

An experimental model for ascending acute pyelonephritis caused by *Escherichia coli* or proteus in rats

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SUMMARY Experimental, ascending acute pyelonephritis in rats was produced by injecting 0.5 ml of 10^9 bacteria/ml into the urinary bladder via the urethra. No traumatic manipulation of the ureters of kidneys was necessary. A grading system for kidney lesions based on macro- and microscopical examination was used.

The capacity of different *Escherichia coli* and proteus strains to induce acute pyelonephritis was tested, and the *E. coli* 06K13H1 strain and the *Proteus mirabilis* 03H1 strain were especially capable of causing urinary tract infection. For the *P. mirabilis* 03H1 strain, a dominance of right kidney lesions was noted in contrast to the *E. coli* 06K13H1 strain which did not show any side preference.

Experimental *in vivo* models are necessary for studies of urinary tract infection (UTI). Several procedures have been developed to study the pathophysiology of kidney damage, the effect of antibiotic treatment, prophylactic immunisation, and other aspects on the host-parasite relationship in UTI.¹⁻¹⁰

Several animal species used do not readily develop UTI, and most experimental models therefore include mechanical manipulations of the urinary tract to ensure a high frequency of pyelonephritic infections. Examples of such manipulations are bladder or kidney massage,^{1 4 11 12} abdominal operations, often with temporary ureteral ligation or puncture of the kidneys or bladder wall,^{2 5 7 13-16} and introduction of foreign bodies into the bladder.^{12 17-19} Forced diuresis by the addition of glucose to the drinking water also facilitates the development of pyelonephritis.^{6 13} Hormone treatment for induction of pyelonephritis has given contradictory results.^{20 21}

Urinary tract infection in man is considered to be of ascending origin, except in the very young and very old. The experimental methods mentioned above hardly resemble the development of ascending UTI in man. Pyelonephritis after introduction of bacteria via the urethra into the bladder without additional manipulations would be preferable. As

rats have spontaneous ureteric reflux, allowing for easy passage of bacteria up to the renal pelvis, such animals seem to be suitable for experimental ascending pyelonephritis.^{4 18} Several reports have dealt with methods for establishing retrograde UTI in rats but have mostly included manipulations of the urinary tracts.^{2 5 12 16 17 19 22}

The aim of the present investigation was to develop and evaluate a model for ascending acute pyelonephritis in rats, without special manipulations of the urinary tract, suitable for studies on the immune response, and the protective effect of vaccination against UTI.

Material and methods

ANIMALS

Female Sprague-Dawley rats weighing 200 to 300 g (Anticimex, Stockholm, Sweden) were used. They were fed with pellets and tap water *ad libitum*.

BACTERIAL STRAINS

The following *Escherichia coli* standard strains from the Collaborative Centre for Reference and Research on Escherichia (WHO), Statens Serum Institut, Copenhagen, Denmark, were used (WHO designation in parentheses): 01K1H7 (U5/41), 02K1H4 (U9/41), 04K12H- (Su65/42), 06K13H1 (Su4344/41), 07K1H- (Bi7509/41). Most experiments were performed with the *E. coli* 06K13H1 strain, as it had

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previously been found regularly to cause pyelonephritis in animal models.^{7,8} The following *Proteus mirabilis* and *vulgaris* strains were used (Kauffmann and Perch designation²³ in parentheses): 01H1 (XL), 03H1 (XK), 010H3 (F73). The bacterial strains were cultivated in nutrient broth at 37°C overnight. The bacteria were washed once with saline and then adjusted to the desired concentration. In early experiments the bacterial dose was determined by viable counts on agar plates and optical density, and later with optical density alone.

INFECTION PROCEDURES

Ascending pyelonephritis

The bacteria were introduced via the urethra into the urinary bladder of ether anaesthetised rats using a blunt, slightly bent needle. Different volumes (0.1-2.0 ml) and concentrations of bacteria (10⁷-10¹⁰ bacteria/ml) were tested for infection but the standard dose was 0.5 ml of 10⁹ bacteria/ml in phosphate buffered saline (PBS). The bacteria were injected into the bladder for about a 5-second period.

Blood samples were obtained from 50 rats 15-30 minutes after bacterial injection of 0.25-2.0 ml and cultivated in standard blood culture bottles containing solid and liquid media.

After one week of infection the animals were killed and the kidneys removed under sterile conditions. Cut kidney surfaces were inoculated on modified Drigalski²³ and/or CLED agar plates (Oxoid) for confirmation of the infecting strain. Serotypings of *E. coli* O antigen,²⁴ K antigen,²⁵ and proteus O antigen²⁶ were performed as earlier described.

Microscopical examination was performed blindly on formalin-fixed (10%) kidney specimens.

Grading of kidney lesions

Kidney lesions were graded, based on gross examination and light microscopy using a classification modified from MacLaren:²⁷

- 0 = unaffected kidney
- 1 = microscopical pyelitis
- 2 = microscopical pyelonephritis with no or one to two discrete macroscopical pinpoint abscesses
- 3 = several macroscopical pinpoint abscesses showing coalescence
- 4 = confluent macroscopical lesions occupying less than half of the kidney surface
- 5 = confluent macroscopical lesions occupying more than half of the kidney surface.

Control experiments

- (1) Increased pressure in the renal pelvis caused by

the injected volume has been considered to cause endothelial damage which might facilitate the development of a local pyelonephritis due to bacteria originating from the urinary tracts or the bloodstream.^{4,22} This was tested by injecting sterile PBS (0, 0.25, or 0.5 ml) into the urinary bladder of 60 rats. Immediately afterwards an intracardiac injection of *E. coli* (10³ or 10⁵ bacteria in 0.5 ml) was given. This procedure did not cause haematogenous pyelonephritis. However, 12 animals died within two days, presumably of bacteraemia.

(2) The following control experiment was performed in order to analyse whether serum antibodies can leak into the urine via damaged kidneys and thus interfere with studies on local immunity. Rat anti-*E. coli* 02 serum was injected intravenously into 10 rats, in whom ascending *E. coli* 06K13H1 pyelonephritis had been induced one week previously. In four of these rats, who were later found to have severe acute pyelonephritis (grades 4-5), serum and urine samples were analysed for anti-*E. coli* 02 antibodies. In urine samples obtained one day and one week after the serum transfer, no 02 antibodies of the IgG, IgA, or IgM classes could be detected with the enzyme-linked immunosorbent assay using boiled 02 antigen for coating of the tubes.²⁸ A significant rise in serum antibodies could be recorded, however.

Statistical calculations were made using the χ^2 test and the Kolmogorov-Smirnov test.²⁹

Result

PYELONEPHRITIS CAUSED BY *E. coli* 06K13H1
 Intravesical introduction of large volumes (0.6-2.0 ml) caused bacteraemia with the 06K13H1 strain (Table 1), as determined with blood cultures 15-30 minutes after the bacterial injection. As 0.5 ml caused no bacteraemia, this was used as the standard volume in subsequent ascending pyelonephritis experiments. The standard procedure with 0.5 ml of 10⁹ bacteria/ml gave rise to acute pyelonephritis in about 60% (grades 2-5, value of worst damaged

Table 1 *Bacteraemia in rats as determined with blood cultures obtained 15-30 minutes after intravesical injection of E. coli 06K13H1 (10⁹ bacteria/ml)*

Volume injected (ml)	Total no. of animals	No. of animals with positive blood culture
2.0	7	7
1.0	8	8
0.75	9	4
0.6	6	1
0.5	10	0
0.25	10	0

Table 2 Frequency, grading, and side of renal lesions in rats intravesically infected with *E. coli* 06K13H1 or *P. mirabilis* 03H1. Grading system as in Material and methods

Bacteria injected	No. of rats with kidney lesions*	No. of rats with pyelonephritis localised to:			No. of rats with pyelitis	Grading of kidney lesions						
		Right kidney	Left kidney	Both kidneys		0	1	2	3	4	5	
<i>E. coli</i> 06K13H1	113	16	19	47	31	Right	9†	40	16	18	13	17
						Left	23	23	19	10	22	16
<i>P. mirabilis</i> 03H1	135	63	12	59	1	Right	8	5	23	26	36	37
						Left	56	8	13	22	19	17

*Grades 1-5. †No. of kidneys.

kidney). The distribution of renal lesions is shown in Table 2. Reduction of the bacterial dose to 10^7 bacteria/ml in 0.5 ml gave a pyelonephritis rate of only 11%. In control experiments, the same number of bacteria as in the standard procedure, but given in a smaller volume (0.1 ml), caused no pyelonephritis.

PYELONEPHRITIS CAUSED BY OTHER BACTERIAL STRAINS

Injection of the other *E. coli* strains using the standard procedure (0.5 ml; 10^9 bacteria/ml) gave attack rates less than 40%. Infection with the *P. mirabilis* 03H1 strain, standard procedure, gave an attack rate similar to that of *E. coli* 06K13H1 (70-80%). The other proteus strains gave lower frequencies of pyelonephritis (< 50%).

PYELONEPHRITIS IN LEFT AND/OR RIGHT KIDNEY

Analysis of ascending pyelonephritis in rats infected with *E. coli* 06K13H1 or *P. mirabilis* 03H1 revealed a significant difference concerning the distribution of kidney lesions (Table 2). Pyelonephritis caused by *P. mirabilis* 03H1 developed significantly more often in the right kidney than in the left ($p < 0.001$, χ^2 test and Kolmogorov-Smirnov test). Kidney lesions caused by the *E. coli* 06K13H1 strain showed no side preference.

Discussion

The importance of the volume injected into the urinary bladder to cause ascending pyelonephritis has been much discussed.^{2 4 16 18 22 30} The ideal is to use volumes large enough to give reflux without damaging the renal epithelium and causing bacteraemia and haematogenous pyelonephritis.¹⁶ In the present study, intravesical injection of large volumes (0.6-2.0 ml) of *E. coli* 06K13H1 caused bacteraemia. Similar findings were reported earlier.^{4 22} Even a volume of 0.5 ml has been shown to give positive blood cultures.^{4 22} In the latter study, this was

found in rats deprived of drinking water. In the former, several bladder squeezes were performed immediately after the bacterial injection.

In adult rats with free access to drinking water, reflux regularly occurs at 0.5-0.6 ml, although it has also been shown to occur at lower volumes.^{18 22 30} In rats deprived of water, reflux occurs regularly at lower volumes (0.25 ml).²²

In the present investigation, the optimal volume for developing pyelonephritis was 0.5 ml, with which about 60% of the animals contracted disease. Similar attack rates were reported by Vivaldi *et al.*,¹⁷ Andersen and Jackson,³⁰ and Fierer *et al.*²² In the two first studies, however, manipulations or larger volumes were used.

The work by Morgan *et al.*³¹ showed that intravesical injection of sterile saline caused a decrease in the ability of rats to clear the kidneys of bacteria injected intravenously. These experiments were terminated after 24 hours, and the frequency of pyelonephritis was not determined. In the present investigation, intravesical injection of up to 0.5 ml of sterile PBS, followed by intracardiac injection of *E. coli* 06K13H1, caused no pyelonephritis. This suggests that the volume of 0.5 ml caused no severe epithelial damage. In the report by Fierer *et al.*²² 1.0 ml of sterile broth infused into the bladder, followed by *E. coli* injected intravenously, caused pyelonephritis.

The optimal number of bacteria injected to cause pyelonephritis was in this series determined as 10^9 bacteria/ml for the *E. coli* 06K13H1 and the *P. mirabilis* 03H1 strains. Reports from experiments using other bacterial strains have shown differing doses and attack rates.^{2 5 12 16 20 30} This might be explained by the fact that bacterial strains with different virulence factors such as O and K antigens and serum resistance have been used, or that differences in susceptibility to bacteria exist among rat strains. Another explanation could be that, although a defined inoculum is injected, the rats often void shortly afterwards, making the exact infecting dose thus hard to define.

As pointed out by Heptinstall,³² organisms residing in the rat urethra may be carried up to the kidney during injection and cause infection. To exclude this possibility, the identity of the infecting strain should be confirmed for the renal isolates. In the present work this was done with serological methods.

To compare renal damage in immunised and control groups of animals, a relevant grading system is important. Often only grading of pyelonephritis into damaged-non-damaged kidneys has been used.^{16 22 30} Statistical calculations have also been performed on mean values of arbitrary units.^{5 27} A more elaborate grading system based on both macro- and microscopical kidney appearances was adopted in this study, using the non-parametric Kolmogorov-Smirnov test for the statistical calculations.

In this work a marked predominance of *P. mirabilis* 03H1 pyelonephritis in the right kidney was found, compared to *E. coli* 06K13H1 induced pyelonephritis. No obvious explanation for such side preferences could be found. In the work of Adler *et al.*¹² no such side differences were shown for *E. coli* and *proteus*. In most studies in which no manipulation of one of the kidneys or ureters has been done, separate values for right and left kidneys are not given. The possibility of different attack rates on right and left kidneys favours the use of the graded value of the worst damaged kidney for statistical calculations.¹⁰

In conclusion, the method presented for inducing ascending acute pyelonephritis in rats is reliable and simple and requires no special manipulations of the urinary tracts. An appropriate volume and number of bacteria should be injected and virulent bacteria should be used.

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