching to opposite direction fig. 2. Treatment with cytochalasin B up to 100 µg/ml failed to inhibit the movement. Preliminary experiments on M. gallisepticum and M. pulmonis showed, that the cells of these species too were moving ahead with a tip-like structure. The mechanism of the movements seems not to be related to gliding mechanisms of animal cells, which are known to be inhibited by cytochalasin B.

max speed	0,75 µm/sec
Longest distance measured	161 µm
Direction of movement	irregular, mostly circular

La séance du Mardi après-midi (9 janvier 1973) était présidée par Michael F. Barile, Ph.D. Mycoplasma Section, Division of Biologic standards National Institutes of Health, Bethesda, Maryland.

Titre de la séance :

MYCOPLASMA-CELL INTERACTIONS

21 - IDENTIFICATION OF MYCOPLASMA SPECIES ISOLATED FROM CONTAMINATED CELL CULTURES AND COMMERCIAL BOVINE SERA by Michael F. Barile, Ph.D., R.A. DelGiudice, Hope E. Hopps, M.S., M.W. Grabowski, B.S., and D.B. Riggs, Bureau of Biologics, FDA, and HEM Research, Inc., Rockville, MD.

The Bureau of Biologics requires a test for the presence of mycoplasmas in viral vaccines for human use which are prepared in cell cultures. Accordingly, we have maintainéd a continuing study for the past fourteen years to establish and to survey the incidence and sources of mycoplasma contamination of cell cultures. Of >6600 cell cultures examined, 1374 mycoplasmas were isolated and sero-identified by either the growth inhibition and/or the epi-immunofluorescence procedures. The cell culture contaminants include seventeen distinct species of mycoplasmas. Of these, 99% are human, bovine and swine species, thus, the major sources of contamination originate from these three animals. The source of bovine mycoplasma contamination of cell cultures is commercial bovine serum. Of 888 serum lots examined, 285 mycoplasmas have been isolated and 193 have been sero-identified. The serum contaminants include at least 8 distinct bovine species of mycoplasmas. The significance of these findings will be discussed.

22 - PROBLEMS CONCERNING "NON-CULTIVABLE" MYCOPLASMA CONTAMINANTS IN TISSUE CULTURES by Hope E. Hopps, M.S., Barbara C. Meyer, M.A., Michael F. Barile, Ph.D. and Richard A. DelGiudice, Bureau of Biologics, FDA, and HEM Research, Inc., Rockville, Md.

The formation of typical colonies on agar is a major criterion for identification of mycoplasma organisms. Nevertheless, recent studies in this laboratory indicate that some mycoplasmas cannot be isolated by conventional techniques. A WI-38 cell culture being used for virus studies was found to contain a mycoplasma-like agent as determined by observation of Giemsa-stained preparations. However, no organisms were isolated in standard mycoplasma broth or agar even after several serial passages. The agent grew luxuriantly in primary rabbit kidney cell cultures producing almost complete destruction of the culture in 7-10 days and attaining titers of 10^6 to 10^7 $TCID_{50}$ per 0.1 ml. Identification of the organism as Mycoplasma hyorhinis was achieved in this cell culture system using fluorescent antibody and classic neutralization test procedures.

Growth of the organism on agar was eventually achieved only after serial passage (4 times) in broth supplemented with heat-inactivated horse serum. The data suggest that in certain instances the use of cell cultures may be a useful and important adjunct in the isolation of mycoplasmas.

23 - ORGAN CULTURE TECHNIQUES WITH MYCOPLASMAS. by Albert M. Collier, M.D. and Joel B. Baseman, Ph.D., University of North Carolina School of Medicine, Chapel Hill, N.C.

For study of mycoplasma-host cell relationship at the cellular level, tracheal organ culture provides a means of maintaining living, organized, differentiated, respiratory epithelium in an easily observable and manipulable environment. The pathogenesis of respiratory infections can be analyzed in this system, including pathways of host cell parasitism and injury. Using this model, Mycoplasma pneumoniae has been demonstrated attached to epithelial cell membranes by a specialized terminal structure; the parasite remains extracellular, although the intercellular spaces may be invaded. The dynamic aspects of the attachment process can be studied with liquid scintillation spectrometry and radioautography using either organisms or host cells labeled with ^{3}H-thymidine and -amino acids.
Evidence of host cell damage accompanies organism attachment and replication, and includes ciliary dysfunction, cytopathology and exfoliation of epithelium. Tracheal pathophysiologic changes observed were unique to M. pneumoniae among human mycoplasma species studied, but the techniques discussed may be applicable to other organ culture systems and pathogenic microorganisms.

24 - MYCOPLASMA PATHOGENICITY STUDIES IN ORGAN CULTURES. James D. Cherry, M.D. and David Taylor-Robinson, M.D., St Louis Univ., School of Med., St. Louis, Mo. and Clinical Research Centre, Harrow, Middlesex, England.

Chicken tracheal organ cultures and specifically their functioning ciliated epithelium offer a quantitative living system for the investigation of mycoplasma pathogenicity factors. Three mycoplasmas, M. gallisepticum, M. mycoides var. capri (M. capri) and M. gallinarum have been extensively studied. The rapidity of the cilia-stopping-effect (CSE) resulting from M. capri infection was directly related to the concentration of multiplying organisms in the culture. On the other hand, the CSE of M. gallisepticum was not closely related to dose. Medium containing tetracycline inhibited M. gallisepticum was found to be mildly cilio-toxic; a similar finding was not noted with M. capri. Cytadsorption did not appear to contribute to the CSE of either M. capri or M. gallisepticum infections. M. capri was found to liberate more peroxide than other mycoplasmas and this contributed to the CSE, as the adverse effect could be partially reversed by the addition of catalase to the system. Peroxide did not contribute to the CSE of M. gallisepticum infection. M. gallinarum (a non-pathogenic mycoplasma) infection of the organ cultures had an inhibiting effect on the CSE of M. gallisepticum.

25 - MIXED MYCOPLASMA-VIRUS INFECTIONS IN CELL CULTURES by Stanley H. Singer, M.D., M. F. Barile, Ph.D., and R.L. Kirschstein, M.D., Bureau of Biologics, Food and Drug Administration, Rockville, Maryland.

Deliberate infection or covert contamination of cell cultures with mycoplasma can effect subsequent virus replication in these cultures. This effect may result in either an increase or decrease in virus yield. The mechanisms of decreased virus yield may be due to either a partial destruction of the cell matrix by the mycoplasma or to a more complex mechanism such as the utilization by the mycoplasma of a chemical substrate, such as arginine, which is a necessary component for replication of certain DNA viruses. One mechanism involved in increased virus yield appears to be related to the ability of viruses to produce interferon. The presence of mycoplasmas in cell cultures decreases interferon production. Since interferon itself acts to decrease virus yield, the decrease in interferon production caused by the mycoplasma will lead to a higher virus yield. The cellular substrate is also of importance in determining the effect on virus yield. For example, using the same virus and mycoplasma, increased virus yield was obtained in a primary mouse cell culture system, whereas decreased yield was obtained in a continuous mouse cell line.

26 - MYCOPLASMA AND ACHOLEPLASMA IN PLANTS by Arthur H. McIntosh, Sc.D., and Karl Maramorosch, Ph.D., Boyce Thompson Institute, Yonkers, N. Y.

Experiments were conducted to determine whether or not *Mycoplasma* and *Acholeplasma* species could be recovered from plants which had been exposed to these microorganisms. *Nicotiana rustica* (tobacco) and *Callistephus chinensis* (aster) were exposed to approximately 10^7 colony forming units per ml of *Mycoplasma gallisepticum* and *Acholeplasma laidlawii* for 45–120 min. Methods of exposure included roots (intentionally and unintentionally damaged), cut leaves, mechanical abrasion of leaves and injection. Controls consisted of plants exposed to growth medium only. Samples of both petioles and leaves were taken 7–8 hr following exposure to the microorganisms. *A. laidlawii* was recovered in 6 out of 15 experiments (40 %). Of these 20 % were from root exposure experiments. The remaining 20 % were equally distributed among the other categories. *M. gallisepticum* was recovered in only 1 out of 11 experiments (9 %, root exposure). No Mycoplasmatales were recovered from control plants. Isolates were identified by growth inhibition and immunofluorescent tests. These observations indicate that healthy appearing plants can incorporate animal mycoplasmas and that such microorganisms are recoverable by cultural techniques.

La séance du mercredi matin (10 janvier 1973) était présidée par Leonard Hayflick, Ph.D. Stanford University, School of Medicine, Stanford, California.

Titre de la séance :

ISOLATION, PATHOGENICITY AND CHEMOTHERAPY I

27 - RECOVERY AND IDENTIFICATION OF MYCOPLASMAS FROM ANIMALS by Merwin L. Frey, D.V.M., Ph.D., Gail B. Thomas, B.A., M.Sc., and Patricia A. Hale B.Sc., Veterinary Medical Research Institute, Iowa State University, Ames, Iowa.

In thorough attempts to isolate mycoplasmas from disease processes of unknown etiology or to survey the mycoplasmal flora of an animal host species, the basal medium/serum combination(s) utilized should be capable of propagating good growth of reference strains of all known mycoplasmas previously isolated from man and lower animals. Any single combination which fulfills this criterion is necessarily a compromise, and often is not the best available medium for antigen production with a given mycoplasma species. One such medium is prepared from dehydrated peptones, yeast autolysate and extract, $MgSO_4$, KCl, HEPES buffer, vitamins and cholesterol, and is supplemented with reduced NAD and rabbit or swine serum at time of use. Water quality, glassware preparation, conditions of medium storage and selection of agar should be as for cell culture cloning media, or as close thereto as possible. Serum should be acid treated or heat inactivated. The simplest identification procedure is disc colony growth inhibition, but it is so specific that some strains can only be identified at the subspecies level. CF and FA procedures described suffer occasionally from the same drawback, but to a lesser extent.

28 - PATHOGENICITY OF SWINE MYCOPLASMAS by R.F. Ross, D.V.M. Vet. Med. Res. Inst., Iowa State University, Ames, Iowa.

Three species of mycoplasmas known to be pathogenic for swine are M. hyorhinis, M. hyopneumoniae and M. hyosynoviae.

M. hyorhinis causes polyserositis and arthritis in 3 to 10 week old swine. The organism can be isolated from nasal secretions of normal as well as diseased swine and is a frequent isolate from pneumonic swine lungs. Experimental production of the disease is achieved by intraperitoneal inoculation. Tissue and serum/synovia changes in M. hyorhinis arthritis resemble those in rheumatoid arthritis.

M. hyopneumoniae causes a chronic, nonsuppurative pneumonia in young swine characterized by a 2 to 3 week incubation period and 3 to 6 weeks of coughing, roughened hair coats and poor weight gains. The organism can be isolated from pneumonic lungs and nasal secretions of affected pigs. The disease can be produced by intranasal or intratracheal inoculation of lung tissue suspensions or broth cultures.

M. hyosynoviae causes an acute nonsuppurative polyarthritis in young swine over 10 weeks of age. During the acute stage, the organism can be isolated from arthritic and some nonarthritic joints, lymph nodes, blood and various mucosal secretions; in convalescence, it persists in the nasopharynx and tonsils. The disease can be produced in certain types of swine by intravenous or intranasal routes.

29 - THE PATHOGENICITY OF BOVINE MYCOPLASMAS by Julius Fabricant V.M.D., Ph.D., Dept. of Avian Diseases, N.Y. State Veterinary College, Cornell Univ., Ithaca, N. Y.

Mycoplasma mycoides var mycoides in cattle is known to produce bovine contagious pleuropneumonia and is also capable of causing arthritic lesions in cattle. This review is primarily concerned with the occurence of other mycoplasma species in cattle and an evaluation of the evidence relating to their pathogenicity. Various species or serotypes of mycoplasma have been isolated from the bovine respiratory tract (nose, trachea, lungs), the male reproductive tract (prepuce, semen, seminal vesicles), the female reproductive tract (vagina, uterus, oviduct, aborted fetuses), the mammary gland and from joints. Existent data clearly demonstrates that mycoplasma can cause clinical mastitis, arthritis and seminal vesiculitis in cattle. Mycoplasma can cause less severe pneumonic and endometrial lesions. Their relationship to other bovine lesions is still not proven.

30 - IMMUNOELECTROPHORETIC COMPARISON OF MYCOPLASMA MYCOIDES ISOLATED FROM CATTLE AND GOATS WITH FOUR MYCOPLASMA ISOLATED FROM GOATS IN THE UNITED STATES
by S.S. Stone and R.J. Yedloutschnig, Plum Island Animal Disease Laboratory, Agricultural Research Service, US Dept. of Agriculture, Greenport, New York, and National Animal Disease Laboratory, P.O. Box 70, Ames, Iowa.

Four virulent mycoplasma recently isolated from goats in the United States of America (USA) were compared primarily by immunoelectrophoresis to Mycoplasma mycoides var capri (Vom, Nigeria) and to bovine Mycoplasma mycoides var mycoides.

Using bovine, pig, and rabbit anti-M. mycoides var mycoides sera and rabbit anti-M. mycoides capri serum several similar precipitin bands between the USA isolates and the M. mycoides var mycoides were found. Cross reactions were also observed between rabbit anti-M. mycoides var capri serum and the USA goat mycoplasma isolates. Complement fixation tests using the same antisera showed a similar relationship. It is concluded that these USA goat mycoplasma isolates have an antigenic relationship to M. mycoplasma var capri and to M. mycoplasma var mycoides.

31 - MURINE MYCOPLASMA RESPIRATORY DISEASES by Gail H. Cassell, M.S., J. Russell Lindsey, D.V.M., M.S., and Ronald G. Overcash, D.V.M., Depts. of Comparative Medicine and Microbiology, Univ. of Alabama in Birmingham, Birmingham, Ala.

Important advances have been made recently in understanding the mycoplasmal respiratory diseases of laboratory rats and mice. All principal lesions of the natural "chronic respiratory disease" in these species have been reproduced by inoculating pure cultures of Mycoplasma pulmonis intranasally into animals known to be free of other pathogens. The experimental disease in mice has proved to be the more consistently reproducible model system, permitting quantitation of host response to a wide range of doses. Specific antibodies of the G_1, G_2, M and A classes were detected in serum and bronchial secretions. These antibodies were shown to be produced locally in the lung and regional nodes. Active immunization with live organisms and passive immunization with immune mouse serum have been shown to protect against pneumonia. The experimental disease in the rat is less well characterized because of poor reproducibility, particularly of lesions in the lower respiratory tract. Based on preliminary comparative studies using immunofluorescence, a possible explanation appears to be a more efficient clearance mechanism in rat lung.

32 - PATHOGENESIS STUDIES IN EXPERIMENTAL MYCOPLASMA DISEASE by Wallace A. Clyde, Jr., M.D. and Lewis Thomas, M.D., Dept. Pathology, Yale Univ. Sch. Med., New Haven, Conn.

Neurologic and arthritic disease syndromes produced by Mycoplasma gallisepticum strain S6 in turkeys were analyzed by collation of clinical, microbiologic, pathologic, and immunofluorescence data. After intravenous inoculation birds developed fatal encephalopathy after a dose-dependent incubation period. In disease evident at 3-7 d, but not that at 1-2 hr, organisms were found on the endothelium or within the walls of cerebral arteries. Concurrently : 1) subclinical renal disease was manifest by glomerular injury including serum protein leakage and deposition of immune complexes (these changes reversed in surviving birds); and 2) organisms were found in vessels of clinically normal joints. After 30 d polyarthritis developed and progressed, but the tissues became sterile after 47 d. The arteriotropism of M. gallisepticum represents an unique host-parasite relationship. Temporal-pathologic correlations suggest that disease mediation is via products from infected arterial foci acting upon cerebral capillaries, glomeruli and synovium where organisms may be absent. These findings have implications for the study of human collagen-vascular diseases whose etiology is unknown.

33 - RESEARCH AND DEVELOPMENT OF MYCOPLASMAL VACCINES by Norman L. Somerson, Ph.D., Ohio State University College of Medicine, Laurence B. Senterfit, Sc.D., Cornell University Medical College, and Vincent V. Hamparian, Ph.D., Ohio State University College of Medicine.

A potent, formalin inactivated vaccine was prepared with Mycoplasma pneumoniae for clinical trials in man. In animals, this latest vaccine (designated OSU 1A) was considerably more antigenic than the original prototype vaccine. Extinction potency tests in hamsters based on serologic conversion showed vaccine OSU 1A to be at least a hundred times more potent. Improvements in the manufacturing process of vaccine OSU 1A include a better seed inoculum, an improved medium, and detachment of organisms by trypsinization and glass beads. No adverse reactions were observed when the vaccine was administered to thirteen volunteers.

34 - EFFICACY OF INACTIVATED MYCOPLASMA PNEUMONIAE VACCINE DEMONSTRATED BY PROTECTION IN LARGE FIELD TRIALS by William J. Mogabgab, M.D., Tulane University School of Medicine, New Orleans, La.

Inactivated Mycoplasma pneumoniae vaccine stimulated antibody responses that were of sufficient magnitude for protection in most individuals and that were comparable to those produced by natural infection in amount and duration. Antibody persisted for twenty months or longer. There were no adverse local or systemic reactions of consequence. Protective efficacy of the vaccine was determined in a controlled study in 13,892 airmen during 1969-70 at Keesler Air Force Base, Mississippi where mycoplasma pneumonia has been present for several years with epidemic occurrences. The protective efficacy of the vaccine against bronchitis caused by Mycoplasma pneumoniae was 87 percent and against pneumonia 66 percent. Persons who developed Mycoplasma pneumoniae infections in spite of vaccination did not experience more severe illnesses.

La séance du mercredi après-midi (10 janvier 1973) était présidée par Floyd W. Denny, M.D., the University of North Carolina School of Medicine, Chapel Hill, North Carolina en remplacement de E.A. Freund (déjà cité).

Titre de la séance :

ISOLATION, PATHOGENICITY AND CHEMOTHERAPY II

35 - PATHOGENICITY OF MYCOPLASMALIKE AGENTS IN PLANTS by Karl Maramorosch, Ph.D., Boyce Thompson Inst., Yonkers, N. Y.

Mycoplasmalike organisms (MLO) induce sterility, stunting, axillary proliferation, breaking of dormancy or death in over 60 different plant diseases. Coconut palms die rapidly when affected by lethal yellowing. Aster yellows diseased plants live longer than healthy plants and may become attractive and palatable to certain insects that cannot survive on normal individuals. The mechanism of MLO action in plants is unknown but suggestive of hormonal imbalance. Mechanical inoculation of crude plant or insect vector extracts into vectors, but not into plants, provides a sensitive MLO bioassay. Systemic MLO infection has been studied by electron microscopy of phloem sections during various stages of disease. Mechanical clogging of vessels, as well as passage through sieve plate pores, and later degeneration of MLO have been observed. MLO-associated viruses have been detected but there is no indication that such viruses can overcome chronic MLO diseases. Heat therapy has provided permanent cure of certain MLO diseases, whereas tetracyclines caused temporary remission only. Mechanical inoculation of plants with MLO, reportedly isolated and cultured on artificial media, has not been confirmed.

K. Maramorosch presented photographs of yellowing of several species of plants : Aster yellow, corn stunt, rice yellow dwarf, yellowing of coconut plants (occuring in Caribbean, Jamaica, Africa), Papaya bunchy Top (cured by surgery) and Opuntia. In the case of corn stunt, MLO elongated particules were observed, in the vector and in the plant but rounded particles ressembling those of Acholeplasma and Mycoplasma were observed only in the insect.

36 - CHARACTERIZATION OF THE MYCOPLASMA LIKE ORGANISM ASSOCIATED WITH STUBBORN DISEASE OF CITRUS. J.M. Bové, Ph.D., Centre de Recherches de Bordeaux, INRA, Bordeaux, France.

By optical dark-field examination of log-phase broth cultures, the organism is a motile, helical filament 2-4 µm long fig. 3. Helical shape is preserved by molybdate, but not by phosphotungstate, for negatively-stained EM exam ; and by glutaraldehyde in medium, but not in cacodylate, for fixation followed by sectioning, metal shadowing, freeze-etch, or scanning EM. Filaments are 100-120 nm wide, and amplitude of helices varies from 200-300 nm. In old broth cultures or in colonial growth from agar, helical shape is lost. An outer layer or nap on the single limiting membrane is present, to which attaches a tailed type B bacteriophage which can also be seen developing intracellularly. Unexplained components include random striated structures seen by negative staining and an interrupted or inconstant submembranous layer seen in sections. No cell wall nor organelles are present. Both strains (Morocco and California) are identical and resemble the uncultured corn stunt agent, but show new features. Shape, motility, and presence of classic phage obscure taxonomic position as a mycoplasma as suggested by other studies, but appear to warrant establishment of a new genus to be placed later in higher taxa.

37 - FAILURE TO ISOLATE MYCOPLASMAS FROM ASTER YELLOWS DISEASED PLANTS AND LEAFHOPPERS by Leonard Hayflick, Ph.D., Stanford Univ. Sch. of Med., Stanford, Ca. and Sumio Arai, M.D., Tohuku Univ. Sch. of Med., Sendai, Japan.

Seventeen different agar media were employed in attempts to isolate mycoplasmas from aster yellows disease and from leafhoppers that had fed on these diseased plants. The media contained a variety of supplements including coconut, soil and malt extracts ; lobster hemolymph, glycine, and extracts of uninfected plants. No mycoplasmas were isolated from homogenized infected plants or leafhoppers when placed directly on agar, in fluid culture, or in diphasic medium after 14 days of incubation and after several blind passages. Negative results were also obtained when the cut stems of infected plants were imbedded directly into agar media. We conclude from these and other data that typical mycoplasma species are probably not etiologically involved in aster yellows.disease. The forms associated with this disease have properties not only suggestive of mycoplasmas but also similar to the L-Phase of Bacteria, the Rickettsiaceae, the Chlamydiaceae, some protozoa and, perhaps, might be regarded as a new group of phytopathogens. Consequently these entities should be called yellows disease agent (YDAA) until Koch's postulates are fulfilled.

38 - COMBINED MYCOPLASMA AND VIRUS INFECTIONS IN PLANTS AND INSECTS by Ernest E. Banttari, Ph.D., and Richard J. Zeyen, Ph.D., Department of Plant Pathology, University of Minnesota, St. Paul, MN. 55101.

There are at least three reported cases of dual infections of plants with viruses and mycoplasmalike organisms (MLO). One report deals with Likuben disease of Citrus ponki and C. tankan infected with tristeza virus and an unidentified MLO. The second is the report of MLO and rhabdovirus in tissues of naturally infected Cajanus Cajun. The other report is that of Linum usitatissimum infected with oat blue dwarf virus (OBDV) and aster yellows (AY). In Linum, AY and OBDV are phloem limited and cause hyperplasia and obliteration of phloem elements and hypertrophy of adjacent tissues. Extensive leakage of a dark staining substance into fibers, cortex, and pith indicated a severe disruption of phloem function. Although both disease agents were found in the same phloem cells, there appeared to be no qualitative or quantitative effect of one upon the other. OBDV multiplies in immature phloem cells having a full complement of cellular constituents whereas AY apparently multiplies in more mature enucleate elements. Both disease agents are propagative in the vector, Macrosteles fascifrons. The leafhopper can acquire both disease agents from doubly infected plants and transmit them simultaneously to plant hosts.

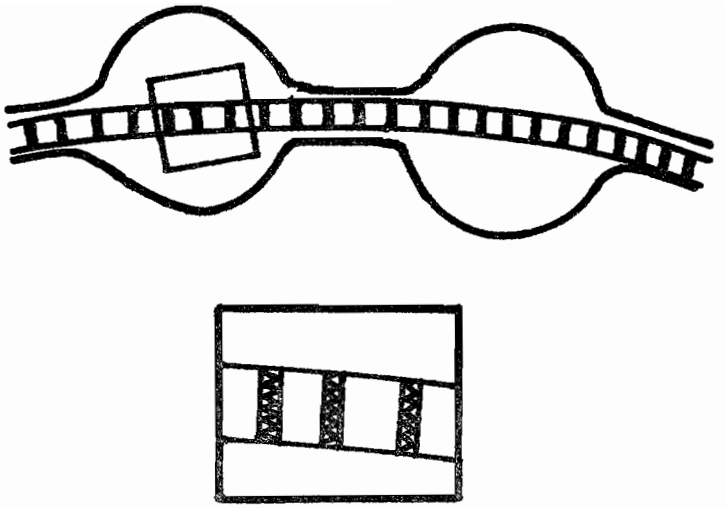
39 - EFFECTS OF TETRACYCLINE COMPOUNDS ON PLANT DISEASES CAUSED BY MYCOPLASMA-LIKE AGENTS by T.T. Iida, Institute for Plant Virus Research, Chiba, Japan.

Effects of tetracyclines to several "yellows" type diseases suspected to be caused by mycoplasma-like agents were tested in a cooperative work by a group from 13 institutions in Japan. Mulberry dwarf, rice yellow dwarf, (Japanese) potato witches' broom (potato and tomato), (Japanese) aster yellows (tomato, carrot, potato, and cosmos), and Cryptotaenia witches' broom were tested. Tetracycline, chlortetracycline, dimethylchlortetracycline, and oxytetracycline were used in most tests. A few antibiotics other than tetracyclines were also included in some tests. Plants were treated by dipping the roots or by spraying the leaves, usually with 10-100 ppm solutions. The treatments were made for various durations at various times before inoculation, after inoculation, or after symptom appearance. The tetracyclines had distinct but only temporary effect in causing remission, depending on dose, but not cure of the diseases. Other antibiotics were ineffective.. In mulberry dwarf and rice yellow dwarf, infective vector leafhoppers were either administered per os or injected with tetracyclines, and tested for their infectivity on test plants. It resulted in prolonging the incubation period within insect, or sometimes complete suppression of infectivity.

40 - ASTER YELLOWS : EFFECT OF SULFA DRUGS by R.J. Frederick, MS, Warner Lambert Company, Morris Plains, New Jersey.

Four sulfa drugs were applied individually or in combination with tetracycline-HCl to healthy and aster yellows-affected China asters. Recovery from severe stunting was obtained with sulfadiazine, sulfisoxazole, and sulfisomidine. Disease expression was suppressed more and for longer periods by the combined application of sulfadiazine and tetracycline-HCl than by either compound alone. The mean tetracycline-HCl concentrations in diseased plants treated by a cut-leaf immersion technique decreased when the antibiotic was administered in combination with sulfisomidine and sulfanilamide. Transmission by vectors of the aster yellows agent increased or remained unchanged when the sulfa drugs were applied to infected plants. Disease transmission efficiency of infective insects fed intermediately on healthy plants treated with sulfadiazine and/or tetracycline-HCl was significantly lower than the transmission efficiency of insects fed on healthy plants treated with sulfadiazine alone. All of the treated healthy plants used as intermediates for these vectors became infected, although expression of the disease was delayed.

K. Maramorosch a fait une conclusion générale en remerciant les mycoplasmatologistes "survivants" qui ont assisté à la dernière séance ; et la clôture du congrès a été faite par le Président de l'Académie des Sciences de New York : Kenneth W. Thompson, M.D.



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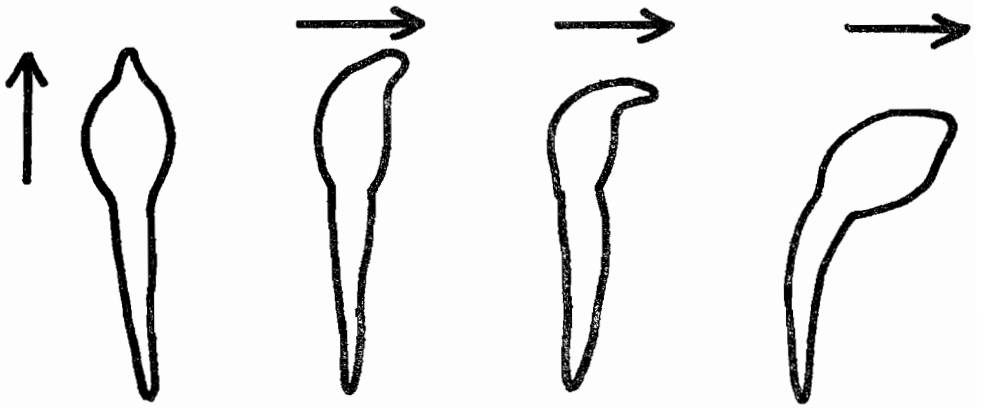

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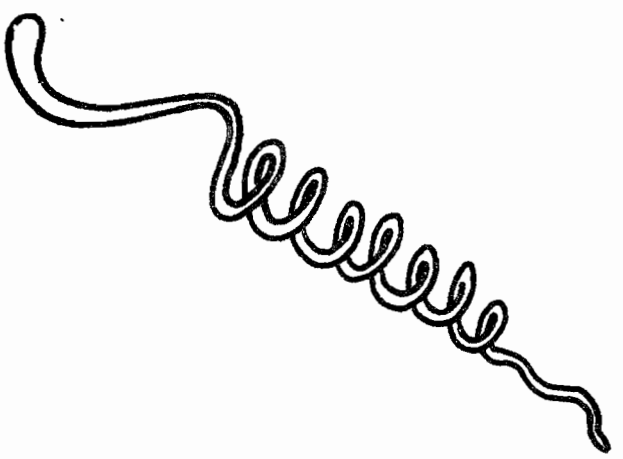

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