# A COMPARISON OF NITROFURANTOIN AND DIHYDROSTREPTOMYCIN IN THE TREATMENT OF EXPERIMENTAL FELINE ESCHERICHIA COLI UROCYSTITIS

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Donald Loss Piermattei

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# A COMPARISON OF NITROFURANTOIN AND DIHYDROSTREPTOMYCIN IN THE TREATMENT OF EXPERIMENTAL FELINE *ESCHERICHIA COLI* UROCYSTITIS

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DONALD LOSS PIERMATTEI

AN ABSTRACT

Submitted to the College of Veterinary Medicine
Michigan State University of Agriculture and
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ABSTRACT

The cystitis-urolithiasis syndrome is an important problem in feline medicine. Infection plays a part in this syndrome. Urocystitis was experimentally induced in eighteen cats by traumatizing the bladder mucosa and injecting a culture of E. coli into the bladder lumen. The cats were divided into three groups of six, designated nitrofurantoin, dihydrostreptomycin, and untreated control.

Treatment with nitrofurantoin proved to be more effective than dihydrostreptomycin in producing bacteriologically negative post-treatment urine specimens. The control cats were not as severely affected and did not remain infected as long as was anticipated. Suggestions for further study were made.

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# I

## INTRODUCTION AND REVIEW OF THE LITERATURE

The practice of feline medicine and surgery has attracted increased attention and interest in recent years. The cat is more and more becoming a family pet and cat owners are usually quite willing to consult a veterinarian when the animal is sick. Most practitioners agree that the cystitis-urolithiasis syndrome is one of the most difficult problems encountered in feline medicine. McClellan and McClellan (31) reported that this condition was the most common medical problem of the urogenital system, and accounted for 13 percent of 700 feline cases over a period of three years. Glover (18) reported an incidence of four percent in 457 cases.

Cystitis in the cat may be a primary condition due to bacterial infection or, in the male, may be associated with urethral obstruction due to "calculi". These obstructions are usually precipitates of magnesium ammonium phosphate (27) rather than true calculi. True cystic calculi have been reported in the cat, however (17). Cystitis without blockage of the urethra is a serious, although not often fatal, condition. With the presence of a urethral obstruction, however, death due to uremia can result. Uremia, in this case, is a consequence of kidney damage due to back pressure of urine in the bladder. The entire complex of urethritis, cystitis, bladder atony, acute urethral block and uremia are called the cystitis-urolithiasis syndrome by Lumb (27).

Case histories from the files of the Department of Surgery and Medicine, College of Veterinary Medicine, Michigan State University, were reviewed to gain information on this condition. A review of 33 cases of cystitis or cystitis and urolithiasis revealed the median age for affected animals was three years. The sex distribution was 33 percent females, six percent spayed females, 36 percent males, and 25 percent castrated males. Sixty-seven percent of the affected animals were domestic short-hair breeds, 30 percent were domestic long-hair breeds, and three percent were purebred Siamese. There was a greater incidence of these cases during the winter and early spring months.

Symptoms observed are dependent on the type of condition present: cystitis alone, or cystitis and urethral obstruction. In uncomplicated cystitis, the constant symptoms are hematuria and dysuria. The cat makes frequent trips to the litter-box and voids small quantities of urine at a time. The bladder is usually contracted and thickened. Abdominal palpation is often painful to the patient. The body temperature may be elevated or normal, depending upon the severity of the infection.

In urethral obstruction, the first symptom noted is dysuria, with urine voided a drop at a time or in short spurts. As the bladder continues to fill, the cat becomes more irritated and restless. The animal will spend several minutes in the litter-box and strain in a manner that suggests constipation to the average owner. Priapism may be noted at this time,

and if the obstruction is near the urethral orifice, the tip of the penis distal to the obstruction may be inflamed and discolored due to licking of the area and interference with circulation. If the obstruction continues for several days, uremia develops.

Much of the literature available on this subject is concerned with possible causes of this syndrome. Bloom (4) has listed as predisposing causes for urocystitis the following: trauma to the mucosa, cystic calculi, tumors, parasites of the bladder, obstruction of the urethra due to calculi and inflammation, obstruction of the prostatic urethra due to prostatitis, and neurogenic disturbances resulting in bladder paralysis. Smith (46) theorized on possible general causes and predisposing factors. He thought that females were as often affected as males and noted that closely confined house cats were more often affected than cats that were allowed complete freedom. The incidence of this syndrome was increased during the colder seasons of the year. Inactivity due to closer confinement and consequent urine retention were considered to have increased during these seasons. Other possible reasons listed for the seasonal incidence were decreased fluid intake during cold weather and lack of opportunity for varying the diet by hunting.

Possible avenues for infection of the bladder have been discussed by Bunce (7). These included bacterial infection from kidneys, from the intestines via local lymphatic channels, and ascending infection from the urethra. Trauma from instrumentation and surgery was noted to facilitate infection of the bladder.

The normal pH of cat urine is slightly acid. Bacterial infection with urea-splitting organisms causes an increase in the pH of the urine (4). The end product of urea-splitting is free ammonia, which is very irritating to the vesical mucosa (46). Nielsen (37) concluded that most cases of urolithiasis in farm mink were associated with infection of the urinary tract. The most common infection was a urease positive, hemolytic Micrococcus pyogenes var. albus. Mosier and Coles (36) reported a series of urine cultures from cats with urinary tract infections. Their findings are summarized in Table I. Many practitioners feel that bacterial infection is present in the usual case of acute urethral blockage, as antibiotic therapy is a routine part of their treatment. However, Fishler (14) has called attention to the fact that penicillin and streptomycin have been much less effective in the treatment of this syndrome in recent years. He speculated that antibiotic residues in foods, particularly in dairy products, might be causing an increase in the number of antibiotic resistant bacteria.

Concerning possible causes of calculus formation in the bladder and urethra, Bunce (7) noted that bacteria, mucus, and desquamated epithelial cells from the bladder or kidney may serve as a nucleus for stone formation. Thus, chronic cystitis could influence the growth of stones by furnishing many of these nuclei.

A high-ash diet, as is provided by many commercially available cat foods made from whole fish, has been considered as a possible cause of calculus formation. Stansbury and

Truesdail (47) have stated that they could find no evidence of increased vesical calculi formation as a result of feeding this type of diet for 33 months to a group of cats. Dickinson and Scott (11) attempted to produce calculi in kittens by feeding high levels of bone meals, but the kittens showed no abnormalities of the urinary tract at the conclusion of the experiment.

Armistead (1) has considered several factors related to the formation of uroliths. Those listed included avitaminosis A, urinary tract infection, urine pH, urine volume, lack of protective colloids in the urine, and the anatomy of the male cat.

Urinary colloids were considered by Butt and Hauser (9) to be the basis of the problem of urolith formation in man. They stated that the protective action of urinary colloids is of major importance in preventing precipitation, agglomeration and conglomeration of urine crystalloids. Boyce et al. (6) determined that the total quantities of proteins and other particles of crystalloid size in the urine of humans with calculi were increased three to thirteen times above normal. Butt and Hauser (9) also pointed out the increase in urinary colloids in humans following subcutaneous injection of hyaluronidase. Puntriano (41) thought hyaluronidase had a possible use in preventing urolithiasis in livestock. There have been reports related to the successful use of hyaluronidase in the treatment of canine urolithiasis (19, 48).

Vitamin A deficiency has been demonstrated by Higgins (20) to cause inflammation of the urinary tract of rats and dogs.

This deficiency caused abnormal keratinization of the epithelium and predisposed these tissues to infections. Higgins also demonstrated that a diet low in vitamin A predisposes rats and dogs to calculus formation and, in the absence of infection, administration of vitamin A will cause dissolution of some stones.

The importance of adequate fluid intake in cats that are subject to calculi formation was cited by Lieberman (25). He was of the opinion that increasing the fluid intake prevented the formation of calculi in most cases. The significance of urine volume as related to fluid intake was reported by Grove et al. and cited by Lumb (27). Small zinc discs were surgically placed in the lumen of the bladder in a group of rats. The discs acted as foreign bodies which enhanced stone formation. Intake of large amounts of water was encouraged by adding glucose to the drinking water. This procedure resulted in a five-fold increase in urine volume. None of the rats that received this treatment developed stones, whereas 63 percent of those fed stock diet and 78 percent of those fed stock diet with glucose powder developed stones. The effect of urine pH on stone formation was also demonstrated in this experiment. Some of the animals receiving the glucose in water developed urinary tract infections with urea-splitting organisms. The pH of the urine rose above 7.5 in these rats and stones formed despite diuresis.

There are many reports concerning the clinical management of the cystitis-urolithiasis syndrome. Most of these are de-

voted to the relief of acute urethral block, and prevention of subsequent stone formation. The patient with acute urethral obstruction must be provided immediate relief. Occasionally the only procedure necessary is the application of pressure over the distended bladder, this pressure being sufficient to expel the obstruction from the urethra.

Usually anesthesia is required to permit the necessary treatment and ultra-short-acting barbiturates or ether are recommended (24). Following induction of anesthesia, the penis is extruded and an attempt is made to break down soft calculi by massaging the penis. This is usually followed by irrigation of the urethra with saline or mineral oil. A blunt 20 gauge needle attached to a syringe is introduced into the urethra and the penis and urethra are compressed around the needle. By applying pressure on the syringe, it is usually possible to flush the calculous material back into the bladder. This procedure has been described by several authors (5, 24, 49, 50). It may be necessary to repeat this treatment daily for several days. If the urethra cannot be immediately opened, it is necessary to empty the bladder by paracentesis. Leighton (24) has stressed the necessity of using a needle no larger than 22 gauge to minimize the possibility of leakage through the hole produced in the bladder wall.

Fishler (14) and Burroughs (8) have advocated the use of indwelling catheters of polyethylene tubing. These catheters were inserted after the urethra had been cleared and were left in place for several days. Free flow of urine was thus insured during this period. Burroughs also stressed instilla-

tion of local anesthetics into the bladder as a part of his treatment. He believed this technique provided relief from urethral spasm present in most cases of obstruction. If the urethra cannot be cleared, Crawford (10) has recommended urethrotomy above the penile bone. Since the obstruction is usually found in the penile bone, the opening produced allows free flow of urine. Beamer (2) has described a procedure for ureterocolostomy and urethralcolostomy. This procedure was recommended for relief of intractable urethral obstruction. He found urethralcolostomy to be the more successful technique.

Schein (44) and Hoffman (21) have reported good clinical results with the use of an anti-spasmodic drug to aid in the treatment of urethral block. They recommend injection of a deproteinated nitrogenous extract of pancreas[a]. This material caused relaxation of the urethral wall, and thus allowed for easier catheterization and passage of calculous material. Irwin, Clark, Konde and Taylor have been quoted by Lumb (27) as having successfully used methyliso-octenylamine[b] for its antispasmodic effect.

Following relief of the obstruction, many medical treatments have been recommended. Bloom (5) and Fishler (14) have used urecholine to stimulate contraction of the bladder. They both stressed the necessity of having the urethra open before this powerful parasympathetic stimulant is used.

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[a]Depropanex (R), Merck, Sharp and Dohme, Philadelphia, Penn.

[b]Octin (R), Knoll Pharmaceutical Co., Orange, New Jersey

Changing the pH of the urine, if it proves to be alkaline, has been advocated. This was done by changing the diet to fresh meat (5, 45) or by administration of acidifying agents. Sodium phosphate, five grains three to five times a day, was recommended by Bloom (5). Frick (15) used methenamine, five grains a day. The use of ethylenediamine dihydrochloride[c] or ammonium chloride was also reported (27). Administration of therapeutic amounts of vitamin A (1, 5) and testosterone therapy (45) have also been advised.

Cystitis without obstruction has been treated mainly with antibacterial agents. Bunce (7) stressed the need for antibiotic sensitivity tests to choose the most effective agent. Leighton (24) advised chloramphenicol[d] and nitrofurantoin[e] as the most effective drugs. Piermattei (39) has found nitrofurantoin to have the broadest spectrum of *in vitro* effect against microorganisms found in feline urinary tract infections. Oxytetracycline[f] was recommended by Armistead (1) and a combination of sulfadiazine and streptomycin by Schnelle (45).

Prevention of recurrence of the cystitis-urolithiasis syndrome has been approached from several viewpoints. Lieberman (25) recommended increasing the fluid intake by salting the food enough to cause the cat to drink appreciable quanti-

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[c]Chlorethamine (R), Pitman-Moore Co., Indianapolis, Indiana

[d]Chloromycetin (R), Parke-Davis & Co., Detroit, Michigan

[e]Furadantin (R), Eaton Laboratories, Inc., Norwich, New York

[f]Terramycin (R), Pfizer Laboratories, Brooklyn, New York

ties of water. Sternfels (49) advocated that the cat receive only distilled or boiled water. He thought minerals in the drinking water were a factor in the formation of calculi. He also recommended a low-ash diet and the use of a urinary antiseptic for six months after the occurrence of a urinary stoppage. Despite the work of Higgins (20), Bloom (5) has not been impressed by the effectiveness of vitamin A in preventing calculus formation in the cat. Bloom recommended a meat diet as the most satisfactory long term prophylaxis. Leighton (24) advised the use of meat such as hamburger and liver, fresh cooked fish, and the prescription diet C/D(g).

Despite all of these measures, many cats have recurrent attacks of cystitis-urolithiasis which often lead to their death. This thesis presents the results of an experiment designed to increase the knowledge of one phase of this problem -- namely, efficient treatment of infection of the urinary bladder. Specifically, this research was undertaken to compare the efficacy of treatment of bacterial urocystitis with nitrofurantoin and dihydrostreptomycin. A secondary aim of the experimental work was to find a means of instituting and maintaining a lower urinary tract infection.

Nitrofurantoin (5-nitro-2-furfurylidene aminohydantoin) is a synthetic antibacterial compound of the nitrofuran group. The mode of action of the nitrofurans is considered to be interference with carbohydrate utilization by bacterial cells (38). Nitrofurantoin has been extensively employed in human

(g)(R), Hill Packing Co., Topeka, Kansas

medicine for treatment of various urinary tract infections such as pyelonephritis, pyelitis and cystitis (26, 29, 32, 51). Infections due to the following organisms are reported (30) to be particularly sensitive to nitrofurantoin: Escherichia coli, Aerobacter aerogenes, Proteus rettgeri, Proteus morganii, and Staphylococcus aureus. Nitrofurantoin has also been reported as effective in the treatment of chronic purulent rhinitis refractory to the usual antibacterial therapy (28).

In veterinary medicine, nitrofurantoin has been used for treatment of epizootic tracheobronchitis (kennel cough) in dogs (3, 13, 34, 35, 42) and urinary tract infections in both large and small animals (3, 30, 36). On oral administration to dogs and cats, about 40 percent of the drug is excreted in the urine (3). The recommended dose for urinary tract infections in small animals is one to two milligrams per pound of body weight three times daily (3).

Streptomycin is biosynthesized by several species of actinomycetes, belonging principally to the genus Streptomyces or related genera. Dihydrostreptomycin is produced by hydrogenation of the parent compound streptomycin. Both products have equivalent antibiotic activity (23). In human medicine, streptomycin and dihydrostreptomycin are used in the treatment of tuberculosis, urinary tract infections, tularemia, undulant fever, pulmonary infections, and various enteric infections (40).

In veterinary medicine, these drugs have been used in the treatment of pneumonia, leptospirosis and cystitis, as well as

gastrointestinal, ocular, and wound infections (23). The aqueous sulfate salt is the most commonly employed form of dihydrostreptomycin. The minimum dose recommended by Jones (22) is five milligrams per pound of body weight intramuscularly at twelve to twenty-four hour intervals.

II

# MATERIALS AND METHODS

## A. Preparation of Experimental Animals

All cats in this experiment were procured in the vicinity of East Lansing, Michigan. Most of them were from farms, although a few had been city pets. Ages varied from young mature (eight to ten months), to rather aged animals. All cats were given two doses of feline distemper vaccine[h] at seven to ten day intervals. They were also given one injection of feline pneumonitis vaccine[i] and wormed with Vermiplex (R)[j]. Urine samples were bacteriologically examined to insure all cats were negative for bacteriuria at the start of the experiment. Three cats were rejected because of bacteriuria on initial cultures. During the experiment, the animals were given an exclusive diet of C/D and water. The cats were housed in individual stainless steel cages and were furnished a plastic pan filled with wood chips for their eliminations.

## B. Selection of Test Organism

The first consideration was selection of a representative microorganism to be used in the experimental infection of the

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(h) Fromm Laboratories, Inc., Grafton, Wisconsin

(i) Pneumovax (R), Pitman-Moore Co., Indianapolis, Indiana

(j) Pitman-Moore Co., Indianapolis, Indiana

animals. On the basis of a report by Mosier and Coles (36) that E. coli was the most frequent microorganism found in feline urinary tract infections, a hemolytic strain of E. coli isolated from a clinical case of feline urocystitis was selected. This organism was sensitive to chloramphenicol, dihydrostreptomycin, neomycin, polymyxin B and nitrofurantoin, and resistant to chlortetracycline, penicillin, oxytetracycline, tetracycline and novobiocin, as determined by the paper disc method. Commercially prepared discs [k] were used in the following strengths: dihydrostreptomycin and erythromycin, two micrograms; chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, neomycin and novobiocin, five micrograms; nitrofurantoin, 100 micrograms, penicillin, two units; and polymyxin B, 50 units.

## C. Infection Procedure

Eighteen cats were divided into three groups of six each, with males and females equally distributed. Exact ages were not available; however, their approximate age was estimated. They were classified as young (under two years), middle-aged (two to five years) and old (over five years). The age brackets were equally represented in the groups. Weights were recorded to the nearest ounce before the experiment started and at its conclusion. These three groups were designated as nitrofurantoin, dihydrostreptomycin and untreated control, respectively.

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[k] Sensi-discs (R), Baltimore Biological Laboratories, Baltimore, Maryland

A cystotomy was performed and the epithelial lining of the bladder was abraded over a one to two cm. square area. This abrasion was done with the scalpel blade. A specimen of the bladder wall was also taken during the operation for microscopy. After the bladder incision was sutured, two ml. of a 24 hour broth culture of the bacterium ($9.06 \times 10^8$ organisms/ml.) were injected through the bladder wall into the lumen. Feed and water were withheld for 48 hours after surgery to subject the animal to stress and to concentrate the urine.

## D. Treatment

A cat was considered infected and treatment was started if the urine culture was positive on the eighth postoperative day. Nitrofurantoin in tablet form was given per os at the rate of 10 mg. at eight hour intervals. This represented a range of 1.17 to 2 mg. per pound of body weight. Dihydrostreptomycin was given intramuscularly, as the aqueous sulfate solution, once a day at the rate of 20 mg. per pound. Treatment was continued for 24 hours after two consecutive daily urine samples were bacteriologically negative.

## E. Bacteriological Procedure

Urine samples were collected by catheterization daily during the treatment period and every fourth day thereafter. Catheterization was performed while the cat was under very light thiamylal sodium anesthesia[1]. Size four French, woven

[1] Surital (R), Parke, Davis and Co., Detroit, Michigan

nylon ureteral catheters with a whistle tip were used. They were cut to a length of ten inches to simplify handling. Sterilization was accomplished by autoclaving at 15 pounds pressure for 18 - 20 minutes, and no chemical disinfectants were used at any time on the catheters. Males were catheterized by extruding the penis from the sheath and inserting the catheter directly into the urethra. The procedure was slightly more involved in the female and required greater care to avoid contamination. The cat was placed on her right side, and the assistant grasped the skin lateral to the vagina. While the assistant pulled this skin laterally, the operator grasped the ventral tip of the labia and pulled posteriorly. The combined traction dilated the vestibule and allowed visualization of the urethral orifice on the floor of the vagina. The catheter was then inserted in the urethra, with care taken to avoid touching the vagina with the catheter.

Whenever possible, a ten ml. urine sample was obtained of which five ml. was centrifuged. If less than five ml. was obtained, the entire amount was centrifuged. The sample was spun at 2500 r.p.m. for twelve to fifteen minutes. The supernatant was then decanted or pipetted off and a loopful of the sediment was streaked on a tryptose agar plate enriched with five percent defibrinated bovine blood. The agar plate was incubated at 37° C. for 18 to 24 hours, at which time it was observed for growth. The specimen was considered bacteriologically positive and uncontaminated if all the colonies were of the same typical gross appearance as the stock culture. If

more than one type of colony was observed, a representative of each type was picked and identified by biochemical reactions.

## F. Necropsy Procedure

On the 43rd day following the start of treatment (51st post-infection day), the cats were sacrificed and a necropsy was performed. Bladder and kidney specimens were collected for histopathological examination. Kidney tissues and urine were examined bacteriologically.

The tissues collected were fixed in formalin prior to sectioning. Sections were cut at right angles to the vesical mucosa. Sections to be stained with hematoxylin-eosin were cut six microns thick, and those to be stained by the Gram-Wiegert technique were cut four microns thick. Changes in thickness of the various cell layers of the bladder wall were determined by measuring the thickness of the cell layers at several areas in each biopsy and necropsy section for each cat. Measurements were made with an ocular micrometer.

# III

# RESULTS

## A. Progress of the Infection

Of the 26 originally infected cats, three died post-operatively within the first 48 hours. It was considered that one of these cats died from bacteremia due to excessive abrasion of the bladder epithelium. This cat had a high fever the first postoperative day but showed no evidence of peritonitis at necropsy. The other two cats developed peritonitis from leakage of the bacterial culture through the bladder incision. These three cats had fevers of 103 to 106 degrees Fahrenheit and showed signs of depression. The remaining 23 animals showed great variation in response, with temperatures ranging between 101 and 105.2 during the first week. The degree of depression and anorexia varied consistently with the temperature. Hematuria and dysuria present for the first two or three days disappeared rapidly in most cases and by the eighth day only a few showed gross hematuria. Five cats showed no bacteriuria on the eighth post-infection day and were therefore withdrawn from the experiment.

Cats in the infected untreated control group lost an average of 1.6 ounces in body weight, and those in the dihydrostreptomycin group lost an average of 5.6 ounces, whereas those in the nitrofurantoin group had gained an average of 1.8 ounces at the end of the experiment.

## B. Bacteriologic Results and Statistical Analysis

The results of urine cultures and a notation of the number of days each cat was treated are compiled in Table III. Comparison of the numbers of bacteriologically negative urine in each group of cats is demonstrated graphically in Figure 5. The nitrofurantoin group was treated an average of eight days and the dihydrostreptomycin group an average of nine days.

Statistical analysis for significance was directed to a comparison of the percentage occurrence of bacteriologically negative post-treatment urine specimens in each experimental group. The number of cultures that could be considered for each animal was determined by the number available from the animal that had been treated the longest (cat 634, Table III) since all cats were terminated on the same day. Therefore, a series consisting of six urine cultures was compared for each cat. This series for the treated groups consisted of cultures taken at four day intervals beginning with the fifth to the eighth day following cessation of treatment. The average series of cultures from the two treated groups covered the period from the 15th to the 35th day from start of treatment. Since the control group was not treated, the same period was used to obtain the sample series from this group.

Using this sampling plan, there were 36 urine specimens for comparison in each group. The occurrence of bacteriologically negative specimens among the experimental groups was: control, 66.66 percent; dihydrostreptomycin, 66.66 percent; nitrofurantoin, 88.88 percent.

Comparison of these percentages for significant difference was accomplished by "Student's" t-test (12). The occurrence of sterile urine cultures in the nitrofurantoin group was significantly higher than in either of the other groups. The test showed a P value between .05 and .02 (95 to 98 percent level) for significant difference.

## C. Necropsy Findings

There were no gross changes noted on necropsy except in the urinary bladders and surrounding tissues. Adhesions of omentum and mesentery to the bladder surface in the incision area were usually observed. In a few cases, the bladder was adherent to the ventral abdominal wall, with no significant variation among the three groups. The epithelial lining of the bladder generally showed scattered areas of hemosiderosis and in several cases (three control, one dihydrostreptomycin, and three nitrofurantoin), small vesicles one to two mm. in diameter were noticed within the epithelium. These vesicles were filled with a clear, serous fluid. In most cases, the epithelium was intact and free from any breaks in the surface. Cat 633 (dihydrostreptomycin) had a shallow ulcer of the mucosa, five mm. in diameter, at one side of the incision, and cat 635 (dihydrostreptomycin) showed a similar, although healed, area.

Comparison of the biopsy and necropsy bladder sections revealed changes in the character and thickness of certain cell layers. In a normal bladder[m] (Figures 1 and 2), the

[m] Bladder section from normal cat. Anatomy Department, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

demarcation between the lamina propria and the submucosa was easily distinguishable, since the submucosa was less dense than the lamina propria. In most of the post-infection bladders, this differentiation was much more difficult, mainly because of an increase in density of the submucosa (Figure 3) as a result of hyperplasia of connective tissue. Capillary proliferation in the lamina propria, and to a lesser extent in the submucosa, was marked in these bladders. Figure 4 shows the increased number and size of blood vessels in the lamina propria.

As shown in Table II, there was a fairly consistent increase in thickness of both the lamina propria and the submucosa. These changes were less in the nitrofurantoin group than in either of the other two groups, with no appreciable difference between the control and the dihydrostreptomycin group. Following infection, the transitional epithelium was consistently reduced in height in all three groups, and the cells of this layer appeared shrunken in comparison to those of the normal bladder.

Examination of Gram-Wiegert stained bladder and kidney tissues failed to demonstrate any bacteria in situ and post-mortem cultures of kidney tissue did not reveal any ascending infection.

# IV

## DISCUSSION

The technique used in this experiment to establish and maintain infection in the bladder was not entirely adequate. Evidence of infection was lacking in 22 percent of the infected cats by the eighth day. Urine from control cat 557 was bacteriologically negative after the 15th day (seventh post-treatment day, Table III) and two others (174 and 209) were negative after the 23rd day (15th post-treatment day, Table III). A means to prevent spontaneous recovery from experimental infection was needed. The method of Miller *et al.* (33), in which a sterile zinc disc was dipped into a culture of the test organism and then surgically placed in the lumen of the bladder, may fulfill this purpose. The disc would act as a source of irritation to prevent spontaneous recovery from the infection.

As originally conceived, urine specimens were to be obtained by paracentesis. Unfortunately this could not be accomplished during the first few days following surgery because the bladder was very thickened and the cats were voiding urine frequently. Thus, there was never a very large quantity of urine present. As a result, it was necessary to resort to catheterization to obtain the necessary urine samples. Contrary to common belief, the collection of urine samples by catheterization proved to be a bacteriologically acceptable technique. Contamination of the urine was virtually nil.

Of the first 143 specimens collected, only two were bacteriologically contaminated. However, careful collection technique was necessary, particularly in the female.

It was thought that the infection should have a chance to become established before any treatment was initiated. The period of eight days was arbitrarily selected. It is interesting to speculate on the possible results had treatment been delayed several weeks.

The dose range of nitrofurantoin used is now considered to be fairly low. The latest labeling information on Furadantin advises a minimum dose of two mg. per pound at eight hour intervals. Emesis following the oral dose of nitrofurantoin was observed in one animal (209) for the first two days of treatment. Feeding a small amount of food with the medicine seemed to prevent this. The problem solved itself by the fourth day, and no further feeding was required with the medication.

Treatment was continued in both groups for a total of 72 hours after the urine first became negative. It is a general concept of chemotherapy that treatment should continue for 48 to 72 hours after disappearance of the cardinal sign or symptom. The fact that three animals (066 and 633 - dihydrostreptomycin, and 634 - nitrofurantoin) showed positive cultures within three to four days following cessation of treatment may indicate a need for more prolonged treatment.

The average weight changes in the three groups presented a confusing picture. Although it might have been expected

that the untreated control group would lose weight, the reason for an even greater loss in the dihydrostreptomycin treated group was puzzling. Perhaps this was a manifestation of toxicity of the antibiotic. The author can present no reason for the weight gain in the nitrofurantoin group other than that this group apparently was less severely affected by the infection and recovered more quickly. Evidence of these two points has been previously mentioned under "Results".

The most striking fact noted in this study was the extreme inconsistency in bacteriological results. As noted in Table III, cat 774 (dihydrostreptomycin) had a series of nine consecutive negative samples over a period of 36 days. On the 43rd day, however, the culture was positive. Other examples of this tendency were noted in animals 560, 074 and 195.

During the late stages of the experiment, an attempt was made to determine the cause of inconsistent urine cultures in certain animals. Out of curiosity and not as an integral part of the experiment, certain urine samples were cultured using an enrichment technique. The sample was inoculated into brain heart infusion broth containing 0.5 percent agar and incubated 24 hours prior to streaking on blood plates. In some cases, growth was obtained by this procedure when none was found by the previously outlined method. All of the urine samples included in the results were cultured in the same manner, however, and the method of comparing the occurrence of sterile samples remains valid.

It has been strongly suggested that the only valid criterion of a cure in urinary tract infections is the absence

of bacteriuria, and this can be determined only by culture (43). The author also considered this fact of primary importance and constructed the statistical evaluation on this basis. There seems to be no other way to evaluate a problem of this nature. McCrea et al. (32) have stressed that credence cannot be placed on the routine urinalysis, and interpretation based on the presence or absence of leukocytes in the sediment can be in direct opposition to the true bacterial picture.

An experiment of this nature would probably be more significant if the animals could be kept for a longer period of time. As evidenced in Table III, many of the cats were in an equivocable position in regard to status of infection when the experiment was terminated. Ideally, the animals should be held long enough to establish a definite pattern for each animal. McCrea et al. (24) have not considered their human patients cured until they were clinically free of symptoms and had five consecutive negative post-treatment cultures taken at weekly intervals.

# V

## SUMMARY AND CONCLUSIONS

Urocystitis was induced in 18 cats by traumatizing the bladder and introducing a culture of E. coli. Nitrofurantoin was given orally to six cats at a dose of 1.17 to 2 mg. per pound of body weight at eight hour intervals, for an average of eight days. Dihydrostreptomycin sulfate was given intramuscularly to six cats at a dose of 20 mg. per pound of body weight once a day, for an average of nine days.

There were significantly more bacteriologically negative urine specimens following treatment with nitrofurantoin than with dihydrostreptomycin. There was no difference in the occurrence of bacteriologically negative urine specimens between the dihydrostreptomycin group and the control group.

Inflammatory reaction of the bladder wall to the experimental infection was considerably less in the nitrofurantoin treated cats than in either the dihydrostreptomycin treated cats or the infected untreated controls.

The methods used in this experiment to produce and maintain infection in the urinary bladder were not entirely satisfactory. Twenty-two percent of the cats originally infected had sterile urine cultures in eight days, and the untreated controls did not stay as uniformly infected as was expected. In future experiments, it would seem useful to provide means of continued bladder irritation in order to prevent spontaneous cures of experimental infections.

Nitrofurantoin was well-tolerated by five of six cats. One cat required feeding before medication to prevent vomiting.

Bacteriuria was not always detected by the usual bacteriologic laboratory procedures. A cultural method to increase the probability of detecting bacteriuria was discussed.

TABLE I

MICROORGANISMS CAUSING URINARY TRACT INFECTIONS IN CATS

Mosier and Coles (36)

| Bacteria | Cases* | Percent of Total |
|---|---|---|
| E. Coli | 16 | 61.5 |
| Proteus sp | 1 | 3.9 |
| Streptococci | 6 | 23.1 |
| Staph. aureus | 3 | 11.5 |
| Total | 26 | 100.0 |

*Dual infections were present in 3.7 percent of these cases.

TABLE II

PERCENT CHANGE IN BLADDER WALL THICKNESS FROM PRE-INFECTION (BIOPSY) TO POST-INFECTION (NECROPSY)

| | Cell Layers | | |
|---|---|---|---|
| Group | Transitional Epithelium | Lamina Propria | Submucosa |
| | percent | percent | percent |
| Control | -23.5 | +60 | +46 |
| Dihydrostreptomycin | -26 | +33 | +74 |
| Nitrofurantoin | -22.5 | -2 | +19 |

TABLE III

BACTERIOLOGICAL RESULTS AND SCHEDULE OF TREATMENT DAYS

| Animal No. | Days From Start Of Treatment: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 23 | 27 | 31 | 35 | 39 | 43 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CONTROLS | | | | | | | | | | | | | | | | | | | | | | | | | |
| 188 | + | + | + | + | + | | + | | | | + | | | | + | | | | + | - | - | + | - | + | - |
| 174 | + | + | + | + | + | | X | | | | - | | | | + | | | | - | - | - | - | - | - | - |
| 209 | + | - | - | - | - | | - | | | | - | | | | + | | | | - | - | - | - | - | - | - |
| INFECTED | | | | | | | | | | | | | | | | | | | | | | | | | |
| 674 | + | + | + | + | + | | - | | | | + | | | | + | | | | + | - | + | + | + | + | + |
| 557 | + | - | - | + | + | | + | | | | - | | | | - | | | | - | - | - | - | - | - | - |
| 560 | + | + | + | + | + | | + | | | | - | | | | - | | | | - | - | + | - | + | - | - |
| DIHYDROSTREPTOMYCIN | | | | | | | | | | | | | | | | | | | | | | | | | |
| 066 | + | + | - | + | - | + | - | - | * | | + | | | | + | | | | - | - | - | - | - | - | - |
| 074 | + | + | + | + | + | + | + | + | * | * | - | * | | | - | | | | - | - | - | + | - | - | - |
| 774 | + | + | - | + | + | | - | + | * | * | - | | | | - | | | | - | - | + | - | - | - | - |
| 207 | + | + | - | - | + | | - | - | * | * | - | | | | + | | | | - | - | - | + | - | - | - |
| 633 | + | + | + | + | + | + | - | + | * | * | - | * | * | | - | | | | - | - | - | - | + | - | - |
| 635 | + | + | + | + | + | + | + | + | * | * | - | * | - | * | - | | | | + | + | - | - | - | - | - |
| NITROFURANTOIN | | | | | | | | | | | | | | | | | | | | | | | | | |
| 189 | + | + | - | X | - | | - | | | | - | | | | - | | | | - | - | - | - | - | - | - |
| 195 | + | + | - | X | - | | - | | | | + | | | | - | | | | - | - | - | + | - | - | - |
| 206 | + | + | + | + | + | + | + | + | + | + | - | * | | | - | | | | - | - | - | - | - | - | - |
| 634 | + | + | - | - | + | - | + | + | + | + | - | * | | | + | | | * | - | + | + | - | - | - | - |
| 773 | + | + | + | + | + | - | - | + | | | - | | | | - | | | | - | - | - | - | - | - | - |
| 672 | + | - | - | - | - | | - | | | | - | | | | - | | | | - | - | - | - | - | - | - |

Key: + = Urine Culture Positive; - = Urine Culture Negative;
X = No Sample; * = Treated

Figure 1
NORMAL FELINE BLADDER

X90

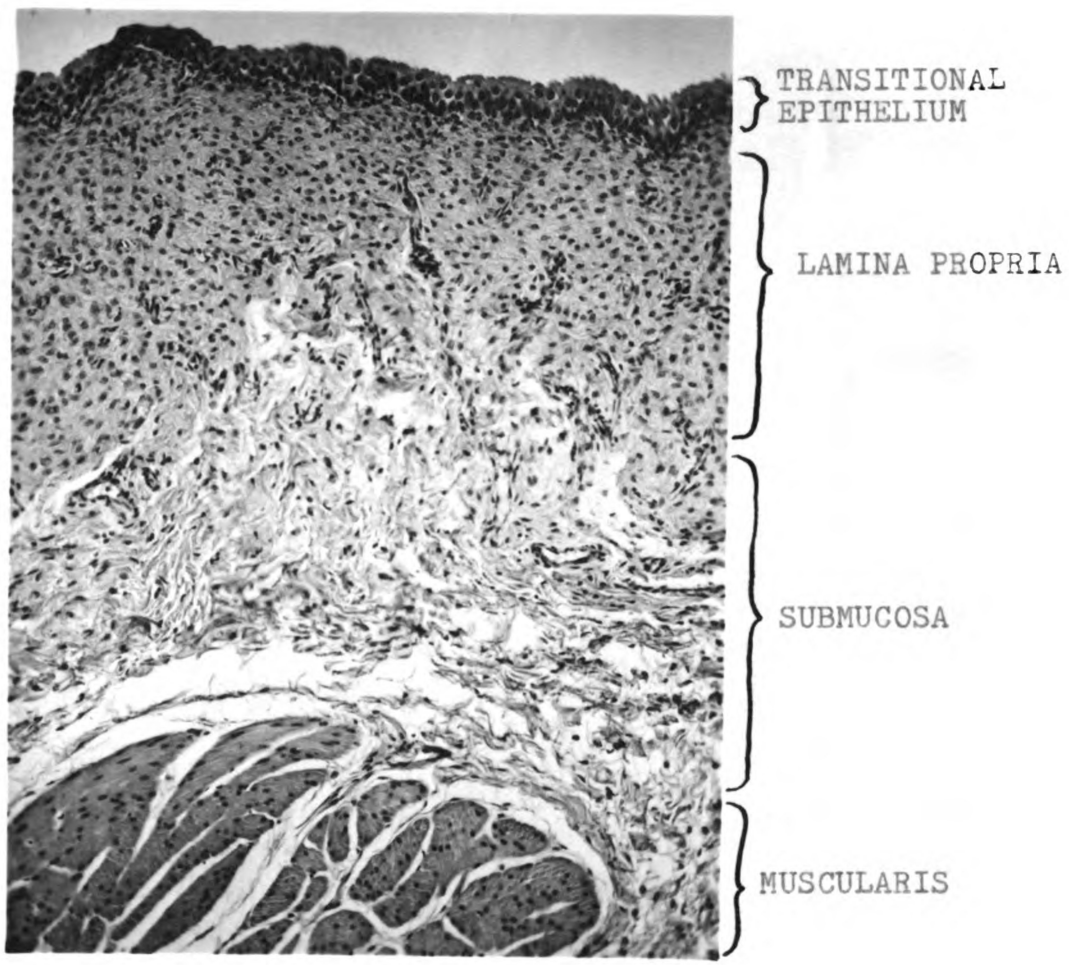


Figure 2
NORMAL FELINE BLADDER

X235

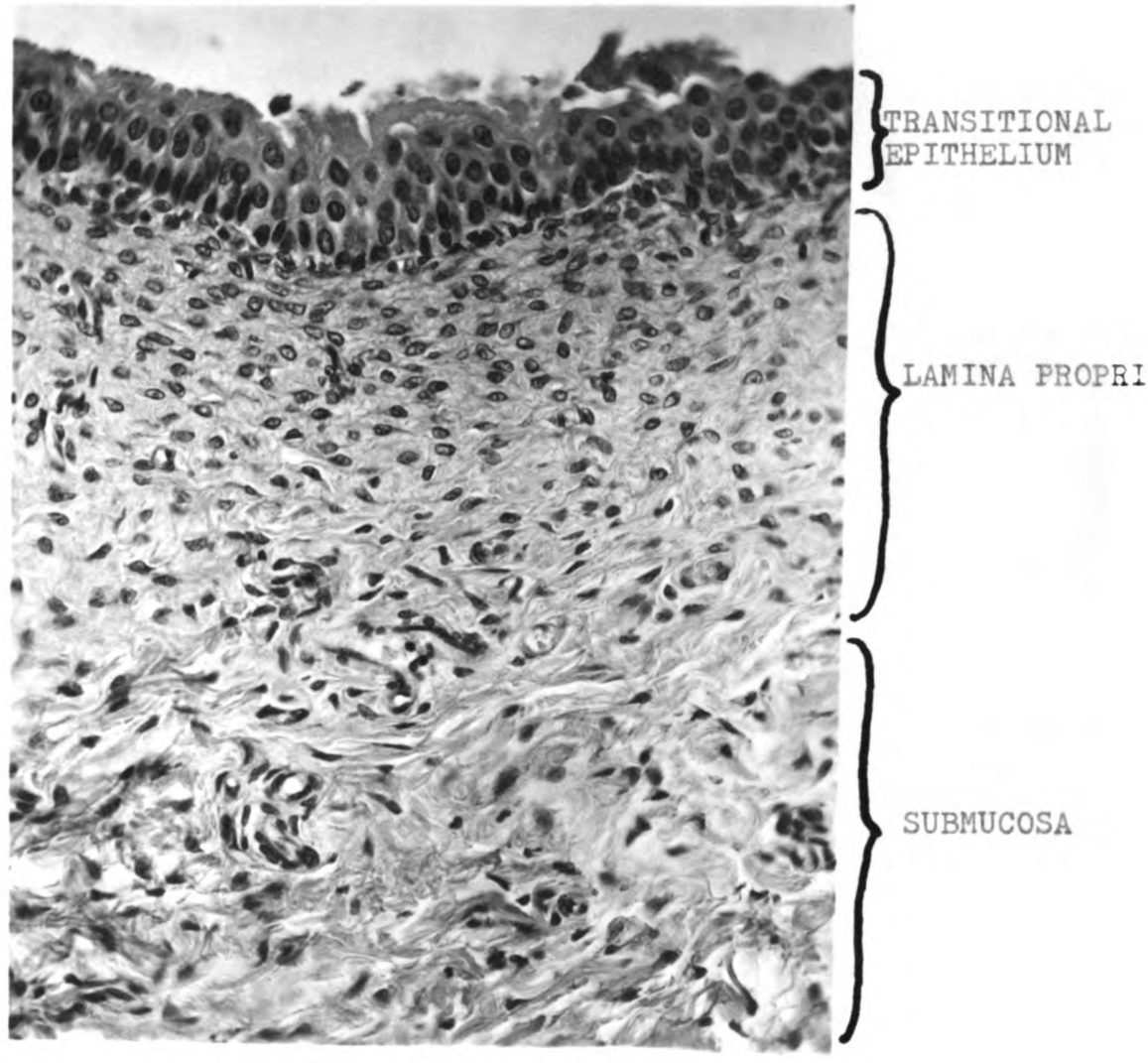


Figure 3
INCREASED DENSITY OF THE LAMINA PROPRIA AND SUBMUCOSA - POST-INFECTION. CAT 633

X235

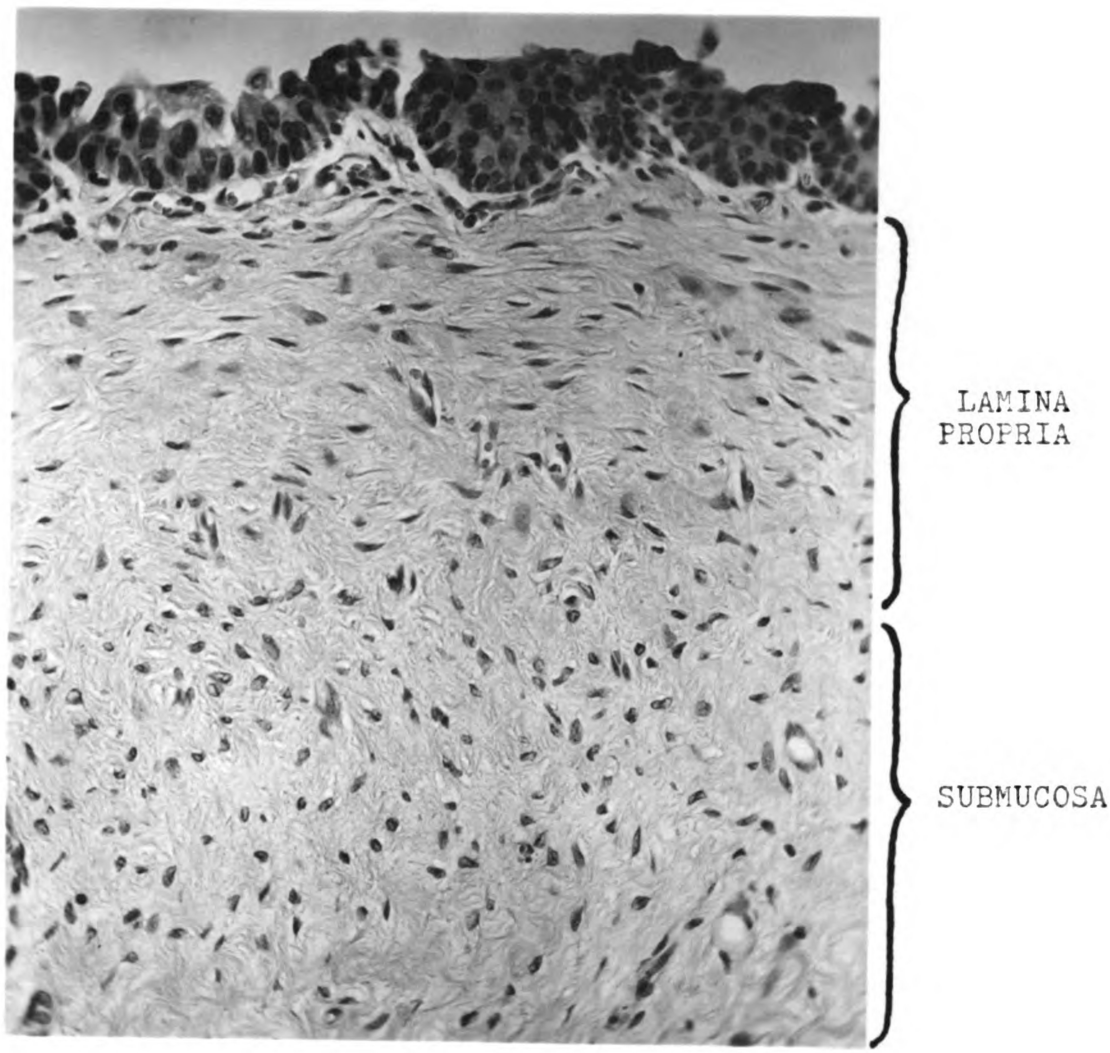


Figure 4
INCREASED VASCULARITY OF THE LAMINA PROPRIA - POST-INFECTION. CAT 209

X235

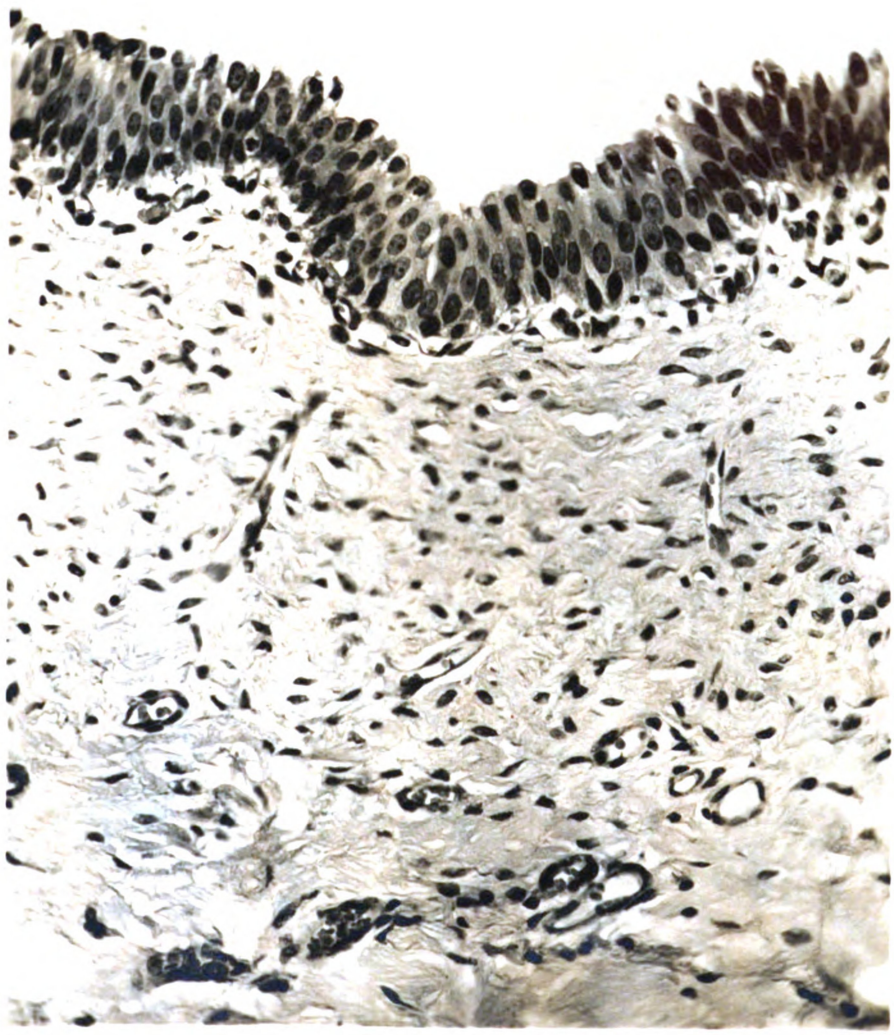
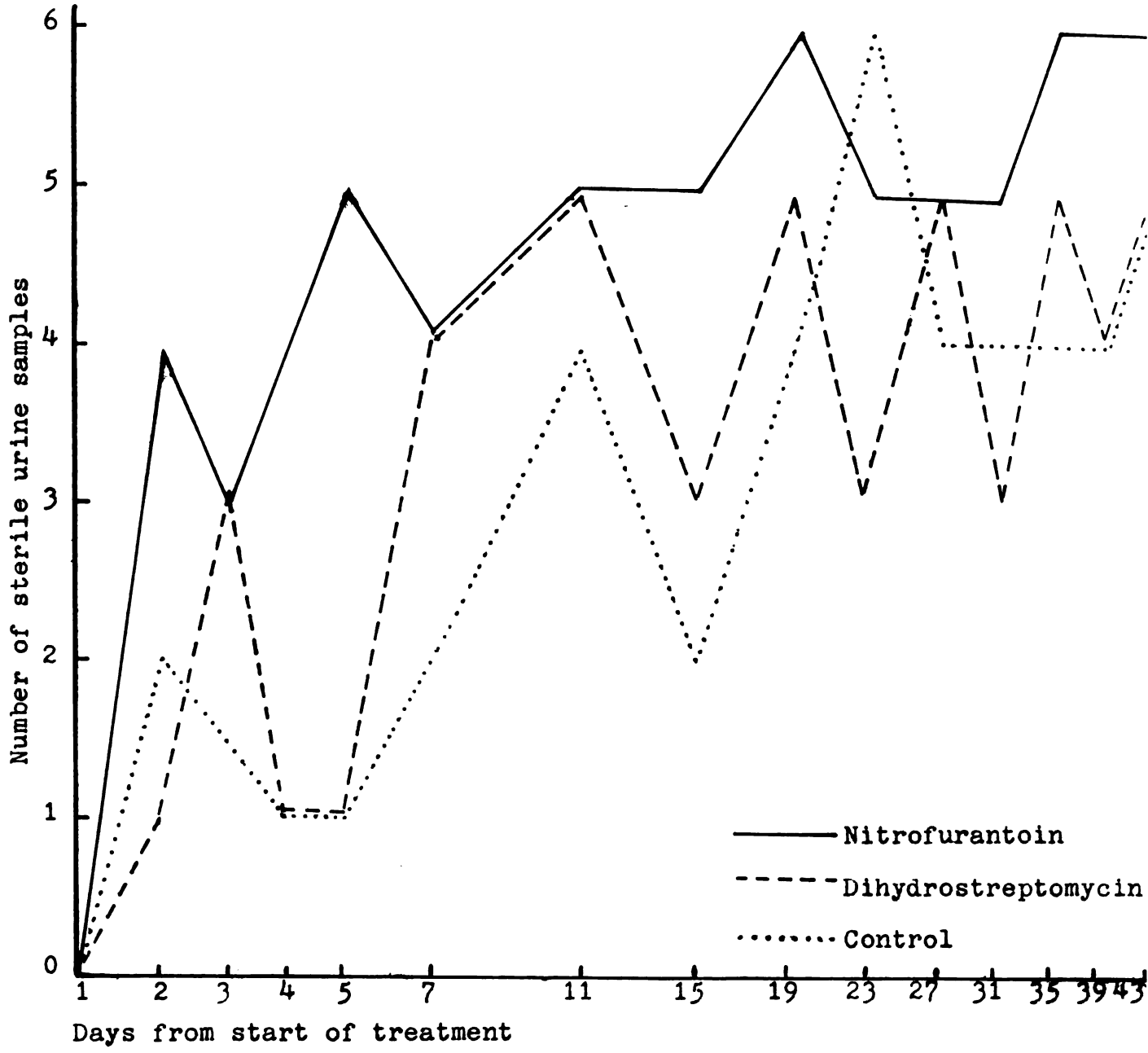


Figure 5
COMPARISON OF THE NUMBER OF BACTERIOLOGICALLY NEGATIVE URINE SPECIMENS OCCURRING IN EACH EXPERIMENTAL GROUP



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