# THE ROLE OF IMMUNITY IN THE PATHOGENESIS OF EXPERIMENTAL RETROGRADE PYELONEPHRITIS*, ‡

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Immunity has been demonstrated to be a significant determinant in the pathogenesis of experimental hematogenous pyelonephritis in rats infected with strains of *Escherichia coli*, *Klebsiella pneumoniae*, or *Proteus mirabilis* (1). Specifically, either active or passive preimmunization resulted in decreased infection following subsequent hematogenous challenge by the homotypic strain. However, preimmunization could confer resistance to hematogenous pyelonephritis by promoting the more rapid removal of bacteria from the circulation, thereby diminishing the inoculum to which the kidney was exposed, rather than by retarding the localization and multiplication of bacteria within the kidney. If resistance to hematogenous pyelonephritis were the consequence of accelerated vascular clearance, the phenomenon would be of less biological significance, as there is considerable evidence that the ascending route of infection is of major importance in the pathogenesis of pyelonephritis (2, 3).

The studies which form the basis of this report demonstrate that preimmunization resulted in the development of acquired resistance in experimental retrograde pyelonephritis following intravesical challenge by *Proteus mirabilis*. Hence, accelerated intravascular clearance of a bacterial inoculum could not be the sole mechanism whereby immunization afforded protection against reinfection. Furthermore, late recrudescence of infection initially suppressed by means of preimmunization was not observed.

## *Methods*

Experimental retrograde pyelonephritis was produced in male rats of the Sprague-Dawley strain obtained from Holtzman Farms (Madison, Wisconsin), weighing 200 to 300 gm main-

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tained on "basic standard diet" obtained from Uncle Johnny Mills (Houston) and water *ad libitum*. Infection was produced by the intravesical injection of the strain of *Proteus mirabilis* employed in earlier hematogenous studies (1). This strain was originally isolated from a patient with the clinical diagnosis of pyelonephritis. The bladder was exposed through a suprapubic incision, any urine present was aspirated, then 0.2 ml of a 1:50 dilution of an 18 hour trypticase soy broth (Baltimore Biological Laboratories, Baltimore) culture was injected, and the suprapubic incision was sutured. The inoculum ranged from $10^8$ to $10^{10}$ viable bacteria.

*Evaluation of Renal Infections.*—Experimental animals were sacrificed at varying intervals and through a suprapubic incision urine was obtained for culture. The kidneys were removed aseptically and the extent of abscesses, presence of hydronephrosis, hydro-ureter, urinary tract calculi, and infection of seminal vesicles were semiquantitated on the basis of 0 to 4+. The individual kidneys were weighed, then ground with trypticase soy broth and sand using a mortar and pestle. Tenfold serial dilutions of urine and the tissue suspension were made and pour plates made utilizing MacConkey agar (Baltimore Biological Laboratories). Numbers of bacteria were expressed per gram wet weight of renal tissue.

Determinative bacteriology was performed on representative colonies from each urine or kidney specimen. Identification of reisolates as the original strain of *Proteus mirabilis* was made by agglutination of at least 3 representative colonies with specific hyperimmune rabbit antiserum.

Blood urea nitrogen determinations were performed by the urease method of Karr (4) on blood specimens using sodium versenate as an anticoagulant. The mean normal value was 26.4 mg per cent ± 9.5 mg per cent.

*Serologic Techniques.*—*Proteus mirabilis* antigen was prepared by inoculating flasks containing 250 ml of trypticase soy broth with a stock culture of *P. mirabilis*, then incubating 18 hours at 37°C. The cultures were boiled for 2 hours, centrifuged, and the supernatant discarded. The sediment was resuspended in an equal volume of saline solution (250 ml), formalinized (0.5 per cent), and stored at 4°C prior to use.

Antibody levels were determined by the following agglutination procedure: Twofold serial dilutions using 4-drop quantities of saline and an initial 1:5 dilution of rat serum were made. An equal volume (4 drops) of antigen solution was added. Final dilutions ranged from 1:10 to 1:10240. Tubes were incubated at 37°C for 3 hours, refrigerated for 48 hours at 4°C, and macroscopic agglutination noted.

*Immunization Procedures.*—

*Immunization by previous renal infection:* Acute pyelonephritis was produced by the hematogenous injection of *P. mirabilis* followed by light renal massage according to a technique previously described in detail (1). One week later the infection was eradicated by a 2 week course of antibiotic therapy consisting of chloramphenicol 50 mg per kg twice daily, and kanamycin 50 mg per kg twice daily (5). The animals were then allowed a 7 day period for any residual antibiotics to be excreted or degraded. This therapeutic regimen was effective in eradication of the antecedent infection.

*Immunization by killed bacteria: P. mirabilis* antigen was prepared by boiling an 18 hour trypticase soy broth culture for 2 hours, followed by centrifugation, and resuspension in 0.9 per cent saline solution to $\frac{1}{10}$ of the original volume. 1.0 ml of this antigen was injected subcutaneously on days 1 and 5; on day 7, 0.25 ml of the antigen was injected intravenously.

*Passive immunization:* Rabbits weighing 3 kg received increasing quantities (0.5 to 10 mg per injection) of killed organisms daily by intravenous injection over a 3 week period. The animals were bled and antisera preserved with 1 ml of 1:5000 merthiolate per 10 ml of serum and stored at −4°C. The rabbit antisera were administered intravenously prior to retrograde bacterial challenge.

## RESULTS

*Course of Retrograde Proteus mirabilis Pyelonephritis.*—Acute retrograde pyelonephritis uniformly was produced in rats by the intravesical injection of *P. mirabilis*. During the first 2 days hemorrhagic cystitis was evident. Organisms were isolated only from the bladder, renal and blood cultures being negative (Table I, Figs. 1 and 2). On the 3rd day gross infiltrates became evident in the kidneys. Concomitant with this, the mean viable bacterial counts in the kidneys rose to $\log_{10}$ 4.7 organisms per gm wet weight tissue. By 1 week gross abscesses increased to a score of 1.4+ with an associated bacterial count of $\log_{10}$ 5.9 per gm of kidney. At 6 weeks gross abscesses had further increased (mean 2.8+) and the viable bacterial count had risen to $\log_{10}$ 8.3 organisms

TABLE I

*Course of Experimental Pyelonephritis Following the Intravesical Injection of Proteus mirabilis*

| Duration of Infection | No. rats | Gross abscesses (0 to 4+) | Log of No. organisms per gm kidney | Log of No. organisms per ml urine | Mean titer of antibody | Renal calculi (0 to 4+) | Blood urea nitrogen |
|---|---|---|---|---|---|---|---|
| | | | | | | | *mg per cent* |
| 10 min. to 4 hrs. | 4 | 0 ± 0 | 0.50 ± 0.96 | 8.1 ± 0.71 | 0 | 0 ± 0 | —(29.0*) |
| 1 day | 1 | 0 ± 0 | 0.75 ± 1.1 | >4.7 | 0 | 0.2 | — |
| 2 days | 1 | 0 ± 0 | 0 ± 0 | >6.3 | 0 | 0.5 | 29.0 |
| 3–4 days | 6 | 0.87 ± 3.0 | 4.7 ± 3.5 | >7.7 ± 0 | 1:80 | 0.63 ± 0.53 | 93.0 ± 89.2 |
| 5–7 days | 12 | 1.4 ± 1.7 | 5.9 ± 4.6 | — | 1:160 | 0.92 ± 0.72 | 112.5 ± 132.0 |
| 2 wks. | 12 | 1.4 ± 1.2 | 6.8 ± 3.8 | — | 1:320 | 1.6 ± 0.75 | 82.4 ± 100.3 |
| 6 wks. | 5 | 2.8 ± 1.1 | 8.3 ± 1.6 | — | 1:1280 | 3.3 ± 0.84 | 66.2 ± 12.0 |

* 29.0 mg per cent is mean of 40 normal rats tested.

per gm wet weight tissue. As observed in animals with proteus pyelonephritis of hematogenous origin, circulating antibodies became detectable at either the 3rd or 4th day. Renal calculi became apparent during the 1st week. By 6 weeks calculi were present in large numbers both in the bladder and in the renal pelvis and were associated with severe hydronephrosis. Blood urea nitrogen levels were increased by the 3rd day and ranged from 66 to 112 mg per cent during the remaining 6 weeks period of observation. Cumulative mortality rates varied from 20 to 50 per cent in separate experiments.

*Evaluation of Immunization.*—The effectiveness of various methods of active preimmunization in protecting against retrograde pyelonephritis is seen in Table II and Fig. 3. The first method of active immunization evaluated was antecedent infection which had been eradicated by antibiotics. At the time of challenge by means of intravesical inoculation of homotypic organisms, mean antibody titers in 2 groups of immunized animals were 1:640 and 1:320. Both groups exhibited marked protection against infection with gross abscesses

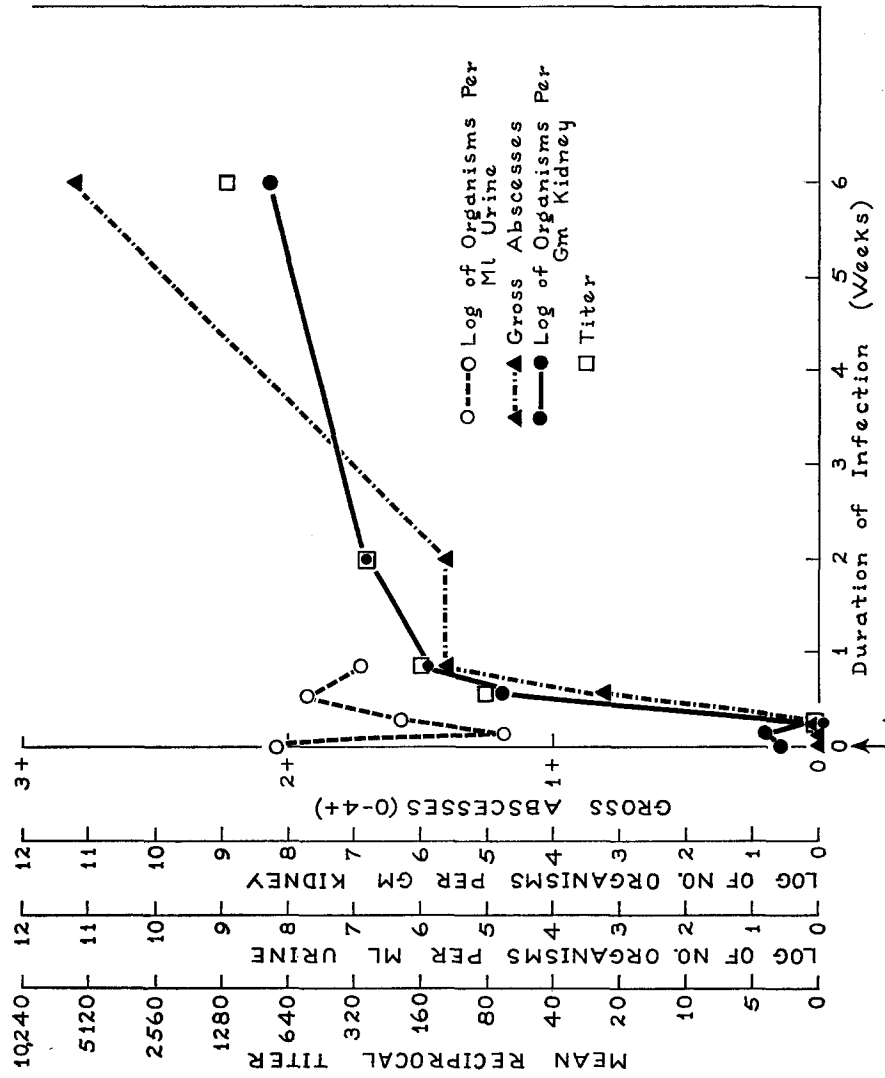


FIG. 1. Course of development of abscesses, bacterial multiplication, and agglutinin response in normal rats following retrograde challenge with a strain of *Proteus mirabilis*.

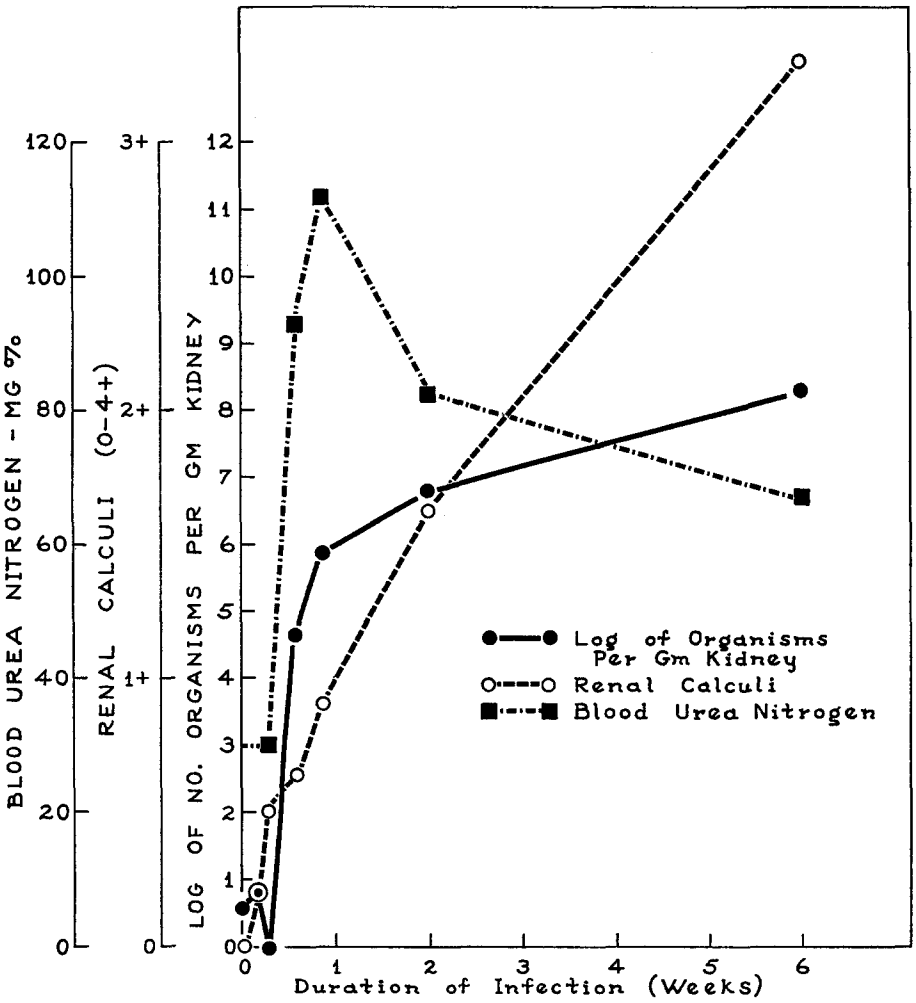


FIG. 2. Course of retrograde *Proteus mirabilis* pyelonephritis in rats. Correlation between bacterial multiplication, blood urea nitrogen levels, and development of urinary tract calculi.

averaging 0.1+ and 0 as compared to 1.6+ and 2.8+ in controls ($P < 0.01$). Concomitantly, the $\log_{10}$ of the number of organisms per gm kidney in the 2 immunized groups were 1.2 and 0 as compared to 8.1 and 8.3 in controls ($P < 0.01$). As would be expected, corresponding to the lesser degree of infection in the immunized groups, the degree of renal calculus formation and the blood urea nitrogen levels in immunized animals were lower than in controls. Animals autopsied at 1 week did not exhibit as marked a difference in degree of stone formation as those autopsied at 6 weeks, inasmuch as even in controls

relatively few stones were formed at 1 week. Recrudescence of infection was not apparent in animals studied 6 weeks after retrograde challenge.

The second method of active immunization involved the administration of heat-killed organisms as antigen. Prior to the intravesical inoculation of organisms the mean titer of the immunized group was 1:160. Again, preimmunization afforded a marked degree of protection against infection. Gross abscesses were 0.06+ in immunized animals as compared to 1.3+ in controls ($P < 0.02$). The $\log_{10}$ of the number of organisms per gm kidney was 1.5

TABLE II

*Effectiveness of Active Immunization in Experimental Pyelonephritis following Intravesical Injection of Proteus*

| Type of active immunization | Group | Mean titer before challenge | No. rats | Gross abscesses* (0 to 4+) | Log of No. organisms per gm kidney* | Renal calculi* (0 to 4+) | Blood urea nitrogen* |
|---|---|---|---|---|---|---|---|
| | | | | | | | *mg per cent* |
| Previous infection‡ | | | | | | | |
| Exp. I (Autopsied at 1–2 wks.) | Immunized | 1:640 | 11 | 0.12 ± 0.39 | 1.2 ± 2.3 | 1.1 ± 1.1 | 27.7 ± 2.8 |
| | Controls | 0 | 11 | 1.6 ± 1.3 | 8.1 ± 2.8 | 1.2 ± 0.71 | 84.4 ± 104.7 |
| | *P*§ | | | <0.01 | <0.01 | >0.50 | >0.05 <0.10 |
| Exp. II (Autopsied at 6 wks.) | Immunized | 1:320 | 7 | 0 ± 0 | 0 ± 0 | 0.57 ± 1.5 | 26.1 ± 6.5 |
| | Controls | 0 | 5 | 2.8 ± 1.1 | 8.3 ± 1.6 | 3.3 ± 0.84 | 66.2 ± 22.4 |
| | *P* | | | <0.01 | <0.01 | <0.01 | <0.01 |
| Antigen (Autopsied at 2 wks.) | Immunized | 1:160 | 4 | 0.06 ± 0.18 | 1.5 ± 1.9 | 1.0 ± 0.37 | 28.3 ± 7.2 |
| | Controls | 0 | 5 | 1.3 ± 1.4 | 6.0 ± 3.9 | 1.7 ± 3.9 | 59.6 ± 38.2 |
| | *P* | | | <0.02 | <0.01 | >0.01 <0.02 | >0.01 <0.05 |

* Mean ± SD.

‡ Animals infected 3 weeks previously and then infection eradicated with antibiotics.

§ *P* based on student's method.

in immunized animals and 6.0 in control animals ($P < 0.01$). Renal calculi and blood urea nitrogen levels were reduced in the immunized groups.

Passive prechallenge immunization (Table III, Fig. 3) also protected animals against retrograde pyelonephritis. Rats given 1 ml of hyperimmune rabbit antiproteus serum 4 hours before challenge had a mean antibody titer of 1:80 at the time of challenge. At 1 week gross abscesses in this group were 0.01+ as compared to 0.7+ in a control group of animals ($P = 0.05$). Correspondingly, the $\log_{10}$ of the number of organisms in the immunized group was 1.2 as compared to 3.7 in the control group ($P = 0.05$).

## DISCUSSION

These observations demonstrated that preimmunization, whether the result of pyelonephritis which had been eradicated, the result of administration of

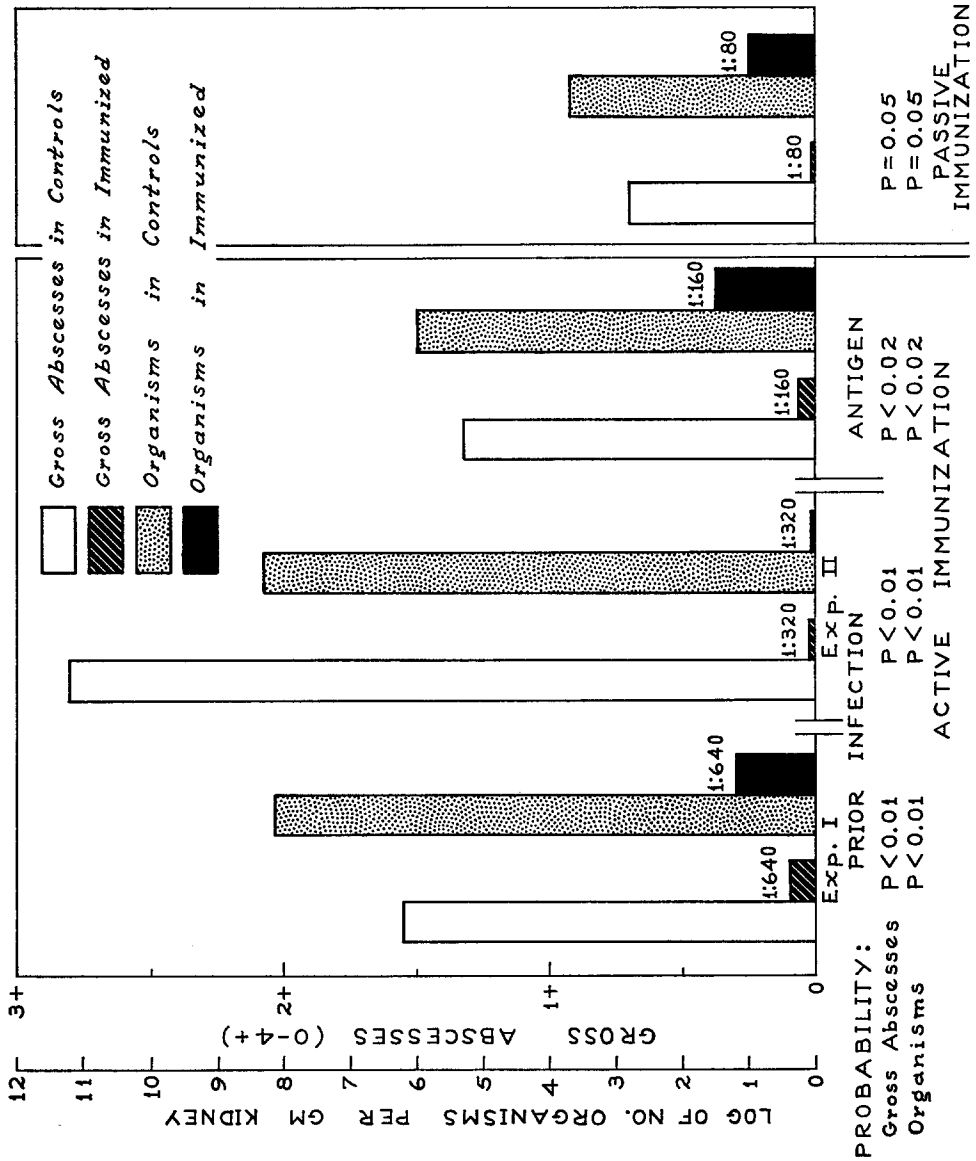


FIG. 3. Effect of active and passive preimmunization on the development of pyelonephritis following retrograde challenge with a strain of *Proteus mirabilis* based upon comparison of abscess formation and bacterial multiplication. Prior infection was eradicated by means of antibiotics. In experiment I animals were sacrificed 1 week following retrograde challenge and in experiment II, 6 weeks following challenge.

heat-killed antigen, or the result of the passive administration of specific rabbit antiserum, protected rats against retrograde pyelonephritis when challenged with homotypic organisms. The evidence from the present studies strongly suggests that accelerated vascular clearance of organisms could not be the sole mechanism of protection. Thus, during the 6 weeks interval of experimental observation, preimmunization must either have prevented or retarded the multiplication of *P. mirabilis* within the renal parenchyma.

The concept that accelerated vascular clearance is not the mechanism of protection is substantiated by the findings of Braude and Siemienski, who reported that clearance of *E. coli* from the blood during the first 5 minutes was not increased by immunization (6). Furthermore, in agreement with our ob-

TABLE III

*Effectiveness of Passive Immunization* in Experimental Pyelonephritis Following Intravesical Injection of Proteus (Animals Autopsied at 1 Week)*

| Group | Mean titer before challenge | No. rats | Gross abscesses‡ (0 to 4+) | Log of No. organisms per gm kidney‡ | Renal calculi‡ | Blood urea nitrogen‡ |
|---|---|---|---|---|---|---|
| | | | | | | *mg per cent* |
| Immunized.... | 1:80 | 7 | 0.01 ± 0.03 | 1.2 ± 2.7 | 0.33 ± 0.45 | 39.7 ± 18.7 |
| Controls..... | 0 | 7 | 0.72 ± 1.34 | 3.7 ± 3.7 | 0.24 ± 0.17 | 51.7 ± 33.3 |
| *P*§..... | | | 0.05 | 0.05 | >0.50 | >0.10<br><0.50 |

* Given 1 ml of hyperimmune rabbit antiproteus serum 4 hours before challenge.

‡ Mean ± SD.

§ *P* based on Student's method.

servations, Arana, Kozij, and Jackson reported that preimmunization against *E. coli* O-22 had an early protective effect against retrograde pyelonephritis in rats. However, they reported that preimmunized animals developed chronic pyelonephritis several weeks after the initial retrograde challenge (7). Andersen and Jackson previously had demonstrated that pyelitis represented the initial phase in the pathogenesis of retrograde pyelonephritis in rats (8, 9). Pyelonephritis was the result of subsequent cortical extension of chronic pyelitis. Their data would suggest that preimmunization may have suppressed but did not prevent the establishment of low-grade pyelitis which later progressed. In contrast, during 6 weeks observation we did not observe the delayed development of pyelonephritis after an initial interval of protection. An explanation for this discrepany is not readily apparent although different bacterial species were employed. An alternative possibility could be suggested from the experiments of Beeson and Rowley, who demonstrated that renal homogenates exert an anticomplementary action involving inactivation of the

fourth component of complement, probably through the formation of ammonia (10). An increase in acid-ash residue in the diet would increase glutaminase activity of renal tubular cells and as a consequence of the countercurrent mechanisms would result in a marked increase in tissue ammonia content in the renal papillae (11). Variation in tissue ammonia levels with concomitant variation in complement activity could significantly influence the effectiveness of preimmunization.

From review of earlier studies, three patterns of hematogenous pyelonephritis emerged to suggest that immunity was a significant determinant (1). First, the course of pyelonephritis due to strains of *Escherichia coli* was acute, self-limited, and associated with the development of circulating agglutinins. Following healing, this pattern of infection was associated with acquired resistance to reinfection with the same bacterial strain. Second, the course of pyelonephritis due to a strain of *Klebsiella pneumoniae* type C was chronic. This infection was not associated with the production of circulating agglutinins against encapsulated strains. Acquired resistance to reinfection by the homotypic strain could not be demonstrated following eradication of infection but was produced by the passive transfer of concentrated antiserum. Third, pyelonephritis due to *Proteus mirabilis* was associated with circulating agglutinins and resistance to reinfection with the same organism following eradication of infection. Yet the course of infection was chronic when uninterrupted by specific antibacterial therapy (1, 5). The chronicity appeared to be the consequence of obstructive uropathy resulting from calculi which developed during the course of the infection.

The experimental animal data of Jackson and coworkers indicate that immunization after establishment of infection did not eradicate bacteria from the pelvis or medulla although extension into the cortex was decreased (9, 12). We would concur in their conclusions that: "Little support is gained therefore for any benefit to be derived from vaccine administration in the treatment of established urinary infection with a similar strain." However, there are clinical data which suggest that recurrent urinary tract infections often represent reinfection with serologically distinct bacteria rather than recrudescence of an initial infecting organism (13, 14). Under such circumstances preimmunization by prior infection may be an important determinant to the course of reinfection or even parenchymal progression and renal destruction in non-obstructive pyelonephritis.

Current studies correlating the antibody response of patients with urinary tract infections to the infecting organisms are preliminary and do not enable unequivocal evaluation of the hypothesis that immunity represents a significant determinant in pyelonephritis (15, 16). Furthermore, clinical interpretation of the role of immunity as a determinant in pyelonephritis will require appreciation of the experimental observation that "obstructive uropathy" may either modify or nullify the protective action of antibody (1).

The earlier demonstration that in experimental pyelonephritis preimmunization had a significant role in resistance to reinfection by the hematogenous route of challenge strengthened by the present observation that preimmunization protected rats against retrograde pyelonephritis when challenged by homotypic organisms emphasizes the need to attempt further to elucidate the role of immunity in clinical pyelonephritis.

## SUMMARY

Retrograde pyelonephritis was produced in rats by the intravesical injection of *Proteus mirabilis*. When animals were preimmunized against *Proteus mirabilis* by (*a*) prior infection, (*b*) administration of antigen, or (*c*) passively transferred antiserum, they were resistant to infection by proteus when challenged by the retrograde route.

The protective effect of specific preimmunization in retrograde pyelonephritis indicates that a major site of action is retardation of bacterial growth within the parenchyma of the kidney.

## BIBLIOGRAPHY

1. Sanford, J. P., Hunter, B. W., and Souda, L. L., The role of immunity in the pathogenesis of experimental hematogenous pyelonephritis, *J. Exp. Med.*, 1962, **115,** 383.
2. Beeson, P. B., Factors in the pathogenesis of pyelonephritis, *Yale J. Biol. and Med.*, 1955, **28,** 81.
3. Kass, E. H., Bacteriuria in pyelonephritis of pregnancy, *Am. Med. Assn. Arch. Int. Med.*, 1960, **105,** 194.
4. Determination of urea; Method of Karr, *in* Practical Physiological Chemistry, (P. B. Hawk, B. L. Oser, and W. H. Summerson, editors), New York, Blakiston Co., 1954, 554.
5. Hunter, B. W., Souda, L. L., and Sanford, J. P., Antibiotic therapy in established experimental *Proteus mirabilis* pyelonephritis, in Antimicrobial Agents and Chemotherapy—1963, (J. L. Sylvester, editor), in press.
6. Braude, A. I., and Siemienski, J., The influence of endotoxin on resistance to infection, *Bull. New York Acad. Med.*, 1961, **37,** 448.
7. Arana, J. A., Kozij, V., and Jackson, G. G., Effect of alterations in humoral and cellular host defense mechanisms on the pathogenesis of retrograde pyelonephritis, *J. Lab. and Clin. Med.*, 1962, **60,** 856, (abstract).
8. Andersen, B. R., and Jackson, G. G., Pyelitis, an important factor in the pathogenesis of retrograde pyelonephritis, *J. Exp. Med.*, 1961, **114,** 375.
9. Andersen, B. R., and Jackson, G. G., Persistent pyelitis and pyelonephritis from retrograde urinary tract infection with a strain of klebsiella in rats, *J. Lab. and Clin. Med.*, 1962, **60,** 457.
10. Beeson, P. B., and Rowley, D., The anticomplementary effect of kidney tissue. Its association with ammonia production, *J. Exp. Med.*, 1959, **110,** 685.
11. Rector, F. L., Discussion, *in* Biology of Pyelonephritis, (E. L. Quinn and E. H. Kass, editors), Boston, Little, Brown and Co., 1960, 443.

12. McCabe, W. R., and Jackson, G. G., The natural course of retrograde infections of the urinary tract of rats with different serotypes of *Escherichia coli* or enterococcus, *in* Biology of Pyelonephritis, (E. L. Quinn and E. H. Kass, editors), Boston, Little, Brown and Co., 1960, 39.
13. Turck, M., Browder, A. A., Lindemeyer, R. I., Brown, N. K., Anderson, K. N., and Petersdorf, R. G., Failure of prolonged treatment of chronic urinary-tract infections with antibiotics, *New England J. Med.*, 1962, **267**, 999.
14. Kunin, C. M., Microbial persistence versus reinfection in recurrent urinary tract infections, *in* Antimicrobial Agents and Chemotherapy—1962, (J. L. Sylvester, editor), Ann Arbor, Braun-Brumfield, Inc., 1963, 21.
15. Carter, M. J., and Ehrenkranz, N. J., A comparison of bacteriological and immunological studies of induced urinary tract infection in man, *Bact. Proc.*, 1962, 87, (abstract).
16. Brainerd, H., Williamson, J., and Scaparone, M., Antibacterial immunity in urinary tract infections, *Clin. Research*, 1963, **11**, 117, (abstract).