

T-STRAIN MYCOPLASMA IN NON-GONOCOCCAL URETHRITIS*

PATHOGEN OR COMMENSAL?

BY

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The term "T-strain" was applied by Shepard (1954) to a strain of mycoplasma which, when cultured on solid agar, shows morphological differences from other members of the species (Shepard, 1956). Shepard (1954) recovered T-strain mycoplasmas from urethral scrapings taken from cases of non-gonococcal urethritis (NGU) and later found that this organism was present in some 70 per cent. of such cases (Shepard, 1956, 1959). Subsequently he postulated that T-strain mycoplasma was responsible for a specific type of venereal urethritis in which the incubation period was about 4 weeks with a range of 3 to 5 weeks; the symptoms were urethral itching, dysuria, and frequency of micturition, and the discharge was usually mucopurulent in character and scanty to moderate in amount (Shepard, Alexander, Lunceford, and Campbell, 1964).

Support for this view was supplied by Ford and his associates. In their initial study these workers recovered T-strain mycoplasmas from 60 per cent. of 45 cases of NGU (Ford, Rasmussen, and Minkin, 1962). Later they isolated the organism in 79 per cent. of 100 cases of NGU (Ford and Duvernet, 1963). In both studies T-strain mycoplasmas were recovered from the genito-urinary tract of normal men but significantly less frequently than from men suffering from NGU.

After a comprehensive study, Csonka, Williams, and Corse (1966) were unable to support the hypothesis that T-strain mycoplasma produced a specific type of urethritis, but they did agree that this organism could be of aetiological significance in NGU. They isolated T-strain mycoplasmas from 70 per cent. of 101 cases of NGU and from 76.9 per cent. of thirteen cases of gonorrhoea in which post-gonococcal urethritis had developed, but from only 28 per cent. of fifty male cases of uncomplicated gonorrhoea, and from 12.5 per cent. of 95 normal males. They found also that T-strain mycoplasmas

were present in 61 per cent. of 21 female contacts of men suffering from NGU but in only 40 per cent. of normal women. Csonka and others (1966) noted that cases of NGU in which T-strain mycoplasmas had been isolated responded better to treatment with tetracycline than cases in which the organism was not recovered; they regarded this as additional evidence of relationship between the T-strain mycoplasma and NGU.

On the other hand, Ingham, Macfarlane, Hale, Selkon, and Codd (1966) and Black and Rasmussen (1968) were unable to support the hypothesis that T-strain mycoplasmas are of aetiological significance in NGU. Ingham and others (1966) isolated the organism from 60 per cent. of 45 cases of NGU, from 61 per cent. of 36 male cases of gonorrhoea, and from 48 per cent. of normal males. Black and Rasmussen (1968) recovered the organism from 46.6 per cent. of 56 patients with NGU, from 35 per cent. of sixty patients with gonorrhoea, and from 54.3 per cent. of 46 healthy contacts of the latter.

The purpose of this paper is to report the incidence of isolation of T-strain mycoplasma in the cases of patients under our care and to discuss the possible role of the organism in the light of these findings.

Clinical Material

There were 841 males and 211 females.

Among the males there were 314 cases of NGU and 404 cases of gonorrhoea; 123 men presented no evidence of disease of the lower genito-urinary tract and were used as controls.

Among the females there were 72 cases of gonorrhoea and 48 of trichomonal vaginitis; 91 females presented no evidence of disease of the lower genito-urinary tract.

Of these female patients 86 were consorts of males included in this investigation (55 of men with gonorrhoea and 31 of men with NGU).

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Method

Cultures for the T-strain mycoplasma were prepared at the initial examination in every case and were repeated after treatment in 210 male cases (100 with gonorrhoea and 110 with NGU). In all but 291 male cases the method of collecting specimens from the urethra for culture was as described by Csonka and others (1966), and the culture medium was that used by these workers after March, 1965 (Method 1). The same culture medium was used in the female cases, but here a sterile cotton-wool swab, and not a platinum wire loop, was used to obtain a specimen from the cervix.

In 291 males (135 with NGU and 156 with gonorrhoea), the urethral specimen was collected on a sterile cotton wool swab, cultured in the liquid medium,* and sub-cultured, if there was any colour change, on the solid medium** of the Clinical Research Centre of the Common Cold Research Unit and also on the solid medium used in the other cases (Method 2).

Liquid Medium for T-strains*

- 70 ml. PPLO broth
- 1 ml. penicillin G, 100,000 units (final concentration 1,000 units/ml)
- 1 ml. thallium acetate 2.5 per cent. (final concentration 0.05 per cent. *i.e.* 1:2000)
- 1 ml. urea 10 per cent. (final concentration 0.1 per cent.)
- 10 ml. yeast extract 25 per cent.
- 20 ml. horse serum
- 2 ml. phenol red 0.1 per cent. (final concentration 0.002 per cent.)
- pH adjusted to 7

Solid Medium for T-strains**

- 70 ml. 6.0 agar-broth
- 0.4 ml. N.H.C.L.
- 1 ml. penicillin G, 100,000 units
- 1 ml. thallium acetate 2.5 per cent.
- 10 ml. yeast extract 25 per cent.
- 20 ml. horse serum
- 1 ml. urea 10 per cent.
- 2 ml. phenol red 0.1 per cent.
- pH 6.5

With both methods the colonies were studied after staining with alcoholic methylene blue, as described by Deines (1939).

Urease Inhibition Using the methods of Purcell, Taylor-Robinson, Wong and Chanock (1966), sera collected at the initial examination from 64 T-strain positive cases (38 males and 26 females) were tested

against two strains of T-strain mycoplasma isolated during this investigation.

Cold Agglutinins In 88 T-strain positive cases (63 males and 25 females) sera were examined for cold agglutinins.

Results

Tables I and II show the rates of isolation of T-strain mycoplasma in the various categories of male and female patients listed above.

TABLE II
INCIDENCE OF T-STRAIN MYCOPLASMA IN 211 FEMALES (METHOD 1)

Diagnosis	No. of Cases	T-strain Positive	
		No.	Per cent.
Gonorrhoea	72	37	51.3
Trichomonal vaginitis	48	23	47.9
Control	91	39	42.8

Sensitivity of Tests

It will have been noted that a higher percentage of positive results was obtained with Method 2 than with Method 1. To check the sensitivity of Method 2, cultures were taken 3 days after penicillin treatment in 100 male cases of gonorrhoea, and negative results were obtained in twelve (19.3 per cent.) of the 62 cases in which T-strain mycoplasmas had been isolated before treatment. On the other hand, T-strain mycoplasmas were recovered after treatment in nine (23.6 per cent.) of the 38 cases in which the organism had not been found initially.

Post-gonococcal Urethritis This may manifest itself in one of two ways:

(A) Signs of urethritis persist for longer than 7 days despite successful treatment of the gonococcal infection as shown by repeated failure to isolate the gonococcus in films or cultures.

(B) Within a few days of treatment being given for gonorrhoea the signs of urethritis disappear but then recur after a varying period, usually about 3

TABLE I
INCIDENCE OF T-STRAIN MYCOPLASMA IN 841 MALES (METHODS 1 AND 2)

Diagnosis	Gonorrhoea				NGU				Control			
	Method	No. of Cases	T-strain Positive		No. of Cases	T-strain Positive		No. of Cases	T-strain Positive			
			No.	Per cent.		No.	Per cent.		No.	Per cent.		
	1	248	120	48.3	179	95	53.0	123	50	41.2		
	2	156	90	63.4	135	82	60.7					

weeks; failure to demonstrate the gonococcus microscopically or by culture shows that the urethritis is not due to this organism.

Cases of post-gonococcal urethritis were encountered in this study as follows:

Method 1 121 male cases of gonorrhoea were under observation for longer than 3 weeks and eight cases developed post-gonococcal urethritis. Four of these had been T-strain positive initially, out of 65 T-strain positive cases followed.

Method 2 81 cases were followed for longer than 3 weeks and post-gonococcal urethritis developed in five of them; three of these had been T-strain positive before treatment out of 44 T-strain positive cases followed.

Non-gonococcal Urethritis

(A) CLINICAL FEATURES

For over 2 years the results of our various studies of non-gonococcal urethritis have been analysed by computer. The information submitted for analysis

is shown in the Appendix. Forms were completed for all cases of non-gonococcal urethritis included in the present investigation, but unfortunately, due to a mishap, only 61 T-strain positive and 93 T-strain negative cases were analysed. The results are shown below:

(1) *Possible Incubation Period* It was obviously impossible to give a reliable incubation period in the cases of married men who denied extramarital coitus, or of single men who admitted to regular coitus with only one consort. Also, in other cases symptoms had obviously been present for some time before promiscuous coitus, and in other cases coitus was denied. Table III shows the interval, where this was known, between promiscuous intercourse and the appearance of symptoms or signs of urethritis in 154 males.

(2) *Symptoms* These are shown in Table IV.

(3) *Urethral Discharge* This is shown in Table V.

(4) *Urethroscopic Appearances* These are shown in Table VI (overleaf).

TABLE III
INCUBATION PERIOD IN 154 CASES OF NGU

T-Strain	Not defined	Up to 1 wk	1-3 wks	Up to 1/12	Up to 2/12	Up to 3/12	Up to 6/12 +	Total
Positive	15	19	19	5	2	1	—	61
Negative	35	19	27	7	2	2	1	93

TABLE IV
SYMPTOMS IN 154 CASES OF NGU

T-Strain	Nil		Irritation, Scalding, or Pain on Micturition		Frequency		Haematuria	Total Cases
	No.	Per cent.	No.	Per cent.	No.	Per cent.		
Positive	35	57.0	20	32.8	5	8.0	1	61
Negative	43	45.1	41	43.9	8	8.5	1	93

TABLE V
URETHRAL DISCHARGE IN 154 CASES OF NGU

T-Strain	Discharge		Scanty	Moderate	Profuse	Total
Positive	Mucoid		19	4	0	23
	Mucopurulent		4	8	3	15
	Purulent		5	4	14	23
	Total	No. Per cent.	28 46.0	16 26.2	17 27.8	61
Negative	Mucoid		47	3	1	51
	Mucopurulent		6	13	2	21
	Purulent		5	6	10	21
	Total	No. Per cent.	58 62.3	22 23.5	13 14.0	93

TABLE VI
URETHROSCOPIC APPEARANCES IN 154 MALES

T-Strain	Positive	Negative
No examination	35	54
Not possible	1	1
Normal	6	9
Congestion	12	13
Soft infiltration	5	13
Stricture	—	—
Sago grain	2	2
Warts	—	—
Congenital abnormality	—	1
Total	61	93

(5) *Subsequent History* Of the 61 T-strain positive cases of urethritis, three are known to have recurred more than 3 months after the apparent cure of the initial attack. There were similar recurrences in four of the 93 T-strain negative cases.

(B) RESPONSE TO TREATMENT

Tetracycline 250 mg. was given four times a day for 4 days. Treatment was regarded as successful if signs of urethritis cleared within 14 days of starting therapy and did not recur during the subsequent observation period, or if they were absent at the last examination of those who defaulted after only one or two post-treatment inspections. The results are summarized in Table VII.

TABLE VII
RESPONSE TO TREATMENT

Method	T-strain	No. of Cases	Cured	Failed	No follow-up
1	Positive	95	49	25	21
	Negative	84	42	22	20
2	Positive	82	44	22	16
	Negative	53	29	14	10

T-strain Mycoplasmas after Tetracycline Treatment 110 cases of NGU were investigated for T-strain mycoplasmas 7 days after starting tetracycline treatment; positive cultures were obtained in 27 cases, including ten of the 45 cases which had been T-strain negative before treatment. Of these 27 cases, eight showed no evidence of urethritis and the remainder still showed signs of urethral inflammation.

T-strain Mycoplasmas in Consorts In the cases of 38 couples genital secretions from both partners were either T-strain negative or T-strain positive. In 48 couples, one partner's secretions were T-strain positive and the other T-strain negative.

Cold Agglutinins Cold agglutinins were not demonstrated in any case.

Urease Inhibition Negative results were obtained in all patients.

Discussion

Table I shows that a higher incidence of isolation of T-strain mycoplasma was obtained when the primary inoculation was made in a liquid medium and then sub-cultured on to solid media (Method 2) than when the inoculum was plated directly on to a solid medium (Method 1). As a cotton-wool swab was used to collect the inoculum in Method 2 and a platinum wire loop in Method 1 the slightly superior results obtained by the former cannot be attributed with certainty to the liquid medium.

Judging from the results obtained in the 100 male cases of gonorrhoea and the T-strain negative cases of non-gonococcal urethritis in which the investigations were repeated after treatment, it seems that the incidence of T-strain mycoplasma could well have been some 20 per cent. higher than appeared from these initial findings (Tables I and II). It will be appreciated that this does not invalidate any conclusions which might be reached from a comparison of the incidence of T-strain mycoplasma in the various groups in Tables I and II. Nor does it prevent the comparison of the findings in these Tables with those of other workers, as the incidence of T-strain mycoplasmas has usually been based on the results of investigations carried out on one occasion only in each case studied.

The rates of isolation of T-strain mycoplasmas in male cases of gonorrhoea, male cases of NGU and normal males (Table I), like those found by Ingham and others (1966) and Black and Rasmussen (1968), provide no support for the view that there is a causal relationship between the T-strain mycoplasmas and NGU. Likewise there is no support in our rates of isolation in the 86 couples, in the females with vaginitis or cervicitis, and in normal females (Table II). In fact, the findings in Table II are very similar to those of Csonka and others (1966) who recognized that the frequency with which T-strain mycoplasmas were recovered from normal females and the comparative infrequency of NGU were hardly compatible with the belief that the disease was caused by the T-strain mycoplasmas and led them to wonder whether special circumstances were necessary for the organism to become pathogenic.

If T-strain mycoplasmas only became pathogenic under certain conditions, then the urethritis produced might well present special features, as

was suggested by Shepard and others (1964). We have to agree with Csonka and others (1966) who could find no evidence in favour of this belief because we found no significant difference in the incubation period, symptomatology, or clinical signs, including urethrosopic appearances, between cases in which T-strain mycoplasmas had been isolated and those in which this organism had not been recovered (Tables III to VI). Nor did it appear that T-strain positive cases differed from T-strain negative cases in their response to tetracycline (Table VII) or in their tendency to recur.

We found no indication that the T-strain mycoplasma was influential in the production of post-gonococcal urethritis. This is in keeping with our other findings because it is impossible to distinguish post-gonococcal urethritis from non-gonococcal urethritis on clinical grounds.

Little importance can be attached to the results of our serological investigations. The demonstration of cold agglutinins would have been important, but no significance can be attached to our negative findings. Nor can any significance be attached to our failure to demonstrate antibodies by the urease inhibition method, as the test was carried out in the initial stages of the disease only. However, it is pertinent to note that Purcell and others (1966) did test sera during the course of the illness and could demonstrate no rise in titre. This finding is in agreement with our results and supports our opinion that there is no causal relationship between the T-strain mycoplasma and non-gonococcal urethritis.

Summary

Genital secretions from 841 males and 211 females were investigated for the presence of the T-strain mycoplasma. The males included 314 cases of NGU and 404 of gonorrhoea; there were also 123 males with no evidence of disease of the lower genito-urinary tract who were used as controls. The females included 72 cases of gonorrhoea and 48 of trichomonal vaginitis; there were also 91 females with no evidence of disease of the lower genito-urinary tract who were used as controls.

T-strain mycoplasmas were recovered in 51.3 per cent. of males with gonorrhoea, in 47.9 per cent. of males with trichomonal vaginitis, and in 42.8 per cent. of the female controls.

Depending on the technique used, the incidence of T-strain mycoplasma varied from 48.3 to 63.4 per cent. in males with gonorrhoea and from 53 to 60.7 per cent. in males with non-gonococcal urethritis. In the male controls the incidence was 41.2 per cent.

So far as clinical features, response to treatment, and the tendency to recurrence were concerned, it was impossible to distinguish between T-strain positive and T-strain negative cases of non-gonococcal urethritis. Nor was there any evidence that T-strain mycoplasma had any influence on the production of post-gonococcal urethritis. It is concluded that there is no causal relationship between the T-strain mycoplasma and non-gonococcal urethritis.

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La souche T de mycoplasme, dans les urétrites non gonococciques, est-elle pathogène ou saprophytique?

RÉSUMÉ

La présence de la souche T de mycoplasme fut recherchée dans les sécrétions génitales de 841 hommes et de 211 femmes. Parmi les hommes, 314 étaient atteints d'UNG, 404 de gonococcie; figuraient aussi, à titre de témoins, 123 hommes dont les voies urogénitales basses ne montraient aucun signe d'infection. Parmi les femmes, il y avait 72 cas de gonococcie, 48 de vaginite à trichomonas. Il y avait également, à titre de témoins, 91 femmes dont les voies urogénitales basses ne montraient aucun signe d'infection.

Des souches T de mycoplasme furent isolées chez 51,3 pour cent des femmes gonococciques, chez 47,9 des femmes atteintes de vaginite à trichomonas et chez 42,8 pour cent des femmes-témoins.

Selon la technique employée, l'incidence de la souche T alla de 48,3 à 63,4 chez les hommes gonococciques, de 53 à 60,7 pour cent chez ceux atteints d'UNG. L'incidence chez les témoins masculins fut de 41,2 pour cent.

Il fut impossible de distinguer les cas d'UNG positifs ou négatifs vis-à-vis de la souche T en ce qui concerne

l'allure clinique, la réponse au traitement ou la tendance aux récives. Il n'y fut pas non plus évident que la souche T de mycoplasme eut une influence sur la survenue d'urétrite post-gonococcique. Il est conclu qu'il n'y a pas de relation entre la présence de la souche T de mycoplasme et les urétrites non gonococciques.

APPENDIX

Non-Gonococcal Urethritis

Trial No.		<input type="text"/>	1
Registration No.		<input type="text"/>	2
Date of First Attendance		<input type="text"/>	8
		Day Month Year	
Marital Status:	0 Not known, 1 Single, 2 Married, 3 Widowed, 4 Divorced, 5 Separated, 6 Remarried	<input type="text"/>	14
Race	0 Not known, 1 U.K., 2 Eire, 3 Other European, 4 West Indian Negro, 5 African Negro, 6 Other Negro, 7 Asian, 8 Mediterranean (Non-European), 9 Other	<input type="text"/>	15
Age (yrs)		<input type="text"/>	16
Past History	0 Nil, 1 One attack NSU, 2 More than one attack NSU, 3 GC, 4 GC + NSU, 5 Reiter's disease	<input type="text"/>	18
		<input type="text"/>	19
		<input type="text"/>	20
Consort	0 Nil, 1 Marital, 2 Regular, 3 Acquaintance, 4 Pick-up, 5 Pro, 6 Same sex	<input type="text"/>	21
	1 Trichomonal vaginitis, 2 Thrush, 3 Other, 4 Normal, 5 Not examined	<input type="text"/>	22
		<input type="text"/>	23
		<input type="text"/>	24
Possible Incubation Period		<input type="text"/>	25
		mths wks days	
Duration before Treatment		<input type="text"/>	28
		mths wks days	
Symptoms	0 Nil, 1 Irritation, 2 Scalding, 3 Pain, 4 Diurnal frequency, 5 Nocturnal frequency, 6 Diurnal + nocturnal frequency, 7 Haematuria	<input type="text"/>	31
		<input type="text"/>	32
		<input type="text"/>	33
Character of Discharge	1 Scanty, 2 Moderate, 3 Profuse, 4 Watery, 5 Mucoid, 6 Mucopurulent, 7 Purulent	<input type="text"/>	34
		<input type="text"/>	35
Urine	0 Not examined, 1 Clear, 2 Clear + threads, 3 Haze, 4 Haze + threads, 5 Blood, 6 Albumin, 7 Sugar, 8 pH less than 6.5, 9 pH 6.5, 10 pH 7 or over	<input type="text"/>	36
		<input type="text"/>	37
		<input type="text"/>	38
Films	1 Sterile, 2 <i>T. vaginalis</i> , 3 Coliform, 4 <i>Strep. faecalis</i> , 5 Haemophilus, 6 Thrush, 7 Inclusions, 8 Other	<input type="text"/>	39
		<input type="text"/>	40

Rectal Films	(a) Before Treatment 1 Normal, 2 Fungal, 3 Not done	<input type="checkbox"/>	41
	(b) After Treatment 1 Normal, 2 Fungal, 3 Not done	<input type="checkbox"/>	42
Fluorescing Antibodies	1 Not done, 2 Present, 3 Absent	<input type="checkbox"/>	43
Cultures	1 Sterile, 2 <i>T. vaginalis</i> , 3 Mycoplasma T-Strain, 4 TRIC agent, 5 Other	<input type="checkbox"/>	44
Urinary Deposit	0 Not examined, 1 Pus cells, 2 No pus, 3 Sterile, 4 <i>Staph. albus</i> , 5 <i>Staph. aureus</i> , 6 Coliform, 7 <i>Strep. faecalis</i> , 8 <i>B. proteus</i>	<input type="checkbox"/>	45
		<input type="checkbox"/>	46
Deposit after Treatment	0 Not examined, 1 Pus cells, 2 No pus, 3 Sterile, 4 <i>Staph. albus</i> , 5 <i>Staph. aureus</i> , 6 Coliform, 7 <i>Strep. faecalis</i> , 8 <i>B. proteus</i> , 9 Other	<input type="checkbox"/>	47
		<input type="checkbox"/>	48
		<input type="checkbox"/>	49
Complications	0 None, 1 Before treatment, 2 After treatment, 3 Both	<input type="checkbox"/>	50
Genito-urinary Complications	0 None, 1 Periurethral abscess, 2 Prostatitis, 3 Epididymis, 4 Stricture, 5 Upper urinary tract, 6 Other	<input type="checkbox"/>	51
		<input type="checkbox"/>	52
Systemic Complications	0 None, 1 Arthritis, 2 Conjunctivitis, 3 Iritis, 4 Keratoderma, 5 Balanitis, 6 Other	<input type="checkbox"/>	53
		<input type="checkbox"/>	54
		<input type="checkbox"/>	55
Urethroscopic Examination	0 Not done, 1 Not possible, 2 Normal, 3 Congestion, 4 Soft infiltration, 5 Stricture, 6 Warts, 7 Sago grains, 8 Congenital abnormality	<input type="checkbox"/>	56
		<input type="checkbox"/>	57
		<input type="checkbox"/>	58
Urethral Meatus	1 Normal, 2 Inflamed, 3 Hypospadias, 4 Epispadias, 4 Circumcised, 5 Uncircumcised	<input type="checkbox"/>	59
		<input type="checkbox"/>	60
Treatment	0 Nil, 1 Lactose, 2 Tetracycline × 4 days, 3 Tetracycline × 14 days, 4 Streptomycin + sulphonamides, 5 Vitamin C, 6 Other, 7 Tetracycline + Nystatin	<input type="checkbox"/>	61
		<input type="checkbox"/>	62
Results	1 Cleared, 2 Failed, 2 Relapsed	<input type="checkbox"/>	63
Relapse Time after Treatment		<input type="checkbox"/>	64
		mths wks	
Re-treatment	1 Tetracycline, 2 Other	<input type="checkbox"/>	66
Response to Re-treatment	1 Cleared, 2 Failed, 3 Relapsed	<input type="checkbox"/>	67
Relapse Time after Re-treatment		<input type="checkbox"/>	68
		mths wks	
Follow-up	1 Nil, 2 Incomplete, 3 Complete	<input type="checkbox"/>	70
Number of Attendances		<input type="checkbox"/>	71
Subsequent History	0 Not known, 1 NSU, 2 Reiter's disease, 3 Stricture	<input type="checkbox"/>	74
		<input type="checkbox"/>	75
		<input type="checkbox"/>	77