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The persistence of mycoplasmas in the urogenital tract of men in the Antarctic

By M. J. HOLMES, PATRICIA M. FURR and D. TAYLOR-ROBINSON

M.R.C. Clinical Research Centre, Division of Communicable Diseases, Watford Road, Harrow, Middlesex, HA1 3UJ

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SUMMARY

A series of meatal swabs, taken from 17 men over a period of 17 months during their tour at an Antarctic base was examined for mycoplasmas. The number of organisms isolated never exceeded 10^4 and not every specimen from each man yielded mycoplasmas. Nevertheless, *Mycoplasma hominis* was isolated from 71 % and T-mycoplasmas from 59 % of the men at some time during their stay. *M. hominis* persisted in the presence of serum IHA antibody titres of 1/64. Three subjects yielded only *M. hominis* and one only T-mycoplasmas.

Six men had already spent a year at the base when the study began and mycoplasmas were still being isolated from some of them at the end of a 31 month period of isolation. The persistence of mycoplasmas in the male genital tract can therefore be independent of sexual contact. Two modes of persistence are suggested; either a few men act as carriers and reinfect the others by contaminating their environment, or as seems more likely, most men have chronic infections.

INTRODUCTION

Mycoplasmas are found in the genital tracts of neonatal infants (Klein, Buckland & Finland, 1969; Foy, Kenny, Levinsohn & Grayston, 1970), but isolation during childhood is rare. Subsequently, it is possible to isolate these organisms from a proportion of adolescent persons at, and following, puberty (Mårdh & Weström, 1970). In addition, the proportion of children with serum antibodies to mycoplasmas begins to increase at about the time of puberty (Purcell, Chanock & Taylor-Robinson, 1969). In adult life, T-mycoplasmas have been isolated from the genital tract of about 50 % of apparently healthy men and from about the same proportion of non-pregnant women (Taylor-Robinson & Furr, 1973), while *Mycoplasma hominis* has been isolated somewhat less frequently. Isolation of mycoplasmas at puberty suggests that changes in the genital tract and possibly sexual activity are conducive to the establishment of infections. The hypothesis that their subsequent spread is largely the result of sexual activity is reinforced by the findings of Archer (1968), and Kundsin, Parreno & Kirsch (1973), who succeeded in isolating mycoplasmas from the urogenital tracts of only 8 % and 3 % respectively, of two groups of nuns. However, it is unknown whether mycoplasma infections of the male genital

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tract persist or whether they are transient. In the latter case, reinfections from repeated sexual encounters could simulate persistence. In an attempt to resolve this problem a group of men were studied during their stay at a British Antarctic Survey base.

MATERIALS AND METHODS

Subjects

The group studied was the wintering party of 1971–72 at Stonington Island, a base on the Antarctic Peninsula in Marguerite Bay. There were 17 men, six of whom had completed 1 year of a 2-year tour. The remaining 11 arrived during the relief of 1970–71 to stay for one or two years. The last ports of call of the ships where social contacts could be made were Montevideo, and Port Stanley in the Falkland Islands. None of the incoming group had any overt venereal disease after leaving these ports. They were studied for a total period of 17 months at the base and on board the Survey's ships.

Specimens and storage

Meatal swabs were collected at 6- to 10-week intervals from January 1971 to May 1972. Each man took his own swabs before micturition. The final samples were taken aboard ship from the six men returning to England. Each swab, of plain cotton wool (Stayne labs), was snapped off in 1·5 ml. of mycoplasma medium (Manchee & Taylor-Robinson, 1968), which contained glucose. On base these specimens were stored at $-28°$ C., during the voyage home at $-40°$ C., and in England at $-70°$ C. Serial blood samples were also taken during the study and the sera shared the same storage conditions.

Mycoplasma isolation

Medium expressed from the swabs was diluted 10^{-1}, 10^{-2}, and 10^{-3} in duplicate portions of three liquid mycoplasma media which contained phenol red and 0·1 % of glucose, arginine and urea, respectively. Detection of mycoplasmas was based on the occurrence of a change in colour of the media (Taylor-Robinson & Purcell, 1966). Medium containing glucose was included not only for the detection of *M. fermentans* but also for *M. canis* and other canine mycoplasmas. In addition, each sample was inoculated onto agar media containing, respectively, arginine and urea. All cultures were incubated at 37° C. for up to 14 days and specimens containing organisms at the 10^{-3} dilution were retested, being diluted up to 10^{-6}.

Where a series of specimens from one subject yielded arginine-metabolizing organisms, a single isolate was selected for identification by the disk inhibition technique (Clyde, 1964) using *M. hominis* (PG21) antiserum.

Serological tests

M. hominis serum antibody titres were determined by the indirect haemagglutination (IHA) micro-technique (Taylor-Robinson *et al.* 1965). Each of the serial sera taken during the year was inactivated at 56° C. for 30 min. and adsorbed with unsensitized tanned sheep red cells at 37° C. for 2 hr. The sera were then titrated at an initial dilution of 1/8 against sensitized tanned cells. Since many of the sera

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Table 1. *Persistence of* M. hominis *and T-mycoplasmas in the urogenital tracts of* 17 *men wintering at Stonington Island*, 1971

	Mycoplasmas isolated for at least (months)	Number of men yielding *M. hominis*		T-mycoplasmas	
1st	0	5		7	
year	6	0		1	
men	11	2		3	
	13	0		2	
	16	5		0	
	17	2	71 %	0	59 %
2nd	23	0		2	
year	24	1		0	
men	29	2		1	
	31	0		1	
	Totals	12		10	

in the lower dilutions agglutinated unsensitized tanned cells, controls consisted of duplicate dilutions of the sera tested against them. The sensitized and unsensitized tanned cells had been used successfully in a previous study (Taylor-Robinson *et al.* 1965) and had been stored at $-70°$ C. for the past 9 years. Titres of serum antibody against a T-mycoplasma were determined by the metabolism-inhibition method (Purcell, Taylor-Robinson, Wong & Chanock, 1966). Strain T-27 was used because it had been found previously that 82 % of 50 adult sera inhibited its metabolism to some degree (L. Q. Sang & D. Taylor-Robinson, unpublished observations). The sera were used at an initial dilution of 1/2.

RESULTS

Isolation of mycoplasmas

M. hominis

This mycoplasma was found on one or more occasion during the isolation period in 12 (71 %) of the 17 men (Table 1). However, mycoplasmas were not isolated from every specimen taken from the men harbouring these organisms. Of 72 swab specimens examined, only 23 (32 %) yielded mycoplasmas, and these were present in low numbers since they were not isolated from specimens diluted more than 10^{-3} (Table 2). In nine cases mycoplasmas were isolated from only the lowest dilution of one of the duplicate cultures and of these they were re-isolated from only five of the original specimens, probably due to loss of organisms during freezing and thawing. In only one case, that of subject no. 3, were mycoplasmas isolated from every specimen (Table 2).

The number of positive cultures from each series of samples increased towards the end of the isolation period (Fig. 1). Thus, whereas *M. hominis* was recovered from only three of 15 men in March–April 1971, it was recovered from eight of 17 men sampled in February 1972.

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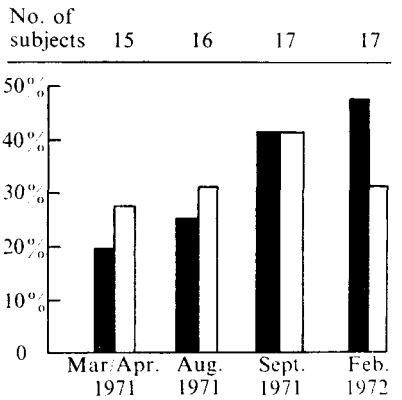

Fig. 1. Percentages of subjects from whom mycoplasmas were isolated at Stonington Island, March 1971–February 1972. ■, *M. hominis*; □, T-mycoplasmas.

T-mycoplasmas

These were recovered on one or more occasions from 10 (59 %) of the 17 men (Table 1). However, of 72 swab specimens examined, T-mycoplasmas were isolated from only 24 (33 %) of them, a result similar to that for *M. hominis*. The number of organisms on each swab was also similar, since they were cultured from only one specimen at a dilution of 10^{-4} (Table 2). Six of these 'positive' specimens yielded mycoplasmas at the lowest dilution in only one of the duplicate cultures. Subject no. 3 (Table 2) was again the only person whose swabs yielded mycoplasmas on every occasion. The number of isolations made from a series of swabs seemed to be unrelated to the particular time of the year during which they were taken (Fig. 1).

Comparison of M. hominis *and T-mycoplasma isolation*

The number of swabs which were positive for *M. hominis* (23) was similar to that for T-mycoplasmas (24) (Table 2). In addition, the numbers of organisms of the two mycoplasma species that were isolated from each swab were similar. However, both mycoplasma species were not always isolated from each swab. Thus, *M. hominis* but no T-mycoplasmas were isolated from subjects nos. 1, 6 and 17, and T-mycoplasmas but not *M. hominis* from subject no. 7. The remaining men from whom mycoplasmas were isolated carried both species at some time during the isolation period.

Antibody to mycoplasmas

M. hominis

The titres of antibody measured by IHA ranged from < 8 to 512 (Table 3). There were fourfold or greater rises in the titres of antibody in the sera of subjects nos. 2, 8 and 13, and one eightfold fall in the sera of subject no. 17. Mycoplasmas were isolated from subjects nos. 2, 13 and 17 but not from subject no. 8. The other 13 subjects had antibody titres of < 8 to 64 and these were maintained without significant change over the period of isolation. *M. hominis* was cultured from nine of these men on one or more occasion; from subjects nos. 1, 3 and 9 at times when their serum antibody titres were 1 in 64, but not from subject no. 5 who had antibody titres of 256–512. There would, therefore, appear to be no correlation between changes in, or the presence of, serum antibody and the isolation of *M. hominis*.

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Table 2. *Numbers of organisms isolated, March* 1971 *to April* 1972

		M. hominis						T-mycoplasmas					
	Subject no.	Mar. 71	Apr. 71	Aug. 71	Sept. 71	Feb. 72	Apr. 72	Mar. 71	Apr. 71	Aug. 71	Sept. 71	Feb. 72	Apr. 72
1st year men	1			–	10^1	–	–			–	–	–	–
	3	10^1		10^1	10^2	10^2	10^3	10^2		10^1	10^3	10^2	10^2
	8		–	–	–	–	–	–	–	–	–	–	10^2
	9			–	–	10^3				–	10^1	10^1	
	11	–		10^3	10^1	–		–		10^3	10^3	–	
	13		–	–	–	10^1			–	–	–	–	
	14		10^3	–	10^1	10^2			10^3	–	10^3	10^4	
	15		–	–	–	10^1			10^1	–	10^1	10^1	
	17		–	–	–	10^1			–	–	–	–	
2nd year men	2	10^1	–	–	–	–	–	–	–	–	10^1	10^1	–
	4	–		10^2	10^1	10^1		–		10^2	–	–	
	6		–	–	10^1	10^1			–	–	–	–	
	7	–		–	–	–	–	10^2		10^1	–	–	–
	10	–		10^3	10^1	–		–		10^3	10^3	–	

– = less than 10 organisms/ml. Blank = not swabbed.

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Table 3. *Titres of serum antibody to* M. hominis *and T-mycoplasma strain* 27, *March* 1971 *to February* 1972

	Antibody to *M. hominis* measured by IHA							Antibody to T-27 measured by MI						
Subject no.	Mar. 71	Apr. 71	Aug. 71	Sept. 71	Dec. 71	Feb. 72	Rise or Fall	Mar. 71	Apr. 71	Aug. 71	Sept. 71	Dec. 71	Feb. 72	Rise or Fall
1	–	–	32	64	64	64		–	–	< 2	< 2	< 2	< 2	
2	16	32	32	32	64	64	↑	< 2	< 2	8	<	< 2	< 2	↑ ↓
3	64	–	64	32	32	32		< 2/2*	–	4	< 2	< 2	4	↓ ↑
4	64	64	32	32	32	32		< 2	4	4	< 2	< 2	4	↑ ↓ ↑
5	–	256	–	256	512	256		–	8	–	2	4	2	↓
6	–	< 8	< 8	< 8	< 8	< 8		–	2	4	< 2	< 2	< 2	↓
7	< 8	–	8	8	8	8		< 2	–	< 2	< 2	< 2	< 2	
8	–	16	16	32	64	128	↑	–	2/4	4/2	< 2	2/< 2	4	
9	–	32	32	32	64	64		–	2/< 2	< 2	< 2	< 2	2	
10	16	–	32	32	32	32		< 2	–	< 2	< 2	2	2	
11	32	–	32	32	32	32		< 2	–	< 2	< 2	< 2	2	
12	64	–	64	32	32	32		< 2/2	–	2	4/2	< 2/2	< 2	
13	–	< 8	< 8	8	8	16	↑	–	4	2/4	2	4	2	
14	–	16	16	16	32	16		–	< 2	< 2	< 2	2	< 2	
15	–	< 8	< 8	8	8	8		–	< 2	< 2	< 2	2	< 2	
16	–	8	8	8	8	8		–	4/8	2	2	8	8/4	↑
17	–	64	32	32	8	8	↓	–	2	4	< 2	< 2	< 2	↓

– = Serum not available. * Results of duplicate tests.

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T-mycoplasmas

Antibody titres to strain T-27 measured by metabolism inhibition were low, ranging from < 2 to 8. There were 5 fourfold or greater rises and six falls in the titre of antibody (Table 3). T-mycoplasmas were isolated from three of the four men who had rises in serum antibody titre, and from three of the seven subjects who had falls in titre. There was no correlation between the changes in antibody titre and the isolation of T-mycoplasmas.

Studies on dogs

Mycoplasmas were not isolated from pharyngeal and genital swabs taken on three occasions over a period of 6 months from 12 of the 140 Husky dogs at Stonington Island. A single set of sera from the same animals did not specifically inhibit the metabolism of *M. hominis*, indicating the absence of antibody. The sera were not tested for antibody to T-mycoplasmas, nor by IHA against *M. hominis*.

DISCUSSION

The average rate of isolation of mycoplasmas from the men, based on swabs taken on any single occasion, is similar to the rate of isolation reported by other workers who studied larger numbers of men without disease (Taylor-Robinson, Addey, Hare & Dunlop, 1969; Taylor-Robinson & Furr, 1973). However, although repeated isolations were made from a series of swabs taken from each individual, these isolations were often intermittent. This was not due to variation in medium because the same batch was used throughout. Failure to isolate mycoplasmas consistently might have been due to a series of transient infections of the genital tract. Each reinfection might have originated in one of three ways. The first, from handling Husky dogs infected by T-mycoplasmas, is unlikely since the available evidence suggests that these dogs did not carry T-mycoplasmas, nor was anything known about the feasibility of dog to man transmission. A second possibility, auto-reinfection of the genital tract by mycoplasmas infecting the oropharynx, is unlikely because only about 5% of persons have the same mycoplasmas in their throats as in their genital tracts (Ford, 1967). Another alternative explanation is the persistence of mycoplasmas in the environment due to the primitive facilities for personal hygiene. Despite the possibility of consecutive reinfections, it is true to say that mycoplasma infections tend to be chronic rather than acute. *M. hominis* is well known to cause chronic contamination of tissue cultures (Stanbridge, 1971). *M. pneumoniae* may persist in the respiratory tract even after the institution of apparently adequate antibiotic therapy (Smith, Chanock, Friedewald & Alford, 1967), and T-mycoplasmas persist for many months in the genital tracts of isolated dogs (D. Taylor-Robinson & P. M. Furr, unpublished observations). It is not unlikely that the same situation exists in the genital tract of man. If this is so, inability to isolate mycoplasmas consistently from the swabs might have been due to inconsistent swabbing, or to the presence of mycoplasmas in small numbers only, so that those on some swabs might not have survived the variable storage conditions ($-28°$ C. for several months before lower temperatures).

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It was to be expected that organisms on swabs taken in the early phase of the study would not survive as well as those taken later; in this respect it seems that T-mycoplasmas survived better than *M. hominis*. A third explanation might be that some of the men were given antibiotics for short periods only. These included tetracyclines but, unfortunately, no records of administration are available. Tetracyclines are effective in inhibiting the growth of mycoplasmas; suboptimal doses suppress multiplication rather than eradicate the organisms (Shepard, 1972).

It was hoped that the assessment of antibody titres would help to determine whether or not re-infections had occurred. However, since there was no evident correlation between the presence of, or changes in, serum antibody titres and the isolation of *M. hominis* or T-mycoplasmas, there was nothing to indicate whether or not the intermittent isolations were due to re-infections. The small variations in the titres of antibody to the T-mycoplasmas are difficult to interpret. Apparent rises and falls in titre could be due to the presence of antibiotics in the subjects' sera, or responses to serial infections by different strains.

Most antibody titres persisted unchanged. This would be expected if persistent organisms provided a continuous antigenic stimulus for antibody production. Although the titres of IHA antibody recorded in the present study were similar to those found to mitigate symptoms in volunteers following intranasal inoculation of *M. hominis* (Mufson *et al.* 1965), they are clearly insufficient to eradicate organisms established in the genital tract. It is, therefore, likely that local antibodies and cell mediated immunity are the most important protective mechanisms controlling urogenital mycoplasma infections.

It is likely that T-mycoplasma infections can occur simultaneously with more than one serologically distinct strain, some persisting and others transient. Howard, Gourlay & Brownlie (1973) have observed variable persistence in experiments with bovine strains in the bovine udder. Whether mycoplasma infections of the human urogenital tract, once established, persist for life, obviously cannot be assessed from the present study. It is quite clear, however, that both *M. hominis* and T-mycoplasmas can persist in the male genital tract for at least two and a half years independent of sexual contact.

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