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ANTIBACTERIAL MECHANISMS IN THE URINARY BLADDER†

The pathogenesis of urinary tract infection frequently involves entrance of bacteria into the urinary bladder through the urethra and subsequent spread via the ureters to the kidneys.[2] However, it is likely that small numbers of bacteria frequently reach the bladder and do not result in infection. In fact, Cox and Hinman[3] inoculated large numbers of *Escherichia coli* in the urinary bladders of healthy males and noted sterilization of the urine over a period of three days. These investigators demonstrated the importance of urine flow as a defense against bacterial infection; in addition, they postulated an intrinsic antibacterial activity of the urinary bladder independent of urine flow.

Vivaldi, Munoz, Cotran, and Kass[4] observed a decrease in the number of bacteria following direct application of *E. coli* to the mucosa of an exteriorized flap of rabbit urinary bladder. However, Paquin, Perez, Kunin, and Foster[5] and Mulholland, Foster, Gillenwater, and Paquin[6] reported that *E. coli* grew following inoculation into rabbit bladders.

The present study was undertaken to investigate antibacterial mechanisms in the urinary bladder of the rat. In order to avoid the variables of urine flow and interaction of urine with the bacteria, the bladder was isolated from urine flow by bilateral ureteral ligation.

METHODS

Bacteria.

Three strains of *Escherichia coli* and one strain of *Proteus mirabilis* were studied. *E. coli* strain 1 (serotype 0111 B4) was obtained from the New York City Department of Health. *E. coli* strains 2 (serotype 0139) and 3 (non-typable) and the strain of *P. mirabilis* were isolated from urine of different patients with pyelonephritis. Stock

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cultures were maintained by storing aliquots of an 18-hour culture in Todd Hewitt broth* at —20° C. For each experiment, an aliquot of the stock culture was subcultured in trypticase soy broth and incubated at 37° C. for 18 hours. Inocula were prepared by diluting the bacteria in standard strength trypticase soy broth* (*i.e.* 3 per cent trypticase soy broth in distilled water).

Inoculation of animals.

White male or female Sprague-Dawley strain rats** weighing 150 to 250 g. were used in all experiments. They were fed Rockland Laboratory Animal Diet† and tap water *ad libitum* prior to the experiments. This diet was free of antimicrobial drugs. Rats were anesthetized with intraperitoneal pentobarbital sodium. Using sterile technique the peritoneal cavity was exposed by a midline abdominal incision. The ureters were doubly ligated with 000 silk sutures and divided. Urine within the urinary bladder was aspirated with a syringe and 30 gauge needle placed directly through the bladder wall, and was saved for study of bactericidal activity. Residual urine was removed from the bladder lumen by alternately injecting through the needle and expressing through the urethra five times, fresh 0.3 ml. aliquots of trypticase soy broth. Varying numbers of bacteria were then injected (through the same needle) into the empty bladder in 0.01 ml. of trypticase soy broth. The needle was removed and the abdominal cavity was closed. The animals were maintained without food or water for the remainder of the experiment. In other experiments basal medium Eagle with Earle's balanced salt solution‡ subsequently referred to as Eagle's medium, isotonic saline solution, or rat urine diluted 1 : 2 in distilled water was used to remove residual urine and to suspend the bacteria for inoculation.

Bacterial enumeration.

In each experiment two or more rats were studied immediately after inoculation of bacteria and the rest were studied at intervals up to 24 hours. Animals that had recovered from anesthesia were anesthetized again with pentobarbital sodium. The urinary bladder was exposed using sterile technique and the base of the bladder was ligated. The bladder with its contents was removed and homogenized in a teflon tissue grinder¶ in 1.0 ml. of trypticase soy broth. Blood was collected from the heart by needle aspiration with the chest opened and the serum was separated for study of bactericidal activity.

Serial 100-fold dilutions of each bladder homogenate were made in sterile saline solution and 1- and 0.1- ml. aliquots of each dilution were plated in trypticase soy agar pour plates. Exposure to saline solution for this period did not change the number of viable bacteria. With *P. mirabilis* the agar was allowed to harden and a second layer of agar was poured to prevent swarming. The total number of viable bacteria per bladder homogenate was calculated from colony counts after incubation of the plates for 24 hours at 37° C. Representative colonies were identified to assure identity with the species inoculated.

Experiments with radioactive labeled bacteria.

To determine the fate of bacteria after inoculation into the bladder, *E. coli* strain 1

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† Distributed by Teklad Incorporated, Monmouth, Illinois.
‡ Microbiological Associates, Inc., Bethesda, Maryland.
¶ Scientific Glass Apparatus Co., Inc., Bloomfield, N. J.

was labeled with ^{51}Cr by the method of Braude, Carey, Sutherland, and Zalesky[7] which labels the endotoxin of the bacteria. About 10^4 *E. coli* strain 1 were washed three times in large volumes of isotonic saline solution by centrifuging at 3,200 rpm (2250 x *g*) for 20 minutes at 4° C. These bacteria were then subcultured into 10 ml. trypticase soy broth to which was added 1 mc of ^{51}Cr in the form of Na_2CrO_4.* After 18 hours incubation at 37° C., the bacteria present were washed five times (using 40 ml. trypticase soy broth for each wash) and then were suspended in 10 ml. trypticase soy broth. This suspension was used to inoculate rat bladders in the same way as previously described for unlabeled bacteria.

To determine if the washing was adequate to remove free $^{51}CrO_4$ = ion, 0.1 ml. aliquots of the bacterial suspension were filtered through a 0.45 u filter** and then 10 ml. of sterile saline solution was poured over the filter to further wash the bacteria on the filter. The filter was removed and the radioactivity of the filter and of the wash were determined. In each experiment about three per cent of the total radioactivity on the filter could be detected in the wash. Some of the bacterial suspension was also incubated and then filtered to determine the stability of the radioactive tag. Following incubation at 37° C. for twenty-four hours, about seven per cent of the amount of radioactivity that was on the filter was present in the wash.

Some rats were studied immediately after inoculation of radioactive labeled bacteria and others were studied at intervals up to 24 hours. The intact urinary bladder was removed and homogenized in 1 ml. of trypticase soy broth. In addition, the liver, spleen, kidneys, lungs, retroperitoneal lymph nodes, psoas muscle, sternum, and abdominal skin were removed with separate sterile instruments, weighed, and individually homogenized with sufficent saline solution to make 10 per cent suspensions. Aliquots of heart blood and of each homogenate were serially diluted and plated for determination of viable bacteria. Other aliquots were used for determination of radioactivity. The remainder of the carcass was dissolved by boiling in concentrated hydrochloric acid. The radioactivity of aliquots of the dissolved carcass was determined. All counting of radioactivity was done with a well type scintillation detector.† Rats inoculated with radioactive bacteria were kept in individual glass jars. At the end of each experiment these jars were rinsed with water and the radioactivity in the rinse determined.

Antibacterial activity of the homogenized bladder.

An 18 hour trypticase soy broth culture of *E. coli* strain 1 was heated for one hour at 63° C. (resulting in sterilization of the culture) and 10^7 of the heat-killed *E. coli* were inoculated into the bladders of anesthetized rats in the manner already described. Four or 24 hours later the intact bladders were removed and homogenized in 0.75 ml. sterile distilled water. Normal bladders were also homogenized in 0.75 ml. water. One-tenth milliliter of water containing 10^4 viable *E. coli* strain 1 was inoculated into each homogenate and into 0.75 ml. of trypticase soy broth as a control. Samples were removed for determination of numbers of viable bacteria before and four and twenty-four hours after incubation at 37° C. Numbers of bacteria were determined by serial dilution and pour plate techniques.

Examination of rat serum or urine for bactericidal activity.

The urine that had been aspirated from the urinary bladder prior to inoculation of

* E. R. Squibb and Sons, New Brunswick, N. J.
** Millipore Filter Corporation, Bedford, Mass.
† Model C 120-1, Nuclear-Chicago Corporation, Chicago, Ill.

bacteria, and serum from heart's blood was studied immediately or stored at —20° C. until study. An inoculum of 10^6 bacteria in 0.1 ml. trypticase soy broth was added to 0.9 ml. serum or 0.3-0.9 ml. urine. The same bacterial strain was studied in the urine and serum of a rat as had been inoculated into the bladder of that animal. One-tenth milliliter of serum or urine was removed for determination of the number of bacteria. Each sample was then incubated for four hours at 37° C. and the number of bacteria was again determined.

Examination of the rat bladder by light microscopy.

Rats were anesthetized, the ureters ligated and 10^7 viable *E. coli* strain 1 were inoculated into the bladder.

Immediately after inoculation and at one-half hour, 1, 2, 3, 4, 5, 6, and 24 hours later bladders were removed and rapidly frozen in two per cent methyl cellulose, 15 centipoise* in saline solution. This was done to preserve the relationships of the intraluminal contents. Frozen sections were cut at 5 to 8 u in a cryostat and stained with hematoxylin and eosin. Normal rat bladders were also examined histologically.

RESULTS

Demonstration of antibacterial activity in the bladder

Striking antibacterial activity of the rat bladder was observed *in vivo* against *E. coli* strains 1 and 2. Figures 1 and 2 demonstrate the results of

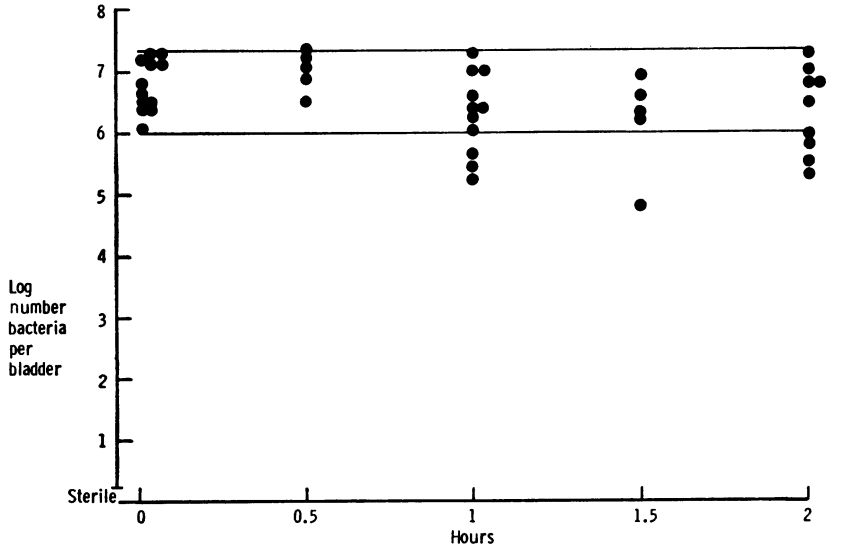

FIG. 1. Number of bacteria recovered from urinary bladders of rats immediately and ½, 1, 1½ and 2 hours after inoculation of 10^6-$2x10^7$ *E. coli* strain 1. Each point represents one animal.

* Fisher Scientific Co., Fair Lawn, N. J.

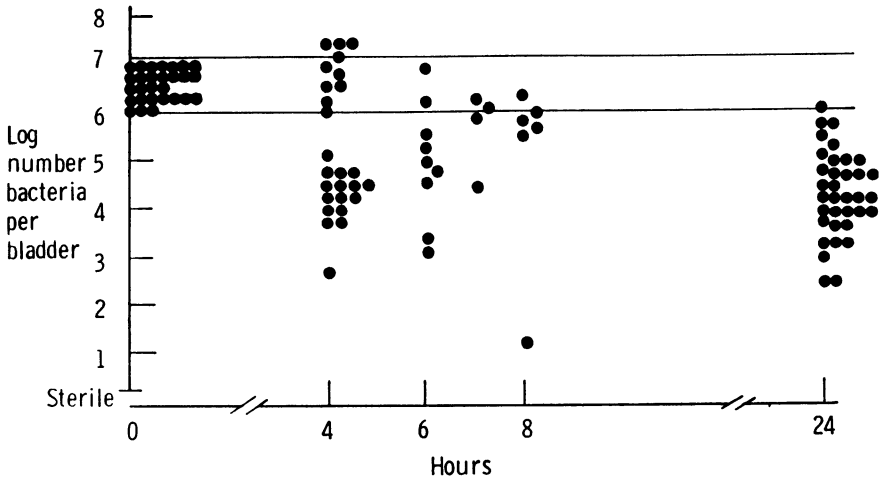

FIG. 2. Number of bacteria recovered from the urinary bladders of rats immediately and 4, 6, 7, 8 and 24 hours after inoculation of 10^6-$2x10^7$ *E. coli* strain 1. Each point represents one animal.

experiments in which 10^6 — 2 x 10^7 *E. coli* strain 1 were inoculated into the bladders of rats and the numbers of bacteria remaining in the bladders determined by killing rats at intervals. In each experiment some rats were killed immediately after inoculation and the number of bacteria in the bladders of these animals was used to calculate the delivered inoculum. Figure 1 shows results from rats killed at intervals up to 2 hours and Figure 2 shows results from rats killed at intervals up to 24 hours. In these experiments there was usually little or no decrease in the total number of bacteria that could be recovered from the bladders until four hours after inoculation of bacteria. However, by four hours after inoculation there was a significant decrease in the number of bacteria recovered from the bladders of the majority of animals. By 24 hours a 10 to 1,000-fold decrease had occurred in the total number of bacteria recovered from 29 of 35 bladders studied.

Similar results were obtained in experiments in which 10^6-10^7 *E. coli* strain 2 were inoculated into bladders. Studies with *E. coli* strains 1 or 2 in inocula of 10^3-10^6 demonstrated similar decreases in recoverable bacteria. With inocula of 10^3-10^4 of either of these two strains some bladders were sterile after 24 hours. Experiments were not continued past 24 hours because after this time rats with ureters ligated and divided began to appear moribund regardless of whether or not bacteria had been inoculated into the bladder. Deaths started to occur after 36 hours.

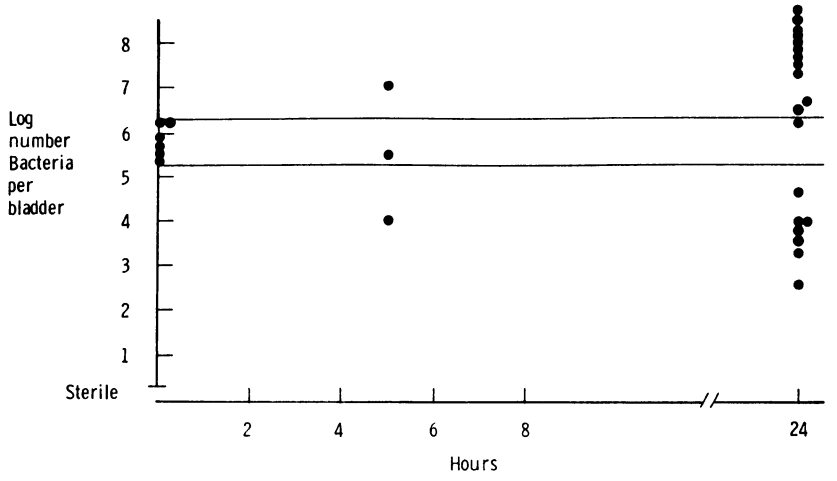

FIG. 3. Number of bacteria recovered from the urinary bladders of rats immediately and 5 and 24 hours after inoculation of $2x10^5$-$2x10^6$ *P. mirabilis*. Each point represents one animal.

Antibacterial activity against *E. coli* strain 3 and the strain of *P. mirabilis* was less potent than that against *E. coli* strains 1 and 2 and could be "overcome" when 10^5 *E. coli* strain 3 or *P. mirabilis* were inoculated. For example, Figure 3 demonstrates the results of experiments with the strain of *P. mirabilis* in an inoculum of 2 x 10^5 to 2 x 10^6. An increase in the number of recoverable bacteria occurred in 11 of 19 animals after 24 hours. However, with inocula of 10^4 or less *E. coli* strain 3 or *P. mirabilis*, significant decreases in recoverable bacteria occurred in most bladders.

TABLE 1. FATE OF BACTERIA AS A FUNCTION OF INOCULUM

Bacterial strain	*Inoculum*	*Fate of bacteria in the majority of bladders*
P. mirabilis	10^5 or more	Growth
	10^4 or less	Decrease in number of recoverable bacteria
E. coli (Strain 1)	10^7 or less	Decrease in number of recoverable bacteria
E. coli (Strain 2)	10^7 or less	Decrease in number of recoverable bacteria
E. coli (Strain 3)	10^5 or more	Growth
	10^4 or less	Decrease in number of recoverable bacteria

A summary of results of experiments with *P. mirabilis* and the three strains of *E. coli* is shown in Table 1. It is clear that the antibacterial activity of the rat bladder varied with the strain of bacteria tested and the inoculum size. All strains decreased in numbers if 10^4 or fewer bacteria were inoculated. *P. mirabilis* or *E. coli* strain 3 usually increased in titer when 10^5 organisms were inoculated whereas following inoculation of 100-fold more *E. coli* strains 1 and 2 there was usually a decrease in titer.

Results similar to these were obtained when urine was not washed from the bladder prior to inoculation of bacteria or when Eagle's medium, isotonic saline solution, or rat urine was used as a suspending medium for inoculation of bacteria. There was no difference in results with male or female rats.

Possible factors related to antibacterial activity of bladder

There are several possible explanations for the decrease in numbers of bacteria observed in the rat bladder in these experiments: 1) loss of the inoculum through the urethra; 2) clumping of bacteria resulting in falsely low viable counts; 3) lack of adequate nutrient material in the bladder lumen or presence of an inhibitory or bactericidal substance in the bladder (such as residual urine or serum factors); and 4) phagocytosis and killing of bacteria by leukocytes. Experiments were designed to investigate each of these possibilities.

Experiments with radioactive labeled bacteria. The possibility that the decrease in numbers of recoverable bacteria was secondary to loss through the urethra was investigated by using radioactive chromium tagged *E. coli*. *E. coli* strain 1 labeled with ^{51}Cr was inoculated into rat bladders and the fate of viable bacteria and of the radioactive label was followed. By six to eight hours after inoculation, when the number of viable bacteria had decreased tenfold or more in most rat bladders, the amount of radioactivity was usually equivalent to the starting value or had decreased only slightly. By 24 hours after inoculation both the numbers of viable bacteria and the total bladder radioactivity had decreased more than tenfold. The results of a typical experiment are shown in Figure 4.

Up to 8 hours after inoculation of radioactive labeled *E. coli,* little or no radioactivity was detected in liver, spleen, kidneys, lungs, retroperitoneal lymph nodes, psoas muscle, sternum, skin, blood, or carcass. However, by 24 hours after inoculation, at least 50 per cent of the injected radioactivity could be demonstrated in these tissues and the carcass. Radioactivity was occasionally present in blood, liver, or spleen and was consistently present in muscle. In contrast to these results, viable *E. coli* were never isolated in large numbers from any specimen other than the

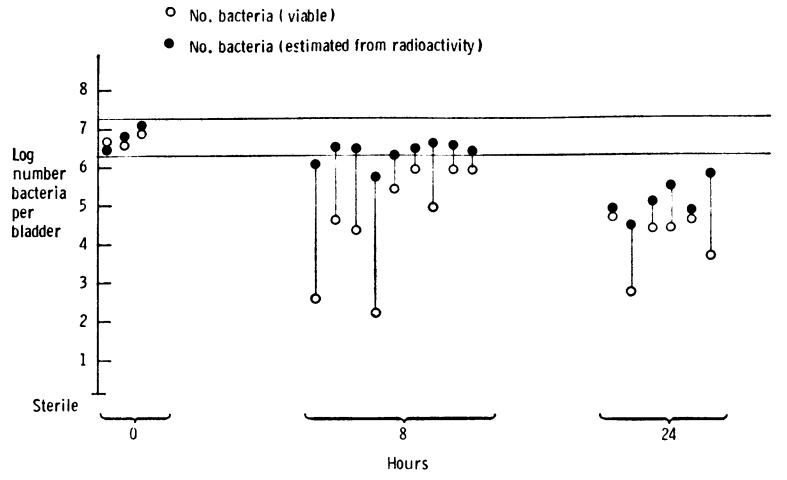

FIG. 4. Number of bacteria recovered from the urinary bladders of rats following inoculation of 10^7 *E. coli* strain 1. The hollow circles represent viable counts and the solid circles represent numbers of bacteria determined by radioactivity. Each paired hollow and solid circle represents one animal.

bladder; occasionally (following inoculation of 10^7 *E. coli*), 1-50 viable *E. coli* were isolated from total liver, spleen, or lung.

In no instance could more than 50 per cent of the injected radioactivity be recovered from the jar in which a rat had been kept following inoculation of the bacteria.

Investigation for bacterial clumping. To determine if clumping of bacteria was partly responsible for the apparent decrease in viable bacteria, numbers of bacteria were determined before and after trypsinization of bladder homogenates. Bladders were homogenized in 1 ml. of trypticase soy broth 4 and 24 hours after injection of *E. coli* strain 1. To half of each homogenized bladder was added 0.1 ml. of 2 per cent trypsin* in phosphate buffer, at pH 7.5. To the other half of each specimen was added 0.1 ml. of phosphate buffer without trypsin. After incubation at 37° C. for 30 minutes the number of bacteria was determined in each specimen. There was no significant difference in the number of bacteria recovered from the trypsinized and the non-trypsinized half of each bladder. Two per cent trypsin in phosphate buffer will not inhibit growth of *E. coli* strain 1.

Additional experiments were designed to evaluate clumping of bacteria. In these studies, 10^5-10^7 *E. coli* strain 1 were inoculated into the bladder,

* Trypsin 1:250, Difco Laboratories, Detroit, Mich.

(after ligation and division of the ureters), and 4, 6, or 24 hours later the contents of the bladder were removed by vigorously injecting and withdrawing the same 0.3 ml. aliquot of trypticase soy broth five times with a 30 gauge needle placed through the wall of the bladder. Aliquots of "bladder aspirates" were heat-fixed on glass slides, Gram-stained, and examined microscopically. Numbers of viable bacteria were determined in other aliquots of the aspirates and in homogenates of the bladder. Despite 10 to 1,000-fold decreases in the number of bacteria recovered from the bladders, most extracellular bacteria seen were single. When clumps were present, there were rarely more than 2-3 bacteria in a clump. Leukocyte associated bacteria will be described below in the discussion of leukocytes in "bladder aspirates." Similarly hematoxylin-stained and eosin-stained sections of bladders prepared as described in METHODS did not demonstrate clumps of bacteria.

Growth supporting or inhibiting characteristics of the bladder in vitro. Several experiments demonstrated that the nutrient material in the bladder lumen was sufficient to support bacterial growth. Following inoculation of 10^4 *E. coli* strain 1 into the bladders of rats, (with ureters ligated and divided as described in METHODS), the rats were killed and left at room temperature or at 37° C. There was significant growth in all bladders within 6 hours.

In additional experiments, the contents of the bladder were removed by injecting and withdrawing the same 0.3 ml. aliquot of sterile distilled water five times. These "bladder aspirates" were obtained from bladders of normal rats and from bladders into which 10^7 heat-killed *E. coli* strain 1 had been injected four hours previously. All of these "bladder aspirates" supported growth of *E. coli* strain 1 following inoculation of 10^4 microorganisms suspended in .01 ml. sterile distilled water. Furthermore, all of the bacterial strains studied (i.e. *E. coli* strains 1, 2 and 3 and *P. mirabilis*) were inoculated in the bladder in trypticase soy broth and as little as 1 per cent standard strength trypticase soy broth in water (i.e., .03 per cent trypticase soy broth powder in water) will support growth of all of the bacterial strains studied.

The fate of *E. coli* strain 1 was also studied in homogenized bladders. Normal rat bladders and bladders into which heat-killed bacteria had been inoculated were homogenized and the homogenates studied for antibacterial activity, as detailed in METHODS. Bacteria grew as well in the homogenates of the bladders as in trypticase soy broth.

Examination of rat serum or urine for bactericidal activity. Although there was no evidence of an inhibitory or bactericidal substance in aspirates

from bladders or in homogenized bladders, it was of interest to examine rat serum and urine for bactericidal activity as detailed in METHODS.

Serum was obtained from normal rats, from uninfected rats with ureters ligated 24 hours previously and from infected rats with ureters ligated 24 hours previously. All serum was bactericidal for all four strains of bacteria. In each experiment, following inoculation of 10^5 bacteria, there was usually a tenfold or more decrease in the number of bacteria after four hours of incubation at 37° C. A total of 10 or more serum samples were studied against each bacterial strain. Heating serum at 63° C. for five minutes eliminated the bactericidal activity.

When rat urine was inoculated with 10^5 *E. coli* strains 1, 2, or 3 or *P. mirabilis* and incubated for four hours at 37° C., variable results were obtained. *P. mirabilis* usually multiplied in the urines tested. Each of the *E. coli* strains multiplied in some urine samples, whereas in other urine samples there was either no growth or a tenfold or greater decrease in the number of bacteria. The fate of a bacterial strain in a urine sample did not correlate with the antibacterial activity of that rat's urinary bladder against the same bacterial strain. For example, *E. coli* strain 1 grew in urine specimens from some bladders in which a decrease in the numbers of the same bacterial strain had occurred.

Determination of leukocytes in "bladder aspirates." "Bladder aspirates" were obtained by vigorously injecting and withdrawing five times from the bladder the same 0.3 ml. aliquots of 1 per cent acetic acid. To determine the number of leukocytes in "bladder aspirates," counts were performed in a bright line hemocytometer. The "bladder aspirate" was also smeared on clean slides and stained with Wright's stain for differential counts and Gram's stain for identification of bacteria. As shown in Table 2, "bladder aspirates" obtained from normal rats or from rats immediately after ligation of the ureters contained from 15,000 to 75,000 cells (mean 42,250), 10 per cent of which were polymorphonuclear leukocytes and 90 per cent of which were mononuclear cells. Four hours after inoculation of 10^7 *E. coli* strain 1, counts ranged from 78,000 to 315,000 per total "bladder aspirate" (mean 138,830) and 50 per cent were polymorphonuclear. Six hour counts ranged from 126,000 to 687,000 (mean 417,214) and 80 per cent were polymorphonuclear. At 24 hours the counts ranged from 96,000 to 1,500,000 (mean 634,185) with 50 per cent polymorphonuclear.

Bacteria were frequently observed intracellularly within polymorphonuclear leukocytes in "bladder aspirates" obtained two to six hours after inoculation of bacteria into the bladder.

Examination of the rat bladder with light microscopy. Hematoxylin and

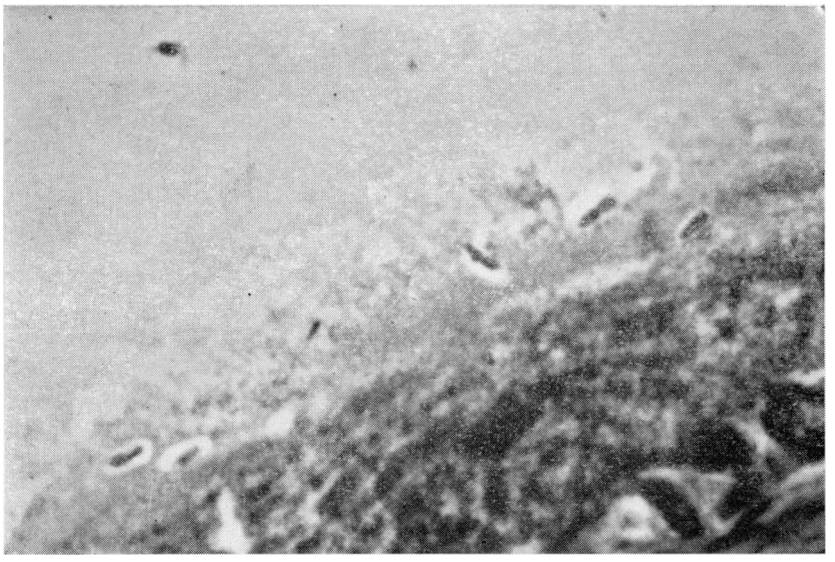

FIG. 5. Bacteria (*E. coli* strain 1) in mucous material lining the bladder lumen, one hour after inoculation. Hematoxylin and eosin stain, phase contrast, x 2000.

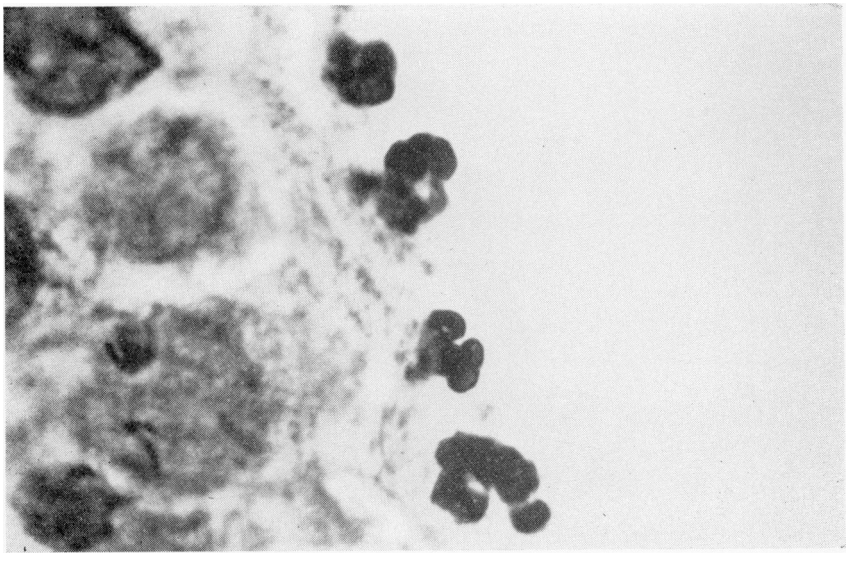

FIG. 6. Polymorphonuclear leukocytes lining the mucosal border of the bladder 2 hours after inoculation. Hematoxylin and eosin stain, phase contrast, X 2000.

TABLE 2. NUMBERS OF CELLS PRESENT IN "BLADDER ASPIRATES"

		Total cells in "bladder aspirate"		
Time	*No. of determinations*	*Mean* (x 1000)	*Range* (x 1000)	*Per cent Polymorphonuclear leukocytes*
Before inoculation of bacteria	12	42	15-75	10
After inoculation of 10^7 *E. coli* strain 1 (hours)				
1	6	69	33-135	10
2	3	40	30-58	10
3	6	103	27-201	10
4	9	139	78-315	50
5	6	88	30-201	60
6	7	417	126-687	80
24	7	634	96-1,500	50

eosin stained sections of bladders were prepared one-half, 1, 2, 3, 4, 5, 6 and 24 hours after inoculation of 10^7 viable *E. coli* strain 1, as described in METHODS. One-half hour and one hour after inoculation, bacteria were seen free in the lumen and at the mucosal border in all bladders. Some of the bacteria appeared to be embedded in the mucous material lining the bladder lumen (Figure 5). Polymorphonuclear leukocytes were not seen in the mucosa or within the lumen at this time.

Starting at one-half hour after inoculation of the bacteria, there was a moderate degree of acute inflammatory change in the wall of the bladder as evidenced by vascular dilation, hyperemia, and margination of polymorphonuclear leukocytes in blood vessels. These changes progressed with time. By two hours after inoculation of bacteria polymorphonuclear leukocytes were occasionally observed in the mucosa and along the mucosal border lining the lumen (Fig. 6).

By four hours after inoculation, polymorphonuclear leukocytes were consistently seen in the mucosa and lining the mucosal border. At this time bacteria were observed in only some of the bladders and when present were seen along the mucosa and inside polymorphonuclear leukocytes.

The inflammatory reaction subsequently increased in intensity and by 24 hours after inoculation all bladders showed large clumps of polymorphonuclear leukocytes in the mucosa and lumen. Bacteria were not seen at this time.

No histologic changes or bacteria were observed in normal bladders.

Experiments with the bladder obstructed

In some experiments after inoculation of bacteria into the bladder (with ureters ligated and divided as described in METHODS), the bladder outflow tract was obstructed by one of two methods: 1) the base of the bladder (including ureters and blood vessels supplying the bladder) was ligated; or 2) the urethra was exposed subcutaneously and was ligated approximately 1 cm. proximal to the external urethral meatus. Ligation of the base of the bladder not only prevented bladder emptying but also interrupted the blood supply to the bladder and caused necrosis of the bladder within a few hours. Urethral ligation alone did not interrupt the blood supply to the bladder and necrosis of the bladder did not occur.

The antibacterial activity of the rat urinary bladder was abolished for all the bacterial species tested if the bladder outflow tract was obstructed, either by ligation of the urethra or by ligation of the base of the bladder. Figure 7 shows results of experiments in which 10^4-10^5 *E. coli* strain 1 were inoculated and the bladder outflow then obstructed by ligation of the urethra. In most animals bacterial multiplication occurred and in no animal was there a significant decrease in the number of bacteria recovered. Results were identical when the base of the bladder was ligated.

In all rats in which ligation of the urethra or base of the bladder was

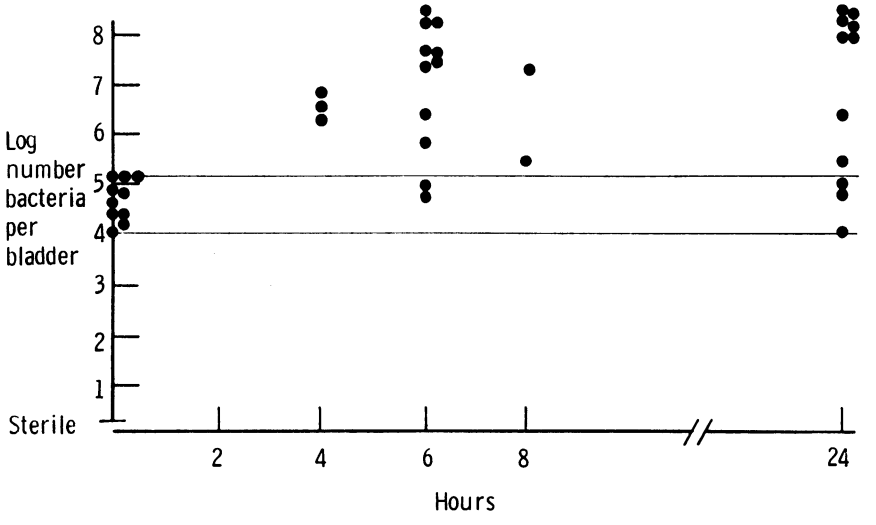

FIG. 7. Number of bacteria recovered from the urinary bladders of rats with urethras ligated immediately and 4, 6, 8 and 24 hours after inoculation of 10^4-10^5 *E. coli* strain 1. Each point represents one animal.

performed, about 0.1-0.2 ml. of fluid was present in the bladder after 24 hours (which was enough to slightly distend the bladder). This was in contrast to rats without obstruction of bladder outflow tract where the bladder did not contain fluid after 24 hours.

In an attempt to determine the mechanism by which ligation of the urethra abolishes the antibacterial activity of the bladder, the effect of urethral ligation on leukocytic infiltration into the bladder was studied. An inoculum of 10^7 *E. coli* strain 1 was injected into bladders of rats with the ureters and urethras ligated. Leukocyte counts were periodically performed on bladder aspirates as described above in *"Determination of leukocytes in bladder aspirates."* There was no impairment of leukocytic infiltration in obstructed bladders as compared with unobstructed bladders (Table 2). In fact, the numbers of polymorphonuclear leukocytes in aspirates from bladders with ureters and urethras ligated were higher at each time interval than in aspirates from unobstructed bladders. Smears of aspirates from bladders of rats with the urethra ligated revealed many bacteria at all times. Most of the bacteria were extracellular but many were within polymorphonuclear leukocytes.

Sections of bladders prepared following inoculation of 10^7 *E. coli* into bladders of rats with ureters and urethras ligated demonstrated intense leukocytic infiltration more marked at each time interval than in bladders of rats without urethral ligation. Many bacteria were seen within the lumen at all times.

DISCUSSION

The present study has demonstrated an antibacterial defense mechanism in the rat urinary bladder unrelated to urine flow. In two abstracts, Paquin and co-workers[5] and Mulholland and co-workers[6] reported that no antibacterial activity could be demonstrated in the bladders of rabbits against two strains of *E. coli* and one strain each of *P. mirabilis* and *Streptococcus fecalis.* It is possible that antibacterial mechanisms are not effective within the rabbit bladder or that the bacterial strains studied by these workers were resistant to the antibacterial mechanisms of the rabbit bladder.

There are several possible explanations for the antibacterial activity observed in the rat bladder and some of these were investigated in the present study. Experiments with radioactive-chromium-labeled *E. coli* provided evidence against loss of bacteria through the urethra serving as a major mechanism. In these experiments, 6 to 8 hours after inoculation the viable bacterial count had decreased tenfold or more in most rat blad-

ders but the amount of radioactivity was usually equivalent to the starting value. Furthermore, at 24 hours at least one-half of the initial radioactivity was still associated with the rat in tissues and carcass. In addition, jars in which animals were maintained post-operatively were not found to contain more than one-half of the injected radioactivity in any instance. Therefore, while there was some loss of radioactive label through the urethra, this alone could not explain the 10 to 1,000-fold decrease in the number of bacteria that could be recovered from the bladder.

There is convincing evidence that decreases in viable bacterial counts did not result from clumping of bacteria in the bladder. Despite 10 to 1,000-fold decreases in viable counts. no large clumps of bacteria were seen either in smears of "bladder aspirates" or in histological sections. Also, trypsinization of bladder homogenates did not increase viable counts. Furthermore, in the absence of bacterial killing, 24 hours of incubation in the bladder should have been sufficient to result in net increases in numbers of bacteria even if bacterial clumping did occur. Experiments with 10^4 or less *E. coli* strains 1 or 2, in which some bladders were sterile after 24 hours, also serve as evidence against clumping as the sole or major contribution to the antibacterial activity.

It is likely that nutrient material in the bladder lumen was sufficient to support bacterial growth. *E. coli* strain 1 grew in bladders of dead rats and in aspirates from bladders. In addition, *E. coli* strain 3 and the *P. mirabilis* strain usually grew when 10^5 or more were inoculated, and all of the bacterial strains grew when the urethra was ligated. Furthermore, all strains were inoculated in trypticase soy broth and as little as 1 per cent standard strength trypticase soy broth in water (i.e., .03 per cent trypticase soy broth powder in water) will support growth of all of the bacterial strains studied.

Attempts to demonstrate an inhibitory or bactericidal substance in the bladder were unsuccessful even after injection of heat-killed bacteria into the bladder. Although rat serum was bactericidal for all four bacterial strains studied, and although certain urine samples were bactericidal for some of the bacterial strains, the activity of serum or urine from a rat against a bacterial strain did not correlate with the fate of that bacterial strain in the bladder of the same rat. However, the possibility still exists that serum factors or urine factors (even though urine had been removed from the bladder) played an important role in the antibacterial activity of the bladder.

Evidence suggests that phagocytosis and killing of bacteria by leukocytes constitutes the major antibacterial mechanism of the rat bladder in the

model studied (i.e. isolated from urine flow). The numbers of polymorphonuclear leukocytes in aspirates from the bladder and in histological sections of the bladder increased following injection of bacteria into the bladder. Furthermore, smears of aspirates from the bladder and histological sections of bladders demonstrated bacteria intracellularly within polymorphonuclear leukocytes. It seems likely from microscopic examination of the sections that bacteria initially become embedded in the mucous lining the bladder lumen and then later are phagocyted by polymorphonuclear leukocytes.

Obstruction of the bladder outflow tract either by urethral ligation or by tying the base of the bladder abolished the antibacterial activity of the bladder. Ligation of the base of the bladder obstructed the blood supply and caused necrosis of the bladder which would be expected to severely compromise many possible mechanisms of antibacterial activity. However, ligation of the distal urethra did not cause necrosis, but still abolished antibacterial activity. It is not clear how urethral ligation decreased the antibacterial activity of the bladder. This procedure did not interfere with leukocytic infiltration. It is possible that the accumulation of secretions in the obstructed bladder (up to 0.2 ml. after 24 hours) was sufficient to separate the mucosa from some of the bacterial inoculum and allow bacterial multiplication in the lumen at a site where phagocytosis may not be optimal (i.e., where close contact between leukocytes, bacteria, and the bladder mucosa is not possible).

The observation that obstruction of the bladder outflow tract predisposes to urinary tract infection correlates well with experience in humans and observations in experimental animals. In one study Cox and Hinman[8] were unable to produce persistent infection in the normal human urinary tract by inoculation of ten million *E. coli* into the urinary bladder. However, with obstruction to outflow of the bladder, urinary tract infection is common in humans.[8, 9] Fiveash, Foster, and Paquin[10] demonstrated in rabbits that the incidence of persistent bacteriuria was much higher following injection of *E. coli* into urinary bladders with the outflow tracts partially obstructed than into bladders with unobstructed outflow tracts. One of the ways by which urinary tract obstruction predisposes to infection may be by elimination of the antibacterial mechanism of the urinary bladder.

SUMMARY

The present study was undertaken to investigate antibacterial mechanisms in the rat urinary bladder. In order to avoid the variables of urine

flow and interaction of urine with the bacteria, the bladder was isolated from urine flow by bilateral ureteral ligation.

The fate of each of three strains of *Escherichia coli* and one strain of *Proteus mirabilis* was studied after inoculation into the bladder. Striking antibacterial activity of the rat bladder was observed against *Escherichia coli* strains 1 and 2. Following inoculation of up to 10^7 *Escherichia coli* strains 1 or 2 a significant decrease in the total number of bacteria that could be recovered from the bladders was noted as early as four hours after inoculation in the majority of animals. By 24 hours a 10 to 1,000-fold decrease had occurred in the total number of bacteria recovered from most bladders.

The antibacterial activity of the bladder was also present but less potent against *Escherichia coli* strain 3 or the strain of *Proteus mirabilis*. When 10^5 or more of either of these strains were inoculated into the rat bladder, growth occurred in the majority of bladders by twenty-four hours. However, with inocula of 10^4 or less, significant decreases in recoverable bacteria occurred in most bladders.

Histologic evidence suggested that phagocytosis and killing of bacteria by polymorphonuclear leukocytes constituted the major antibacterial mechanism of the rat bladder in the model studied.

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