Pyelonephritis XV. Long-term Study of Ascending
Escherichia coli Pyelonephritis in Mice

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Keane and Freedman (1) demonstrated that diuresis produced by feeding 5% glucose rendered mice susceptible to acute ascending pyelonephritis with Escherichia coli. We reported the use of this model in therapeutic studies of E. coli (2) and Proteus mirabilis (3) pyelonephritis. The present investigation, a bacteriologic, immunologic, and pathologic study of long-term infection with E. coli shows that pyelonephritis persisted and progressed for the duration of the study (32 wk).

MATERIALS AND METHODS

Animals. Random-bred male Swiss-Webster mice weighing 20–25 g were obtained from local commerical sources.

Bacteria. Escherichia coli strain Yale, originally isolated from a patient with urinary tract infection, was used.

Media. Brain–heart infusion broth and agar (Difco) were used. Blood agar base (BBL) with 5% defibrinated sheep erythrocytes was used to grow the E. coli directly from infected kidneys during mouse passage to maintain virulence.

Production of diuresis. Experimental animals were fed 5% glucose in water ad lib.; controls were given tap water ad lib. In addition, restriction of food to 4 g per day per mouse (Purina-Ralston) was found to result in a lower urinary specific gravity in diuresed mice. Therefore, both diuresed and nondiuresed mice were provided only 4 g per day. Growth was normal; the mice approximately doubled their body weight over the 32 wk of study. Diuresed animals were started on glucose 1 wk before infection and continued so until sacrifice.

Production of pyelonephritis. Pyelonephritis was produced as previously described (2). At time of sacrifice both kidneys were removed, homogenized separately, serially diluted, and quantitatively assayed by plate count. Urine was as-
pirated from the bladder, cultured, and specific gravity determined. Blood was obtained from the heart at time of sacrifice, allowed to clot, serum separated, and frozen at \(-20^\circ\text{C}\) within 3 hr.

**Immunologic studies.** Antibacterial antibody was measured by agglutination and hemagglutination. Agglutinating antibodies were determined by serial dilution of serum with live *E. coli* as antigen. Hemagglutinating antibody was measured using as antigen sheep erythrocytes coated with extract prepared by boiling the bacteria. In both studies, sera were diluted in 0.85% NaCl. End point in each instance was the highest dilution of serum giving definite agglutination.

Serum bactericidal activity was done as previously described (4) by mixing in final concentration per milliliter, \(10^8\) bacteria, 1:25 guinea pig complement (Hyland) and serial serum dilutions in minimal medium (5). Surviving bacteria were determined by plate count after 1 hr incubation at 37°C.

Antikidney antibody was measured against both saline (0.85% NaCl) extract and specific soluble substance prepared from normal mouse kidney. Saline extract was prepared by rinsing kidney slices in saline until free of visible blood, followed by homogenization in 2 vol of saline. After overnight storage at 4°C, debris was removed by low-speed centrifugation and supernatant stored at \(-20^\circ\text{C}\). Extract was coated on tanned sheep erythrocytes and agglutination measured with incubation of serum serially diluted in 2% normal rabbit serum in 0.85% NaCl. Specific soluble substance was prepared by the method of Cole et al. (6) and similarly tested by coating on sheep erythrocytes.

**Histologic study.** At time of sacrifice, a slice of each kidney was placed in neutral formalin and sections stained with hematoxylin eosin and periodic acid–Schiff.

**Blood urea nitrogen (BUN):** The blood urea nitrogen (BUN) was determined by urea test kit (C. F. Boehringer and Soehne GMH).

**RESULTS**

Mice were sacrificed in groups of five to six from 1 to 32 wk after infection. The results of bacteriologic examination are shown in Table 1. Overall one-half

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**TABLE 1**

**BACTERIOLOGIC FINDINGS AFTER INTRABLADDER INOCULATION OF *Escherichia coli* YALE INTO MICE UNDERGOING DIURESIS**

| Time of sacrifice (wk) | Number animals | Log number bacteria/g kidney* | Proportion kidneys with bacteria | Log number bacteria/ml urine* | Proportion urine with bacteria |
|------------------------|----------------|-------------------------------|---------------------------------|-----------------------------|-------------------------------|
| 1                      | 6              | 4.34 ± 0.46                   | 11/12                           | 4.62 ± 1.18                 | 6/6                           |
| 4                      | 6              | 4.14 ± 0.70                   | 9/12                            | 7.79 ± 0.06                 | 3/6                           |
| 8                      | 6              | 4.67 ± 0.42                   | 5/12                            | 7.53 ± 0.35                 | 3/6                           |
| 12                     | 5              | 4.25 ± 0.05                   | 2/10                            | 3.21 —                      | 1/5                           |
| 16                     | 5              | 6.10 ± 0.58                   | 4/10                            | 6.01 ± 2.54                 | 3/5                           |
| 20                     | 5              | 4.30 ± 0.53                   | 6/10                            | 6.89 ± 0.45                 | 3/5                           |
| 24                     | 6              | 7.16 ± 0.86                   | 6/12                            | 6.98 ± 0.44                 | 3/6                           |
| 28                     | 6              | 4.42 ± 0.34                   | 2/12                            | 5.90 ± 3.01                 | 2/6                           |
| 32                     | 6              | 5.73 ± 0.52                   | 6/12                            | 8.09 ± 0.04                 | 3/6                           |
| Overall times          | 51             | 5.02 ± 0.52                   | 51/102                          | 6.76 ± 1.00                 | 27/51                         |

*Infected kidneys or urine only. At 12 wks only one urine had bacteria, no standard deviation could be calculated.*
of the kidneys were infected. Bacteria persisted in high numbers in the infected kidneys and urine and the proportion of infected kidneys was unchanged for the duration of the study, 32 wk. Twenty three of 51 mice had both kidneys infected and five had unilateral infection. Among the 28 animals with bacteria in one or both kidneys, 25 had bacteriuria while three (all with unilateral infection) had sterile urine. Two animals had bacteriuria without bacteria in the kidney.

The earliest pathological change observed in the infected kidneys was infiltration of the pelvic epithelium and subepithelial connective tissue by mononuclear cells and polymorphonuclear leukocytes at 1 wk (Fig. 1). These cells were predominantly in the region of the fornix, with occasional microabscesses. No changes were seen in the renal parenchyma. At 4 wk, the pathologic picture had changed. Predominant changes were found in the renal parenchyma. Collections of polymorphonuclear leukocytes were seen in the tubules in both cortex (Fig. 2) and medulla. Necrotizing lesions with collection of polymorphonuclear leukocytes obliterating the cortical architecture were not rare. Periarteriolar infiltration with mononuclear cells were frequently seen. Tubular atrophy and interstitial scarring were noted.

From 8 to 32 wk, the pathologic picture was similar to that seen at 4 wk after inoculation. The only changes were a progressive increase in the severity of the lesions, and a relative increase in mononuclear infiltration; and the chronic lesions, tubular atrophy, and interstitial scarring became more obvious. Colloid casts and hyalinized glomeruli were seen only rarely. Acute lesions with intratubular masses of polymorphs as well as areas of necrosis, however, were readily observed in about
FIG. 2. Infected kidney, 4 wk. Section from renal cortex demonstrating intratubular collection of polymorphonuclear leukocytes in the center of photograph. To the left, part of a poorly delineated necrotizing abscess is observed. ×160.

half of the animals (Fig. 3) coexisting with chronic changes. Polymorph and mononuclear cells were always observed in the renal pelvis.

Lesions in kidneys found to be noninfected at the time of harvest were variable. On occasions, a few polymorphs were seen in the pelvic region. Moderate tubular atrophy was seen in approximately one-fourth of the kidneys and rarely collections of mononuclear cells were observed interstitially and around the vessels.

Measurement of the BUN showed that *E. coli* pyelonephritis leads to renal impairment. The BUN of 16 control mice was 24.0 ± 3.1 mg%. In 18 mice with pyelonephritis the BUN was 41.0 ± 5.0 mg%. In 26 animals which had been injected with bacteria but had negative kidney cultures at sacrifice, the BUN was 36.7 ± 3.5 mg%. The BUN of mice with infection did not differ significantly (*P* < .20) from the BUN of mice injected with *E. coli* and found to have sterile kidneys at sacrifice. As indicated above, however, many of these kidneys showed histologic changes consistent with moderate renal damage.

Antibody titers are shown in Table 2. Test animals which were bacteria-free at sacrifice nevertheless demonstrated antibacterial antibody. When hemagglutination titers were compared overall, noninfected animals had significantly less antibody than infected animals (*P* < .001). On the other hand, agglutination titers of noninfected were not significantly different from those of infected animals (*P* > .90). Agglutination titers were higher than hemagglutination titers overall (*P* < .01).

Ideally, serum bactericidal activity studies should have been done with the infecting strain. However, the Yale strain of *E. coli* used in this study was not susceptible
to the bactericidal effect of serum with complement. It could not be made sero-
susceptible by treatment with diphenylamine (7). Therefore, in order to study
effect of infection on serum bactericidal activity, *E. coli* 0127:B8 (kindly supplied
by Dr. M. Landy) was used. This strain is known to be very susceptible to human
serum but we were unable to demonstrate any bactericidal activity of mouse serum
from infected or noninfected animals at the lowest dilutions tested, 1:32 to 1:64.
We were also unable to demonstrate antibody against homologous kidney saline ex-
tract or specific soluble substance at serum dilution of 1:40 in infected or nonin-
fected mice.

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\text{TABLE 2} \\
\text{ANTIBACTERIAL ANTIBODY RESPONSE AFTER INTRABLADDER INOCULATION OF} \\
\text{Escherichia coli Yale into Mice Undergoing Diuresis}
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| Time of sacrifice (wk) | Hemagglutination | Agglutination |
|------------------------|------------------|---------------|
|                        | Infected at sacrifice | Not infected at sacrifice | Infected at sacrifice | Not infected at sacrifice |
| 4                      | 2.39 (6)         | —             | 2.81 (6)         | —             |
| 8                      | 2.12 (2)         | 0.00 (4)      | 3.11 (1)         | 3.08 (4)      |
| 12                     | 1.90 (1)         | 1.25 (2)      | 3.11 (1)         | 2.81 (2)      |
| 20                     | 2.56 (3)         | 0.00 (2)      | 3.81 (3)         | 2.81 (2)      |
| 24                     | 2.81 (1)         | 0.06 (1)      | 2.81 (1)         | 1.20 (1)      |
| 28                     | 1.80 (1)         | 0.45 (5)      | 3.41 (1)         | 3.17 (5)      |
| 32                     | 2.25 (3)         | 2.55 (2)      | 2.05 (3)         | 2.81 (2)      |
| Overall                | 2.39 ± 0.12 (16) | 0.70 ± 0.20 (18) | 2.92 ± 0.67 (16) | 2.80 ± 0.07 (18) |

\(a\) Bacteria present in one or both kidneys.
\(b\) Log geometric mean of reciprocal of serum dilution titer. The lowest serum dilution tested was 1:40. A negative at that dilution was scored zero. The number of animals tested is shown in parentheses.
DISCUSSION

This study has confirmed and extended the observations of Keane and Freedman (1). They showed that abscesses developed in the kidneys of mice when E. coli was injected into the bladder of mice during diuresis and that bacteria were cultured from the kidneys and urine up to 3 wk after infection. The present studies show that bacteria persisted in kidneys and urine, progressive histological changes of pyelonephritis occurred, and E. coli antibodies were elevated for the duration of the study, 32 wk. This infection even when no longer present at time of sacrifice as judged by the absence of bacteria, led to a moderate elevation of the BUN.

At 1 wk after infection histological lesions were seen in the renal pelvis. Others have also noted the early lesions in the pelvis in experimental ascending pyelonephritis (10–12). Severe changes of pyelonephritis were observed 4 wk after infection with polymorphonuclear casts, necrotizing abscesses, mononuclear infiltration together with moderate tubular atrophy, and interstitial scarring. These lesions persisted and progressed up to 32 wk. The pathological changes in the kidneys were those of progressive pyelonephritis. Acute inflammation at 32 wk raised the possibility that recurrent renal infection was occurring from infected bladder urine.

Immunologic studies indicated that agglutination titers did not distinguish between animals found to be infected and noninfected at sacrifice. This suggests that the agglutinating antibody response may have been primarily to the original injection. On the other hand, it would appear that further antigenic stimulation secondary to infection was necessary for hemagglutinating antibody response. In any case, presence of antibody did not eliminate the infection, as there was persistence of infection in animals with a mean agglutinating titer of 1:840. In this respect it was of interest that no bactericidal activity could be demonstrated in sera of infected mice although, as indicated above, we could not test below 1:32 due to lack of serum. The results of this study are similar to those reported from this laboratory on elderly men with pyelonephritis where there was no relation between antibody titer and serum bactericidal activity (4). Alternatively, the antibody produced might be potentially effective, but its activity is interfered with by the unique metabolic milieu of the kidney as previously demonstrated (8).

Anti-tissue antibody was sought in this model because of the possibility of autoimmune phenomena occurring in pyelonephritis. As was the result in man (9), no antitissue antibody could be demonstrated.

Previous studies of experimental pyelonephritis with E. coli and other gram-negative bacilli have required manipulation of the urinary tract in order for the infection to persist in the kidney. Manipulation of the kidney or urinary tract has usually introduced some form of obstruction which itself has influenced host defense mechanisms and has made the study of immune mechanisms and virulence factors difficult to interpret. We feel that the present model of Keane and Freedman (1) more nearly represents the ascending E. coli infection resulting in pyelonephritis that is seen clinically and that this makes it a useful model to study the natural history and the effects of therapy.

SUMMARY

The natural history of pyelonephritis produced by inoculation of E. coli into the bladder of mice undergoing diuresis was studied for 32 wk. Bacteria were present in half the kidneys in high numbers and persisted for the duration of the
study. Early histological changes of pyelonephritis were seen at 1 wk after infection with infiltration of the pelvic epithelium by polymorphonuclear leukocytes. Polymorphonuclear leukocyte casts, necrotizing abscesses, mononuclear infiltration together with tubular atrophy, and interstitial scarring had developed at 4 wk and increased in severity up to 32 wk. The persistence of acute lesions until the thirty-second week suggested that recurrent infection may have been occurring from a reservoir of bacteria in bladder urine. The renal lesion resulted in a moderate elevation of the BUN (41.0 ± 5.0 mg%). Serum agglutination titers did not distinguish between animals found to be infected and noninfected at sacrifice. On the other hand, hemagglutination titers of infected animals were significantly higher than those which did not become infected.

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