# EXPERIMENTAL PYELONEPHRITIS

## I. EFFECT OF URETERAL LIGATION ON THE COURSE OF BACTERIAL INFECTION IN THE KIDNEY OF THE RAT*

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It has been known for many years that the susceptibility of the kidney to infection by bacteria injected intravenously is greatly enhanced if the flow of urine has been obstructed by ligating the ureter. This usually results in rapidly progressing pyogenic infection in the affected kidney, whereas the opposite, unobstructed, organ remains normal (1). The procedure has been employed in investigations of the pathways of infection in pyelonephritis, the histology of acute and chronic pyelonephritis (2), and experimental hypertension (3). However, little attention has been given to elucidation of the mechanism by which obstruction affects the susceptibility of the kidney to infection. The work reported here was undertaken in an attempt to identify some factors which may bear on that point.

## Materials and Methods

*Animals.*—White male Sprague-Dawley strain rats, weighing 200 to 300 gm. were used.

*Bacterial Strains.—Escherichia coli.* The culture employed was a strain of *E. coli* originally isolated from the urine of a patient with pyelonephritis. Before using it in the experiments to be described, the organism was "passed" through a series of animals by injecting the culture intravenously into rats with obstructed ureters. One week after inoculation the affected kidney was removed, homogenized, and cultured, and the bacteria obtained were then injected into another prepared animal. After twelve such animal passages, the organism was grown in beef heart infusion broth for 3 hours; 5 ml. portions then being frozen and stored at $-20°$C. Each week one tube was warmed to $37°$C., incubated for 24 hours, then "passed" once more through a rat. The culture recovered, after proper identification, was used for all experiments during the next week.

*S. marcescens:* A stock strain of *Serratia marcescens*, which produced good pigmentation on incubation at room temperature, was obtained from the Department of Microbiology, Yale Medical School. It was maintained by weekly inoculation of nutrient agar.

Identification of these organisms was by colony characteristics, Gram stain, pigment pro-

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duction, and characteristic reactions on Simmons' citrate and Kligler's iron agar. In the case of staphylococci encountered in the course of the experiments, coagulase tests were done, using freshly collected human citrated blood. A portion of a colony was mixed with 0.5 ml. of plasma; the occurrence of a firm clot at 24 hours was interpreted as a positive result. The medium employed for isolation of staphylococci from feces contained 0.5 ml. of a 1:500 solution of potassium tellurite and 0.25 ml. of a 5 per cent solution of chloral hydrate per 20 ml. of agar.

*Injection of Organisms.*—The tail vein was usually employed for intravenous injections; however when blood cultures were to be taken from the tail veins inoculations were made into foot veins, to avoid the possibility that extravasation into the subcutaneous tissues might influence the subsequent cultures.

Bacteria to be injected intravenously were incubated in beef heart infusion broth at 37°C. for 4 hours. The volume of culture injected was 0.5 ml. Tenfold dilutions in 0.85 per cent sodium chloride solution were incubated in agar pour plates to enumerate each inoculum.

*Ligation of Ureters.*—Preliminary observations revealed no difference whether the right or the left kidney was obstructed by ureteral ligation. Since the operative procedure is easier on the left, this side was used in all experiments. The animal was anesthetized by intraperitoneal injection of 10 to 12 mg. of pentobarbital sodium, supplementing as necessary. The hair of the abdomen and left flank was removed with an electric clipper, and the operative site cleansed with 70 per cent ethanol. Using sterile instruments, a 2 cm. incision was made over the kidney just below and parallel to the costal margin. A small segment of the ureter, approximately 2 cm. distal to the ureteropelvic junction, was freed from the surrounding tissues and ligated with silk thread. The peritoneum was approximated with silk sutures and the skin incision closed with metal clips.

*Removal of Organs for Enumeration of Bacterial Content.*—Under pentobarbital anesthesia, the abdomen or thorax was opened and the organ to be examined removed and placed in a TenBroeck grinder. In the case of kidney or spleen, the whole organ was employed, whereas with lung or liver, approximately 1 gm. of tissue was removed. The tissue was ground in 9 ml. of 0.85 per cent sodium chloride solution until a smooth suspension was obtained. This represented the $10^{-1}$ dilution, subsequent tenfold dilutions being made in the saline solution. Agar pour plates were made from several of these dilutions, depending on the expected number of microorganisms. After incubation for 48 hours, colony counts were made; the one containing between 30 and 300 colonies being taken as most nearly representative. When the plate containing the greatest dilution contained too many colonies to be counted the number was recorded as greater than 1000.

*Blood Cultures.*—An area on the tail was treated one or more days in advance with a commercial depilatory (neet, Whitehall Pharmacal Co., New York, N. Y.). At the time of culture, the animal was placed in a cylindrical plastic holder, and the tail immersed for 1 minute successively in tincture of merthiolate® and tincture of iodine, then dried with a sterile gauze pad. A small cut was made in the skin and 0.1 ml. blood drawn up into a 0.2 ml. pipette previously coated with heparin. The blood was then mixed with 0.9 ml. 0.85 per cent sodium chloride solution and the mixture incorporated in an agar pour plate, for determination of the colony count. Blood cultures on succeeding occasions were obtained by cutting the tail approximately 1 cm. proximal to the site used previously.

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*Course of the Infection in Normal Animals.*—In order to evaluate the role of ureteral obstruction it was first necessary to obtain some information about the distribution and persistence of the test organism in normal animals.

Observations were therefore made on the bacterial content of blood and various organs of approximately 300 normal rats at various intervals after intravenous injection of *E. coli*. It was not technically possible to follow these serially in individual animals, or even to sample all tissues in the same animal at one time because of the time required for grinding, preparing dilutions, and making pour plates. In the following paragraphs, therefore, the findings are presented in general terms, since they were obtained in the course of many separate experiments over a period of 2 years. The actual numbers of organisms found in various tissues at different times would doubtless differ somewhat with other strains of *E. coli*. We believe, however, that the pattern of disposition of this organism can be depicted satisfactorily from these studies.

*Bacteriemia.*—2 to 6 minutes after the intravenous injection of 150 to 200 million organisms in normal rats, blood cultures revealed 1 to 4 million colonies per ml. A rapid decrease then ensued, so that by the end of 30 minutes there were 1 to 4 thousand per ml. Subsequent cultures demonstrated a further reduction to a few hundred at 2 to 6 hours. This seemed to be followed by a slight rise at 24 hours and then another gradual decline toward negative cultures. At 48 hours, 10 of 12 animals still showed bacteriemia, but at 4 days, all 7 animals tested had negative blood cultures, as was also the case with 8 animals examined between the 7th and 14th day.

*Kidneys.*—Approximately equal numbers of organisms were arrested in the two kidneys. At 6 minutes the number of viable units ranged between 13,700 and 188,000, the wide range probably being attributable to variations in bacterial content of the blood circulating through the kidneys at this period. At 1 hour, counts were 90 to 420 and at 2 hours, 10 to 310. Eight hundred to 2,300 viable units were found at 6 hours. On the 4th day, 7 animals tested had 550 to 16,900 units per kidney. At 7 days all but 1 of 8 animals were found to have positive cultures in both kidneys, the numbers ranging from 10 to 2870. At 13 and 14 days only 3 of 14 kidneys gave positive cultures, all with fewer than 250 colonies.

*Spleen.*—At 6 to 20 minutes the counts ranged from 4 to 22 million viable units. At 6 hours the number was 1 to 4 million. By the end of the 2nd day there were 7 to 170 thousand. A gradual diminution continued so that by 7 days the numbers were comparable to those found in the kidney: 60 to 5,200. At 13 and 14 days 3 of 7 spleens tested gave no growth, and the remaining 4 showed 40 to 560 colonies.

*Liver.*—Large numbers of bacteria were arrested in the liver following intravenous inoculation, there being more than 20 million viable units per gm. of tissue at 6 minutes in 4 animals sacrificed at this time. In the succeeding 18 hours the number fell to 4,100 to 42,000 colonies. At 48 hours the counts ranged between 26,900 and 40,800 and by the 4th day 3 of the 7 animals examined had no demonstrable organisms, the remaining 4 being between 100 and 6,300. Three animals sacrificed at 7 days were found to have 4 to 25 colonies per gm. of liver tissue, and 5 others examined at 14 days were also positive but contained fewer than 100 colonies of *E. coli* per gm. of liver.

*Lungs.*—Lung tissue showed 50,000 to 3,700,000 bacteria per gm. of tissue at 6 minutes. Counts after 48 hours were less than 1,000 in 6 of 7 animals sampled (90 to 980), and the seventh had 7,000 viable units per gm. of lung. All 5 animals sacrificed on the 4th day had fewer than 100 colonies (20 to 70). At 7 days, 4 animals were tested; all had fewer than 250 colonies per gm. Five animals tested at 14 days showed no *E. coli* in lung tissue. It should be mentioned that occasionally Gram-negative organisms other than *E. coli* were found in lung tissue.

*Other Organs.*—Small numbers of *E. coli* were found in brain and adrenals up to 48 hours after inoculation, *i.e.* during the period of bacteriemia, but none were recovered from brain or adrenal from the 4th day onward.

To summarize: with the strain of *E. coli* employed, an inoculum containing 150 to 200 millions of viable units disappears rapidly from the circulating blood, large numbers being arrested in the liver, spleen, and lungs. The kidneys play no large role in the initial clearing of bacteria from the circulation; nevertheless the few organisms which do lodge there persist for several days after bacteriemia has terminated, without any notable tendency to multiply or to be destroyed.

*Ability of E. coli to Persist in the Kidney after a Second Inoculation.*—The persistence of relatively small numbers of bacteria in the normal kidney for 1 week, and their disappearance during the 2nd week, suggested the possibility that the development of antibody played a part in the final eradication. Accordingly an experiment was carried out to determine whether there would be an acceleration in rate of disappearance of a second inoculum, given 1 week after the first.

Nineteen normal rats were injected intravenously with 150 to 200 million organisms. Seven days later they were given a second similar injection. One week afterward the kidneys were removed for quantitative culture.

All the animals in this group had *E. coli* in one or both kidneys. Thirty of 38 kidneys sampled had between 10 and 5,900 viable units, 2 had more than 100,000 and 6 had negative cultures. These population estimates are of the same magnitude as had been found in animals receiving only 1 injection of bacteria, despite the fact that the 2nd injection was given at a time when the bacteria from the 1st injection would be expected to be disappearing from the kidney. Thus the mechanism of ridding the kidney of bacteria is not apparent, but does not seem to depend upon the formation of antibody.

*Comparative Numbers of Organisms in Obstructed and Unobstructed Kidneys at Different Intervals after Intravenous Injection of E. coli.*—Observations were now made in animals in which one ureter had been ligated 24 hours prior to injection. The magnitude and duration of the bacteriemia appeared to be similar to those previously observed in normal animals. Table I shows the findings in the kidneys of 62 animals. These are spaced in the table to indicate the groups of animals in individual experiments. Of particular interest is the finding that at the end of 1 or 2 hours there appeared to be no difference in the number of organisms present in the two kidneys. At the 4th and 6th hours, however, a difference began to be apparent, the number of organisms in the obstructed organ being higher; and between the 1st and 14th days the bacterial content of the obstructed organ was consistent with that to be expected in a heavily infected tissue. Sometimes between the 2nd and 6th week, a gradual "burning out" of the infection occurred, this being at a time when the obstructed kidney was only an enlarged sac of pus with little recognizable renal parenchyma.

In the unobstructed kidney, the number of organisms remained relatively constant for the first few days. Macroscopic and microscopic examination did

## TABLE I

*Colony Counts in Obstructed and Unobstructed Kidneys at Different Intervals after Intravenous Injection of E. coli*

| Time interval | Obstructed kidney | Unobstructed kidney | Time interval | Obstructed kidney | Unobstructed kidney |
|---|---|---|---|---|---|
| *hrs.* | | | | | |
| 1 | 6,000 | 7,000 | 1 day | 1,140,000 | 170 |
| | 5,900 | 1,360 | | > 10,000,000 | 390 |
| | 7,500 | 7,000 | | > 10,000,000 | 100 |
| | 13,500 | 6,000 | | | |
| | | | | 1,100* | 700 |
| 2 | 2,300 | 3,100 | | 1,100* | 8,900 |
| | 3,120 | 5,800 | | 118,200 | 600 |
| | 3,140 | 6,000 | | | |
| | 1,800 | 2,800 | | > 1,000,000 | 8,100 |
| | | | | > 1,000,000 | 1,510 |
| | 3,420 | 1,600 | | > 1,000,000 | 1,150 |
| | 5,280 | 2,700 | | | |
| | 7,460 | 1,000 | 2 days | > 10,000,000 | 4,900 |
| | | | | > 10,000,000 | 9,800 |
| | 6,000 | 1,200 | | > 10,000,000 | 19,200 |
| | 6,600 | 3,300 | | | |
| | 1,300 | 230 | 5 days | > 10,000,000 | 970 |
| | | | | 9,000 | 2,960 |
| | 3,800 | 3,900 | | > 10,000,000 | 60 |
| | 11,400 | 6,300 | | > 10,000,000 | 6,580 |
| | 7,600 | 5,500 | | | |
| | | | | > 100,000,000 | 13,000 |
| 4 | 28,000 | 860 | | > 100,000,000 | 28,600 |
| | 5,200 | 620 | | > 100,000,000 | 0 |
| | 3,200 | 330 | | | |
| | 5,600 | 430 | 2 weeks | 139,000 | 0 |
| | | | | 154,000 | 0 |
| 6 | 11,000 | 920 | | 4,640,000 | 0 |
| | 4,900 | 1,580 | | | |
| | 14,400 | 480 | 4 weeks | 0 | 0 |
| | | | | 1,880,000 | 0 |
| | > 100,000 | 7,600 | | 620,000 | 0 |
| | > 100,000 | 5,700 | | | |
| | > 100,000 | 9,100 | 6 weeks | 0 | 0 |
| | | | | 0 | 0 |
| | 15,000 | 540 | | 0 | 0 |
| | 2,000 | 350 | | | |
| | 1,400,000 | 960 | | | |
| | 29,400 | 730 | | | |
| | 10,700 | 850 | | | |
| | 17,900 | 980 | | | |
| | 14,600 | 930 | | | |

\* In this one group of 3 animals examined 24 hours after injection few bacteria were found in the obstructed kidney. The result is so out of line with all other experience in the course of this work that a technical error is believed to be responsible, *e.g.*, incomplete ureteral ligation, faulty dilution, etc.

not disclose evidence of inflammation, and the cultures taken on the 14th day gave no growth despite the occurrence of destructive pyogenic infection in the opposite kidney.

Thus, in the rat in which one ureter had been ligated prior to injection of bacteria, the course of the bacteriemia and the number of organisms in the unobstructed kidney were found similar to those in the normal animal. However, in the obstructed kidney the number of bacteria began to increase rapidly 4 to 6 hours after injection, resulting in a massive pyogenic infection until the renal parenchyma was destroyed.

*Determination of the Number of Organisms Required to Cause Infection in the Obstructed Kidney.*—Groups of rats in which one ureter had been ligated 24 hours previously were given 0.5 ml. injections of serial tenfold dilutions of *E. coli* culture; 1 week later the obstructed kidneys were homogenized and cultured quantitatively.

TABLE II

*Relation of Number of Bacteria Injected to Incidence of Infection in Obstructed Kidneys*

| Dilution (and approximate No. of organisms) injected | No. of animals | No. in which *E. coli* infection occurred |
|---|---|---|
| Undiluted (150 to 200 million)............. | 7 | 7 |
| $10^{-1}$ dilution (15 to 20 million)............. | 6 | 3 |
| $10^{-2}$ dilution (1.5 to 2 million)............. | 7 | 2 |
| $10^{-3}$ dilution (150 to 200 thousand)......... | 6 | 1 |

As shown in Table II, an intravenous dose of approximately 150 to 200 million organisms caused pyogenic infection in all 7 animals in the group. (Actually in many other experiments wherein this inoculum was employed, involving a total number of more than 200 animals, there were only 2 instances when *E. coli* infection failed to occur.) When the size of inoculum was reduced the proportion of animals infected diminished. At a dilution of 1:1000 (containing approximately 150,000 to 200,000 viable bacteria) only 1 of 6 animals became infected.

The decreased incidence of infection with reduction in number of bacteria injected is in accord with the findings recorded in Table I, in which it was shown that injection of 150 to 200 millions of organisms resulted in only 1,360 to 13,500 being arrested initially in each kidney. If it be assumed that the proportion of injected bacteria lodging in the kidney does not change when the inoculum is reduced then an inoculum of the $10^{-3}$ dilution would result in the deposit of fewer than a dozen viable units in each kidney. It is not surprising then, that infection occurred in only 1 of 6 animals receiving that inoculum.

*Ligation of Ureter at Varying Intervals after the Intravenous Injection of E.*

*coli.*—Since organisms persist in the normal kidney up to 7 days in relatively constant numbers, it might be expected that ligation of the ureter at any time during the week after injection of bacteria would result in pyelonephritis.

Experiments were carried out to test this hypothesis. Ureteral ligation was performed in groups of animals on the 1st, 2nd, 3rd, 5th, and 9th day after intravenous injection of bacteria. One week later the usual quantitative cultures were made, and the results obtained are given in Table III.

When ligation was performed 24 hours after the intravenous injection, 5 of 7 of the animals developed *E. coli* infections. When the interval between injection and ligation was delayed 5 days only 3 *E. coli* infections occurred among 17 animals tested. At 9 days none of the ligated kidneys was found to have *E. coli* pyelonephritis. Suitable controls were included in each of these experi-

TABLE III

*Occurrence of Infection in Obstructed Kidneys When Ureteral Ligation Was Performed at Varying Intervals after Intravenous Injection of Bacteria*

| Interval between injection and ligation | No. of animals | No. infected | | No. not infected |
|---|---|---|---|---|
| | | E. coli | Staphylococci | |
| *days* | | | | |
| 1 | 7 | 5 | 1 | 1 |
| 2 | 6 | 2 | 1 | 3 |
| 3 | 4 | 4 | 0 | 0 |
| 5 | 17 | 3 | 6 | 8 |
| 9 | 9 | 0 | 4 | 5 |

ments and these revealed the presence of bacteria in the kidney through the 5th day. It is reasonable to assume then, that the same held true for the animals subjected to ureteral ligation. The staphylococcal infections noted in the table will be discussed subsequently.

*The Role of Ureteral Obstruction in the Production of Pyelonephritis with S. marcescens.*—In order to further evaluate the nature of the increased susceptibility to infection of the obstructed kidney some observations were made with an organism of feeble pathogenicity, *S. marcescens*.

Repeated attempts to produce pyelonephritis in the normal rat by the intravenous injection of 4 hour cultures of *S. marcescens* resulted in no evidence of persisting infection. In animals with unilateral renal obstruction, however, infection was observed. The technique of this experiment did not differ from that already described in the experiments with *E. coli*. Six animals were employed, and were sacrificed in pairs on the 2nd, 5th, and 9th days. Pyogenic infection was found in all but one of the obstructed kidneys. Colony counts were higher than 1,000,000 organisms in each of 5 obstructed kidneys which

appeared macroscopically infected. In the unobstructed kidneys, 4,480 and 19,000 colonies were obtained at 2 days, 90 and 200 at 5 days, and none in the case of either of the unobstructed kidneys and one obstructed kidney examined at 9 days.

*Naturally Occurring Infection in the Obstructed Kidney.*—In view of the fact that ligation of the ureter increases susceptibility of the kidney to bacteria injected intravenously, it was of interest to determine whether infection would ever develop after ligation without injection of bacteria.

Cultures were made of obstructed kidneys in 93 rats, at intervals of 1 to 19 days after ureteral ligation. These revealed the occurrence of infection in a considerable proportion of animals, as shown in Table IV. Staphylococci were

TABLE IV

*Occurrence of Staphylococcal Infection in the Obstructed Kidney*

| Days after obstruction | No. of animals | No. infected | Colonies per kidney |
|---|---|---|---|
| 1 | 8 | 3 | 350, 130, 260 |
| 2 | 8 | 4 | 200, 260, 10,000, 1,540 |
| 3 | 4 | 1 | 6,000 |
| 4 | 17 | 5 | > 100,000(5) |
| 8 | 18 | 7 | > 100,000(4), 20,000, 10,000, 16,600 |
| 9 | 5 | 2 | > 100,000, 1,600 |
| 11 | 4 | 2 | > 100,000, 65,000 |
| 14 | 19 | 4 | > 100,000(4) |
| 15 | 3 | 2 | > 100,000(2) |
| 16 | 4 | 2 | > 100,000(2) |
| 19 | 3 | 2 | > 100,000(2) |
| Totals............ | 93 | 34 | |

the causative organisms in every case. All of the 34 staphylococcal strains recovered produced yellow pigment; 23 were hemolytic, but only 10 gave a positive coagulase test.

Although spontaneous staphylococcal infection occurred in a considerable proportion of animals with ureteral obstruction this was seldom encountered in rats which received large numbers of *E. coli* intravenously. Thus, in more that 200 animals receiving 0.5 ml. of the undiluted culture only 3 instances of mixed colon bacillus and staphylococcal infection were detected. When the dose of *E. coli* was decreased, resulting in a lower incidence of *E. coli* infection, the spontaneous staphylococcal infections increased in number. For example, 3 of the 13 animals described in Table II, which received the $10^{-2}$ or $10^{-3}$ dilutions of *E. coli*, and failed to become infected by that organism, proved to have staphylococcal infections. Similarly, 12 of the 43 animals included in the experiments summarized in Table III were found to have staphylococcal infection.

In view of the preceding findings an experiment was performed in which the intravenous injection of colon bacillus was delayed until 2 weeks after ureteral obstruction, that is, a time at which some of the animals would be expected to have developed a staphylococcal infection in the obstructed kidney, in order to determine whether colon bacillus infection would occur in organs already infected with staphylococci.

Sixteen animals received undiluted culture of *E. coli* 14 days after ligation. Seven days later the affected kidneys were examined bacteriologically, in the usual manner. All were found to contain large numbers of bacteria. In 7 there was a pure growth of *E. coli*, in 4 only staphylococci were recovered, while in the remaining 5 there was a mixed growth of staphylococcus and colon bacillus.

*Sham Operation and Naturally Occurring Infection.*—In 10 animals sham operation was carried out; *i.e.*, the animal was subjected to anesthesia, incision of the abdominal wall, freeing of the ureter from the surrounding tissue, and closure of the wound; in short, a duplication of the operative procedure except for ligating the ureter. None of these animals developed infection in either kidney.

*Possible Sources of Staphylococci Causing Infection in Obstructed Kidneys.*— Cultures were made of the skin, nose, pharynx, feces, and both kidneys of 4 normal animals. Hemolytic *Staphylococcus aureus*, coagulase-negative was isolated in every instance from the pharynx and stool and from the nose and skin in 3 of the 4 animals tested. No organisms were recovered from the kidneys. In 4 other animals, cultures were taken from the liver, spleen, lung, both kidneys, and feces. Only the fecal cultures were positive, 3 of them containing staphylococci. It should be mentioned in addition, that in the course of various other experiments more than 300 unobstructed kidneys were homogenized and cultured, but staphylococci were not recovered from these except for occasional surface colonies, almost certainly contaminants.

*Attempts to Release Ureteral Obstruction at Varying Intervals after Intravenous Injection of E. coli.*—Since Mallory *et al.* (2) found that in the rabbit the progress of infection could be halted by releasing the ureteral ligature 4 to 5 days after the injection of bacteria, an attempt was made to repeat that observation in the rat. Although it was possible to remove the ligature without further apparent damage to the ureter, flow of urine through the area was not reestablished, and the obstruction persisted.

## DISCUSSION

The results obtained in the present work do not support the hypothesis (1) that in the presence of ureteral obstruction some change in renal blood flow causes more bacteria to lodge in the kidney than is the case with a normal organ, since the number of organisms in the two kidneys did not differ significantly 1 or 2 hours after intravenous injection of bacteria. The difference is in the sub-

sequent course of events in the two kidneys. In the normal organ no apparent change occurs during the first 7 days; then, for reasons which are not obvious, clearing gradually occurs, so that by the end of 14 days only rarely can *E. coli* be recovered. In the obstructed kidney, on the other hand, the organisms which do find lodgement soon begin to multiply, and by the 4th to 6th hour an increase in bacterial population is demonstrable. By the end of 24 hours the organ is usually heavily infected.

The rarity with which infection developed when ureteral ligation was delayed until 5 days after inoculation, despite the persistence of relatively constant numbers of *E. coli* in the normal kidney for 7 days or longer, is difficult to explain. It may be related to the continuing bacteriemia during the first 2 or 3 days. Alternative explanations were considered, such as the findings of Rowley (4) and Pillemer *et al.* (5). They described alterations in susceptibility to *E. coli* infections in mice following the injection of cell walls of virulent *E. coli*. This altered susceptibility to infection paralleled fluctuations in levels of serum properdin, being lowest 24 hours after injection of the cell walls, then rising to higher than normal levels at the 5th or 6th day, at which time the animals were most resistant to infection. In the present work ureteral ligation 24 hours after the intravenous injection of *E. coli* usually resulted in progressive infection, whereas delay of ligation until the 5th day usually failed to do so, despite the fact that the kidney appeared to contain about the same number of viable units throughout that period of time. However, Dr. David E. Rogers has examined our strain of *E. coli* and reports that it shows little evidence of susceptibility to the properdin system. Probably, therefore, other factors are responsible for the results obtained in our experiments. It seems inadvisable to attempt more than speculation whether the difference is attributable to some effect of endotoxin introduced at the time of inoculation (6–8), or possibly to some change in the conditions of the bacteria as they reside in the kidney.

It seems probable that the spontaneous staphylococcal infections following ureteral ligation were instances of blood-borne infection and not the result of contamination at the time of laparotomy. No evidence of the presence of these bacteria in normal kidneys was obtained, nor was staphylococcal infection induced by the sham operation. Staphylococci were, however, easily demonstrable elsewhere in the animals' bodies, from which sites the most probable pathway of infection would be the blood stream.

Other investigatiors have described instances of pyogenic infection occurring in acutely obstructed kidneys.

In 1897, Bradford (9) reported that pyonephrosis occurred in 3 of 12 dogs in which one ureter had been ligated. Pearce (10) noted pyelonephritis in 1 of 5 rabbits subjected to unilateral ligation. Amos (11) found "infection" in the kidneys of 2 of 6 guinea pigs in this condition. Keith and Pulford (12) in studies of experimental hydronephrosis in dogs stated that, "The progress and extent of the pathologic lesion

in the kidney depended on the amount and duration of intra-ureteral pressure, and, in a lesser degree, on the invariable secondary pelvic infection." Scott (13) stated that 1 or 41 animals with complete or partial ureteral obstruction developed pyonephrosis. However, a review of his findings indicates that several other obstructed kidneys became infected; these were thought by the author to be associated with infections elsewhere in the body; e.g., gallbladder. Barney (14) collected 62 cases of accidental ligation or injury to one or both ureters in human beings during surgical operations. He states that infection of the kidney necessitated subsequent nephrectomy in 9 of the patients. Barney was unable to substantiate this clinical evidence with experimental work, since he reported that the hydronephrotic fluids of 3 rabbits and 2 dogs were sterile when cultured. Similarly, Helmholz and Field (15), in studying the effects of ureteral ligation in rabbits, were unable to culture bacteria from the "hydronephrotic urine" in any of their animals, and attributed the marked inflammatory changes observed to "sterile hydronephrosis."

Despite the fact that staphylococcal infections developed in one-third of animals subjected only to ureteral ligation, it was rarely encountered along with E. coli pyelonephritis. Admitting the possibility that staphylococci were not detected in this circumstance, it can be said that all cultures were carefully examined for mixed infection, and several colonies from each plate were identified. Very possibly the environment created by active E. coli pyelonephritis is not a favorable one for arrest or multiplication of the staphylococci. The reverse also may be true, since it was found that obstructed kidneys already the seat of staphylococcal infection had a lessened susceptibility to infection by E. coli injected into the blood stream.

Helmholz had experience of a similar kind (16). When he injected 11 rabbits with mixed cultures containing staphylococci and colon bacilli he was able to recover the staphylococci from the kidneys of all animals, but in only 3 was the colon bacillus found. He further demonstrated that if he gave colon bacilli at intervals after staphylococci (i.e., after enough time had elapsed for the establishment of renal abscesses) he was able to recover the colon bacilli in only 2 of 16 animals.

Complete ureteral obstruction, as employed in the present work, causes severe, rapidly progressive damage to the kidney, so that within a period of 3 to 4 weeks the organ is converted to a thin shell filled with fluid (17, 18). It is not surprising that each injury is associated with increased susceptibility to bacterial infection. However, complete ureteral obstruction, with its attendant rapid destruction of the kidney, may not be requisite to this heightened susceptibility, since there is ample clinical evidence that incomplete obstruction, causing only moderate increase in intrarenal pressure, and compatible with long continued function, predisposes to the development and persistence of pyelonephritis (19, 20). Experiments are now in progress, therefore, to test the effects of incomplete ureteral obstruction on susceptibility to pyogenic infection.

SUMMARY

A study has been made of the effect of ureteral ligation on the susceptibility of the kidney to pyogenic infection. In most experiments a strain of *E. coli* was employed as the test organism, being injected intravenously in varying quantity either before or after ureteral ligation. A few experiments were also carried out with *S. marcescens*.

Preliminary observations were made on the distribution and persistence of *E. coli* following its inoculation into the blood stream of normal rats. Rapid reduction in number of bacteria in the circulation occurred during the first 30 minutes, but bacteriemia persisted at a comparatively low level for at least 48 hours. Large proportions of the inoculated bacteria were arrested and apparently destroyed in the liver, spleen, and lungs. Comparatively small numbers were deposited in the kidneys; nevertheless, these continued to be demonstrable during the 1st week, without notable tendency to increase or decrease, then disappeared during the 2nd week. There was no acceleration in rate of disposal of the bacteria in the kidney when a second injection was made 1 week after the first.

In rats with one ureter ligated the number of bacteria lodging in the kidneys after intravenous inoculation did not differ from that found in normal animals. It appears, therefore, that the increased susceptibility of the obstructed kidney to infection *via* the blood stream is not attributable to an increased trapping of circulating bacteria. 4 to 6 hours after the intravenous injection, however, an increased number of bacteria could be demonstrated in the obstructed kidney, apparently due to local multiplication, and by the end of 24 hours purulent infection was usually obvious.

A comparatively large number of bacteria was required to cause infection, even in the kidney with obstruction. This appeared to be related to the small proportion of the intravenous inoculum which lodged in the kidney initially.

Although bacteria could be demonstrated in the normal kidney for a week or more following intravenous injection it was not possible to induce active infection with equal regularity by ligating the ureter throughout this time. During the first 3 days the majority of obstructed kidneys developed infection, but after 5 or more days this occurred in only a small proportion of animals so treated. The reason for the difference, in relation to interval between intravenous injection and time of ligation, is not apparent.

When the ureter was ligated but no intravenous injection of bacteria was given, staphylococcal infection developed in the obstructed kidney within 2 weeks in about one-third of the animals. Reasons are given for the belief that this was blood-borne infection, and not the result of contamination at the time of operation. Staphylococci were not recovered from the normal rat kidney. These "spontaneous" staphylococcal infections seldom developed when *E. coli* was injected intravenously at the time of ureteral ligation.

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