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Experimental Studies on Hematogenously Induced Renal Damage in the Rabbit Due to Pseudomonas aeruginosa (38688)

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Pseudomonas aeruginosa is an opportunistic organism capable of causing infection in virtually all organs of the body (1); the frequency with which infections are encountered has increased over the past decade. The organism has reached preeminence as a human pathogen in immunosuppressed patients, patients receiving extensive antimicrobial chemotherapy, and those receiving radiation therapy or prolonged treatment with corticosteroids and/or antineoplastic drugs (2, 3).

The kidneys and urinary tract of man are a common site of *Pseudomonas* infections. Bacterial pyelonephritis due to *Pseudomonas aeruginosa* is a well recognized clinical entity, but little is known of the pathophysiological sequence of the development of *Pseudomonas* nephritis. Ascending pyelonephritis accounts for the vast majority of pyelonephritis in man (4–6), but other routes of renal invasion, specifically hematogenous sources are documented (7, 8). Hematogenous pyelonephritis is especially important in the very young child who develops renal infection secondary to septicemia. In addition, the increased use of arterial and venous catheters, urinary catheters and instrumentation of ureters undoubtedly plays a role in initiating systemic and renal infections with *Pseudomonas aeruginosa* (9–11).

Previous studies (12) indicated that it was difficult to establish renal infections in mice using *P. aeruginosa*, since the renal infection dose is approximately equal to the lethal dose in the species. Consequently, we have examined the sequential histological response of hematogenously induced renal damage caused by *Pseudomonas aeruginosa* in rabbits and have attempted to determine if functional changes could be detected by the simple determinations of serum urea nitrogen (SUN) and serum creatinine. In addition, since there are conflicting reports that obstruction to urinary outflow enhances the susceptibility of renal infection due to microorganisms (13, 14), rabbits were also surgically rendered unilaterally hydronephrotic to determine if an increase in susceptibility or degree of severity of renal lesions could be demonstrated following hematogenous introduction of *Pseudomonas aeruginosa*.

Materials and Methods. Bacterial strain. Stock cultures of *Pseudomonas aeruginosa*, strain 19660 ATCC, were routinely cultured on tryptose agar slants and were stored at 25°. The slants were subsequently used to inoculate 125 ml flasks containing 50 ml of 5% peptone (Difco, Detroit, MI) and 0.25% trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, MD). The flasks were placed on a rotary shaker at 37° for 18 hr. Cells were harvested by centrifugation at 27,000 *g* for 20 min at 4°. The resulting pellet was resuspended in 0.9% nonpyrogenic saline (Abbott Laboratories) to a concentration of 4×10^8 colony forming units per ml, as determined by plate counts. A 1.0 ml suspension of cells at a concentration of 4×10^8 cells per ml was used to inject each rabbit intravenously. Prior to these studies, it was determined by titration that chronic infections persisting 12–16 days and leading to renal damage, was best established with this concentration of organisms.

Rabbits. Female Dutch Belt rabbits weighing approximately 2.2 kg, obtained from Oakhill Rabbit Farms, Michigan, were used in all experiments. Rabbits were divided into two groups of seven animals each. One group referred to as "ligated," was surgically rendered unilaterally hydronephrotic 30 days prior to infection, while the other group, referred to as "nonligated," underwent no surgery. Rabbits in both groups each received 1.0 ml of *Pseudomonas*

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aeruginosa in the concentration previously specified. All injections were given intravenously into the marginal ear vein. Both groups were followed with serial SUN and serum creatinine determinations. One rabbit from each group was randomly sacrificed at 2- to 4-day intervals. The kidneys and other major organs were removed and examined grossly and histologically and compared to untreated controls.

Bacterial dose-response. To determine the number of organisms necessary to establish an infection persisting 12–16 days with renal damage in 100% of the animals, various dilutions of organisms were administered. The range of dose-response for "nonligated" and "ligated" rabbits was determined for *Pseudomonas aeruginosa* at concentrations of 10^9 cells/ml, 10^8 cells/ml, and 10^7 cells/ml. At a concentration of 1.0×10^9 cells/ml, given as 1.0 ml iv, death uniformly occurred in all animals within 12–18 hr. Gross examination of these animals revealed extensive hemorrhage of the kidneys and lungs, and large numbers of *Pseudomonas aeruginosa* in pure culture were obtained from samples of heart blood, kidneys, lungs, liver, and spleen. At a concentration of 1.0×10^7 cells/ml, death did not occur up to 41 days at which time animals were sacrificed. With the exception of unilateral hydronephrosis and hydroureter in the ligated rabbits, no significant gross pathological changes were observed in any of these animals. Bacteriological cultures of heart, blood, bladder urine, kidneys, lungs, liver, and spleen were sterile. Animals receiving 1.0 ml of 10^8 cell/ml died within 11–19 days. These animals had no remarkable changes in appearance, behavior, or weight until 24–48 hr before death at which time they became anorexic, lethargic, and moribund. There was no difference in death response between nonligated and ligated rabbits at any of the above bacterial concentrations. From these data a dose of 4×10^8 cells/ml was used in the experiments described in order to study animals over the 12- to 16-day holding period.

Surgical procedures. Under inhalational anesthesia with methoxyflurane (Metafane, Pittman-Moore Co., Washington Crossings, NJ) and intramuscular injection of 0.4 ml ketamine hydrochloride (Ketaset, Bristol-Myers Co., Syracuse, NY), unilateral hydronephrosis was produced under aseptic conditions by ligation of the left ureter approximately 2 cm from the renal pelvis, with 3–0 silk. Care was taken to avoid direct trauma to the renal parenchyma. Incisions were closed in four layers with 3–0 silk sutures. The wounds were cleansed with postoperative iodine solution, and the animals were held in modern animal care facilities for 30 days before being injected with bacteria. Blood cultures, food and water consumption, and urine and feces were observed for signs of illness in the animals.

TABLE I[a]

	SUN (mg/100 ml)		Serum Creatinine (mg/100 ml)	
	Nonligated	Ligated	Nonligated	Ligated
Range	10–22	18–41	1.0–1.8	1.2–1.8
Mean	15.7	26.7	1.4	1.5
Animals sampled	28	13	22	22

[a] Serum urea nitrogen (SUN) and serum creatinine levels in ligated and nonligated female rabbits prior to infection with *P. aeruginosa*. Determinations were made 30 days postsurgery in the ligated group.

SUN and serum creatinine determinations. Blood specimens were drawn from each rabbit by needle puncture of ear veins and 2 ml of blood was collected. The blood was centrifuged at 480 *g* for 10 min and the serum separated. Serum urea nitrogen (SUN) determinations were performed using 0.05 ml of serum following previously described procedures (15). Serum creatinine determinations were made using 0.5 ml of serum according to the Folin–Wu method (16). All preinfection determinations were performed after a "holding" period of 30 days postsurgery in the ligated group. Subsequent serial determinations were made in both groups at 2- to 4-day intervals postinfection.

Histologic specimens. After gross postmortem observations, the kidneys, lungs, heart, liver, and spleen were removed and immediately fixed in phosphate-buffered

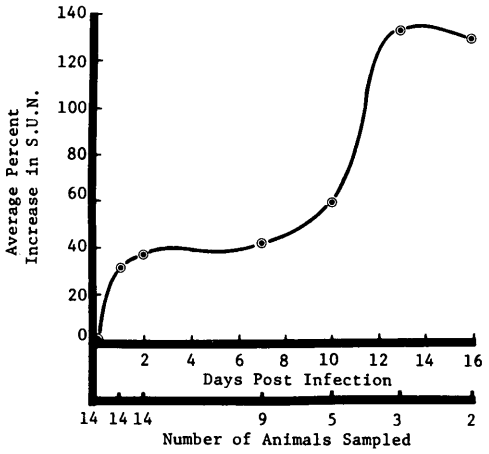

FIG. 1. Sequential serum urea nitrogen (SUN) levels of ligated and nonligated rabbits infected with *Pseudomonas aeruginosa.*

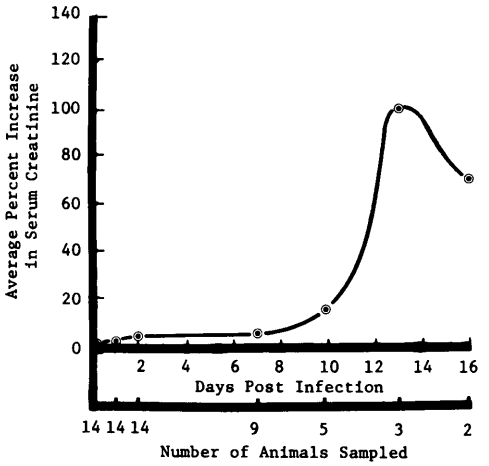

FIG. 2. Sequential serum creatinine levels of ligated and nonligated rabbits infected with *Pseudomonas aeruginosa.*

Formalin at pH 7.2. Tissue specimens were dehydrated, embedded in paraffin, and sections were cut on a rotary microtome and stained with hematoxylin and eosin. The slides were examined by light microscopy.

Results. The SUN and serum creatinine levels for pre-infected, "nonligated" and "ligated" rabbits are shown in Table I. As can be seen, nonligated rabbits have SUN values between 10- and 22 mg/100 ml with a mean of 15.7 mg/100 ml, in contrast to ligated rabbits which have a SUN range between 18 and 41 mg/100 ml and a mean of 26.7 mg/100 ml. However, serum creatinine levels did not show a significant difference between the two groups of rabbits.

Figures 1 and 2 show the sequential rise in SUN and serum creatinine following iv injection of 1.0 ml of 4×10^8 cells/ml of *Pseudomonas aeruginosa.* These data represent two groups of seven ligated and seven nonligated animals. The preinfection mean SUN of 20.27 for ligated animals was significantly higher than the mean SUN of 13.00 for the nonligated group with a T value of 5.37 at the 0.01 level. However, the relative percentage increases above these starting values were not significantly different between the two groups after infection. Therefore, Fig. 1 shows the sequential average percent increases in SUN over a period of 16 days for both groups averaged together. The preinfection mean serum creatinine of 1.5 for ligated animals was not significantly different from the mean of 1.3 for the nonligated group. Figure 2 shows the sequential average percent increases in serum creatinine over a period of 16 days for both groups averaged together.

One animal from each group was sacrificed at 2- to 4-day intervals and histological preparations were made of major organs in order to determine the renal histopathology of non-ligated and ligated rabbits. In both groups, infection was followed by severe hemorrhage in the cortical interstitium with scattered mononuclear cell infiltrates. Hemorrhage often extended to include some glomeruli. Peritubular hemorrhage and early changes of tubular structures within the medulla consisting of cloudy swelling and hydropic degeneration of tubular epithelium were also noted. These changes occurred by the second to fourth day post-infection. By the seventh to tenth day, intense mononuclear cell infiltrates were seen in all areas of the cortex and medulla. Cellular debris casts were also noted by the seventh to tenth day. Degeneration of glomeruli with frank hemorrhage, or shrinkage of the glomerular tuft, and dilation of Bowman's space was also noted. The enlarged Bowman's spaces often contained eosinophilic material. Histologic sections taken from terminal animals showed virtually complete destruction of renal pa-

renchyma in both cortex and medulla with numerous foci of microabscess formation.

The kidneys of ligated rabbits showed marked gross and microscopic hydronephrosis in the ligated kidney with some compensatory hypertrophy in the contralateral kidney. The sequence of pathologic events was essentially identical in the ligated kidney, the contralateral kidney of ligated rabbits, and the kidneys of nonligated rabbits.

Gross and microscopic examination of the other major organs revealed profound changes in the lungs and liver. Grossly, lungs showed little pathology before the tenth day. Microscopically, however, patchy areas of mononuclear cell infiltrates, alveolar wall thickening, and interstitial hemorrhage could be seen by the fifth to seventh days. There was no difference noted between the lungs of nonligated and ligated rabbits. Terminally, lungs from both groups showed evidence of extensive pneumonia. Interstitial changes were marked, with numerous areas of edema, hemorrhage, and mononuclear cell infiltrates. Large areas of atelectasis with intra-alveolar hemorrhage and infiltrates of mononuclear cells were present. Small areas of microabscess formation, similar to those described in the kidneys, were also noted.

The livers of both groups of animals showed essentially similar pathologic alterations. Liver lesions were microscopically apparent by the seventh to tenth days postinfection. Well demarcated areas of lobular necrosis were noted. Extensive portal and periportal mononuclear cell infiltration was present and early signs of fibrosis were evident. The hepatocytes themselves showed marked hydropic vacuolization and evidence of early necrosis. Microabscesses were present throughout the hepatic parenchyma and contained mononuclear cells and necrotic hepatocytes.

The gall bladder after the tenth day postinfection was markedly distended and filled with pus and attentuated light green bile. Microscopic examination of gall bladder contents revealed the presence of numerous gram-negative rods, a few polymorphonuclear leukocytes and a predominance of mononuclear cells. *Pseudomonas aeruginosa* was recovered in pure cultures from all involved organs in the absence of any bacterial contaminants.

Discussion. Artificial manipulations such as surgically induced obstructions (14), focal injury to the kidney (17), vigorous massage of the renal parenchyma (18), or direct microinoculation of organisms into the kidney (19), have been necessary to create renal damage in previous experimental models, since both the upper and lower urinary tracts of experimental animals are resistant to a wide spectrum of infectious agents. However, such maneuvers do not seem to be necessary in rabbits hematogenously infected with *Pseudomonas aeruginosa.*

The presence of surgically induced unilateral ureteral ligation in this system does not seem to enhance the susceptibility to infection or increase the severity of pathologic changes within the kidneys as compared to nonligated animals. These data, at first glance, appear to be in direct contradiction to the work of Guze and Beeson (14), who clearly showed an increase in the susceptibility to infection in rats with unilateral ureteral ligation injected intravenously with *E. coli.* However, in their study, animals were injected with the organism 24 hr prior to the surgical obstruction, while in the present study, rabbits were surgically obstructed for 1 mo prior to the introduction of organisms. The difference in experimental design could certainly account for the contradictory data. Further inconsistencies in data might be explained on the basis of differences in both the organisms and animal models employed. For, in other studies, these investigators were unable to show a difference in susceptibility to *E. coli* pyelonephritis in rabbits with partially obstructed ureters (20). A further explanation for a lack of increased susceptibility to pseudomonas infection in the ligated animals might be attributable to a decrease in renal blood flow following ureteral outflow obstruction. A decrease in renal blood flow in the obstructed kidney could be envisioned to reduce the seeding of bacteria from other systemic foci. Likewise, a reduction in the inflammatory response in the ligated kidney could also occur. Furthermore, Gorrill and De-

Navasquez (12) have reported that previous scarring of mouse kidneys does not increase the incidence of lesions due to *Pseudomonas aeruginosa* injected intracardially. *Pseudomonas*, then, may not be a particularly invasive organism, but once infection begins, it produces marked tissue damage and the organism is difficult to eradicate.

Previous studies with elastase and collagenase from *P. aeruginosa* indicated rapid and striking pathologic changes in mice although the sequence of histopathological damage could never be ascertained (21, 22). However, in the present studies it was possible to establish the sequence of histopathological events that occurred during the infection. Changes in the kidneys began with scattered areas of hemorrhage and nonspecific tubular atrophy. There was extensive round cell infiltration in both cortex and medulla, with progressive glomerular involvement. Cellular infiltration was widespread and lead to microabscess formation with eventual cicratization at 14–16 days. The SUN and serum creatinine levels were observed to rise most dramatically at that time. Consistently, there was a striking paucity of polymorphonuclear leukocytes in the renal lesions throughout the course of infection. The patchy nonrestrictive nature of the hematogenously introduced infection is in keeping with the distribution of lesions observed by others (8). The presence of predominantly mononuclear cell infiltrates, even early in the course of infection raises some interesting possibilities in the pathophysiology of *Pseudomonas* renal damage that deserve further investigation. It would appear that the organism may produce an antineutrophilic factor that prevents neutrophils from migrating to the site of the lesion in this system (23), thus favoring a potent mononuclear cell response. Recent studies (24) indicate that the slime of *P. aeruginosa* exhibits antineutrophilic activity, thereby giving credence to the striking predominence of mononuclear cells also observed in pulmonary and hepatic lesions in both groups of rabbits. Alternately, the large infectious challenge might have resulted in leucopenia contributing to the paucity of neutrophilic leucocytes in the lesions. However, differential cell counts of peripheral venous blood cells were not conducted in these studies.

Although the experimental model described herein does not represent the more common ascending form of pyelonephritis it does establish a model for hematogenously induced renal damage. The importance of hematogenously induced renal infection is particularly important in the pediatric patient who frequently develops pyelonephritis secondary to septicemia, with or without obstructive uropathy. The system described herein appears to be an effective model by which to study renal damage secondary to septicemia.

Summary. Chronic systemic infections of rabbits were established by intravenous inoculation of 4×10^8 *P. aeruginosa* cells in order to study the sequence of events leading to severe kidney damage. Renal lesions were detected by the fifth- to seventh-day postinfection, as were lesions in the liver and lungs. Progressive azotemia led to death by the 12th–16th day. Lesions in the kidneys, lungs and liver were characterized terminally by intense mononuclear cell infiltrates, hemorrhage, and microabscess formation. Mononuclear cells also appeared to be the predominant responsive cell early in infection. There appeared to be no difference in the susceptibility to infection or severity of renal lesions between rabbits with surgically induced unilateral ureteral obstruction and nonobstructed rabbits.

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1. Forkner, C. E., *in* "*Pseudomonas aeruginosa* Infections". Modern Medical Monograph 22, Grune and Stratton, New York (1960).
2. Finland, M., Jones, Jr., W. F., and Barnes, N. W., J. Am. Med. Ass. **170**, 2188 (1959).
3. Iacocca, V. F., Sibinga, M. S., and Barkero, G. J., Amer. J. Dis. Child. **106**, 315 (1963).
4. Beeson, P. B., Amer. J. Med. **24**, 1 (1958).
5. Gillespie, W. A., Linton, K. B., Miller, A., and Slade, N., J. Clin. Pathol. **13**, 187 (1960).
6. Kunin, C. M., and McCormack, R. C., N. Eng. J. Med. **274**, 1155 (1966).
7. Barrington, F. J. F., and Wright, H. D., J. Pathol. Bacteriol. **33**, 871 (1930).

8. Becker, E. L. (Ed.), in "Structural Basis of Renal Disease." Harper and Row, New York, (1968).
9. Lame, E. L., and Chang, H. C., Amer. J. Roentgenol. **71**, 193 (1954).
10. Lame, E. L., Amer. J. Roentgenol. **75**, 938 (1956).
11. Waisbren, B. A., Arch. Intern. Med. **88**, 467 (1951).
12. Gorrill, R. H., and DeNavasquez, S. J., J. Pathol. Bacteriol. **87**, 79 (1964).
13. Bricker, N. S., *in* "Diseases of the Kidney," (Strauss, M. B. and Weet, L. G., ed.), Little Brown and Co., Boston (1963).
14. Guze, L. B., and Beeson, P. B., J. Exp. Med. **104**, 803 (1956).
15. Searcy, R. L., Reardon, J. E., and Foreman, J. A., Amer. J. Med. Tech. **33**, 15 (1967).
16. Folin, O., and Wu, H., J. Biol. Chem. **38**, 81 (1919).
17. Rocha, H., Guze, L. B., Freedman, L. R., and Beeson, P. B., Yale J. Biol. Med. **30**, 340 (1958).
18. Braude, A. I., Shapiro, A. P., and Siemienski, J., J. Clin. Invest. **34**, 1489 (1955).
19. Miller, T. E., and Robinson, K. B., J. Infect. Dis. **127**, 307 (1973).
20. Guze, L. B., and Beeson, P. B., Yale J. Biol. Med. **30**, 315 (1958).
21. Diener, B., Carrick, Jr., L., and Berk, R. S. Infect. Immunity, **7**, 212 (1973).
22. Meinke, G., Barum, J., Rosenberg, B., and Berk, R. S., Infect. Immunity **2**, 538 (1970).
23. Cruickshank, C. N. D., and Lowbury, E. J. L., Brit. J. Exp. Pathol. **34**, 583 (1953).
24. Sensakovic, J. W., and Bartell, P. F., J. Infect Dis. **129**, 101 (1974).

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