Human Polymorphonuclear Leukocyte Function in Urine

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Pyuria is usually present in patients with urinary tract infections. Therefore, it is surprising that the host defense function of polymorphonuclear leukocytes (PMNs) in urine is not well understood. Studies by Chernew and Braude demonstrated that urine suppressed PMN phagocytic function in an environment containing 7–8% serum (1). Similarly, studies by Norden, Green, and Kass suggested that PMNs did not contribute significantly to eradication of bacteria in the experimentally-infected rat bladder (2). Conversely, observations by Kaye and Cobb indicated that PMNs might play an active role in defense against bladder infection (3).

In the present studies, leukocyte migration, adhesion to glass, aggregation by endotoxin, and phagocytosis of bacteria were tested in urine in order to evaluate the host defense function of PMNs in urine. In addition, experiments were performed to differentiate changes in leukocyte behavior attributable to cell injury in urine from changes caused by the absence of plasma proteins. In the latter experiments, leukocyte function was tested in plasma after incubation of leukocytes in urine. The time required for cell injury in urine was assessed. Factors in urine which were tested individually for their toxicity to leukocytes included hyperosmolality, increased hydrogen ion concentration, and increased urea concentration. In addition, studies were performed to determine the effect of opsonins on leukocyte phagocytic function in urine.

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METHODS AND MATERIALS

Blood from normal human donors was heparinized to a final concentration of 3.3 mg/100 ml. Leukocyte-rich plasma (LRP) was prepared as follows. Tubes containing 15 ml of heparinized whole blood were centrifuged for 10 min at 500g. Two-thirds of the red cell layer was removed, remaining cells and plasma were mixed and sedimented at 37°C for about 1 hr. The leukocyte-rich plasma supernatant (LRP) obtained by this method contained approximately 60% polymorphonuclear (PMN) leukocytes by differential count.

Serum was prepared by centrifugation of blood collected in plain vacutainer tubes and allowed to clot. In certain studies, serum was heparinized (3.3 mg heparin/100 ml serum). Fresh, autologous plasma or serum was used in experiments testing leukocyte function.

Urine was supplied by one normal male donor. Specific gravity of specimens was measured at 25°C with a urinometer, and osmolality (mosm/kg H2O) with an osmometer. Specimens were selected for experimentation on the basis of desired specific gravity and osmolality. Urine was sterilized by filtering through a 0.45 μm Millipore field monitor and stored in sterile glass containers at 4°C. Prior to use, specimens were warmed to 37°C in a water bath. If sediment formed at 4°C did not dissolve, urine was re-filtered. In certain studies, the pH of urine specimens was varied by addition of HCl or NaOH. After experiments had been concluded, specimens were centrifuged and supernatant fluids examined to determine osmolality, pH, and ionic strength of urine or other solutions incubated with leukocytes.

Buffered electrolyte solution (BES) used in these studies contained 125 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl2, 11 mM glucose, and 23.8 mM Trizma Base dissolved in distilled water and adjusted to a pH of 7.5 with 6 N HCl. This solution was sterilized by filtration through a 0.45 μm Millipore field monitor.

Leukocyte functions tested in these studies were migration in 32-mm glass capillary tubes, adhesion to 32-mm glass capillary tubes, aggregation after exposure to E. coli endotoxin, and phagocytosis of Staphylococcus aureus. All experiments were performed using a double blind technique.

1. Leukocyte Function in Normal Urine

Migration. Tubes containing 2 ml of LRP were centrifuged at 250g for 3 min. Each cell pellet was suspended in 2 ml of urine (osmolality shown in Table I). Two ml of fresh plasma were added to cells in the control tube. These test mixtures were used to fill 32-mm glass capillary tubes (10 tubes per variable). One end of each capillary tube was flame-sealed. Tubes were centrifuged for 2 min in a Drummond microhematocrit centrifuge and then taped to a large glass microscope slide. The slide was placed on a vertically positioned microscope stage in a 37°C incubator. Measurement of vertical progression of leukocytes from the buffy coat was made with an ocular micrometer after 4 hr of incubation (4). Migration in urine was expressed as percent of control migration in fresh plasma.

Adhesion. Test mixtures prepared like those used for migration studies were loaded into 32-mm glass capillary tubes (10 tubes per variable), and one end was flame-sealed. Tubes were incubated in a horizontal position for 30 min at 37°C, then centrifuged for 1 min in a Drummond microhematocrit centrifuge. Tubes were next mounted with the cell-adherent surface facing upward on a large glass micro-
scope slide. The slide was placed on a vertically positioned microscope stage in a
37°C incubator. Enumeration of cells adhering to a 0.1 × 5.0 mm area in the
midportion of the tubes was performed with an ocular micrometer (5). Adhesion
in urine was expressed as percent of control adhesion in fresh plasma.

Aggregation. LRP was centrifuged in 10-ml aliquots at 130g for 10 min, and
the sedimented cells were suspended in fresh plasma to a final concentration of
approximately 10⁷ leukocytes/ml. Test mixtures were prepared as follows. Tubes
containing 1.8 ml of LRP were centrifuged at 130g for 5 min. Plasma was re-
moved, and each cell pellet was suspended in 1.8 ml of urine (osmolality as indi-
cated in Table 1). Tubes were centrifuged again, urine removed, and cells were
suspended in 1.8 ml of urine. The cell pellet for the control specimen was first
suspended in 1.8 ml of urine (450 mosm/kg, pH 7.5) and then in 1.8 ml of
heparinized serum. All test mixtures were prepared in duplicate and rotated for
30 min at 37°C. One specimen of each pair received 200 μg of E. coli endotoxin
in saline, and the other received an equal volume of isotonic saline (controls).
After rotation on a rotary wheel for 20 min at 37°C, a small sample of test mixture
from each tube was transferred to a clean sterile tube. An equal volume of
heparinized serum was mixed with each sample. Cover slip smears were made im-
mediately after the addition of serum to each sample. Smears were stained with
Wright's stain. The percent of 500 PMNs present in aggregates was determined
microscopically. An aggregate was defined as three or more adjoining leukocytes.

Phagocytosis. The organism used was Staphylococcus aureus, strain 209P. Cul-
tures grown in trypticase soy broth for 18 hr usually yielded 10⁸ bacteria/ml. Test
mixtures were prepared as follows. Tubes containing 2.5 ml of LRP were cen-
trifuged at 130g for 5 min. Each cell pellet was suspended in 2.5 ml of BES. Spec-
mens were centrifuged again, and each cell pellet was suspended in 2.5 ml of urine
(osmolality as shown in Table 1) or plasma (control). The last two steps were
repeated once before addition of bacteria to each specimen in an approximate ratio
of 10 bacteria/PMN. After rotation for 20 min at 37°C tubes were centrifuged

| TABLE 1 |
|------------------|------------------|------------------|------------------|------------------|
| **Leukocyte Function in Normal Urine** |
| Osmolality (mosm/kg H₂O) | Migration (% of control)± | Adhesion index (% of control)± | Aggregation (% PMNs in aggregates)± | Phagocytosis (% of control)± |
| 100–200 | 0 | 1 ± 0.4 | — | 0.5 |
| 300–400 | 0 | 0.7 ± 0.3 | 2.8 ± 0.9 | 0 |
| 400–600 | 0 | — | 0 | 0 |
| 600–800 | 0 | 4 ± 1.3 | — | 0 |
| 800–1100 | 0 | 1 ± 0.3 | 0.3 ± 0.3 | 0 |
| 280 (plasma or serum control) | 100± | 100± | 83.9 ± 1.5 | 100± |

± Means of 3 experiments.
| Means of 2 experiments ± SEM. |
| Mean migration = 1.25 mm. |
| Mean control adhesion index = 538 cells/mm². |
| Control phagocytic index = 1119 bacteria/100 PMNs. |
at 130g for 5 min, and each cell pellet was suspended in 1.8 ml of BES. Four hundred eighty units of lysostaphin, an enzyme which lyse extracellular staphylococci but which does not affect intracellular staphylococci, were mixed with the contents of each tube (6). Test mixtures were incubated for 35 min at 37° C, centrifuged at 130g for 5 min, and each cell pellet was suspended in fresh plasma. Cover slip smears were made and stained with Wright’s stain. Phagocytosis was quantitated microscopically at 1250 X by determining the number of bacteria in 100 PMNs. This number was designated as the phagocytic index. Phagocytic indices were expressed as percent of control.

II. Leukocyte Function in Plasma After Incubation in Urine

Migration and adhesion. Duplicate test mixtures were prepared for each migration and adhesion experiment using methods described above. One set of urine-leukocyte mixtures was rotated for 15 min at 37° C and the other set for 60 min at 37° C. After rotation, tubes were centrifuged for 3 min at 250g, and each cell pellet was suspended in 2 ml of fresh plasma. Capillary tubes were filled for quantitation of migration and adhesion using methods described above. Results were expressed as percent of control.

Phagocytosis. Tubes containing 1.9 ml of LRP were centrifuged at 130g for 5 min, and each cell pellet was suspended in 2 ml of BES. Tubes were centrifuged again, and each cell pellet was suspended in 2 ml of the appropriate incubating solution indicated in Fig. 3 (i.e., urine, BES, or plasma). Centrifugation and suspension were repeated. Test mixtures were prepared in duplicate. One set was incubated for 15 min at 37° C and the second set for 60 min at 37° C. After the incubation period, tubes were centrifuged at 130g for 5 min, and each cell pellet was suspended in fresh plasma. Bacteria were added in a radio of approximately five bacteria per PMN. Specimens were rotated for 30 min at 37° C prior to addition of lysostaphin in a final concentration of 240 U/ml. Following an incubation period of 35 min at 37° C, smears were made and phagocytic indices determined. Phagocytosis was expressed as percent of control.

III. Factors Affecting Cell Injury in Urine

Urine osmolality. Tubes containing 2.5 ml of LRP were centrifuged at 130g for 5 min. Each cell pellet was suspended in 2.5 ml of urine, BES, or of plasma (osmolality and pH as indicated in Table 2). Specimens were rotated for 1 hr at 37° C, centrifuged at 250g for 2 min, and each cell pellet was then suspended in 2.5 ml of fresh plasma. Capillary tubes were filled with test mixtures and migration determined. Results were expressed as percent of control.

Urine pH. Tubes containing 4 ml of LRP were centrifuged at 130g for 5 min. Each cell pellet was suspended in 20 ml of BES at pH 5.5. After centrifugation at 250g for 10 min, each cell pellet was suspended in 2.5 ml of either urine or BES (pH 5.5 or pH 7.5). Cells in the control specimen were suspended in 2.5 ml of fresh plasma. Test mixtures were incubated for 1 hr at 37° C, centrifuged at 130g for 5 min, and each cell pellet was suspended in 2.5 ml of fresh plasma. Migration was determined and expressed as percent of control.

Concentrations of urea or NaCl in BES. Tubes containing 2 ml of LRP were centrifuged at 130g for 5 min, each cell pellet was suspended in 2 ml of BES,
and tubes were centrifuged again. The last two steps were repeated once. Each cell pellet was then suspended in 2 ml of BES containing 170, or 340, or 690 mM urea, or 210, or 295, or 465 mM NaCl (final concentrations). In control specimens, leukocytes were suspended in 2 ml of BES without additive, or in 2 ml of fresh plasma. The sample tubes were rotated at 37°C for 15 min and centrifuged at 130g for 5 min. Each cell pellet was then suspended in 2 ml of fresh plasma and migration determined. Results were expressed as percent of control (migration of cells incubated in BES without additive).

### IV. Phagocytosis of Opsonized and Nonopsonized Bacteria in Urine

Bacteria were opsonized by rotating 2 ml of an 18-hr culture of *S. aureus* (strain 209P) with 4 ml of normal human serum for 30 min at 37°C. After centrifugation at 3000g for 20 min at 4°C, bacteria were suspended in 10 ml of fresh trypsin-soy broth and centrifuged again. This step was repeated twice. Sedimented bacteria were then suspended in 2 ml of fresh trypsin-soy broth. Nonopsonized bacteria were rotated in trypsin-soy broth instead of serum but were otherwise treated the same as opsonized bacteria. Cultures were sonicated for 10 sec before use. Specimens from both opsonized and nonopsonized procedures contained about 10⁷ bacteria/ml when diluted and quantitated by trypsin-soy agar pour plates. Leukocyte mixtures were prepared as follows. Ten milliliters of BES were mixed with each 2-ml sample of LRP. Specimens were centrifuged at 500g for 10 min, each cell pellet was suspended in 20 ml of BES, and specimens were centrifuged again. Each cell pellet was then suspended in 1.8 ml of urine (477 mosm/kg) or plasma. Duplicate specimens were prepared for each urine or plasma mixture. After incubation for 30 min at 25°C, a leukocyte-urine and a leukocyte-plasma mixture were inoculated with 0.2 ml of a 10⁻² dilution of either opsonized or nonopsonized bacteria. (Bacteria/PMN ratios were approximately 5:1). These mixtures were rotated for 20 min at 37°C, transferred by sterile pipet to larger tubes, and then mixed with 18 ml of fresh BES. Tubes were centrifuged at 500g for 10 min. Each cell pellet was suspended in 1.8 ml of BES to which 480 U of lysostaphin were added. Following incubation for 35 min at 37°C, specimens were centrifuged at
130g for 5 min. Each cell pellet was suspended in 1.8 ml of fresh plasma, and smears were made. Phagocytic indices were determined as previously noted.

**REAGENTS AND EQUIPMENT**

Preservative-free heparin (Connaught, Toronto, Canada) and 0127:B8 *E. coli* endotoxin (Difco Laboratories, Detroit, MI) were prepared in sterile isotonic saline. Heparin was sterilized by filtration through a 0.45 µm Millipore filter (Millipore Corp., Bedford, MA). Lysostaphin (Schwarz-Mann, Orangeburg, NY) was dissolved in a mixture of 0.145 M NaCl and 0.05 M Trizma Base (Sigma Chemical Co., St Louis, MO) adjusted to a pH of 7.5, sterilized by Millipore filtration, and stored at —20°C. Becton-Dickinson and Co., supplied trypticase soy broth and agar, vacutainer tubes, and sterile, disposable Falcon test tubes used in most experiments. *Staphylococcus aureus* strain 209P was obtained from Meade Johnson, Evansville, IN. White blood cell counts were made on a Model F Coulter Counter (Coulter Electronics, Hialeah, FL). Osmolalities were measured on an Advanced Instruments Osmometer (Advanced Instruments, Newton Highlands, Newton, MA). Sonication of bacterial cultures was done with a sonic oscillator (MSE, Crawley, Sussex, England). Glassware was sterilized by heating to 190°C for 2 hr in a dry oven. All glassware used for leukocyte studies except capillary tubes was siliconized (Siliclads, Clay-Adams).

**RESULTS**

**I. Leukocyte Function in Normal Urine**

As shown in Table 1, leukocyte migration and adhesion to glass were virtually abolished in urine. Aggregation of cells observed after rotation of *E. coli* endotoxin with PMNs in plasma was not observed after rotation of endotoxin with PMNs in urine. Similarly, phagocytosis of *S. aureus* by PMNs was suppressed in urine. Thus, all leukocyte functions tested were significantly inhibited by urine.

**II. Leukocyte Function After Incubation in Urine**

Cell injury caused by incubation of leukocytes in urine was reflected by impairment of subsequent leukocyte function in plasma. Injury appeared to be correlated with urine osmolality and length of the incubation period. Changes in leukocyte migration are shown in Fig. 1. Migratory capacity in plasma was reduced markedly after incubation of leukocytes in urine of high or low osmolality. Urine specimens with osmolalities of 500–600 mosm/kg were least toxic to leukocytes. In fact, incubation for 15 min in such urine actually stimulated subsequent leukocyte migratory capacity in plasma. Figure 2 illustrates the changes in leukocyte adhesive capacity after incubation in urine. Adhesion was abolished by urine with osmolalities greater than 950 mosm/kg. Reduction of urine osmolality to 350–800 mosm/kg or reduction of the incubation period to 15 min produced less injury. Figure 3 shows the effect of incubation in urine on PMN phagocytic capacity. Phagocytosis of *S. aureus* by PMNs in plasma was virtually abolished by prior incubation in urine of 570 mosm/kg for 1 hr. However, incubation for 15 min in urine with osmolalities of 300–400 mosm/kg stimulated subsequent phagocytosis. Although not shown, incubation of leukocytes in BES for either 15 or 60 min did not significantly alter phagocytic capacity of PMNs.
III. Factors Affecting Cell Injury in Urine

Urine osmolality. Cell injury during incubation in urine or other solutions was assessed by testing leukocyte migratory capacity in plasma after incubation. Changes in migration after incubation in plasma-free solutions of different osmolality are illustrated in Table 2. Cells exposed for 1 hr to iso-osmotic BES or iso-osmotic urine at physiological pH migrated normally in plasma. However, incubation in urine specimens with an osmolality of 500 or greater markedly inhibited subsequent migration in plasma.

Urine pH. Leukocyte migration in plasma after incubation of cells for 1 hr in iso-osmotic solutions adjusted to different pH is shown in Table 3. BES at pH
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FIG. 3. Phagocytosis in plasma after incubation in urine for (●) 15 min and (○) 60 min. Suppression of this cell function correlated with the osmolality of urine and the length of the incubation period. Results are means of two experiments. Mean control phagocytic index of 15-min incubation = 554; mean control phagocytic index of 60-min incubation = 616.

TABLE 3

| Incubating solution | Migration in plasma after incubation in urine (% of control) |
|----------------------|------------------------------------------------------------|
| Type of solution     | pH     | Osmolality (mosm/kg H₂O) |                                |
| Urine                | 5.5    | 313                      | 16 ± 2.8                       |
| Urine                | 7.3    | 332                      | 105 ± 5                        |
| BES                  | 5.6    | 288                      | 111 ± 1.4                      |
| BES                  | 7.3    | 287                      | 100 ± 2.7                      |
| Plasma               | 8.0    | 273                      | 100c                            |

a Buffered electrolyte solution.

b Means of 2 experiments ± SEM.

c Mean control migration = 1.33 mm.

5.6 had no effect on the cells' subsequent ability to migrate in plasma. However, urine specimens at comparable pH markedly reduced this function.

Concentrations of urea and NaCl in urine. Figure 4 illustrates the effect of incubating leukocytes for 15 min in BES supplemented with different concentrations of urea or NaCl prior to testing migration in plasma. Cell injury increased in proportion to the hypertonicity of the incubating solution. However, injury produced by urea was first evident at a slightly lower osmolality than that observed with NaCl.

IV. Phagocytosis of Opsonized and Nonopsonized Bacteria in Urine

The effect of serum opsonins on phagocytic capacity of PMNs in urine is shown in Table 4. Phagocytosis of opsonized bacteria was comparable to that observed in plasma, even in urine at pH 5.6. Nonopsonized bacteria were much less readily ingested by PMNs in similar urine specimens.

DISCUSSION

There is a well established relationship between the rapidity and magnitude of the leukocyte response in the kidney and the susceptibility of the renal cortex or
FIG. 4. Leukocyte migration in plasma after incubation of cells in buffered electrolyte solution supplemented with urea or NaCl. Incubation period was 15 min. Cell injury increased in proportion to the concentration or either agent. Results are means of two experiments. Mean control migration = 1.64 mm. Brackets represent standard error of the mean.

TABLE 4

PHAGOCYTOSIS OF OPSONIZED AND NONOPSONIZED Staphylococcus aureus by PMNs in Urine

| Phagocytic mixture | Solution | mosm/kg H2O | Bacteria       | Phagocytic index a |
|--------------------|----------|-------------|----------------|-------------------|
| pH 5.6             | Urine    | 477         | Nonopsonized   | 13                |
| pH 5.6             | Urine    | 477         | Opsonized      | 455               |
| pH 7.8             | Plasma   | 388         | Nonopsonized   | 431               |
| pH 7.8             | Plasma   | 388         | Opsonized      | 443               |

*Phagocytic index = number of bacteria/100 PMNs (Means of 2 experiments).*

medulla to infection (7, 8). Such a relationship has not been established for the lower urinary tract. In fact, no relationship has been established between the presence of pyuria and the resistance of the urinary tract to infection. Sanford and co-workers have suggested that leukocytes in the urine may represent a passive spillover of cells from the infected medullary areas (9). Similarly, Norden, Green, and Kass found that leukocytes played virtually no role in killing of bacteria within the bladder (2). Cobbs and Kaye demonstrated a prompt leukocyte response to instillation of bacteria into the urinary bladder, but their studies did not substantiate ingestion or killing of bacteria by PMNs present in urine (3).

Certain characteristics of urine can suppress leukocyte function in vitro. A number of investigators have shown that a hyperosmotic environment markedly inhibits the phagocytic capacity of leukocytes (1, 5, 10). Chernew and Braude were the first to demonstrate that acid pH, extremes of osmolality, and increased concentrations of urea, potassium, ammonia, creatinine, and citrate inhibited phagocytosis by PMNs (1). Similarly, Lancaster and Allison found that high concentrations of sodium chloride and urea impaired phagocytosis of bacteria by PMNs as well as clumping of such cells after ingestion of bacteria (10). These authors also found that the toxic effects of urea on leukocytes were not abolished by restoring cells to a normal plasma environment. Knoll, Johnson, Pearce, and Rebuck observed that urine inhibited immigration of leukocytes into Rebuck chambers. In addition, urine impaired the phagocytic capacity of PMNs within the chambers (11). Urine
also caused greater cell destruction and alteration of cell morphology than comparable concentrations of hypertonic urea or saline (12).

The extent of the inhibitory effects of urine on leukocytes has not been adequately evaluated. Previous studies did not test the effect of urine on leukocyte function in a plasma-free environment (1, 5, 10). Therefore, it is difficult to estimate the effect of plasma proteins, such as bacterial opsonins, on the results obtained. In addition, techniques employed did not differentiate ingested bacteria from those merely adhering to leukocyte surfaces (1, 10). Furthermore, previous studies usually examined PMN function shortly after cells had been exposed to urine. Therefore, the toxic effects of urine on leukocytes were minimized, and results did not simulate conditions present in vivo in which leukocytes might be present in urine for several hours before excretion. Experimental studies of phagocytosis which employ rotation of leukocyte-bacterial mixtures in test tubes also artifically optimize opportunities for phagocytosis. Thus, it is likely that such studies overestimated the ability of leukocytes to phagocytize bacteria in urine.

The present studies were designed in an attempt to evaluate host defense functions of leukocytes in urine. Factors which might artificially enhance PMN phagocytic capacity were excluded as much as possible. Phagocytosis tests were done in urine containing less than 1% plasma, and lysostaphin was used to eliminate extracellular bacteria. Other leukocyte functions were also examined. These included the ability of leukocytes to adhere to glass, migrate in capillary tubes, and to interact with endotoxin. All of these functions were virtually abolished in urine. In addition, studies were performed to determine how rapidly leukocyte function decreased after exposure of the cells to urine. All functions tested were quickly lost once leukocytes were exposed to urine. Urine specimens least toxic to leukocytes were those with osmolalities of less than 700 mosm/kg. It is conceivable that the loss of renal concentrating mechanism in pyelonephritis or reduced medullary osmolality during water diuresis might potentiate leukocyte function in infected urine, albeit ever so slightly.

Since leukocyte function in plasma was not significantly impaired by previous incubation of cells in buffered electrolyte solution, urine appears to have direct toxic effects on leukocytes which are independent of effects caused by removal of leukocytes from the plasma environment. Cell injury attributable to extremes of osmolality or to increased concentrations of urea or hydrogen ion are readily demonstrable. However, relatively normal leukocyte function after incubation of cells in iso-osmotic urine adjusted to pH 7.5 suggests that the toxic factors(s) in urine are inactive at a neutral pH. Studies by Chernew and Braude demonstrated that the toxic factor(s) appears to be dialyzable (1). Demonstration that leukocytes are not aggregated by endotoxin in urine helps to exclude the possibility that impaired leukocyte function in urine is mediated by endotoxin.

Retention of leukocyte phagocytic capacity has been observed in certain adverse circumstances which completely suppress PMN migration (14), adhesion to glass, and aggregation with other leukocytes (5). Such circumstances include a hyposmotic environment and divalent cation depleted plasma (13–15). However, such studies demonstrating persistence of phagocytosis were performed with leukocytes suspended in plasma. In contrast, the present studies show comparable suppression of phagocytosis, migration, adhesion, and aggregation after incubation of leukocytes in urine. Although the explanation for this finding is not clear, the absence of plasma may enhance the adverse effects of extremes of pH or osmolality on
the phagocytic capacity. That other factors are also involved is indicated by the
greater degree of cell injury in acid urine than in buffered electrolyte solution of
comparable pH.

Although prolonged (1 hr) exposure to normal urine may result in irreversible
injury to leukocytes, impaired phagocytic function observed shortly after addition
of leukocytes to urine is attributable to the lack of serum opsonins in normal urine.
The presence of opsonins dramatically enhances the phagocytic capacity of leuko-
cytes in urine. This enhancement can be demonstrated in urine of relatively high
osmolality and is clearly separable from a nonspecific effect of proteins on leuko-
cyte viability. It might be theorized that such opsonins would play little if any
role in defense against ascending infection because they are unlikely to be present
in urine. Perhaps they would contribute significantly to containment of infection
late in the course of established pyelonephritis. The existence and significance of
opsonins in the urine of patients with pyelonephritis should be determined. Simi-
larly, the effect of proteinuria, polyuria, and bacilluria on leukocyte function in
urine should be determined. Although such factors may alter leukocyte function
in urine, it is more likely that the adverse environment of the urinary bladder
abolishes all leukocyte host defense function.

The main consideration raised by the present studies is that leukocyte function
is virtually abolished in the plasma-free environment of normal urine. This suppres-
sion of function in urine is mediated in part by direct toxic effects of urine on
leukocytes and, in part, by the absence of plasma proteins necessary for optimal
leukocyte function. Cell injury is rapid and so profound that it appears likely that
intact leukocytes in urine do not contribute significantly to host defense against
ascending infection.

SUMMARY

Leukocyte migration, adhesion to glass, aggregation by endotoxin, and phagocy-
tosis of bacteria were virtually abolished in urine. Suppressed leukocyte function
in urine appeared to be mediated by direct toxic effects of urine on leukocytes.
Such effects were, in part, attributable to extremes of osmolality, pH, urea concen-
tration, and to an as yet unidentified toxic factor(s) present in normal urine. Leu-
cocyte function in plasma was not impaired by incubation in buffered electrolyte
solution or in iso-osmotic urine adjusted to pH 7.5. Thus, cell injury in urine was
not attributable to removal of leukocytes from the plasma environment. Under
conditions which minimized cell injury, it was possible to demonstrate that the im-
paired phagocytic capacity of leukocytes in urine was in part due to lack of serum
opsonins necessary for optimal phagocytosis.

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