LAWRENCE R. FREEDMAN*

Department of Internal Medicine, Yale University School of Medicine

EXPERIMENTAL PYELONEPHRITIS

VI. OBSERVATIONS ON SUSCEPTIBILITY OF THE RABBIT KIDNEY TO INFECTION BY A VIRULENT STRAIN OF STAPHYLOCOCCUS AUREUS**

For many years it has been known that the intravenous injection of large numbers of virulent staphylococci into normal mice or rabbits regularly results in abscess formation in the kidneys.[2,7] These infections occur also in the rat.[6] Gram-negative bacilli of the coliform group, on the other hand, very rarely produce such infections when similarly administered to normal animals;[10,11] some form of renal injury is usually required for the establishment of experimental pyelonephritis by that group of bacteria. It seemed likely, therefore, that there might be significant differences in the pyelonephritis caused by these two kinds of microorganisms. The present article is a report of experiments designed to compare the course of staphylococcal infection in the rabbit kidney with that previously noted in experiments with coliform bacterial infections.

MATERIALS AND METHODS

Except where specified, the materials and methods were the same as those described in preceding reports from this laboratory.[4,9,12] All experiments were carried out in white New Zealand male rabbits weighing between 2 and 3 kilograms.

Staphylococcus aureus. This organism, known as Giorgio, was supplied to us by Dr. David Rogers of Vanderbilt Medical School. It had originally been isolated from a staphylococcal abscess in man. It is hemolytic and coagulase positive, and has been characterized in some detail by Smith and Dubos.[15] Intravenous injection of 10^7 organisms (18-hr. culture) into mice was found to cause the death of approximately two-thirds within 7 days. The organism was maintained at room temperature on agar slants. In the beginning of this study 6 agar slants were inoculated from a broth culture, and, after overnight incubation, were sealed with paraffin. A new slant was taken each month as the source of the organism. A broth subculture was made, and from that, a plain agar plate was streaked and incubated overnight. This served as the source of organisms for the cultures used that week. The present series of experiments was completed within a six-month period.

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* Markle Scholar in Medical Science and Assistant Professor of Medicine.

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RESULTS

Infectivity of different numbers of bacteria injected intravenously

The intravenous inoculation of 10^8 staphylococci in normal rabbits was followed by the death of over 50 per cent of the animals within 72 hours. All of those which survived 48 hours were found to have visible kidney infections, but no gross sign of infection was found in other organs.

The ease with which the rabbit kidney can be infected by staphylococci suggests that the kidney might be peculiarly susceptible to this organism. It was decided, therefore, to determine the size of inoculum necessary for renal infection by way of the blood stream. Serial 10-fold dilutions of culture were injected into a group of rabbits. The results are given in Table 1.

TABLE 1. RELATION OF SIZE OF INTRAVENOUS INOCULUM TO INCIDENCE OF RENAL INFECTION* IN NORMAL RABBITS

No. of organisms injected	*Incidence of renal infection*†
10^4	0/7
10^5	0/6
10^6	2/5
10^7	8/8
10^8	12/12

* The recovery of 10^5 or more staphylococci per kidney is taken to indicate infection. Those kidneys not infected contained fewer than 100 organisms. Gross abscesses were visible in most infected kidneys.

† Number of infections/number of tests made.

It can be seen that 10^6 organisms were the smallest inoculum which produced renal infection. This is comparable to the number of staphylococci needed to infect the mouse kidney[7] and is similar to the number of *E. coli* given intravenously necessary to infect the rat or rabbit kidney following ligation of the ureter.[5,9] The same relationship seems to pertain to enterococcal infections of the rat kidney.[8] It would appear then that a large number of organisms must be injected intravenously in order to produce infection of the kidney.

Staphylococci could be demonstrated in the blood of about three-fourths of the animals 24 hours after they had received 10^8 organisms. Urine cultures were positive only when there was kidney infection.

Kidney lesions produced by intravenous inoculation of staphylococci

The gross lesions found in infected kidneys were of three types: (1) Isolated disc-shaped abscesses, 1.0-2.0 mm. in diameter, similar in appear-

ance to a bacterial colony growing in a solid medium (Fig. 1). These lesions were found only in the medulla and were surrounded by a comparatively thick wall of inflammatory reaction. Although they appeared to obstruct nephrons draining through that area, there was little or no evidence of spread of infection throughout the obstructed nephrons in a manner similar to that observed in other forms of intrarenal hydronephrosis.[4, 12] (2) Wedges of infection with the apices in the papilla, forming confluent abscesses on the surface of the kidney (Fig. 2). (3) Severe papillary injury associated with wedge lesions, leading to necrosis of the papilla (Fig. 3).

Effect of age of culture on virulence

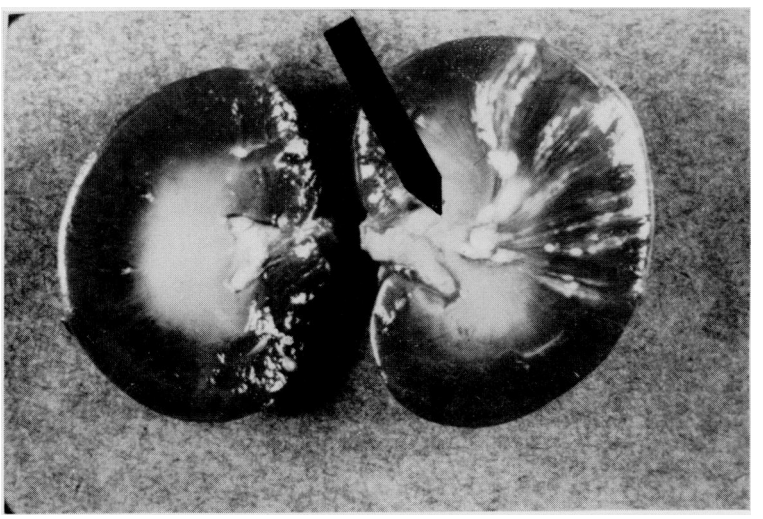
It has been demonstrated by Rogers that intravenous injection of an 18-hour culture of *E. coli* is lethal to a large percentage of rabbits, whereas the same number of organisms from a 4-hour culture produces neither death nor infection.[13] It was thought worthwhile, therefore, to determine whether the ability of staphylococci to infect rabbits varied according to age of the culture used. Using an inoculum of 10^8 organisms, no difference was found between a 4-hour and 18-hour culture in either the lethal effects or the renal lesions produced.

Effect of staphylococcal culture supernate on virulence of staphylococci and E. coli

The toxic effects of filtrates obtained from staphylococcus cultures are well known.[8] It seemed possible therefore that the ability of staphylococci, when given intravenously, to infect the normal kidney was dependent upon some effect of the simultaneously administered toxins.

To test this hypothesis, an 18-hour broth culture of staphylococci containing 10^8 bacteria per ml. was centrifuged at 3,500 revolutions per minute for 30 minutes at 10° C. This reduced the number of bacteria in the supernatant fluid to 10^6 organisms/ml. A 4-hour culture of *E. coli* (10^8/ml.) was similarly centrifuged, but the supernatant fluid in this instance was discarded. The *E. coli* were then resuspended in the original volume of staphylococcal supernate in a concentration to provide 10^8 *E. coli* per ml. Five-tenths ml. of this mixture was given intravenously to each of three rabbits. One week later the animals were sacrificed. All six kidneys appeared normal, but one of them yielded 10^5 staphylococci; the remainder were sterile. All urine cultures were negative save for the sample from the infected animal; this urine contained 10^5 staphylococci per ml.

This experiment tended to indicate that the simultaneous administration of staphylococcal culture medium did not enable *E. coli* to infect the



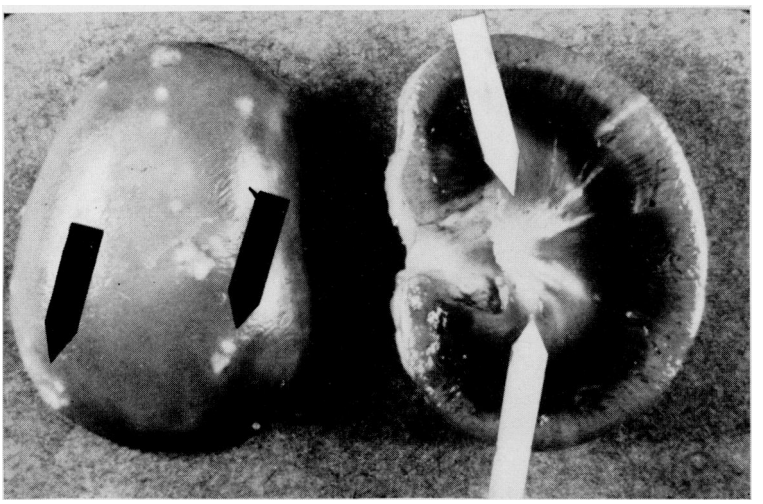
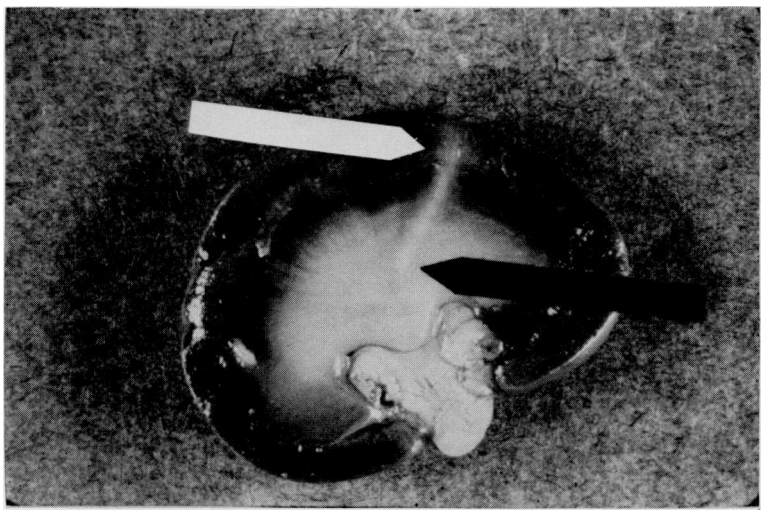


FIG. 1. Disc-shaped abscess resulting from the intravenous injection of 10^8 staphylococci.

FIG. 2. Wedge of infection resulting from the intravenous injection of 10^8 staphylococci. A normal kidney is demonstrated on the left.

FIG. 3. Papillary necrosis resulting from the intravenous injection of 10^8 staphylococci. Abscesses visible on the surface are shown on the left.



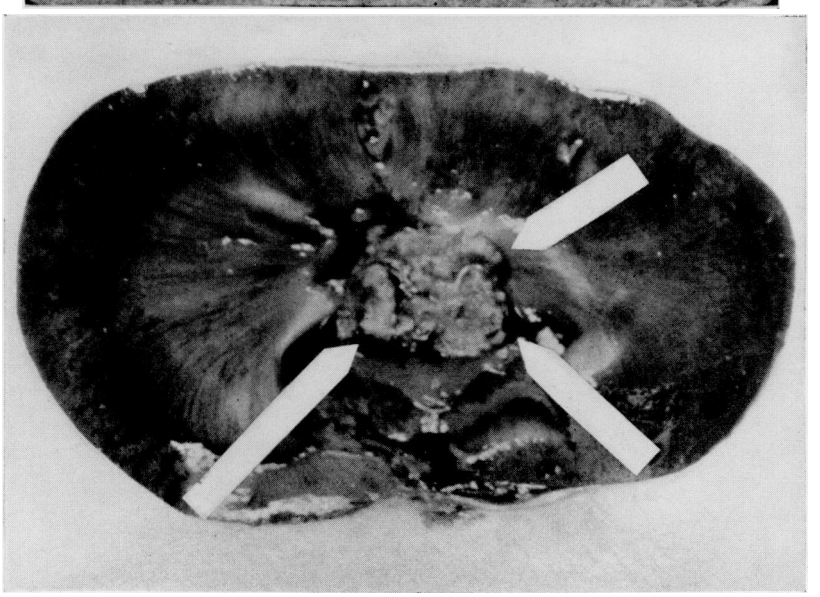


FIG. 4. Sterile linear scar one month after the direct injection of 10^4 staphylococci into the renal papilla.

FIG. 5. Renal stone one month after the direct injection of 10^4 staphylococci into the renal papilla.

normal kidney, nor was a relatively small inoculum of staphylococci able to produce more infections than it had in previous experiments.

In a related experiment, three rabbits were injected with *E. coli* centrifuged as before and resuspended in a 1:10 dilution of an 18-hour culture of staphylococci. This mixture contained the same number of *E. coli* with 1/10 the amount of staphylococcal culture medium and ten times as many staphylococci as in the previous experiment. One week after injection two animals had infection in one kidney, the remaining rabbit had bilateral renal infection. All urine cultures were positive. The positive cultures revealed staphylococci, but no *E. coli* could be identified.

TABLE 2. INCIDENCE OF INFECTION RESULTING FROM INJECTION OF BACTERIA INTO CORTEX OR MEDULLA OF THE NORMAL KIDNEY

No. of organisms injected	*Site of injection†*		*Colonies Staphylococci per kidney*
	Cortex	*Papilla*	
10		1/4	10^5
10^2		5/6	10^5, 10^5, 10^6, 10^6, 10^6
10^4	0/6		
10^5	1/3		10^5
10^6	1/1*		10^5
10^7	1/1*		10^5

* The contralateral kidney in these rabbits also became infected.
† Number of infections/number of tests made.

This experiment indicates that even when given at the same time as an infecting dose of staphylococci, *E. coli* infection cannot be detected. The findings are in agreement with those reported by Helmholtz.[10]

Direct inoculation of staphylococci into different zones of the kidney

Previously reported experiments with coliform bacteria had provided evidence of a great difference between the cortical and medullary zones of the kidney, in respect to susceptibility to infection. On direct inoculation, the number of bacteria required to infect the cortex was 100,000 or more; whereas as few as 10 organisms injected into the medulla were capable of causing infection. The same techniques were used to determine the number of staphylococci necessary to infect the two zones of the kidney. Animals were examined four days after injection of bacteria. The findings were similar to those which had been obtained with *E. coli*. See Table 2.

At least 10^5 staphylococci are required to establish infection when placed directly in the cortex of the kidney. The abscesses produced by cortical

inoculation of 10^5 staphylococci are localized to the area of injection and do not form wedges extending into the papilla. With larger numbers of organisms wedge-shaped infections are produced. These abscesses are, however, not limited to the site of injection but instead are found in both kidneys. It is evident that sufficient bacteria escaped into the blood stream at the time of injection to infect *both* kidneys.

In the case of the medulla, infection could easily be produced with small numbers of bacteria. An inoculum estimated to contain fewer than 10 viable units caused infection in one of four animals tested, and an inoculum containing between 10 and 100 viable units caused infection in five of six animals. The infection produced by the direct injection of staphylococci into the renal medulla also follows a course like that observed with *E. coli* infection. The process remains confined to a wedge of tissue in which tubular drainage had been interrupted by the needle puncture. This is remarkable in view of the capacity of staphylococci to infect the normal kidney via the blood stream. One might have expected the same infectivity to be apparent by either direct extension of infection through the kidney substance, or by the bathing of normal papillary tissue in heavily infected urine.

Rate of healing of staphylococcal abscesses

Further similarity between the lesions caused by the two organisms was found in the rate of healing of staphylococcal abscesses. Ten rabbits which had received papillary injections of from 10^2 to 10^4 staphylococci were examined two months afterward. Seven of them had but a single linear scar to mark the site of previous infection (Fig. 4), and cultures of whole kidneys and urine in these animals were sterile. In the remaining three, there was still an active infection, but this appeared to be related to the formation of renal calculi. These concretions, in the pelves of the kidneys, were yellow-brown, coarsely corrugated, oval in shape, with the longest dimension measuring 0.2-1.0 cm. (Fig. 5).

Inoculation of staphylococci into bladder urine

The staphylococcus used in the present experiments grows about as well in rabbit urine as it does in broth. It was decided therefore to test the effects of inoculating staphylococci into the bladder urine of 10 normal rabbits. Twelve to 14 days after the injection of 10^8 staphylococci the kidneys and bladders of all animals appeared normal. The left kidney of one rabbit contained 10^5 staphylococci, the remaining kidneys were sterile. In addition to the animal with the positive kidney culture, three other rab-

bits had staphylococci in the bladder urine. It is difficult to interpret the one positive kidney culture in an organ which appeared normal. Conceivably the bacteria were found in this organ as a result of reflux of infected urine from the bladder. It can be said at any rate that staphylococci inoculated into bladder urine are rarely able to establish infection in the normal kidney of the rabbit.

In other experiments, not published, we have found that coliform bacteria also do not infect the rabbit kidney following inoculation into bladder urine.

DISCUSSION

The present studies, utilizing a virulent strain of *Staphylococcus aureus,* have demonstrated that the susceptibility to infection of different zones of the kidney on direct inoculation of this organism is similar to that previously demonstrated for *E. coli;* infection is produced with as few as 10 organisms in the medulla, whereas approximately 100,000 are required to infect the cortex. The infections produced by the direct inoculation of either of these organisms are similar in appearance, method of spread and rate of healing. These results are surprising in view of the fact that when sufficient bacteria are injected into the blood stream of normal animals staphylococci regularly produce renal infection whereas the coliform bacteria do not. Factors such as age of the culture and the effect of culture fluid did not appear to be of importance. The question had to be considered whether transport through the blood stream either enhanced the virulence of the staphylococcus or diminished that of *E. coli.*

There is no information indicating that the virulence of staphylococci is enhanced when these organisms are inoculated into the blood stream. There is considerable evidence, however, which indicates that "avirulent" *E. coli* are more vulnerable to phagocytosis and destruction by leukocytes than are "virulent" staphylococci. This has been demonstrated *in vitro* by Cohn and Morse using normal rabbit leukocytes.[1] Of particular interest, however, are observations in normal rabbits[14] which demonstrate that following initial rapid clearance, staphylococci persist in the blood stream for many hours in a concentration 10 to 1,000 times that of *E. coli.* Some of our experiments in rats also lend support to this observation. Two hours following the injection of similar inocula, the number of staphylococci recoverable from the kidneys is 10 to 100 times as great as *E. coli.*[8] It is inconceivable that multiplication of staphylococci within two hours could account for this difference. Clearly then, staphylococci are much more

likely than coliform bacteria to arrive at the proper place in the kidney in a concentration sufficient to produce infection.

While one would expect to find great differences in pathogenicity of a "virulent" staphylococcus and an "avirulent" strain of coliform bacteria, the surprising result of these studies has been the remarkable similarities in their ability to produce infections when injected directly into the kidney. *This would seem to indicate that the factor most decisive in determining virulence for these two organisms is the effectiveness of host defense mechanisms as they operate in the circulating blood.*

Evidence has previously been presented pointing to the medulla of the kidney as the site of initial bacterial multiplication in experimentel pyelonephritis.[4, 12] The present work offers further confirmation of this thesis in the study where injection of 10^6 staphylococci into the cortex of one kidney produced wedge-shaped infection involving the medulla of both kidneys. A concentration of 10^5 bacteria per 0.05 ml. injected directly into the cortex had been shown necessary to produce local infection. This concentration of bacteria would not be achieved in the blood stream when only 10^6 organisms were injected. Since only 10 or fewer staphylococci per .05 ml. are required to produce medullary infection, it is likely that under these circumstances renal infection resulted from blood stream dissemination of organisms and had its beginning in the medulla of the same and the opposite kidney.

SUMMARY

Renal infection by the intravenous route in normal rabbits requires 10^6 or more virulent staphylococci. Direct injection of the organisms into the cortex and medulla of the kidney indicates a great difference in susceptibility of these two zones, since fewer than 10 bacteria produced infection in the medulla whereas 100,000 or more were necessary to cause an abscess in the cortex.

A virulent staphylococcus and the coliform bacteria differ in their capacity to infect the normal kidney following intravenous injection. Aside from this one feature, however, the present studies based on direct inoculation of bacteria into the kidney emphasize the remarkable similarities in the capacity of these organisms to produce infection. It is suggested that the difference in virulence demonstrable only upon intravenous inoculation is dependent upon the effectiveness of host defense mechanisms in the blood stream, thereby determining the fate of bacteria which have been injected and the proportion of the inoculum which is able to lodge in the kidney.

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