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# Acute and chronic bacterial prostatitis due to *E. coli*

## Description of an animal model

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**Summary.** Inoculation of *Escherichia coli* (serotype O:6) into the bladder of male and female Mastomys (Praomys) natalensis produced severe prostatitis. In this rodent both male and female animals possess a well developed prostatic gland. The histologic and microbiologic course of the prostatic infection resembled strongly the human disease. Acute bacterial prostatitis was followed by the development of chronic bacterial or nonbacterial prostatitis. The infection persisted in some animals for up to six months. Prostatitis was observed histologically in all animals sacrificed six months postinfection. Animals responded to the infection with a rise of anti-lipopolysaccharide antibodies. No major morphologic differences were detected in the histologic pattern of the inflammatory process between animals with positive and negative bacterial cultures and between male and female animals.

**Key words:** Prostatitis – *E. coli* – Mastomys natalensis

The term prostatitis is not a definite nosologic entity but includes various inflammatory conditions affecting the prostate. A widely accepted clinical classification system of "benign diseases associated with prostatic pain" had been based on cytologic and microbiologic examinations of the expressed prostatic secretion (EPS) [4]. Recognition of additional etiologic agents necessitated a modification and extension of the scheme to include 1. acute bacterial prostatitis; 2. chronic bacterial prostatitis caused by a) common urinary tract pathogens, b) Ureaplasma urealyticum, c) Mycobacterium tuberculosis; 3. mycotic prostatitis; 4. urethro-prostatitis caused by Neisseria gonorrhoeae, Trichomonas vaginalis, or Candida spp.; 5. nonbacterial prostatitis; 6. prostatodynia [3].

Despite the fact that some of these conditions are widespread diseases affecting every fifth man during his lifetime, our knowledge of their etiology, pathogenesis, pathophysiology, morphology, systemic and local immune response, appropriate therapy, clinical course and prognosis is still incomplete [10, 14, 18]. This lack of knowledge is partly due to the fact that this complex pattern of diseases has not yet been studied in detail in an adequate animal model.

Most previous studies of experimental prostatitis have used either unusual ways of infection, e.g., by intraprostatic or intraarterial injection of pathogens, or rarely implicated bacteria, e.g. Klebsiella pneumoniae or Proteus spp. Clinical course, final outcome of longterm experiments and immune response have not been addressed [1, 6, 19].

We tried a different approach using Mastomys natalensis. Mastomys is a small rodent intermediate in size between the mouse and rat. It is the only species of small rodents in which all female animals possess a well-defined prostatic gland [2, 16]. The male prostate consists of three different lobes and is analogous to the prostate of the rat. The female gland resembles the ventral lobe of the male prostate and responds readily to androgens. In the field of prostatic research interest in Mastomys was raised by the observation of spontaneous prostatic hyperplasia and adenocarcinoma [7, 17]. The low androgen environment of the female prostate makes it a useful model for the study of hormone effects on prostatic disease [11].

## Materials and methods

### *Animals*

Male and female Mastomys natalensis (Giessen colony) were kindly provided by the Institute of Parasitology, Justus Liebig University Giessen, and bred in our own colony. Animals were kept in a natural day-night system and had free access to food and water. They were used for infection at 14 to 16 weeks of age, weighing 50 to 80 g. A total of 63 male and 68 female Mastomys were infected. 6 male and 6 female animals served as controls.

### *Bacterial strain and inoculum properties*

The *E. coli* strain (serotype O:6) used was a clinical isolate from a patient with chronic bacterial prostatitis. For infection, *E. coli* was

**Table 1.** Bacterial recovery from the prostate, kidneys and urine in male Mastomys natalensis ($n = 63$)

| Time after infection | *E. coli* O:6 isolated from | | |
|---|---|---|---|
| | Prostate | Kidneys | Urine |
| 1- 3 days | 11/11 | 4/11 | 11/11 |
| 6–15 days | 15/17 | 4/17 | 8/17 |
| 18–27 days | 10/15 | 2/15 | 6/15 |
| 1 month | 3/5 | 3/5 | 3/5 |
| 2 months | 3/6 | 1/6 | 1/6 |
| 3 months | 1/5 | 0/5 | 1/5 |
| 6 months | 2/4 | 1/4 | 1/4 |

**Table 2.** Bacterial recovery from the prostate, kidneys and urine in female Mastomys natalensis ($n = 68$)

| Time after infection | *E. coli* O:6 isolated from | | |
|---|---|---|---|
| | Prostate | Kidneys | Urine |
| 1- 3 days | 16/16 | 5/16 | 15/16 |
| 6–15 days | 9/13 | 1/13 | 4/13 |
| 18–27 days | 6/17 | 0/17 | 1/17 |
| 1 month | 1/6 | 0/6 | 6/6 |
| 2 months | 2/8 | 0/8 | 1/8 |
| 3 months | 0/4 | 0/4 | 0/4 |
| 6 months | 1/4 | 0/4 | 0/4 |

grown in trypticase soy broth (TSB), adjusted to a final concentration of $10^8$ CFU/ml, and stored on ice until use.

### *Infection procedure*

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (5 mg/kg). From a lower abdominal midline incision the bladder was exposed and emptied by manual compression. The inoculum containing $10^8$ organisms per ml, was injected into the bladder through a 25 gauge needle until one drop of the suspension appeared at the orificium externum urethrae. In the control animals sterile TSB was injected. Finally, the abdominal wall was closed in a double layer with 5/0 chromatic catgut.

### *Bacteriologic and histopathologic examinations*

Animals were sacrificed by an overdose of pentobarbital at days 1, 2, 3, then every third day within the first month, and 1, 2, 3, and 6 months after infection. Blood was obtained by heart puncture. Urine specimens were collected from the bladder with a 25 gauge needle. Kidneys and prostatic lobes were removed aseptically. One half of each prostate was immediately fixed in 10% buffered formalin. After embedding in paraffin, tissue sections were stained with hematoxylin and eosin. Kidneys and the second half of the prostate were used for bacteriologic examination. Before homogenization, each specimen was rinsed twice with sterile saline solution. Tissue samples were weighed and homogenized in 1 ml of TSB. 100 µl of serial tenfold dilutions of the homogenate, and 100 µl of urine were plated in duplicate on CLED (Sandys) agar, incubated overnight at 37 °C before counting the number of colonies. Bacterial counts were expressed as colony forming units (CFU) per gram tissue or ml urine, respectively.

### *Antibodies against the lipopolysaccharide (LPS) of E. coli*

An indirect hemagglutination assay was performed using the lipopolysaccharide (LPS) of the *E. coli* strain as antigen. LPS was extracted by the phenol-water method, dialyzed and lyophilized. Suspensions (1%) of glutaraldehyde fixed Mastomys erythrocytes in phosphate buffered saline (PBS) were sensitized with five volumes of LPS diluted 1:200 in PBS. A 1% suspension of sensitized erythrocytes was used for the test. The hemagglutination assay was performed in microtiter plates. Serial twofold dilutions of 25 µl inactivated serum from each animal were incubated with 25 µl of sensitized erythrocytes. Sera from noninfected animals and nonsensitized erythrocytes served as controls. The reciprocal of the highest dilution of sera causing complete agglutination of erythrocytes (carpet formation) was considered the titer.

## Results

### *Bacteriology*

During the observation period of 6 months, *E. coli* was isolated from the prostates of 45/63 male (71%) and 35/68 female (51%) animals (Tables 1 and 2).

The prostates of all male and female animals sacrificed within the initial 3 days, were infected. *E. coli* was cultured in numbers ranging from $10^3 - 10^{11}$CFU/g tissue.

During the second week and later on, the rate of infection was found to decline, but selflimitation of the infection was not observed since 2, 3 and 6 months postinfection the prostates of 3 female and 6 male animals were still infected (range of CFU/g: $10^2 - 10^9$).

The numbers of CFU/g prostatic tissue differed in each group of animals. Therefore no mean and standard deviation were calculated.

Urine cultures showed heavy growth of *E. coli* with $> 10^5$ CFU/ml in 11/11 male and 15/16 female animals within the initial 3 days. Later on, corresponding to the infection rate of the prostates, the rates of positive urine specimens declined. Bacteria were cultured from urine in 4/9 animals with persistent prostatic infection for more than 2 months. In 16/45 male and in 12/35 female animals with positive prostatic cultures, urine cultures were sterile.

In contrast to the frequent occurrence of prostatitis and cystitis, pyelonephritis developed in less than 50% of animals within the first observation period. In all female animals homogenates of the kidneys were sterile by day 12, while in some of the male animals pyelonephritis persisted for up to 6 months. Prostatitis was associated with pyelonephritis in 21 animals (27%).

Epididymitis was observed in 5/63 (8%) male animals. In one animal it developed unilaterally after 12 days. Bilateral epididymitis was detected in three animals after one, and in one animal after 6 months. In all cases urine cultures were positive for *E. coli*.

At no time bacteria were cultured from the prostates, kidneys and urine specimens of the control animals.

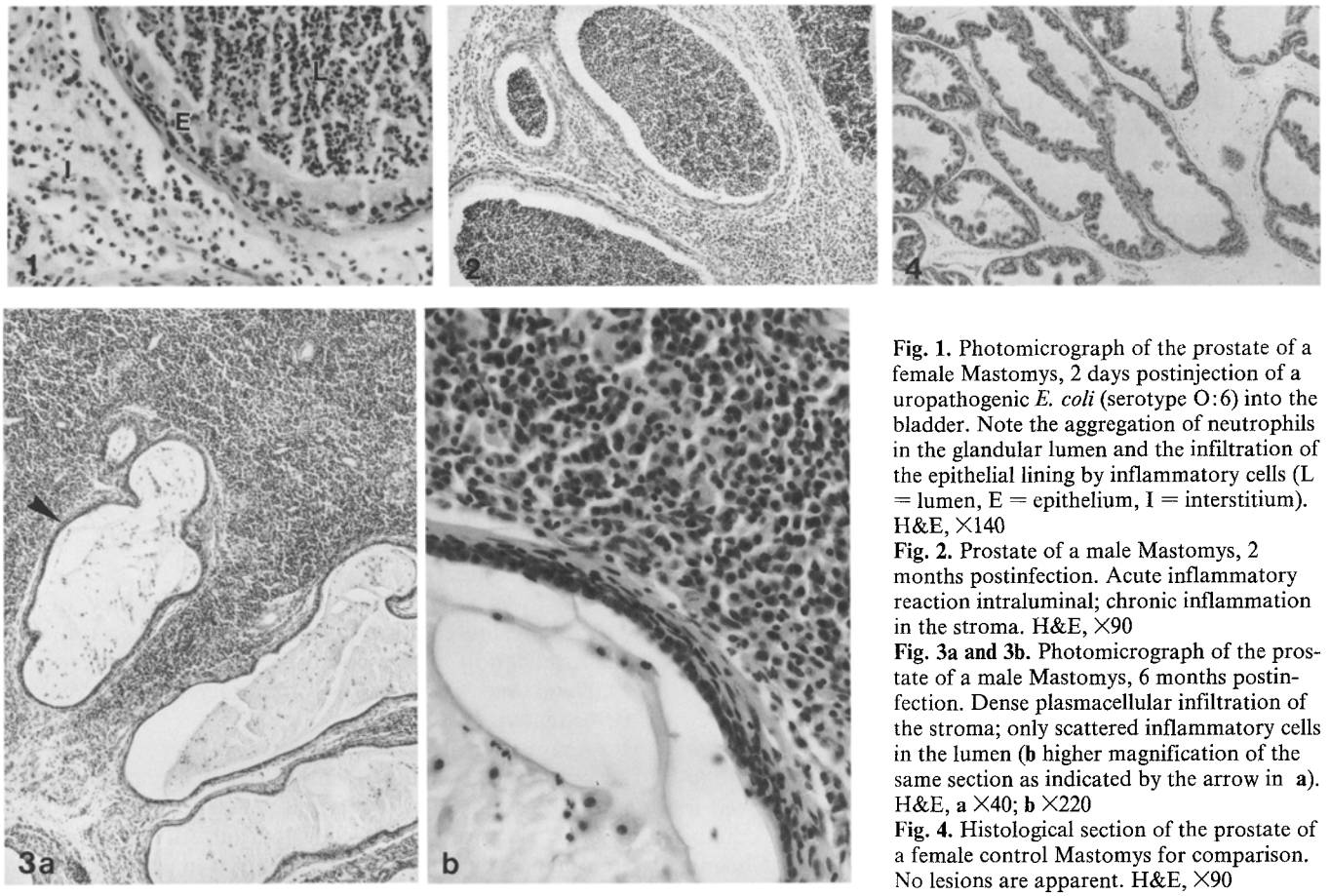


**Fig. 1.** Photomicrograph of the prostate of a female Mastomys, 2 days postinjection of a uropathogenic *E. coli* (serotype O:6) into the bladder. Note the aggregation of neutrophils in the glandular lumen and the infiltration of the epithelial lining by inflammatory cells (L = lumen, E = epithelium, I = interstitium). H&E, ×140

**Fig. 2.** Prostate of a male Mastomys, 2 months postinfection. Acute inflammatory reaction intraluminal; chronic inflammation in the stroma. H&E, ×90

**Fig. 3a and 3b.** Photomicrograph of the prostate of a male Mastomys, 6 months postinfection. Dense plasmacellular infiltration of the stroma; only scattered inflammatory cells in the lumen (**b** higher magnification of the same section as indicated by the arrow in **a**). H&E, **a** ×40; **b** ×220

**Fig. 4.** Histological section of the prostate of a female control Mastomys for comparison. No lesions are apparent. H&E, ×90

**Table 3.** Serum antibody titers in male and female animals

| Time after infection | titer | |
|---|---|---|
| | median | range |
| 1– 3 days | 0 | 0– 64 |
| 6–15 days | 128 | 0–16,000 |
| 18–27 days | 128 | 8– 1,024 |
| 1 month | 192 | 8– 4,096 |
| 2 months | 32 | 8– 256 |
| 3 months | 24 | 8– 512 |
| 6 months | 16 | 4– 4,096 |

*Histopathology*

In the infected animals at day 1 postinfection, emigration of inflammatory cells from the vessels of the prostatic stroma was noted. At 2 days postinfection, the epithelium of the prostatic acini was heavily infiltrated with neutrophil granulocytes and showed local destruction and desquamation. The lumen was filled with an exudate of neutrophil granulocytes. In the stroma a moderate infiltration of plasma cells, lymphocytes and, rarely, neutrophils was seen. In the following an increase of periacinar and intraluminal inflammation was noted. The lumina became dilated and filled with numerous granulocytes, macrophages, and cellular debris. The epithelial lining was nearly completely destructed, and abscesses had formed. At 15 days, wide areas of the prostatic tissue were replaced by granulation tissue. Remaining acini were filled with purulent exudate. The epithelium had lost its secretory activity and was cuboidal in shape. At 2 and 3 months, histology showed a granulomatous inflammation. Also focal epithelial regeneration was seen. In all animals sacrificed 6 months postinfection, inflammatory changes were still present. Most epithelium was regenerated showing localized hyperplasia and metaplasia. The surrounding stroma was filled with an infiltrate consisting of plasma cells and lymphocytes. In animals with sterile prostatic specimens some scattered intraluminal leucocytes were observed, while in the still infected animals the lumina of some acini were dilated and filled with neutrophils. No major morphologic differences in the histologic pattern of the inflammation between animals with positive and negative bacterial cultures and between male and female animals were noted.

In the prostatic tissue of all female and male controls, no lesions were observed.

*Immune response*

Anti-LPS antibodies were detected first at day 3 postinfection in the infected animals. From day 6 through the end of the study, antibody response was observed in 92/94

(98%) animals. Hemagglutination titers rose rapidly during the second week and remained at high levels within the first month. After 2–6 months titers returned to low levels (Table 3). During the 6 months of study, maximal titers were 128 in 13, 256 in 17, 512 in 11, 1024 in 4, 2048 in 3, 4096 in 2, and 16000 in 1 animal.

In the control animals, antibodies directed against the lipopolysaccharide of the *E. coli* strain were not detected.

## Discussion

Bacterial prostatitis is caused in up to 80% of patients by gramnegative bacteria. Among those *E. coli* is the main etiologic agent. Reflux of infected urine is considered the major cause of prostatitis [14]. Therefore, an animal model for bacterial prostatitis should imitate this way of infection.

In our model the *E. coli* suspension was injected into the urinary bladder and, by reflux, infected the prostate. Friedlander and Weidner reported on a similar model of experimental prostatitis using *Proteus vulgaris* and *Klebsiella pneumoniae* which, however, are uncommon pathogens of human prostatitis [6, 19]. Longterm results (> 1 month) have not yet been reported.

Clinically, bacterial prostatitis can be divided into an acute and chronic inflammation. In cases of acute bacterial prostatitis the whole prostatic gland is involved in the inflammatory process. The etiologic agent can be recovered in all cases from the midstream urine. Chronic bacterial prostatitis is characterized by focal inflammatory processes in the peripheral zone of the prostate. Midstream urinary specimens are usually sterile [10, 18].

*E. coli* was recovered from the prostate and urine specimens of all infected animals within the initial 3 days.Histologically an acute purulent inflammation was observed. Intraprostatic abscesses which are a well-known complication of acute bacterial prostatitis in man, occurred as soon as six days postinfection. Thus these stages of experimental prostatitis resemble strongly the condition of acute human bacterial prostatitis.

Self-limitation of the infection was not observed. In some animals, chronic bacterial prostatitis developed and persisted until the termination of the experiment. The reason why some animals were able to clear their prostates of bacteria and some were not, is unclear and needs further investigation. Probably, we are dealing with the same disorders leading to the development of chronic bacterial prostatitis in humans.

In this study, acute bacterial prostatitis was followed by chronic bacterial prostatitis, i.e., a similar situation as discussed in humans [14]. Thus this model allows the study of pathogenesis and treatment of both acute and chronic bacterial prostatitis.

In a total of 28 animals, the pathogen was recovered exclusively from the prostate while kidney and urine specimens remained sterile. An identical situation is found in men suffering from chronic bacterial prostatitis.

In one animal, urinary tract infection combined with bacterial prostatitis was still observed after 6 months. This corresponds to the observation that chronic bacterial prostatitis is a common source of recurrent bacteriuria [14].

The correlation between clinical symptoms and histopathology in prostatitis is unknown.

In 1979 Kohnen and Drach reported on the morphologic classification of prostatic inflammation [9]. Based on the histopathologic examination of hyperplastic prostates, six morphologic patterns of inflammation were described: 1. segregated glandular, 2. periglandular, 3. diffuse stromal, 4. isolated stromal, 5. acute necrotizing, and 6. focal granulomatous inflammation. All these patterns were observed in our animal model. Similar to the situation in humans, no relation between inflammation of the prostate and prostatic infection could be defined. Based on the course of prostatic inflammation leading to nonbacterial prostatitis after 6 months in the majority of cases, it may be assumed that nonbacterial prostatitis in humans may be a sequela of an episode of bacterial prostatitis. This theory has been discussed by various investigators previously [10, 14].

The question whether an infectious injury of the prostate results in a self-perpetuating process, even after the eradication of the causative agent, and the role of humoral and cell-mediated immunity must further be investigated.

Acute and chronic bacterial prostatitis in man are accompanied by a rise of antigen-specific agglutinating antibodies [12]. Titers remain elevated during bacterial infection and return to low levels after sufficient antibiotic treatment. Healthy men have no humoral antibodies directed against gramnegative enterobacteriaceae [13]. A similar antibody response was observed in our model. A correlation between positive bacteriologic findings and humoral antibody response was not noted. Antibodies reached highest levels 2–4 weeks postinfection and returned to low levels 2 to 6 months p.i. The role of humoral immunity in the resolution of bacterial prostatitis is unknown. The presence of specific antibodies in the EPS of patients with chronic bacterial prostatitis indicates that humoral immunity may play a significant role in the resolution of bacterial prostatitis [20]. Disturbances of humoral and cellular immunity are probably responsible for the persistence of chronic bacterial prostatitis. The presence of humoral antibodies in nearly all animals indicates that all animals, even those with sterile prostatic cultures, were infected.

A hormonal influence on the development of experimental bacterial prostatitis has been emphasized by Kaplan and coworkers [8]. In their experiments castration of male rats shortened the period of prostatic infection in comparison to noncastrated animals. Generally, male animals seem to be more susceptible to bacterial infections than females [6]. The experimental infection of female Mastomys offers the opportunity to study bacterial prostatitis in a low androgen milieu [11]. As concerns microbiology, immunology and histology of bacterial prostatitis, no differences were observed between male and female animals. Even in the prostate of female animals, bacterial infection persisted for up to 6 months.

Sex differences were noted in the development of pyelonephritis. The kidneys of all female animals were

sterile by day 12 while in male animals kidney infection persisted until the end of the study.

Pyelonephritis with concomitant prostatitis developed in 22% of animals. Whether it was induced by ascension of bacteria from the bladder or by reflux of the inoculum to the kidney is unclear. The low incidence of pyelonephritis is in contrast to the observations of Friedlander who noticed pyelonephritis in nearly all rats with experimental prostatitis induced by *Proteus vulgaris* [6]. Different nephritogenic pathogenicity of both bacterial species may have caused these differences.

Epididymitis is a rare complication of prostatitis [14]. In our model, the infection spread from the prostate to the epididymis in a small proportion of animals. In the majority of cases the inflammation of the epididymis developed bilaterally.

Many aspects concerning pathogenesis, pathohistology and etiology of acute and chronic bacterial and nonbacterial prostatitis are still little understood. Our animal model may help to clarify some important aspects of this widespread disease.

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