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MYCOPLASMAS AND 'NON-SPECIFIC' GENITAL INFECTION*

II. CLINICAL ASPECTS

BY

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A study of genital infection has been in progress since 1963, carried out jointly at Moorfields Eye Hospital, the Institute of Ophthalmology, and The London Hospital.[1]

Studies for mycoplasmas have been undertaken in parallel with those for chlamydiae, trichomonads, candida, and bacteria; the name chlamydia is used because it has been recommended again (Page, 1966) as the correct term for the Bedsonia group of agents. Initially the isolation of mycoplasmas was carried out by Dr Ruth Lemcke at the Lister Institute, London. After she went to Australia they were undertaken from July, 1965, at the Clinical Research Centre, Harvard Hospital, Wiltshire. Because of the changes in methods it is not valid to consider both sets of results together; accordingly only the latter series is now reported.

The object of these investigations has been to study the causation of "non-specific" genital infection and the relationship of that condition, or group of conditions, to disease of the eye and to Reiter's disease.

Material

The following groups of patients have been studied:

(1) Men presenting because of "non-specific" urethritis (NSU), and their sexual contacts.
(2) Patients presenting because of possible Reiter's disease, and their sexual contacts.
(3) Babies suffering from ophthalmia neonatorum due to the agent of trachoma-inclusion conjunctivitis (TRIC agent), and their parents.
(4) Adults suffering from infection of the eye by TRIC agent, and their sexual contacts.

In addition, joint fluids obtained from patients suffering from rheumatoid arthritis with positive latex tests were cultured.

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Methods

All specimens for culture for mycoplasmas were taken by the physicians concerned in this study, most of them by one person (EMCD). They were taken before the collection of material for examination for chlamydiae; each specimen, consisting of a Stuart's swab (Moffett, Young, and Stuart, 1948) placed in mycoplasma medium (Manchee and Taylor-Robinson, 1968), was sent by post on the day it was obtained to the Clinical Research Centre.

Genital and Ano-rectal Material

(*a*) *Males* Urethral specimens were obtained before the patient passed urine; a swab was inserted into the urethral meatus and rotated.

(*b*) *Females* Specimens were obtained under direct vision using an operating microscope. Vaginal specimens from each adult were collected by rubbing a swab on the epithelium of the vaginal vault after a "Cusco" speculum had been passed. In a few cases, at the beginning of the study, cervical material was also collected; however, mycoplasmas were isolated less frequently from this than from vaginal material. In each case a specimen from the ano-rectal canal was collected by rubbing a swab on the mucosa after a small proctoscope had been passed. Specimens from babies were taken in similar fashion from the vulva and, if possible, from the rectum.

Conjunctival Material

Each specimen was obtained by rubbing a swab on the conjunctiva of the lower lid and fornix.

Joint Fluid

A swab was soaked in the fluid obtaneid by aspiration. Surgical masks were worn by the doctor and his assistant who "scrubbed up" but did not wear gloves. Using a careful "no touch" technique, the skin overlying the affected joint was cleaned with Weak Iodine Solution BP,

and sterile towels were applied to the surrounding area. After infiltration of the subcutaneous tissues with Procaine and Adrenaline Injection BP, the specimen was obtained by aspiration with another needle and syringe.

Results

"Non-specific" Urethritis (NSU)

Thirty men who had presented because of NSU were tested (Table I). T-strain mycoplasmas were isolated from nineteen (63 per cent.) and *M. hominis* from twelve (40 per cent.). In eleven cases T-strain mycoplasmas and *M. hominis* were found together. There was nothing clinically distinctive in the cases of men from whom mycoplasmas were isolated.

TABLE I
MYCOPLASMAS IN THIRTY MEN PRESENTING BECAUSE OF NSU

Site tested		Urethra
Number tested		30
Mycoplasmas isolated	T-strain	19
	M. hominis	12
	Both	11

Tests were also carried out on 22 female contacts of men who had presented because of NSU (Table II). T-strain mycoplasmas were found in vaginal material from twenty women, *M. hominis* from ten and both agents together from nine. T-strain mycoplasmas were found in rectal material from ten women and *M. hominis* in rectal material from nine.

TABLE II
MYCOPLASMAS IN 22 FEMALE CONTACTS OF MEN PRESENTING BECAUSE OF NSU

Site tested		Vagina	Rectum
Number tested		22	22
Mycoplasmas isolated	T-strain	20	10
	M. hominis	10	9
	Both	9	5

The relationship between the finding of mycoplasmas and the incidence of clinical proctitis and of proctitis as shown by a maximal polymorphonuclear leucocyte count of ten or more per high-power field (HPF) is shown in Table III.

TABLE III
MYCOPLASMAS AND PROCTITIS IN 22 FEMALE CONTACTS OF MEN PRESENTING BECAUSE OF NSU

Results of Cultures	No.	Clinical Proctitis	Pus 10/HPF
T-strain +	10	0	0
M. hominis +	9	1	1
Both +	5	0	0
Total Mycoplasma +	14	1	1
No Mycoplasma	8	0	0

Reiter's Disease

Results of tests in 34 cases of possible Reiter's disease are shown in Table IV. The 32 men and two women were suffering from polyarthritis; in no case had a latex test given a positive result; in all cases there was genital inflammation of varying degree. Conjunctival specimens from nine patients suffering from conjunctivitis were tested but mycoplasmas were not isolated; T-strain mycoplasmas were isolated from urethral material from twelve of 27 patients and *M. hominis* in association with T-strain mycoplasmas in five of 25. In the cases of the two women, T-strain mycoplasmas with *M. hominis* were grown from the vagina and *M. hominis* from the rectum in one; there was gross proctitis in the other (Mrs AC) but mycoplasmas were not isolated from rectum or vagina. Joint fluids from sixteen patients were tested: a glucose-fermenting

TABLE IV
MYCOPLASMAS IN 34 PATIENTS WITH SERO-NEGATIVE POLYARTHRITIS AND GENITAL INFLAMMATION (32 men and 2 women)

Site tested		Conjunctiva	Urethra (males)	Vagina	Rectum (females)	Joint Fluid
Number tested		9	29*	2	2	16†
Mycoplasmas isolated	T-strain	0	12/27	1	0	2‡
	M. hominis	0	5/25	1	1	0
	Both	0	5/25	1	0	0

*Some specimens unsuitable owing to bacterial contamination.
†From one synovial fluid a glucose-fermenting mycoplasma was isolated.
‡Both patients suffering from psoriasis (Mr KK, Mrs AC).

mycoplasma was isolated from one; T-strain mycoplasmas were grown from two (Mr KK and Mrs AC), both of whom suffered from psoriasis; a T-strain mycoplasma had been isolated from the urethra of Mr KK.

Joint fluids from eighteen patients suffering from rheumatoid arthritis with positive latex tests were examined for comparison with the findings in seronegative polyarthritis; mycoplasmas were not isolated from patients in this comparison group.

Twenty female contacts of men with probable Reiter's disease were tested (Table V). T-strain mycoplasmas were isolated from vaginal material from fourteen of fifteen patients; *M. hominis* was isolated together with T-strain mycoplasmas from five of fourteen patients. T strain mycoplasmas were grown from rectal material from six of nineteen patients and *M. hominis* from two of eighteen. The relationship between the finding of mycoplasmas and the incidence of proctitis is shown in Table VI.

TABLE V

MYCOPLASMAS IN TWENTY FEMALE CONTACTS OF PROBABLE REITER'S DISEASE

Site tested		Vagina	Rectum
Number tested		19*	20*
Mycoplasmas isolated	T-strain	14/15	6/19
	M. hominis	5/14	2/18
	Both	5/14	1/18

*Some specimens unsuitable owing to bacterial contamination.

TABLE VI

MYCOPLASMAS AND PROCTITIS IN TWENTY FEMALE CONTACTS OF PATIENTS WITH PROBABLE REITER'S DISEASE

Results of Cultures	No.	Clinical Proctitis	Pus 10/HPF
T-strain +	6/19	1	1
M. hominis +	2/18	1	1
Both +	1/18	0	0
Total Mycoplasma +	7	2	2
No Mycoplasma	12	1	1

Again there was no close association between the finding of the organisms and the incidence of proctitis.

Ophthalmia Neonatorum due to TRIC Agent

The findings in eight families, in each of which the baby had been found to have ophthalmia neonatorum due to TRIC agent, are shown in Table VII. The conjunctiva of one baby was tested for mycoplasmas with negative result. T-strain mycoplasmas were isolated from vulval material from three babies tested. There was congestion of the vulva in the cases of two of the three babies, and marked vulvitis in the third. Examination of vulval smears showed many bacteria in all three; *E. coli* was grown from one and *B. proteus* from two. It is possible that the inflammatory changes were of bacterial origin or that the presence of bacteria was secondary to the changes produced by another agent.

T-strain mycoplasmas were isolated from urethral material from two fathers, one of whom had urethritis; mycoplasmas were not isolated from two but there was urethritis in one of these.

T-strain mycoplasmas were isolated from vaginal material from all seven mothers tested and from rectal material from four of them. *M. hominis* was isolated from vaginal material from two mothers and from rectal material from one.

The relationship between the finding of mycoplasmas and the incidence of proctitis is shown in Table VIII.

TABLE VIII

MYCOPLASMAS AND PROCTITIS IN SEVEN MOTHERS OF BABIES WITH OPHTHALMIA NEONATORUM

Results of Cultures	No.	Clinical Proctitis	Pus 10/HPF
T-strain +	4	1	2
M. hominis +	1	0	0
Both +	0	0	0
Total Mycoplasma +	5	1	2
No Mycoplasma	2	0	0

TABLE VII

MYCOPLASMAS IN TRIC OPHTHALMIA NEONATORUM GROUP (8 familes)

Site tested		Conjunctiva (baby)	Vulva (baby)	Urethra (father)	Vagina (mother)	Rectum (mother)
Number tested		1	3	4	7	7
Mycoplasmas isolated	T-strain	0	3	2	7	4
	M. hominis	0	0	0	2	1
	Both	0	0	0	2	0

TABLE IX

MYCOPLASMAS AND TRIC OCULAR SYNDROMES: 26 PATIENTS AND 9 CONTACTS
(16 men and 19 women)

Site tested		Conjunctiva	Urethra (males)	Vagina	Rectum (females)
Number tested		6	15	18	17
Mycoplasmas isolated	T-strain	0	7	13	5
	M. hominis	0	0	6	8
	Both	0	0	5	4

Adults with Ocular Infection due to TRIC Agent

26 adults suffering from infection of the eye by TRIC agent were tested, as were nine of their sexual contacts (Table IX). Mycoplasmas were not isolated from the eye of any patient tested but all had already received local treatment that probably affected the results of tests. T-strain mycoplasmas were grown from urethral material from seven of fifteen men, from vaginal material from thirteen of eighteen women, and from rectal material from five of seventeen women. *M. hominis* was not isolated from urethral material from fifteen men but was isolated from vaginal material from six of eighteen women and from rectal material from eight of seventeen women.

The relationship of the presence of T-strain mycoplasmas to the incidence of urethritis in this group is shown in Table X. Eleven of the fifteen men, in this group of patients and their sexual contacts, were found to be suffering from NSU with a maximal count of 20 or more polymorphonuclear leucocytes per HPF in the urethral secretion. T-strain mycoplasmas were isolated from urethral material in seven of the fifteen cases and there was urethritis in five of that seven. T-strain mycoplasmas were not isolated in eight but urethritis was present in six of that eight.

TABLE X

MYCOPLASMAS AND TRIC OCULAR SYNDROMES: PATIENTS AND CONTACTS: RELATION OF T-STRAIN TO URETHRITIS IN FIFTEEN MEN

Results of Cultures	No.	Urethritis
T-strain +	7	5
T-strain—	8	6

The lack of close relationship between the presence of mycoplasmas and the incidence of proctitis in women in this group is shown in Table XI.

TABLE XI

MYCOPLASMAS AND PROCTITIS IN TRIC OCULAR SYNDROMES IN SEVENTEEN WOMEN
(Patients and Contacts)

Results of Cultures	No.	Clinical Proctitis	Pus 10/HPF
T-strain +	5	2	2
M. hominis +	8	3	4
Both +	4	2	2
Total Mycoplasma +	9	3	4
No Mycoplasma	8	2	2

Proctitis in Women

The incidence of each mycoplasma in rectal material, and of the finding of proctitis, are shown for the total of 68 women in Table XII. The proportion of cases in which there was proctitis, diagnosed clinically or on the presence of ten or more polymorphonuclear leucocytes per HPF, was similar irrespective of the presence or absence of T-strain mycoplasmas, but was rather higher in the presence of *M. hominis*. Mycoplasmas were present in 36 cases in all, including seven of clinical proctitis; they were not found in 31 cases, including four of clinical proctitis.

TABLE XII

MYCOPLASMAS AND PROCTITIS IN ALL 68 WOMEN*

Results of Cultures	No.†	Clinical Proctitis	Pus 10/HPF
T-strain +	25	4	5
T-strain—	42	7	8
M. hominis +	21	5	6
M. hominis—	45	6	7
Both +	10	2	2
Total Mycoplasma +	36	7	9
No Mycoplasma	31	4	4

*Patients and contacts of patients.
†Some specimens unsuitable owing to bacterial contamination.

In this group of patients, some of whom were suffering from chlamydial infection, it would be expected that proctitis due to that cause might occur. If the presence of mycoplasmas is related to proctitis they should be present more commonly in

TABLE XIII

MYCOPLASMAS: INCIDENCE IN SEXUAL PARTNERS*

Mycoplasmas isolated	No. of Pairs	Female } + Male }	Female+ Male —	Female— Male +	Female } — Male }
T-strain	39	23	14	0	2
M. hominis	37	4	11	6	16

*In urethra of male, vagina of female.

that condition than in the normal rectum. There was no evidence for such a relationship for T-strain mycoplasmas. *M. hominis*, however, was recovered more commonly from the clinically inflamed rectum (approximately 1 in 4) than from the normal rectum (approximately 1 in 8).

The presence of T-strain mycoplasmas in the rectum appears to be a reflection of their presence in the vagina. In 56 cases in which results of tests at both sites were available, whenever T-strain mycoplasmas were isolated from the rectum they were also isolated from the vagina. In seven of the 56 cases, however, *M. hominis* was detected in the rectum without an accompanying vaginal isolation.

Mycoplasmas in Sexual Partners

The patients who were studied included 39 couples, for both of whom valid results of cultures for T-strain mycoplasmas were obtained (Table XIII). The agent was isolated in 23 pairs from both partners; in fourteen pairs from the female partner only, and in none from the male only. Only two pairs were apparently free from T-strain mycoplasmas.

Results of cultures for *M. hominis* were available for 37 couples. It was isolated in four pairs from both partners, in eleven pairs from the female partner only, and in six from the male only. Sixteen couples were apparently free from *M. hominis*.

TABLE XIV

MYCOPLASMAS AND CHLAMYDIAE IN THE URETHRA (54 men)*

Chlamydia		Found	Not found
Number tested		8†	46‡
Mycoplasmas isolated	T-strain	5	30
	M. hominis	1	12
	Both	1	11

*Patients and contacts of patients from whom valid tests were obtained.
†Urethritis in all.
‡Urethritis in forty.

Association with Infection by Other Agents

The possibility that mycoplasmas might produce disease in association with other agents was investigated:

(a) Chlamydiae The results of culture of urethral material for mycoplasmas from 54 men, most of whom were suffering from urethritis, are shown in Table XIV. Chlamydiae were found in urethral material from eight patients and were not found in material from 46. T-strain mycoplasmas were isolated from approximately two-thirds of each group. *M. hominis* was present in one of the eight positive cases and in twelve of the 46 negative cases.

No close association between mycoplasmas and chlamydiae is apparent from these results, nor from those obtained in the cases of 49 women (Table XV).

TABLE XV

MYCOPLASMAS IN VAGINA AND CHLAMYDIAE IN CERVIX (49 women)*

Chlamydia		Found	Not found
Number tested		11	38
Mycoplasmas isolated	T-strain	10	30
	M. hominis	6	13
	Both	5	10

*Patients and contacts of patients.

(b) Bacteria The lack of significant relationship between the incidence of mycoplasmas and that of certain bacteria in the urethra in NSU is shown in Table XVI (opposite).

(c) Trichomonads The incidence of each mycoplasma in the cases of ten women with trichomoniasis is shown in Table XVII. T-strain mycoplasmas were grown from vaginal material from

TABLE XVII

MYCOPLASMAS IN TEN WOMEN* WITH TRICHOMONAL INFESTATION

Site tested		Vagina	Rectum
Mycoplasmas isolated	T-strain	8	2/7
	M. hominis	10	6/7

*Seven patients in study, three additional patients with trichomonal vaginitis

TABLE XVI
MYCOPLASMAS AND BACTERIA IN URETHRA
(26 men presenting because of NSU)

Bacteria isolated	T-strain + *M. hominis* +	T-strain + *M. hominis* —	T-strain — *M. hominis* +	T-strain — *M. hominis* —	Total
Staph. sap. alone	3	4	1	6	14
Staph. sap. and Diphtheroids	4	1	—	—	5
Staph. sap. and *Strep. viridans*	1	—	—	—	1
Staph. pyogenes	—	—	—	1	1
E. coli	2	2	—	—	4
Strep. faecalis	—	1	—	—	1
Total	10	8	1	7	26

eight and *M. hominis* from ten. Five of the male contacts of these women were examined: there was no urethritis in one case but T-strain mycoplasma was grown from the urethra; urethritis was present in four cases, mycoplasmas were not found in three of them but T-strain mycoplasmas and *M. hominis* were grown in one case. Mycoplasmas were isolated commonly from women harbouring *T. vaginalis*; again there was no significant correlation between their presence and urethritis in the male.

Discussion

T-strain mycoplasmas were grown from genital material from most women in all groups that were tested and from rectal material from some women. They were grown from urethral material from a smaller proportion of men. As these agents are probably sexually transmitted, it is perhaps surprising that they were not found in urethral material from men even more often, because they were present in most sexually-active women.

The presence of mycoplasmas is not always associated with clinical disease in the genital tract. Three possibilities that arise are:

(1) That they are never pathogenic in this site.

(2) That they are pathogenic under certain circumstances: perhaps only when particular concentrations of mycoplasmas have been reached, or perhaps in association with other organisms. However, there is no evidence to support such an association with chlamydiae or bacteria. Mycoplasmas are also found with *T. vaginalis* and this association is further considered in Part III (Hare, Dunlop, and Taylor-Robinson, 1969), which includes an account of studies of the quantities of mycoplasmas found in the urethra and the development of NSU.

(3) That only some strains are pathogenic. The present findings suggest that, if this is so, such strains are uncommon.

The conjunctiva and the genital tract are susceptible to infection by the same organisms; thus gonococci, chlamydiae—particularly TRIC agent, *Candida albicans*, and even *T. vaginalis*, may all be recovered from the conjunctiva. It is surprising that mycoplasmas were not isolated from the conjunctiva in this series, but these negative findings may be explained by the fact that most patients suffering from conjunctivitis had already received local treatment when material was taken for tests for mycoplasmas.

The chlamydial disease of the eye of a baby born with that infection demonstrates the pathogenicity of the TRIC agent that it has acquired from its mother's genital tract; moreover, the mother usually has characteristic changes in the cervix and the father usually has NSU (Dunlop, Freedman, Garland, Harper, Jones, Race, du Toit, and Treharne, 1967). Infection of the eye with isolates of chlamydia has been produced many times in man since the initial experiments reported by Collier and Sowa (1958) and by Collier, Duke-Elder, and Jones (1958). No such clear clinical evidence exists to show the pathogenicity to man of *M. hominis* or of T-strain mycoplasma. However, nasopharyngeal inoculation of male volunteers with very large numbers of *M. hominis* has produced pharyngitis and enlargement of lymph nodes in the neck (Mufson, Ludwig, Purcell, Cate, Taylor-Robinson, and Chanock, 1965). Because of the general lack of clinical evidence of pathogenicity, findings have to be compared with those in "control" groups: but the main difficulty in assessing the role of mycoplasmas in genital disease lies in obtaining suitable control groups.

The rate of isolation may well depend on the amount of material used as an inoculum. For this reason a specimen of genital material obtained from a person without genital inflammation is not strictly comparable with a specimen from a patient with discharge from the genital tract. It seems particularly unlikely that the results of examining urine from female "controls" without symptoms can be compared with the results of tests of vaginal specimens from patients with discharge. T-strain

mycoplasmas are found in vaginal material from most women examined; although they are likely to be spread by sexual intercourse, they do not necessarily cause disease. Evidence has been presented in Part I that these mycoplasmas are resistant to adverse conditions (Taylor-Robinson, Addey, Hare, and Dunlop, 1969) so that they are likely to be spread also by means such as towels, face-flannels, and baths.

Csonka, Williams, and Corse (1966) studied patients suffering from gonorrhoea as a comparison group. They found T-strain mycoplasmas in fourteen (28 per cent.) of fifty men who did not develop non-gonococcal urethritis (NGU) after the treatment of gonorrhoea, and in ten (77 per cent.) of thirteen who did. This compared with an incidence of 70 per cent. in 101 cases of NGU. This approach to the problem was valuable but the numbers of patients were small. Also certain questions remain to be answered: if such patients were to be followed up for a month after treatment for gonorrhoea, would all or most of those harbouring mycoplasmas develop NSU? Would tests of the overnight urethral secretions show that all those harbouring mycoplasmas had low-grade urethritis? Could it be that urethritis would only develop when the organisms reached a certain concentration? Careful attention to these further points might indicate whether mycoplasmas, and particularly T-strain mycoplasmas, cause NSU. An attempt has been made to answer these questions in Part III of this study (Hare and others, 1969).

Summary

Tests for mycoplasmas were carried out as part of a planned study, in parallel with tests for other organisms, in the following groups of patients:

(1) Men presenting because of "non-specific" urethritis (NSU), and their sexual contacts.
(2) Patients presenting because of possible Reiter's disease, and their sexual contacts.
(3) Babies suffering from ophthalmia neonatorum due to TRIC agent, and their parents.
(4) Adults suffering from infection of the eye by TRIC agent, and their sexual contacts.

In addition, joint fluids, obtained from patients suffering from rheumatoid arthritis with positive latex tests, were tested.

Mycoplasmas were found in the genital tract in the cases of most women in all groups and in the rectum in some women. They were present in the urethra in the cases of a smaller proportion of men. Mycoplasmas were obtained from synovial fluid in a few cases. No correlation was found between infection with mycoplasmas and with chlamydiae in men or women, or with bacteria in men presenting because of NSU. There was no strong clinical evidence of pathogenicity; accordingly, study of a comparison group was planned to assess the relationship to NSU (reported in Part III).

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Les mycoplasmes et l'infection génitale "non spécifique"

II. Aspects cliniques

SOMMAIRE

Des tests concernant les mycoplasmes furent effectués lors d'une étude planifiée, en parallèle avec des tests pour d'autres organismes, chez les groupes suivants de malades:

(1) Hommes consultant à cause d'une urétrite non gonococcique (UNG), et leurs partenaires sexuelles.
(2) Malades consultant à cause d'une éventuelle maladie de Reiter, et leurs partenaires.
(3) Nouveau-nés souffrant d'une ophtalmie due à l'agent TRIC, et leurs parents.
(4) Adultes atteints d'une infection de l'oeil par l'agent TRIC, et leurs partenaires.

En plus, des liquides articulaires, recueillis chez

des malades atteints d'arthrite rhumatoïde avec tests au latex positifs, furent examinés.

Des mycoplasmes furent trouvés dans les voies génitales chez la plupart des femmes dans tous les groupes et dans le rectum de quelques-unes d'entre elles. Ils furent présents dans l'urètre dans une plus petite proportion des cas masculins. On trouva des mycoplasmes dans le liquide synovial pour quelques cas. Il ne fut pas constaté de corrélation entre l'infection à mycoplasmes et celle appartenant au groupe des *Chlamydozoon* chez les hommes ou chez les femmes, ou avec les bactéries trouvées chez les hommes consultant pour UNG. Il n'y a pas de preuve clinique nette de pathogénicité; en conséquence on projeta l'étude d'un groupe de comparaison pour établir la relation avec UNG (voir partie III).