Renal Lysozyme Levels in Animals Developing
Proteus mirabilis-Induced Pyelonephritis

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In animals developing experimentally induced unilateral pyelonephritis, both the infected kidney (IK) and the contralateral noninfected kidney (NIK) showed an immediate increase in renal lysozyme activity of about 5 days' duration after the unilateral injection of viable Proteus mirabilis into the renal cortex. Lysozyme activities of the NIK were consistently higher than those of the IK. This initial increase was followed by a second increase which lasted throughout the period of observation (17 days), and enzyme activities of the NIK were consistently higher than those of the IK. In saline punctured kidneys of control animals, both the saline punctured kidney (SP) and the non-saline punctured kidney (NSP) showed only the immediate increase in renal lysozyme activity, which persisted until the SP was completely healed. These enzyme activities were less than those observed in the infected animals, but the response of the NSP was greater than that of the SP. Trauma not directed to the kidney does not produce a similar response of renal lysozyme. The elevated renal lysozyme of the NIK could not be shown to protect it from bacterial infection.

To increase our ability to discover better therapeutic agents for pyelonephritis, we have been attempting to acquire basic information concerning some of the specific chemical events which underlie the gross and microscopic tissue and cellular changes which occur during the course of infection and subsequent healing. Urinary levels of lysozyme (mucoprotein N-acetyl-muramyl hydrolase, EC 3.2.1.17) and various acid hydrolases in patients with urinary levels of these enzymes appear to have limited value in diagnostic procedures (3, 4, 14, 20). Lysozyme levels of urine are elevated in animals by a number of experimental procedures, including renal ischemia in dogs (7), ingestion of mercuric chloride (14), renal homograph rejection in dogs (10), classical runt disease in mice (17), and the injection (intrapertoneal) of egg white lysozyme in rats (9, 13). Recently, the intrarenal distribution of lysozyme in normal rat kidneys (12, 16) and induced changes in the concentrations of renal lysozyme (6, 9, 12, 15) have been reported. The function of renal lysozyme in pyelonephritis and other urinary-tract infections remains unknown, although some authors have speculated that lysozyme acts as a protective antibacterial agent (2, 9, 11). With the development of a rat model in which reproducible unilateral bacterial pyelonephritis can be produced without systemic infection (1), levels of renal and urinary lysozyme associated with Proteus mirabilis pyelonephritis could be determined, and their diagnostic and functional significance could be investigated. The results presented here include some of our efforts in these areas.

MATERIALS AND METHODS

Experimental animals. CFN rats of either sex weighing 160 to 184 g were used in all experimental procedures. These animals were housed five per wire mesh cage (38 by 35 by 18 cm) and maintained on Purina Laboratory Chow and water. Experimentally induced unilateral P. mirabilis (strain Pr-91) pyelonephritis was established by methods previously described (1). Appropriate noninfected controls were prepared similarly by injecting sterile saline into the left kidney of each control animal.

Preparation of lysozyme-containing extract. Kidneys were immediately removed from CO2-killed animals and the pelvis was teased away. These kidneys were placed in 9.0 ml of normal saline at 0 C. All subsequent procedures were carried out in an ice bath. The kidneys were homogenized by three passes in a Potter-Elvehjem tissue homogenizer, and the homogenate was centrifuged at 48,200 × g for 10 min. The resulting supernatant fluid served as the source of lysozyme.

Assay of lysozyme. Lysozyme activity was assayed by modifications of the method of Miller et al. (9). The substrate was prepared by suspending 30 mg of lyophilized Micrococcus lysodeikticus cells in 20 ml of 1% NaCl and adding 80 ml of 0.057 M phosphate buffer (pH 6.2). The reaction mixture contained 2.5 ml of substrate and 0.25 ml of kidney homogenate super-


nant. 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 at 650 nm by use of a Guilford 300N spectrophotometer. Activity as $\Delta A_{650}$/minute was calculated from the slope of the $A_{650}$ recording. Since kidneys weighing approximately 1 g were homogenized in 9.0 ml of normal saline and activity was calculated for 0.25 ml, total renal lysozyme was calculated by multiplying the activity by 40. One unit of activity was defined as the amount of enzyme which causes a change of 1.0 $A$ per minute. Specific activity was defined as units per milligram of protein. Under these conditions, change in optical density of the substrate was linear with respect to cell density. Changes in optical density were linear with respect to time, and activity was linear with respect to enzyme.

Experimental hip injury. Rats were ether-anesthetized and the hip was cleaned with alcohol. A small section of skin (1 cm$^2$) was removed, exposing the muscle. Little bleeding was associated with the injury, and by 9 days the wounds generally were closed.

Bacterial titers. Bacterial titers of the infected kidneys were determined according to the procedure of Burrous and Cavein (1).

Protein determination. Protein concentrations were determined by the method of Lowry et al. (8).

RESULTS

Urinary lysozyme. Lysozyme could not be detected by our methods in normal rat urine or in infected rat urine during any stage of the infection. The failure to detect urinary activity apparently was not due to the presence of urinary lysozyme inhibitors, since renal lysozyme could be diluted in urine without loss of activity.

Renal lysozyme levels in experimental pyelonephritis. Levels of renal lysozyme were assayed during the 17-day course of infection (Fig. 1). Normal values for total renal lysozyme were found to be 4.36 ± 0.73. In the saline punctured (SP) control, the lysozyme level rose rapidly for 4 days and returned to normal by 5 days after the insult. The contralateral nonmanipulated kidney of the saline punctured animal (NSP) showed a similar pattern. The lysozyme content of the P. mirabilis-infected kidney (IK) reached a maximum 4 days after infections, and then dropped into the normal range on day 6. A second increase occurred which lasted throughout the remaining 10 days of observation. The nonmanipulated contralateral kidney of the Pr-91 infected animal (NIK) showed a similar pattern. During the last 10 days of these experiments, renal lysozyme content of the NIK was significantly higher than that of the NSP (Fig. 2). The lysozyme activity of the IK was higher than that of the SP, although these differences were not as great as those between the NIK and the NSP. Figure 3 illustrates the experimental observation that the NIK almost always had a higher lysozyme content than the IK. Generally speaking, these results confirm the observation (6, 9, 16) that bacterial infection or trauma to a kidney elicits an increase in lysozyme activity in that kidney, and they further demonstrate that injury to one kidney elicits a similar and greater
response in the contralateral, nonmanipulated kidney.

**Specific activities of renal lysozyme.** Specific activities of the kidney supernatants were determined for kidneys at 9, 11, and 17 days after infection (Fig. 4). Normal values for specific activity were 0.044 ± 0.012. As was found in the determinations of total activity, the values for the SP and contralateral NSP kidneys were normal during this period. The IK and NIK, on the other hand, showed significant elevation above normal values. They also showed a substantial increase during this period of time.

**Effect of hip injury on renal lysozyme.** To ascertain the specificity of the observed lysozyme response, animals were injured on the hip. Renal lysozyme content was determined over a 9-day period (Table 1). Lysozyme content in the injured animals varied more than in normal animals, but most of the values fell within or overlapped the normal range of 4.36 ± 0.73. It appears that general trauma may cause a slight elevation of renal lysozyme concentration which can be detected 5 days after traumas. Trauma not directly related to the kidney does not, however, evoke a response of the same magnitude as does trauma to or infection of the kidney.

**Infection of both kidneys.** To determine whether
the high concentration of lysozyme protected the kidney from bacterial infection, the following experiment was conducted. Animals were infected as previously described (1). The NIK was infected on day 2 when its lysozyme content was maximal. Lysoyme content and bacterial titer of both kidneys were determined at 24-hr intervals for 9 days. The lysozyme data and bacterial titers of both of the infected kidneys are presented in Fig. 5. No unusual changes in bacterial titer could be attributed to high lysozyme concentrations.

**TABLE 1. Renal lysozyme activity of traumatized animals**

| Days after trauma | Total renal lysozyme |
|-------------------|----------------------|
| Normal            | 4.36 ± 0.69          |
| 1                 | 4.29 ± 1.50          |
| 2                 | 3.80 ± 0.90          |
| 3                 | 4.10 ± 1.20          |
| 4                 | 5.27 ± 0.53          |
| 5                 | 6.50 ± 0.60          |
| 6                 | 5.10 ± 1.70          |
| 7                 | 4.60 ± 1.90          |
| 8                 | 5.20 ± 1.10          |
| 9                 | 3.90 ± 1.60          |

*Animals were injured on the hip, and renal lysoyme levels were assayed during the healing process. Each value represents the average lysoyme activity and standard deviation of at least 10 kidneys.*

**DISCUSSION**

The data presented here indicate that in rats with experimentally induced pyelonephritis, renal lysozyme is elevated in both the infected and non-infected kidney. We have no evidence that the elevation of lysozyme content of the NIK is associated with hypertrophy. Only soluble lysozyme has been considered in this study, and its relation to lysozyme of lysosomal origin (19) is unknown. Using bruised poultry tissue, Hamdy (5) demonstrated that lysozyme activity increased with the severity of the bruise. Also, complete healing coincided with the absence of free lysosomal activities in the traumatized tissues. In the procedures reported here, trauma is associated with injection of saline into the kidney in a control animal or an animal with infection. The lysozyme content of SP kidneys increased slightly during the first 4 days after trauma and rapidly returned to normal values at a time which coincided with complete healing of the kidney tissue (1). The results obtained in infected kidneys during the first 5 days were similar to those obtained with trauma alone. Hamdy (5) noted that concentrations of free lysozyme were over fourfold greater in Escherichia coli-infected bruised tissue than in noninfected bruised tissue. We too have noted higher and more persistent lysozyme levels in the IK than in the saline punctured control.

The concomitant response of the contralateral kidney lysozyme activities in both the saline punctured and infected animals showed results similar

**FIG. 5. Bacterial titers (left graph) and total renal lysozyme (right graph) for left and right kidneys.** The left kidneys were infected at time zero, and 48 hr later the contralateral (right) kidneys were similarly infected.
to those discussed above. The NSP shows an increase in soluble renal lysozyme concentration of similar magnitude and duration to that observed in the SP. The NIK lysozyme levels rose rapidly to three times normal and to almost twice that of the NSP. In addition, the NIK lysozyme activity was consistently higher than that of the IK. These last results remain completely unexplained and have not previously been reported. They might reflect increased serum concentrations of enzyme or increased transport of enzyme from the injured kidney into the circulation. Two conclusions appear warranted. Traumatization of one kidney elicits a lysozyme response in that kidney, as indicated by Sussman et al. (16). Trauma also elicits a concomitant and tissue-specific lysozyme response in the nonmanipulated kidney. The data presented here do not rule out the possibility that trauma to other tissues may induce slight increases in renal lysozyme. The data of Perri et al. (12) indicate that certain manipulations not directly involving the kidney can cause elevations of renal lysozyme activity.

Six to seventeen days after infection, when the infected kidney becomes pyelonephritic, renal lysozyme activities of both IK and NIK remain higher than normal. According to Simmonds et al. (15), an increase in renal lysozyme appears to accompany various aspects of the immune response. This may also be true in the case of pyelonephritis (18). It has previously been reported that the incidence of infection in the NIK in this model is less than 1% (1). During the course of these experiments (about 1 year), the incidence of bacteria in the NIK was observed to be somewhat higher than that previously reported, but pathological examination of the NIK showed no inflammatory response during the 17 days of observation. These observations raise the question of how greatly the presence of bacteria in the NIK influences the results obtained with the infected animals. Experiments with sterile pyelonephritis are currently underway to answer this question. During this same period, the specific activities of the soluble lysozyme in the infected and noninfected contralateral kidneys increase (Fig. 4).

The significance of this observation remains obscure.

Experiments have been conducted to evaluate the role of high lysozyme levels in preventing P. mirabilis infection in the NIK. In numerous experiments, the bacterial titers follow a course similar to that illustrated in Fig. 5. The logarithm of the geometric mean of the kidney titer is consistently elevated by 0.5 to 1.2 above the initial value of 6.0 to 7.5 during the first 24 hr; then it gradually declines to 5.0 ± 0.9 by the end of 17 days. Any protection afforded the contralateral kidney might prevent the increase of titer during the first 24 hr or hasten the reduction observed thereafter. However, the NIK showed no increased resistance to infection as indicated by bacterial titers, in agreement with the finding of Miller et al. (9).

In our system, the greatest increase in renal lysozyme was found in the noninfected contralateral kidney of the infected animal. Susman et al. (16) have observed that unilateral nephrectomy causes elevation of the lysozyme content of the remaining kidney which they attributed to an increased glomerular filtration rate. When both kidneys are intact, unilateral ligation of the ureter causes a significant reduction in lysozyme content of the kidney with the ligated ureter. This decrease in renal lysozyme was attributed to a decreased rate of glomerular filtration of lysozyme. In the results presented here, the filtration theory does not appear to be adequate. If blood is the source of renal lysozyme, one would assume that lysozyme content of the nonmanipulated NIK and NSP might be elevated. However, the SP and IK kidneys would be expected to show decreased activity. From the data presented here, it appears more likely that an activation or induction phenomenon is involved. It is not necessary, however, that one mechanism must account for both sets of observations.

The implications of these observations for chemotherapy of urinary-tract infections are under investigation.

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