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THE PREVALENCE AND IMPORTANCE OF PPLO AND L FORMS

FINAL REPORT

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ABSTRACTS

1. Expertise in techniques for mycoplasmas has been developed. Isolation, propagation, identification and storage of both classical and T strain mycoplasmas have been successfully accomplished. Serologic techniques in microtiter complement fixation and metabolic inhibition have been studied, implemented, and are currently used. Rabbits have been immunized with six human classic species.

2. Pharyngeal carriage of mycoplasma species among healthy young adults has been studied to provide a base line for comparison with isolates from hospitalized patients.

   M. pharyngis and M. salivarium appear to be the organisms carried by healthy individuals. M. hominis I and M. pneumoniae have only been isolated from patients with respiratory disease.

3. M. pneumoniae has been isolated from 16 patients out of a total of 30 with lower respiratory tract infection at the Peter Bent Brigham Hospital; a rise in titer has been found in 11. M. hominis has been isolated from two people with pharyngitis with no obvious bacterial etiology. No association between pharyngeal carriage and other disease states has been found. One-hundred-forty-six cultures of patients with asthma, one hundred cultures of patients with malignancy have led to no species correlation of carriage. One-hundred-sixty cultures of patients with upper respiratory tract infections have also been negative as to correlation of mycoplasma species isolated from the nasopharynx with symptoms.

4. Mycoplasmas appear to be responsible for a proportion of genitourinary tract infections seen by physicians at the Peter Bent Brigham Hospital. Treatment for mycoplasmas has resulted in subsidence of symptoms and eradication of mycoplasmas. The infection most frequently seen is urethritis in male patients.

5. Mycoplasmas have been found associated with cases of infertility and reproductive failure. Mycoplasma isolations were more frequent from pregnant patients attending a high risk clinic than from pregnant patients attending the normal clinic. Cultures of twenty-two placentas taken from premature births and spontaneous abortions resulted in isolations of mycoplasmas from fifteen.

6. Aerosols of mycoplasmas and L forms have been successfully created and recovered demonstrating that these organisms can remain viable when airborne as droplet nuclei. Particle size was small enough to assign hygienic significance to them as agents of airborne infection.

7. Isolates from bronchi and lungs at autopsy have yielded mycoplasmas. From one third of thirty-three bronchial cultures and from three of eight lung cultures, the organisms recovered were M. salivarium, M. hominis I, M. pneumoniae, and M. pharyngis.

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I. TECHNIQUES FOR MYCOPLASMAS

Five years of investigations on the PREVALENCE AND IMPORTANCE OF MYCOPLASMAS AND L FORMS has resulted in expertise in isolation, propagation, identification, and storage of these microorganisms. Serologic techniques have also been developed, complement fixation for classic mycoplasmas and metabolic inhibition for T strains, both done in microtiter, are currently used for detection of antibodies. Over 500 serologic tests have been performed to date. A fourfold rise or drop in titer is sought for M. pneumoniae. Classic mycoplasmas forming large colonies and capable of being serologically and biochemically differentiated have been isolated in specimens taken from healthy and diseased individuals. T strain, or small colony mycoplasmas have been isolated from over 250 patients from the genitourinary tract. Undoubtedly serologic types of T strains exist but techniques for such identification have not been developed because of the difficulty in propagating these organisms. Their identification is based on small colony size at maturity 10-40 microns, possession of a urease system, inhibition by thallium acetate, and preference of a low pH6 medium, all characteristics described by Shepard (1).

Metabolic inhibition tests done with 50 T strains isolated from the genitourinary tract of patients with a history of infertility or reproductive failure, have not yielded high titers when tested against the serum of individuals from whom the T strain was isolated. The highest titers were 1 to 16 in one patient while positive controls with commercial goat anti-serum against the homologous T-strain 960 were over 1 to 2048. Eight other patients had titers between 1:2 and 1:8 against homologous T strains. Most patients' serum against their own isolates, however, were negative. The value of the test for evidence of T strain infection is therefore equivocal.

Both types of mycoplasmas have been sought in isolations from clinical material during the last year of our investigations. L forms were not found but large bodies were isolated on numerous occasions; these reverted to bacterial forms on subculture.

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(1) Maurice C. Shepard, Carl D. Lunceford: Occurrence of Urease in T Strain of Mycoplasma, J.Bact., 93, 1513-1520, 1967.

The high rate of isolations of mycoplasmas which we have reported, may be attributed to the use of the Fortner plate which utilizes Serratia marcescens furnishing a microaerophilic environment providing moisture and carbon dioxide. Most strains prefer microaerophilic to aerobic conditions of growth resulting in a higher rate of isolation from the nasopharynx than other investigators using the conventional anaerobic techniques.

Rabbits were immunized with standard human strains and these antisera used for complement fixation and growth inhibition testing. Methods of inoculation combining foot pad inoculation with algivant as well as intravenous inoculation gave demonstrable titers as well as zones of inhibition with homologous mycoplasmas - useful in species identification until the advent of commercially prepared antisera. The M. hominis I antiserum prepared in our laboratory has proved to be superior to the commercial product, producing larger zones in growth inhibition tests and consequently has been consistently used for identification of this species. This suggests that several serologic types of M. hominis may exist.

II. PHARYNGEAL CARRIAGE OF MYCOPLASMAS BY HEALTHY YOUNG ADULTS

The investigation of mycoplasma carrier rates and the species of mycoplasma carried among medical students over a period of 5 months, and repeated one year later together with a study of carriage in healthy adolescents was made to yield a base line for comparison with isolations from hospitalized patients with pneumonia.

Pharyngeal carriage of Mycoplasma pharyngis and M. salivarium is common among healthy young adults. Ninety-four per cent of the medical students were found to carry either or both species; the mere presence of either Mycoplasma species could not be correlated with respiratory tract symptoms. Among high school students the carrier rate was also high (77%) and the mycoplasmas recovered were either M. salivarium, M. pharyngis, or both.

M. hominis I was isolated once from a medical student with acute upper respiratory tract symptoms.

M. pneumoniae and M. hominis I were not found in the throat cultures of healthy adults.

Species identification of the mycoplasmas is essential in evaluating significance of isolates. M. pneumoniae has been isolated in this study only from patients with clinical symptoms of respiratory tract infection. M. hominis I, currently suspect as a respiratory tract pathogen, may be another significant isolate.

A comparison of species carried by normal healthy individuals and species isolated from patients with clinically diagnosed viral pneumonia showed a highly significant difference ($p = .001$). M. pharyngis isolations were significantly lower among patients than among healthy young adults. There were no significant differences of species carried between the two student groups.

Table I

Mycoplasma species isolated from the pharynx

	Second year medical students positive (% of 32)	Senior high school students positive (% of 22)	Patients w.clinical viral pneumonia positive (% of 29)
Number positive for mycoplasma	30 (94%)	17 (77%)*	23 (79%)*
M. pharyngis	23 (72%)	11 (50%)	1 ( )
M. salivarium	5 (16%)	12 (55%)	10 (34%)
M. pneumoniae (Eaton Agent)	0 -	0 -	10 (34%)
M. hominis I	1 (3%)	0 -	0 -
Unidentified	1 (3%)	- -	3**(13%)

* Percentages do not add up to total per cent as some carry mixed species.

** Species other than M. pneumoniae

III. PHARYNGEAL MYCOPLASMA CARRIAGE ASSOCIATED WITH DISEASE

Pneumonia patients referred to the laboratory were cultured by swabs taken from the pharynx for mycoplasmas. Acute and convalescent sera were drawn for complement fixation testing. Unfortunately laboratory results are obtained too late in the course of the infection to be of clinical importance - nevertheless confirmatory evidence of a diagnosis of primary atypical pneumonia is sought with interest by most physicians. Out of a total of 30 patients with suspected primary atypical pneumonia, M. pneumonia was isolated from 16 (53%). A fourfold rise in titer was secured in 11 of these patients.

One-hundred-forty-six cultures of 52 patients with asthma have been done but no correlation of mycoplasmas found with symptoms could be made. Both M. pharyngis and M. salivarium were isolated most frequently. An attempt was made in collaboration with Dr. Ralph Schumacher Jr. to culture viruses, mycoplasmas, or bacteria from early synovial lesions in systemic rheumatic diseases. No positive cultures were obtained from synovial membranes and fluid from 15 patients with synovitis of from 4 days to 3 months' duration. Over 100 nasopharyngeal cultures from patients with varying types of malignancies have also resulted in no correlation with species of mycoplasmas isolated from the nasopharynx.

One-hundred-sixty nasopharyngeal cultures were taken from patients with miscellaneous diagnosis, and upper respiratory tract infection among hospital personnel and patients yielded isolations of either M. salivarium or M. pharyngis or both, M. salivarium predominating. M. hominis I was isolated from two people with a sore throat.

IV. MYCOPLASMAS AND GENITOURINARY TRACT INFECTIONS WITHIN THE PAST YEAR

Within the past year, seventy-four patients were studied with genitourinary tract symptoms. Either genital or urine cultures or both were taken in each patient of the seventy-four. Of these, thirty-three were negative and forty-one (55.4%) were positive for T strain mycoplasmas, five (6.8%) had both T strain and M. hominis I. The symptoms were urethritis, prostatis, or cystitis. Treatment with erythromycin resulted usually in negative cultures as well as elimination of symptoms. In most

instances treatment of the husband and wife was also done after cultures were taken. Culture results were usually identical in husband and wife. On several occasions sexual contacts were studied and their mycoplasma isolates were comparable. In one instance both the male and female patient involved had M. hominis I and T strains isolated. When both urine and genital cultures from the same person were done and compared both were usually identical in culture results. On rare occasions one was positive and the other negative. These patients were referred to us for culture when treatment for bacterial infection did not result in subsidence of symptoms. It would appear therefore that mycoplasmas are responsible for some proportion of genitourinary tract infections, although what proportion of the total number of patients seen by our physicians with genitourinary tract infection are found to carry mycoplasmas, is not known at this time, but the infection obviously exists.

V. MYCOPLASMAS IN INFERTILITY AND REPRODUCTIVE FAILURES

In collaboration with a maternity hospital, the cervix of 104 patients was cultured. Fifty-four women were cultured in the High Risk Clinic (a clinic with aberrant reproductive histories). Mycoplasmas were found in 36 (67%), T strains in 34 (63%), M. hominis I in 15 (28%). Of the 54 women, 13 had both types of mycoplasmas. Of the comparison or control group, fifty women were cultured; 24 (48%) yielded mycoplasmas, 21 (42%) had T strains, 10 (20%) had M. hominis I. Of these, seven had both types.

Statistical analysis of the two groups for the presence of mycoplasma, using χ^2, results in a $P < .05$ indicating that a significant difference exists between the two groups in the number of total mycoplasma isolations. A comparison of T strains, using the same analysis, also results in statistical significance - $P < .05$. The difference in isolation of M. hominis I between the two groups is not statistically significant with the numbers studied. The bacteriology on blood agar plates was not remarkable. The conclusion therefore is that the presence of T strains probably does have a role in human reproductive failure. The outcomes of pregnancies of patients with positive and negative mycoplasma cultures are currently being investigated and compared .

Cultures of the placenta were studied to determine whether these organisms could also be recovered from the membranes associated with spontaneous abortion, premature births, or neonatal deaths. Twenty-two placental membranes were studied. Of the twenty-two, fifteen were positive for mycoplasmas (68%). Seven placentas of the twenty-two were negative for mycoplasmas. Of the fifteen positive placentas, twelve were positive for T strains only, one for M. hominis I only, and two for M. hominis I and T strains. One interesting finding was that two placentas of twins delivered prematurely were cultured and both were positive for M. hominis I and T strains. Histologically, chorioamnionitis was evident in both. The babies died within a few hours following birth. The bacteriology was negative. No bacteria were recovered on aerobic and anaerobic cultures on blood agar plates. Of the twenty-two specimens (the twin placentas were counted as one since they resulted from one conception), nine were premature births, nine were spontaneous abortions, and four were neonatal death. To compare cultures from placentas obtained under conditions other than association with a high risk population, four placentas were cultured: (1) an elective caesarian section at 36 weeks with the result, a well infant; (2) a first trimester therapeutic abortion by hysterotomy; (3) a normal delivery at term; and (4) a premature delivery at 37 weeks. All membranes were negative for mycoplasmas and showed no histologic evidence of inflammation.

Of the nineteen placentas, which were divided into amnion, chorion, decidua, and villous tissue and cultured separately, twelve were positive in cultures of the decidua, ten were positive in amnion culture, nine in chorion, and eight were positive in cultures from the villous tissue, suggesting from this few number of cultures that the decidua and amnion are more likely to yield positive cultures.

Nongonococcal urethritis has been described as a sixth venereal disease of T strain etiology in a male military population (2). Our observations have suggested a female counterpart to this male infection. The female is asymptomatic but with the onset of pregnancy the infection is

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(2) Shepard, M.C., Alexander, C.E., Lunceford, C.D., Campbell, P.E.: Possible Role of T Strain Mycoplasma in Nongonococcal Urethritis, J. of the A.M.A., 188, 729-735, 1964.

expressed in some form of reproductive abnormality. The high prevalence of mycoplasmas in the cervix of women with histories of pregnancy wastage supports this observation. To date there have been successful outcomes of pregnancy in seven women with repeated spontaneous abortions following antibiotic therapy. All delivered healthy infants at or near term following a regimen of either erythromycin, tetracycline, or demethylchlortetracycline. Both husband and wife require simultaneous treatment to eliminate the possibility of reinfection.

VI. AEROSOLS

Studies of mycoplasmas and L forms as aerosols, and determination of sizes of particles formed following nebulizing indicates that aerial survival of the four species of mycoplasmas and the two species of L forms tested remained suspended as droplet nuclei for periods of time long enough for them to be of hygienic significance. The bacteria tested, E. coli, N. meningitidis, S. aureus, also formed viable aerosols capable of surviving for several hours in suspension.

Aerosols of mycoplasmas formed the smallest particles tested and differed significantly in size from aerosols of L forms, bacteria, and Candida. L forms and bacteria had particles of the same size. The droplet nuclei of C. albicans were the largest of the organisms tested.

Aerial survival of all microorganisms was demonstrated. All can survive as airborne droplet nuclei for one hour or more at the relative humidities tested (45% and lower), and consequently have the potential for airborne transmission. At 77% relative humidity, M. pneumoniae survived for one hour as an aerosol. The lack of a cell wall did not preclude formation of viable aerosols by mycoplasmas or L forms.

Ultraviolet radiation destroyed droplet nuclei of bacteria and mycoplasmas instantly with very few survivors. L forms and C. albicans were not destroyed as efficiently. When spraying, testing, and irradiation were simultaneous, 13% of S. aureus L form, 31% of diphtheroid L form, and 11% of the C. albicans droplet nuclei remained viable. They did not appear as vulnerable as mycoplasma and bacterial forms, probably because of clumping or budding. The S. aureus aerosol was destroyed more efficiently by ultraviolet

radiation than its L-form aerosol.

Whereas bacteria and L forms did occasionally form submicron particles, 19% of aerosol particles formed by mycoplasmas were submicron in size. This finding has epidemiological implications in airborne infections due to mycoplasmas.

Table II

	Count Median Diameter in microns
Mycoplasmas	2.1 ± 0.5
S. aureus L form	4.6 ± 1.7
Diphteroid L form	3.4 ± 0.3
Escherichia coli	5.4 ± 2.5
Neisseria sicca	3.3 ± 0.5
Neisseria meningitidis	3.4 ± 0.2
S. aureus (parent strain of L form)	3.9 ± 1.2
Candida albicans	5.9 ± 1.4

The implications of this work are that aerosols of the bacteria tested, mycoplasmas, L forms and Candida remain viable and can be agents of airborne infection. Particle size consistent with aerodynamic dimensions for lower respiratory tract has been demonstrated.

VII. AUTOPSY

Autopsy specimens have been investigated and reported earlier. Identification of the species isolated from bronchial cultures taken at the bifurcation of the trachea have been done. At autopsy 11 isolates were found, four were M. salivarium, and one was M. hominis I from a person who was dead on arrival with death due to a myocardial infarct and a congested left lung. An isolate from a patient dying with fulminating pneumonitis was identified as a mixture of M. pneumoniae and M. pharyngis. Five isolates were unidentified. Isolation rate is therefore 11 out of 33 or 33%. From eight lung specimens taken at autopsy, three were positive, two for M. pneumoniae, one for M. salivarium. Of the M. pneumoniae, one isolate was mixed with M. pharyngis. This same combination was found in isolations from the same patient's bronchi.

The interesting finding is that two patients with deaths attributable to pneumonitis yielded M. pneumoniae on lung culture.

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13. ABSTRACT

Mycoplasmas appear to be carried by normal healthy adults. M.salivarium and M. pharyngis are species commonly carried. M. hominis and M. pneumoniae have been found only in individuals with respiratory tract infections.

Genitourinary tract infection can be caused by mycoplasmas; in male patients it is expressed as nongonococcal urethritis, in female patients as infertility and reproductive wastage.

No association can be found between the species of mycoplasmas carried in the nasopharynx of patients with asthma, malignancy, rheumatoid arthritis.

Mycoplasmas can form viable aerosols of droplet nuclei with a particle size having a significant potential in airborne infection.

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