Chlamydial infection

Role of Chlamydia Subgroup A in non-gonococcal and post-gonococcal urethritis

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Members of the genus *Chlamydia* Subgroup A are obligate intracellular parasites possessing many of the structural and metabolic characteristics of bacteria rather than of viruses (Moulder, 1964), and are recognized by the compact intracytoplasmic iodine-staining inclusions they form in host cells. They include the causative agents of trachoma and inclusion conjunctivitis (TRIC agents).

In countries where trachoma is not endemic, it is believed that in TRIC ocular disease (inclusion conjunctivitis, TRIC punctate kerato-conjunctivitis, trachoma and ophthalmia neonatorum), the organism is acquired from the genital tract of the individuals or their consorts, or from the birth canal of the mother in cases of ophthalmia neonatorum, and that the reservoir of chlamydial infection is in the genital tract (Schachter, Rose, and Meyer, 1967). However, it is not yet established whether these organisms cause non-specific genital infection, though they have been found in association with non-gonococcal urethritis (NGU) and post-gonococcal urethritis (PGU) in males and with cervicitis in females (Jones, Al-Hussaini, and Dunlop, 1964; Dunlop, Al-Hussaini, Garland, Treharne, Harper, and Jones, 1965; Dunlop, Hare, Darougar, Jones, and Rice, 1969; Philip, Hill, Greaves, Gordon, Quan, Gerloff, and Thomas, 1971). Moreover, a closely related Subgroup A *Chlamydia* is known to be the cause of the sexually-acquired disease lymphogranuloma venereum.

Chlamydia was first isolated from the male urethra by Jones and others (1964) by inoculation of the yolk sac of the fertile hen's egg. By this method, *Chlamydia* was then isolated from two out of nine unselected cases of NGU (Dunlop and others, 1965). Recently a method of isolating *Chlamydia* in irradiated McCoy cells has been developed (Gordon and Quan, 1965); this has proved to be quicker and more sensitive than

the yolk sac method. The presence of *Chlamydia* in the urethra of a proportion of men presenting with NGU and PGU has been confirmed by this method (Dunlop and others, 1969; Dunlop, Hare, Darougar, and Jones, 1971; Philip and others, 1971; Gordon and Quan, 1971).

In the present study, the incidence of *Chlamydia* in the urethra of men presenting with NGU was determined and compared with the incidence of *Chlamydia* in (i) men with gonorrhoea (G), and (ii) men attending venereal disease clinics without urethritis, in an attempt to elucidate the role of *Chlamydia* in NGU. A further objective was to discover whether initial *Chlamydia* isolation from men with G bore any relation to the subsequent development of PGU in these patients.

Patients and Methods

PATIENTS

The patients were Caucasian males presenting with urethritis at the venereal disease clinics of the Bristol Royal Infirmary and the Royal United Hospital, Bath, between June 28, 1971, and March 29, 1972. Urethritis was diagnosed if ten or more pus cells per high power field were seen in a smear taken with a loop from the urethra, and G was diagnosed when Gram-negative intracellular diplococci were seen in the smear. The other cases of urethritis were termed NGU, and since the presence of *Trichomonas vaginalis* in the urethra was not excluded a small proportion of these cases of urethritis may have resulted from this parasite (Weston and Nicol, 1963). When the patient had no visible discharge but a bead of pus was expressed from the urethra, he was described as 'dry'.

A group of men not suffering from urethritis, also attending the clinics during the same period, were used as control patients. They either had no local complaints, or had noted spots on the genitalia or body, or had symptoms commonly ascribed to the venereal diseases. There may or may not have been exposure to infection in the preceding weeks or years. A very few patients had scabies, pediculosis pubis, or warts, but those with herpes genitalis were excluded. Early morning examination was not carried out in all the control cases. Some may therefore have had a low grade urethritis (Rodin, 1971).

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In order to compare the sexual behaviour of the men in the various groups, each patient was assigned to a promiscuity category on the basis of his marital, premarital, or extramarital relationships. These categories were as follows:

- (A) No sex contacts within the last 3 months;
- (B) Wife only;
- (C) 'Regular' consort only;
- (D) Wife and casual consort(s);
- (E) 'Regular' consort and casual consort(s);
- (F) Only casual consort(s).

The NGU group included one known homosexual and one patient with epididymo-orchitis, but no patient with Reiter's syndrome, and the G group included three known homosexuals. No antibiotics had been given to the patients in the three groups in the 5 weeks before testing, with the exception of two patients, one who had had penicillin and one who had had tetracycline. G was usually treated with procaine penicillin, 1.2 m.u., and probenecid, but eight patients had gentamicin and four patients, suspected of being allergic to penicillin, had kanamycin.

Patients with G were followed up for several weeks whenever possible. If urethritis was present 7 days or more after the initial examination, with no relapse of the gonococcal infection, a diagnosis of PGU was made. This diagnosis was made without knowledge of which patients were *Chlamydia*-positive.

All initial examinations were carried out by one physician (A.L.H.) but this was not always possible at follow-up sessions. The diagnosis of PGU was made by examination of the urethral smear; early morning examination was not carried out routinely. *Chlamydia* isolation was not attempted at follow-up.

COLLECTION OF SPECIMENS

Specimens were taken by means of a sterile swab inserted half to one inch inside the urethra, before the patient passed urine. The swabs were prepared from plain, non-absorbent cotton-wool and were supplied by the Bristol Public Health Laboratory. Each patient was swabbed twice, for *Chlamydia* isolation and for culture of gonococci. Plain swabs were also used for culture of gonococci, in association with thallium acetate transport medium. Since cultivation of gonococci was not attempted in the control subjects, they were swabbed only once and the swab was sometimes moistened in the transport medium to minimize the discomfort experienced by the patient.

The swab for *Chlamydia* isolation was placed in 2 ml. transport medium consisting of Earle's saline with 10 per cent. foetal calf serum, 10 per cent. sorbitol, and 0.3 per cent. sodium bicarbonate. Specimens were kept at 4°C. until being inoculated into cell cultures, or snap frozen at -70°C. until inoculated. No specimens were kept for more than 48 hrs at 4°C. Preliminary tests showed that chlamydial isolates retained their viability in this medium for 48 hrs at 4°C. and when frozen at -70°C. Unlike the sucrose-phosphate solution previously described for *Chlamydia* isolations (Gordon, Harper, Quan, Trehan,

Dwyer, and Garland, 1969), it was not toxic to the culture cells, so that replacement of the medium after inoculation was not necessary.

CELL CULTURE

The method used was essentially that described originally by Gordon, Dressler, and Quan (1967), slightly modified and simplified by Darougar, Kinnison, and Jones (1971). McCoy cells, irradiated with about 5,000 rads 5 to 7 days previously, were seeded onto 13 mm. diameter coverslips in flat-bottomed Conway tubes. Specimens were inoculated onto these monolayers 2 to 3 days later, and the cultures were spun at 2,500 g. for 1 hr before incubation at 35°C. 0.25 ml. of each specimen was inoculated into each of two tubes. Clinical specimens were not sonicated. After 68 hrs in culture, one coverslip was fixed in methanol, stained in Giemsa, and scanned at $\times 200$ magnification for the typical intracytoplasmic inclusions with the use of a wide-field darkground condenser. All initial pass coverslips were read blind. If a good preparation was obtained, and no inclusions were found, the second culture was discarded and the specimen was recorded as negative. If the first coverslip did not provide a good preparation, or if there was any doubt about the presence of an inclusion, the second culture was passed into two further cell cultures. When preparations contained one or more definite inclusions the specimen was recorded as positive. Each isolate was passed at least once before storage, and one coverslip was always stained with iodine to confirm that the inclusions were of the *Chlamydia* Subgroup A type. Since this method of isolation does not distinguish between TRIC agents and other Subgroup A *Chlamydia*, isolates are referred to as *Chlamydia* rather than as TRIC agents.

Two specimens proved toxic to the cells and, as it was impossible to obtain preparations from these, they were excluded from the series. Six preparations were slightly contaminated with yeasts, but not to the extent that they interfered with the scanning of the cells for intracytoplasmic inclusions. They were therefore included. Contamination was not a problem, possibly because all these specimens were from males.

NUMBER OF INCLUSION-FORMING UNITS

The number of inclusions found on initial passage was counted for each isolate and, since the same volume of inoculum was used for each specimen and the organisms complete only one growth cycle by this method of culture, inclusion counts per positive inoculum were roughly proportional to the number of inclusion-forming units per specimen.

Results

During the period of study, 103 men with NGU, 99 men with G, and 92 control men without urethritis were examined. G was confirmed by culture of gonococci in 85 out of the 99 cases (86 per cent.).

Chlamydia was isolated from forty NGU cases (39 per cent.), from 32 G cases (32 per cent.), and from five of the controls (5 per cent.) (Table I). There is no statistically significant difference between the isolation rates in the NGU and G groups, whereas the difference between the isolation rate in patients with urethritis and the rate in the controls is highly significant ($P < 0.001$).

TABLE I *Chlamydia isolations in NGU, G, and control subjects*

Group studied	NGU	G	Controls
Total patients	103	99 ^b	92 ^c
<i>Chlamydia</i> positive	40 (39 per cent.) ^a	32 (32 per cent.)	5 (5 per cent.)

^aIncludes 1 homosexual and 1 patient with epididymo-orchitis

^bIncludes 3 homosexuals all *Chlamydia* negative

^cIncludes 1 patient treated with tetracycline 5 days before examination, who was *Chlamydia* negative

AGE

The age distribution (Table II) was similar in the NGU and G groups, though the mean age of the G group (26.4 yrs) was slightly lower than that of the NGU group (28.7 yrs). In the control group the mean age was 29.9 yrs, but there was a higher proportion of men under 20 and over 30 than in the urethritis groups. Age appears not to affect the *Chlamydia* isolation rate in NGU, G, or in the urethritis group as a whole.

TABLE II *Age distribution in NGU, G, and control subjects*

Group studied		Age group (yrs)				No record
		<20	20-24	25-29	>29	
NGU	Total patients	8	36	24	35	0
	<i>Chlamydia</i> positive	2	16	11	11	0
G	Total patients	12	45	19	22	1
	<i>Chlamydia</i> positive	3	17	6	6	0
Controls	Total patients	21	19	14	38	0
	<i>Chlamydia</i> positive	2	2	0	1	0

TABLE III *Amount of urethral discharge at time of examination*

Group studied		Amount of discharge				
		Dry ^a	Slight	Moderate	Profuse	No record
NGU	Total patients	10	66	17	9	1
	<i>Chlamydia</i> positive	5	29 (44 per cent.)	4 (24 per cent.)	2 (22 per cent.)	0
G	Total patients	1	25	11	61	1
	<i>Chlamydia</i> positive	1	14 (56 per cent.)	4 (36 per cent.)	12 (20 per cent.)	1

^aPatients with no visible discharge but a bead of pus could be expressed

SEVERITY AND DURATION OF URETHRITIS

In the NGU series there was no statistically significant relation between the amount of urethral discharge present at the time of examination (which was influenced by the interval since the patient last passed urine, as well as by the severity of the urethritis) and the *Chlamydia* isolation rate. In the G group, the isolation rate was significantly lower in men with a profuse urethral discharge than in men with a slight or moderate discharge (Table III). *Chlamydia* was isolated from significantly greater numbers of men with NGU who had had a urethral discharge for more than 7 days when they presented at the clinic than from men who presented within 7 days of onset of the discharge ($P < 0.02$) (Table IV, overleaf).

A similar trend was observed with G, except that a much larger proportion of patients presented within a few days of onset of urethral discharge owing to the greater severity of the urethritis. There was no difference in isolation rate between men with G who had had a urethral discharge for 3 days or less and those who had had symptoms for between 4 to 7 days, but a longer-standing untreated discharge appeared to be associated with a higher isolation rate ($P < 0.01$) (Table IV).

PAST HISTORY

The *Chlamydia* isolation rate was compared in those patients who admitted to a past history of urethral discharge of any cause (since it was not always possible to distinguish accurately between a past

TABLE IV *Duration of urethral discharge before initial visit*

Group studied	Duration of discharge (days)		Not complaining of urethral discharge	Dysuria, irritation, or no record
	0-7	>7		
NGU Total patients	70	23	5	5
<i>Chlamydia</i> positive	22 (31 per cent.)	14 (61 per cent.)	2	2
G Total patients	84	12	3	0
<i>Chlamydia</i> positive	22 (26 per cent.)	8 (67 per cent.)	2	0

history of G and NGU) and in those with no past history (Table V). The overall incidence of a past history was significantly greater in the men with NGU (47 of 103—46 per cent.) than in those with G (24 of 99—24 per cent.) ($P < 0.01$). This difference was not related to *Chlamydia* isolations. There was no significant difference either between the proportion of *Chlamydia*-positive NGU patients with and without a past history, or between the proportion of *Chlamydia*-positive G patients with and without a past history. In the control group, the incidence of patients with a past history was low (12 of 92—13 per cent.) and none of these was *Chlamydia* positive during the present investigation.

TABLE V *Past history of urethritis*

Group studied		Past history of urethritis	No past history
NGU	Total patients	47	56
	<i>Chlamydia</i> positive	19 (40 per cent.)	21 (38 per cent.)
G	Total patients	24	75
	<i>Chlamydia</i> positive	11 (46 per cent.)	21 (28 per cent.)
Controls	Total patients	12	80
	<i>Chlamydia</i> positive	0	5

PROMISCUITY

The grouping of the patients into promiscuity categories, based on their marital, premarital, and

extramarital histories, is shown in Table VI. If these categories are used as criteria, the NGU and G groups appear to be reasonably well matched, whereas the control group was less promiscuous. If, in order to get better matched controls, only patients from categories D, E, and F are compared (Table VII), there is still a highly significant difference in the *Chlamydia* isolation rate between men with and without urethritis ($P < 0.001$).

TABLE VII *Chlamydia isolations: categories D, E, and F only*

Group studied	NGU	G	Controls
Total patients	69	75	50
<i>Chlamydia</i> positive	25 (36 per cent.)	23 (31 per cent.)	3 (6 per cent.)

POST-GONOCOCCAL URETHRITIS

Follow-up studies on patients with G are shown in Table VIII. The incidence of PGU in the total group was 21 of 99 (21 per cent.) but, if those who defaulted or were treated within 1 week are excluded, the incidence was 21 of 67 (31 per cent.). The *Chlamydia* isolation rate in those who subsequently developed PGU is significantly higher than the rate in those with no PGU after 1 week or more ($P < 0.001$).

Out of the 46 patients who were clear at 1 week, fifteen were followed for 1 month or more. Two of

TABLE VI *Recent sex contacts*

Group studied		Type of sex contact					
		A	B	C	D	E	F
		Nil	Wife only	Regular consort only	Wife and casual(s)	Regular consort and casual(s)	Casual(s)
NGU	Total patients	3	5	26	29	12	28
	<i>Chlamydia</i> positive	1	2	12	10	4	11
G	Total patients	0	3	21	21	8	46
	<i>Chlamydia</i> positive	0	0	9	10	3	10
Controls	Total patients	25	5	12	29	3	18
	<i>Chlamydia</i> positive	1	0	1	1	0	2

TABLE VIII *G* patients: follow-up and incidence of PGU

Follow-up findings	Immediate defaulter	Followed less than or treated within 1 wk	PGU at 1 wk or more	Clear at 1 wk or more
Total patients	10	19	21	46 ^b
<i>Chlamydia</i> positive	2	3	17 (81 per cent.) ^a	10 (22 per cent.)

Excluded: 1 G re-infection (confirmed by culture), 2 G relapse (1 confirmed by culture)

^aincludes 3 patients treated with gentamicin, and 1 patient treated with kanamycin

^bincludes 2 patients treated with kanamycin (both *Chlamydia* negative), and 4 patients treated with gentamicin (all *Chlamydia* negative)

these, both *Chlamydia*-positive, developed a non-gonococcal 're-infection' 15 and 16 weeks after the initial treatment. Of the remaining 31 patients, 24 had routine smears performed 1 week or more after treatment, and three of these patients, one of whom was *Chlamydia*-positive, were later treated because of the presence of Gram-negative diplococci or scanty pus cells.

CONJUNCTIVITIS

Two patients were suffering from obvious conjunctivitis at their initial visit. *Chlamydia* was isolated from the urethra of both and the conjunctiva of one of these patients. One *Chlamydia*-positive G patient had conjunctivitis as well as PGU 11 days after his initial visit, but *Chlamydia* isolation was not attempted at that stage.

INCLUSION COUNTS

The distribution of the number of inclusions per initial isolation is shown in Table IX. The range of infectivity in the clinical specimens was very great, varying from 1 to about 2,000 inclusions per coverslip (equivalent to between 8 and 16,000 inclusion-forming units per specimen). There was no difference between the count distribution in isolates from NGU and G and no relationship was found between the inclusion count and the time the urethral discharge had been present. In the NGU and G cases, no relationship was found between the inclusion counts and the amount of urethral discharge.

TABLE IX *Distribution of inclusion counts*

Inclusions per coverslip	<50	50-500	>500
NGU	14	17	9
G	19	7	6
Controls	5	0	0

Discussion

A *Chlamydia* isolation rate of 39 per cent. in NGU agrees well with the rates found by others using the

same cell culture method. Gordon and Quan (1971) in the U.S.A. isolated *Chlamydia* from nineteen of 84 NGU cases (32 per cent.). In Great Britain, Dunlop and others (1971) reported eighteen isolations from 41 NGU cases (44 per cent.), and Oriel, Reeve, Powis, Miller, and Nicol (1972) reported 35 out of 105 (34 per cent.). Ford and McCandlish (1971), however, obtained a much lower rate in Canada (8 of 151—5 per cent.).

Until recently, urethral specimens for *Chlamydia* isolation have usually been collected with a Dunlop-Jones curette, and scraping has also been used to obtain conjunctival specimens (Dunlop, Jones, and Al-Hussaini, 1964). However, conjunctival swabbing is now believed to be at least as efficient as scraping (Darougar and Jones, 1971). From the results reported here, urethral swabbing seems to be as effective as scraping. Furthermore, it is quicker, involves the patient in less discomfort, and allows isolation to be attempted in conditions in which scraping may not be possible (in G and in men without urethritis). Slight visible urethral discharge or none at the time of examination did not reduce the *Chlamydia* isolation rate (Table III) and it therefore seemed justifiable to compare isolation rates in men with urethritis with those in men with no urethritis.

The McCoy cell method is at present the most sensitive method available for detecting chlamydial infection in the genital tract, but the relation of the *Chlamydia* isolation rate to the actual incidence of this infection is unknown. It is probably reasonably efficient in detecting active infection, where large numbers of infectious extracellular particles are produced. It may not, however, detect a latent infection of the urethra if the agent persists predominantly in its non-infectious intracellular form with little or no production of infectious particles.

Latency can occur in birds and mammals, including man, with other members of the *Chlamydia* genus (Storz, 1971). Latent ocular infections with TRIC agents in the *Chlamydia* Subgroup A have been shown in man by direct immunofluorescent staining of conjunctival cells. Positive fluorescence was

obtained without clinical signs of eye infection and the agent could not be isolated in eggs. It was suggested that such subclinical TRIC eye infections were common (Hanna, Dawson, Briones, Thygeson, and Jawetz, 1968). A similar state of quiescent chlamydial infection may well exist in the male urethra.

In the present study, NGU and G appeared to occur in fairly similar populations, though the G group was overall slightly younger and slightly more promiscuous. Age was found not to influence *Chlamydia* isolation rates (Table II) and, when only those men admitting to recent casual sex contacts are compared (Table VII), the *Chlamydia* isolation rates are still very similar.

This finding is not evidence that *Chlamydia* is the primary cause of NGU. NGU is thought to be a sexually-transmitted infection; some mixed G and NGU infections might therefore be expected to occur. If there were no mixed infections, no isolation of the primary causative organism of NGU should be obtained from G cases. If, however, some of the G cases show mixed infections, some isolations of the primary causative agent would be found but, since it is extremely unlikely that all the G cases are mixed infections, the isolation rate should be higher in the NGU group of patients than in the G group. The observation (Table I) that the *Chlamydia* isolation rates in the two groups are very similar argues therefore that *Chlamydia* is seldom the primary cause of cases of NGU but is rather sexually acquired and usually non-pathogenic in the genital tract of these populations.

The serological work of Philip and others (1971) also provides no evidence that *Chlamydia* is a primary cause of NGU. Using the complement-fixation test and the radioisotope precipitation test with a group antigen, and a serum neutralization test against TRIC and LGV agents, they found a similar prevalence and levels of antibody in NGU, G, and PGU. Moreover, the occurrence and titres of antibodies in *Chlamydia*-positive NGU did not differ from those in *Chlamydia*-negative NGU. They were able to demonstrate only one rise in antibody titre to the group antigen, in a patient with NGU, from whom *Chlamydia* was not isolated.

There is, however, in the present work, the striking association between *Chlamydia* and urethritis when compared with the isolation rate found in men without urethritis, matched as far as was possible on the basis of their sex contacts (Table VII); also the evidence presented which indicates that chlamydial infections in G predisposed these patients to the development of PGU (Table VIII). Moreover, *Chlamydia* was isolated from a greater number of

men with a long-standing untreated urethral discharge than from NGU and G cases of recent onset (Table IV). A urethral discharge persisting after an initial untreated attack of NGU may represent a situation similar to that in which PGU develops after G.

Both NGU and PGU respond to treatment with tetracyclines, to which *Chlamydia* is sensitive *in vitro*, and Holmes, Johnson, Floyd, and Kvale (1967) found that patients with G, who were treated initially with tetracyclines rather than with penicillin, were far less prone to PGU. The fact that males presenting with active chlamydial eye infection are often found to be suffering from *Chlamydia*-positive urethritis is further evidence associating *Chlamydia* with urethritis (Dunlop, Freedman, Garland, Harper, Jones, Race, du Toit, and Treharne, 1967).

These conflicting observations can be reconciled if one postulates that NGU is primarily due to some other agent or trauma and that the primary cause of the urethritis, whether G or NGU, 'stirs up' a latent chlamydial infection in the urethra. During this activated phase of infection, *Chlamydia* would be isolated by the McCoy cell method and the *Chlamydia* reactivation itself might cause a urethral discharge. Then *Chlamydia*-positive G patients would be more likely to develop PGU than *Chlamydia*-negative G patients, and NGU patients presenting with a long-standing untreated urethral discharge would have a higher proportion of *Chlamydia* isolations than those presenting soon after the onset of the urethritis. *Chlamydia*-negative NGU might respond spontaneously without treatment, but the urethral discharge might become chronic if an underlying *Chlamydia* infection was reactivated by the initial urethritis. If this theory is true, it is still possible that a small proportion of the *Chlamydia*-positive NGU cases might be primary infections actually causing the initial urethritis.

The methods available for distinguishing between a primary chlamydial infection and a reactivation of an already existing infection in the urethra have been unsatisfactory up to the present. The wide range in number of inclusion-forming units found in the positive specimens in this study is in agreement with the range found by Gordon and Quan (1971), but we found no correlation between the number of particles and the severity or duration of the urethritis in either *Chlamydia*-positive G or NGU patients. Serological studies on individual patients using a group antigen are not satisfactory, both because antibodies detected in this way are not type specific and because ocular and genital TRIC infections are not usually systemic infections and do not give rise to reliable or high titres of antibodies to the group

antigen. The indirect immunofluorescent method recently described for detecting serum antibodies to TRIC infections is more type specific and more sensitive than methods for detecting group-reactive antibodies (Hanna, Jawetz, Nabli, Hoshiwara, Ostler, and Dawson, 1972). Paired sera from NGU and G patients tested by this method may help to elucidate which chlamydial infections in the urethra are primary infections.

Detailed studies of the consorts of patients in the present series were not attempted. *Chlamydia* has been isolated from the cervixes of mothers whose babies have TRIC ophthalmia neonatorum, and from NGU consorts. These *Chlamydia*-positive females are often found to be suffering from cervicitis (Dunlop and others, 1967). The incidence of *Chlamydia* in the female genital tract of a group of unselected females attending venereal disease clinics, and its relation to cervicitis in the female and urethritis in the male, has not been investigated by the McCoy cell method of culture. This approach to the problem should be helpful. If *Chlamydia* is reactivated by other infections, then a higher isolation rate might be expected in those females in whom there is a concomitant infection with organisms such as the gonococcus, *Trichomonas vaginalis*, and perhaps mycoplasmas.

The microimmunofluorescent test now allows classification of the majority of *Chlamydia* Subgroup A isolates into serotypes (Wang and Grayston, 1970; Treharne, Davey, Gray, and Jones, 1972). Longitudinal studies of *Chlamydia*-positive patients combined with the typing of isolates obtained from the same patient at different times should give information on the natural history of genital chlamydial infections in both the urethra and cervix, correlated with the clinical picture and any treatment that may be given.

The low incidence of a past history of urethritis in control patients (Table V) is probably related to the lower promiscuity of this group as a whole, compared with the G and NGU groups (Table VI). The high incidence of a past history in NGU patients found in this survey is in agreement with the finding of Weston (1965) that patients with NGU are very liable to recurrent attacks. This might be because NGU patients are more susceptible to urethritis than G patients, although they are no more exposed to risk of infection. This high incidence of a past history appears to be unrelated to the *Chlamydia* isolations in these patients.

The finding of one patient with *Chlamydia*-positive conjunctivitis out of forty cases of *Chlamydia*-positive NGU supports the view that the *Chlamydia* reservoir may be situated in the genital tract and not

the eye (Schachter and others, 1967) and indicates that overt ocular infection occurs relatively infrequently in men with urethral chlamydial infections.

Although this study has provided no evidence to suggest that *Chlamydia* is the primary cause of NGU, a definite association between *Chlamydia* and urethritis has been demonstrated, whether the urethritis is initially gonococcal in origin, or due to some undetermined cause. Since *Chlamydia* appears to predispose G patients to the development of PGU and is more frequently found in long-standing untreated cases of NGU than in cases of recent onset, it may be causing prolongation of a urethral discharge initiated by some other agent. This could occur if the initial urethritis reactivated a latent chlamydial infection in the urethra. The method of isolation used does not distinguish between primary infections and reactivations, so it is not known how many of the *Chlamydia* isolations were made from primary infections, though some of the evidence presented suggests the majority were made from reactivations of an already existing chlamydial infection.

Summary

Irradiated McCoy cell cultures were used to investigate the incidence of *Chlamydia* in the urethras of men with non-gonococcal urethritis (NGU), of men with gonorrhoea (G), and of men without urethritis, attending venereal disease clinics. *Chlamydia* was isolated from forty of 103 NGU cases (39 per cent.), from 32 of 99 G cases (32 per cent.), and from five of 92 control cases (5 per cent.). Isolation rates were higher in men with NGU who had a long-standing untreated urethral discharge than in those who appeared for treatment early. Follow-up studies of men with G showed that those who were initially *Chlamydia*-positive were more prone to develop post-gonococcal urethritis than those who were *Chlamydia*-negative. It is suggested that *Chlamydia* is not often the primary cause of NGU, but that urethritis of any origin may reactivate a quiescent chlamydial infection in the urethra and that this reactivated infection may itself give rise to a urethral discharge. Further studies that might test this hypothesis are discussed.

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Infection chlamydiale

Le rôle du *Chlamydia*, sous-groupe A, dans l'urétrite non gonococcique et post-gonococcique

SOMMAIRE

On utilisa les cultures en cellules McCoy irradiées pour rechercher l'incidence de *Chlamydia* dans les urèthres d'hommes atteints d'urétrite non gonococcique (UNG), d'hommes atteints de gonococcie (G) et chez des hommes sans urétrite, consultants dans des dispensaires anti-vénériens. Le *Chlamydia* fut isolé chez 40 des 103 cas d'UNG (39 pour cent), chez 32 des 99 cas de G (32 pour cent) et chez 5 des 92 cas témoins (5 pour cent). Les taux d'isolement furent plus élevés chez les hommes atteints d'UNG qui avaient depuis longtemps un écoulement urétral non traité que chez ceux qui se présentaient tôt au traitement. Les études de surveillance des hommes atteints de G montrèrent que ceux qui étaient initialement *Chlamydia*-positifs étaient plus prédisposés à une urétrite non gonococcique que ceux du groupe *Chlamydia*-négatif. On considère que le *Chlamydia* n'est pas souvent la cause primaire des UNG mais qu'une urétrite de n'importe quelle origine peut réactiver une infection chlamydiale quiescente dans l'urètre et que cette infection réactivée peut elle-même donner un écoulement urétral. On discute des études ultérieures qui pourraient juger de cette hypothèse.