EXPERIMENTS WITH INDUCED BACTERIURIA, VESICAL EMPTYING AND BACTERIAL GROWTH ON THE MECHANISM OF BLADDER DEFENSE TO INFECTION

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That the normal bladder is inherently resistant to infection was repeatedly expressed in urologic writing previous to 1925. However, recent publications on urinary tract infections have overlooked this concept which once was so well accepted. Accordingly, we designed a sequence of four experiments to determine, if possible, the existence of such intrinsic resistance of the bladder to infection. This resistance, which we have termed "the bladder defense mechanism," will be shown to be the result of two factors: vesical emptying, and destruction of bacteria by an antibacterial action of the bladder.

The earlier studies by Guyon (1889) and Rovsing (1898) showed that non-obstructed, non-traumatized animal bladders could not be infected by the introduction of pathogenic microorganisms. These observations supported their clinical impression that the normal bladder is inherently resistant to infection. Hess (1913), although unable to produce infection by the introduction of Escherichia coli into normal clog bladders, was readily able to produce cystitis if an irritant substance such as paraffin or turpentine was introduced at the same time. In the early part of this century, other observers also concluded that the normal bladder could not be infected merely by the introduction of pathogenic bacteria. Cabot (1911) in 1916, summarized the previous work on the natural resistance of the bladder and brought the concept into proper focus. He and his co-workers repeated much of the previous animal experimentation and added pertinent clinical observations. He demonstrated that following cystoscopy, the normal bladder would often contain bacteria for as long as 6 days without evidence of cystitis and stated, "This evidence appears to us to require the conclusion that, both experimentally and clinically, the introduction of bacteria and even moderate traumatization of the posterior urethra and bladder are incapable of producing cystitis" (p. 33).


tract infections, on the contrary, have concentrated on the harmful effects of introducing bacteria by instrumentation and have neglected the natural defense capabilities of the normal bladder. A statistical relationship between single catheterization and established infection has been suggested. 3 As the defenders of the catheter 3 on the other hand, have been slow to point out the role of intrinsic vesical resistance. Instrumentation has been primarily defended on the basis that urologic disorders require it, 4-6 and that few harmful late effects follow instrumentation. 7-9 However, as we ex-

[References and citations are omitted for brevity.]

...fected with the normal bladder has a natural resistance: provided by certain defense mechanisms, and appears able to defend itself.

An initial study by the authors 10 showed that infection of the bladder was difficult to produce by indwelling catheter in normal subjects. This finding is in distinct contrast to the impression gained from previously published observations and opinions. 11-13 In our study of 80 subjects in whom catheters were placed for periods ranging from 18 to 72 hours, the incidence of bacteriuria was only 6.6 per cent after 24 hours. Positive cultures were obtained from only 45 per cent of the bladders at the end of 72 hours.
of continuous catheter drainage. Cultures taken
days or weeks after removal of the catheter
showed clearing of the bacteriuria in all subjects
with initially positive cultures. These observa-
tions suggested to us that the normal bladder
has a strong intrinsic protective mechanism.

In order to expose the defense mechanisms at
work, the following complementary experiments
were done. Two defense mechanisms were
studied and their relative importance was evalu-
ated: mechanical emptying (voiding) in the
absence of significant residual urine, and an
intrinsic "antibacterial" factor or factors in the
bladder. The experimental series was designed to
investigate in sequence: 1) the growth in vitro
of bacteria in urine, 2) the change in a bacter-
ial population as a result of mechanical emptying
(voiding), 3) the effect of antibacterial in vivo
factors upon bacterial growth; and 4) the com-
bined effects of both mechanical emptying and
vesical antibacterial activity.

EXPERIMENTAL SERIES

Experiment 1. The purpose of the first set of
experiments was to determine the growth curve
of bacteria in vitro in normal urine and to see if
this growth was similar to the rapid multiplicat-
on of bacteria that occurs in usual nutrient
media.

Methods: Sterile urine obtained from a nor-
mal subject was inoculated from an 18 to 24
hour broth culture of E. coli. An equal volume
of brain heart infusion broth (Difco) was ino-
culated simultaneously with an equal number of
E. coli. The urine and broth were incubated at
37°C and bacterial colony counts obtained at
1, 2, 4, 6, 8, 12 and 24 hours. The same pro-
cedure was repeated with three other specimens
of urine.

Results: Mean bacterial counts rose essen-
tially the same in urine as in broth (fig. 1).
These curves follow the classic curve of bacterial
growth (fig. 2). Urine from these subjects then

*Quantitative bacterial counts in this and the
following experiments were car
ried out by th
ee (or four) successive tenfold dilutions of the ur
ne or culture medium in sterile 0.9 per cent saline
solution. One ml of aliquots of the dilutions were
then pipetted into sterile Petri dishes. Ten ml of
melted, cooled agar was added and mixed by
rotation of the Petri dishes.

7 Wilson, G. S. and Miles, A. A.; Topley and
Wilson's Principles of Bacteriology and Immunity,
100.

is a medium in which the growth of E. coli is
uninhibited. That urine, within the physiologic
range of normal, will readily support bacterial
growth has been previously demonstrated, and
it has also been shown that the growth curve of
E. coli in urine closely approximates that in
nutrient broth.

Interpretation: Urine, under the con-
ditions of our present experiments, is an excel-
ten culture medium for E. coli and does not interfere
with bacterial multiplication under ordinary
circumstances. That urine under other circum-
cstances may contain inhibitory substances will
be commented upon below.

Experiment 2. This experiment was to deter-
mine if mechanical emptying (voiding) is a de-
fense mechanism and if so, to what extent will
it remove bacteria from the bladder. By the use
of a simulated bladder, an attempt was made to
isolate the effect of voiding from the effect of
other bladder defense mechanisms.

Methods: A sterile 500 ml flask was used to
simulate a bladder. The subject voided directly
into this flask upon arising in the morning. The
flask was then inoculated from an 18 to 24
hour broth culture of E. coli and an aliquot was
immediately withdrawn for counting of bac-
terial colonies. The flask was carried in such a
way as to maintain it at approximate body tem-
perature. Prior to each voiding during the next
24 hours, the subject inverted and drained the
flask, discarded the contents, and then voided
directly into it, so that the filling and emptying
of this extracorporeal container roughly paral-

Kass, E. H.: The role of asymptomatic bac-
teriuria in the pathogenesis of pyelonephritis. In:
Biology of Pyelonephritis, ed. by E. L. Quinn and
E. H. Kass, Boston: Little, Brown & Co., 1960,
chap. 28, pp. 399-412.
eled that of the bladder. The following morning, 24 hours after inoculation, a bacterial colony count was again obtained before emptying the flask. This entire procedure was done 5 times.

In 2 cases, colony counts were also obtained at 48 hours and in 1 case, at 72 hours. In 1 other case, 2 colony counts were obtained at each voiding during a 24-hour period, one just prior to emptying the flask and one just after refilling the flask.

Results (figs. 3 and 4): Bacterial counts made after 24 to 72 hours, during which time the flask was periodically drained and refilled, closely approximated the initial counts in all cases.

Interpretation: The results show that periodic emptying does not completely remove bacteria from the flask. However, emptying does restrict the rapid accumulation of bacteria which occurred in standing urine (experiment 1, fig. 1). The fore, draining of a simulated bladder acts as a defense mechanism, but a limited one. It will interfere with a bacterial population, but will not totally rid the bladder of bacteria.

That bacteria are not progressively reduced to a vanishing point by frequent emptying in the absence of "residual urine" requires emphasis. The flask, although stated to have been "drained completely," undoubtedly contained a small amount of residual urine, enough to wet the inside walls. This residuum, although small contained bacteria in the same concentration as the urine drained from the flask, and each time their concentration was reduced by the dilution effect of refilling the flask. However, the number of bacteria per milliliter rapidly increased between periods of voiding. This process whereby bacteria in high concentrations contained in a small residual of urine are diluted to relatively few bacteria per milliliter only to achieve again the higher concentration after a period of incubation is illustrated in figure 4.

The same activity may be attributed to the normal bladder. Although the absence of residual urine is assumed in normal micturition, some urine is undoubtedly present after voiding, if only the small amount required to wet the mucosa (probably from 1 to 5 ml). This "insignificant" residual, as this experiment shows, prevents voiding from completely "ridinge the

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**FIG. 4.** Changes in bacterial population by periodic emptying and refilling (dilution) in simulated bladder, showing importance of "insignificant" residual urine in maintaining bacteriuria at relatively constant level.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Hours of Incubation</th>
<th>Bacterial Counts per Milliliter</th>
<th>Total Bacterial Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Bladder (vol. 100 ml)</td>
<td>2</td>
<td>10^5</td>
</tr>
<tr>
<td>Container (vol. 200 ml)</td>
<td>2</td>
<td>6 X 10^4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^9</td>
</tr>
<tr>
<td>Subject 1</td>
<td></td>
<td>2 X 10^9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5 X 10^5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2 X 10^5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^9</td>
</tr>
<tr>
<td>Subject 2</td>
<td>3</td>
<td>5 X 10^5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2 X 10^5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^9</td>
</tr>
<tr>
<td>Subject 3</td>
<td>4</td>
<td>10^9</td>
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<td>10^9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5 X 10^8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10^7</td>
</tr>
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</table>

*Calculated by assuming 5 ml bladder residual.
bladder of bacteria. In 1934, Helmholtz\textsuperscript{20} stated that, "It is known that the sterility of the urine is not dependent on bactericidal power of the urine, but is probably the result of constant washing out of the urinary passages; so probable is this that it is stated that the normal bladder cannot be infected by the introduction of organisms" (pp. 173-4). However, it has been demonstrated in experiment 2 that "constant washing" will not totally defend the bladder, and as we shall show, the normal bladder is resistant to infection, so some additional antibacterial factor must be present.

Experiment 3. The purpose of this experiment was to compare the in vitro growth of bacteria in urine simultaneously with the in vivo growth of bacteria in the bladder in an attempt to demonstrate the presence of an antibacterial or natural inhibitory factor within the bladder.

Methods: Four healthy young male subjects were selected on a voluntary basis. All were free of symptoms and findings of vesical neck obstruction and urinary tract infection. In all cases, urinalysis, including methylene blue stain of the sediment, showed no abnormalities and significant residual urine as determined by the phenolsulfonphthalein\textsuperscript{29} test was not present. The subject was asked to void completely into a sterile container and the volume was noted. Five milliliters of a prepared dilution of an 18 to 24 hour broth culture of E. coli, containing 2 million organisms per milliliter, were then placed both in the container and into the subject's bladder through a IOF Robinson catheter. The catheter was immediately removed. The container was then incubated at \textdegree C. The subjects were instructed to void at either 2, 3, or 4 hours after the introduction of bacteria. Before each voiding, the glans penis was cleansed with pHisoHex. The volume of each specimen was noted. A bacterial colony count was then immediately obtained both from the container and from the subject's freshly voided urine.

Results (table 1, figs. 5 and 6): Subject 1 voided 2 hours after the introduction of bacteria and essentially no bacterial multiplication was noted in vivo (in the bladder) or in vitro (in the container).

Subjects 2, 3 and 4 voided 3, 4, and 4 hours respectively after the introduction of bacteria, and total bacterial counts were made. Although the initial in vitro counts of 10 million increased to over 400 million after 3 to 4 hours of incubation in the container (fig. 5), the initial in vivo counts of 10 million organisms increased only slightly or decreased during the 3 to 4 hours of incubation in the bladder.

A comparison was also made of the change in bacterial counts per milliliter. The initial in vivo bacterial counts of one million per milliliter (calculated from the assumption of a 5 ml. bladder residual) decreased to 500,000 per ml. or less; whereas initial in vitro bacterial counts increased from 50,000 per ml. to over 2 million per milliliter (fig. 6).

Interpretation: These experiments demonstrate that bacteria introduced into a normal bladder are inhibited in their growth when compared to similar bacteria introduced in vitro into
urine from the same bladder. This inhibitory or antibacterial factor appears to be an effective part of the bladder defense mechanism. It is not contained in the urine, and seems to become more effective the longer the invading bacteria are in contact with it.

Experiment 4. This experiment demonstrated the combined action of both defense mechanisms on bacteria introduced into the bladder.

Methods: The same 4 subjects used in the third experiment were studied further. Ten million E. coli were introduced into the subjects' bladders; they were allowed to remain for 2 to 4 hours, and bacterial colony counts were obtained when the subjects voided. The subjects then voided at 3 hour intervals for 9 hours. Bacterial colony counts were obtained at each of these intervals, and again in 72 hours. One to 2 months later, urine was also obtained and cultured.

Results (fig. 7): In 3 of the 4 cases, the initial colony counts were above the currently accepted level of significant bacteriuria (fig. 6). Succeeding colony counts made at 3 hour intervals rapidly decreased in all the subjects. After 6 hours, all counts were below 20,000 per ml. No subject was found to have E. coli in the urine at 72 hours and no E. coli were present 1 to 2 months later. No pyuria was found and no symptoms of cystitis developed.

These results confirm the observations of Cabot, as well as everyday clinical experience, that the normal bladder will dispose of bacteria. Since bladder defense mechanisms were able to dispose of the 10 million experimentally introduced bacteria in a comparatively short time (72 hours or less), it is not surprising that the normal bladder is capable of removing the relatively few (10-100) bacteria which may be introduced by routine instrumentation.

Interpretation: Relatively high levels of induced bacteriuria were shown to decline steadily and rapidly during a 6 to 9 hour period. This steady fall in bacterial counts cannot be explained by the defense mechanism of voiding alone, as was demonstrated in the second experiment. In addition, it was shown in the third experiment that the bladder, not the urine, contained an antibacterial factor. Therefore, it must be concluded that this initial rapid reduction of bacterial counts and the total removal of bacteria in 72 hours were the result of the combined action of at least two factors in the bladder defense mechanism, voiding and vesical inhibition of bacterial growth.

DISCUSSION

Host defense mechanisms. The presence of bacteria in the body results in a conflict between these bacteria and various available host defense mechanisms: principally 1) physiologic barriers such as skin and mucous membrane; 2) phagocytosis; 3) inflammatory response; 4) antibody production and 5) possibly other antibacterial factors. Vesical antibacterial activity could be the result of one or a combination of these basic host defense mechanisms in addition to mechanical emptying. It could be a component of the urine, be inherent in the bladder lining or transported to the bladder in times of stress by the bloodstream. Previous published reports have not localized the mechanism of vesical antibacterial activity, nor has the total defense mechanism of the bladder been clarified.

Conditions lower defense barriers. The literature contains much indirect evidence of the manner in which the urinary tract succumbs to bacterial invasion. Many conditions and disease have been shown or postulated to be associated with an increased incidence of urinary tract infections. Diabetes, pregnancy, debilitated individuals, and some other conditions increase the susceptibility of the urinary tract to infection.

states/ potassium deficiency, and neurologic disturbances are but a few of these. Although these observations are pertinent from a clinical standpoint, it is difficult to determine how these conditions alter the defense mechanisms. A neurogenic bladder often is deprived of one of the mechanisms, that of efficient emptying. Also, the gravid uterus may be associated with obstructive states which do not permit efficient emptying of both the upper and lower urinary tract, but Kass has demonstrated an increased incidence of bacteriuria early in pregnancy, probably before the obstructive factors appear. He suggests that the increased incidence of bacteriuria may be related to the hormonal changes in pregnancy. Apart from changes in vesical emptying, these conditions and diseases apparently have in common the same end result, a reduction of antibacterial or natural inhibitory factors which normally participate in the defense of the urinary tract.

**Vesical defense factors.** Specific references to factors which participate in the bladder defense mechanism are difficult to find in the literature. The general proposition that the normal bladder is difficult to infect was accepted long ago, strengthened by the observations of Guyon in 1889 and later by Cabot. In 1902 Brown, in a discussion of the causes of acute and chronic cystitis, attempted to explain why the colon bacillus, a bacteria he considered to be of low pathogenicity, became an infecting organism. He pointed out associated factors in his series of cases which "prepared the bladder for the reception of these germs and rendered it susceptible to their low pathogenic powers." In all of his cases he found one or more of the following factors to be present: anemia, pressure on the bladder which produced congestion, relaxation of the vesical outlet, trauma to the bladder, childbirth, catheterization with poor technique or direct extirpation of infection. Thomson in 1910, discussing urinary infections in infants with presumably non-obstructed urinary tracts, stated, "A very important point in the etiology of this condition (pyelitis of infancy) is the question as to the precise nature of the local or predisposing causes which have so weakened the natural defenses of the urinary tract as to make it liable to be invaded by its normal neighbor, the Bacillus coli. Probably the weakening influence may be something in the nature of a chill; possibly some chemical change in the urine may have occurred." (p. 2531).

In recent years, apparently as a result of the failure of antibiotics to significantly lower the incidence of chronic urinary infections, new interest in these infections has arisen. In 1960 Kass, in an attempt to explain how the normal bladder clears itself of bacteria, theorized that, "The implication here is that there is an antibacterial mechanism in the lower urinary tract that is not a function of serum protein, that is not mediated by inflammatory exudates, and that is presumably related to the metabolic activity of the bladder mucosa. A metabolic product such as metabolically produced acid might inhibit the multiplication of bacteria that were in close apposition to the mucosa. The effect of obstruction would then be to keep the bacteria from close contact with the mucosa and its metabolic products" (p. 116). It is this protective mechanism which our present experiments exposes.

**Inhibitory substances in urine.** In 1917, Shoholl and Janney demonstrated that human urine adjusted to a pH of 4.5 to 5 and 9.2 to 9.6 would not support the growth of Bacillus coli. Davis and Hain in 1918 reported the occurrence of antiseptic properties in dog urine. This was an inconsistent finding and was not related to pH or specific gravity variations within the physiologic limits of the urine. The antiseptic properties were generally effective only against E. coli. An unsuccessful attempt was made to remove this antibacterial substance with various solvents, but they did not note a tendency for growth to occur more often in unfiltered than in filtered urine. No similar effect was observed with the

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filtration of human urine. Bloomfield\textsuperscript{10} in 1920 demonstrated two mechanisms by which the upper air passages disposed of bacteria which had been introduced into a series of human subjects. One method was a prompt antibacterial action by the secretions and the other a "normal mechanical flushing process in the mouth and nose." He pointed out essentially the same defense mechanisms which we find to be present in the bladder.

That the urine of humans may at times exhibit an antibacterial effect was noted by Kass.\textsuperscript{11, 12} He described a "natural inhibitor" which is most effective at a urinary pH of 5, is non-volatile, alcohol-soluble and ether-insoluble, and loses much of its activity as the pH of the urine is raised. This suggested that it is a weakly ionizable acid. It has also been demonstrated\textsuperscript{13} that changes in urinary pH or specific gravity within physiologic limits usually have no effect upon bacterial growth in the urine.

Jackson and Grieble\textsuperscript{14} considered the possibility that infection might be encouraged in some instances by the excretion of urine, which is a better medium for bacterial growth. They observed the in vitro growth of bacteria in normal urine, in pooled urine from patients with urinary tract infections, in balanced salt solution and in enriched bacteriologic media. No significant difference in final growth was noted in normal urine and urine from patients with urinary tract infection; however, the growth rate seemed significantly greater in the latter group. Interestingly, they demonstrated that the addition of serum to urine did not enhance bacterial growth, but its addition to salt solution greatly enhanced bacterial growth in that substance. They concluded that possibly bacterial growth in urine was restricted by an inhibitory substance which was not neutralized by serum. These authors also found that urea in a concentration of 0.5 gm. per cent produced slight, but not significant, depression of bacterial growth in urine. More recently, Schlegel and associates\textsuperscript{15} have noted bactericidal effects of higher (2 to 4 per cent) concentrations of urea in urine in vitro, levels which can be attained during dehydration.

Organic substances excreted in the urine, such as lactic, acetic and mandelic acid, are known to have a bacteriostatic action. In addition, hippuric acid, related chemically to mandelic acid, has recently been shown to be bacteriostatic when present in urine in suitable concentration.\textsuperscript{16} However, the effect of all of these acids appears to be greatly reduced as the pH of the urine is raised much above 5.

It has recently been suggested by Boyce and Edwards\textsuperscript{17} that urinary mucosubstances may potentiate bacterial invasion. They stated "These mucosubstances which are presumed to be 'protective' for the mucosa may actually form protective blankets for urinary pathogens. Such mucosubstances adhering to the urinary passages may thus prevent bacteria from being carried away in the urinary stream" (p. 733).

Role of immune mechanisms and hormones. The manner in which these factors contribute to the defense of the urinary tract is poorly understood. Serum immune globulin deficiency\textsuperscript{18} and properdin deficiency\textsuperscript{19} have been considered as possible factors which increase human susceptibility to infection. Rowley\textsuperscript{20} feels that the most fundamental antibacterial mechanism by which the urinary tract defends itself is the combination of complement with natural or acquired antibody which acts on bacteria and prepares them for phagocytosis. Although Hughes\textsuperscript{21} was unable to find agglutinating antibodies in the sera of patients with pyelonephritis, other observers,\textsuperscript{22} using a more sensitive test, demonstrated high antibody titers in these patients.

That resistance to infection is closely related to hormonal changes is well recognized. This relationship is obvious in some instances, such as insulin deficiency and postmenopausal estrogen depletion, but is obscure, particularly in relation to subtle changes in adrenocortical and thyroid function.\textsuperscript{35, 45} In addition, the mechanism by which hormones exert their influence is unclear. It would seem that a more thorough understanding of normal hormonal action is necessary before their function in abnormal states can be properly evaluated.

\textbf{Effect of obstruction on defense mechanisms.} The association of urinary tract obstruction and infection is well documented in the literature. That obstruction often results in measurable amounts of residual urine is common clinical experience. The concept of a stagnant pool of urine teeming with bacterial growth is frequently presented. We have pointed out that the normal bladder contains a small residualum, yet it is resistant to infection. However, with significant residual urine, as in the presence of obstruction, it is unable to combat bacteria. An inefficient emptying mechanism, therefore, permits bacterial invasion and continuing infection.

In addition, there is some evidence to suggest that the deleterious effects of obstruction are greater than can be accounted for by residual urine alone. Campbell\textsuperscript{59} has postulated a decreased phagocytic activity of the urinary epithelium when backpressure is applied. Mehrotra\textsuperscript{60} repeated some of the earlier work on the natural resistance of the non-obstructed animal bladder. In addition, he demonstrated by an ingenious technique that in the presence of increased intravesical pressure, intravenously injected particulate matter and bacteria would become localized in the bladder wall. He also pointed out, as did Hess\textsuperscript{3} in 1913, that introduction of irritating substances such as xylol had but a mild effect on the bladder mucosa, but if bacteria were simultaneously introduced, an intense inflammation resulted. Irritating substances apparently do not "call out" the natural defenses, but rather decrease their effectiveness.

More recently, workers\textsuperscript{13, 15, 47, 61} studying

experimental pyelonephritis have postulated that the association of upper urinary tract infection and obstruction may not be entirely the result of a residual pool of "stagnant urine." Obstruction, in some manner, may interfere with the "natural inhibitory factor" or "antibacterial factor" in the kidney.

SUMMARY AND CONCLUSIONS

That the normal bladder is inherently resistant to infection was pointed out before the turn of the century. This natural resistance has, however, been overlooked in recent work on urinary infections, as is particularly evident in the recent controversy over the role of instrumentation in the etiology of infection. Therefore, an attempt has been made to investigate this resistance, which we have termed the "bladder defense mechanism."

This investigation has demonstrated, as was suggested by a previous study by the authors on retention catheterization, that the normal bladder is resistant to infection. It is shown that this inherent resistance is provided by at least two defense mechanisms: one, the mechanical factor of voiding; and, two, an antibacterial factor or factors, inherent in the bladder, and not contained in the urine.

Four complementary experiments were carried out. The first showed that the in vitro multiplication of bacteria in random urine is similar to the rapid bacterial multiplication that occurs in nutrient broth; thereby demonstrating that urine under ordinary circumstances is not antibacterial.

The second experiment showed that vesical emptying (voiding) reduced bacterial counts but was insufficient to completely rid the simulated bladder of bacteria.

The third experiment indicated that the growth of bacteria in vivo was limited by an antibacterial factor, as demonstrated by a comparison of the growth of bacteria introduced into a subject's urine in vitro and the simultaneous growth of bacteria introduced into the same subject's bladder.

The last experiment showed that bacteria introduced into the normal bladder rapidly decreased in number in 6 to 9 hours, and completely disappeared in 72 hours. This demonstrated the combined effect of both bladder defense mechanisms, each increasing the effectiveness of the other. These two defense mechanisms and their relative effects can be schematically illustrated (fig. 8) to show that mechanical emptying levels the normal bacterial growth curve, but the presence of an intravesical antibacterial factor is necessary to reduce the curve to zero.

The advice of Dr. Ernest Jawetz, professor of microbiology, has been invaluable throughout the investigation. The technical assistance of Miss Joyce Amluxen is gratefully acknowledged.