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Acute upper genital-tract disease in female monkeys provoked experimentally by *Mycoplasma genitalium*

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Summary. The oviducts of two grivet monkeys and three marmosets, all sexually mature animals, were inoculated with *Mycoplasma genitalium* at laparotomy. The mycoplasma was not recovered from the grivet monkeys, nor from the oviducts of the marmosets although it was isolated intermittently from the vagina of two of the latter animals up to 4–6 weeks after inoculation. In contrast, all of the animals developed antibody to *M. genitalium* measured by a micro-immunofluorescence technique. It developed rapidly in the grivet monkeys but slowly in the marmosets, being detected first about 1 month after inoculation with a maximal response by 2 months. Furthermore, despite an absence of vaginal discharge or cytological response, all the animals developed a moderate to severe endosalpingitis characterized by the infiltration of acute inflammatory cells into the tubal epithelium, together with a lumenal exudate and adhesions between the mucosal folds. The changes are similar to those produced by *Chlamydia trachomatis* in simian models and naturally in women.

Keywords: simians, salpingitis, Mycoplasma genitalium

Chlamydia trachomatis, Neisseria gonorrhoeae and *Mycoplasma hominis* are considered to cause pelvic inflammatory disease (PID), the results of several studies indicating that these miro-organisms account for about three-quarters of all the acute cases (Mårdh 1980). The aetiology of the remainder, however, is unknown.

Recently, a hitherto unknown mycoplasma, *M. genitalium*, was recovered from men with non-gonococcal urethritis (NGU) (Tully *et al.* 1981). The detection of a significant change in the titre of antibody to this mycoplasma in consecutive serum samples from women with acute PID who did not have antibody to *C. trachomatis* or *M. hominis* suggested that *M. genitalium* was a cause of the disease in at least some of these women (Møller *et al.* 1984).

The grivet monkey has proved suitable as an experimental model for studying upper genital-tract infections with other human mycoplasmas, that is *M. hominis* and *M. fermentans* (Møller & Freundt 1983). Moreover, experimental inoculation of the lower genital tract of female marmosets with *M. genitalium* produced a persistent vaginitis, indicated by an inflammatory cell response, and accompanied by an antibody response (Taylor-Robinson *et al.* 1982).

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In an attempt to determine whether *M. genitalium* has the capacity to produce disease of the female upper genital tract, the oviducts of grivet monkeys and marmosets were inoculated with this mycoplasma. In this communication we describe the macroscopic and histopathological changes, as well as the serological antibody response, which occurred in the monkeys.

Materials and methods

Animals. Three sexually mature female grivet monkeys (*Cercopithecus aethiops*), each weighing about 2 kg, and four mature female marmosets (*Callithrix jacchus*) weighing 200–300 g, were used. The grivet monkeys had been captured in East Africa and were kept in quarantine for at least 6 weeks before use, whereas the marmosets were bred at the Clinical Research Centre. The animals were caged individually in an isolation room during the investigation period and were maintained as described previously (Møller *et al.* 1978; Furr *et al.* 1976).

Preparation of inoculum. Mycoplasma genitalium (strain G37) had been isolated originally from the urethra of a patient with NGU. The strain was recovered in SP4 broth medium (Tully *et al.* 1979), and had been cloned three times and subsequently passed twice in SP4 medium to produce the inoculum for the grivet monkeys. The inoculum for the marmosets was prepared in a similar way. The same cloned strain, passed only once in SP4 medium, was grown in a glucose-containing medium (Manchee & Taylor-Robinson 1968) in which the horse serum was replaced by fetal calf serum and the thallium acetate was omitted. The two different inocula were stored in 1-ml aliquots at −70°C. After rapid thawing an aliquot was found to contain 5×10^6 viable organisms (colour-changing units: ccu) and was bacteriologically sterile.

Pre-inoculation sampling for micro-organisms. Vaginal swabs were taken to be examined for *M. genitalium*, other mycoplasmas and ureaplasmas by conventional techniques (Taylor-Robinson & Furr 1981). In addition, for marmosets, chlamydiae were sought by staining vaginal smears with a fluorescein-labelled monoclonal antibody (Thomas *et al.* 1984) and *Gardnerella vaginalis* by a cultural method (Totten *et al.* 1982). Furthermore, at laparotomy but before inoculation, swabs were taken from the oviducts and parametria to be examined for *M. genitalium* and ureaplasmas.

Operative technique and inoculation procedure. Grivet monkeys were anaesthetized by intramuscular (i.m.) injection of phencyclidine hydrochloride (Sernylan, 20 mg/ml), 0.15 ml; chlorpromazine (0.25% solution), 0.5 ml; and atropine (0.1% solution), 0.2 ml. Marmosets were anaesthetized by i.m. injection of Althesin (Glaxo; 1–1·5 ml/kg body weight). Laparotomy was performed under aseptic conditions. The abdominal wall was shaved and disinfected and the abdomen opened by a paramedian incision. The suspension of organisms was inoculated by means of a 26-gauge needle into the lumen of the oviduct through the lateral wall. Both oviducts of each animal were inoculated, a 0.2 ml volume of suspension being used for each side in the grivet monkeys and 0.1 ml of the suspension in the marmosets.

Assessment of changes in vivo and collection of specimens. Laparotomies were performed on each of the grivet monkeys 7, 14, 21, and 28 days after inoculation. Laparotomies were performed on the marmosets as follows: 7 days after inoculation of animal no. 1, 15 days after inoculation of no. 2 and 25 days after inoculation of no. 3. The marmoset which served as a control (no. 4) underwent surgery 7 and 25 days after inoculation. At laparotomy, the lumen of the oviduct, the parametrium, and sometimes the uterine cavity were swabbed (Nasopharyngeal swabs; Medical Wire and Equipment Co. Ltd, Horsham, UK). In addition, biopsy specimens

for histology were taken from the same sites. Swabs from the vagina were taken at the same time intervals for culture and for preparing smears for cytological examination.

Attempts to recover M. genitalium. After collection of material, the swabs were expressed in 1.8 ml of either SP4 broth or the modified glucose-containing medium mentioned above. This was deemed to provide a 10-fold dilution. In the case of grivet monkeys, the diluted specimens were stored at −70°C. Further serial 10-fold dilutions were made subsequently in the respective media and these were incubated at 37°C until the colour of the medium changed from pink to yellow, indicative of mycoplasmal growth. The highest dilution at which there was a colour change was considered to contain 1 ccu and the number of organisms in a swab specimen was expressed as ccu/0·2 ml. The organisms which were recovered were confirmed as *M. genitalium* by using a specific rabbit antiserum in the metabolism-inhibition test (Taylor-Robinson *et al.* 1966).

Cytological examination. Vaginal swabs were rolled on glass microscope slides, the smears fixed in methanol, stained with Giemsa reagent and examined for polymorphonuclear (PMN) leucocytes and other cells.

Histological examination. The biopsied tissues were fixed in 10% formol–saline, embedded in paraffin and stained with haematoxylin and eosin.

Detection of antibody. Blood was obtained on the days of operation prior to inoculation and at 1- to 2-week intervals thereafter for several months. The sera were stored at −20°C and antibody against *M. genitalium* was measured by a micro-immunofluorescence (MIF) technique (Furr & Taylor-Robinson 1984).

Control animals. One grivet monkey (no. 3) and one marmoset (no. 4) were used for control purposes. The oviducts of the grivet monkey were inoculated with phosphate-buffered saline, whereas those of the marmoset received glucose-containing mycoplasmal medium. Blood samples and specimens for culture, cytology and histology were taken as described previously.

Results

General health of the animals

The general condition of the three grivet monkeys during the experimental period was unimpaired. Marmoset no. 2, which had a laparotomy 15 days after inoculation of *M. genitalium*, lost about 15% of its body weight during the 4 weeks after inoculation. It was killed after 42 days. The other three marmosets showed no clinical signs of infection, remaining in an apparent healthy condition during the investigation period.

Isolation of micro-organisms

M. genitalium, other mycoplasmas, ureaplasmas, chlamydiae or *G. vaginalis* were not detected in the lower genital tract of any of the animals before experimental inoculation. Nor was *M. genitalium* isolated from the oviducts or parametria. However, as in previous studies (Furr *et al.* 1976), ureaplasmas were isolated from the throats of all four marmosets.

After inoculation of *M. genitalium*, attempts to recover the mycoplasma from the lower and upper genital tract of the grivet monkeys were unsuccessful (Table 1). However, it should be noted that frozen specimens were thawed during transportation from Denmark to the UK and were at room temperature for at least 8 h. Attempts to recover *M. genitalium* from the marmosets were more successful. As shown in Table 2, it was isolated from the vagina 7 and 32 days after inoculation of marmoset no. 1, and 25, 32 and 42 days after inoculation of marmoset no. 2. However, the mycoplasma was not recovered from the vagina of marmoset no. 3

Table 1. Serological and histopathological responses of two grivet monkeys after intraoviduct inoculation of *M. genitalium*

Grivet monkey no.	Type of test	Result of test on indicated day after inoculation*				
		0	7	14	28	35
1	Serology	<2	64	512	512	128
	Histology†	−	+	+++	++	ND
2	Serology	<2	32	128	64	64
	Histology	−	+	+++	++	ND
3 (control)	Serology	<2	<2	<2	<2	<2
	Histology	—	—	—	—	ND

* *M. genitalium* was not recovered at any time after inoculation.
† Of upper genital-tract specimens: − no, + slight, ++ moderate, +++ severe inflammation.
ND, Not done.

despite seven attempts, nor from the upper genital tract of any of the animals at the time specimens were also taken for histological examination (Table 2).

Antibody response

Antibody to *M. genitalium* was not found before inoculation of any of the animals. It was detected, however, in both grivet monkeys inoculated with *M. genitalium*, the response being seen first after 7 days and being maximal after 14 days (Table 1). Furthermore, antibody was detected in all three marmosets given *M. genitalium* but not until 25 to 32 days after inoculation; the titres appeared to be maximal about 8 weeks after inoculation.

Vaginal cytological findings

No PMN leucocytes or only a few were found in vaginal smears before inoculation. Subsequently, there was no significant increase in the number of these cells and none of the animals developed a vaginal discharge.

Upper genital tract changes

Macroscopic findings. There were obvious signs of inflammation 14, 21 and 28 days after inoculation of the two grivet monkeys (nos 1 and 2) with *M. genitalium*. Thus, both oviducts were swollen and there was moderate hyperaemia; the parametria were moderately oedematous, but without redness. Marmoset no. 1, examined at laparotomy 7 days after inoculation, had minimal hyperaemia of both oviducts, whereas marmosets nos 2 and 3, which were examined 15 and 25 days after inoculation, respectively, had marked inflammation characterized by pronounced redness and oedema of the oviducts and slight to moderate oedema of the parametria.

Microscopic findings. The severity and time of occurrence of the inflammatory changes are indicated in Tables 1 and 2. The two grivet monkeys (nos 1 and 2) had obvious signs of endosalpingitis and parametritis which were most pronounced 14 days after inoculation. The changes were characterized by a moderate infiltration of the tubal epithelium by PMN leucocytes with some inflammatory cell exudate also in the lumen of the tubes (Fig 1). There were no adhesions with the tubes which were not occluded. The parametria were infiltrated with a few PMN leucocytes and numerous mononuclear cells (Fig

Table 2. The recovery of *M. genitalium* from three marmosets after intraoviduct inoculation and their serological and histopathological responses

Marmoset no.	Type of test	Result of test on indicated day after inoculation							
		0	7	15	25	32	42	56	66
1	Culture*	–	10^5	ND	–	$\geqslant 10^6$	–	–	–
	Serology	<2	<2	ND	8	16	32	128	64
	Histology†	–	+	ND	ND	ND	ND	ND	ND
2	Culture	–	–	–	10^4	10^4	10^3		
	Serology	<2	ND	<2	2	32	64		
	Histology	ND	ND	++	ND	ND	–‡		
3	Culture	–	–	–	–	–	–	–	–
	Serology	<2	ND	<2	2	32	64	128	64
	Histology	ND	ND	ND	+++	ND	ND	ND	ND
4	Culture	–	–	ND	–				
(control)	Serology	<2	<2	ND	<2				
	Histology	–	–	ND	–				

* No. of organisms recovered from vaginal specimens; upper genital-tract specimens negative.

† Of upper genital-tract specimens: – no, + slight, ++ moderate, +++ severe inflammation.

‡ Only uterine tissue remaining which was normal.

ND, Not done.

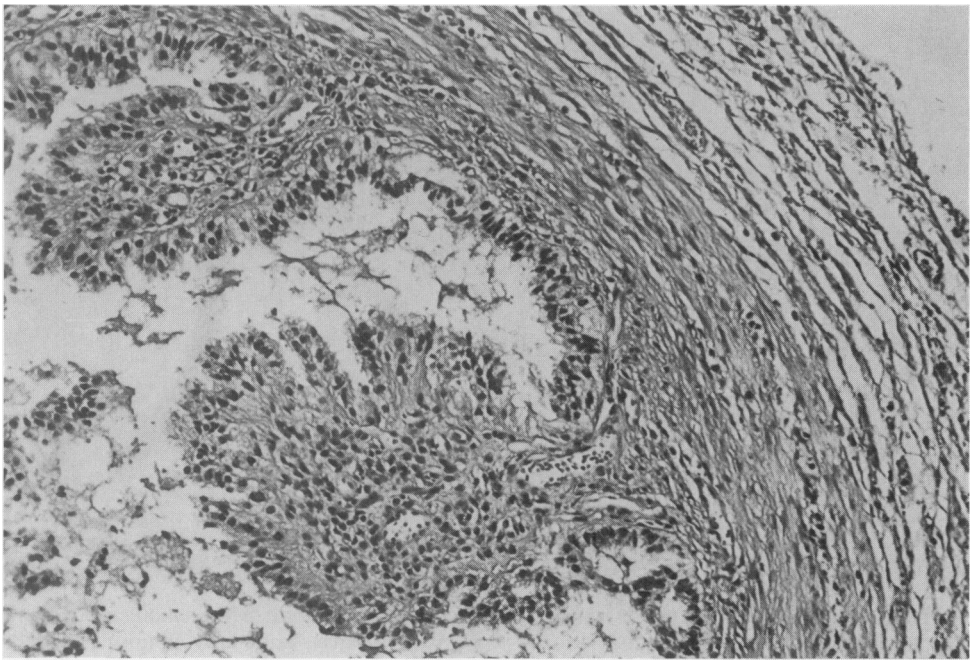

Fig. 1. Section of the oviduct of a grivet monkey 21 days after inoculation of *M. genitalium*. Moderate infiltration of the epithelium by PMN leucocytes with some lumenal exudate. H & E. ×150.

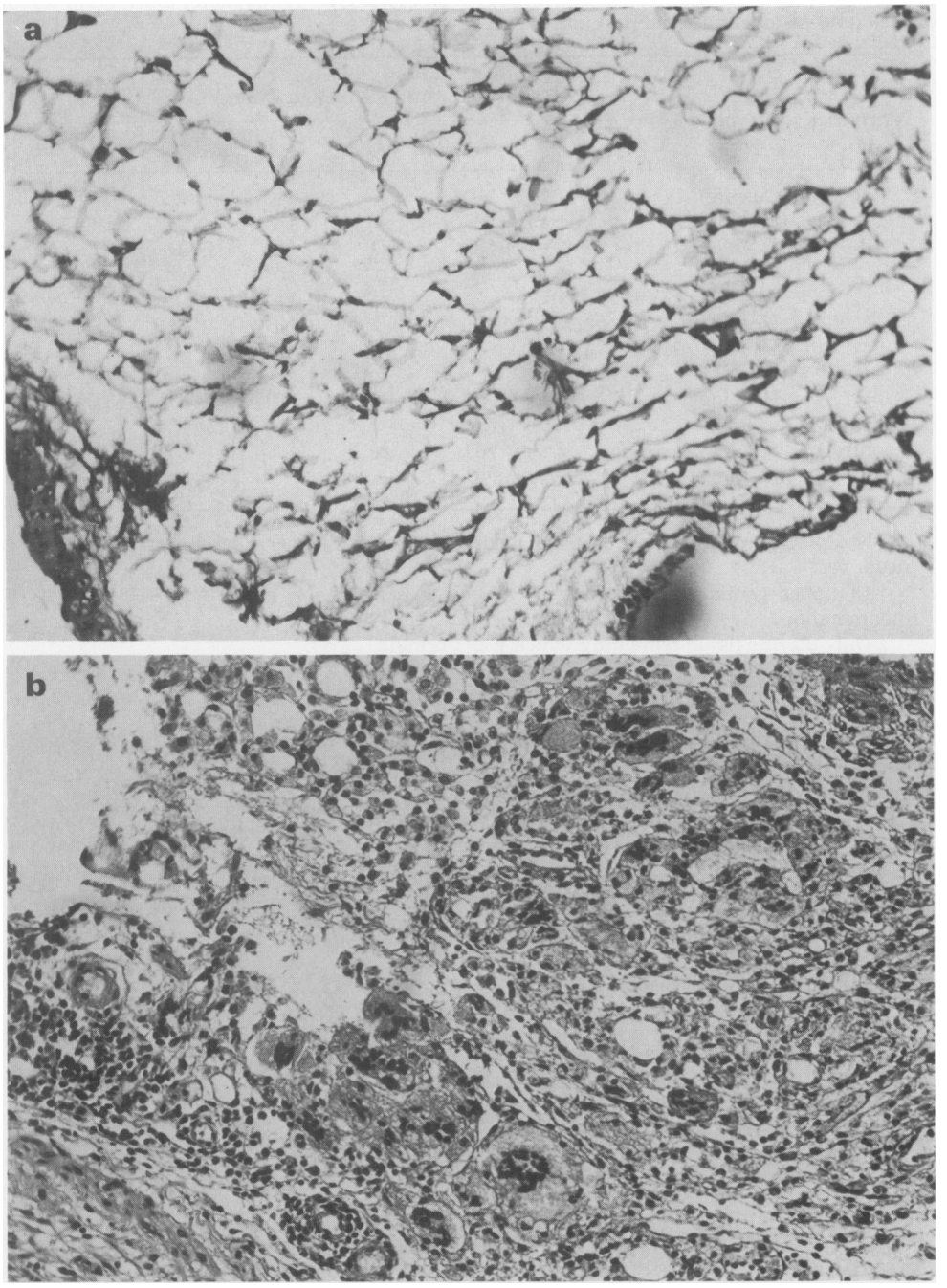

Fig. 2. (*a*) Section of the parametrium of a grivet monkey before inoculation of *M. genitalium*. H & E. × 150. (*b*) The parametrium 14 days after inoculation. A few PMN leucocytes and many mononuclear cells are seen with some fat necrosis. H & E. × 150.

2*a*,*b*). In some areas slight fat necrosis had occurred.

The tubal epithelium of marmoset no. 1, examined 7 days after inoculation, was infiltrated with a few PMN leucocytes and there were a few inflammatory cells in the tubal lumen. The parametrium was normal. Marmoset no. 2, examined 15 days after inoculation, exhibited a more severe but, nevertheless, moderate endosalpingitis and slight parametritis. Marmoset no. 3, examined 25 days after inoculation, had a very severe endosalpingitis in which there was a heavy infiltration of PMN leucocytes and some mononuclear cells into the tubal epithelium and the muscular layers and, in some areas, the subserosa also (Fig. 3*a*,*b*). The lumen was occluded by an exudate containing inflammatory cells and some desquamated cells and adhesions between the mucosal folds occurred frequently. In addition, there was slight to moderate inflammatory cell infiltration of the parametrium and some fat necrosis.

Marmoset no. 2 was killed 42 days after inoculation. There were no obvious signs of peritonitis, perihepatitis or respiratory-tract inflammation. Furthermore, histological examination of tissue biopsies from the liver and lungs showed that they were normal.

Observations on animals serving as controls

As indicated in Tables 1 and 2. *M. genitalium* was not recovered from either of the control animals (grivet monkey no. 3; marmoset no. 4), nor was antibody to the mycoplasma detected either before or after inoculaton of the control materials. Furthermore, no significant cytological signs of inflammation of the lower genital tract or histological evidence of inflammation of the oviducts or parametria of either animal developed.

Vaginal inoculation with M. genitalium

Marmosets nos 1 and 3 were inoculated intravaginally with 5×10^5 ccu of *M. genitalium* 75 and 65 days, respectively, after their original tubal inoculations. An increase in the number of PMN leucocytes was seen in vaginal smears from both animals 1 week after inoculation, but organisms were recovered only from marmoset no. 3, 14 days (10^3 ccu) and 47 days (10^2 ccu) after inoculation. There were no further increases in the antibody titres.

Discussion

Two types of PID have been described. One of them is endosalpingitis, caused by such micro-organisms as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* which spread canalicularly from the cervix via the uterine endometrium to the lumen of the Fallopian tubes (Møller & Mårdh 1980). The other type of PID is predominantly a parametritis and exosalpingitis, the lumen of the tubes being unaffected. *M. hominis* has been incriminated as a cause of this type of disease which is believed to occur as a result of organisms spreading from the lower to the upper genital tract via blood vessels and lymphatics (Møller *et al.* 1980).

In the present study, inoculation of *M. genitalium* directly into the lumen of the oviducts of grivet monkeys and marmosets provoked a moderate to severe endosalpingitis characterized by the infiltration of acute inflammatory cells into the tubal epithelium, together with a lumenal exudate and adhesions between the mucosal folds. These changes are similar to the alterations in the oviducts of grivet monkeys caused by experimental inoculation of *C. trachomatis* and similar also to the histopathological changes seen in patients with chlamydial salpingitis. Although a very mild parametritis was found in some of the animals in the present study, probably caused by the inoculation procedure which allows a little of the inoculum to penetrate into the parametrium, the *M. genitalium*-induced inflammation was different from the severe parametritis and exosalpingitis caused experimentally in grivet monkeys by other genital mycoplasmas, namely *M. hominis* and *M. fermentans.*

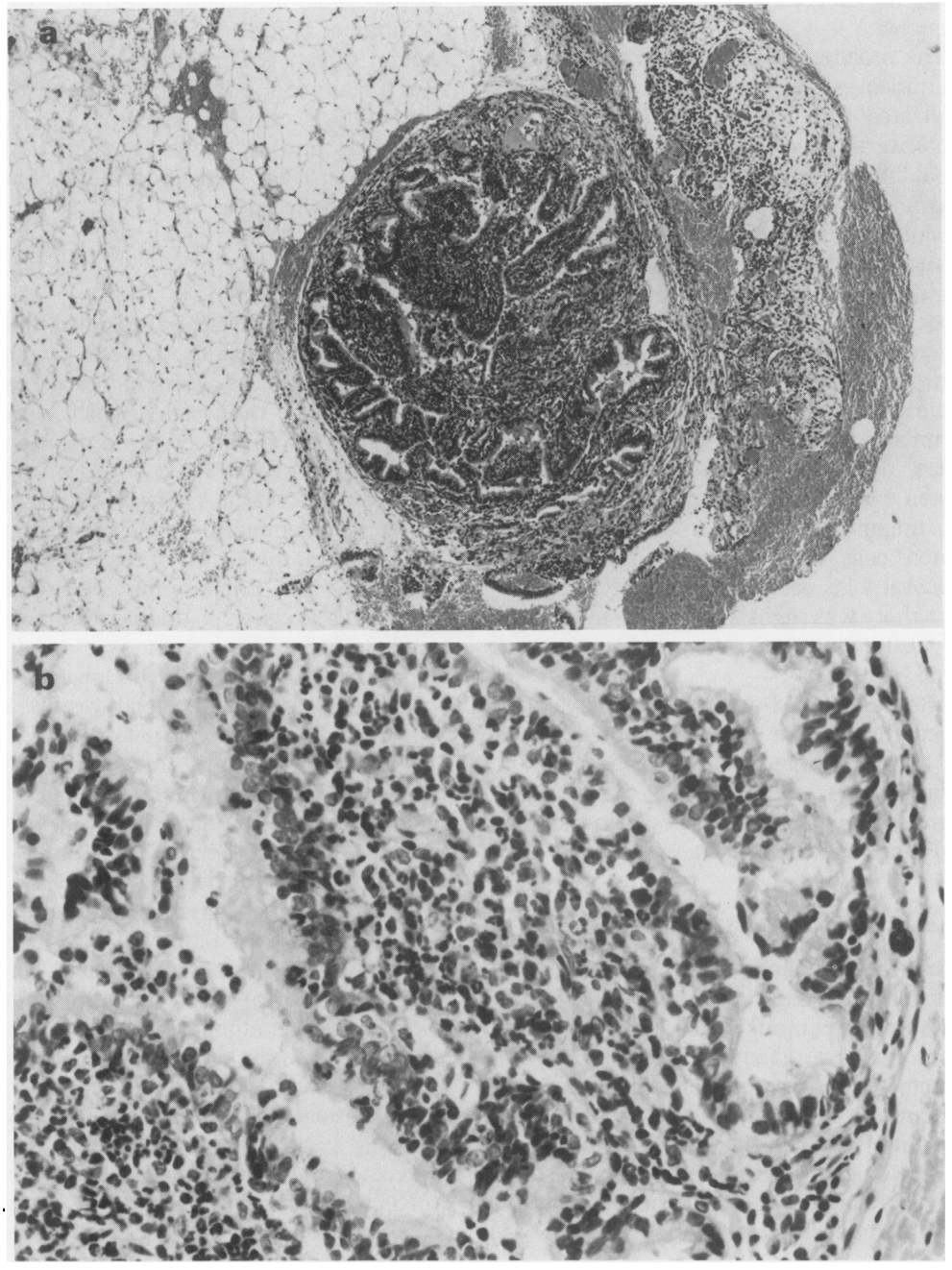

Fig. 3. (*a*) Section of the oviduct of marmoset no. 3. examined 23 days after inoculation of *M. genitalium*. Marked acute inflammatory cell infiltration through all the layers. H & E. ×70. (*b*) Same as (*a*). Infiltration with PMN leucocytes and some mononuclear cells. The lumen is filled with an exudate containing inflammatory cells and some desquamated cells. H & E. ×420.

The histopathological changes seen in the current experiments did not occur in the control animals which indicates that they were caused by *M. genitalium*. This contention is supported by the prominent antibody response of each animal inoculated with *M. genitalium* but to a far less extent by reisolation of the mycoplasma. Indeed, it was not recovered from the upper genital tract of any of the animals. This may have been due to the known difficulty of growing the microorganism, coupled with the small amount of material available for study. Furthermore, vaginal culture was unsuccessful in one marmoset and only positive intermittently in the two other animals. It is possible that the difficulty in growing *M. genitalium*, the hormonal status of the vaginal epithelium due to the oestrus cycle and secretory antibodies against the mycoplasma may have influenced its recovery. The latter two factors were not assessed. It is noteworthy that in a previous study (Taylor-Robinson *et al.* 1982), inoculation of marmosets intravaginally with *M. genitalium* resulted in persistent vaginal infection for more than 9 weeks with a concomitant PMN leucocyte response. However, an inoculum of 5×10^5 ccu was required to initiate the persistent vaginitis. In the present investigation, such a leucocyte response was not seen and it is possible that 'leakage' of organisms from the upper genital tract was insufficient to provoke a prolonged infection in which there was an inflammatory cell reaction. Nevertheless, it seems that the tubal inoculation produced some immunity to reinfection of the vagina. Thus, the inoculum mentioned above, known to cause persistent infection and vaginitis (Taylor-Robinson *et al.* 1982), produced a minimal infection only in one of two marmosets when introduced intravaginally 9 weeks after tubal inoculation.

The results of the present study suggest that the way in which *M. genitalium* produces disease in the upper genital tract of simians resembles that of *C. trachomatis* more than that of the other genital mycoplasmas. This may be due to the adhesive property of *M. genitalium*, associated with the terminal portion of its flask-shaped structure, which may help to confine it to the epithelium. However, infection by direct inoculation of the oviducts is, of course, artificial and further experiments are needed to determine whether similar disease occurs following inoculation of the endocervix, that is via a portal of entry to the upper genital tract which might be breached in women. There is already some serological evidence that *M. genitalium* produces PID in women (Møller *et al.* 1984). Apart from the simian model as a whole providing some confidence that this is a reality, two aspects of the model are worth noting in relation to human disease. First, failure to recover the organisms from the simian oviducts suggests that it may be extremely difficult or impossible to isolate them from fallopian tubes and that evidence of infection in women may have to rely on serology alone. Second, the late occurrence of an antibody response in the marmosets indicates that it might be unwise to look for such a response too early in women.

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