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Staphylococcus-induced Urolithiasis in Estrogen-treated Ovariectomized Nude Mice

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Nine of 24 ovariectomized nude mice developed ulcerative skin lesions 28 d after implantation with human breast cancer cells and slow-release estrogen pellets. Aerobic culture of samples from the skin lesions yielded *Staphylococcus intermedius*. By day 45 postimplantation, all mice displayed ulcerative skin lesions, and 5 mice exhibited hunched posture, listless behavior, cyanosis, anorexia, and dehydration. This subset was euthanized but not necropsied. When additional animals became moribund, the investigator elected to end the study at day 51. At necropsy, all 20 but 1 had cystitis and urolithiasis, characterized by relatively large struvite stones and crystals that had filled the urinary bladders of the research animals and caused severe thickening of the bladder walls. Gram-positive cocci were observed microscopically in both the kidneys and bladders of the necropsied animals. Spontaneous urolithiasis has not previously been documented to occur in association with infection of female nude mice by *S. intermedius*.

Struvite urolithiasis has been reported to occur in a wide variety of animals,[5,11,12,30,31] including humans.[4,16] Multiple etiologies have been described; the most common include high-protein diets[25] and the presence of urease-producing bacteria.[3] Although diverse rodents with struvite uroliths have been reported, they most commonly have been described in rats, which have been established as an animal model for human struvite urolithiasis,[2,9,19,22,28] and guinea pigs.[11,17,34] Uroliths in mice typically are restricted to male mice, in association with mouse urologic syndrome,[1,6] and consist of either oxalate and phosphate or struvite. Here we describe a case of spontaneous struvite urolithiasis in estrogen-treated ovariectomized female nude mice with *Staphylococcus intermedius*-induced cystitis.

Case Report

A colony of 4-mo-old ovariectomized female nude mice (Crl: Nu-*FOXn1*^{nu}, Charles River Laboratories, Wilmington, MA) was imported for use in a cancer intervention study. On day 0 of the experiment, each mouse was implanted subcutaneously with a 60-d slow-release estrogen tablet (1.7 mg 17β-estradiol/pellet; Innovative Research of America, Sarasota, FL). On day 7 of the experiment, each mouse was injected subcutaneously over the flank with 2 to 20 million human breast cancer cells from established human cell lines (either BT-20 or HBL-100; American Type Culture Collection, Manassas, VA). The mice then were monitored every other day for tumor growth and progression. They were to be euthanized at day 60 of the experiment, unless they exhibited clinical signs suggesting that earlier euthanasia would be appropriate. All procedures and endpoints were described in an animal use protocol approved by the Portland VA Medical Center Institutional Animal Care and Use Committee.

All mice were housed in polycarbonate shoebox cages with filter tops (Thoren Caging Systems, Hazelton, PA) and corncob bedding (Bed-o'cobs, Maumee, OH). Cages were changed at least once weekly in a laminar-flow changing station (Lab Products, Seaford, DE). The animal caretakers wore latex gloves while changing cages and sprayed their gloves with bleach solution (1:30 dilution) between cages. Soiled cages were sanitized in a mechanical cage washer with a final rinse temperature of 180 °F (82.2 °C). All equipment in contact with the animals was autoclaved prior to use in the animal rooms. The rooms were kept on a 12:12-h light:dark cycle (lights on, 0600), and animals were provided autoclaved rodent chow (LabDiet 5010, Brentwood, MO) ad libitum. The diet had a guaranteed analysis of 23% minimum crude protein, 4.5% minimum crude fat, 6.0% maximum crude fiber, and 8% maximum ash. Acidified tap water was provided in polycarbonate bottles (Thoren Caging Systems, Hazelton, PA) ad libitum. Temperature and humidity were maintained at 72 °F (22 °C) and at least 30%, respectively.

Indirect-exposure sentinel mice were used to screen the colony for pathogens on a quarterly basis. Two 5-wk-old female ICR mice (Taconic, Germantown, NY), were provided for every 100 cages of mice. Sentinel mice had been exposed to pooled dirty bedding from colony cages for a minimum of 21 d. Serum samples collected from these sentinel mice by cardiac exsanguination under isoflurane anesthesia were submitted to the University of Missouri Research Animal Diagnostic Laboratory (St Louis, MO) for serologic testing. Internal and external parasite screens were performed inhouse. At the time of this experimental manipulation, this colony of mice was determined to be free of Sendai virus, mouse parvovirus, minute virus of mice, ectromelia virus, reovirus type 3, pneumonia virus of mice, murine adenovirus, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse rotavirus, mouse encephalomyelitis virus, polyoma virus, murine cytomegalovirus, mouse coronavirus, and rodent pinworms and mites.

At approximately day 28 of the experiment, 9 of the 24 mice were presented for clinical examination because they had developed skin lesions originating at the perineal area and cranial to the tail base. The skin lesions were superficial and ulcerative, with reddened centers and serous discharge. Most of the 9 mice had multiple small (diameter, less than 10 mm) ulcers. Overall, the body condition of the mice appeared to be normal. The use of bleach to disinfect hands during cage changing was discontinued due to concern that the bleach had caused a chemical irritation and predisposed the animals to the ulcerative lesions. Dilute chlorhexadine diacetate (Nolvasan, Fort Dodge Animal

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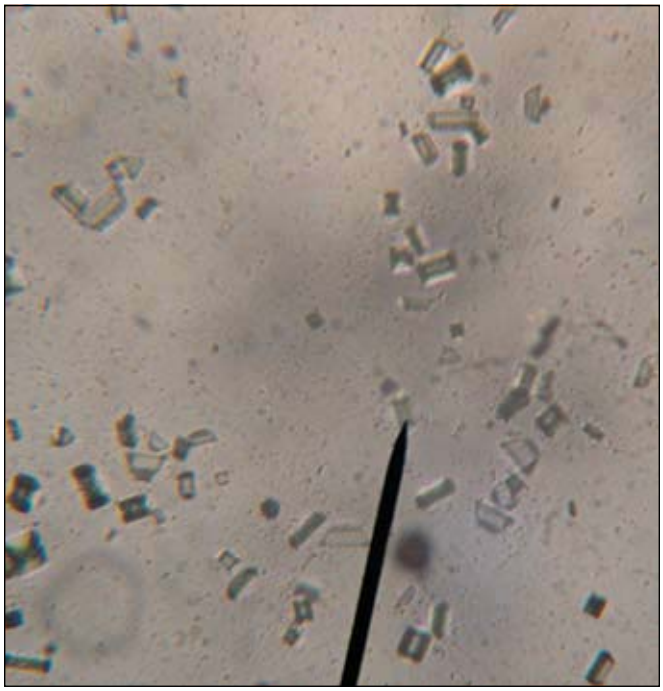

Figure 1. Struvite crystals dissolved in water and examined under light microscopy. Unstained; magnification, ×100.

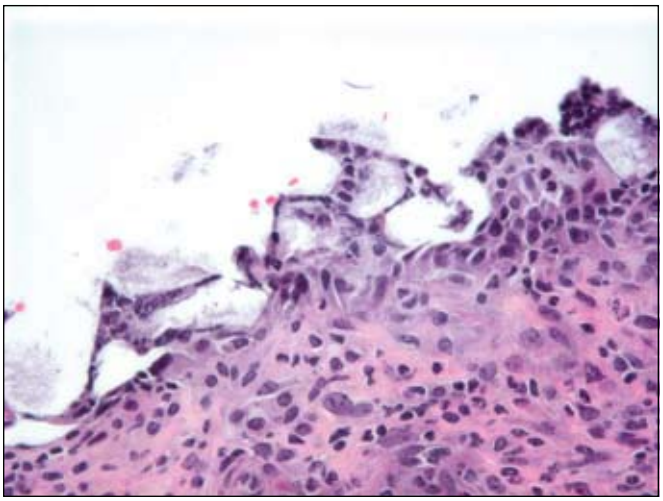

Figure 2. Photomicrograph of bladder wall with neutrophil infiltration into the transitional epithelium, lamina propria, and muscularis. Bacteria and crystals also are present in the lumen. Hematoxylin and eosin stain; magnification, ×400.

Health, Fort Dodge, IA) and forceps were used instead.

The initial cases were treated topically with neomycin, polymixin B, and bacitracin zinc ophthalmic ointment (Darby Drug Companies, Westbury, NY) at least once daily. The serous discharge decreased, and the lesion sizes seemed to stabilize in most cases, but overall there was no significant improvement. In some cases, the smaller ulcers began to coalesce. Over the next 2 wk of treatment, 10 additional cases were reported. Prior to beginning topical antibiotic treatment for the newly identified cases, swabs of the ulcers were submitted for microbiologic assessment. *S. intermedius* was identified and determined to be sensitive to neomycin and polymyxin B. By the time of euthanasia, all 24 animals had developed at least 1 ulcerative lesion.

At day 45 of the experiment, 5 of the mice began to exhibit hunched posture, listless behavior, cyanotic pallor of the skin, anorexia, and dehydration. In addition, their induced tumors were growing more slowly than predicted, by extrapolation from previous experiments. After 3 mice were euthanized due to the described clinical signs and additional mice began to develop similar signs, the investigator elected to euthanize all remaining mice. They were euthanized by carbon dioxide asphyxiation on day 51 of the experiment, and the cadavers were presented to the veterinary staff for necropsy.

Necropsy. At necropsy, 24 adult female ovariectomized nude mice were examined. All were in good post-mortem and fair nutritional condition. All had a 4-mm–diameter white estrogen tablet placed subcutaneously between the shoulders. All had various degrees of perianal ulceration, ranging from 4 to 20 mm in diameter. The ulcers tended to be dry, although some were associated with serous discharge. The bladders of all but 1 mouse were distended and contained chalky white uroliths, ranging from 1 to 4 mm in diameter, which crumbled easily on handling. The mucosal surfaces of the bladders were diffusely reddened, and the bladder walls were thickened. On 2 animals, the kidneys were slightly pale. No other gross lesions were noted in the examination of the remaining viscera. Microscopic examination of the uroliths revealed struvite crystals (Figure 1). Swabs were taken from the interior of the bladders of 2 mice and submitted for microbiological analysis. *S. intermedius* was isolated from these swabs.

The kidneys and bladders of 2 additional mice were saved and submitted to University of Missouri Research Animal Diagnostic Laboratory for histopathology. Both bladders were described as having moderate to severe bacterial cystitis characterized by marked multifocal transitional cell epithelial hyperplasia and ulceration. Neutrophil infiltration into the transitional epithelium, lamina propria, and lamina muscularis also was noted (Figure 2). Lumenal coccal bacteria were identified in the lumen of the bladders, as were crystals (Figure 3). Perivascular calcification was present in the lamina propria of the bladder. In addition, both mice had evidence of pyelonephritis, characterized by moderate multifocal pelvic epithelial hyperplasia and mild vacuolation of pelvic epithelial cells. Bacterial cocci were present in the lumen, with associated degenerate neutrophils and proteinaceous debris. Multifocal neutrophilic infiltration to the pelvic and tubular epithelium also was present (Figure 4). A gram stain revealed the coccal bacteria to be gram-positive, consistent with the microbiologic identification of *S. intermedius*.

The gross and histopathologic lesions were consistent with an acute to chronic pyelonephritis. The perivascular mineralization in the lamina propria of the bladder suggests uremia secondary to an ascending bacterial infection. Because only a limited number of tissues was examined microscopically, the extent of mineralization cannot be determined. We were unable to conclusively determine whether the bacterial infection was initiated as cystitis that resulted in secondary perianal dermatitis or if chemical irritation from the dilute bleach allowed an opportunistic dermatitis that resulted in a secondary cystitis.

Discussion

Estrogen supplementation in ovariectomized female mice is an established animal model for breast cancer research. Tumor cells with estrogen receptors that grow slowly in an ovariectomized mouse will grow rapidly if the same mouse is given exogenous estrogen, usually in the form of a subcutaneously implanted estrogen tablet or capsule.[7,43] Because of this attribute, investigators can modulate breast cancer tumor growth through the administration of exogenous estrogen.

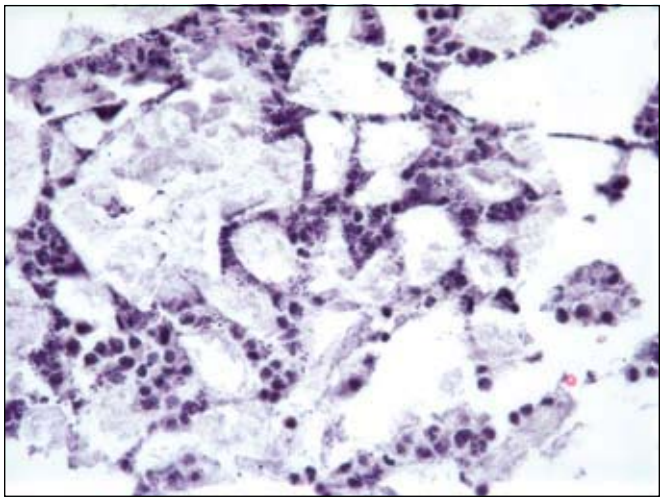

Figure 3. Photomicrograph of lumen of bladder containing multiple inflammatory cells, bacteria, and crystals. Hematoxylin and eosin stain; magnification, ×400.

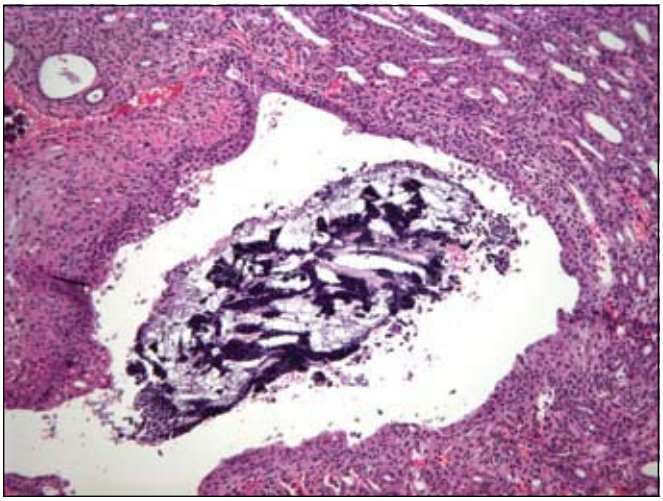

Figure 4. Photomicrograph of the renal pelvis of a mouse with severe pyelonephritis. Hematoxylin and eosin stain; magnification, ×100.

Ascending urinary tract infections have been induced in female New Zealand black × New Zealand White mice treated with 6 mg of estrogen delivered continuously through a silastic capsule for 24 wk.[40] The authors described crystalline concretions but did not characterize them. Other authors have reported that uroliths of mice usually are oxalate and phosphate.[2] Ascending infections of the urinary tract also have been documented in female nude mice housed on nonautoclaved corncob bedding and implanted with 0.36-mg estrogen pellets for 9 wk.[35] Chronic treatment with estrogens has been associated with urine retention and vesicoureteral reflux,[23,35,40] a recognized predisposing factor leading to cystitis and ascending urinary tract infections.[41] The case reported here is unique in that it is the first documented case of infectious struvite urolith formation in a large group of ovariectomized and estrogen-implanted female nude mice housed in an autoclaved microenvironment.

We hypothesize that the use of dilute bleach to disinfect hands between cages of animals contributed to the skin lesions seen in this case. No other haired strains or stocks of mice in the facility, regardless of immune system function, have developed similar lesions associated with this standard operating procedure. In haired mice, the hair provides some protection to the skin of the animals. Nude mice lack this protection, allowing the dilute bleach solution to contact the skin directly and potentially leading to chemical irritation and secondary bacterial infections. Although we were unable to determine definitively whether the pyelonephritis was due to an ascending infection, the history, clinical findings, and gross necropsy suggest that this etiology is the most likely. The concurrent factors of a moist, ulcerative dermatitis with associated *S. intermedius* infection and a urinary system predisposed to urine retention and vesicouretal reflux could result in ascending cystitis and subsequent urolith formation.

Sporadic reports of urolithiasis in male mice in association with mouse urologic syndrome appear in the literature.[1,6] However, reports of these cases often do not include characterization of the urolith. In 1 report, a single male B6C3F1 mouse used in a 2-y carcinogenicity bioassay developed subacute cystitis and struvite uroliths.[45] The literature contains only 1 report of struvite urolithiasis in a single male mouse in conjunction with mouse urologic syndrome.[13] Genetically manipulated mice have been reported to develop cystine uroliths[8] and are used for examination of Dent disease, a calcium-channel disruption disorder of humans that is believed to lead to nephrolithiasis.[10,37,38]

Struvite uroliths are composed of magnesium ammonium phosphate ($MgNH_4PO_4$). *S. intermedius* and *Proteus* sp. are urease-producing bacteria commonly associated with struvite urolith formation,[9] although struvite uroliths also have been reported to form in the absence of bacteria.[19] Urease acts on urea to increase urine pH and ammonium ion concentration, and alkaline urine increases trivalent phosphate ion availability and increases the likelihood of struvite urolith formation by decreasing the solubility of struvite.[3] Although not required for struvite urolith formation, a high-protein diet facilitates this process by increasing the amount of urea available for metabolism by urease-producing bacteria.[25] *Proteus mirabilis* and *Escherichia coli* were associated with the ascending urinary tract infections in New Zealand black × New Zealand White female mice,[40] suggesting that the uroliths present may have been struvite. Other factors that have been implicated in urolithiasis include anatomic defects, neurologic dysfunction, foreign bodies, urethral obstruction by accessory sex gland secretions, and genital trauma.[13,41] In our case, *S. intermedius* was cultured from the bladders of the affected mice, and we hypothesize that the *S. intermedius* colonization contributed to the struvite formation in this case.

Struvite urolithiasis has been identified or induced in multiple species including cats,[3] dogs,[3] dolphins,[25] pygmy sperm whales,[12] sand tiger sharks,[42] western spiny softshell turtles,[26] rabbits,[5] ferrets,[11,31] water buffalo,[44] sheep,[30] calves,[33] and a colt.[39] In addition, struvite uroliths are common in people,[4,16] but is reported less frequently in rodents. Rats and guinea pigs manifest the disease most frequently,[2,11,17,19,22,27,29,32,34] and rats are an established an animal model for the human disease.[9,28]

The role of estrogen in the immune function of rodents is not well characterized. The literature suggests that estrogen may have a protective function in the face of some diseases such as *Coxiella burnetii*[21] and other infectious agents.[18,36] However, other investigators have reported that sustained estrogen may facilitate and maintain infections.[15] Inhibitory[14,20,46] and stimulatory[24] effects of estrogen on the formation of uroliths have been described for rats.

In this case, we were unable to determine the initial factor that predisposed the mice to development of urolithiasis. The treatment with estradiol may have led to cystitis, which in turn

may have caused ulcerative dermatitis. Alternatively, the use of dilute bleach during cage changes may have caused a chemical irritation that predisposed animals to ulcerative dermatitis and subsequent ascending cystitis and urolithiasis. The pyelonephritis was less severe than the cystitis, suggesting that the pyelonephritis was an ascending infection.

These mice had been treated topically with neomycin, polymyxin B, and bacitracin zinc without apparent effect despite the observation that the *S. intermedius* cultured was sensitive to all of these antibiotics. Although topical antibiotics may be indicated in dermatitis, systemic antibiotics generally are more effective therapeutics for systemic infections. Systemic antibiotics should be considered whenever dermatitis that is in association with the genitourinary system requires treatment. Perhaps delivery of a different antibiotic compound by another route and for a longer period of time would have slowed the progression of disease.

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