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Role of Urinary Solutes in Natural Immunity to Gonorrhea

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Natural resistance of the male urethra to gonococci has not been explained by classical immune mechanisms but could result from antibacterial properties of urine. Accordingly, we measured survival in midmorning urine of 10^7 F-62 T2 gonococci per ml by serial dilutions and plate counts. Fifteen killer urines from eight people all killed >3 logs (average, 5.3), and 13 of 15 were sterilized. Fourteen nonkiller (inhibitor) urines from seven subjects allowed no growth. Killer urines were more acidic (pH 5.4 versus 6.4) and more concentrated (861 versus 717 mosmol/kg) than nonkillers. Upon addition of hydrogen ion, urea, and sodium chloride to urines and broth, pH proved to be the major killing factor, but urea and NaCl were also bactericidal. Susceptibility to urine bactericidal power did not vary with colony type (T2 versus T4) or strain (F-62 versus two fresh isolates). Killing was rapid (0.5 to 3 h) and not bacteriolytic. *Escherichia coli* multiplied 10-fold in urines that inhibited growth of gonococci. Thus, the bacteriostatic effect of urine may explain why gonococci do not infect the bladder and kidney during gonorrhea. The bactericidal properties of urine may contribute to resistance against gonococcal urethritis.

After exposure to gonorrhea, the urethras of most men resist infection (11), and in patients with gonococcal urethritis the bladder and kidney are never infected. This intriguing natural immunity of the male urethra and the urinary tract of men and women has not been explained by conventional immunological concepts (13)-but could be explained by antibacterial properties of urine. Normal urine contains only trivial amounts of immunoglobulins and complement (20) and inhibits phagocytosis (4), but it contains solutes, which might kill gonococci and thereby explain natural resistance.

The survival of gonococci in human urine has been examined by investigators interested in the diagnosis of gonorrhea. Allison et al. recovered gonococci from urines at low pH (5.0 to 5.5) less often than urines at pH 6.0 to 6.5 (42 versus 90%), suggesting that pH determined viability of gonococci in urine (1). More recent studies have confirmed the value of culturing the urinary sediment in the diagnosis of both symptomatic gonorrhea and asymptomatic carriage of gonococci (3, 6, 16). On the basis of results in asymptomatic patients, culture of the urinary sediment has been proposed for noninvasive screening for asymptomatic gonorrhea (16).

Because killing of gonococci by urine might influence resistance to gonorrhea, as well as diagnostic screening, we studied the factors governing survival of gonococci in urine.

MATERIALS AND METHODS

Urines. Midmorning urines were collected from healthy young men and women on the day of each experiment. The pH was determined with a bipolar electrode (Ingold Electrodes, Inc.) and a Coleman pH meter (Coleman Instruments, Maywood, Ill.). After filtration through a 0.45-μm filter (Millipore Corp., Bedford, Mass.) to remove cells, bacteria, and debris, urines were held at room temperature until they were examined. Osmolality was determined with a vapor pressure osmometer (Wescor Inc., Logan, Utah), and urea nitrogen was measured by the carbamido-diacetyl reaction on a Technicon autoanalyzer (Ardsley, N.Y.). Urea concentration was calculated from urea nitrogen.

Bacteria. The F-62 strain of *Neisseria gonorrhoeae*, described by Kellogg (14), was obtained from Jerry Brown, Center for Disease Control, Atlanta, Ga. Recent clinical isolates of *N. gonorrhoeae* N-17 and N-24 were obtained from the San Diego County Public Health Laboratory, subcultured several times to purify colony type, stored by freezing in citrated rabbit blood at −70°C, cultured on colony-type media (GC agar base supplemented with IsoVitaleX [BBL, Cockeysville, Md.]), and examined under indirect substage illumination for colony type. T4 colony types of F-62 were derived from spontaneously appearing colonies on T2 plates. Two *Escherichia coli* strains (O6 Riffle and O6 Williams) were handled similarly to gonococci.

Agar plate cultures of bacteria grown for 16 to 20 h at 37°C in 5% CO_2 were suspended in Dulbecco minimal essential medium (Grand Island Biological Co. Grand Island, N.Y.) with a platinum-wire loop. After vortex blending to disperse organisms, the suspension was centrifuged at 500 × *g* for 1 min to deposit clumps and agar, and the supernatant suspension was diluted in Dulbecco medium to an optical density equivalent to 5×10^8 organisms/ml.

Bactericidal assay. Survival of bacteria in urine or broth was determined by serial dilutions and plate counts. Killing was calculated from the log of the 0-time inoculum (measured in each experiment) minus log of survivors. Preliminary experiments in which bacteria were counted microscopically showed that the inoculum consisted of viable diplococci without significant clumping. A 6-h incubation period was used in most experiments after we found that killer urines were usually sterile by that time. Dulbecco medium was used to suspend gonococci because it did not kill. By measuring pH before and after 6 h of incubation, we have shown that gonococci suspended in Dulbecco medium do not change the pH of the mixture. The effect of inoculum size on killing by urine was determine with serial 10-fold dilutions of bacteria. When killer urines were shown to sterilize as many as 10^7 gonococci, we chose this inoculum for most experiments, so that unequivocal distinctions between killer and nonkiller urines could be made.

A 0.1-ml portion of the bacterial suspension was mixed by vortex blending with 0.9 ml of urine or broth in plastic tubes. The mixture was incubated at 37°C in 5% CO_2. The number of viable gonococci at zero and subsequent times was determined by plating on agar 0.1 ml of serial 10-fold dilutions of a 0.1-ml portion of the mixture. Surviving organisms were calculated from colony counts after incubation for 20 h.

Buffered liquid medium. To analyze the action on gonococci of various urinary solutes, we added varying concentrations of HCl, NaOH, urea, and NaCl (as an osmotic agent) to a buffered liquid gonococcal growth medium. The medium contained the ingredients of GC medium base (Difco) without agar or corn starch and was supplemented with IsoVitaleX and 0.05 M citrate buffer (0.025 M citric acid and 0.025 M sodium citrate). It did not kill gonococci and maintained stable pH after addition of gonococci suspended in Dulbecco medium.

RESULTS

Killer and nonkiller urines. Twenty-nine urines from 15 subjects fell into two distinct groups. Fifteen urines from eight people killed more than 99.9% of the inoculum, and 13 of the 15 completely sterilized it in 6 h. This "killer" group of urines reduced the number of viable gonococci by an average of 5.25 logs. In 14 "nonkiller" (inhibitor) urines from seven subjects, the average decline in viable gonococci was less than 1 log (average, 0.69; maximum, 1.7) and no growth occurred.

To find differences between these two groups that could account for their striking difference in ability to kill gonococci, we compared their pH, urea concentration, and osmolality. Table 1 shows the highly significant differences in mean killing, pH, urea concentrations, and osmolality between the two groups. The killer group had the properties of concentrated urine: higher acidity, urea concentration, and osmolality. The range of values for these three factors overlapped considerably, indicating that none of the three fully accounted for the difference in ability to kill. For example, the killer urines were all below pH 5.8, but three nonkiller urines also fell into that pH range. Since none of these properties by itself could fully account for the observed difference, either a combination of them or an unidentified factor in concentrated urines must account for bactericidal activity.

Because low pH appeared important for killing, we investigated the effects of changing the pH of urines. The pH of three killer and five nonkiller urines was adjusted with concen-

TABLE 1. *Gonococcal killing, pH, urea, and osmolality in killer and inhibitor urines*

Determination	Killer	Inhibitor	Level of significance (*P*)[a]
Logs killed at 6 h			
Average	5.25	0.69	<0.001
Range	3.1–7.8	(−0.3)–1.7	
pH			
Average	5.4	6.5	<0.001
Range	4.9–5.8	5.3–7.5	
Urea concentration (mmol/liter)			
Average	358	241	<0.001
Range	221–496	111–400	
Osmolality (mosmol/kg)			
Average	861	717	<0.05
Range	700–1,118	310–850	

[a] Comparison of group means for 14 inhibitor and 15 killer urines by Student's *t* test for unpaired samples.

TABLE 2. *Effects of adjusting urinary pH on killing on gonococci by inhibitor (I) and killer (K) urines*

Urine	pH	Adjusted pH	Logs killed
I-1	7.1		0.3
		5.5	0
I-2	6.8		−0.7
		5.4	>6.6
I-3	6.6		1.3
		5.6	>6.6
I-4	7.1		0.1
		5.7	>6.6
I-5	6.5		1.8
		5.5	>6.6
K-1	5.4		>6.3
		7.0	0.4
K-2	5.1		>6.4
		6.9	0.5
K-3	5.8		>5.3
		7.4	1.4

trated HCl or NaOH. The killing of F-62 T2 by the adjusted urines was compared with an unadjusted sample of the same urine. In Table 2, five inhibitor urines were near neutral pH. When acidified, four were converted into killers and sterilized the inoculum. The three acid urines lost their killing ability when neutralized with NaOH. These results confirmed the permissive role of hydrogen ion by showing that acidity was essential, but not always sufficient, for killing gonococci in urine.

In Fig. 1, four of five nonkiller urines became bactericidal when their urea concentrations were increased. The most acidic urine (pH 5.9) showed a linear increase in killing capacity with each increment in urea concentration. Three other urines (pH 6.4 to 7.6) killed more than 2 logs within the upper physiological range of urea concentrations. The ability of high urea concentrations within the physiological range to render both acid and neutral urines bactericidal for gonococci shows that urea, by itself, can kill these organisms.

Joint action of hydrogen ion, urea, and NaCl. The three urinary solutes (H^+, urea, NaCl) were added in varying concentrations to GC medium supplemented with IsoVitaleX and 0.05 M citrate buffer. Without addition of the solutes, the osmolality of this broth was 440, it contained no measurable urea, and it did not kill gonococci at neutral pH. Without supplemental urea or NaCl, killing increased linearly with increasing hydrogen ion concentration (Fig. 2). As in most urines, less than 2 logs of killing occurred at pH 6 or above, but at pH 5.5 or less more than 3 logs were killed. Increasing the osmolality of the broth with either NaCl or urea produced increased killing at each pH. At most pH values, increasing osmolality with either urea or NaCl produced similar enhancement of killing. At pH 6.0, urea produced consistently less effect than NaCl. At pH 5.0 the effect of osmolality is obscured by the almost complete sterilization of the inoculum by hydrogen ion alone.

The relative contribution of hydrogen ion and osmolality to killing is shown by comparing the bactericidal effect of each determinant at nonlethal values of the other. For example, at pH

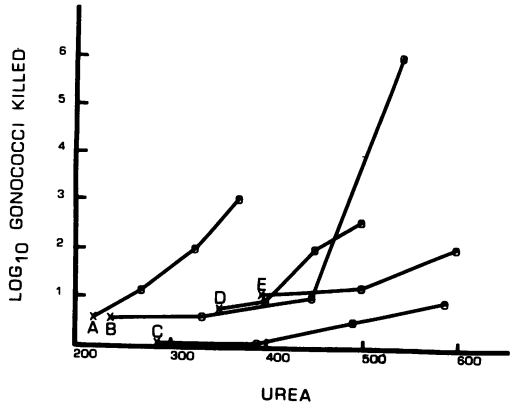

FIG. 1. *Killing of gonococci by addition of urea to nonkiller urines. The $\log_{10}$ of F-62 T2 gonococci killed in 6 h by five urines is shown before (×) and after (O) adding urea. The most acidic (A, pH 5.9) urine increased killing by more than 2 logs when 150 mmol of urea per liter was added. Urine B (pH 7.4) sterilized the inoculum when urea exceeded 500 mmol/liter. In the remaining urines killing was increased by about 1 log when urea exceeded 500 mmol/liter.*

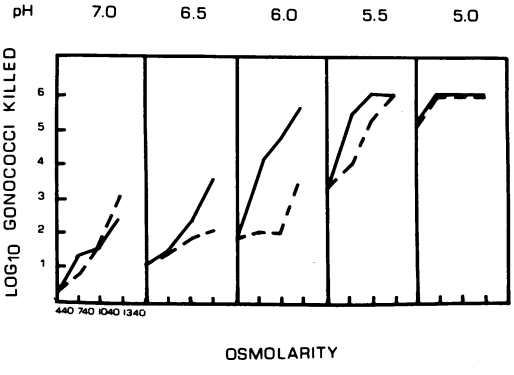

FIG. 2. *Effect of hydrogen ion and osmolality on killing of gonococci by urine. F-62 T2 gonococci (10^7/ml) were incubated in buffered GC broth (440 mosmol/liter), which was not bactericidal for gonococci. Broth osmolarity was adjusted to 740, 1,040, and 1,340 mosmol/liter with either urea (broken line) or NaCl (solid line) at each of five different pH levels. Each point on the graph is the average from three experiments.*

TABLE 3. *Effect of physiological concentrations of urinary organic acids on survival of gonococci in PBS at pH 5.0*

Organic acid	Range in human urine (mg/liter)	Concn in PBS (mg/liter)	Log_{10} killing at		
			30 min	60 min	180 min
PBS, neutral (pH 7)				−0.1	−0.2
PBS, acid (pH 5)			1.0	1.5	5.4
Oxalate	20–40	40	0.5	1.3	3.0
Citrate	128–1,128	1,000	0.4	1.5	5.4
Urate	80–976	1,000	0.5	1.5	4.4
Lactate	100–600	850	0.5	2.2	2.5

5.5, near the average for killer urines, broth with osmolality of 440 kills 3.5 logs. Likewise, neutral broth with osmolality near 850 kills 1.5 logs at pH 7.0. When acting together in broth, pH 5.5 and osmolality of 850 reproduce the killing (5.3 logs) seen in the killer urines, suggesting that the effects of hydrogen ion and osmolality are additive. Thus, hydrogen ion is the primary factor responsible for killing gonococci and can account for the differences between killer and nonkiller urines. Urea and sodium chloride produce additional killing at concentrations found in human urines.

Other antigonococcal factors in human urine. To evaluate the possibility that gonococci are killed by heat-labile proteins (e.g., antibody or enzymes), we heated samples of four killer urines to 90°C for 10 min without changing pH. Killing ability was unchanged after heating, indicating that heat-labile proteins are not necessary to kill gonococci.

Although hydrogen ion is a key factor in killing gonococci by urine, its action could be explained either by direct or indirect effects mediated via another urinary constituent. Several urinary organic acids have pK_a values in the range of pH 6. We postulated that hydrogen ion might convert one of these acids to the nonionized form, which could penetrate the cell and become injurious upon ionizing intracellularly. We tested this possibility by observing the rate of killing of F-62 T2 in acid (pH 5.0) phosphate-buffered saline (PBS) to which oxalate, citrate, urate, and lactate were added at concentrations near their physiological maxima in urine. In Table 3, killing at 30, 60, and 180 min in the PBS control and the organic acids is shown. Gonococci survive well in neutral PBS and are killed in acid PBS. The organic acids produce no increase in killing and lactate and oxalate appear to protect gonococci at 180 min. Thus, hydrogen ion alone (at pH 5) kills gonococci in 3 h.

Rate of killing in urine. The rate of killing by urine varied remarkably within the killer group, ranging from 6 logs in 30 min (Fig. 3, E)

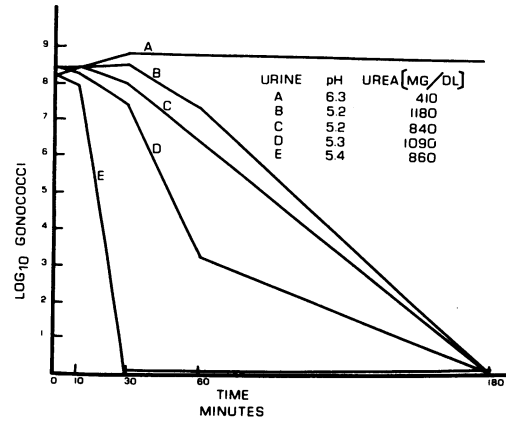

FIG. 3. *Survival of 3 × 10^8 F-62 gonococci per ml in an inhibitor (A) and four killer (B–E) urines. Among killer urines the striking differences in bactericidal kinetics cannot be attributed to large differences in pH or urea concentration. Urine E sterilized the inoculum in 30 min, and all killer urines were sterile by 3 h.*

to 1 log in 60 min (Fig. 3, B) and requiring from 0.5 to 3 h for sterilization. The pH was similar and urea was closely matched for each of two pairs (B and D, C and E) of killer urines which killed at different rates. Thus, speed of killing varied despite similar solute composition.

Observation of bacteriolysis. A killer urine that sterilized the inoculum of 10^8 gonococci/ml (F-62 T2) in 1 h was examined for bacteriolysis. Despite the striking fall in viable gonococci in urine as measured by colony-forming units, bacterial cell counts performed in a hemocytometer under dark-field illumination at 0, 1, 3, and 6 h were unchanged and identical to the Dulbecco medium control, in which the viable count remained unchanged. Urine killing, in contrast to serum killing (15), is not a bacteriolytic process.

Comparative survival of gonococci and *E. coli*. Kaye has shown that the usual urinary pathogens may be inhibited, but are seldom sterilized, by human urine (12). We compared

survival of F-62 T4 gonococci with two standard laboratory strains of *E. coli* originally isolated from urine. In Table 4, the acid urine inhibited *E. coli* but sterilized identical inocula of gonococci. In the same urine neutralized to pH 6.8, both *E. coli* strains increased by 1 log in 6 h, whereas gonococci decreased by 1 log. In an acid urine that kills gonococci, *E. coli* is merely inhibited. Neutral pH permits growth of *E. coli* in urine but inhibits the gonococcus.

Effect of colony type and strain on susceptibility to killing. The susceptibility to killing of T2 and T4 colony types (F-62 of Kellogg) and of two clinical isolates of *N. gonorrhoeae* (T2 colony type) was compared after 1 and 3 h in nine urines. We found no consistent difference in susceptibility of T4 and T2 colony types to killing (Table 5). Upon challenge with F-62 colonies, urine 2 killed more T4 than T2, but urine 3 killed more T2 than T4. Urine 1 killed fewer N-24 at 3 h than any other strain. There appears to be only occasional differences by strain and colony type in susceptibility to some urines. In contrast to serum (15), urine usually kills both virulent and avirulent colony types equally well.

TABLE 4. *Survival of F-62 T4 gonococci and two strains of E. coli in a urine at acid and neutral pH*

Organism	Log_{10} killed at:		
	3 h	6 h	24 h
pH 5.4			
F-62 T4	>6.5	>6.5	>6.5
E. coli O6 Riffle	0.4	0.5	1.5
E. coli O6 Williams	0.7	−0.3	1.6
pH 6.8			
F-62 T4	0.8	0.8	>6.5
E. coli O6 Riffle	−0.2	−1.1	−0.9
E. coli O6 Williams	−0.8	−1.0	−1.3

DISCUSSION

The major findings of this study are that human urine can kill gonococci rapidly and that the main determinant of killing is urinary pH. We have shown that urea and sodium chloride at physiological concentration in urine also contribute to killing of gonococci. Since each of these lethal factors is increased, concentrated urine can rapidly sterilize a large inoculum of gonococci. Human urines can be sharply divided into killer and nonkiller groups, because concentrated urine contains more of each bactericidal component.

In contrast to gonococci, the ordinary urinary pathogenic bacteria can grow well in most urines. Whereas large inocula of gonococci (10^8/ml) were killed or inhibited by all urines, small inocula of enteric bacteria usually grew well in urine. Kaye found that 10^2 to 10^3 broth-grown *E. coli* per ml increased 1 to 4 logs in 6 h in most urines and multiplied slightly more in water (12). He showed that urines at pH 6 or below could inhibit *E. coli* and that urine at pH 5.0 sterilized small (less than 10^2/ml), but not larger, inocula. Supplementation of urine with urea enhanced inhibitory properties, but ammonia and sodium chloride did not have much effect. Removal of urea removed inhibitory properties. We have found that the same factors (pH and urea) that merely inhibit *E. coli* are lethal for the gonococcus and that urine that supports growth of *E. coli* inhibits growth of the gonococcus.

The mechanism of antibacterial action by hydrogen ion, urea, and sodium chloride are not well understood. Microorganisms, like mammalian cells, must maintain stability of their internal milieu, because of constraints on the pH and osmolality at which vital enzyme systems function. For example, Neal et al. showed the effects of pH changes inside cells of bakers yeast (17). Phosphate buffer (2 M, pH 3) pro-

TABLE 5. *Urine killing of three strains of gonococci by nine human urines*

Urine	pH	Log_{10} killing at:							
		1 h				3 h			
		F-62 T2	F-62 T4	N-17 T2	N-24 T2	F-62 T2	F-62 T4	N-17 T2	N-24 T2
1	5.7	0.5	0	0.1	−0.5	>6.5	5.7	>6.4	2.0
2	5.2	2.7	>6.5	>6.1	>5.6	>6.2	>6.5	>6.4	>5.6
3	5.3	2.0	0.8	0.7	0.3	>6.2	>6.5	>6.4	>5.6
4	5.9	0	−0.3	0.1	−0.3	0.7	0.8	0.8	0.3
5	6.2	−0.2	−0.3	−0.1	−0.1	0	0	0	−0.2
6	5.8	−0.1	0.1	0	−0.2	0.2	0.1	0	−0.1
7	5.2	1.1	1.1			>6.7	>6.7		
8	5.7	0.2	0.2			2.5	2.5		
9	5.5	0.3	0.2			0.8	0.6		

duced no change in internal pH, no loss of activity of six glycolytic enzymes, and no decrease in rate of glycolysis. Acetate buffer of the same pH and molarity did acidify the interior of the yeast, resulting in inactivation of glycolytic enzymes and decreased glycolytic rate. In addition to these metabolic effects, low pH might kill microbial cells by producing structural changes in proteins other than enzymes.

Gonococci grow at pH 6 to 8 with optima around neutrality, depending on the medium used (5). Survival of gonococci as a function of hydrogen ion has not been systematically studied until now. Our results show pH is a critical determinant of survival in both urine and broth. Urine above pH 6.0 is inhibitory, but not lethal, for gonococci. Broth showed an almost linear relationship of pH and killing between pH 7.0 and 5.0. The site of toxicity of hydrogen ion for gonococci is unknown, but killing by acid PBS and broth indicates hydrogen ion alone is bactericidal.

Sodium chloride and other membrane-impermeable solutes produce an osmotic effect on bacteria. Because of their rigid cell walls many bacteria can survive low external osmotic pressures. When the cell wall is removed (e.g., by penicillin treatment), the spheroplast formed is susceptible to lysis in hypoosmolar solutions. The ability of salts and other solutes to prevent lysis of spheroplasts is evidence for an osmotic effect on the cell. The ability of gram-negative bacteria to withstand the effects of hyperosmolar solutions is dependent on maintenance of the intracellular osmolarity above that of the environment. Osmolality increases within bacterial cells largely through accumulation of intracellular potassium (8). For example, *E. coli* can elevate intracellular potassium fourfold when placed in a hyperosmolar environment. When this mechanism fails to compensate for a hyperosmolar environment, the cell dies from plasmolysis, an osmotic separation of the cell membrane from the rigid cell wall. Plasmolysis, without cell lysis, may be involved in hyperosmolar killing of gonococci.

Although urea accounts for about half of the osmotic pressure of urine, it is not clear that its osmotic effect accounts for bacterial killing. The inability of urea to protect *E. coli* spheroplasts from hyposmolar lysis suggests that urea does not act osmotically on bacterial cells. This property of urea may result from its diffusion across the plasma membrane. However, in the presence of low concentrations of sodium (0.100 M Na^+) inadequate to prevent hyperosmolar lysis, urea does prevent lysis of *E. coli* spheroplasts, suggesting either a partial permeability barrier to urea or a stabilizing effect of urea on the cell (15). An alternative mechanism for urea toxicity is change in physical and chemical properties of bacterial proteins. For example, the low concentrations found in urine (0.5 M) produce changes in diffusion rates and viscosity of serum albumin by apparent changes in tertiary structure (18). Similar effects on enzymes or structural proteins could account for killing of gonococci by urea.

Our finding that all human urines inhibit or kill gonococci could help explain two striking features of gonorrhea. First, many individuals exhibit natural resistance to infection. Second, gonococci almost never invade the urinary tract despite heavy urethral infection.

Natural resistance to gonorrhea is inferred from studies of the attack rates of exposed individuals. Holmes et al. studied the crew of an aircraft carrier exposed to a population of prostitutes with a known frequency of gonorrhea and calculated an attack rate of 22% (11). The rates of infection of women exposed to men with gonorrhea has been used to estimate attack rates in women. Two large studies have found rates of 59 and 60%, but this method of estimation has several sources of serious error (3, 21). The important point is that many exposed individuals resist infection.

Urine is given credit for flushing gonococci out of the urethra, but the value of postcoital urination for prophylaxis is not established (10). The ability of pili, found only on the virulent colony types, to promote adherence of gonococci to urethral epithelium may help gonococci resist mechanical removal (19). Our results suggest that urine may further reduce the number of urethral gonococci by its bactericidal actions. The urine adhering to the urethral mucosa could eliminate those adherent gonococci that survive urination.

The inability of gonococci to invade the urinary tract is surprising in view of its ability to produce posterior urethritis in men and to infect the short urethra of women (6). Occurrence of low-grade cystitis in gonorrhea has not been carefully investigated, but gonococcal pyelonephritis is virtually unknown. The ability to grow in urine is an important property of urinary pathogens infecting the bladder, since urination periodically removes large numbers of bacteria (9). There are several facts that suggest a relationship between urine composition, bacterial growth rates, and susceptibility to infection. The common urinary pathogens grow exuberantly in most urines, with doubling times of about 1 h (2, 12). *E. coli*, for example, grows well in urines over a wide range of pH, urea, and osmolarity and is inhibited only at

physiological extremes for these factors (e.g., pH 5.0). Asscher et al. have shown that the same order exists for rate of *E. coli* urinary infections, ability of urine to support growth of *E. coli*, and urinary pH in three groups of patients (pregnant women > other women > men) (2). High protein intake, which favors production of a concentrated acid urine, has been shown to protect dogs from experimental pyelonephritis (J. D. K. North and T. E. Miller, Abstr. 3rd Int. Congr. Nephrol., Washington, D.C., vol. 2, p. 248, 1966). Finally Kaye demonstrated that common urinary pathogens like *Enterobacter, Proteus,* and *E. coli* grow more rapidly in urine than infrequent urinary pathogens like *Staphylococcus aureus* and *Staphylococcus albus* (12). We have shown that gonococci are unable to grow in urine that supports growth of *E. coli*. We postulate that gonococci are unable to invade the urinary tract because they cannot grow in urine.

Our observations lead us to two conclusions. Non-immunological factors, especially pH, may be the major determinants of natural resistance to gonorrhea in the urethra and other sites of low pH such as the bladder, kidney, and vagina. Isolation of gonococci might be improved if urine sediments are neutralized at the time of culturing.

ACKNOWLEDGMENTS

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