Experimental Rat Model for *Corynebacterium renale*-Induced Pyelonephritis

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Received for publication 16 June 1977

The laboratory rat was able to serve as a model for ascending pyelonephritis after implantation of a zinc disk coated with *Corynebacterium renale* into the urinary bladder because it satisfied three different criteria for infection. The production of an alkaline urine and the presence of significant numbers of *C. renale* in the kidneys, as well as distinct pyelonephritic lesions as revealed by histological examination, were observed in all rats infected with *C. renale*. Control rats that harbored sterile disks in their urinary bladders exhibited none of the above effects.

*Corynebacterium renale* is the etiological agent of a naturally occurring pyelonephritis in cattle. This is a commonly encountered urinary tract disease, with cases being reported throughout agricultural areas of North America and Europe (19). The study of the pathogenesis of this disease has been hampered by the lack of a good experimental model. Until recently (20), experimental infections have been achieved in laboratory animals, notably mice, only by the hematogenous route (13, 15–17).

There has been controversy as to whether bovine pyelonephritis is hematogenous in origin or is strictly localized in the urinary tract, which has been invaded via the urethra (3, 4, 11, 16). Most investigators in this area now agree that evidence favors the view that *C. renale*-induced pyelonephritis in cattle is an ascending infection (8). In addition, attempts to produce the disease in cattle have failed when the bacterial cells have been administered by routes other than the urogenital tract. When cultures are injected into the urinary bladder by way of the urethra, success is not always attained, but the disease unquestionably has been established in this way by several groups of workers (9–12).

The purpose of this investigation was to develop an experimental animal model for the study of ascending pyelonephritis caused by *C. renale* by a method (22) originally designed to induce the formation of infected urinary bladder stones.

**MATERIALS AND METHODS**

**Bacteria.** The strain of *C. renale* used for this study was isolated from the urine of a pyelonephritic cow at the Teaching Hospital, College of Veterinary Medicine, University of Georgia, and designated as 1321.

These bacterial cells were cultivated on 10 ml of brain heart infusion broth at 37°C with shaking at 150 rpm on a New Brunswick model G-2 gyrotary shaker. When the *C. renale* cells were to be used to infect rats, they were washed twice with 0.85% sodium chloride by centrifugation at 1,085 × g for 10 min and suspended in 1.0 ml of saline. These suspensions were found to contain 1.45 × 10⁶ to 2.7 × 10⁶ colony-forming units (CFU) per ml by serially diluting these suspensions and plating them onto brain heart infusion agar.

**Animals.** Charles River outbred male albino rats weighing between 150 and 250 g were housed in standard metabolic cages in which fine mesh screening and filter paper were placed between the animal and the urine-collecting vessel to prevent solid waste and food particles from coming in contact with the excreted urine. The rats were fed standard chow pellets ad libitum, and fluids were administered either ad libitum or in fixed amounts by the use of graduated cylinders in an attempt to control fluid intake.

Attempts to induce pyelonephritis in these rats was initiated by the method of Vermeulen and Goetz (22). After anesthetization of a rat with sodium pentobarbital by the intraperitoneal route, a sterile zinc disk (6 mm in diameter) that had been dipped in a saline suspension of *C. renale* and carried approximately 1 × 10⁶ cells was inserted directly into the urinary bladder by way of a suprapubic incision. The noninfected controls were treated in precisely the same manner except that a sterile disk that had not been dipped into a suspension of *C. renale* was implanted in the urinary bladder. The zinc disks were prepared by Ralph Morton of the Instrument Shop, University of Georgia, from a sheet of commercially available zinc by the use of a Whitney hand punch. The rats were deprived of food and water for 6 to 8 h after the operation.

Estimation of the number of *C. renale* in the blood...
after insertion of C. renale-coated zinc disks in the urinary bladders of test rats was attempted at 3, 5, and 7 days postinfec- tion by removing approximately 0.5 ml of blood via cardiac puncture, distributing the blood into five 0.1-ml samples, and incubating each of the samples in 10 ml of brain heart infusion broth at 37°C for 2 weeks. Samples of blood were plated directly onto brain heart infusion agar. The broth and the agar were examined daily to detect growth of C. renale.

Urine samples were collected by surgically exposing the urinary bladder, retracting it, and removing the urine with a 1.0-ml syringe. Plate counts of C. renale in the urine of infected as well as control rats were made by plating the samples onto brain heart infusion agar and incubating the plates at 37°C.

Detecting infection. Fresh 6- to 8-h urine samples were collected from each infected as well as noninfected rat on a daily basis, and the pH of each sample was measured with a Corning model 12 pH meter equipped with an expanded scale. Statistical analysis was performed by the Student t test, and the data were expressed as the mean ± the standard error of the mean.

All rats were necropsied either after ether euthanasia at 7 days postinfection or after dying of the infection, whichever occurred first. Both kidneys were removed and bisected longitudinally. For histopathological examination, one-half of each kidney, as well as the entire urinary bladder, was fixed in 10% buffered Formalin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin. Renal sections were coded so that the histopathological evaluations were done without prior knowledge of the identity of the specimen in regard to the status of bacterial infection.

The remaining half of each bisected kidney was used for the quantitation of C. renale in the renal tissue. The specimen that had been weighed and immersed in 18 ml of saline was mixed with a Virtis homogenizer model 23 (no. 6-105 AF). A portion of the homogenate was serially diluted onto brain heart infusion agar. The agar plates were incubated at 37°C and examined after 24 and 48 h for C. renale colonies.

RESULTS

Urine pH. The mean urine pH of 10 rats that had sterile zinc disks implanted in their urinary bladders was slightly below neutrality (pH 6.85 ± 0.40). This was well within the range of the urinary pH often reported for normal rats, that is, noninfected animals on which surgery had not been performed. This should be contrasted with the distinctly alkaline urine (pH 8.14 ± 0.30) of the 18 C. renale-infected rats. The difference in the pH of the urine between the infected and control rats was significant (P < 0.01).

Bacteriological data. At 7 days after infection of rats with C. renale strain 1321, plate counts of serially diluted kidney homogenates showed that these rats harbored an average of 6.73 ± 0.68 log10 colony-forming units of C. renale per g of kidney. A high C. renale content of the kidney was usually correlated with a high urinary pH. However, plate counts of the kidney homogenates of rats that had sterile zinc disks inserted in their urinary bladders did not reveal detectable numbers of C. renale.

A few hours before the rats were necropsied, urine samples of C. renale-infected rats, which were removed from the urinary bladder after a surgical procedure, were found to contain 6.3 log10 colony-forming units of C. renale per ml of urine. The only feasible method for obtaining a sufficient quantity of uncontaminated urine appeared to require the removal of the urine directly from the bladder after exposing the bladder surgically. Consequently, to avoid any unnecessary trauma to the rats, plate counts were made on urine samples only at one time interval postinfec- tion. Absolutely no C. renale cells were detected in the urine of the control animals.

No C. renale cells were recovered from the blood of the infected rats at any of the time intervals tested (3, 5, or 7 days postinfection).

Gross pathology of the kidney. A comparison was made of the gross appearance of the kidneys of rats that had C. renale-coated zinc disks in their urinary bladders with those that had sterile zinc disks in their bladders. At the end of 7 days, the kidneys of the infected rats were enlarged. The renal pelvis was dilated and contained a purulent exudate. There was a necrotic and ulcerative papillitis and complete or partial disappearance of the tip of the papilla (Fig. 1).

Histopathology. The kidneys and urinary bladders of rats that had C. renale-coated zinc disks implanted in their bladders were examined microscopically. Of the 24 rats harboring the disks impregnated with C. renale, 18 developed pyelonephritis as well as urinary bladder infections. Four of the remaining six animals developed lesions of the urinary bladder, whereas two of them exhibited no lesions whatsoever. Histological examination of the 18 rats that were

FIG. 1. Comparison of a kidney of a rat infected with C. renale and a normal rat kidney. The injected kidney is enlarged and contains a dilated pelvis. There is necrosis of the renal papilla (arrow).
assessed as demonstrating renal involvement revealed important and severe changes in the renal pelvis and medulla (Fig. 2). The tip of the renal papilla was necrotic, and calcareous deposits were randomly distributed in necrotic and adjacent tissues. A zone of polymorphonuclear leukocytes and intense collateral hyperemia divided necrotic areas from living tissues of the pyramid. Tubules in the medulla adjacent to necrotic tissue had undergone granular degeneration, were sometimes dilated, and occasionally contained casts. There were dense concentrations of leukocytes in and around these tubules. Bacteria were present in the medulla of some kidneys. As the lesion progressed, immature granulation tissue was observed in the medulla. The inflammatory process extended into the cortex in some kidneys. The renal lesion was characterized as an acute necrotizing pyelonephritis. The control rats that had only sterile disks implanted in the urinary bladder had no remarkable lesions in their kidneys with the exception of an occasional randomly distributed calcareous deposit (Fig. 3).

The microscopic features of urinary bladders from rats infected with *C. renale* included massive necrosis and erosion of transitional epithelium of the mucosa (Fig. 4). Inflammatory cells, principally polymorphonuclear leukocytes with lesser numbers of lymphocytes, extended into the submucosa. Squamous metaplasia of transitional epithelium was observed in some areas adjacent to mucosal ulceration. Calcareous deposits were scattered throughout necrotic tissue, and occasional bacteria were observed. This pathological process was diagnosed as an acute necrotizing cystitis. The urinary bladders of rats into which sterile zinc disks had been implanted were characterized by focal mucosal ulceration and focal necrosis (Fig. 5). Inflammatory cells were often present in the mucosa and submucosa, and/or foreign body granulomas were induced as a result of the trauma of disk implantation.

**DISCUSSION**

The availability of a suitable laboratory model is essential for the study of the pathogenesis of *C. renale*-induced pyelonephritis. Some success has been achieved by the intravenous injection of *C. renale* into mice. However, considering the ascending nature of the infection in the natural host, the hematogenous route does not appear to satisfy all of the criteria for a good model of bovine pyelonephritis. The initiation of infection in the urinary bladders of rats by means of the

**FIG. 2. Renal papilla of a rat infected with *C. renale*. Observe the zone of necrosis, granular degeneration of tubular epithelium, and polymorphonuclear leukocytes in the medulla. Hematoxylin and eosin. x115.**

**FIG. 3. Renal papilla of a normal rat. Hematoxylin and eosin. x65.**
insertion of *C. renale*-coated zinc disks followed by pyelonephritis proved to be a suitable model for the study of this disease. In the majority of cases, infected rats became listless and tended to roll themselves into a ball and die by day 6 or 7 of infection.

All three parameters tested, namely, urine pH, bacteriological plate counts of *C. renale* in the kidneys, and histological examination of renal tissue, revealed the presence of *C. renale*-induced pyelonephritis. It has been shown that *C. renale* possesses a powerful urease (14, 18), and it has been suggested that the alkalinity associated with the liberation of ammonia from the urease-catalyzed hydrolysis of urea may be, at least in part, responsible for the necrosis of kidney tissue associated with pyelonephritis (2). Consequently, it seems quite conceivable that the alkaline urine of the *C. renale*-infected rats may be due to the production of ammonia resulting from the breakdown of urea. The gross appearance and histological condition of the renal tissue of infected rats bore a very close resemblance to descriptions given of the renal tissue of pyelonephritic cows. The criteria for determining histopathological evidence of acute pyelonephritis in rats consisted of purulent inflammation and necrosis that involved the renal pelvis, collecting ducts, and renal parenchyma (21). Likewise, the urinary bladders of infected rats showed signs of an acute necrotizing purpurative cystitis, whereas the rats that had sterile disks inserted in their bladders displayed some focal necrosis and/or foreign body granulomatous tissue due to the trauma of disk implantation. Boyd (1) noted that bladders of cows infected with *C. renale* are thickened and the mucosa is superficially ulcerated and covered with a slimy secretion mixed with shreds of tissue and fibrin. The kidneys are greatly enlarged, papillae are necrotic, and abscesses occur throughout the kidney structure. Similar conditions were observed in the rats infected with *C. renale* in this investigation.

It should be noted that, quite recently, a retrograde infection has been produced in mice by inoculation of *C. renale* into the urinary bladder (20). However, the rat model described in this communication has already been used to study the pathogenesis of *C. renale*-induced pyelonephritis (R. J. Jerusik, et al., Can. J. Microbiol., in press). Pyelonephritis produced in the rat as an experimental model could be prevented by the daily oral administration of the commonly used urease inhibitor acetohydroxamic acid. Also, a urease-negative mutant of the same strain of *C. renale* used to produce pyelonephritis was not capable of initiating this infection in rats, suggesting the possible involvement of the powerful urease possessed by *C. renale* (14, 18) in the pathogenesis of this disease. Moreover, the rat model was found to be suitable for studying the mechanism of antibody coating of bacteria in the kidney (J. A. Appleton, M.S. thesis, University of Georgia, Athens, 1977). Thus, it is apparent that the rat model described here, as

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**FIG. 4. Urinary bladder of a rat infected with *C. renale*. The transitional epithelium is eroded, and there is necrosis of the mucosa and submucosa. Hematoxylin and eosin. x115.**

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**FIG. 5. Urinary bladder of a rat receiving only a surgically implanted sterile zinc disk. The erosion of transitional epithelium and focal necrosis are the result of trauma from the implanted disk. Mononuclear inflammatory cells, polymorphonuclear leukocytes, and histiocytes are present in urinary bladder wall. Hematoxylin and eosin. x220.**
well as the mouse model reported earlier (20), could serve as a valuable tool for studying various aspects of the pathogenesis of *C. renale*-induced pyelonephritis.

At no time was there evidence of stone formation around the zinc disks at necropsy. Histological examination of the urinary bladder did on occasion reveal calcareous deposits within the bladder tissue itself in both *C. renale*-infected and control rats. Kuzdas et al. (13), using mice intravenously injected with *C. renale*, noted necrotic areas in the kidneys of these mice at necropsy and observed, "When such kidneys were minced with scissors, a slight grating sound was produced indicating the presence of minute calculi. A similar condition, with the presence of calcareous deposits, has been observed in bovine pyelonephritis."

Nevertheless, the lack of the definite struvite (magnesium ammonium phosphate) stone formation around the zinc disks of *C. renale*-infected rats is somewhat surprising. It has been shown that the implantation of *Proteus mirabilis*-coated zinc disks in the urinary bladders of rats resulted in urinary tract infection with an abundant precipitation of struvite (6). Subsequent studies have revealed that urease-induced supersaturation appears to be the primary cause of *P. mirabilis*-induced urinary stones (7), and in vitro perfusion of struvite crystals with undersaturated urine caused crystal dissolution (5). Since *C. renale* is a more active urea hydrolizer than *P. mirabilis*, one might expect stone growth in the urinary bladder of *C. renale*-infected rats within the 7-day duration of the experiments described herein. No immediate explanation can be given for the apparent discrepancy between the behavior of *P. mirabilis* and that of *C. renale* in this regard. Of course, the lack of stone formation in the *C. renale*-infected rats could be correlated with the fact that frank urolithiasis is not a conspicuous feature of bovine pyelonephritis at the time of necropsy.

ACKNOWLEDGMENT

This study was supported by a grant from the University of Georgia Veterinary Medical Experiment Station.

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