HEMATOGENOUS PYELONEPHRITIS IN RATS

III. RELATIONSHIP OF BACTERIAL SPECIES TO THE PATHOGENESIS OF ACUTE PYELONEPHRITIS

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In most accounts of the pathology of human pyelonephritis, no consideration has been given to variations in the disease that could be attributed to differences in the bacterial etiology of the infections. This failure to study the etiological background can be explained by the fact that most pyelonephritic kidneys at autopsy are either no longer infected or at the stage where the organism responsible for the initial infection has been replaced by a succession of other bacteria during the course of recurrences. These limitations to the study of human pyelonephritis led us to examine the problem in rats experimentally infected with those bacterial species usually responsible for human pyelonephritis. Earlier experiments had succeeded in developing a technique for producing a form of hematogenous pyelonephritis in rats that closely resembled the type of human disease occurring in the absence of hydrenephrosis (Braude et al., 1955). Because this form of experimental pyelonephritis was produced without resorting to the conventional technique of ureteral ligation, it was possible to analyze the evolution of renal infection under circumstances that were not confused by the effects of urinary obstruction.

METHODS

The procedures have been reported elsewhere for producing pyelonephritis, for bacteriological examination of tissues and blood, for measurement of blood pressure, and for histological examinations (Braude et al., 1955; Shapiro et al., 1956). The bacteria used in the present study were all isolated from patients with urinary infections. For the production of pyelonephritis, 0.5 ml of an 18-hr culture in tryptose broth was inoculated intracardially into female albino rats (Sprague-Dawley or Holtzman) weighing approximately 250 g. This inoculum contained approximately 10^6 viable bacteria per ml as determined by plate counts and produced an average count of 292,000 viable bacteria per g of renal tissue in the massaged kidneys during the first hr after intracardiac injection (Braude et al., 1955). The 18-hr cultures were inoculated without dilution except for strains of Proteus and pseudomonads; these were diluted 1:5 with tryptose broth in order to minimize the initial mortality rate from overwhelming bacteremia. The 18-hr cultures were refrigerated at 4°C during the period of 90 to 120 min required to inoculate the rats. This precaution was necessary to prevent the excessive mortality that otherwise occurred at the end of this period of time if the cultures remained at room temperature. Immediately after bacterial inoculation, the rats were subjected to bilateral renal massage through the intact abdominal wall for 5 min as previously described (Braude et al., 1955; Shapiro et al., 1956).

EXPERIMENTAL RESULTS

Two strains of Proteus mirabilis, 2 strains of Proteus morganii, 1 strain of Proteus vulgaris, 1 strain of Proteus retgeri, 4 strains of Escherichia coli, and 4 of enterococci (Streptococcus zymogenes or Streptococcus faecalis) were inoculated into groups of 4 or more rats (for each strain) and survivors sacrificed at the end of 1 week. For purposes of more prolonged observation, a second series of 100 rats was divided into 5 groups. Each of 3 groups was inoculated with 1 representative strain of E. coli, Pseudomonas aeruginosa, or P. morganii and rats in a 4th group inoculated with
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1 of 3 different strains of enterococci. In addition, a 5th group of 15 rats inoculated with E. coli was reinoculated after 2 or 4 weeks with an enterococcus. Survivors in the 5 groups from the second series were then sacrificed in 4 to 6 weeks. In order to determine the importance of renal massage in the production of pyelonephritis, a 3rd series of rats was divided into 3 groups for inoculation with either S. zymogenes, E. coli, or P. morganii. Half of the rats in each of these groups were subjected to renal massage for 5 min immediately after the bacterial inoculation and, at the end of 1 week, their kidneys were compared with those of the rats receiving no massage. The systolic blood pressure was measured on the day before sacrifice in 90 of the 129 surviving animals. Blood urea nitrogen was determined on cardiac blood obtained at sacrifice.

General condition of rats after inoculation. In preliminary experiments, the average mortality rate after inoculation of the gram-negative bacteria was 40 per cent and varied from 10 to 95 per cent. The highest mortality rate occurred when the 18-hr cultures were allowed to stand at room temperature for several hr during experiments requiring renal massage of many animals. The mortality rates were sharply reduced by chilling the 18-hr culture at 4 C during the period when the rats were awaiting inoculation. Even with this precaution all survivors became ill following inoculation of gram-negative bacteria and for 3 to 5 days exhibited marked weakness, anorexia, roughening of fur, and failure to resist handling.

In contrast to the gram-negative bacteria, however, the enterococci never disturbed the apparent well-being of the inoculated rats, and the acute mortality rate was zero.

Comparison of pyelonephritic lesions at 1 week. Lesions produced by the inoculated bacteria were always limited to the kidney. In figures 1A, 1B, and 1C, the size and shape of the lesions throughout each kidney are diagrammatically represented as though they occurred in a single plane.

The most severe lesion resulted from Proteus inoculation. All members of this genus produced highly destructive infections with the exception of P. rettgeri. The strains of P. rettgeri used in this study differed from the other species of Proteus by the failure to swarm at 37 C on culture plates. They were capable of establishing only 3 tiny lesions in the 8 kidneys examined in this experiment; in subsequent experiments, other strains

Figure 1A. Lesions produced at 1 week by 5 different strains of Proteus
of this nonswarming species proved incapable of establishing more than a few tiny pyelonephritic lesions. Photographs of the kidneys were made upon sacrifice 1 week after infection by 4 different strains of Proteus and are shown in figure 2. The infected kidneys were characteristically swollen and on sectioning presented yellowish-gray, wedge-shaped areas of inflammation with their bases at the surface of the organ and their apices extending to the pelvic mucosa. This process was not accompanied by suppurative necrosis and softening; instead, there was a marked increase in resistance to section. Before sectioning, the pyelonephritic kidney could be identified by its enlarged size and by the concentration of elevated inflammatory foci along the convex portion of the
surface. In animals sacrificed at 24 and 48 hr, the lesions were observed to begin as small circumscribed foci in the outermost portion of the cortex and by 72 hr to extend downward into the pelvis. There was a tendency for the lesions to be most extensive at the upper and lower poles of the kidneys.

On microscopic examination, the fully developed lesion at 1 week was found to be composed of an intense interstitial infiltration of leukocytes which surrounded the tubules and frequently destroyed and replaced them. The glomeruli were injured less frequently and often stood intact in the midst of widespread necrosis and inflammation. The cellular reaction was chiefly mononuclear but sometimes there was an equal number of polymorphonuclear cells. Within the tubules, on the other hand, polymorphonuclear cells predominated in the numerous pus casts. In addition to the infiltration of inflammatory cells, the interstitial reaction was characterized by fibrosis as determined by the Mallory stain. The degree of fibrosis ranged from slight to severe and paralleled the intensity of the cellular infiltration and necrosis.

Stones were present in the bladder of 2 of the 20 rats. No evidence of hydronephrosis was present in these kidneys.

Large numbers of Proteus organisms were cultured from both kidneys of all animals examined. A few Proteus organisms were also cultured from the liver and spleen of almost every animal, but the blood cultures were positive in only 4 rats. Azotemia, as indicated by elevation of blood urea nitrogen above 33 per cent, was present in 12 rats. Polymorphonuclear leukocytes in the urinary sediment were found whenever it was possible to obtain urine for microscopic examination.

E. coli produced marked renal injury but less than that observed with Proteus. The microscopic changes were qualitatively similar to those produced by Proteus and consisted essentially of a mononuclear interstitial reaction which destroyed the tubules but usually spared the glomer-
The ratio of mononuclears to polymorphonuclears was about 4:1 in the interstitial areas, but the numerous pus casts within the tubules contained a predominance of polymorphonuclears. In those areas where destruction was most marked, there was interstitial fibrosis along with early periglomerular fibrosis. Luxuriant growth of *E. coli* was obtained from kidney and urine, and pyuria was consistently demonstrated. The blood urea nitrogen was slightly elevated in most animals.

Enterococci (*S. faecalis* and *S. zymogenes*) produced the least renal destruction at 1 week, but pyelonephritic lesions were present in every inoculated animal. The enterococcal lesion was characteristically limited to the cortex or outer medulla and only rarely extended to the pelvis. This lack of pelvic involvement was the main anatomic feature distinguishing enterococcal renal infections at 1 week from those by Proteus and *E. coli*. The histologic reaction in the kidneys infected by enterococci, although less severe, was qualitatively indistinguishable from that described for *E. coli* and Proteus, and large numbers of enterococci were cultured from the kidneys and urine. Unlike the renal infections by gram-negative bacteria, however, infections by enterococci were invariably accompanied at 1 week by a normal blood urea nitrogen.

**Comparison of pyelonephritic lesions at 4 to 6 weeks.** Among animals infected by Proteus and *E. coli*, the extent and character of the pyelonephritic lesions were not much different from the lesions produced by these gram-negative bacteria at 1 week. In addition, the severity of the pyelonephritis produced at 4 to 6 weeks by the one

![Figure 2. Gross appearance at 1 week of kidneys infected with 4 different strains of Proteus. C, G, D, and W correspond, respectively, to Collins, Gustavsen, Dixon, and Wolford in figure 1A.](image)

### TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Rats</th>
<th>Total Kidneys Examined by Culture</th>
<th>No. of Infected Kidneys*</th>
<th>No. of Sterile Kidneys</th>
<th>No. of Azotemic Rats (Blood Urea Nitrogen &gt; 30)</th>
<th>Mean Blood Urea Nitrogen (Mg Per Cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris,</em> <em>P. mirabilis,</em> and <em>P. morganii</em></td>
<td>22</td>
<td>40</td>
<td>2</td>
<td>1</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><em>Streptococcus zymogenes</em> and <em>S. faecalis</em></td>
<td>16</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>32</td>
<td>0</td>
</tr>
</tbody>
</table>

* The system for grading the intensity of infection (+ to ++++) is given in figure 1D. The numbers indicate how many kidneys exhibited each grade of infection.

† Only 12 rats examined for blood urea nitrogen.
strain of *P. aeruginosa* was approximately the same as that with *E. coli*. The lesions produced by enterococci, on the other hand, had now extended through the medulla to reach the pelvis and assumed the wedge-shaped configuration found at 1 week in the gram-negative infections. Despite this extension of enterococcal disease to the pelvis, it remained the mildest of the 4 infections, so that the difference in severity observed at 1 week still existed among the species.

A unique finding again was that of renal stones in 4 of 14 rats infected with Proteus. In 3 of these rats, the stone was in the pelvis of the right kidney; and in the 4th, stones were in the bladder and the left kidney. In all 3, hydronephrosis was present. Stones from 2 rats were analyzed by Dr.

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**Figure 3.** Pyelonephritic kidney 6 weeks after infection with Proteus illustrating crowding of glomeruli (×70).
E. L. Prien of Boston, Massachusetts, who sent the following report:

Bladder stone: Irregular gray calculus, beautifully crystallized; composed of magnesium ammonium phosphate with a trace of calcium phosphate.

Renal (pelvic) stone: Dirty gray, irregular in shape, 0.5 by 0.4 by 0.3 cm; also composed of magnesium ammonium phosphate but poorly crystallized (less perfect crystals).

In each case the stone was accompanied by severe pyelonephritis, extremely heavy infection, and azotemia. The total incidence of stones, including those at 1 week, was 6 in 54 rats or 11.1 per cent. The incidence was zero in normal rats and in those infected by bacteria other than Proteus.

Histologically, there was surprisingly little change from that observed at 1 week. The pyelonephritic process consisted of an interstitial mononuclear infiltration which showed a marked tendency to spare the glomeruli but destroy the tubules. The collapse of tissue resulting from this selective tubular destruction produced a crowding together of glomeruli (figure 3). The degree of fibrosis, observed with Mallory's stain, paralleled the intensity of inflammation and tissue destruction and was therefore most marked in Proteus infections. Characteristically, the fibrosis began in the center of the pyelonephritic process and spread concentrically up to the glomeruli and from cortex to pelvis (figures 4 and 5). Those tubules escaping destruction frequently contained pus casts composed of polymorphonuclear leukocytes.

The results of culture of all kidneys at 4 to 6 weeks are summarized in table 2 and of the pyelonephritic kidneys in table 3. It is clear from these results that despite the milder grade of renal destruction resulting from enterococcal pyelonephritis, cultures of one-half of each kidney yielded heavier growth of enterococci than of gram-negative bacteria. A remarkable difference was observed both in the degree and incidence of infection. All but one of the kidneys showing pyelonephritic lesions were still heavily infected at 4 to 6 weeks in those rats originally inoculated with enterococci. On the other hand, many of the pyelonephritic kidneys had become sterile by this...
The inflammatory reaction in the sterile pyelonephritic kidney is illustrated in figure 5. This reaction, which persisted after infection had disappeared, could not be distinguished from the inflammatory process in infected pyelonephritic kidneys. There was an intense interstitial mononuclear cell reaction, with prominent pus casts in the tubules.

An exception to the occurrence of heavy renal infection by enterococci at 4 weeks was observed...
in those rats inoculated with enterococci after pyelonephritis had already been established with *E. coli*. Enterococcal infection could be established in only 33 per cent of such kidneys, regardless of whether the duration of the existing *E. coli* infection had been 2 weeks or 4 weeks at the time of the enterococcal challenge. These results indicated that existing pyelonephritis due to *E. coli* may have prevented superinfection by enterococci.

**Effect of renal massage on development of pyelonephritis.** The results summarized in table 4 demonstrate that renal massage was essential for a high incidence of pyelonephritis with the gram-negative bacteria; thus, with *E. coli*, gross lesions were never observed without massage, and only rarely with Proteus. Enterococci, by contrast, readily established both renal infection and, to a lesser extent, renal lesions in animals not subjected to renal massage.

**Blood pressure.** Despite the extensive pathological changes of acute pyelonephritis and the frequent finding of azotemia, the systolic blood pressure exceeded 140 mm Hg in only 4 of the 90 rats examined. Two had been infected for 6 weeks and 1 for 4 weeks with Proteus. The 4th had received a mixed infection consisting of *E. coli* for 4 weeks and *S. zymogenes* for 2. In 3 of these 4 animals, there was severe unilateral damage (atrophy in 2 instances and hydronephrosis in the 3rd) with a relatively intact contralateral kidney. These data clearly demonstrate that hypertensive vascular disease does not accompany bilateral acute pyelonephritis, regardless of the severity of renal damage.

**DISCUSSION**

Although a basic similarity was observed in the histological appearance of the renal lesions pro-

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**TABLE 2**

Results of culture of kidneys 4 to 6 weeks after inoculation of bacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Kidneys Examined</th>
<th>No. of Infected Kidneys*</th>
<th>No. of Sterile Kidneys</th>
<th>Per Cent of Kidneys with Heavy Infection (Greater than +++)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>24</td>
<td>13</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><em>Proteus morganii</em></td>
<td>28</td>
<td>1</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus zymogenes</em> and <em>S. faecalis</em></td>
<td>52</td>
<td>3</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

* The system for grading the intensity of infection is given in figure 1D. The numbers indicate how many kidneys exhibited each grade of infection.

**TABLE 3**

Presence of infection at 4 to 6 weeks in kidneys with pyelonephritic lesions

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Pyelonephritic Kidneys</th>
<th>Sterile Pyelonephritic Kidneys</th>
<th>Per Cent of Pyelonephritic Kidneys with Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>22</td>
<td>5</td>
<td>77</td>
</tr>
<tr>
<td><em>Proteus morganii</em></td>
<td>22</td>
<td>5</td>
<td>77</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td><em>Streptococcus zymogenes</em> and <em>S. faecalis</em></td>
<td>41</td>
<td>1</td>
<td>98</td>
</tr>
</tbody>
</table>

**TABLE 4**

Effect of renal massage on incidence of gross pyelonephritis 1 week after inoculation of bacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>Renal Massage</th>
<th>No. of Kidneys Examined</th>
<th>No. of Kidneys with Gross Pyelonephritis</th>
<th>Per Cent with Pyelonephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Yes</td>
<td>22</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus morganii</em></td>
<td>Yes</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td><em>Streptococcus zymogenes</em></td>
<td>Yes</td>
<td>12</td>
<td>10</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>12</td>
<td>6</td>
<td>50</td>
</tr>
</tbody>
</table>

4 The average systolic blood pressure in a large series of normal rats in our laboratory is 110 ± 10 mm Hg. Spontaneous elevations to 140 mm Hg are rare and are taken to indicate hypertension when sustained. The present study, however, was designed to include only a single determination.
duced by the various bacteria in this study, certain differences in the patterns of infection could be clearly related to differences in the bacterial species. Proteus not only produced the most severe pyelonephritis, but also was the only species responsible for stone formation, a finding present in more than 10 per cent of the animals. Marked renal destruction also resulted during infection by *P. aeruginosa* and *E. coli* but relatively little by enterococci. Yet infections by enterococci were heaviest and of longest duration despite the mild degree of renal damage and could even produce pyelonephritis in kidneys that had not been massaged.

Some of these differences in the pattern of experimental renal infection seem to have their counterpart in human pyelonephritis. The association between renal stone in man and infection by urea-splitting bacteria has been observed repeatedly (Braasch, 1938; Chute and Suby, 1940). Proteus possesses the most active bacterial urease and through the decomposition of urea creates an alkalinity favorable to the precipitation of magnesium ammonium phosphate, calcium phosphate, and calcium carbonate (Prien, 1955). In the rats, the crystals could be seen forming in their earliest stage as a mineral deposit at the tips of the renal papillae. Such deposits may be comparable to the calculous plaques observed by Randall (1939) on the papillae of human kidneys. Randall postulated that submucosal lesions led to the formation of plaques which eroded through the epithelium and grew into calculi as urinary salts were deposited upon them. This chain of events leading to stone formation could be traced easily in Proteus-infected rat kidneys but not in those infected by the other bacteria even though submucosal pyelonephritic lesions were produced in the papillae of kidneys infected by each of the four species examined. This would suggest that in addition to the submucosal lesion, the necessary conditions for salt precipitation must be provided in order for stones to develop.

The self-limited nature of *E. coli* infections observed in most of the experimental animals is also characteristic of human infections. In patients with acute pyelonephritis due to *E. coli* complete recovery usually occurs within a few weeks even without antibacterial chemotherapy (Brainerd, 1956). On the other hand, several reports have suggested that enterococcal infections characteristically persist for years in patients with chronic pyelonephritis (Kleeman and Epstein, 1957; Beeson, 1955). These tendencies for various bacteria to maintain their differential pathological effects in infections of both human and animal kidneys suggest that an explanation for these differences may be found in an analysis of the bacteria themselves. Aside from the special capacity of Proteus to decompose urea, two other species' differences may have a bearing on the evolution of pyelonephritis. One of these concerns the bacterial cell walls; those of gram-positive cocci, including enterococci, are tough and difficult to disrupt, whereas the walls of gram-negative bacteria are thinner and easily lysed (Salton, 1956). The other difference is found in the presence of a toxic lipopolysaccharide in the gram-negative bacteria and their absence in the gram-positive bacteria. The damaging action of the toxic lipopolysaccharide was prominent during the early stages of the infection after inoculation of gram-negative bacteria when all animals became extremely sick and many died; but such toxemia was never seen after inoculation of enterococci. It is possible, therefore, that the greater renal inflammatory injury from gram-negative bacteria and its persistence after infection disappears may also be due to the direct action of endotoxin in the tissues, while the persistence of enterococcal infection could be accounted for on the basis of its ability to resist the lytic action of tissue fluids.

The intense inflammatory reactions elicited by the gram-negative bacteria may also be a factor in their earlier disappearance from the kidney. This possibility is supported by the observation that pyelonephritis due to *E. coli* seemed to prevent superinfection by enterococci. Similar observations have been recorded by Guze and Beeson (1956) on the prevention of renal staphylococcal infections by *E. coli* pyelonephritis. They suggested that the environment created by *E. coli* pyelonephritis is unfavorable for multiplication of gram-positive cocci.

**SUMMARY**

The pathogenesis of hematogenous pyelonephritis in rats was influenced by the species of infecting bacteria. Strains of Proteus not only established the most severe lesions but also produced renal stones (*MgNH4PO4*). Marked renal destruction also resulted from infections by *Escherichia coli* and *Pseudomonas aeruginosa* and least from enterococci. Yet renal cultures at 4 to 6 weeks yielded a far heavier growth of entero-
cocci than gram-negative bacteria; in fact, the gram-negative bacteria had frequently disappeared from the kidneys by then. The inflammatory reaction initiated by gram-negative bacteria often persisted, however, after infection had vanished.

It is postulated that these differences were related to the following properties of the bacteria studied: (a) the urease of Proteus; (b) the endotoxin of the gram-negative bacteria; (c) the tough cell wall of enterococci.

REFERENCES


