INFECTION AND IMMUNITY, Jan. 1993, p. 8–12
0019-9567/93/010008-05$02.00/0

Vol. 61, No. 1

Effect of pH and Osmolality on In Vitro Phagocytosis and Killing by Neutrophils in Urine

R. A. GARGAN,* J. M. T. HAMILTON-MILLER, AND W. BRUMFITT

Department of Medical Microbiology, The Royal Free Hospital and School of Medicine, Pond Street, Hampstead, London NW3 2QG, United Kingdom

Received 3 February 1992/Accepted 6 October 1992

Phagocytosis and intracellular killing of two strains of *Escherichia coli* and a *Staphylococcus saprophyticus* by polymorphonuclear neutrophils (PMN) in pooled sterile urine at three osmolalities (800, 485, and 200 mosM/kg of H_2O) between pHs 5 and 8 was investigated. Urine at 800 mosM virtually abolished phagocytosis of both *E. coli* strains, regardless of pH, and reduced the phagocytosis of *S. saprophyticus* to 30%; no killing of any organisms took place at this osmolality. On the other hand, phagocytosis was as good in urine as in Hanks balanced salt solution at both 485 and 200 mosM between pHs 6 and 8. Phagocytosis of all three strains was virtually abolished at pH 5. Killing of the strains by PMN was optimal between pHs 6.5 and 7.5 in urine at 485 mosM (being at least 90% of the control values in Hanks balanced salt solution), whereas at 200 mosM killing was reduced to 50 to 70% of these values. Reduced killing of all three strains occurred at pH 8, whereas at pH 6 only *S. saprophyticus* was killed. Thus, the bactericidal activity of PMN in urine was more sensitive than phagocytic function to alterations in pH. The dominant modulating factor affecting PMN function in urine of 500 mosM or less was pH, but osmolality had a greater influence at 800 mosM. Thus, raising the pH of urine and reducing the osmolality may increase the ability of natural defense mechanisms to eliminate infecting organisms.

Phagocytosis and killing of microorganisms by polymorphonuclear neutrophils (PMN) are major defense mechanisms; although they have been studied in many respects, little is known about their function in urine even though pyuria is common in urinary infections.

Fukushi and Orikasa observed that in experimental infections PMN in the bladder mucosa showed evidence of having phagocytosed bacteria (11). This supports the observations of Cox and Hinman on the antibacterial activity of the bladder wall (7). Other workers have reported that PMN contribute to the defense of the bladder in experimental infections (15) and that PMN in the urine of patients with urinary tract infections contain phagocytosed bacteria (20); these reports do not establish whether the phagocytosis observed takes place in or at the surface of the bladder wall or in the urine.

A few reports show that phagocytosis does occur in urine in vitro, where one of the main modulating factors is osmolality (6, 24). pH has been also reported to affect phagocytosis in solutions other than urine (6), but, apart from the observation of Bryant et al. on phagocytosis at pH 5.6 (3), little experimental work with urine has been carried out. The urine pH can range from 4.6 to 8 (23); it is therefore desirable to obtain a more complete understanding of the effect of pH on phagocytosis.

To our knowledge, the effects of osmolality and pH of urine on the bactericidal activity of PMN, which is another important defense feature of these cells, have not been studied. Thus, we investigated the effect of a wide but physiological pH range (pH 5 to 8) on phagocytosis and killing by PMN in urine at two osmolalities at the extremes of the range (200 and 800 mosM) within which phagocytosis has been reported (6, 25). Comparison was made with a midrange osmolality (485 mosM).

* Corresponding author.

Phagocytosis and killing of bacteria by PMN were investigated because these functions may be pivotal to the clearance of bacteria from the bladder. The voiding during micturition of PMN that have ingested bacteria contributes to the rapid elimination of the organisms from infected urine. If PMN can also inactivate or kill bacteria intracellularly in the urine, they contribute a further means of eradicating the infection.

MATERIALS AND METHODS

Preparation of urine. Three urine pools (dilute, midrange, and concentrated) from healthy individuals were made as described below. All urines were without antibacterial activity and contained fewer than 10^3 bacteria per ml. Sediment and bacteria were removed by sequential filtration of the three pools with coarse and fine filters and finally with a 0.22-μm-pore-size filter (Flow Laboratories, London, England). The pH was measured with a Corning 120 pH meter, and osmolality was determined twice with a microosmometer (model 3MO; Advanced Instruments Inc.). Urine pools were divided into 20-ml volumes and stored at −36°C.

Dilute urine (190 mosM) was from three individuals (one male and two females) who had consumed an excess of water. Midrange urine (504 mosM) was from 16 healthy volunteers (8 males and 8 females). The concentrated pool (850 mosM) consisted of early-morning urines of four volunteers (two males and two females).

After the addition of 10% serum, which was used in all experiments, the final working osmolalities of the dilute, midrange, and concentrated urines became 200, 485, and 800 mosM, respectively. The urea concentrations in these three pooled urines were 72, 195, and 365 mM, respectively.

Volumes of 4 ml of pooled urine containing 10% serum at the required osmolality were adjusted under sterile conditions to pHs 5, 6, 6.5, 7, and 8 with NaOH or HCl. This caused a negligible change in osmolality.

Isolation and preparation of PMN. Samples of 4 ml of venous blood were taken from healthy donors and anticoagulated with potassium EDTA. After erythrocytes were lysed in isotonic ammonium chloride containing sodium bicarbonate, PMN were isolated by low-speed differential centrifugation in Hanks balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} (Gibco Ltd.). This method was previously described and compares favorably with other standard separation techniques in its preservation of the PMN functions (9). PMN viability, assessed by testing the integrity of the cell membrane with ethidium bromide and acridine orange (10), was greater than 95%. The purified PMN were centrifuged from HBSS at 160 × *g* for 5 min and resuspended in the same volume of pooled sterile urine containing 10% serum to give a concentration of approximately 4×10^6 cells per ml. The viability of the PMN remained unchanged (greater than 95%) after 1 h of exposure with rotation in each of the three urine pools (200, 485, and 800 mosM) with 10% serum at 37°C.

Bacteria and culture methods. Two strains of serum-resistant *Escherichia coli*, 398 (serotype O6) and 441 (serotype O18:K5), from urinary tract infections were supplied by A. Roberts, Department of Microbiology, West London Hospital. A strain of *Staphylococcus saprophyticus*, isolated at the Royal Free Hospital, London, from a urinary infection, was also used. This was identified by novobiocin resistance and ApiStaph (Biomerieux, La Balm Les Grottes, Montalieu-Vercieu, France) profile. These species were chosen because they are among the commonest organisms involved in urinary infections.

All bacteria were grown in 5 ml of brain heart infusion broth (CM225; Oxoid Ltd., Basingstoke, United Kingdom) statically for 18 h at 37°C. Bacteria were washed once in HBSS, resuspended in 1 ml of HBSS, and kept at 4°C until used the same day.

Opsonization. A 5-μl sample of an *E. coli* suspension was added to 995 μl of pooled urine containing 10% serum (from the same donor as the PMN), giving approximately 2×10^7 organisms per ml. Then 200 μl of this suspension was added to the same volume of a PMN suspension in urine, giving a final bacterium/PMN ratio of approximately 5:1. A 15-μl *S. saprophyticus* suspension was added to 985 μl of urine containing 10% serum to give approximately 6×10^7 organisms per ml. This gave a bacterium/PMN ratio of about 15:1 when mixed as described above. Controls for all three organisms in urine (pH 7) without serum were prepared by the same procedure, as were HBSS controls with serum, to assess maximum phagocytosis.

The organisms were not preopsonized, and were only briefly exposed to urine before they were added to the PMN. This is because we have found that stationary-phase *E. coli* exposed before phagocytosis to a nutrient source such as serum or urine can develop resistance within 20 min to subsequent intracellular killing (14).

Phagocytosis and killing. Two different methods (described below) to determine phagocytosis were used in conjunction to avoid the problem of distinguishing organisms that were attached to the cell surface (membrane associated but not ingested) from organisms that had been phagocytosed. The use of crystal violet to quench the fluorescence of bacteria not ingested by the PMN overcomes this problem. Crystal violet does not penetrate the living PMN, allowing the differentiation of ingested (fluorescent) bacteria from extracellular cell-associated (nonfluorescent) organisms.

Stained Cytospin preparation. PMN were cytocentrifuged and prepared as described by Horwitz and Silverstein (17). After incubation for 1 h, 100-μl samples of the mixtures of PMN and bacteria were cytocentrifuged onto glass slides with a Cytospin 2 centrifuge (Shandon Southern Products Ltd., Cheshire, United Kingdom), fixed, and stained with Prodiff 1 and 2 (Braidwood Labs). Then 100 PMN were examined microscopically to determine the percentage of PMN that had phagocytosed bacteria and the mean number of bacteria phagocytosed by each PMN.

Fluorescent extracellular quenching. The fluorescent extracellular quenching procedure used was based on the method of Goldner et al. (16). A 10-μl sample of acridine orange (0.005 mg/ml in HBSS) was added to 25 μl of the mixture of PMN and bacteria at the end of the incubation period (1 h). This caused intra- and extracellular bacteria and the PMN nuclei to fluoresce under UV light. After 45 s, 10 μl of crystal violet (0.5 mg/ml in HBSS) was added, and a coverslip was applied. The number of PMN that had phagocytosed bacteria and the number of organisms ingested per PMN were estimated as described above. With this method, phagocytosis could be assessed rapidly.

The cytocentrifuged preparation was used in the final determination of the percent phagocytosis of PMN and the number of ingested organisms per PMN. There was little difficulty in distinguishing organisms that had been ingested from those that had not. Cytocentrifuged slides of suspensions of PMN and bacteria in which phagocytosis had not taken place (for example, in urine of high osmolality) showed no bacteria within the boundaries of the PMN. These large clear areas of PMN were in stark contrast to the distribution of bacteria over the rest of the surface of the slide. Thus, bacteria were not centrifuged into the PMN or onto the cell surface but were dispersed to the periphery of the cell during cytocentrifugation. This is an extremely useful artifact of preparation with the Cytospin. When phagocytosis had occurred, the majority of bacteria were clearly within the boundary of the PMN membrane and there were usually organisms within vacuoles. The results obtained by this technique were compared with those obtained with extracellular quenching.

Bacterial killing. Immediately after the organisms were added to the PMN, 20 μl of the reaction mixture was removed and added to 4.98 ml of distilled water (Travenol) at pH 11 for 5 min at 37°C. This completely lyses PMN without harming the microorganisms (13). The sample was vigorously shaken, and viable counts were determined in duplicate with MacConkey agar (CM7b; Oxoid) for *E. coli* and blood agar for *S. saprophyticus*. Distilled water (pH 5 to 6) was used as the diluent. The reaction mixture was rotated at 120 rpm in a water bath for 1 h at 37°C, and viable counts were determined as described above. Bacterial killing is expressed as the percent reduction of the initial viable count. All experiments were carried out in triplicate. Control experiments in HBSS were carried out to give optimal conditions, and without PMN in urine to eliminate the possibility that killing was due to serum, urine, or water at pH 11.

Where pH and/or osmolality had a detrimental effect on the morphology of the PMN after 1 h, for example, at pH 5 in hyperosmolar urine, preparations for phagocytosis were made after 15 min with a separate reaction mixture.

RESULTS

Verification of methods used to determine phagocytosis. The results of phagocytosis determined with stained Cytospin preparations agreed well with those determined with extracellular quenching. A minimum of three experiments were carried out with each method in urine samples from pH 5 to

pH 8 for each of the three bacterial strains and analyzed statistically with Student's t test. There was no difference observed ($P \geq 0.16$) between the two methods for *E. coli* 441. The phagocytosis of *E. coli* 398 and *S. saprophyticus* also showed no difference between the two methods ($P \geq 0.07$), except in two instances. These were at pH 8 (484 mosM, $P = 0.04$) for *E. coli*, where the means ± standard deviations were 96% ± 3% (Cytospin) and 87% ± 5% (extracellular quenching), and at pH 6 (200 mosM, $P = 0.03$) for the staphylococcus, where the means ± standard deviations were 93% ± 7% and 82% ± 8%, respectively. Thus, from a total of 30 paired sets of experiments in which the Cytospin method was compared with extracellular quenching, 28 showed no significant difference between the two techniques.

Effect of pH on phagocytosis at 485 and 200 mosM. Good phagocytosis of both strains of *E. coli* and *S. saprophyticus* occurred over a wide pH range (6 to 8) in urine at 485 and 200 mosM. There was no significant difference between phagocytosis in urine and that in HBSS ($P > 0.05$, Student's t test) (Fig. 1 and 2). The mean numbers of bacteria ingested per PMN between pHs 6 and 8 were 5.9 for *E. coli* (both strains) and 14.1 for *S. saprophyticus*. At pH 5, virtually no bacteria were phagocytosed (Fig. 1). At this pH in urine, PMN were reduced in size and pyknotic at 485 mosM but appeared normal at 200 mosM.

Effect of pH on intracellular killing by PMN at 485 and 200 mosM. Killing by PMN in urine of all three strains was optimal at 485 mosM at either pH 6.5 or 7 (Fig. 1), reaching at least 90% of control values in HBSS ($P > 0.05$, with the exception of strain 441 at pH 6.5). Killing by PMN in dilute urine (200 mosM) at pHs 6.5 and 7 was significantly reduced to between 50 and 70% of the control values ($P < 0.05$, except for strain 398 at pH 7).

At pH 8, killing was significantly less at both osmolalities, ranging from 27 to 57% of that of the control ($P < 0.05$, except for strain 398 at 485 mosM). At pH 6, PMN could not kill either strain of *E. coli* at either osmolality (despite good phagocytosis). *S. saprophyticus*, however, was killed by PMN in urine at pH 6 at 485 mosM (88% of the control value, mean of six experiments, $P > 0.05$) (Fig. 1c) but not at 200 mosM. No killing of any of the organisms took place at pH 5.

These results are summarized in Fig. 2, which shows that optimum intracellular killing in the majority of experiments lay between pHs 6.5 and 7.5.

Controls without PMN showed no killing of any of the bacterial strains in urine throughout the pH range at 200 and 485 mosM.

Phagocytosis and killing at 800 mosM. Phagocytosis of both *E. coli* strains was virtually eliminated at 800 mosM throughout the pH range from 5 to 8 (Fig. 1). Phagocytosis of *S. saprophyticus*, maximal at pHs 6.5 and 7, was only 30% of that occurring in HBSS. In urine of this osmolality, the PMN appeared shrunken and pyknotic at pH 5 and no killing occurred (Fig. 1).

Controls without PMN showed no killing of any strain in urine at this osmolality at any pH.

Phagocytosis in urine without serum. Phagocytosis of both strains of *E. coli* was abolished, and that of *S. saprophyticus* was reduced to a mean of 13% (SD, ±11%; $n = 4$), when serum was omitted from urine samples at 485 and 200 mosM (pH 7). No phagocytosis of any of the three organisms in urine of 800 mosM without serum took place. This would be expected, because phagocytosis was minimal at this osmolality even with serum.

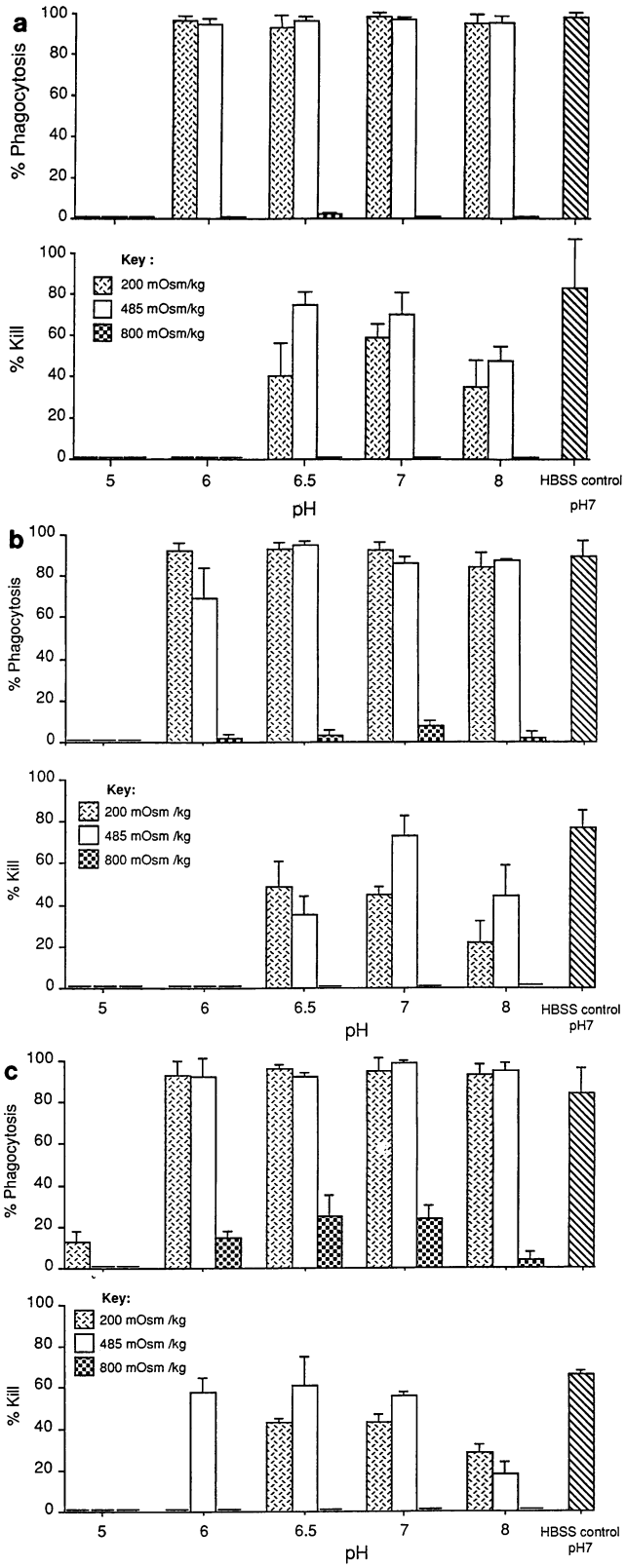

FIG. 1. Effect of pH on phagocytosis and killing by PMN of *E. coli* 398 (a), *E. coli* 441 (b), and *S. saprophyticus* (c) in urine at three osmolalities compared with controls in HBSS. Results are the means ± standard deviations of a minimum of three different experiments.

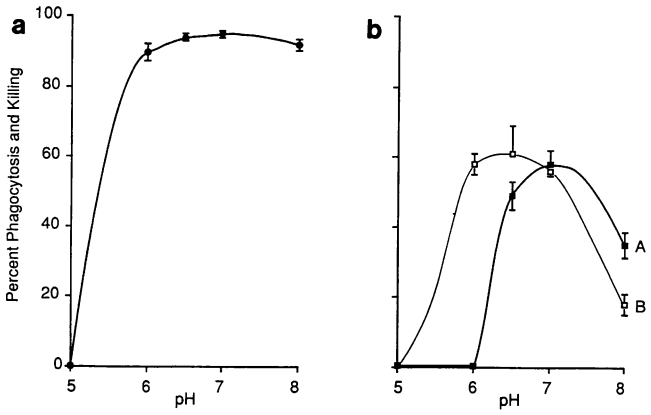

FIG. 2. (a) Phagocytosis by PMN of two *E. coli* strains and *S. saprophyticus* at 200 and 485 mosM between pHs 5 and 8 (at least 20 experiments for each pH value). (b) Intracellular killing by PMN in urine of all three strains at 200 mosM and both *E. coli* strains at 485 mosM ($n \geq 15$) (A) and *S. saprophyticus* at 485 mosM ($n = 6$) (B). Bars indicate the standard errors of the means.

Bacterial growth in urine controls. Bacterial counts after 1 h in the absence of PMN were greater than those of the starting population except at pH 5, where the counts remained static.

DISCUSSION

Phagocytosis and killing of bacteria by PMN are efficient in urine under appropriate conditions of pH and osmolality. Under these circumstances, PMN could play a major role in the elimination of infecting organisms from bladder urine.

The effect of pH on phagocytosis and killing by PMN was apparent in urine of 485 and 200 mosM. Phagocytosis occurred across a wide pH range; it was lost or severely inhibited only at pH 5. This phenomenon has not been properly addressed before, and reports are scanty and misleading. For example, loss of phagocytosis of opsonized *E. coli* by PMN in buffer of pH 5.5 was reported by Chernew and Braude (6). On the other hand, Bryant et al. demonstrated good phagocytosis of opsonized *Staphylococcus aureus* in urine at pH 5.6 (3). This can be explained by our findings that the cutoff point where phagocytosis of *S. saprophyticus* and *E. coli* ceases lies between pHs 5 and 6.

Killing by PMN was optimal only within a narrow pH range, 6.5 to 7.5, at 485 and 200 mosM. PMN were able to kill *E. coli* at pH 8 and *S. saprophyticus* at pHs 6 and 8, but in the majority of cases the killing was less effective. Thus, killing by PMN was more susceptible to pH change than was phagocytosis.

pH was the dominant factor affecting PMN function in urine of 485 mosM or less, but a high osmolality (800 mosM) was a greater suppressor of PMN function than was pH, almost completely preventing phagocytosis and abolishing killing at all pHs. The osmotic contribution of the urea (365 mM/liter [365 mosM]) in our hyperosmolar urine (800 mosM) could have been sufficient to interfere with PMN function. Matsumoto et al. reported suppression of phagocytosis and killing in hyperosmolar HBSS at 690 mosM, of which the osmotic contribution of urea was 400 mosM (21).

Our observations of effective phagocytosis in urine at both 200 and 485 mosM agree with the results of others (20, 24). Suzuki and colleagues showed that there was only a one-third loss in phagocytic activity when PMN exposed to urine with an osmolality of less than 200 or greater than 550 mosM were removed from the urine for phagocytosis studies (25).

We found no reports on the effect of pH and osmolality on the bactericidal activity of PMN in urine. The present results show that although urine with a high osmolality (800 mosM) abolishes killing by PMN, at lower osmolalities (200 to 485 mosM) killing does occur and is pH dependent. Dooley and Takahashi noted that PMN in HBSS could withstand exposure to osmolalities of 150 to 700 mosM with only a 20 to 30% loss of killing ability at the extremes of this range (8). However, their experimental conditions were very different from ours. PMN were osmotically stressed in hypo- or hyperosmolar HBSS before phagocytosis; the PMN were then removed to isoosmotic HBSS, where opsonized *E. coli* cells were added and bacterial killing was recorded.

The observations of Matsumoto et al. may explain in part the mechanisms whereby osmolality can interfere with PMN function (22). These workers showed that in HBSS raised to 515 and 715 mosM with NaCl, inhibition of phagocytosis occurred and killing was abolished. Superoxide production, an integral part of oxygen-dependent killing, was greatly reduced and absent, respectively at these two osmolalities. This correlated with a decrease in PMN intracellular ATP, suggesting that the Na^+-K^+ pump depletes cellular ATP, resulting in energy loss. Hyperosmolar urea caused a similar inhibitory effect on PMN function by an unexplained mechanism that was not ATP related (21). The inhibitory action of NaCl on phagocytosis has been shown to be reversible, whereas that of urea is permanent (19).

The way in which pH inhibits intracellular killing by PMN could be explained by the observation of Gabig and colleagues (12), that respiratory burst activity is much lower when PMN are exposed to acidic conditions. This is a result of a decline in superoxide anion production, which at pH 6.0 is 10 to 20% of that at pH 7.5. Phagocytosis was unaffected by the reduction in pH in their experiments. This is in agreement with our observations of the lack of killing by PMN in urine at pH 6.0 despite good phagocytosis.

All experiments in this study were carried out in 10% serum in urine to provide bacterial opsonins. This is not an entirely artificial situation, since intrinsic urinary opsonins are present in urine after experimental bladder infection (15) and in infected urine from patients (24). Immunoglobulins, particularly immunoglobulin G, a known bacterial opsonin for which PMN have Fc receptors, can be detected at a higher level in infected urine than in noninfected urine (1, 4, 26). This suggests that bacterial opsonins are present. Bryant and coworkers found that phagocytosis of *S. aureus* did not occur in normal urine without serum opsonins (3). However, urine in their experiments was from a single uninfected source and may well have been devoid of opsonins. Suzuki et al. reported that the level of opsonins for *E. coli* in noninfected urine was lower than that in infected urines (24). We found that phagocytosis was poor or nonexistent in pooled urine from a large number of healthy individuals, without serum opsonins added.

Urinary pathogens usually grow very well in urine; however, their multiplication has been shown to be affected by pH and extremes in osmolality with marked differences in strain susceptibility (2, 18). The growth of *E. coli* stops below pH 5.5 and is reduced at pH 7.5 or above (2). Urine in our study was not bactericidal and was bacteriostatic only at pH 5 for the strains tested. Although an acidic environment can slow or stop bacterial growth, it unfortunately concurrently stops PMN activity as well. On the other hand, phagocytosis and killing by PMN occur at pH 7.5. If such

activity is accompanied by a slowing of the growth of the bacteria, this could further hasten the eradication of the organism.

In conclusion, it is apparent that phagocytosis is extremely efficient in urine. Killing by PMN also occurs, but only in a narrower pH range. A high osmolality, a low pH, or a combination of both of these factors is highly detrimental to bacterial killing by PMN in urine and, to a lesser extent, to phagocytosis. Alkalinization of the urine is used in clinical practice, as is the lowering of osmolality by increased fluid intake (5). Hence, by altering a patient's urine pH and osmolality it could be possible to obtain optimal function of PMN in vivo. This may be highly relevant to treatment of urinary infections that are confined to the lower urinary tract.

REFERENCES

1. **Akerlund, A. S., S. Ahlstedt, L. A. Hanson, and U. Jodal.** 1979. Antibody responses in urine and serum against *Escherichia coli* O antigen in childhood urinary tract infection. Acta Pathol. Microbiol. Scand. Sect. C **87**:29–36.
2. **Asscher, A. W., M. Sussman, W. E. Waters, R. Harvard Davis, and S. Chick.** 1966. Urine as a medium for bacterial growth. Lancet **ii**:1037–1041.
3. **Bryant, R. E., M. C. Sutcliffe, and Z. A. McGee.** 1973. Human polymorphonuclear leukocyte function in urine. Yale J. Biol. Med. **46**:113–124.
4. **Burdon, D. W.** 1970. Quantitative studies of urinary immunoglobulins in hospital patients, including patients with urinary tract infection. Clin. Exp. Immunol. **6**:189–196.
5. **Cattell, W. R., I. Kelsey Fry, F. I. Spiro, J. M. Sardeson, M. B. Sutcliffe, and F. O'Grady.** 1970. Effect of diuresis and frequent micturition on the bacterial content of infected urine: a measure of competence of intrinsic hydrokinetic clearance mechanisms. Br. J. Urol. **42**:290–295.
6. **Chernew, I., and A. I. Braude.** 1962. Depression of phagocytosis by solutes in concentrations found in the kidney and urine. J. Clin. Invest. **41**:1945–1953.
7. **Cox, C. E., and F. Hinman.** 1961. Experiments with induced bacteriuria, vesical emptying and bacterial growth on the mechanism of bladder defense to infection. J. Urol. **86**:739–748.
8. **Dooley, D. C., and T. Takahashi.** 1981. The effect of osmotic stress on the function of the human granulocyte. Exp. Hematol. **9**:731–741.
9. **Eggleton, P., R. Gargan, and D. Fisher.** 1989. Rapid method for the isolation of neutrophils in high yield without the use of dextran or density gradient polymers. J. Immunol. Methods **121**:105–113.
10. **Ford, W. L.** 1978. The preparation and labelling of lymphocytes, p. 23.1–23.22. *In* D. W. Weir (ed.) Handbook of experimental immunology, vol. 2. Blackwell Scientific Publications, Oxford.
11. **Fukushi, Y., and S. Orikasa.** 1981. The role of intravesical polymorphonuclear leukocytes in experimental cystitis. Invest. Urol. **18**:471–474.
12. **Gabig, T. G., S. I. Bearman, and B. M. Babior.** 1979. Effects of oxygen tension and pH on the respiratory burst of human neutrophils. Blood **53**:1133–1139.
13. **Gargan, R. A., W. Brumfitt, and J. M. T. Hamilton-Miller.** 1989. Failure of water to lyse polymorphonuclear neutrophils completely: role of pH and implications for assessment of bacterial killing. J. Immunol. Methods **124**:289–291.
14. **Gargan, R. A., W. Brumfitt, and J. M. T. Hamilton-Miller.** 1991. Pre-opsonisation of *Escherichia coli* induces resistance to neutrophil killing in serum and urine: relationship to growth phase. J. Med. Microbiol. **35**:12–17.
15. **Gillon, G., M. Small, O. Medalia, and M. Aronson.** 1984. Sequential study of bacterial clearance in experimental cystitis. J. Med. Microbiol. **18**:319–326.
16. **Goldner, M., H. Farkas-Himsley, A. Kormendy, and M. Skinner.** 1983. Bacterial phagocytosis monitored by fluorescence and extracellular quenching: ingestion and intracellular killing. Lab. Med. **14**:291–294.
17. **Horwitz, M. A., and S. C. Silverstein.** 1980. Influence of *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. J. Clin. Invest. **65**:82–94.
18. **Kaye, D.** 1968. Antibacterial activity of human urine. J. Clin. Invest. **47**:2374–2390.
19. **Lancaster, M. G., and F. Allison.** 1966. Studies on the pathogenesis of acute inflammation. VII. The influence of osmolality upon the phagocytic and clumping activity by human leukocytes. Am. J. Pathol. **49**:1185–1200.
20. **Maeda, S., T. Deguchi, Y. Kanimoto, M. Kuriyama, Y. Kawada, and T. Nishiura.** 1983. Studies on the phagocytic function of urinary leukocytes. J. Urol. **129**:427–429.
21. **Matsumoto, T., J. Kumazawa, and P. Van der Auwera.** 1989. Suppression of leukocyte function and intracellular content of ATP in hyperosmotic condition comparable to the renal medulla. J. Urol. **142**:399–402.
22. **Matsumoto, T., P. Van der Auwera, Y. Watanabe, M. Tanaka, N. Ogata, S. Naito, and J. Kumazawa.** 1991. Neutrophil function in hyperosmotic NaCl is preserved by phosphoenol pyruvate. Urol. Res. **19**:223–227.
23. **Smith, E. L., R. L. Hill, I. R. Lehman, R. J. Lefkowitz, P. H. Handler, and A. White.** 1983. Nature and composition of urine, p. 199–202. *In* Principles of biochemistry: mammalian biochemistry. McGraw-Hill International Book Co., Auckland, New Zealand.
24. **Suzuki, Y., Y. Fukushi, S. Orikasa, and K. Kumagai.** 1982. Opsonic effect of normal and infected human urine on phagocytosis of *Escherichia coli* and yeasts by neutrophils. J. Urol. **127**:356–360.
25. **Suzuki, Y., S. Toyota, K. Magima, Y. Fukushi, and S. Orikasa.** 1986. Studies of anti-bacterial defense mechanism of urinary bladder. Jpn. J. Urol. **77**:574–580.
26. **Thomas, V. L., M. Forland, and A. Shelokov.** 1975. Immunoglobulin levels and antibody-coated bacteria in urines from patients with urinary tract infections. Proc. Soc. Exp. Biol. Med. **148**:1198–1201.