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Nonbacterial prostatitis caused by partial urethral obstruction in the rat

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Abstract The pathogenesis of nonbacterial prostatitis (NBP) is not understood mainly due to the lack of appropriate experimental models. We developed a new experimental model of NBP by inducing a partial obstruction of the urethra (PUO) in the rat. Male Wistar rats aged 12 weeks were used. PUO was produced by a nylon ligature on the urethra over a rubber tube. The tube was slipped out after the ligature had been tied. Two rats were examined histologically 6 h, 1 day, 3 days and 7 days after PUO. In another group, two rats were killed at 1, 3 and 7 days after the release of the PUO that had been left in place for 3 days. On day 3, another eight rats with PUO and eight control rats had 2 ml of urine in the bladder replaced by the same volume of lucifer yellow (LY; 10 μg/ml, MW 500), microperoxidase (MP; 20 μg/ml, MW 1900), horseradish peroxidase (HRP; 10 μg/ml, MW 40 000), or saline as control, respectively. Lymphocytic infiltration and interstitial edema were noted in the prostate following PUO, being most prominent on day 3. After the release of the PUO, these inflammatory changes gradually disappeared. Only LY was noted within the prostatic stroma of the rats 2 h after bladder instillation. Intraprostatic urinary reflux may be an etiologic factor in NBP. The present study showed that lower urinary tract obstruction caused NBP in the rat. Penetration of prostatic tissue by low-molecular-weight substances in the urine may trigger NBP.

Key words Nonbacterial prostatitis · Animal model · Partial urethral obstruction · Rat

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Introduction

Nonbacterial prostatitis (NBP) is defined as prostatic inflammation in the absence of identifiable pathogens [11]. Although NBP is responsible for as many as 90% of cases of prostatitis [2], its etiology and pathogenesis remain unclear. Recent evidence suggests that reflux of urine into the prostate may lead to NBP [6, 9]. However, the role of intraprostatic reflux of urine in the development of NBP is unclear, mainly because of the lack of appropriate experimental models. The present study investigated the prostate glands of rats in which we had induced partial obstruction of the urethra (PUO).

Materials and methods

The experiments were done according to Japanese legislation on animal care and approved by the local ethics committee on animal protection. A total of 43 12-week-old male Wistar rats (body mass 200–250 g) were purchased from Japan Crea Co., and housed under conditions of controlled temperature and light. They were allowed free access to food and water. They were divided into three groups as follows: group 1 was used for histologic examination, and included 14 rats with PUO and two rats subjected to a sham operation. Two rats each were killed at 6 h, 1 day, 3 days and 7 days after PUO. Two rats each were killed 1 day after the release of PUO for 3 days, 4 days and 7 days, respectively. Sham-operated rats were killed 3 days after the operation. Urine samples were aspirated from the bladder of each rat in a sterile manner to permit bacteriologic examination. Group 2 was used for cystometric evaluation and included 15 rats. Five rats underwent cystometry 3 days after PUO and 4 days after the release of PUO. Five other rats underwent cystometry 3 days after sham operation. Group 3 included 12 rats, six of which underwent PUO while six underwent a sham operation. Two of the experimental rats and two of the sham-operated rats received an intravesical instillation of lucifer yellow (LY; 1 mg/ml, MW 500), microperoxidase (MP; 2 mg/ml, MW 1900) and horseradish peroxidase (HRP; 2 mg/ml, MW 40 000), respectively.

Partial urethral obstruction and release

A small longitudinal skin incision was made in the skin at the base of the penis of the animal after it had been anesthetized with ether.

A 4-0 nylon ligature was placed over a rubber tube of 3 mm outer diameter and 2 mm inner diameter, which had been placed over the proximal urethra. After gentle ligation, the tube was slipped off to create a partial obstruction of the urethra. Animals in the control group underwent a sham operation under ether anesthesia. Release of the obstruction was achieved by removing the suture 3 days after the creation of PUO, under ether anesthesia.

Bladder instillation

Group 3 rats underwent laparotomy to expose the bladder 3 days after PUO. Two milliliters of urine were aspirated and replaced by the same volume of LY, MP, HRP or saline (control) in two rats each. Two hours after the instillation the animals were killed and the prostate was harvested. Two rats that underwent the sham operation each received an instillation of LY, MP or HRP, and were killed 2 h later.

Histologic studies

Group 1 rats were killed by cervical fracture at the times specified. After the prostate gland and the seminal vesicles had been examined, they were dissected out and removed. The ventral and dorsal lobes of the prostate, and the coagulating gland of the prostate were each obtained separately. Specimens were fixed in 10% neutral formalin, embedded in paraffin, and stained with hematoxylin and eosin. Corresponding slides were prepared for staining with Masson trichrome [8].

Immunohistochemical, histochemical and fluorescent studies

Tissue specimens were fixed for 1 h with 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, that contained 7% sucrose. After rinsing with the buffer, frozen sections 10 μm thick were prepared and MP or HRP was stained using 3,3′-diaminobenzidine (10 μg/ml in phosphate-buffered saline) as the substrate by the method of Graham and Karnovsky [3]. Hematoxylin was used for counterstaining. LY was examined directly by fluorescence microscopy. Rat Tamm-Horsfall glycoprotein (THG) was prepared and purified according to the method of Dawnay and St Cottell [1]. Anti-rat THG sera were obtained from rabbits that had been immunized with rat THG biweekly over a 2-month period. The specificity of the antibody was ascertained by Western blotting and immunohistochemistry (data not shown). Staining for THG was done on formalin-fixed slides of tissue obtained from group 1 rats using the ABC method (Dako LSAB Kit, Dako Co.).

Cystometry

Cystometry was performed 3 days after PUO and 4 days after release of the obstruction, to determine the increased capacity and pressure of the bladder and its normalization. The bladder of rats anesthetized with ether was exposed through a small laparotomy. The bladder was emptied with a 24-gauge needle that was connected to an infusion pump (Digital Syringe Pump, KDS100, Neuroscience Co., USA) and a transducer (Blood Pressure Monitoring Kit, Omeda Co., Japan). The urine was examined for bacteriologic culture (Uromedium kit, Nissui Co., Japan). Warm isotonic saline was infused at a rate of 10 ml/h in the sham-operated rats and at the rate of 25 ml/h in the experimental rats according to Malmgren et al.'s method [7]. Cystometry was discontinued in the animal when saline was seen to leak through the urethra. The intravesical pressure was recorded with a polygraph system (RM-6000, Nihon-Koden Co., Japan.).

Statistical analysis

Data are given as mean ± SD. The statistical significance of the differences between the means was determined using Welch's test and StatView software (Abacus Concepts). A level of $P < 0.05$ was considered statistically significant.

Results

The bladder became markedly distended following PUO. No hydronephrosis was noted. The volume of urine aspirated when the animals were killed ranged from 3.0 to 5.0 ml in the experimental rats, and was consistently less than 2 ml in the rats in which the PUO had been relieved and the control rats. The dorsal and ventral lobes of the prostate and the seminal vesicles appeared grossly edematous during PUO. Following relief of the obstruction, the appearance of the bladder returned rapidly to normal, whereas the prostate and the seminal vesicles exhibited mild edema.

Six hours after PUO, the edematous prostatic stroma was infiltrated by histiocytes and neutrophils. These inflammatory changes progressed gradually during PUO, reaching a peak after 3 days. Seven days after PUO, the cellular infiltration decreased and was replaced by an increase in the number of fibroblasts. These changes were most marked in the ventral prostate, followed next in severity by the dorsal lobe and the coagulating gland. The number of fibroblasts showed a further increase 7 days after relief of the obstruction, despite a decrease in cellular infiltration (Fig. 1). Masson trichrome staining demonstrated that fibrosis of the prostatic stroma appeared immediately after the urethral obstruction and progressed even after the obstruction was released (Fig. 2).

Histochemical examination for MP and HRP revealed no significant staining in the prostatic epithelium or stroma of the experimental or control rats. Application of polyclonal antibody against rat THG did not detect THG in the prostatic stroma of any animal in group 1. Of the rats in group 3, two rats received an intravesical injection of LY 3 days after PUO. LY was noted in the interstitial space of the prostate 2 h later (Fig. 3). No fluorescence activity was observed in the control rats or the rats administered saline instillation.

In the rats in group 2, PUO for 3 days led to a significant increase in the capacity of the bladder and in the maximum leakage pressure ($P < 0.05$). Findings returned to normal 4 days after relief of the obstruction (Table 1).

All urine samples proved negative for aerobic and anaerobic bacteria.

Discussion

Prostatitis is classified as acute bacterial prostatitis, chronic bacterial prostatitis and nonbacterial prostatitis (NBP), and as prostatodynia [11]. NBP, which is defined as prostatic inflammation without the identification of pathogens, is responsible for as many as 90% of the cases of prostatitis. *Chlamydia trachomatis* [10], *Myco-*

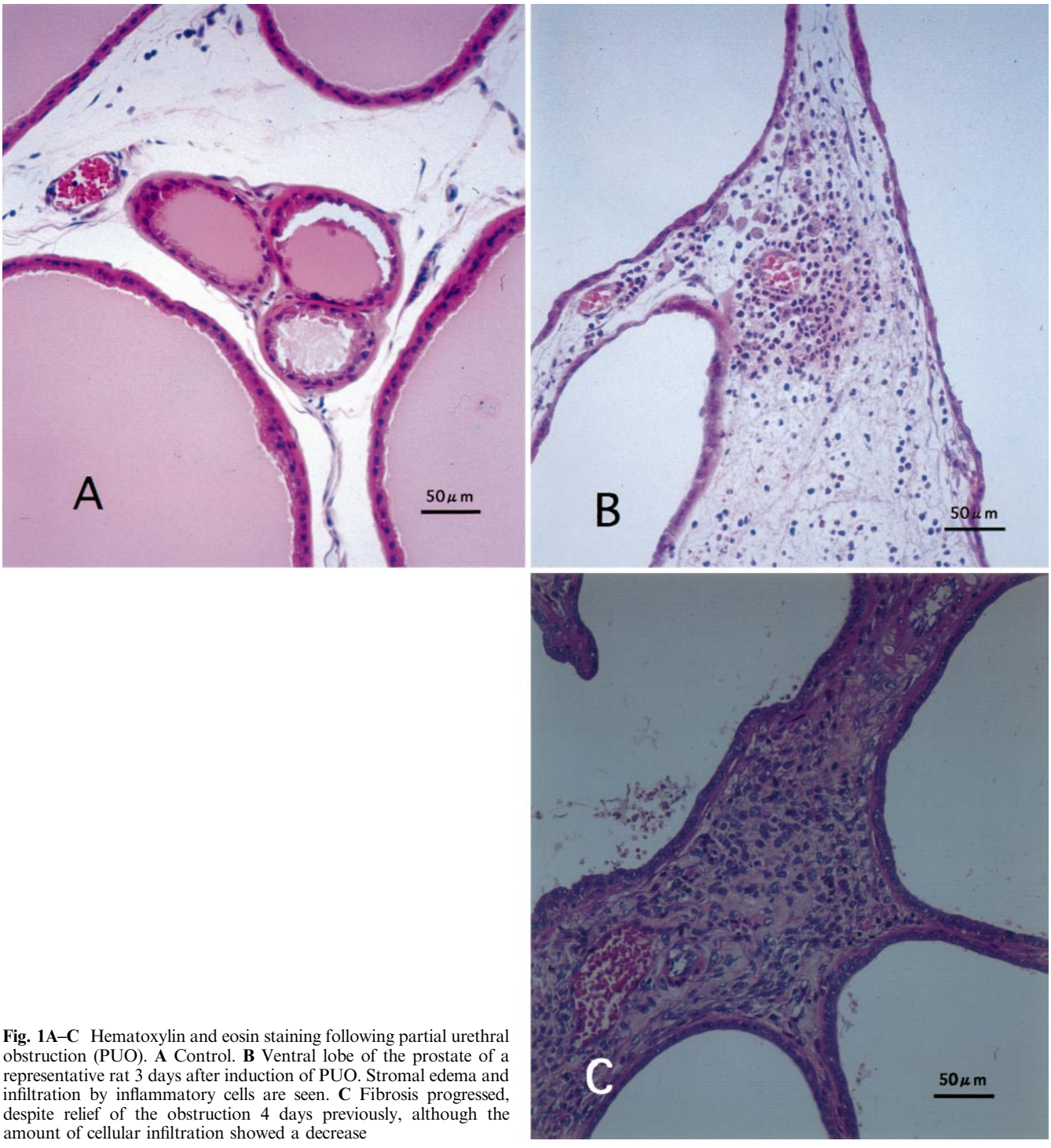

Fig. 1A–C Hematoxylin and eosin staining following partial urethral obstruction (PUO). **A** Control. **B** Ventral lobe of the prostate of a representative rat 3 days after induction of PUO. Stromal edema and infiltration by inflammatory cells are seen. **C** Fibrosis progressed, despite relief of the obstruction 4 days previously, although the amount of cellular infiltration showed a decrease

plasma hominis, *Ureaplasma urealyticum* [12], and other unidentified infectious organisms, have been implicated in NBP but have not been proven to be causal agents in this disorder. The etiology and pathogenesis of NBP remain unclear, mainly because of the lack of appropriate experimental models. Taguchi et al. [13] reported that mice thymectomized at 3 days of age developed chronic prostatitis followed puberty. Keetch and associates [5] reported that the parenteral administration of prostatic homogenates to synergic mice led to the development of NBP. These animal models have failed to clarify the pathogenesis of clinical NBP.

Recent evidence suggests that the intraprostatic reflux of urine may lead to NBP. The injection of carbon particles into the bladder of patients with NBP was followed 72 h later by the finding of such particles in the macrophages in the expressed prostatic secretions [6]. Persson and Ronquist [9], who corroborated

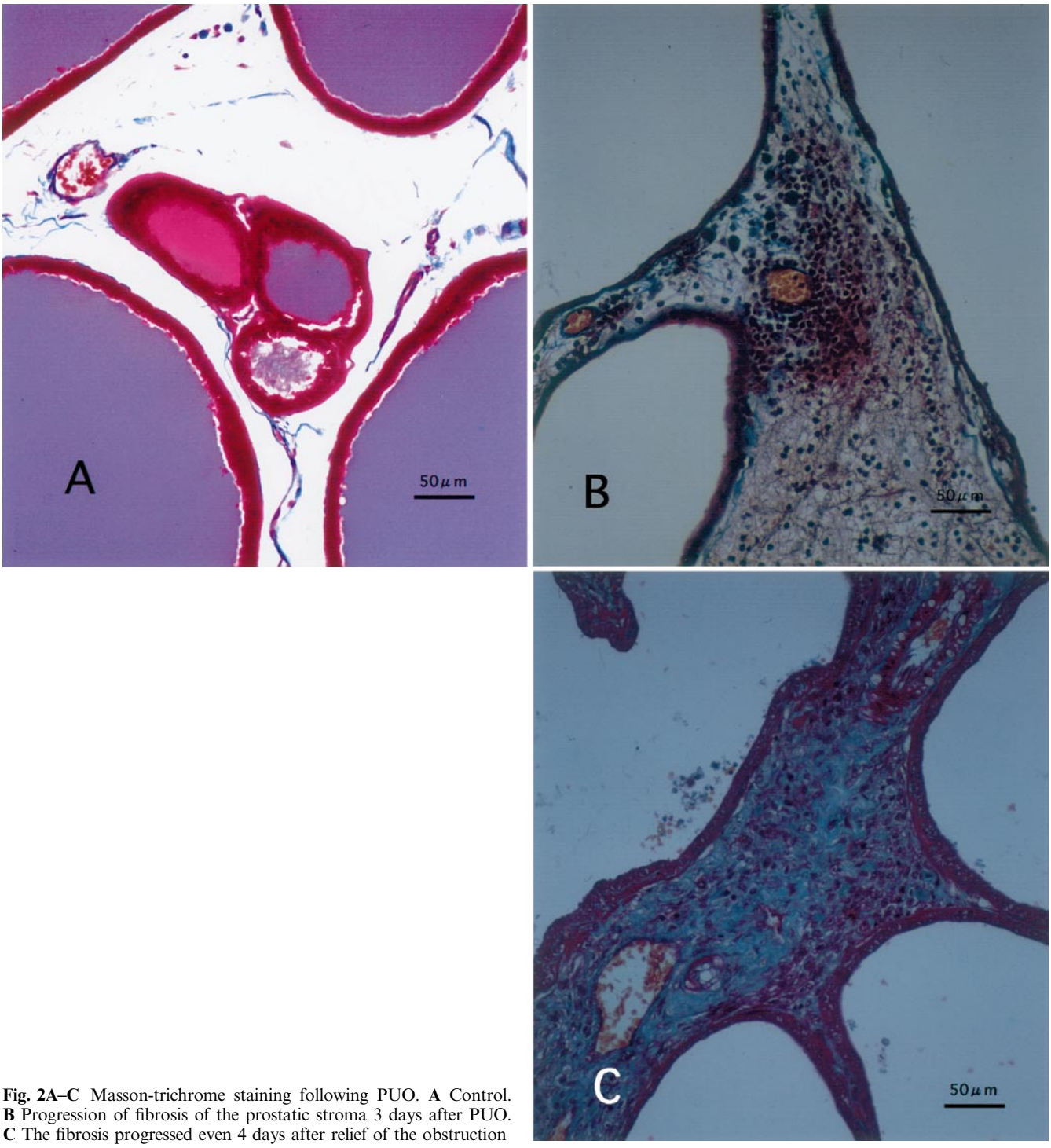

Fig. 2A–C Masson-trichrome staining following PUO. **A** Control. **B** Progression of fibrosis of the prostatic stroma 3 days after PUO. **C** The fibrosis progressed even 4 days after relief of the obstruction

this finding, have suggested that NBP may represent an inflammatory response to the intraprostatic reflux of urate. Those investigators showed that the symptoms of patients with NBP were related to the concentration of urate or creatinine in the expressed prostate secretion. High pressure in the prostatic urethra due to a spasm of the muscles of the pelvic floor may contribute to the intraprostatic reflux [4]. However, there is no evidence that any substance in urine can penetrate the prostatic stroma beyond the basement membrane.

The present study examined the prostate of rats with partial obstruction of the urethra and observed histologic changes. We showed that only substances with a low molecular weight could penetrate the prostatic interstitial space and, hence, are possible causal agents in this model of NBP. We believe that LY (MW 500) achieved a passive permeation of the epithelial layer.

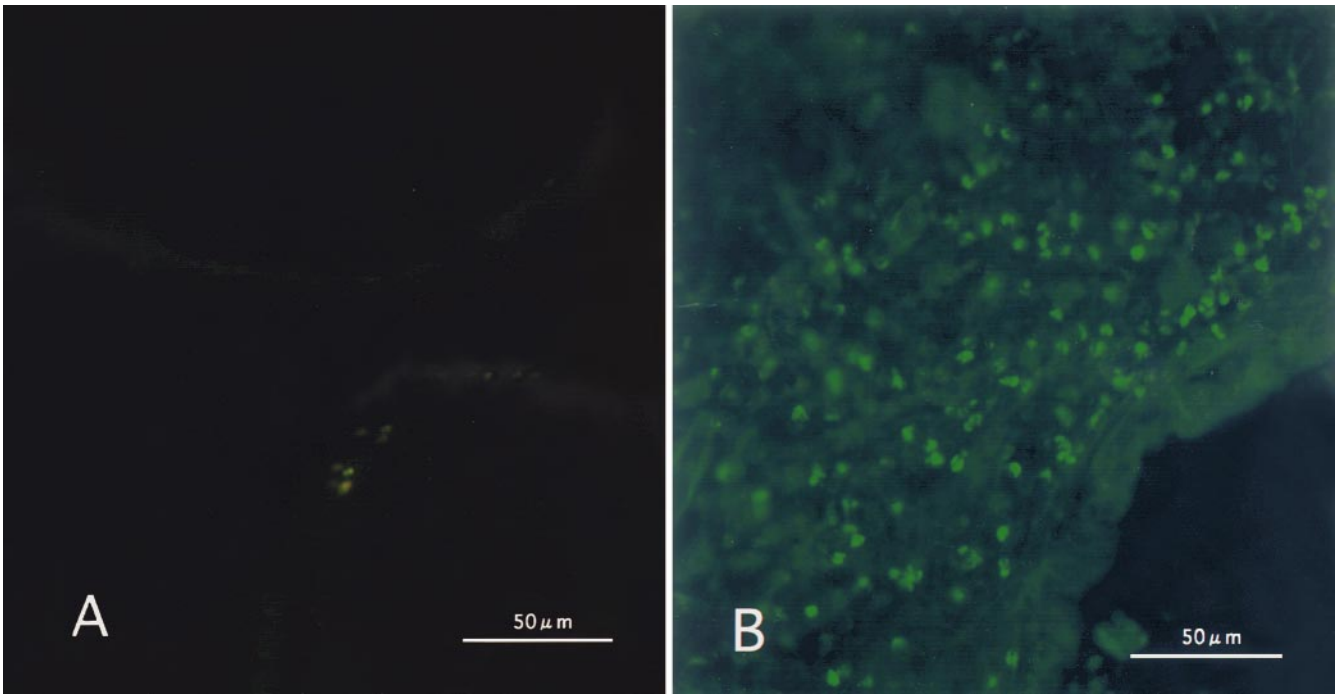

Fig. 3A, B Fluorescence microscopy using lucifer yellow (LY) following PUO. **A** Ventral lobe of the prostate of a control rat, in which LY had been instilled into the bladder 2 h earlier. **B** Ventral lobe of the prostate of a rat 3 days after PUO, in which LY had been instilled into the bladder 2 h earlier. LY was present in the interstitial space of the prostatic stroma. The epithelial layer also exhibited staining for LY

Table 1 Analysis of cystometric parameters (Statistical analysis was done using Welch's test. *PUO* partial obstruction of the urethra, *ROU* release of urethral obstruction)

	Leak pressure (mmHg)		Capacity (ml)	
Control ($n = 5$)	36.8 ± 3.9	* *	2.08 ± 0.36	* NS
3 days PUO ($n = 5$)	110.0 ± 10.0		4.30 ± 0.79	
4 days ROU ($n = 5$)	30.0 ± 7.1		2.00 ± 0.14	

*$P < 0.05$; *NS* no significant difference

The present data support the hypothesis of Persson and Ronquist [9] that NBP is the result of an inflammatory response to urate (MW 190). Osmolar change caused by water permeation is another possible cause.

In conclusion, by inducing PUO in the rat we developed a model of NBP that may be useful in studying the cause of this disorder.

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