Renal Lysozyme Levels in Animals Developing
"Sterile Pyelonephritis"

W. W. EUDY, S. E. BURROUS, AND F. W. SIGLER

Research and Development Department, The Norwich Pharmacal Company, Norwich, New York 13815

Received for publication 4 May 1971

The induction of sterile unilateral pyelonephritis in rats with heat-killed Proteus mirabilis cells is described. The lesions were identical to those produced with viable bacteria. Lysozyme levels in both kidneys of rats developing unilateral sterile pyelonephritis underwent biphasic elevations similar to those produced with viable bacteria. In the injected kidney, the first elevation, associated with the trauma of injection, could be produced by injecting sterile saline. The second elevation was associated with the onset of chronicity in the injected kidney. The nonmanipulated, contralateral kidney showed a similar biphasic elevation, of equal duration but of greater magnitude.

Previous work in our laboratory indicated that trauma to a kidney causes an increase in its soluble lysozyme content (10). This observation agrees with the results of Hamdy (11), who demonstrated that trauma causes elevated levels of soluble lysozyme in poultry tissue. However, in unilateral renal trauma, a simultaneous elevation of the soluble lysozyme content of the contralateral kidney was also observed which was of greater magnitude than and equal in duration to that of the injured kidney (10). Furthermore, in rats developing chronic, unilateral Proteus mirabilis-induced pyelonephritis (7), the levels of renal lysozyme in both kidneys exhibit a second elevation with the contralateral kidney showing the higher lysozyme activity (10).

Incidence of infection in the experimentally uninfected kidney is low (7), and pathological indications of infection are absent (10). These findings do not eliminate the possibility that viable bacteria in the experimentally uninfected kidney are responsible for the observed results. To investigate this possibility and furthermore to investigate the role of the inflammatory response in the tissue destruction characteristic of chronic pyelonephritis, a sterile pyelonephritis model induced with heat-killed bacteria has been developed. This communication concerns the description of this model and the levels of soluble renal lysozyme in the kidneys of rats developing experimentally induced, sterile, unilateral, chronic pyelonephritis.

MATERIALS AND METHODS

Experimental animals. CFN rats (Wistar derived, Carworth Farms) of either sex weighing 160 to 184 g were used. Animals were housed five per wire mesh cage (38 by 35 by 18 cm) and maintained on Purina laboratory chow and water. The cage rooms were lighted from 6 AM to 6 PM. Animals were sacrificed between 9:00 and 11:00 AM.

Injection of dead cells into the kidney. P. mirabilis (Pr-91) was grown overnight in Brain Heart Infusion broth at 37 C on a shaker. The cells were harvested by centrifugation (12,000 x g for 10 min), washed twice with sterile 0.85% saline, suspended in saline at three times the original concentration, and autoclaved at 121 C for 15 min. The dead cells were cooled to room temperature and immediately injected into the kidney cortex by using a previously described technique (7, 10). Kidneys were titered for viable bacteria as previously described (7).

Urease assays. P. mirabilis cells were prepared as described above, and the suspension was divided into two samples. One sample was autoclaved for 15 min at 121 C. Both live and dead bacteria were assayed for urease activity as follows. The cells were harvested by centrifugation at 3,000 x g for 15 min, washed, and suspended in 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 8.0) that was 0.1 M in sodium chloride. The reaction mixture contained 2.4 ml of the cell suspension and 0.1 ml of urea (final concentration, 0.15 M). The reaction mixture was incubated for 10 min at 37 C, and ammonia nitrogen was determined by the method of Seligson and Seligson (18). Activity was calculated as micrograms of ammonia nitrogen liberated per minute, and specific activity was calculated as activity per milligram of cells.

Preparation of renal lysozyme. Kidneys (approximate weight, 1 g) were removed immediately from CO2-killed rats and, after the pelvis was teased away, were placed in 9.0 ml of 0.85% saline at 0 C. All subsequent procedures were carried out at 0 to 4 C. The kidneys were homogenized in a Potter-Elvehjem tissue homogenizer, and the homogenate was centrifuged at 48,200 x g for 10 min. The resulting supernatant fluid served as the source of lysozyme.
Preparation of kidneys for pathological examination. Animals were injected with heat-killed bacteria as previously described. Seventeen days after injection, the rats were sacrificed and their kidneys were fixed in 10% neutral buffered Formalin, dehydrated, cleared, and infiltrated with paraffin. The tissues were then embedded in paraffin, and 5-μm sections made with a rotary microtome were stained with a routine hematoxylin and eosin stain and examined microscopically.

Lysozyme assay. Lysozyme activity was assayed by modifications of the method of Miller et al. (16). The substrate was prepared by suspending 30 mg of lyophilized Micrococcus lysodeikticus cells in 20 ml of 1% NaCl and adding 80 ml of 0.066 M phosphate buffer, pH 6.2. The reaction mixture contained 2.5 ml of substrate and 0.25 ml of kidney homogenate supernatant fluid. The reactions were carried out at room temperature in a 3.0-ml cuvette with a 1-cm light path. The progress of the reaction was monitored for 3 min by measuring absorbance at 650 nm (A650) with a Guilford 300N recording spectrophotometer. Activity as ΔA650/minute was calculated from the slope of the A650 recording. Since kidneys weighing approximately 1 g were homogenized in 9.0 ml of 0.85% saline and activity was determined for 0.25 ml of homogenate supernatant fluid, total soluble renal lysozyme was calculated by multiplying the determined activity by 40. Under these conditions, changes in A650 were linear with respect to enzyme concentration.

RESULTS

Bacteriological evaluation of kidneys. Kidneys injected with heat-killed *P. mirabilis* remained sterile throughout the 17-day observation period.

Urease activity of dead cells. No detectable urease activity was found in the autoclaved *P. mirabilis* cells. The live culture had a specific activity of 0.42.

---

**Fig. 1.** Typical lesion produced by heat-killed *Proteus mirabilis* as seen in sections of the outer medullary zone 3. Hematoxylin and eosin. X 150.
PATHOLOGY OF INJECTED KIDNEYS. Grossly, the lesions were small, pale pits in the cortex (presumably produced by the multiple injections) which were wedge-shaped in cross section. The broad portion of the wedge was located in the outer cortical zone, which Snell (19) refers to as zone 1. The lesions extended through the inner cortical zone 2 and terminated in the outer medullary zone 3. In the outer cortical zone 1, the proximal convoluted tubules displayed epithelial hyperplasia as evidenced by the presence of large basophilic cells containing large irregular hyperchromatic nuclei and showing mitotic figures. Additionally, these hyperplastic epithelial cells occluded the tubular lumina, and there was a slight mononuclear cell infiltration in the neighboring interstitium. The lesion in the inner cortical zone 2 evidenced numerous dilated, empty tubules with minimal cellular infiltration of the neighboring interstitium. In the outer medullary zone 3 (Fig. 1), chronic inflammatory cellular infiltration abounded in the interstitium among the dilated and empty tubules. Since there was no glomerular involvement, these pathologic changes represent those Snell (19) calls chronic nephritis.

Gross and microscopic examination of the contralateral noninjected kidney revealed no visible changes.

RENAL LYSOZYME LEVELS. The lysozyme levels of both kidneys of animals injected with heat-killed *P. mirabilis* were determined for 17 days after injection (Fig. 2). Soluble lysozyme levels showed an immediate increase of 5 days of duration followed by a second increase of 7 days of duration. Fourteen days after the injection of dead cells into the kidney cortex, lysozyme levels in both kidneys had returned to a normal value of 4.36 ± 0.73. In saline-percuted kidneys of control animals, only the initial response was detected (Fig. 3).

DISCUSSION

Recently, the restriction of the term "chronic pyelonephritis" to conditions produced by bacterial infections which demonstrate pelvicalyceal and parenchymal lesions has become fashionable (2, 3, 12). There is no clear evidence that all progressive chronic pyelonephritis has a bacterial etiology (8), and slightly over one-half of the chronic pyelonephritis diagnosed at autopsy is of unknown etiology (9). Much of the experimental work on pyelonephritis models has employed hematogenous or direct infection of the kidney. In neither case can lesions of the pelvicalyceal elements be demonstrated (12). The interstitial nephritis reported here has been evoked by using heat-killed *P. mirabilis* cells, is microscopically and grossly identical to that produced with viable
P. mirabilis (7), and is similar in pathological detail to experimental pyelonephritis resulting from hematogenous infection (12). It also resembles the sterile models of pyelonephritis described by Thelen et al. (20) and Ueda et al. (21), which were produced by subjecting presensitized kidneys to dead bacterial cells or cell products, and to the model described by Braude et al. (6), which was produced with Proteus urease.

Our results support the finding of Thelen et al. (20) and Ueda et al. (21), who demonstrated that pyelonephritis could be induced with dead cells. However, the data reported here indicated that it is not necessary to presensitize or immunize the host before intrarenal injection of the heat-killed cells to produce pyelonephritis. Braude et al. (5) have also demonstrated the production of sterile acute pyelonephritis by using Proteus urease, acetone-killed cells, and acetone-killed Proteus cells with the urease inactivated by mercuric acetate. These authors implicated urease in the pathogenesis of pyelonephritis but did not exclude other equally important factors. The experiments reported here do not exclude urease as a factor in the pathogenesis of P. mirabilis-induced pyelonephritis, but they clearly demonstrate that urease activity is not required for the production of the cortical lesions described.

The data on soluble renal lysozyme reported here support work done in this laboratory (10), which indicated that unilateral renal infection causes a biphasic elevation of lysozyme activity in both kidneys. The first elevation, which can be produced by renal trauma, lasts 4 to 5 days, the time required for the injured kidney to heal and corresponding to a period of severe loss of concentrating ability (15). The second elevation corresponds to the onset of chronicity in the infected kidney. In both cases, the lysozyme responses are greatest in the contralateral kidney.

The persistence of bacterial antigens in pyelonephritic kidneys has been explored in great detail over the past few years. The work of Sanford et al. (17), Cotran (8), and Aoki et al. (4) indicates that bacterial antigen may persist in an experimentally infected kidney as long as 20 weeks after the kidneys have become sterile. Furthermore, with human renal tissue obtained at autopsy or biopsy, Aoki et al. (5) have demonstrated bacterial antigen in six of seven pyelonephritic kidneys which were free from bacterial infection. Similarly, Angell et al. (1) have reported that, in clinical cases of abacterial chronic pyelonephritis, the progressive nature of this disease does not require the presence of demonstrable bacteria. Kass (14), in a discussion of abacterial pyelonephritis, concluded that progressive renal disease in rats occurred only when there was continued bacterial proliferation. By use of intrarenal injection of killed bacteria, Aoki et al. (4) have induced what appear to be chronic inflammatory lesions. The predominant inflammatory infiltrate consisted of round cells rather than polymorphonucleocytes.

The work reported here and elsewhere (6, 21) indicates that lesions typical of chronic pyelo-
Sterile Pyelonephritis

nephritis can be produced in the rat in the absence of viable bacteria.

In conclusion, the work reported here indicates that chronic interstitial nephritis with renal lesions similar to those of chronic pyelonephritis and identical to those of experimental pyelonephritis in animal models produced by hematogenous and direct renal puncture can be produced by heat-killed bacteria. The pathogenesis of the disease does not depend on viable bacteria nor on the presence of active bacterial urease. Soluble renal lysozyme in these injected kidneys is elevated bi-phasingly with the second elevation in lysozyme corresponding to the onset of chronicity in the injected kidney. The soluble renal lysozyme activities of the contralateral kidney show a similar increase, of equal duration but of greater magnitude.

Acknowledgments

The authors gratefully acknowledge the expert assistance, patience, and attention to detail of Roberta Alexander, Barbara Bensko, Carol Dubiel, and Nancy Gallo. Urease assays were kindly provided by Frank Kopko and Jon Andersen. Statistical evaluations were conducted by C. T. Tu.

Literature Cited