URETHRAL INFECTION OF CHIMPANZEES BY *UREAPLASMA UREALYTICUM*

D. TAYLOR-ROBINSON*, R. H. PURCELL†, W. T. LONDON‡ AND D. L. SLY§

* *Clinical Research Centre, Watford Road, Harrow, Middlesex,*

† *Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland,*

‡ *National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Maryland, and*

§ *Meloy Laboratories Incorporated, Rockville, Maryland, USA*

ORGANISMS of the genus *Ureaplasma urealyticum* were previously known as T-strain or T mycoplasmas (Shepard *et al.*, 1974) and are referred to trivially as ureaplasmas. In non-specific urethritis (NSU) in man, the role of ureaplasmas has been contentious for many years. Many comparative studies of men with and without the disease have failed to implicate these micro-organisms conclusively as a cause (Taylor-Robinson, 1976). However, the results of a placebo-controlled trial of minocycline in the treatment of NSU (Prentice, Taylor-Robinson and Csonka, 1976) suggested strongly not only that chlamydiae had an important role but also, albeit less convincingly, that ureaplasmas were implicated. This idea is supported by the results of a study in which certain antibiotics differentially affected chlamydiae and ureaplasmas (Bowie *et al.*, 1976) and by observations on a patient infected by a tetracycline-resistant ureaplasma (Ford and Smith, 1974). In addition, the development of urethritis in two subjects after self-inoculation of ureaplasmas (Taylor-Robinson, Csonka and Prentice, 1977) is a further clear indication of the ability of these organisms to cause disease. Whether all ureaplasmas are pathogenic in this way is unknown and this question is unlikely to be explored by further human urethral inoculation, as the possibility of undertaking human volunteer experimentation is very limited. In view of this, we inoculated chimpanzees intra-urethrally with human genital-ureaplasma strains known to produce disease in man.

MATERIALS AND METHODS

Ureaplasmas. Two ureaplasma strains (38 α and 43 α) that had been used previously for human intra-urethral inoculation (Taylor-Robinson *et al.*, 1977) were used. Each belonged to serotype V and had been cloned three times on artificial media. Strain 38 α was used at subculture 14 and strain 43 α at subculture 11 for inoculation. Each ureaplasma pool in complete medium without thallium acetate was diluted 10-fold in PPLO broth before urethral inoculation.

Chimpanzees. Four adult male chimpanzees (nos. 1-4) weighing between 25 and 50 kg were caged individually. All had been held in captivity for several years. The care and feeding of the animals was as described previously (London *et al.*, 1972).

Media and culture techniques. Media for growth and isolation of ureaplasmas and for the isolation of mycoplasmas were as described previously (Manchee and Taylor-Robinson, 1968; Taylor-Robinson *et al.*, 1971). A urethral swab expressed in 2.7 ml of liquid medium was regarded as a 10-fold dilution. This and urethral washings were further diluted in 10-fold steps to assess the number of ureaplasma organisms present in the original sample. The highest dilution that produced a colour change after incubation at 37°C was considered to contain one colour-changing unit (CCU) (Taylor-Robinson and Purcell, 1966). Washings and swabs were also spread over blood agar and the plates were incubated aerobically at 37°C for at least 48 h.

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Inoculation and sampling procedures. The chimpanzees were anaesthetised with ketamine hydrochloride. Ureaplasma suspension or medium without organisms was introduced into each urethra in a 0.75-ml amount by means of an Eppendorf pipette. Urethral washings were obtained by introducing 0.75 ml of PPLO broth without additives in a similar manner and "milking" back the fluid. Urethral swabs, both before and after inoculation of ureaplasmas, were obtained by passing nasopharyngeal swabs 3-5 cm into the urethra. The swabs were rolled on clean microscope slides for cytological examination as described below, and were then expressed into 2.7 ml of ureaplasma medium. Preputial swabs before inoculation and throat swabs, taken before and after urethral inoculation, were also expressed into 2.7 ml of medium.

Cytological examination. A volume of approximately 0.5 ml of urethral washings was centrifuged at 8000 *g* for 5 min. and the deposit smeared on a microscope slide. The smears and those obtained from the urethral swabs were fixed with heat or absolute methanol and stained with Giemsa. Each smear was given a code number so that subjective bias could be avoided in the subsequent examination for epithelial and inflammatory cells.

RESULTS

Observations before urethral inoculation

Ureaplasmas were not isolated from the throat, prepuce or urethra of the four chimpanzees before inoculation. However, *Mycoplasma salivarium* was isolated from the throats of chimpanzees 1 and 4. Polymorphonuclear (PMN) leucocytes (two or less per high-power field) were observed in urethral smears from chimpanzees 1 and 3 but not in those from the other animals.

Observations after ureaplasma inoculation

Chimpanzees 1 and 2 were inoculated intra-urethrally with ureaplasma strain 43 α , chimpanzee 3 received ureaplasma strain 38 α and chimpanzee 4 was a control inoculated with ureaplasma medium alone.

Evidence for urethral infection. Three days after inoculation the number of ureaplasma organisms (expressed as CCU) in urethral swabs was at least 1000-fold (chimpanzees 1 and 2) or 10-fold (chimpanzee 3) greater than in comparable swabs taken immediately after inoculation (table I). In chimpanzees 1-3 the numbers of organisms detected on the 3rd day were maintained for up to 14 days after inoculation. A 7-day course of neo-terramycin therapy was then instituted and ureaplasmas could no longer be isolated from the urethra 7 days after termination of the therapy (table I).

Transmission to other sites. Despite the presence of large numbers of ureaplasmas in the

TABLE I
Infection of the urethra of chimpanzees with Ureaplasma urealyticum after experimental inoculation

Inoculum	Chimpanzee no.	Numbers of ureaplasmas (CCU) in urethral swabs on the stated days after inoculation					
		0*	3	6	10	14	28†
Strain 43 α	1	10 ²	10 ⁵ or >	10 ⁶	10 ⁵	10 ⁵	0
Strain 43 α	2	< 10 ²	10 ⁵ or >	10 ⁵	10 ⁴	10 ⁶	0
Strain 38 α	3	10 ³	10 ⁴	10 ⁴	10 ³	10 ⁴	0
Medium	4	0	0	0	0	0	...

* Immediately after urethral inoculation.

† Seven days after the conclusion of a 7-day course of neo-terramycin.

TABLE II

Cells in smears from the urethras of chimpanzees inoculated with Ureaplasma urealyticum

Chimpanzee no.	Type of cell	Number of cells per high-power microscope field on the stated days						
		before inoculation		after inoculation				
		-11	-3	0	3	6	10	28
1	Epithelial	20	10	10	10	15	12	7
	PMN*	2	<1	<1	1	<1	1	<1
2	Epithelial	10	5	10	7	15	10	10
	PMN	0	0	0	0	0	0	0
3	Epithelial	NT	7	5	5	10	10	10
	PMN	NT	<1	0	0	0	0	0
4†	Epithelial	NT	5	5	7	10	5	10
	PMN	NT	0	0	0	0	0	0

* PMN = Polymorphonuclear leucocyte.

† Uninoculated control.

NT = Not tested.

urogenital tracts of three chimpanzees, the organisms were not isolated from the throats of these animals during the 14-day period after intra-urethral inoculation. Furthermore, ureaplasmas could not be isolated from the urethra or throat of chimpanzee 4 (a control animal) during the same period.

Efficacy of ureaplasma isolation procedure. In all instances in which a urethral swab was taken immediately before a urethral wash, at least 100-fold more ureaplasma organisms were isolated from the swab than from the wash. However, when a second swab was taken immediately after the first, it contained either as many as did the first, or no less than one-tenth.

Cytological assessment. Urethral washings were unsatisfactory as very few cells were recovered. However, as shown in table II, many epithelial cells were present in smears prepared by rolling cotton-wool swabs on glass slides. There was no evidence that these cells increased in number after infection. PMN leucocytes were detected in smears from one animal (no. 1) both before and after inoculation but there was no increase in the number of these cells after infection and a PMN response was not detected in the other chimpanzees.

DISCUSSION

Each of two human-ureaplasma strains infected the urethra of chimpanzees. Strain 43 α clearly multiplied and persisted for at least 14 days, and strain 38 α persisted for a similar period. Washing the urethra was a less satisfactory procedure than swabbing for the recovery of organisms. There was no evidence that the organisms produced a urethritis in the chimpanzees, although the same strains are known to produce urethritis in man (Taylor-Robinson *et al.*, 1977). There are three possible explanations why this should be so. (1) As used for the infection of chimpanzees, each strain had undergone several additional subcultures *in vitro*; thus the organisms may have become more attenuated, or non-pathogenic. (2) There is some evidence for host specificity among ureaplasmas. For example, certain bovine-ureaplasma strains will not infect the mouth or genital tract of marmosets (P. M. Furr, C. M. Hetherington and D. Taylor-Robinson, unpublished observation). In the same way human ureaplasma strains may not be able to produce disease in chimpanzees, despite their ability to infect the urethra. (3) Removal of PMN

leucocytes by urination may have led to a failure to detect an inflammatory response. Urine was not examined and it may be difficult over a prolonged period to collect urine that is uncontaminated by faecal and other matter.

Apart from man, the chimpanzee seems to be the only animal that is susceptible to *Neisseria gonorrhoeae* (Lucas *et al.*, 1971; Brown, Lucas and Kuhn, 1972) and the question arises as to whether this species might provide a useful model for studying NSU. If it is possible to produce urethritis in the chimpanzee with unpassed material from the urethra of men suffering from NSU, the way is open for studying not only chlamydiae and ureaplasmas but other organisms that may have an aetiological role in NSU. The present study has shown that swabbing rather than washing the urethra enables the cell response and the presence of organisms to be easily monitored. It also provides a base line for further studies on NSU with materials containing ureaplasmas and other micro-organisms that have undergone few, if any, subcultures on artificial media.

SUMMARY

Two strains of *Ureaplasma urealyticum* serotype V that had produced urethritis in human volunteers were, after a number of subcultures in artificial media, introduced intra-urethrally into three chimpanzees. One strain given to two chimpanzees rapidly multiplied 1000-fold whereas there was less evidence that organisms of another strain multiplied in a third animal. Over a 14-day period the ureaplasmas persisted in all animals, did not spread to the throat and did not produce an inflammatory response. After this time they were eliminated by tetracycline therapy.

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