Pyelonephritis IV. Role of Serum Bactericidal Activity and Antibody in Chronic Enterococcal Pyelonephritis in the Rat.* (28531)

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The significance of immune factors in the pathogenesis of pyelonephritis is poorly understood. Since pyelonephritis appears to be the direct consequence of bacterial infection of the kidney, it would be expected that antibacterial immune factors could play some role in the natural history of this disease. Speculation has been made about protective mechanisms which might be operative and a deficiency of these mechanisms which might predispose to infection. Alternatively, the question has been raised concerning the nature of the renal injury in chronic pyelonephritis. Could this be an auto-immune type of disease(1)?

Only limited observations have been made on the possible roles played by immunological phenomena in pyelonephritis. Needell *et al.*(2) found there was a rise in antibacterial antibodies, demonstrable by the indirect hemagglutination technique, in patients with pyelonephritis. The biological significance of these antibodies is not clear. Jacobson and Braude(3) demonstrated that sera of some patients with pyelonephritis had a reduced bactericidal effect on some of the organisms commonly causing human pyelonephritis. They suggested such patients may have had a primary disturbance in the bactericidal system, although it was, also, possible that the development of resistant bacteria played a role. Beeson and Rowley(4) reported studies which may indicate a partial explanation of the peculiar susceptibility of the kidney to infection by coliform bacteria. These investigators found that the kidney, in contrast to other tissues, interfered with the bactericidal activity of normal serum. They thought this was due to destruction of the fourth component of complement by ammonia produced by renal glutaminase activity.

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Weyrauch *et al.*(5) observed the course of experimental pyelonephritis established in rabbits by intravenous injection of *Escherichia coli* after partial ligation of one ureter. Previous vaccination with heat-killed organism appeared to lessen the severity of infection, judged by study of the pathology of infected kidneys. McCabe and Jackson(6) studied chronic pyelonephritis in rats produced by the ascending route (*i.e.*, instillation of either enterococci or coliform bacilli into the bladder). They found that specific agglutinating antibody appeared when proliferation of bacteria in the kidney was most evident and, therefore, "does not have a protective or remedial influence upon the local renal lesion, but it may prevent systemic or metastatic infections."

Anderson and Jackson(7) performed a similar study in rats, using a strain of *Klebsiella* to produce an ascending infection. This infection began as a pyelitis, and it was only after approximately 6 weeks that the disease spread to the medulla and, thence, to the cortex of the kidney. Progression of the disease occurred despite development of antibacterial antibodies demonstrable by the hemagglutination procedure. Neither sera of uninfected animals, nor those obtained from animals which developed high titer antibodies subsequent to infection demonstrated any bactericidal activity against the infecting organism.

Sanford *et al.*(8) studied hematogenous pyelonephritis in rats, using several strains of *Escherichia coli*, a strain of *Klebsiella pneumoniae* and a strain of *Proteus mirabilis*. Infection was produced by intravenous inoculation of the organism and renal massage. It was found that *Escherichia coli* produced an acute self-limited infection, associated with vigorous antibody response. After recovery, the animals were resistant to intravenous reinfection with the same strain of organism.

However, *Klebsiella pneumoniae* produced chronic infection; no antibody formation occurred; and no resistance to reinfection could be demonstrated when the infection was eradicated by antibiotic treatment. A third pattern of response was noted with *Proteus mirabilis*. This organism produced a chronic and prolonged infection despite the presence of circulating antibody. Nevertheless, if the infection was eliminated by antibiotic treatment, the animals were resistant to reinfection. These data suggest that circulating antibody plays some role in elimination of bacteria from the kidney during infection as well as in protecting the host, following repeated intravenous challenge. In *Proteus* infection, chronicity was thought by Sanford *et al.*(8) to be due to development of areas of intrarenal obstructive uropathy (secondary to calculus formation) which interfered with the ability of the immune mechanism to clear the infection. The resistance of animals to reinfection might have been due to exposure of the *Proteus* to circulating antibody before the organism became established in the kidney within areas of intrarenal obstruction.

It has been previously reported from this laboratory(9) that it is possible to produce chronic pyelonephritis in the rat by intravenous inoculation of a strain of *Str. faecalis*. Although this organism is not frequently isolated in human disease, this experimental model has the advantage that no manipulation of the animal is required (*i.e.*, renal massage or ureteral ligation). Thus, it is not necessary to distinguish effects of trauma from those of infection *per se*. Furthermore, the infection is characterized by persistence of bacteria in relatively constant numbers during evolution of the disease from acute to chronic stage over a period of 2 years. For these reasons it was thought worthwhile to study the role of immunological phenomena in the pathogenesis of this experimental model.

Materials and methods. The animals and bacterial strain used, method of injection of organism, and estimation of bacterial content of organs have been described(9). Blood for serological study was obtained by cardiac puncture from rats at time of sacrifice. Serum was promptly separated and stored at −20°C until used. For normal rat serum, uninfected rats were bled at random by cardiac puncture and the serum similarly stored.

Agglutination test. The antigen used in the agglutination test was prepared from surface growth of an 18-hour culture of *Str. faecalis* on trypticase soy agar and diluted in physiological saline to match McFarlane #3 standard. Two-fold serial dilutions of sera were made in physiological saline in 0.5 ml amounts. Equal volume of antigen was added, the mixtures incubated for 2 hours at 37°C, and read after overnight storage at 4°C. The end-point recorded was the highest dilution of serum showing definite macroscopic agglutination.

Hemagglutination test. The method of Lancefield(10), as modified by Kaplan(11), for isolation of "M protein" from group A streptococci was used to prepare an immunologically active protein from 50 liter batches of *Str. faecalis* grown 18 hours in trypticase soy broth. This extract contained 1.63 mg protein/ml. Aliquots of this material were used to immunize rabbits in order to obtain a reference antiserum of known potency for standardization of the hemagglutination procedure. This antiserum had a titer of 1:2560.

The procedure of Stavitsky(12) was followed except that 1:5000 tannic acid was used to tan sheep cells (obtained weekly in Alsever's solution from various commercial sources), since preliminary studies demonstrated that this concentration gave better results than the 1:20,000 concentration used by Stavitsky. Each day's experiment was accompanied by titration of the known rabbit serum and results were accepted only if the rabbit serum demonstrated the expected titer.

Serum bactericidal test. This test was carried out by a slight modification of the method of Beeson and Rowley(4). Since preliminary studies indicated that both physiological saline and "minimal medium" were slowly bactericidal for *Str. faecalis*, 0.5% peptone water was used as diluent. It was found that serum bactericidal activity against *Str. faecalis* was not as marked as that reported against the strain of *Escherichia coli* used by Beeson and Rowley. For

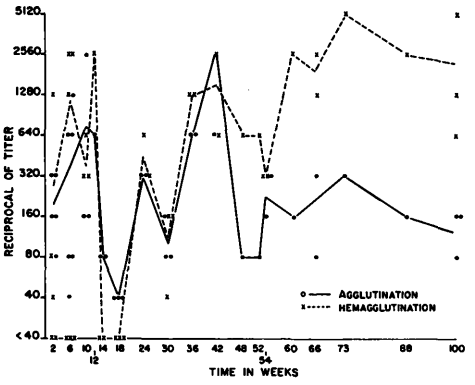

FIG. 1. Bacterial agglutination and hemagglutination titers in rats after I.V. injection with *Str. faecalis*.

this reason the incubation of *Str. faecalis* and serum was prolonged to 2 hours at 37°C. The bacterial inoculum used was approximately 10^3 organisms per ml of reaction mixture. In early experiments, counting of surviving bacteria was carried out by the pour plate method. Subsequently, the calibrated surface drop method of Miles(13) was substituted because it was equally accurate and easier to do. In each experiment, sera from both normal and infected rats as well as a diluent control consisting of bacteria and peptone water were run simultaneously.

Results. Circulating antibody. Preliminary studies of sera of normal (uninfected) rats indicated that none had either agglutinating or hemagglutinating antibodies against *Str. faecalis*. To conserve serum, a final serum dilution of 1:40 was the lowest dilution chosen for testing. Both agglutination and hemagglutination studies were made on sera of rats infected from 2 to 100 weeks. Infection was determined by quantitative enumeration of the renal microbial population and/or demonstration of macroscopic abscesses. The agglutination and hemagglutination titers are shown in Fig. 1. Each point in the figure represents the results obtained in a single animal. During the first 40 weeks the agglutination and hemagglutination titers were similar. Subsequently, the hemagglutination titers were somewhat higher.

Bactericidal activity of sera. The results are shown in Table I and indicate that bacterial survival in sera from uninfected rats was 13 to 77% (average 33%) of the original inoculum. When sera of animals infected for variable periods were similarly tested, it was found that the proportion of bacteria remaining viable at the end of 120 minutes exposure varied, but no significant difference was noted between infected and non-infected animals. No correlation was found between serum bactericidal activity and hemagglutination titer. The percentage of bacteria remaining viable in immune rabbit serum (hemagglutination titer of 1:2560) was 78.

Discussion. It would appear from these results that serum factors do not play a significant role in experimental hematogenous pyelonephritis produced by *Str. faecalis* in the rat. Although normal rat serum is bac-

TABLE I. Agglutination, Hemagglutination Titers and Serum Bactericidal Activity of Rats After Intravenous Injection with *Str. faecalis*.

Duration of infection (wk)	Titer (reciprocal)		% of bacteria surviving at 120 min at 37°C
	Agglutination	Hemagglutination	
None (diluent control)	—	—	317*
Normal (not infected)	<40	<40	33† (range 13-77)
2	320	40	59
6	80	<40	38‡ 42
6	640	2560	54‡ 57
10	2560	640	58
12	640	2560	46
20	40	<40	54
24	320	320	23‡ 35
30	80	40	54
30	160	160	26
36	320	1280	28
42	640	640	54
42	5120	2560	53
52	80	640	50‡ 50 57
54	320	320	20
60	160	2560	33‡ 27
73	320	5120	58
100	160	1280	51
100	160	5120	75

* Avg of 12 separate experiments.
† 17 serum specimens from 7 rats.
‡ Separate determinations on same specimen of serum.

tericidal, infection is readily established in the kidney. While Beeson and Rowley(4) have indicated that kidney inactivates complement and thus destroys the complement-dependent bactericidal activity of serum against *Escherichia coli,* this does not explain the findings in the *Str. faecalis* model. *Str. faecalis* is gram positive and not susceptible to complement since heating sera at 56°C reduces bactericidal activity very little, and guinea pig serum rich in complement has no demonstrable bactericidal effect against this organism.

Once established, renal infection due to *Str. faecalis* persists throughout the normal life span of the rat despite eventual appearance of high titer of circulating antibacterial antibodies. The presence of these antibodies does not enhance bactericidal activity of serum, and thus, would not be expected to contribute to those serum factors involved in elimination of organisms from the kidney. Whether or not these antibodies participate in other host defense mechanisms, such as phagocytosis, is not known.

Studies are in progress to determine whether or not renal metabolic environment has an effect on non-complement-dependent serum bactericidal activity.

Summary. Pyelonephritis produced in rats by intravenous inoculation of *Str. faecalis* becomes established in the presence of normal serum bactericidal activity against the infecting organism. The infection persists throughout the life of the rat despite the eventual appearance of circulating antibacterial antibodies. This finding may be partially explained by the fact that these antibodies do not enhance normal serum bactericidal activity as tested.

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Pyelonephritis V. Role of Serum Bactericidal Activity and Antibody in Acute Pyelonephritis in the Rabbit.* (28532)

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Intravenous inoculation of *Str. faecalis* in the rat produces a pyelonephritis which persists for at least 2 years despite the presence of antibacterial antibodies. It has been suggested that at least a partial explanation for the chronicity of the infection may be the lack of increase of bactericidal activity of serum when antibacterial antibodies are present(1). In the rabbit, in contrast to the rat, preliminary studies revealed that the infection "burned out" in relatively few weeks. Comparative studies of antibody production and serum bactericidal activity were performed to determine whether immunological

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