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IN VIVO REDUCTION OF BACTERIAL POPULATIONS IN THE URINARY TRACT OF CATHETERIZED SHEEP BY IONTOPHORESIS

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ABSTRACT

Purpose: Iontophoresis kills microbes in vitro and, therefore, may be a useful method for eliminating microbial populations associated with catheter-induced urinary tract infections in vivo.

Materials and Methods: Catheters were modified to deliver current to platinum electrodes in the catheter tip. Female sheep were catheterized with this iontophoretic catheter and left ambulatory. In 5 sheep (experimental group) 400 μA was applied to the catheter and withheld in 4 sheep (control group) for 20 to 21 days. The animals were then sacrificed. During the study, types and concentrations of bacteria, and physical and chemical characteristics of the urine samples were determined.

Results: Throughout the study, bacteria levels were reduced in urinary tracts of the experimental group (10^3 to 10^4 microbes per ml.) compared with the control group (10^7 microbes per ml.), without extensive alterations to urine chemistry or the sheep urinary tract.

Conclusions: Since iontophoresis safely reduced bacterial populations in catheterized sheep, this technology may reduce or eliminate nosocomial, catheter-induced urinary tract infections in humans.

KEY WORDS: urine, catheterization, iontophoresis, infection, disinfection

Catheters have long been identified as the leading cause of nosocomial urinary tract infections (UTIs) and the most common predisposing factor in fatal Gram-negative sepsis in hospitals.[1] It has been an important goal for our laboratory to attempt to eliminate catheter-related UTIs. Iontophoresis (the use of current to produce ions of soluble salts) was incorporated into a catheter that delivers 400 μA to urine contained in the urinary bladder as a means of attaining this goal.[2–4] Applied current at this amperage has been shown in in vitro studies to impart antimicrobial properties to the iontophoresed solution,[3,4] but until recently, the mechanism of bacterial killing had not been well described.[5] Our laboratory showed that chloride ions had to be present in the iontophoresed solutions because the current forms chlorine-based biocides (ClO_2 and free Cl) that are probably responsible for the detected antimicrobial effect.[3] The chlorine biocides reduced or eliminated the microbial populations at a rate that was directly proportional to increasing microamperage and inversely proportional to bacterial concentrations in solutions undergoing iontophoresis.[6] The antimicrobial strength of the iontophoresed solution weakened with increasing time after current application had ended.[7] Also, platinum electrodes (as opposed to gold, silver, carbon, nickel and copper electrodes) possessed the best longevity and microbial killing efficacy.[8,9]

To test the ability of iontophoresis to eliminate microbial populations associated with catheter-induced urinary tract infections in vivo, we designed a portable catheter/power supply system that delivered 400 μA to the sheep urinary bladder. From the iontophoretically catheterized sheep, we obtained and analyzed blood and urine samples to determine both the types and concentrations of bacteria and the physical and chemical properties of the samples. The sheep were sacrificed after 20 to 21 days, and the bladder and kidney tissues were microscopically evaluated to detect potential injury from iontophoresis.

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MATERIALS AND METHODS

Iontophoretic catheter. Triple lumen, Foley catheters (size 18 F), purchased from C. R. Bard, Inc. (Covington, Georgia), were modified to deliver 400 μA with a maximum voltage of 6.8 V from an external power source to the urinary bladder. Platinum wire (0.2 mm. diameter) extensions 2.5 cm. long were soldered to 2 polyvinyl chloride insulated (0.1 inch diameter) wire leads. These wires were threaded into the third lumen to the catheter tip, and the platinum extensions of the wires were then threaded out of the lumen through the catheter wall. The catheter wall was sealed with liquid silicon (Dow Corning Corp., Midland, Michigan) and this sealer was then allowed to dry for 24 hours. Catheters, so modified, were then gas sterilized. Small (8 cm. × 11 cm. × 4 cm.) external power supplies were synthesized at the University of Texas Medical Branch, Electronics Department. They were designed to continuously provide 400 μA through the wire leads to the platinum electrodes at the catheter tip. Each device was powered with 2 household 9 V batteries and possessed a warning light to indicate weak batteries (see fig. 1, A).

Sheep. Preliminary studies with dogs were unsuccessful. The animals' agility and temperament let them remove their catheters and power supplies within 24 hours, even if they were housed separately and partially restrained. Sheep were subsequently chosen as representative mammals for this study because they lacked the agility and disposition necessary to remove their catheters or power supplies (see fig. 1, B). Nine female sheep were housed separately and left ambulatory with a closed drainage system. After the animals were anesthetized with approximately 100 mg. of ketamine hydrochloride (intramuscular injection), catheterization was accomplished by swabbing the vaginal os with a 10% povidone iodine solution (Sherwood Pharmaceutical Co., Mahwah, New Jersey) and inserting the modified Foley catheter into the sheep urethra using aseptic techniques (see fig. 1, B for catheter placement in sheep). Current was applied to

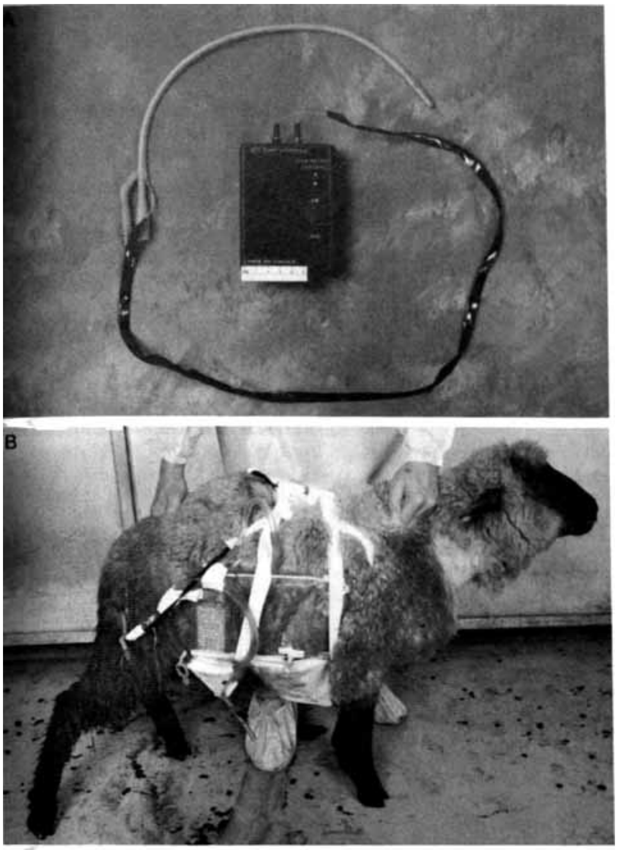

FIG. 1. *A*, power supply (center of photo) provides 400 μA to catheter (top of photo) via wire leads (bottom of photo). *B*, catheter (far left in photo) is in sheep's bladder. Animals were left ambulatory with closed drainage device (center of photo) attached to abdomen by waterproof adhesive tape and umbilical tape tied to animal's wool. Portable battery-powered device is similarly attached to animal's back (top center of photo).

the catheters in 5 of the sheep (experimental group) and withheld in the remaining 4 sheep (control group) for 20 to 21 days. The animals were then sacrificed with a Beauthanasia-D (Schering Corp., Kerrilworth, New Jersey) injection and, within a few minutes, the sheep were autopsied. One animal (control) either had a catheter balloon failure or pulled out the catheter on the seventh day of the experiment. This animal was recatheterized with a conventional Foley catheter (size 18 F) within 24 hours of catheter displacement. A second control sheep also had a catheter balloon failure after 11 days, but this animal was not recatheterized.

Culture and analysis. Blood was collected from the external jugular vein of the sheep approximately once per week, hematocrit and hemoglobin were monitored, and a differential WBC count was performed. Urine samples were taken both from the sample port at the juncture of the closed drainage system every 2 or 3 days during the study and by cystocentesis at the time of necropsy. Also during necropsy, samples of kidney tissue (approximately 1 cc) were obtained from the sheep and aseptically homogenized in sterile phosphate buffered solution (PBS). Bacterial cultures were made by serially diluting these urine and tissue samples with sterile PBS, plating them on brain-heart infusion agar plates and incubating them at 37C for approximately 18 hours. The number of colony forming units (CFUs) in these urine and tissue samples were then determined. The Gram-negative bacterial identities in the samples were ascertained by API 20E Strips (Analytab Products, Plainview, New York) supplied by Dr. J. Reitmeyer of the University of Texas Medical Branch. Also, red and white blood cell counts in each of the urine samples were determined by centrifuging 12 ml. of the sample, removing 11 ml. of supernatant and resuspending the sediment. One hundred microliters of this resuspended sediment was observed under high power microscopic examination (400 × magnification), and the number of WBC and RBC were counted per high power field (hpf). The levels of protein, pH, glucose, ketone, bilirubin, nitrites and urobilinogen of all urine samples were determined with N-Multistix Reagent Strips (Miles Inc., Elkhart, Indiana). The specific gravity of the samples was measured with a refractometer (Reichert TS Meter), and creatinine concentrations of the samples were determined with a Kodak Ectochem Analyzer (Model 700 XR, C series, Eastman Kodak, Rochester, New York). At the time of sampling, all blood and urine samples were cooled in ice and immediately delivered to the Department of Pathology, Division of Clinical Sciences at the University of Texas Medical Branch, where all sample analyses were performed.

Tissue examination. At the time of autopsy, the bladder and kidney were removed and a gross tissue examination was performed to determine the presence of any morphological disparities between the control and experimental groups. Small samples of bladder tissue were then removed from control catheterized, iontophoretically catheterized, and non-catheterized sheep, fixed in 10% buffered neutral formalin, paraffin embedded and sectioned. These samples were subsequently microscopically examined and photographed.

Statistics. All differences between the detected values for sheep catheterized without current application and those with current application were statistically verified by analysis of variance when the data were parametric and the Mann Whitney rank sum test when data were nonparametric.[10] Differences were deemed significant if $p < 0.05$.

RESULTS

Bacterial concentrations and genera. After the fourth day of the study, the numbers of CFUs in the urine samples were significantly lower ($p < 0.05$) in all subsequent sample periods for those animals with current (400 μA) applied to their catheters (experimental group) when compared with those animals in which current to the catheter was withheld (control group), (fig. 2). These differences were also seen at the end of the study when the levels of CFUs found in the homogenized kidney tissue and the urine directly aspirated from the bladder of the experimental group (3.2×10^2 and 2.3×10^4 CFU/ml., respectively) were compared with the significantly higher ($p < 0.05$) CFU levels found in the kidney tissue and bladders of the control group (2.3×10^4 and 2.1×10^8 CFU/ml, respectively). In every urine and tissue sample, the predominant or only microbial genus identified was Escherichia coli.

Urinalysis. In all urine samples of control and experimental groups, Multistix tests for glucose, ketones, bilirubin and nitrites revealed either negative or trace readings. Urobilinogen concentrations, pH and specific gravity ranged from 0.1 to 1.0 mg./dl, 8.5 to 9.0 pH units and 1.005 to 1.029. No significant differences ($p > 0.05$) between the control catheterized and the iontophoretically catheterized sheep for these readings occurred during the 20 to 21 day study. Also, no significant differences ($p > 0.05$) were detected between the mean creatinine concentrations in the urine samples of the experimental group (26.9 mg./dl.) and control group (34.1 mg./dl.). The Multistix readings for urine protein concentrations in sheep catheterized with current (553.8 mg./l.) and without current (564.3 mg./l.) also demonstrated no significant changes ($p > 0.05$) from day 0 to day 15 to 16 of the study. After days 15 to 16 of the study, protein concentrations in the control group's urine drastically increased to 2050 mg./l. by day 20 to 21. However, experimentally catheterized

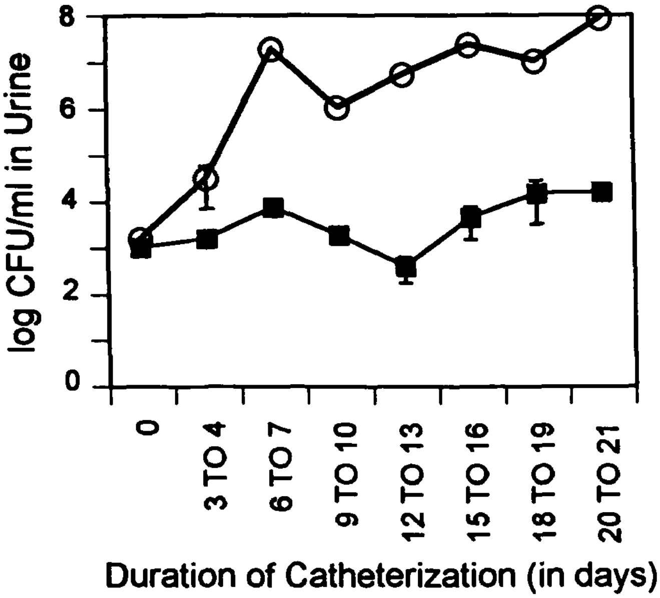

FIG. 2. Number of bacteria (in $\log_{10}$ CFU/ml.) in urine samples drawn from iontophoretically catheterized (■) and control catheterized (O) sheep during 20 to 21 day study. Vertical bars represent standard error of mean.

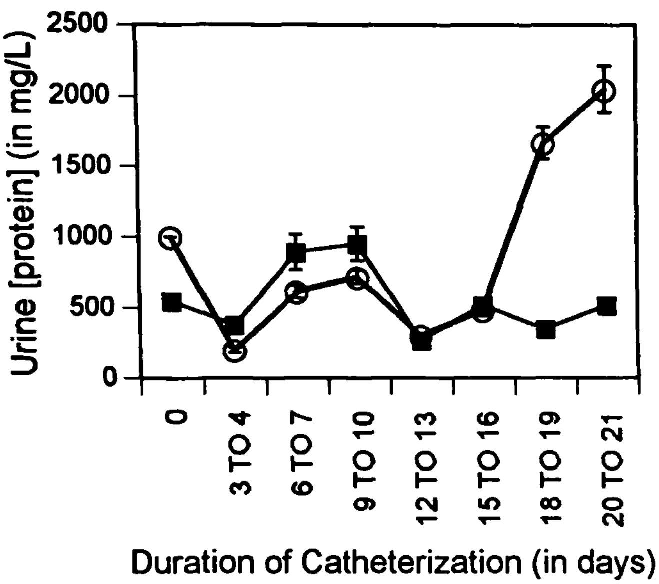

FIG. 3. Concentration of protein (in mg./l.) in urine samples drawn from iontophoretically catheterized (■) and control catheterized (O) sheep during the 20 to 21 day study. Vertical bars represent standard error of mean.

sheep maintained the same levels of protein excretion previously detected (fig. 3). To correct for changes in urine dilution between animals and individual samples, we divided the detected protein concentrations by the creatinine concentrations. The resulting graph of urine protein over creatinine versus duration of catheterization demonstrated a nearly identical plot trend as seen in figure 3. Therefore, the drastic increase in protein excretion demonstrated by control catheterized sheep was probably accurate and not a result of changes in urine dilution. The WBC levels were significantly higher ($p < 0.05$) in urine samples of control organisms (9.3 WBC/hpf) when compared with the experimentally catheterized groups (3.0 WBC/hpf). Also, the numbers of RBC/hpf for the iontophoretically catheterized and control catheterized groups did not differ significantly ($p > 0.05$), but the RBC levels of both groups' samples decreased during the study from 30.65 at day 0 to 10.80 RBC/hpf at day 20 to 21.

Blood analysis. While the concentrations of WBC in the blood of the iontophoretically catheterized and control catheterized sheep revealed no statistically significant changes or differences (7.275×10^3 WBC/mm.^{3}) during the study period, the experimental and control groups equally demonstrated statistically significant increases in the relative concentrations of neutrophils (from 42.4 to 58.6%) with concomitant decreases in lymphocytes (from 58.6 to 42.4%). The sheep blood samples demonstrated no significant differences ($p > 0.05$) between the control and experimentally catheterized sheep for hemoglobin and hematocrit readings, and the overall levels of hemoglobin and hematocrit for both groups remained constant at 11.79 g./100 ml. and 35.1%, respectively.

Histology. While gross tissue examination of the control catheterized group revealed visible pockets of exudate in the right and left kidneys, no such anomalies were present in the experimental group of sheep. Microscopic examination of the experimental and control catheterized groups, however, revealed subepithelial inflammation of the bladder in both groups and polymorphonuclear neutrophil leukocytes (PMNs) located in the bladder epithelium (fig. 4). Therefore, there were no significant differences, as detected by microscope, between bladder tissue samples of the control and experimentally catheterized sheep. However, noncatheterized sheep demonstrated no subepithelial inflammation or PMNs in the bladder tissue samples (fig. 4).

DISCUSSION

Our results support and advance our previous hypothesis that the iontophoretic catheter may be used as a method to safely reduce or eliminate the morbidity and mortality associated with nosocomial, catheter-induced urinary tract infections in humans. Past in vitro studies demonstrated a decrease in microbial populations when current was applied to chloride-containing solutions.[4,5,6,8] We have shown that this antimicrobial effect also occurs in vivo to reduce E. coli populations in catheter-associated UTIs in sheep. The reduction in bacterial concentrations in the urinary tract of iontophoretically catheterized sheep was demonstrated by the ability of the current to maintain bacterial concentrations at 10^3 to 10^4 CFU/ml., well below the 10^5 CFU/ml. criterion that would formally categorize the bacterial presence as a UTI.[1,11,12] However, in the control catheterized sheep (no current applied to their catheters), urine bacteria levels were significantly higher than 10^5 CFU/ml. (10^7 to 10^8 CFU/ml.). Reductions in the bacterial levels of the urine samples drawn from the sample port of the iontophoretically catheterized sheep not only reflected the bacterial concentrations of the urine drawn from the catheter lumen but also reflected the conditions of the urine within the bladder. This bacterial reduction in the bladder was also demonstrated by the low numbers of bacteria (2.3×10^4 microbes per ml.) present in the urine samples directly aspirated from the bladders of the experimental sheep group at the end of the 20 to 21 day study. This level of bacteria found in the bladder aspirate of the experimentally catheterized sheep was much lower than the 10^8 CFU/ml. found in control catheterized sheep. The iontophoretic catheter may also reduce the risk of upper urinary tract infection, as demonstrated by the presence of significantly reduced levels of CFUs in the homogenized kidney samples of the iontophoretically catheterized sheep (as compared with the number of bacteria in the control catheterized kidney samples). Kidney tissue of a normal, healthy, uncatheterized sheep is usually devoid of detectable bacteria. Therefore, once bacteria gain access to the upper urinary tract and infect the renal tissue (away from the antimicrobial

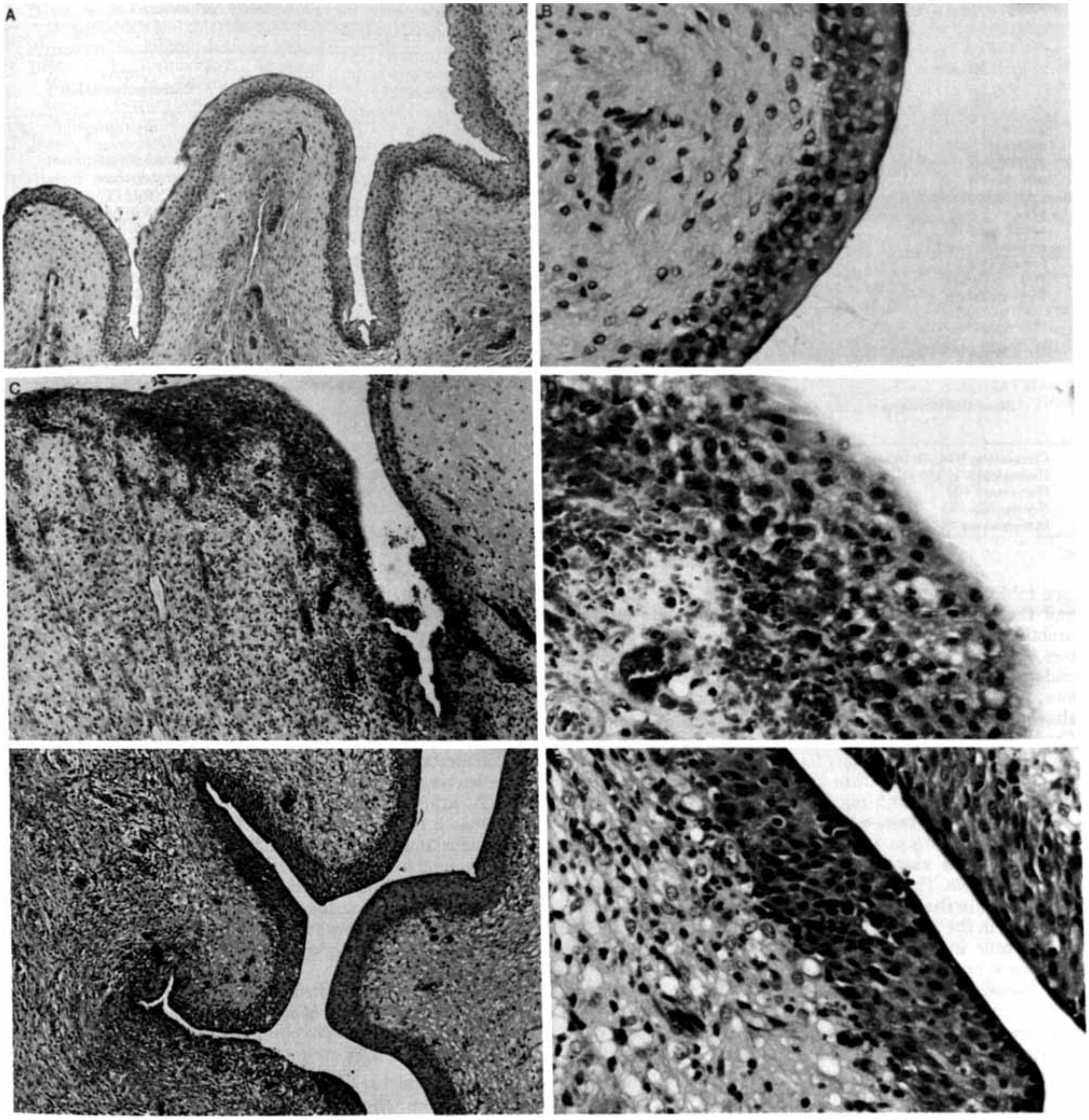

FIG. 4. Photographs of bladder tissue from (*A* and *B*) uncatheterized sheep at 40× (*A*) and 400× (*B*) magnification; *C* and *D*, catheterized sheep without any current application to catheter [note areas of subepithelial inflammation at 100× magnification in (*C*) and presence of polymorphonuclear neutrophil leukocytes (PMNs) demonstrated by dark lobulated nuclei in epithelium at 400× magnification in (*D*)]; and (*E* and *F*) catheterized sheep that had 400 μA current applied to its catheter [note areas of subepithelial inflammation at 100× magnification in (*E*) and presence of PMNs demonstrated by dark lobulated nuclei in epithelium at 400× magnification in (*F*)].

effects of the iontophoretic current in the bladder), both the experimentally and control catheterized sheep might demonstrate approximately equal bacterial growth. However, the bacterial levels are markedly reduced in the iontophoretically catheterized sheep. Therefore, the presence of the iontophoretic current in the bladder may have reduced the effectiveness of bacterial virulence factors, resulting in the reduced microbial concentrations in the experimentally catheterized sheep. Alternatively, our study may also suggest that iontophoresis reduced the number of bacteria in the lower tract and thus, similar to a dose-response curve, fewer bacteria could proceed retrograde to the kidneys. The predominance of E. coli in all urine and tissue samples may have been due to the close proximity of the urethral meatus to the anus allowing sheep feces, known to be colonized with E. coli, to contact the urethral meatus/catheter interface possibly and allow E. coli retrograde invasion.[1]

The reduction in bacterial concentrations in iontophoretically catheterized sheep occurred without extensive alterations in the physical and chemical characteristics of the sheep urine as detected by the creatinine concentrations, specific gravities and Multistix readings of the urine samples

TABLE 1. *Urine properties for sheep from previous studies and from control and iontophoretically catheterized sheep*

Urine Properties	SHEEP READINGS		
	Normal Readings from Previous Studies 1, 12, 13, 14, 15, 16, 17	Iontophoretically Catheterized Sheep	Control Catheterized Sheep
glucose	neg-trace	neg-trace	neg-trace
ketones	neg-trace	neg-trace	neg-trace
bilirubin	neg-trace	neg-trace	neg-trace
nitrites	neg-trace	neg-trace	neg-trace
urobilinogen (mg./dl.)	0.1–1.0	0.1–1.0	0.1–1.0
pH	7.9 ± 0.43	8.5–9.0	8.5–9.0
specific gravity	1.026	1.005–1.025	1.007–1.029
creatinine (mg./l.)	105.9 ± 45.2	269	341
protein (mg./l.)	430–790	553 (Day 0–19), 520 (Day 21)	564 (Day 0–19), 2050 (Day 21)
WBC (cells/hpf)	1–5	3.0	9.3
RBC (cells/hpf)	1	6–24	3–38

TABLE 2. *Blood characteristics for sheep from previous studies and from control and iontophoretically catheterized sheep*

Blood Characteristics	SHEEP READINGS		
	Normal Levels from Previous Sheep Studies[18]	Iontophoretically Catheterized Sheep	Control Catheterized Sheep
Circulating WBC ($\times 10^3$/mm.^{3})	4–12	3.4–9.2	1.3–36
Hemoglobin (g./100 ml.)	9–15	9.9–13.4	9–13.9
Hematocrit (%)	27–45	30–40	27–42
Neutrophils (%)	10–50	33–60	31–65
Lymphocytes (%)	40–75	40–67	35–69

(see table 1). The creatinine concentration, specific gravity, and the Multistix tests for glucose, ketone, bilirubin, pH, urobilinogen, and nitrite were within normal parameters,[13–16] and no differences were seen between the control and experimentally catheterized sheep for these tests. Therefore, no significant renal or urinary tract dysfunctions or alterations in urine chemistry were detected by these tests. The urine protein levels detected in sheep catheterized with and without current from day 0 to day 15–16 were found to be similar, constant and within the normal concentration levels of 430 mg./l.[17] to 791.5 mg./l.[16] However, while the experimental group maintained these normal protein excretion levels after the day 15 to 16 sampling period, the control group demonstrated a sustained 3 to 4 fold increase in protein excretion levels. This sudden increase may be indicative of a compromise in the selectively permeable nature of the kidney glomeruli in the groups catheterized without current, such as would occur in glomerulonephritis.[1] The RBC were found well above normal levels (> 1 cell/hpf),[12] indicating significant hematuria in both experimental and control groups' urine samples. However, the number of RBC in urine slowly decreased with time after catheterization. Therefore, these elevated levels may have actually represented normal responses to recent catheterization and may have had no clinical significance.[1] While the WBC levels in the urine samples of the experimental group were within normal parameters (≤ 1 to 5 WBC/hpf),[1] the levels in the control group were well above normal, suggesting inflammation or infection or both.[1]

Blood analyses also did not detect any significant circulatory or renal abnormalities associated with current application to the catheters placed in sheep (see table 2). The detected levels of hemoglobin, hematocrit percent and numbers of circulating WBC in the sheep blood samples for the control and experimental groups did not significantly change during the study, did not display any differences between each group's measured levels, and were within normal parameters previously detected.[18] Therefore, the introduction of current into the bladders of the experimental group produced no detectable alterations in these clinical blood indices. No evidence of renal dysfunction or hemorrhagic response in either group could be ascertained from these data. The relative increases in circulatory PMNs of both groups were significantly higher than previously detected levels in healthy sheep,[18] which may suggest localized infection and inflammation.[1]

With our results, we have determined that when current was applied to catheterized sheep, bacterial concentrations were maintained at lower levels (approximately 10^3 to 10^4 viable microbes per ml.) when compared with bacterial concentrations detected in sheep catheterized without current (approximately 10^7 to 10^8 viable microbes per ml.). While iontophoretically catheterized sheep may have continued to have bacteria present in their urinary tracts, the microbial concentrations of 10^3 to 10^4 CFU/ml. were well below the threshold levels of 10^5 CFU/ml. that would formally classify the bacterial presence as a UTI.[1] This bacterial reduction was accomplished without any significant alterations in either the physical or chemical characteristics of urine or the tissues of the sheep urinary tract. Therefore, use of the iontophoretic catheter may safely reduce the morbidity and mortality related to catheter-associated UTIs as demonstrated in sheep.

We are pursuing in vitro studies on the microbial killing efficacy of the products of iontophoresed human urine. We then hope to begin human clinical studies to test the practicality of iontophoretic catheter use in humans.

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