Review of Literature
REVIEW OF LITERATURE

Mycoplasmas are the smallest free living microorganisms found in animals, man, plants and insects. Mycoplasmas have long been recognized as pathogens of the respiratory tract, urogenital tract and joints in a variety of animal species. Mycoplasmas produce disease in animals with rare exceptions that are chronic and often of multifactorial origin. The syndromes produced depend on environmental conditions, the genetic predisposition of the host, and to a lesser extent, the nature of the infecting microbe. Of late, mycoplasmas have been incriminated as agents of human disease. Mycoplasma pnemoniae is responsible for primary atypical pneumonia in humans. Other complications and sequale pertaining to M. pnemoniae infections are sinusitis, otitis media and central nervous system complications. Ureaplasma urealyticum has been proved to be a cause of non-gonococcal uretheritis (NGU) in men and is also believed to have a role in infertility, spontaneous abortion and still birth. Mycoplasma hominis has been shown a cause of pelvic inflammatory disease (PID), post partum fever, and a likely cause of pyelonephritis. In addition, new information being accumulated regarding the pathophysiology of Mycoplasmas suggests that these organisms are strong contenders of the aetiology of arthritis in humans.
Nocard and Roux (1880) discovered that Pleuropneumonia in cattle was caused by an organism, then known as the Pleuropneumonia-like organisms (PPLO). The pioneering work of Klienberger and later by Klienberger and Nobel from the mid 1930 onwards threw more light on the nature and pathogenicity of these organisms, particularly for rats and mice.

To date, mycoplasmas have been isolated from a variety of infections of mammalian, avian, insect and plant hosts. Mycoplasmas was first isolated in humans by Dienes and Edsall (1937) from a suppurating Bartholin's gland abscess in a female laboratory worker. In the early 1940's Eaton showed that primary atypical pneumonia had an infectious aetiology and this was later demonstrated to be M. pneumoniae (Clyde, 1961).

The term mycoplasmas describes this group of organisms aptly, 'Myco' (Greek mykes : fungus) referring to the filamentous forms which are frequently seen and 'plasma' (Greek, Latin : formed or moulded) referring to the plasticity of the outer membrane and the pleomorphism that is observed. Mycoplasmas are placed in a separate class Mollicutes (soft skin) which contains only one order - The mycoplasmatales. The subcommittee on the Taxonomy of Mycoplasmatales, International Committee on Systematic Bacteriology (1972) established some characters for a member to be included in the order Mycoplasmatales. They include:
1. Lack of a cell wall or chemical precursors of cell-wall peptidoglycan.

2. Typical colonial appearance

3. Filterability through a 450 nm filter

4. Lack of ability to revert to a bacterium under appropriate conditions.

Included in the order Mycoplasmatales are the families of Mycoplasmataceae, Acholeplasmataceae and Spiroplasmataceae. There are also two genera of uncertain taxonomic position. They include Thermoplasma and Anaeroplasma.

**Fig.1 Classification System for Mycoplasmas**

<table>
<thead>
<tr>
<th>Class</th>
<th>Mollicutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Mycoplasmatales</td>
</tr>
<tr>
<td>Family</td>
<td>Mycoplasmataceae</td>
</tr>
<tr>
<td></td>
<td>Acholeplasmataceae</td>
</tr>
<tr>
<td></td>
<td>Spiroplasmataceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td></td>
<td>Ureaplasma</td>
</tr>
<tr>
<td></td>
<td>Acholeplasma</td>
</tr>
<tr>
<td></td>
<td>Spiroplasma</td>
</tr>
</tbody>
</table>
Anaeroplasmas

The Anaeroplasmas are obligate anaerobic micro-organisms and they are commonly isolated from the rumens of cattle and sheep. They resemble the classical mycoplasmas in their cytology, DNA base composition and in the cholesterol requirement. They are placed in a separate genus because they grow only under anaerobic conditions. The genus Anaeroplasma contains 2 species - Anaeroplasma abactoclasticum and Anaeroplasma bactoclasticum.

Thermoplasmas

Thermoplasmas are isolated from the burning coal refuse piles. They are adopted to extreme conditions of temperature and growth requirements with an optimum temperatures of 59°C and an optimum pH of 1.0 to 2.0. They are placed in the class Mollicutes based on their lack of cell wall. But they differ from the other members of the class in having a flagella, peculiar patterns of DNA, RNA, lipids, minimal nutritional requirements, complex electron transport systems and different pH and temperature requirements. The genus Thermoplasma contains one species Thermoplasma acidophilum.

Mycoplasma Hominis and Ureaplasma Urealyticum

Morphology and Cell Structure

Mycoplasma hominis is commonly found in the genitourinary
The family Mycoplasmataceae contains two genera. They are Mycoplasma and Ureaplasma. There are over 50 species that has been currently identified in the genus Mycoplasma and the type species is M. mycoides subspecies mycoides. The species differences within the genus Mycoplasma is determined by cultural, biochemical characteristics and by antigenecity. The ability of the genus ureaplasma to hydrolyze urea sets it apart from the genus Mycoplasma. Currently, there is one species in the genus ureaplasma - U. urealyticum. However, there are eight serotypes within this species.

The members of the family Mycoplasmataceae commonly infect animals and man and cause disease. They also frequently contaminate cell cultures. The pathogenic role of Mycoplasmas in human disease have been studied especially in the areas of respiratory, genital and joint diseases. Ten species of Mycoplasmas and one species each of ureaplasma and Acholeplasma have been isolated from humans though, only some species have been incriminated in disease. The role of M. pneumoniae in the pathogenesis of human respiratory tract infections is undisputed. M. pneumoniae is responsible for primary atypical pneumonia in humans. Complications and sequale pertaining to M. pneumoniae infections are sinusitis, otitis media, bullous myringitis and erythema multiforme. Central nervous system complications are also not uncommon and these include psychosis, meningitis, meningoencephalitis, cerebellar ataxia, transverse myelitis and Gullian-Barre polyradiculopathy. An association
<table>
<thead>
<tr>
<th>Metabolism of</th>
<th>Frequency of isolation from the</th>
<th>Cause of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Respiratory tract</td>
<td>Genito-urinary tract</td>
</tr>
<tr>
<td>1. M. buccale</td>
<td>Arginine</td>
<td>Rare</td>
</tr>
<tr>
<td>2. M. faecium</td>
<td>Arginine</td>
<td>Rare</td>
</tr>
<tr>
<td>3. M. fermentans</td>
<td>Glucose and Arginine</td>
<td>-</td>
</tr>
<tr>
<td>4. M. genitalium</td>
<td>Glucose</td>
<td>-</td>
</tr>
<tr>
<td>5. M. hominis</td>
<td>Arginine</td>
<td>Rare</td>
</tr>
<tr>
<td>6. M. lipophilum</td>
<td>Arginine</td>
<td>Rare</td>
</tr>
<tr>
<td>7. M. orale</td>
<td>Arginine</td>
<td>Common</td>
</tr>
<tr>
<td>8. M. pneumoniae</td>
<td>Glucose</td>
<td>Rare*</td>
</tr>
<tr>
<td>9. M. primate</td>
<td>Arginine</td>
<td>-</td>
</tr>
<tr>
<td>10. M. salivarium</td>
<td>Arginine</td>
<td>Common</td>
</tr>
<tr>
<td>11. U. urealyticum</td>
<td>Urea</td>
<td>Rare</td>
</tr>
<tr>
<td>12. A. laidlawi</td>
<td>Glucose</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*Except in disease outbreaks.

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of U. urealyticum and M. hominis in human infections is slowly emerging. U. urealyticum has been incriminated in non-gonococcal urethritis, urinary calculi, involuntary infertility, repeated spontaneous abortion and still birth, chorioamnionitis and low birth weight. M. hominis has been incriminated in prostatitis, pelvic inflammatory disease, post-partum fever, post abortal fever and pyelonephritis (Taylor-Robinson and William McCormack, 1980).

Family - Acholeplasmataceae

The members of the family Acholeplasmataceae do not require sterol for growth. They are frequently isolated from sewage, compost and soil and are believed to be saprophytic. However, more recently they have also been isolated from infections of animals such as swine, cattle, sheep, goats, chickens, ducks and rodents. On rare occasions, Acholeplasma laidlawii has been isolated from the respiratory tract of humans. The genus Acholeplasma contains eight species and the type species in A. laidlawii.

Family - Spiroplasmataceae

The family Spiroplasmataceae contains one species - Spiroplasma macitri. Spiroplasmas are isolated from plants and insects and they do not require sterol for growth. These organisms have a helical cellular morphology during some phase of their growth. Recently, an isolate of Spiroplasma from rabbit ticks was shown to cause cataracts in suckling mice.
Anaeroplasmas

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MYCOPLASMA HOMINIS AND UREAPLASMA UREALYTICUM

Morphology and Cell Structure

Mycoplasma hominis is commonly found in the genitourinary
tract of humans and it is also less frequently isolated from the oropharynx or elsewhere. The first strain of Mycoplasma isolated from humans by Dienes and Edsall (1937) probably belonged, in retrospect, to Mycoplasma hominis. This is because of the large colony Mycoplasmas, Mycoplasma hominis is the one most frequently isolated in the genital tract.

U. urealyticum was formerly known as "T-strain of Mycoplasma", "T-strains", and "T-mycoplasmas". Shepard (1954) was the first to describe "Tiny-form PPLO" in primary agar cultures of urethral exudates from male NGU patients. He observed the colonies were of minute size and these organisms had a distinct colonial characteristics in the mycoplasma group. T-mycoplasmas produce urease enzyme which has the ability to hydrolyze urea. This is the most distinguishing and characteristic biochemical property specific for T-mycoplasmas, since no other presently known Mycoplasmas produce urease. This property justified the proposal and acceptance of a new genus-ureaplasmas for T-mycoplasmas (Shepard et al., 1974).

Mycoplasmas are extremely pleomorphic and range in shape from coccoid, coccobacillary ring or signet ring, dumb bell, asteroid forms to long branching beaded or segmented filaments. The minimal reproductive unit is roughly a spherical cell of about 200 to 250 nm in diameter. The cell is limited by a plasma membrane
similar to a cell membrane of a bacterial or animal cell. Chemical analysis also shows the presence of sterols unlike in bacterial membranes. The sterols play an important role in maintaining membrane integrity in the face of varying external osmotic pressures. The presence of cholesterol renders the cell membrane susceptible to damage with agents such as saponin, digitonin and polyene antibiotics that complex with sterols. Since mycoplasmas do not possess a cell wall, as would be expected, they are completely resistant to penicillin and cycloserine which act on various stages of cell wall synthesis. The cytoplasm of the mycoplasma does not contain a endoplasmic reticulum or a defined mesosome. The cytoplasm contains a lot of Ribosomes which are of 70 S variety and protein synthesis is inhibited or modified by antibiotics such as tetracycline, puromycin, tylosin, kanamycin, streptomycin, erythromycin, choloramphenicol, etc. The nuclear material exists in the fibrillary form and there is no nucleolus.

The cells of ureaplasmas exist in the form of round or coccobacillary elements, approximately 0.3 μm in diameter. They may also exist in the form of short bacillary elements, filaments, annualr forms, signet ring forms and bipolar elements. Ureaplasmas are bound by a single triple layered membrane. The outer surface of this membrane has been shown to be covered by an electron dense layer consisting of short radiating hair like structures resembling closely spaced "brush bristles". Ribosomes and intracellular
vacuole like structures were also demonstrated in ureaplasmas. U. urealyticum produce an enzyme urease which hydrolyze urea with the production of ammonia and carbon dioxide. This is an unique property of U. urealyticum among the mycoplasmatales and no other presently known mycoplasmas contain this enzyme.

**Growth Requirements**

Mycoplasmas require proper atmospheric conditions for their growth. M. hominis grow well on agar under aerobic conditions and in an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen. The optimum pH for the growth of M. hominis is 7.0. Horse serum and yeast extract should be supplemented in the medium for the growth of M. hominis. Horse serum provides a source of cholesterol and yeast extract, probably, a source of magnesium and diphosphopyridine nucleotides (Velleca et al., 1980).

U. urealyticum is microaerophilic. A gaseous mixture containing 5 to 15 per cent of carbon dioxide in nitrogen or air is required for best growth in agar cultures. U. urealyticum also grows well under anaerobic conditions. The optimum pH requirement of U. urealyticum for growth in broth or agar cultures is pH 6.0 ± 0.5 (Shepared et al., 1974). U. urealyticum require the supplementation of 20 per cent horse serum for growth. Urea and cholesterol are required for growth of U. urealyticum and these metabolites
are supplied through serum. Alternately, the sterol requirement could also be met by beta-sitosterol (Sphepard et al., 1974). U. urealyticum also require yeast extract which are rich in magnesium for their growth. L. cysteine is beneficial to growth of U. urealyticum and a 10 fold higher numbers of organisms are obtained in broth cultures containing 0.57 m concentrations of L-cysteine hydrochloride (Shepard et al., 1974).

Biochemical Reactions

Mycoplasmas and ureaplasmas of human origin can be speciated based on biochemical reactions. The tests of importance include catabolism of glucose, arginine, urea and phosphatase activity (Freundt and Erno, 1979). The catabolism of glucose, arginine or urea provides a reliable basis for the speciation of mycoplasmas that infect or are commonly encountered as commensals in man. All the species that are encountered in man except M. pneumoniae, M. genitalium and U. urealyticum metabolize arginine. M. pneumoniae, M. fermentans and M. genitalium metabolize glucose, U. urealyticum has the ability to metabolize urea.

Of all the species that are commonly found in man, only M. fermentans has the ability to metabolize both glucose and arginine, phosphatase production has been observed in M. buccale, M. primatum, U. urealyticum and in most strains of M. fermentans.
Table 3

Biochemical Differentiation of Mycoplasmas and Ureaplasmas of Human Origin

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fermentation of glucose</th>
<th>Hydrolysis of Arginine</th>
<th>Hydrolysis of Urea</th>
<th>Phosphatase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. buccale</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. fauricum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. fermentans</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. hominis</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. lipophilum</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>M. orale</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. pnemoniae</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. primatum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. salivarium</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Positive; - = Negative; ± = Variable but most strains positive
ND = Not determined

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Additionally, other tests that help in the differentiation of human mycoplasmas include haemolysis, haemadsorption, aerobic reduction of tetrazolium and production of film and spots in egg yolk medium. *M. pneumoniae* and *U. urealyticum* produce β-haemolysis of guinea pig erythrocytes whereas some strains of *M. hominis* produce α-haemolysis. *M. pneumoniae* haemadsorbs sheep erythrocytes whereas *M. orale* and *M. fauclium* haemadsorbs chicken erythrocytes. *M. pneumoniae* reduces tetrazolium aerobically. *M. fermentans* and *M. salivarium* produce film and spots on egg yolk medium.

**EFFECT OF PHYSICAL AND CHEMICAL AGENTS**

Physical Agents

**Effect of Temperature**

The optimal temperature for growth and multiplication of *U. urealyticum* and *M. hominis* is 36 to 37°C. *U. urealyticum* serotypes do not grow at 42°C and they can be heat inactivated at temperatures of 56°C and 60°C (Shepard et al., 1974). In one study two strains of *U. urealyticum* were heat inactivated at 56°C for 5 minutes (Taylor - Robinson et al., 1968). However, in another study of the eight serotypes of *U. urealyticum*, serotypes I, III and IV were inactivated at 60°C for 10 minutes and treatment at 60°C for 30 minutes resulted in inactivation of all the serotypes (Black, 1973). It has been shown by one study that the mean half life of *M. hominis* is 3.4 hours and 32 seconds at 4°C and 56°C respectively (Kawangkim et al., 1966).
Chemical Agents

Growth of U. urealyticum in vitro is selectively inhibited by 5-iodo-2-deoxyuridine, hydroxyurea, acetohydroxamic acid, sorbyl-, benzoyl- and 3 amino-benzoyl hydroxamic acids. On the contrary, the human classical mycoplasmas are largely unaffected by these agents at concentrations which are completely inhibitory to U. urealyticum (Shepard et al., 1974).

Effect of Thallium Acetate

Thallium acetate is added to the primary culture media of mycoplasmas as an antibacterial agent. The large colony mycoplasmas are not affected by Thallium acetate in the concentrations inhibitory to bacteria and hence it is used in the media for the isolation of mycoplasmas. However, media employed for the isolation of U. urealyticum should be devoid of Thallium acetate since Thallium acetate at a concentration of 1 : 500 is inhibitory to U. urealyticum (Shepard, 1966).

Sensitivity to Digitonin and Sodium-Polyanethol-Sulfonate

The sterol requiring mycoplasmas are differentiated from the sterol non-requiring Acholeplasmas by their sensitivity to digitonin and sodium-polyanethol-sulfonate. U. urealyticum and M. hominis are sensitive to 1.5 per cent digitonin and to 5 per cent sodium polyanethol-sulfonate whereas Acholeplasma species are resistant
to 5 per cent to 20 per cent sodium-polyanethol-sulfonate and are also resistant or a few species are slightly sensitive to 1.5 per cent digitonin (Freundt et al., 1973).

LABORATORY INVESTIGATIONS

Isolation and Identification

U. urealyticum and M. hominis are the prime organisms for disease in the genito-urinary mycoplasmal infections. The source of specimens in males include the following:

a. urethral swabs and urine in cases of NGU.
b. Prostatic massage in cases of prostatitis.

The source of specimens in females includes the following:

a. vaginal swabs in cases of vaginitis
b. cervical swabs in cases of cervicitis
c. urethral swabs and urine in cases of urethritides
d. endometrial washings or biopsy in cases of infertility, endometritis and spontaneous abortion.
e. fallopian tube cultures in cases of infertility, spontaneous abortion and still birth.
f. blood in cases of puerperal infections and septicaemia
g. placenta from cases of spontaneous abortion and still birth
h. foetal parts from cases of spontaneous abortion and still birth.
For optimal isolation, the swabs and other materials should be immediately inoculated into mycoplasma medium. However, in the event of a delay between the collection and processing of specimens, the specimens should be transported to the laboratory in a mycoplasma transport medium (Velleca et al., 1980).

The basic medium employed for the isolation of the genital mycoplasmas contains beef heart infusion and peptone and it is available commercially as PPLO medium. This basic medium is supplemented with horse serum and yeast extract (Velleca et al., 1980). Antibacterial agents in the form of pencillin G and Thallium acetate is added to mycoplasma medium to suppress the bacterial growth. Thallium acetate, however, should be omitted in the media employed for the isolation of U. urealyticum (Velleca et al., 1980). Optimal concentration of agar is added to the basic medium to convert it into a solid medium.

Mycoplasmas can be divided into arginine metabolizers, urea hydrolizers or glucose metabolizers depending on the substrates they use. The basic medium is supplemented with one of these substances depending on the mycoplasmas to be isolated.

The arginine metabolizers of human genital mycoplasmas include M. hominis, M. fermentans and M. primatum. The arginine metabolizers metabolize arginine with the subsequent release of ammonia into the medium. The pH of the medium is originally adjusted to
7.0. Since these organisms produce an alkaline shift in the medium, it results in the colour change in the phenol red indicator from salmon to red.

*U. urealyticum* is the urea hydrolizer of the genital mycoplasmas. These organisms use the urea substrate and release ammonia into the broth medium. The original pH of the medium is adjusted to 6.0, since it is the optimal pH for *U. urealyticum*. The growth of the organisms is indicated by a change in the original yellow to red colour due to shift in the pH.

The genital mycoplasmas that metabolize glucose includes *M. fermentans* and *M. genitalium*. The metabolic end product of glucose is lactic acid, which changes the medium from the original optimal pH of 7.8 to below pH 7.0. A phenol red indicator is included in the medium to demonstrate pH changes. Alkaline to acidic pH shift indicates the growth of glucose metabolizing mycoplasmas.

On isolation of the mycoplasmas and ureaplasmas in a liquid medium, it is subcultured onto a solid medium to observe the typical fried egg colonial morphology. The colonies of *M. hominis*, *M. fermentans* and *M. genitalium* are about 200 to 300 μm in diameter and have a characteristic fried egg appearance due to growth in the agar at the centre of the colony but only on the surface at periphery. The colony size of *U. urealyticum* ranges from 15-30 μm in diameter and hence these organisms were originally known as T-strains or T-Mycoplasmas (T for tiny). Mycoplasmas on isolation
in solid medium can be stained by Dienes stain by 3 different methods (Velleca et al., 1980). Mycoplasma colonies with typical fried egg morphology stain distinctly with dark blue centres and light blue peripheries and appear highly granular. Bacterial colonies also stain by Dienes stain but they are distinguishable from mycoplasmas since they decolourize the stain after 30 minutes. On the contrary mycoplasma colonies, except for the colonies of M. pneumoniae, retain the stain.

M. hominis and M. fermentans are identified and confirmed by a growth inhibition test with the specific antisera (Velleca et al., 1980). Advantage of the unique production of urease by ureaplasmata urealyticum is made use of and these organisms can be identified by the detection of this enzyme by 3 different methods. These include the detection of this enzyme by u-9 urease colour test fluid medium (Shepard and Lunceford, 1970), a spot test for urease (Shepard, 1973) and by differential agar medium (A7) for identification of U. urealyticum (Shepard and Lunceford, 1976). On A7 agar medium which contains urea and a sensitive indicator of ammonia, manganeous sulphate, ureaplasmata colonies develop a dark brown colour, and are therefore, more easily detected. For ureaplasmatas this is the most simple and rapid diagnostic method (Taylor Robinson and Csonka, 1981). U. urealyticum may also be confirmed by growth inhibition with erythromycin (McCormack et al., 1973).
Serological Techniques

Serologic tests are more sensitive indicators of mycoplasma infection than isolation of the organism. This is because an antibody response in many instances, is detected in the absence of isolation of the causative mycoplasma. Furthermore, serologic testing in conjunction with the isolation of the organism in mycoplasma infections helps in determining the role of mycoplasmas in disease rather than if isolation of mycoplasmas alone were sought.

In mycoplasma infections, metabolic inhibition test has been used satisfactorily for the measurement of antibodies in human sera (Purcell and Chanock, 1969). Metabolic inhibition test is used on paired sera for serodiagnosis or on a single serum for serologic surveys. A four fold rise in antibody titer between an acute and convalescent serum, in serodiagnosis is indicative of current or recent infection with the mycoplasma being used in the test (Velleca et al., 1980). metabolic inhibition technique is essentially a growth inhibition technique carried out in liquid media. The principle of the test is that an antigen antibody reaction occurs when a serum containing mycoplasma specific antibody is combined with its homologous organism. This antigen antibody reaction leads to the curtailment of cell metabolism and this is indicated by the failure of the phenol red indicator to change colour. The test is carried out in a microtiter system and the substrates used are urea for U. urealyticum
(Purcell et al., 1966a) and arginine for arginine metabolising mycoplasmas (Purcell et al., 1966b). Metabolic inhibition test was modified and carried out in test tubes to detect the antibodies in patients sera with NGU (Lina Deodhar et al., 1986).

Human mycoplasmas have either poor or no natural haemagglutinins. However, the sheep red blood cell can be used as a carrier so that mycoplasma antigens can be adsorbed on to sheep red blood cells. Specific antibodies present in the sera react with the antigens adsorbed onto sheep red blood cells linking the RBC leading to a haemagglutination pattern. The IHA test has been used in testing for antibodies against mycoplasmas (Dowdle and Robinson, 1964; Niemark, 1968). A four fold or greater rise in titer with paried sera by IHA is suggestive of current or recent infection.

Complement fixation test has also been used for the serodiagnosis of mycoplasmal infections (Purcell and Chanock, 1969). The sensitivity of complement fixation test over metabolic inhibition test is not well established (Velleca et al., 1980). However, for maximum sensitivity homologous strain should be used in infections with M. hominis.

Mycoplasmacidal test may be used for the detection of antibodies against M. hominis and U. urealyticum (Lin and Kass, 1970; 1975). This test involves two stages. In the first stage, antibody and complement are allowed to react with the organisms and kill
them in the absence of the arginine or urea. In the second stage, killing of the organisms is demonstrated by addition of the appropriate substrate. This method is sensitive but whether it is more sensitive than the conventional metabolic inhibition test is not clear.

Radio-immuno precipitation tests (Taylor-Robinson and William McCormack, 1979) can also be used to detect low levels of antibody titers of mycoplasmas, though it is yet to be employed for genital mycoplasmas. Enzyme linked immunosorbent assay (ELISA) has also been used to measure antibodies in patients with NGU (Mary Brown et al., 1983). ELISA is specific when used for the detection of antibodies against U. urealyticum and there is an overall agreement of 82 and 95 per cent respectively in the detection of antibodies in acute and convalescent sera of patients with NGU when compared with metabolic inhibition test. It has also been shown that serum antibody levels in NGU patients were significantly higher than the normal serum standard in the IgG, IgM and IgA classes.

U. urealyticum and M. hominis in Health

Colonization of Infants

Infants become colonized with genital mycoplasmas usually during passage through the birth canal. Ureaplasmas have been isolated from the vulval region in about one quarter to one third of infant girls and M. hominis from a smaller proportion (Klein et al., 1969; Foy et al., 1970a). In infant boys, the mucosa of the
genital tract is less exposed and hence mycoplasmas are less frequently recovered in this group. Neonatal colonization of U. urealyticum and M. hominis does not persist for long but female infants harbour the organisms for a longer time than male infants (Taylor-Robinson and William McCormack, 1979; Taylor-Robinson and Csonka, 1981).

Colonization of Children

Genital mycoplasmas are rarely isolated from prepubertal boys (Lee-H et al., 1974; Foy et al., 1975). Ureaplasmas are found in about 10 per cent of prepubertal girls and M. hominis in a smaller proportion. After puberty the rate of colonization of ureaplasmas and M. hominis rises and this is to some extent dependent on the sexual experience (Taylor-Robinson and William McCormack, 1979).

Colonization of Adults

The proportion of men and women who are colonized by M. hominis and ureaplasmas increases after puberty. This is primarily as a result of sexual experience (McCormack et al., 1972; McCormack et al., 1973a). It has been found that the number of different sexual partners determines the frequency of colonization of ureaplasmas and M. hominis in both the sexes (Taylor-Robinson and William McCormack, 1980). In women, colonization of genital mycoplasmas increases with increasing sexual experience and hence
they are more susceptible to colonization by these organisms than men. Also, the genital mycoplasmas are infrequently isolated in nuns (Archer, 1968; Kunds en et al., 1971), whereas they are isolated more frequently in women attending sexually transmitted disease clinics (Dunlop et al., 1969; Gregory and Payne, 1970).

Genital mycoplasmas have been isolated more frequently from black men and women than from white men and women (Foy et al., 1970b; Braun et al., 1971). The reason for this racial predisposition is not known.

The colonization of genital mycoplasmas is also dependent on the socio-economic status. In one study large colony mycoplasmas were isolated twice as common among women in jail than from patients attending private gynaecologists (Ford, 1967). This socio-economic difference may be due to a difference in sexual activity but other factors such as contraception being involved is only speculative and has not yet been fully evaluated.

Menstruation also affects the colonization of genital mycoplasmas. In one study genital mycoplasmas were isolated more frequently in women after mid cycle attending a family planning clinic than women at mid cycle (Singer and Ivler, 1975). During menopause there is a decrease in the incidence of genital mycoplasmas (Mardh and Westrom, 1970; Csonka et al., 1966).
Based on the anatomical considerations, ureplasmas are more associated with urethral canal than with the glans or prepuce (Hare et al., 1969). On the contrary, M. hominis exists in the prepuce than in the urethral canal. In women, the bladder, uterus and fallopian tubes are usually free of mycoplasmas. In the lower genital tract of women, swabs taken from the vagina of the posterior fornix or even the peri urethral area are more likely to contain mycoplasmas than from the endocervical canal unlike in suspected gonococcal or chlamydial infections (Dunlop et al., 1969).

ROLE OF MYCOPLASMAS IN GENITAL TRACT DISEASE OF MEN

Non Gonococcal Urethritis

There are a number of isolation studies to determine the role of large colony forming mycoplasmas in NGU. Though in the early studies, mycoplasmas were not identified, it is likely that most of the mycoplasmas were M. hominis. It has been shown by different studies that the isolation rate of M. hominis in NGU is not significant from the comparable control group (Hare et al., 1969; Piot, 1976). Bowie et al.; 1977a and 1977b) screened chlamydial positive NGU patients, chlamydia negative NGU patients and patients without urethritis and they isolated M. hominis in 19 to 22 percent in all the groups. Hence these workers are of the opinion that M. hominis is not a cause of NGU. Studies in which differential antibiotics were used by some investigators in their attempt to implicate M. hominis has not supported the view that M. hominis is a cause of NGU (Willcox, 1968; Csonka and Spitzer, 1969).
Several studies were carried out to study the incidence of U. urealyticum in NGU. Shepard (1966) screened 1500 patients with NGU and isolated U. urealyticum from 70 to 80 per cent but only from 26 per cent of control subjects. In another study Suelmann et al. (1971) isolated U. urealyticum in 68 per cent and 25 per cent from NGU patients and controls respectively. However, some investigators did not find any statistically significant difference in the isolation rate of U. urealyticum in NGU than from controls (Fowler and Leeming, 1969; Jansson et al., 1971). According to Taylor-Robinson and Csonka (1981), comparative studies of NGU patients and controls to implicate U. urealyticum in disease should take into account the selection of proper controls with similar sexual experience of the diseased patients.

U. urealyticum can be quantitated from clinical material by virtue of its possession of urease enzyme and consequently its ability to breakdown urea to ammonia. Shepard (1974) showed a quantitative relationship between U. urealyticum and the clinical course of NGU in males. Widner et al. (1978) demonstrated a quantitative association of U. urealyticum and non-specific prostato-urethritis.

Ford (1967) tested a small number of sera and he observed a rise in the titer in 18 per cent of the paired sera. Janson et al. (1971) found a rise or fall in indirect haemaggultinating antibody titer against U. urealyticum in the sera of 24 per cent of the NGU patients they examined.
Some investigators have used antibiotic therapy to evaluate the role of U. urealyticum in NGU. Shepard (1974) demonstrated a disappearance of symptoms and U. urealyticum by administering suboptimal doses of doxycycline. But on completion of treatment, there was a reappearance of symptoms and U. urealyticum in members similar to those found before treatment. Prentice et al. (1976) showed an association between the resolution of symptoms and signs in patients from whom ureaplasmas were isolated and had minocycline therapy.

Experimental intra urethral inoculation of ureaplasmas in human volunteers has also been carried out and Jansch (1972) was the first to demonstrate a pathogenic role for U. urealyticum in human NGU by self inoculation. Two subjects (Taylor-Robinson and Csonka, 1977) inoculated themselves intra-urethrally with $5 \times 10^4$ ccu/ml of different strains of serotype 5 of U. urealyticum. The first subject developed urethritis characterized by dysuria, frequency, urethral discomfort and pyuria. The second subject also developed urethritis, characterized as above and in addition, also developed urinary mucous threads with polymorphonuclear leukocytes suggesting that the prostate also became involved. These findings clearly establish that U. urealyticum has a pathogenic role in the human genital tract.
Acute and Chronic Prostatitis

Many workers have isolated large-colony forming mycoplasmas from patients with prostatitis (Dienes and Smith, 1942; Morton et al., 1951). Hoffstetter and his colleagues (Hoffstetter, 1976; 1977; Marx and Hofstetter, 1975) have investigated more than 4,000 patients with prostatitis. Ureaplasmas were isolated by these investigators more often and in greater than $10^3$ org/ml of prostatic secretions of patients with disease and in less than $10^3$ org/ml in control subjects.

ROLE OF GENITAL MYCOPLASMAS IN DISEASES OF THE GENITAL TRACT IN WOMEN

Pelvic Inflammatory Disease

PID is a complex disorder in which organisms present in the vagina and cervix ascend into the normal sterile areas of the upper genital tract. This may lead to the inflammatory reaction in the fallopian tubes and in adjacent structures. PID can be divided into gonococcal and non-gonococcal types. In gonococcal PID N. gonorrhoea is the primary pathogen and more often than not it is isolated from an endocervical culture. Non-gonococcal PID does not have a single cause and many organisms are held responsible for it.

Many investigators were of the opinion that M. hominis could be one such organism responsible for PID. This was based on the isolation of large colony Mycoplasmas by some investigators
from inflamed fallopian tubes, tubo ovarian abscesses and pelvic abscess or fluid (Hirsch, 1952; Gotthardson and Melen, 1953; Zeltzer and Palti, 1963). The most illuminating observations suggesting M. hominis has a role in PID was made by Mardh and Westrom (1970a). They examined specimens directly from the fallopian tubes of 50 women with salpingitis and isolated M. hominis from 4 patients. Also they did not isolate M. hominis from 50 controls. Furthermore, an increase or decrease in antibody titer to M. hominis by IHA test was detected in 9 out of 16 patients who had M. hominis in the lower genital tract.

Though the role of U. urealyticum in PID is of less importance than M. hominis, it has been isolated directly from the fallopian tubes of 2 of 50 patients with acute salpingitis (Mardh and Westrom, 1970a). U. urealyticum has also been isolated from pelvic fluid (Eschenbach et al., 1975) and from tubo ovarian abscess (Braun and Besdine, 1973).

In some studies carried out to detect antibody response against M. hominis by complement fixation test in cases of PID, antibody titers were greater in sera of some patients with salpingitis than in those from women who served as controls (Melon and Gotthardson, 1955; Lemcke and Csonka, 1962). In another study indirect haemagglutinating antibody to M. hominis was demonstrated in the sera of 53.8 per cent of patients with salpingitis as compared to 10.5 per cent of healthy women (Mardh and Westrom, 1970b). Mardh (1970) detected an increased level of IgM antibody in 34 per cent
of patients with acute salpingitis and this was associated with the isolation of M. hominis and with the presence of indirect haemaggluti-
tinating antibody to the mycoplasma.

Postpartum Fever

M. hominis can cause post partum fever, presumably, by causing endometritis and the patients have low grade fever for a day or two after delivery, are not severely ill and recover unevent-
fully. There were many reports of patients with post partum fever from whom M. hominis was isolated from blood (McCormack et al., 1973; Taylor Robinson and McCormack, 1979). In one study M. hominis has been isolated from 5 to 10 per cent of women with fever after delivery (Wallace et al., 1978). The relation between vaginal colonization with M. hominis or ureaplasmas in puerperal fever is not absolutely clear.

Post Abortal Fever

Some of the post abortal fever cases may be due to M. hominis and has been isolated from the blood of 2 patients with septic abortion (Tully et al., 1965; Harwick et al., 1967). These patients also developed an antibody response to M. hominis. In a study by Jones (1967), tissues from 62 foetuses were cultured and M. hominis was grown from the lungs of 5 of the foetuses. In an another study amniotic fluid taken from 50 patients yielded M. hominis
on 4 occasions, of which 3 of 4 patients were febrile (Harwick et al., 1969).

**Vaginitis and Cervicitis**

It has been found by different workers that *M. hominis* could be isolated more than twice the numbers in patients with vaginitis or cervicitis than from patients who were free of disease (McCormack et al., 1973; Taylor Robinson and McCormack, 1979). However, these data should be interpreted with caution because it is difficult to ensure that the control population for vaginitis and cervicitis were free of disease.

**Bartholins Abscess**

Although mycoplasmas were isolated first in humans from the abscess of Bartholin's gland (Dienes and Edsall, 1937) their role in this disorder is yet to be resolved. In some studies, unidentified mycoplasmas (Dienes and Smith, 1942) and *U. urealyticum* (Solomon et al., 1970) were isolated from the abscess of Bartholin's gland; but these isolations were made where either the abscess were ruptured or surgically incised and hence it is not clear if these organisms were the aetiological agents of abscess of Bartholin's gland.
ROLE OF GENITAL MYCOPLASMAS IN SEXUALLY TRANSMITTED DISEASE

Genital mycoplasmas have also been isolated frequently from the genital tract of patients with sexually transmitted disease (Gregory and Payne, 1970; Eschenbach et al., 1975). U. urealyticum was isolated from the urine of 78 per cent of women attending a sexually transmitted disease clinic (Young et al., 1981). The isolation of the mycoplasmas from the genital tract is dependent on the sexual activity. In a study (McCormack et al., 1972) of students and graduate nurses, it was found that ureaplasmas could be isolated from 75 per cent of women with a history of sexual contact with three or more partners, from 37.5 per cent of women with a history of sexual contact with a single partner whereas women with no history of sexual contact were usually free of ureaplasmas.

ROLE OF GENITAL MYCOPLASMAS IN DISORDERS OF REPRODUCTION

Infertility

The role of mycoplasmas in human infertility is unresolved. As of now, M. hominis has not been implicated in infertility. In a study by Taylor-Robinson and Furr (1973), semen samples from men were examined and were graded into categories of normal or low fertility. Ureaplasmas were isolated from 47 per cent of the seminal specimens from the men with normal fertility and 57 per cent of those from men with low fertility. Toth et al. (1978)
reported a correlation between abnormal seminal cytology and the presence of U. urealyticum. Furthermore, there was an association between the successful treatment of ureaplasmal infection and a considerable improvement in spermatozoal motility with a decrease in certain abnormal features of seminal cytology (Swenson et al., 1979).

U. urealyticum has been recovered more often from endometrial specimens from infertile women than from fertile women (Stray-Pederson et al., 1978). Moreover, in one study, granulomatous endometrial lesions in patients with abnormal reproductive histories were also colonized with mycoplasmas (Horne et al., 1973).

Chorioamnionitis

Since mycoplasmas enter the amniotic cavity, they may be able to cause certain pregnancy related conditions, including chorioamnionitis. Shurin et al. (1975) isolated U. urealyticum twice as frequently from new born infants with an associated histologically severe chorioamnionitis than from infants with less severe disease or no disease at all. The finding of these investigators assumes significance because they took into account the duration of rupture of the membrane and still found a statistically significant association between chorioamnionitis and ureaplasmal infection. Hence, these and other investigators (Tafari et al., 1976) have suggested that some cases of chorioamnionitis might be due to U. urealyticum infection.
Low Birth Weight

There is a strong association between the colonization of mycoplasmas and low birth weight. In a study conducted at the Boston city hospital (Klein et al., 1969), it was found that about 15 per cent of unselected new born infants were found to have nasal or pharyngeal colonization with genital mycoplasmas. The colonized infants had a statistically lower mean birth weight than those who were not colonized. In another study (Brauh et al., 1971), urine and cervical specimens were screened from 484 pregnant women, and it was found that women who were colonized with ureaplasmas gave birth to infants with a statistically lower mean birth weight than those who were not colonized. Furthermore, in some studies carried out to draw an association between prenatal exposure to tetracycline and staining of primary teeth, it was found that women who were treated with a placebo during pregnancy gave birth to infants weighing 2,500 gms or less statistically more often than did women who were treated with tetracycline (Elder et al., 1968; Elder et al., 1971).

ROLE OF GENITAL MYCOPLASMAS IN DISORDERS OF THE URINARY TRACT

Pyelonephritis

Mycoplasmas may ascend from the lower genitourinary tract and infect the kidneys. U. urealyticum so far, is not convincingly
incriminated in pyelonephritis. In a study by Thomson (1978) M. hominis was isolated from the upper urinary tracts of 7 of 80 patients with acute pyelonephritis. In four of these patients M. hominis was isolated in pure culture. Also, antibodies as measured by IHA test were demonstrated in serum, in ureteric and bladder urine from some of these patients. On the contrary, M. hominis was not recovered from the upper urinary tracts of 60 patients with non-infectious urinary disease, nor was antibody detected in their urine. In another study, urine specimens of 702 patients were examined by Thomson and Lindskov (1979), and they found that 9 patients who had pyelonephritis had antibodies to M. hominis. These data suggest that M. hominis may be the cause in some cases of acute pyelonephritis.

Serotyping of U. urealyticum

Human ureaplasmas consists of a heterogenous group and presently, eight serotypes have been recognized by Black (1970). The same investigator employed a growth inhibition test, metabolism inhibition test, an indirect immunofluorescence test and an indirect haemagglutination test for serotyping. In a study by Clyde (1964), growth inhibition test was found to be species specific for the identification of unknown mycoplasmas. Lin et al. (1972) employed complement dependent mycoplasmacidal test for the serotyping of U. urealyticum and identified 5 sero groups on the basis of shared common
antigens and 11 serotypes within these groups. However, they
could not implicate any serotype to be associated with non-gonococcal
urethritis, pregnancy or infancy.

Susceptibility to Antibiotics

The mycoplasmas are susceptible to various antibiotics
in vivo and in vitro but they do not have a cell wall and hence
are completely resistant to penicillin and cycloserine which act on
various stages of bacterial cell wall synthesis.

U. urealyticum is susceptible to the inhibitory action of
the following antibiotics in order of approximate decreasing effect-
iveness in vitro. Doxycycline, minocycline, declomycin, tetracycline,
erythromycin, chlorotetracycline, oxytetracycline, chloramphenicol,
streptomycin, spectinomycin, spiramycin, kanamycin and gentamycin.
U. urealyticum are generally resistant to penicillins including the
semi synthetic penicillins, sulphonamide, rifampin, cephaloridine,
aurothiomalate and lincomycin (Shepard and Masover, 1979).

M. hominis is sensitive to tetracycline, oxytetracycline,
chlorotetracycline, chloramphenicol, streptomycin spiramycin and
neomycin. M. hominis is resistant to penicillin and erythromycin
(Taylor-Robinson, 1967).

Animal Models

Experimental inoculation of animals aids in resolving the
controversy of the pathogenesis of U. urealyticum and M. hominis, especially of U. urealyticum in NGU and of M. hominis in PID.

In one study, intraurethral inoculation of unpasaged ureaplasmas in chimpanzees led to the development of uretheritis and an antibody response (Taylor-Robinson and Csonka, 1981). Hence, chimpanzees seems to be an important animal model for ureaplasma urethraal infection. In an another study, Bovine ureaplasma strains when inoculated into the bovine udder produced mastitis and this helped to substantiate the view of the investigator that ureaplasmas of human origin should also be pathogenic (Taylor-Robinson, 1977). The susceptibility of experimental animals to human ureaplasmas is specific in that strains of human origin can infect marsmoset monkeys while bovine strains do not (Norman Somerson and Barry Cole, 1979).

Moller et al. (1978) showed that M. hominis obtained from the cervix of a patient with acute salpingitis induced a marked inflammatory response of the uterine tubes of grivet monkeys.

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