MYCOPLASMAS AND 'NON-SPECIFIC' GENITAL INFECTION*†

I. PREVIOUS STUDIES AND LABORATORY ASPECTS

BY

DAVID TAYLOR-ROBINSON,§ JEAN P. ADDEY,§ M. J. HARE†‡
AND ERIC M. C. DUNLOP†‡

From the §Clinical Research Centre, Harvard Hospital, Salisbury, Wilts., the
†Whitechapel Clinic, The London Hospital, and
‡Moorfields Eye Hospital

A mycoplasma was isolated before the end of the 19th century from cattle suffering from pleuropneumonia (Nocard and Roux, 1898). Subsequently other mycoplasmas were isolated from different mammals and from birds, but the first report of an isolation from man did not appear until 1937 (Dienes and Edsall, 1937). Although isolated from different species, these organisms, formerly termed pleuropneumonia-like organisms (PPLO), were recognized as having characteristics sufficiently similar to place them within a single genus. The generic term *Mycoplasma* was suggested by Nowak (1929) and was re-proposed by Edward and Freundt (1956).

Nature of Mycoplasmas‡ and Relationship to L-phase of Bacteria

The characteristics of mycoplasmas have been reviewed in detail previously (Freundt, 1958; Klieneberger-Nobel, 1962; Adler, 1965; Hayflick and Chanock, 1965; Edward, 1967; Sharp and Riggs, 1967; Taylor-Robinson, 1968).

These characteristics are shown in Table I and compared with those of bacteria, fungi, protozoa, rickettsiae, chlamydiae, and viruses. Mycoplasmas grow in cell-free media and produce characteristic colonies on solid media. Their smallness has occasionally caused them to be confused with viruses, since both pass filters which retain bacteria. Mycoplasmas do not have rigid cell walls and this means that they are usually less resistant than bacteria to external influences, such as osmotic changes and drying. Their ability to metabolize different substrates may be used in their detection. It also forms the basis of various metabolic-inhibition (growth-inhibition) tests for the quantitative measurement of antibody (Taylor-Robinson, Purcell, Wong, and Chanock, 1966; Purcell, Wong, Chanock, Taylor-Robinson, Canchola, and Valdesuso, 1967). Mycoplasmas may be identified, as may viruses, by inhibition of their growth by specific antibodies (Clyde, 1964). Their growth is inhibited

‡The use of the compound word *Mycoplasma* ("myco" from *μύκης* meaning a fungus, and *πλασμα* "plasma" meaning form or mould) is now accepted. It is strictly correct to use the plural "mycoplasmata" to refer to organisms of the genus *Mycoplasma*, and the usage "mycoplasma" or "mycoplasmas" as the plural is incorrect. However, the latter has become generally accepted, so it will be used in this series of papers.

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TABLE I
CHARACTERISTICS OF MYCOPLASMAS COMPARED WITH THOSE OF OTHER AGENTS

Characteristic	Myco- plasmas	Bacteria	Fungi (yeasts)	Protozoa	Rickettsiae	Chlamydiae*	Viruses
Growth in cell-free medium	+	+	+	some + some —	—	—	—
Smallest forms 100 mμ or less	+	—	—	—	—	—	+
Cell wall absent	+	—	—	some + some —	—	—	+
Contain DNA and RNA	+	+	+	+	+	+	—
Various metabolic systems	+	+	+	+	+	+	—
Growth inhibited by antibody	+	—	—	—	+	+	+
Growth inhibited by antibiotic	+	+	+	—	+	+	—

*Previously termed *Bedsonia* or psittacosis-lymphogranuloma—TRIC group of agents.

by broad-spectrum antibiotics, in particular the tetracyclines. However, growth is not inhibited by penicillins because they do not have cell walls. The addition of penicillin to medium as a bacterial inhibitor (Edward, 1954) has greatly assisted the isolation of mycoplasmas; but it has also led to the criticism that some supposed mycoplasmas are bacteria in their L-phase. This is because most bacteria can be induced to change to the L-phase in the laboratory by repeated culture in medium containing a high concentration of penicillin. Indeed, a few workers believe that all mycoplasmas are the stable L-phases of bacteria. This view is supported by the fact that mycoplasmas, and bacteria in their stable L-phases, have a number of properties in common. Because of this particular controversy and because a few people wrongly use the terms "mycoplasma" (or "PPLO") and "L organism" (or L-form) interchangeably, it is necessary to attempt to clarify the situation.

The occurrence of small colonies that had developed among larger ones of *Streptobacillus moniliformis* was reported by Klieneberger (1935). The small colonies were thought to be due to "PPLO" which were named L1 (L for Lister Institute). Thus the terms "L organism" and "PPLO" were synonymous at this time. However, Dienes (1939) showed that the small colonies were produced by a bacterial variant (L-phase) of *S. moniliformis*. In other words "L organism" and mycoplasma were not synonymous. Unfortunately, other organisms isolated by Klieneberger, which in fact were mycoplasmas, were serially numbered L2 onwards and this led to confusion. Remarkably, the L-phase variant of *S. moniliformis* is the only variant known to arise spontaneously. Other bacteria produce L-phase variants in the laboratory after treatment of the bacterium with glycine, hypertonic salt solution, or, as mentioned previously penicillin. These L-phase variants are normally

unstable in that they revert to their original forms after removal of the added substance. Sometimes reversion does not occur and the organism is then said to be in the stable L-phase. The advent of DNA-DNA and DNA-RNA hybridization techniques (McCarthy and Bolton, 1963; Nygaard and Hall, 1963) has enabled the examination of a mycoplasma and its supposed bacterial parent for genetic homogeneity. There is no evidence as yet that mycoplasmas are bacteria in their stable L-phases (Somerson, Reich, Chanock, and Weissman, 1967; McGee, Rogul, and Wittler, 1967). The present communication is concerned with mycoplasmas and not with L-phase variants of bacteria.

Mycoplasmas isolated from Man

Those mycoplasmas isolated from man are shown in Table II. Only *M. fermentans*, *M. hominis*, and the T-strain mycoplasmas will be considered in detail.

M. fermentans

This glucose-fermenting mycoplasma was first described by Ruiter and Wentholt (1952), but subsequent reports of its isolation have been infrequent. Ford and DuVernet (1966) found that 6 per cent. of their isolates from the urogenital tracts of women fermented glucose and these were probably *M. fermentans*. A low rate of detection may be a reflection of the technique of isolation and identification. The identification of every colony on a solid medium by the immunofluorescence technique, as described by Del Giudice, Robillard, and Carski (1967), might reveal a greater incidence of *M. fermentans*. This would be in keeping with the occurrence of growth-inhibiting antibody to *M. fermentans* in 13.5 per cent. of randomly selected sera from adults (Taylor-Robinson and others,

TABLE II
ISOLATION OF MYCOPLASMAS FROM VARIOUS SITES IN MAN

Site	Mycoplasma									
	<i>M. fermentans</i>	<i>M. hominis</i>	T-strain	<i>M. orale</i> 1, 2, 3	<i>M. salivarium</i>	<i>M. pneumoniae</i>	Navel	<i>M. hominis</i> 2'	<i>M. hyorhinitis</i>	<i>M. laidlawii</i>
Urethra*	+	+	+					+		
Cervix*	+	+	+							
Rectum*		+	+							
Conjunctiva*		+	+							
Joint*†	+	+	+					+	+	
Oropharynx		+	+	+++	+	+				+
Lung		+				+				
Blood										
Bone marrow	+			+						
Umbilicus							+			

*Considered in this study.

†Possible isolations (see text).

1966). On the other hand, this antibody could be due, at least in part, to infection by some other related mycoplasma.

M. hominis

It is difficult to know who first isolated this mycoplasma since organisms cultured many years ago have been lost and cannot be compared with the present reference strains. It is possible that the first mycoplasma isolated from human material was *M. hominis*. It was obtained from an abscess of Bartholin's gland (Dienes and Edsall, 1937). The strains isolated by Beveridge (1943) from the male urethra were probably *M. hominis*. Nicol and Edward (1953) described four distinct serotypes which they considered to be of human origin; the names *M. hominis* Type 1, *M. hominis* Type 2, *M. fermentans*, and *M. salivarium* were given by Edward and Freundt (1956). *M. hominis* Type 2 is now known to be serologically the same as *M. arthritidis* of murine origin (Edward and Freundt, 1965). Recorded isolations of this agent from man are few and might possibly have been spurious due to laboratory misadventures. *M. hominis* Type 1 is now referred to as *M. hominis*. Most strains isolated from the urogenital tract that produce large colonies on solid medium belong to this serotype, although, as indicated previously, identification of every colony that develops might reveal other mycoplasmas. HeLa cells in suspension adhere tenaciously to the surfaces of *M. hominis* colonies (Taylor-Robinson and Manchee, 1967). This property may be used for the differentiation of colonies produced by *M. hominis* and *M. fermentans*, since HeLa cells do not adhere to the latter. A preliminary examination of isolates obtained from the urogenital tract in this study, and considered to be *M. hominis* on serological evidence, has revealed that in nearly every instance all colonies produced on primary isolation adsorbed HeLa cells.

Strains, which on the basis of the disk growth-inhibition technique are grouped together as *M. hominis*, may have minor antigenic differences from one another. This was observed by Nicol and Edward (1953) who used the tube-agglutination technique, by Purcell and others (1967) who used metabolic inhibition, and most recently by Razin (1968) who used the disk growth-inhibition method. It is possible that a particular sub-type might be capable of producing disease while others are not able to do so.

T-strain Mycoplasmas

History T-strain mycoplasmas were first described by Shepard (1954, 1956), who found them

in scrapings from the male urethra. The colonies which they produced were termed "T-form" by Shepard (T for tiny) because they rarely exceeded 20μ in diameter, in contrast with colonies produced by *M. hominis* which may be 200μ or more in diameter. Although it was possible to subculture T-strain mycoplasmas serially in liquid medium, considerable difficulty was experienced in subculturing on solid medium. Confusion arose when Klieneberger-Nobel (1962) apparently identified Shepard's T-strain as a mycoplasma of avian origin, now known as *M. gallisepticum*. This situation arose because Shepard had previously passaged the T-strain mycoplasma in eggs that must have been infected with *M. gallisepticum*. Ford (1962) improved the culture medium for T-strain mycoplasmas so that serial subculture on solid medium soon became possible. In addition, the finding that T-strain mycoplasmas metabolized urea (Purcell, Taylor-Robinson, Wong, and Chanock, 1966; Shepard, 1966) has assisted greatly in their study. T-strain mycoplasmas have a urease enzyme (Shepard and Lunceford, 1967; Ford and MacDonald, 1967) which degrades urea to ammonia. Thus, the pH of medium initially at 6.5 or less, rises to 8.0 or more as the organisms increase in number. Using phenol red as an indicator, the rise in pH can be observed as a change in colour from yellow to purple. As mentioned previously (Taylor-Robinson and Purcell, 1966), this colour-change technique may be used in the isolation of organisms, for titration, and for quantitative measurement of specific antibody.

Nomenclature Shepard (1956) used the term "T-form" to describe the small colonies and the term "T-strain mycoplasma" was first used by him (Shepard, 1957), since when it has come to be generally accepted. Nevertheless, it is open to objection. To have to refer to different "serotypes or strains of a strain" is unsatisfactory. Until agreement is reached upon a suitable generic name, it has been suggested that these organisms should be called T-mycoplasmas (Taylor-Robinson, Williams, and Haig, 1968; Taylor-Robinson, Addey, and Goodwin, 1969). However, because it is desirable that general agreement should be reached before such a change is made, we use the term T-strain mycoplasma in this paper.

Technique of Isolation In this study, a basic medium (Manchee and Taylor-Robinson, 1968) was used, consisting of Difco "PPLO" broth supplemented with a 2 per cent. extract of dried yeast (Distillers Co. Ltd.) and 20 per cent. horse serum (Burroughs Wellcome). For the isolation of

T-strain mycoplasmas, 0.1 per cent. urea and 0.002 per cent. phenol red were added and the pH adjusted with hydrochloric acid to 7.0 or less. Solid medium consisted of the basic liquid medium at pH 6.5 with Ionagar (Oxoid), with or without urea and phenol red. The value of liquid medium containing urea and phenol red for the isolation of T-strain mycoplasmas (Taylor-Robinson and others, 1969) is shown in Table III. Specimens from men and from women were inoculated into the liquid medium, and on to the solid medium at pH 6.5 with and without additives. Use of the liquid medium afforded the most sensitive method for isolating T-strain mycoplasmas. Moreover, it was the easiest and quickest method. The occurrence of the colour change in solid medium (Williams and Taylor-Robinson, 1967) was the least sensitive technique: when the colour change occurred it was possible to observe colonies of T-strain mycoplasmas; but, even in the absence of such colour change, colonies were sometimes present.

Titration By means of the colour-change technique in liquid medium it has been possible to measure growth in stock cultures (Taylor-Robinson and Purcell, 1966). It is also possible to estimate the number of organisms in clinical specimens. This is important since direct inoculation of liquid medium without further titration provides no quantitative estimation of the number of organisms present in a clinical specimen, whereas inoculation of solid medium does provide an opportunity to count colonies, laborious though this may be. Titrations of T-strain mycoplasmas in clinical specimens have been performed easily and rapidly by making serial 4-fold dilutions of the specimens in liquid medium contained in plastic plates*. Takátsy loops† were used to transfer material from cup to cup, the loops being boiled between each transfer. The plates were sealed with adhesive tape and incubated at 37°C. until colour changes ceased to develop. The highest dilution of the specimen which produced a colour

change was the titration end-point. All the specimens in the present study from which isolates were obtained were titrated in this manner during the course of only two sessions with two batches of medium. Thus, problems of reproducibility were reduced to a minimum so that comparison of the number of organisms in various specimens could be made with confidence.

Measurement of Antibody The inhibition by antibody of the colour change produced by T-strain mycoplasmas in media containing urea and indicator has been used to measure antibody quantitatively (Purcell and others, 1966). These authors detected antibody to the T-960 strain of T-strain mycoplasma in randomly selected human sera. Antibody was commonly found after puberty. Rabbits have been immunized with T-strain mycoplasma antigens. By means of the antisera so produced, Purcell and others (1967) and Ford (1967) have established the existence of several serotypes.

Resistance to Drying Because they do not have cell walls, mycoplasmas have been considered to be particularly fragile. However, T-strain and other mycoplasmas may be freeze-dried and a proportion of the organisms remains viable (Shepard, 1967; Taylor-Robinson and others, 1968). Separate cultures in liquid medium of *M. hominis* and of a T-strain mycoplasma were dried on glass in air at room temperature. After 12 hours, when drying was complete, some organisms from both cultures were still viable, although viable organisms in the culture of *M. hominis* were reduced by 90 per cent. and in the culture of T-strain mycoplasma by more than 99 per cent.; there was no further loss after 21 hours. Because of these findings clinical specimens were tested in a similar manner. Thirteen specimens of vaginal secretion were titrated before and after drying on glass for various periods of time up to 42 hours. The isolation rates at different periods of time are shown in Table IV. Failure to recover viable organisms after 18 hours of drying

*Linbro Chemical Co. Inc., New Haven, Conn., U.S.A.
†Microtiter; Cook Engineering Co., Alexandria, Virginia, U.S.A.

TABLE III
VALUE OF LIQUID MEDIUM CONTAINING UREA FOR ISOLATION OF T-STRAIN MYCOPLASMAS*

Specimens Examined	No. of Isolations as determined by					Total Positive
	Colonies on Agar	Colour Change in		Colonies on Agar : No Colour Change in Agar or Liquid	Colour Change in Liquid : No Colonies observed on Agar	
		Liquid	Agar			
Number 38	11†	15	7	1	6	16
Percentage 100	29	40	18	3	16	42

*Data from Taylor-Robinson, Addey, and Goodwin (1969)

†Confirmed by subsequent subculture in liquid medium and/or observing colonies on agar medium

occurred with only one specimen, although there was a 90 per cent. or greater reduction in the number of viable organisms in ten specimens. More surprising was the fact that some organisms persisted for 18 hours in a saturated solution of soap in tap water. Persistence of T-strain mycoplasmas in this way indicates that a mode of spread other than sexual is possible, for example by washing in contaminated water or by using infected towels.

TABLE IV
SURVIVAL OF T-STRAIN MYCOPLASMAS

Isolation from Specimens of Vaginal Secretion					
Condition Time (hrs)	Dried at Room Temperature				In Soapy Water 18
	0	18	24	42	
No. Tested	13	13	9	9	10
No. Positive	13	12	5	3	3

Evidence that they are Members of the Genus Mycoplasma Various facts indicate that "T-strains" are mycoplasmas. They grow in the same kinds of medium, with minor variations, as do other mycoplasmas. Although they produce small colonies on agar, electron-microscopy studies have shown that the individual organism is the same in size and structure as a classical large-colony-forming mycoplasma (Williams, 1967; Taylor-Robinson and others, 1968). The most notable feature is the triple-layered limiting membrane. As with other mycoplasmas, their growth in liquid medium is inhibited by antibody; it has not been possible to inhibit colony formation by the incorporation of antiserum in solid medium. T-strain mycoplasmas are inhibited by broad-spectrum antibiotics but not by penicillin (Taylor-Robinson, 1967). There is no evidence that they are bacterial L-phase variants produced as a result of penicillin in the medium since strains of human origin (Taylor-Robinson and Addey, unpublished) and of bovine origin (Taylor-Robinson and others, 1968) have been isolated in the absence of antibiotics. Strains isolated from man in the presence of penicillin have been repeatedly subcultured in its absence without reversion to a bacterial form (Purcell, 1967).

Differences from Large-colony-forming Mycoplasmas Although similar to large-colony-forming mycoplasmas in many respects, T-strain mycoplasmas may be differentiated from them (Table V). T-strain mycoplasmas grow more rapidly but attain lower maximum titres than most large-colony-producing organisms (Ford, 1962; Taylor-Robinson and others, 1968). Apart from their ability to metabolize urea, T-strain mycoplasmas

are relatively more sensitive to the inhibitory effect of erythromycin (Shepard, Lunceford, and Baker, 1966) and thallium acetate (Shepard and Lunceford, 1967); in isolation procedures it is unwise to incorporate thallium acetate in medium at a concentration greater than 1:4000.

TABLE V
DIFFERENCES BETWEEN T-STRAIN MYCOPLASMAS AND *M. HOMINIS* AND *M. FERMENTANS*

Property	T-strain mycoplasmas	<i>M. hominis</i>	<i>M. fermentans</i>
Colonies small	+	—	—
Rapid growth cycle	+	—	—
Low eventual titre of viable organisms	+	—	—
Slow growth cycle	—	+	+
High eventual titre of viable organisms	—	+	+
Urea metabolized	—	+	±
Arginine metabolized	—	+	±
Glucose metabolized	—	+	±
Inhibition by erythromycin	+	+	+
Inhibition by thallium acetate	+	±	±

Isolation from Mammals other than Man Using the colour-change technique, T-strain mycoplasmas were isolated from 33 per cent. of slaughtered cows (Taylor-Robinson and others, 1968), being isolated more often from the urethra and bladder than from the vagina. Large numbers have been found in bulls in the seminal fluid and in washings from the penile sheath (Taylor-Robinson, Thomas, and Dawson, 1969). It seems possible that seminal fluid is contaminated by the mycoplasmas in the sheath. This finding prompted an investigation of the incidence of mycoplasmas in the urethral meatus in circumcised and uncircumcised men (Part III: Hare, Dunlop, and Taylor-Robinson, 1969). In addition, T-strain mycoplasmas have been isolated from the mouth in squirrel monkeys and from the vagina, semen, and prepuce in dogs. Preliminary evidence (Taylor-Robinson and Addey, unpublished) indicates that these animal T-strains are serologically different from each other and also different from a few T-strains of human origin with which they have been compared. The possible association of the organisms with disease in the various animals has not been investigated. The occurrence of T-strain mycoplasmas in animals suggests that these might provide useful models for studies of potential pathogenicity in man.

Association of Mycoplasmas with Disease

URETHRA: *Non-specific Urethritis (NSU)*

Soon after mycoplasmas were first isolated from the male urethra, attempts were made to determine

whether they were a cause of NSU (Beveridge, Campbell, and Lind, 1946), and many studies of the large-colony-forming mycoplasmas have since been reported. Those studies in which the findings in men with disease are compared with those in men who were apparently healthy are summarized in Table VI; several of these studies were analysed in detail by King (1964). Because *M. fermentans* was found rarely in health or disease it is unlikely that this organism causes NSU. Most of the mycoplasmas that were isolated were not identified, but it is reasonable to assume that the majority were *M. hominis*. If this be so, the frequency of isolation of this organism from men with disease, and from those apparently without disease, is not different. However, a summation of this sort is perhaps unjustified since it brings together studies in which frequency of isolation and criteria for selection of cases differed widely. Indeed, when the studies are considered separately (King, 1964), there are remarkable differences in results. The major reason for this is the use of different "control" groups. To find an acceptable control group for comparison with patients suffering from NSU is difficult. However, there seems little doubt that subjects in the control group should be free from NSU, and have similar sexual habits to those of patients in the group suffering from that disease; such points are discussed further in Part II (Dunlop, Hare, Jones, and Taylor-Robinson, 1969) and in Part III (Hare and others, 1969) of this paper. In the latter, the findings in a contrasted group of men are presented. From the various studies that have been reviewed,

there is no conclusive evidence that *M. hominis* is causally associated with NSU.

In recent years attention has been turned to the possibility that T-strain mycoplasmas are a cause of NSU. Those studies in which the findings in men suffering from NSU have been compared with those in groups free from that disease are presented in Table VII. Some workers have isolated T-strain mycoplasmas more frequently from diseased than from disease-free persons. On the other hand, in the reports by Ingham, MacFarlane, Hale, Selkon, and Codd (1966) and by Black and Rasmussen (1968), the isolation rates for the two groups of persons were almost the same. It seems likely that the differences in the results of the various studies may be attributed to differences in the controls; the reservations regarding the selection of "control" groups in the studies on *M. hominis* may be applied equally to the studies concerning T-strain mycoplasmas. So far no definite conclusions can be drawn about the role of T-strain mycoplasmas in NSU.

CERVIX AND VAGINA: "Non-specific" Inflammation

As in the studies of NSU in the male, a majority of the large-colony-forming mycoplasmas isolated have not been identified, although it is likely that most of them were *M. hominis*. A summary of various studies is presented in Table VI. In these studies, the isolation rate in persons with disease is more than twice that in persons considered to be free from disease. However, in women it is particularly difficult to ensure that "controls" are disease free; furthermore, the possibility cannot be excluded

TABLE VI
SUMMARY OF LARGE-COLONY-FORMING MYCOPLASMAS ISOLATED FROM THE UROGENITAL TRACT
IN 23 SEPARATE STUDIES,* BY SEX

Sex	Mycoplasma isolated	"Subject Ill"		"Subject Not Ill"	
		No. Studied	Per cent. Positive	No. Studied	Per cent. Positive
Male	Not identified	2,252	19.5	839	17.5
	<i>M. hominis</i>	574	22.5	348	12.0
Female	Not identified	1,346	49.0	886	21.0
	<i>M. hominis</i>	128	46.0	135	16.0

*For detailed analysis of some of the studies see King (1964).

TABLE VII
T-STRAIN MYCOPLASMAS ISOLATED FROM THE UROGENITAL TRACT OF MEN IN "CONTROLLED" STUDIES

Reference	Patients with Non-specific Urethritis		"Normal Controls"	
	No. Studied	Per cent. Positive	No. Studied	Per cent. Positive
Ford, Rasmussen, and Minken, 1962	45	60	55	22
Ford and DuVernet, 1963	100	79	200	34
Csonka and others, 1966	101	70	95	13
Ingham and others, 1966	45	66	54	48
Black and Rasmussen, 1968	56	46	46	54

in many cases that another organism is causing the disease, while at the same time providing conditions favourable for growth of mycoplasmas.

Few studies of the isolation rate of T-strain mycoplasmas from women have been reported. Ford (1967) isolated these organisms from 32 (37 per cent.) of 86 patients without leucorrhoea who attended a gynaecologist as private patients. Csonka, Williams, and Corse (1966, 1967) investigated different groups of women. One group consisted of 21 women whose consorts suffered from non-gonococcal urethritis: T-strain mycoplasmas were isolated from five of eleven without symptoms or signs of inflammation and from eight of ten with cervicitis or vaginitis. It is not possible to draw conclusions regarding the role of T-strain mycoplasmas since too few patients were examined. In other groups, urine only was cultured. T-strain mycoplasmas were isolated from 34 (40 per cent.) of 84 healthy women aged 18–25 years and from four of eighteen post-menopausal women: no isolates were obtained from 44 girls aged 13 to 18 years. The authors concluded that T-strain mycoplasmas might be present normally during the sexually-active period of life. Archer (1968) came to a similar conclusion after finding the organisms in 58 per cent. of pregnant women, 51 per cent. of women attending an infertility clinic, 29 per cent. of women in a geriatric unit, and 8 per cent. of nuns.

RECTUM: "Non-specific" Proctitis

Berg, Daggett, Madden, and Dienes (1960) reviewed studies in which mycoplasmas had been isolated from the rectum. The organisms isolated by Nicol and Edward (1953) were identified as *M. hominis*, but other authors did not identify their isolated strains serologically; the total isolation rate from the rectum for men and for women with and without disease was lower than that from the genitourinary tract. Berg and others (1960) considered that the mycoplasmas isolated from the rectum probably originated from the genitourinary tract and that there was no evidence to suggest that they were responsible for proctitis in the small group of patients studied. There are no reports of the isolation of T-strain mycoplasmas from the rectum.

CONJUNCTIVA: "Non-specific" Conjunctivitis and Iritis

Holland (1960) reported that he had grown mycoplasmas from the conjunctiva in the cases of seven of fourteen patients seen with anterior uveitis. These mycoplasmas were not further identified. He also isolated a mycoplasma from the conjunctiva of a patient suffering from Reiter's

disease and from three of 25 untreated patients with acute "simple" conjunctivitis. In contrast, mycoplasmas were not isolated from conjunctival material from thirty non-inflamed eyes of patients presenting for refraction. The number of cases studied was too small to conclude that there was any causal relationship between mycoplasmas and conjunctival disease. Arm, Woolridge, Cheng, and Chang (1966) isolated an unidentified mycoplasma from the inferior conjunctiva of a 4-year-old boy, and Jones and Tobin (1968) isolated *M. hominis* from eight of 250 clinically infected eyes of newborn infants. These authors did not present any evidence that the mycoplasmas were causally related to the disease. Ford (1967) failed to isolate T-strain mycoplasmas from conjunctival material from five patients suffering from Reiter's disease.

JOINT: Arthritis including Reiter's Disease

The isolation of *M. hominis* and "*M. hominis* type 2" (now known as *M. arthritis*) by Bartholomew (1965, 1967) from the synovial fluids of two patients suffering from Reiter's disease was accomplished by means of a cell-culture technique. Because cell cultures are often contaminated by mycoplasmas, the true origin of these isolates is uncertain, although *M. arthritis* has not been commonly found as a contaminant in cell cultures. *M. hyorhinis*, which infects pigs and which has been found in various cell cultures, was isolated from the synovial fluids of patients with rheumatoid arthritis in cell cultures (Bartholomew, 1965, 1967). The source of these isolates is similarly open to doubt. Nevertheless, Jansson and Wager (1967) isolated *M. arthritis* from synovial material from two patients with rheumatoid arthritis using cell-free mycoplasma medium. Williams (1968) has reported the isolation of mycoplasmas related to *M. fermentans* from the synovial fluids of 36 of ninety patients suffering from rheumatoid arthritis. However, Hayflick and Stanbridge (1967) failed to isolate mycoplasmas from sixteen synovial fluids obtained from patients with unspecified diagnoses. It is uncertain how far differences in techniques and the possibility of laboratory contamination could account for such conflicting results.

Dienes, Ropes, Smith, Madoff, and Bauer (1948), using solid medium, isolated mycoplasmas from joint fluid in two cases of Reiter's disease; the organisms produced small colonies. Ford (1967) reported that the incidence of T-strain mycoplasmas in the genital tracts of patients with Reiter's disease was similar to that in patients with NSU; he failed to isolate these organisms from fifteen synovial fluids from patients with Reiter's disease.

This suggests that there is no causal relationship between the organisms and that disease.

Conclusions

There is no definite evidence to suggest a causal association between infection of the genital tract with *M. hominis* and the production of disease. This is a conclusion based on numerous studies each of which in itself would not have been definitive. In the case of T-strain mycoplasmas, fewer studies have been carried out and evidence for a causal association between them and the production of disease is equivocal, principally because the organisms have frequently been isolated from apparently healthy "control" persons. However, the laboratory techniques now available for the study of mycoplasmas, in particular those for the study of T-strain mycoplasmas, are less laborious and enable a quantitative determination of the organisms to be made. For these reasons, studies of certain groups of patients with disease have been undertaken (Part II: Dunlop and others, 1969), and a different approach to the problem of selecting "control" subjects has been made (Part III: Hare and others, 1969).

Summary

An outline of mycoplasma characteristics and the way these compare with those of other agents is presented. The features of *Mycoplasma hominis* and T-strain mycoplasmas are considered in greater detail, and the latest techniques available for study of these organisms, in particular T-strain mycoplasmas, are described. The history of the relationship of mycoplasmas to infection of the human genital tract is presented and it is concluded that there is no definite evidence to suggest a causal association between infection of the genital tract by *M. hominis* and the production of disease. Furthermore, the equivocal nature of the evidence for the pathogenicity of T-strain mycoplasmas indicates the need for further studies with a new approach to the selection of controls.

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Les mycoplasmes et l'infection génitale "non spécifique"

I. Etudes préliminaires et questions de laboratoire

SOMMAIRE

Une silhouette des caractères des mycoplasmes ainsi que la manière dont ceux-ci se comparent avec ceux d'autres agents est présentée. Les caractéristiques de *Mycoplasma hominis* et de la souche T des mycoplasmes sont examinées avec plus de détail et les dernières techniques disponibles pour l'étude de ces organismes, en particulier de la souche T des mycoplasmes, sont décrites. On présente l'historique de la relation entre les mycoplasmes et l'infection des voies génitales humaines; il est conclu qu'il n'y a aucune réelle évidence suggérant qu'il y a une relation de cause à effet entre l'infection de voies génitales par *M. hominis* et l'apparition d'une maladie. Plus encore, la nature incertaine de la preuve de la pathogénicité de la souche T des mycoplasmes réclame des études ultérieures avec une nouvelle sélection de témoins.