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MYCOPLASMAS AND 'NON-SPECIFIC' GENITAL INFECTION*

III. POST-GONOCOCCAL URETHRITIS. A PROSPECTIVE STUDY

BY

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Mycoplasma hominis and T-strain mycoplasmas have been isolated (Part I: Taylor-Robinson, Addey, Hare, and Dunlop, 1969; Part II: Dunlop, Hare, Jones, and Taylor-Robinson, 1969) from the genital tract in the cases of a large proportion of men suffering from "non-specific" urethritis (NSU), and from an even larger proportion of their female sexual contacts. However, to show that these mycoplasmas are a cause of NSU it is important also to demonstrate their absence from a similar group of men without NSU but alike in every other respect to those with NSU. Important factors to match are age, race, and sexual behaviour. In general, volunteer groups of hospital workers are unsuitable as "controls" as are groups comprised of subjects who are not sexually active, for example schoolchildren and celibates.

In a report by Csonka, Williams, and Corse (1966), part of the study was concerned with men suffering from gonorrhoea. These patients were tested for the presence of mycoplasmas and observed after treatment of gonorrhoea for the development of non-gonococcal urethritis (NGU). Thus the men who developed NGU could be compared with those who did not develop this condition, for both groups had previously been treated alike. The present study was planned as a detailed investigation into similar groups of patients.

Patients and Methods

The patients were men who attended the Whitechapel Clinic of The London Hospital because of gonococcal urethritis. From January, 1967, to January, 1968, all men presenting between 10 a.m. and 4 p.m. on Tuesdays and Fridays, and found to have gonorrhoea, were included in the study, excluding only:

(a) Men for whom follow-up examination would be impossible (mainly seafarers);

(b) Men for whom treatment with penicillin was contraindicated.

Many racial groups were represented, with a preponderance of white natives of the United Kingdom and West Indian Negroes (Table I). In each case the diagnosis of gonorrhoea was made by the finding of Gram-negative intracellular diplococci in a smear of urethral discharge. In most cases this was supported by culture of the gonococcus.

The 120 men in this study were seen throughout by one physician (MJH), who took all specimens for mycoplasmas and examined microscopically all smears of genital material. After the diagnosis of gonorrhoea had been established, urethral material for culture for mycoplasmas was obtained on a swab in the manner described previously (Part II: Dunlop and others, 1969). The patient was then treated with 1·2 million units procaine penicillin by intramuscular injection, and given an appointment to return in either 3 or 4 days.

The patient was instructed not to pass urine for 4 hours preceding the second visit. At this attendance the urethra was "milked" from behind forwards, and, whether or not any discharge was seen, the terminal 4 cm. of the urethral mucosa were scraped gently with a platinum loop. Two preparations were made from the

TABLE I
120 PATIENTS CLASSIFIED BY PLACE OF BIRTH

Place of Birth	Number
United Kingdom	46
Caribbean	43
Pakistan	12
Malta	9
Irish Republic	4
India	2
Western Europe	2
Africa	2
Total	120

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material so obtained: one was mixed with normal saline and examined microscopically for *Trichomonas vaginalis*: the other was stained by Gram's method and examined for pus and bacteria. Urethral material was obtained with a swab and sent for mycoplasma culture, and the urine was then examined using the two-glass test. If all was well, an appointment was made to see the patient 10 days later.

At this next visit (on the 14th day after treatment) the patient attended for tests of the overnight urethral secretion. The procedures of the second visit were repeated, and a rectal examination was performed. Fluid expressed by massage of the prostate gland and seminal vesicles was examined microscopically as a wet film for the presence of pus and trichomonads. The final attendance for this study was on the 28th day after treatment, again for tests of the overnight secretion. The procedures of the second visit were repeated, and the anterior urethra was examined with a urethroscope. If there was no evidence of urethritis at this stage the patient returned to routine surveillance.

The maximum period allowed for the resolution of uncomplicated gonococcal urethritis was arbitrarily taken as 7 days. If urethritis was still present at the first follow-up visit and gonococci were observed in urethral smears stained by Gram's method, the patient was re-treated immediately. Only four men needed further treatment at this stage, and all of these were cured by 2·4 million units procaine penicillin intramuscularly, together with probenecid 2 g. by mouth. If, however, urethritis persisted without gonococci being found, the patient was seen again on the 7th day. If abacterial urethritis was still present at this time NSU was diagnosed and treatment started. From the 7th day onwards NSU was diagnosed if examination of urethral smears revealed more than ten polymorphonuclear leucocytes per high-power (1/12) field. Patients suffering from NSU were treated with tetracycline 250 mg. by mouth four times a day for 7 days.

With the aid of the Social Workers attached to the Whitechapel Clinic, an attempt was made to secure the attendance at the clinic of the recent sexual contacts of these men. If, when the consort attended, she was found to harbour *T. vaginalis*, this fact was recorded on the male patient's notes also. At his next visit urethral material was examined microscopically and by culture for this parasite, and, whether or not it was found, he was treated with metronidazole by mouth, 200 mg. three times a day for 7 days. *In vitro* and *in vivo* studies have shown that this drug has no effect on mycoplasmas when given at this dosage; consequently the suitability of these patients for further studies was not impaired.

Throughout the period of observation all patients were instructed to abstain from sexual intercourse. Any patient admitting further intercourse was classified as a defaulter from the series at that point.

This study was planned in such a way as to avoid any possible bias on the part of clinic and laboratory staff. Results of cultures for mycoplasmas were not known by the clinician concerned until the 4-week follow-up period had been completed. All request forms were marked "Gonorrhoea Study" irrespective of other clinical details, so that the laboratory staff were unaware of which patients developed NSU.

Results

Total Incidence of Mycoplasmas

The incidence of mycoplasmas isolated from the urethra at any attendance of the 120 men studied is shown in Table II. T-strain mycoplasmas were found in a total of eighty men (67 per cent.); *M. hominis* was found in a total of fifty men (42 per cent.); both types were found together in 42 men (35 per cent.).

TABLE II
MYCOPLASMAS IN 120 MEN PRESENTING BECAUSE OF GONORRHOEA

		No.	Per cent.
Total Patients		120	100
Mycoplasmas isolated	T-strain	80	67
	M. hominis	50	42
	Both	42	35

From one patient (who did not develop NSU), a third type of mycoplasma was isolated which fermented Arginine much more slowly than *M. hominis*. This organism is at present being fully identified, and will not be discussed further in this report.

Mycoplasmas and Development of NSU

35 (29 per cent.) of the 120 patients developed NSU during the observation period of 28 days. The rate of isolation of mycoplasmas from this group is compared in Table III with the isolation rate from the remaining 85 men who were not observed to develop NSU. Of the 35 men who developed NSU,

TABLE III
MYCOPLASMAS IN PATIENTS DEVELOPING NSU COMPARED WITH THE REMAINDER

Group		Developing NSU	Remainder
Number of Patients		35	85
Mycoplasmas isolated	T-strain	21	59
	M. hominis	15	35
	Both	11	31

T-strain mycoplasmas were isolated from 21 (60 per cent.), *M. hominis* from fifteen (43 per cent.), and both from eleven (31 per cent.). Of the remaining 85 men who were not observed to develop NSU, T-strain mycoplasmas were isolated from 59 (69 per cent.), *M. hominis* from 35 (41 per cent.), and both from 31 (36 per cent.).

The 85 men who were not observed to develop NSU cannot strictly be used as a comparison group, for they include many defaulters in whom it is impossible to be certain that NSU did not develop. A smaller group of 29 men completed the full observation period of 28 days without developing NSU. The rate of isolation of mycoplasmas in this group is shown in Table IV. T-strain mycoplasmas were isolated from 24 men (82 per cent.), *M. hominis* from twelve (41 per cent.), and both from twelve (41 per cent.). When the isolation rates from this group are compared with those in the group of patients who developed NSU, no real difference is found.

TABLE IV

MYCOPLASMAS IN PATIENTS DEVELOPING NSU AND PATIENTS NOT DEVELOPING NSU

Group		Developing NSU	Known free from NSU
Number of Patients		35	29
Mycoplasmas isolated	T-strain	21	24
	M. hominis	15	12
	Both	11	12

An estimate of the quantity of mycoplasmas present in any specimen can be obtained by the titration methods described in Part I (Taylor-Robinson and others, 1969), and this will indicate the number of mycoplasmas in the urethra of the patient from whom the specimen was taken. If mycoplasmas cause urethritis, it would be likely that the higher the concentration in the urethra the greater would be the incidence of urethritis. Of the 42 patients with high concentrations of T-strain mycoplasmas in the urethra (organisms present in dilutions of over 1 in 1,000), ten (24 per cent.) developed NSU (Table VA). Of the 38 patients with a low concentration (organisms present in dilutions of under 1 in 1,000 only), eleven (29 per cent.) developed NSU. Fourteen (35 per cent.) of the forty men in whom T-strain mycoplasmas were not isolated from the urethra developed NSU. In the same way, of the 26 patients with a high concentration of *M. hominis* in the urethra (organism present in dilutions of over 1 in 100), six (23 per cent.) developed NSU (Table VB). Of the 24 men in whom *M. hominis* was present in low concentrations (in dilutions of under 1 in 100 only), nine (38 per cent.) developed NSU. Of the seventy men from whom *M. hominis* was not isolated from the urethra, twenty (29 per cent.) developed NSU.

TABLE VA

CONCENTRATION OF T-STRAIN MYCOPLASMAS AND DEVELOPMENT OF NSU

Urethral Concentration of T-strain Mycoplasma	None	Low[1]	High[2]
Number of Patients	40	38	42
Number developing NSU	14	11	10

[1]"Low" = present at dilutions of less than 1 in 1000 only.
[2]"High" = present at dilutions of over 1 in 1000.

TABLE VB

CONCENTRATION OF *MYCOPLASMA HOMINIS* AND DEVELOPMENT OF NSU

Urethral Concentration of *M. hominis*	None	Low[1]	High[2]
Number of Patients	70	24	26
Number developing NSU	20	9	6

[1]"Low" = present at dilutions of less than 1 in 100 only.
[2]"High" = present at dilutions of over 1 in 100.

If mycoplasmas were a cause of the urethritis, it would also seem likely that inflammation would develop after an increase in the number of mycoplasmas present in the urethra. Eight cases of NSU developed when the concentrations of T-strain mycoplasmas were rising; eleven when the concentrations were falling; two when the concentrations remained stable; and fourteen in the complete absence of T-strain mycoplasmas (Table VI). Four patients developed NSU when the concentrations of *M. hominis* in the urethra were rising; six when the concentrations were falling; five when the concentrations remained stable; and twenty in the complete absence of *M. hominis*.

TABLE VI

RISE OR FALL OF MYCOPLASMA CONCENTRATIONS AND THE DEVELOPMENT OF NSU

Type of Mycoplasma	T-strain	*M. hominis*
Patients developing NSU after a RISE in the concentration of named mycoplasma	8	4
Patients developing NSU when the concentration of named mycoplasma has not varied	2	5
Patients developing NSU after a FALL in the concentration of named mycoplasma	11	6
Patients developing NSU in the absence of named mycoplasma	14	20

Mycoplasmas and Association with *Trichomonas vaginalis*

Seventy of the female contacts of 56 of the men in this study were examined. The contacts of twenty of the 56 men were found to be infested with *T. vaginalis*; although the parasite was found in only three of these men, they were all treated with metronidazole. The incidence of mycoplasmas in the urethras of these twenty men (*T. vaginalis* contacts) is compared in Table VII with the incidence of mycoplasmas in the urethras of the 36 men whose consorts did not seem to be harbouring this parasite. T-strain mycoplasmas were isolated from the urethra in seventeen (85 per cent.) of the "*T. vaginalis* contacts", *M. hominis* in twelve (60 per cent.), and both in twelve (60 per cent.). In the cases of the 36 men whose contacts were not found to be harbouring *T. vaginalis*, T-strain mycoplasmas were isolated in 21 (58 per cent.), *M. hominis* in thirteen (36 per cent.), and both in eleven (31 per cent.).

TABLE VII

MYCOPLASMAS IN MEN CORRELATED WITH TRICHOMONIASIS IN SEXUAL CONSORTS

Group		*T. vaginalis* in Consort	
		+	—
Number of Patients		20	36
Mycoplasmas isolated	T-strain	17	21
	M. hominis	12	13
	Both	12	11

Mycoplasmas and Circumcision

Of the 120 men in the study, 35 had been circumcised (Table VIII); T-strain mycoplasmas were isolated from the urethra in 22 (63 per cent.), *M. hominis* in eight (23 per cent.), and both in seven (20 per cent.). Of the 85 uncircumcised men, T-strain mycoplasmas were isolated from the urethra in 58 (68 per cent.), *M. hominis* in 42 (49 per cent.), and both in 35 (41 per cent.).

TABLE VIII

MYCOPLASMAS IN CIRCUMCISED AND UNCIRCUMCISED MEN

Group		Circumcised	Uncircumcised
Number of Patients		35	85
Mycoplasmas isolated	T-strain	22	58
	M. hominis	8	42
	Both	7	35

Discussion

The results of two studies similar to the present one have been published. Csonka and others (1966) described the findings in 63 men suffering from gonococcal urethritis. Thirteen (21 per cent.) developed non-gonococcal urethritis (NGU), although the period of observation and criteria used for diagnosis of the latter condition were not specified. T-strain mycoplasmas were isolated from ten (77 per cent.) of these thirteen men who developed NGU; *M. hominis* was not isolated from any of them. T-strain mycoplasmas were isolated from fourteen (28 per cent.) of the fifty patients with gonorrhoea alone; *M. hominis* was isolated from five (10 per cent.). These results, together with isolations in cases of NGU, urinary infections, and from "healthy controls", led these authors to conclude that T-strain mycoplasmas played an important role in the causation of NGU.

A similar conclusion was reached by Holmes, Johnson, Floyd, and Kvale (1967), who studied the crew of a warship at sea following a brief visit to a Far Eastern port. In this way all patients were observed over a 30-day period and the possibility of re-infection by heterosexual means was eliminated. The diagnosis of post-gonococcal urethritis (PGU) was made by examining the centrifuged deposit of the first 10 ml. of voided urine: when 20 or more polymorphonuclear leucocytes were found in any of three high-power fields examined, PGU was diagnosed. These authors reported that if a urethral discharge was present pyuria of this degree was always found. Each of 58 men was treated for gonococcal urethritis with 2·4 million units procaine penicillin by intramuscular injection, together with probenecid 2·5 g. by mouth in divided doses. Of this group no less than 37 (64 per cent.) developed PGU during the observation period. Mycoplasmas were isolated from seventeen of the 58 men, but no attempt was made to identify these organisms further. PGU developed in sixteen (94 per cent.) of of the seventeen men from whom mycoplasmas were isolated, but in only 21 (51 per cent.) of the 41 men from whom they were not isolated.

The results obtained in the present study differ from these previous findings. No correlation was found between the presence of *M. hominis* and the likelihood that the patient would develop NSU. In the case of T-strain mycoplasmas, patients infected with these organisms developed NSU less frequently than those from whom they were not isolated, although this tendency did not approach statistical significance. Neither the presence of high concentrations nor an increasing number of mycoplasmas

in the urethra could be correlated with the development of NSU.

Two factors seemed to influence the likelihood of finding mycoplasmas in the male urethra. The male consorts of women with trichomoniasis are probably also infested with *T. vaginalis*, although this can be demonstrated in a minority of cases only. Although *T. vaginalis* was found in only three of the "*T. vaginalis* contacts", it may be assumed that most, if not all, had been infested. Likewise, the 36 men whose contacts were apparently free from this organism were probably also free from the infestation themselves. A much higher percentage of the men in the "*T. vaginalis* contact" group were infected with mycoplasmas than in the group who were presumed free from *T. vaginalis*; the difference between the isolation rates for both mycoplasmas together just reaches statistical significance ($P = 0{\cdot}05$).

Circumcision was also found to be a relevant factor. Although the isolation rate of T-strain mycoplasmas from circumcised men (63 per cent.) is approximately the same as that from uncircumcised men (68 per cent.), the isolation rate of *M. hominis* from circumcised men (23 per cent.) is less than half that from uncircumcised men (49 per cent.). This difference reaches a considerable degree of statistical significance ($P < 0{\cdot}01$), and suggests that *M. hominis* may inhabit the subpreputial sac as well as the urethra. Further studies have been planned to investigate the possible site of infection by each mycoplasma in the urogenital tract.

Although this study provides evidence that the mycoplasmas do not cause NSU, the possibility remains that particular serotypes might be responsible in some cases. This will be studied further, although it appears an unlikely possibility.

Summary

A series of 120 men suffering from gonorrhoea was studied in an attempt to discover any relationship between the presence of mycoplasmas (both T-strain mycoplasma and *M. hominis*) and the development of post-gonococcal "non-specific urethritis" (NSU). The following conclusions were reached:

(1) There was no evidence to implicate either T-strain mycoplasma or *M. hominis* as the causal agent of NSU.

(2) There was a higher incidence of infection by these mycoplasmas of men whose sexual consorts harboured *T. vaginalis*.

(3) *M. hominis*, unlike T-strain mycoplasma, was commoner in uncircumcised than in circumcised men.

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Les mycoplasmes et l'infection génitale "non spécifique"

III. Urétrite post-gonococcique. Etude prospective

SOMMAIRE

Une série de 120 hommes atteints de gonococcie fut étudiée dans l'idée de découvrir une éventuelle relation entre la présence de mycoplasmes (aussi bien la souche T que *M. hominis*) et l'apparition d'une urétrite non spécifique (UNG) post-gonococcique.

On arriva aux conclusions suivantes:

(1) Il n'y eut rien qui permit d'impliquer soit le mycoplasme souche T soit *M. hominis* comme agent causal des UNG.

(2) L'incidence de l'infection par ces mycoplasmes était plus grande chez les hommes dont les partenaires sexuelles hébergeaient des *T. vaginalis*.

(3) Le *M. hominis*, au contraire du mycoplasme souche T, était plus fréquent chez les hommes non circoncis que chez les circoncis.