RE-EPITHELIALIZATION OF THE FELINE URINARY
BLADDER FOLLOWING SURGICAL DE-EPITHELIALIZATION

By *6408*

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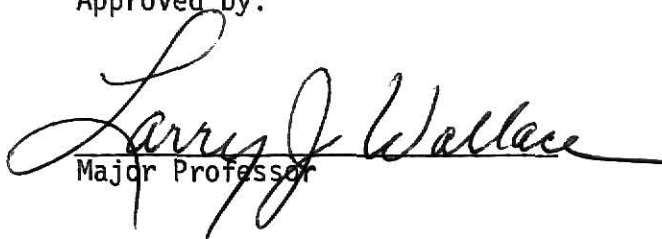submitted in partial fulfillment of the

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INTRODUCTION

Transitional epithelium lining the urinary bladder has been shown to have remarkable capabilities for repairing itself. This tissue has intrigued investigators ever since it was first described in the middle of the nineteenth century. The histology of the transitional cell has been fascinating because of its varied morphology and reaction to certain external stimuli. The tremendous technical advances current in histologic technique, histochemistry, and the use of the electron microscope have allowed researchers to study the relationships between structure and function of the transitional epithelial cell in greater depth.

Since the late 1800's controversy has existed among anatomists in regard to the morphology of transitional epithelium, especially in regard to the number of cell layers that comprise it and whether a true basement membrane exists between the epithelium and lamina propria. Others have investigated the growth and natural renewal of transitional epithelium. Interest in the mechanism of renewal as well as repair following injury has been anything but modest. The response of transitional epithelium to physical, chemical, or neurologic alteration has been explored. In recent years, the regenerative capacity of the whole organ has been examined. Knowledge of this extraordinary covering tissue is gaining. In-depth understanding of how it functions, and how it responds to various aspects of wounding, can increase the scientist's insight into the diseases of the bladder and the nature of its healing phase.

The present investigation was undertaken to study the regenerative

capacity inherent in the transitional epithelium lining the urinary bladder of the cat. The conclusions reached are hoped to lend insight into the response of the feline urinary bladder to specific urologic disease syndromes of the cat. Information gained from this study will have wide application in clinical medicine as well as basic biomedical interest.

REVIEW OF LITERATURE

Attention was drawn to the epithelial lining of the urinary bladder when Henle in 1841 introduced the term transitional cell to designate epithelial cells that are intermediate between stratified squamous and simple columnar (Mostofi, 1954). The transitional cell epithelium which lines the urinary bladder has been a matter of controversy for a long time. Histologically, transitional epithelium is stratified but there is disagreement in regard to the exact type of cells in the vesical mucosa and to the number of layers of strata they compose.

Brauer (1926) was concerned with the renewal of urinary bladder epithelium. He studied this phenomenon in cats and sheep. In the cat he found an epithelium of five to six nuclear rows in the mucosa of the contracted bladder. Each nuclear row was regarded as a stratum. The basal layer was composed of one nuclear row, the middle layer was composed of four to five nuclear rows, and the superficial layer was composed of one row of large cells, rounded on their free surface.

Petry and Amon (1966a, 1966b) conducted light and electron micro-scopic studies of transitional epithelia in the contracted and stretched state in various mammalian species. Analysis of cut sections in different planes led to the conclusion that transitional epithelium is multilayer-ed by not stratified. They described three strata with an intimate, under-lying basement membrane. The cells of the superficial and intermediate strata reach the basement membranes by means of slender cytoplasmic processes. Regele and Lunglmayr (1969) confirmed the results of Petry and Amon with the use of tritium-labelled thymidine.

Harvey (1909) studied the decrease in bladder wall thickness between

the contracted and distended bladder. He noted variations in the mucosa and described three cell layers; the basal cell layer rested in the tunica propria without an intervening basement membrane. He observed that when the bladder was distended, the basal layer was so flattened that it became somewhat confused with the connective tissue cells of the tunica propria.

Vacek and Schück (1960) studied the response of the rat bladder to experimental filling. They found the transitional epithelium of the empty rat bladder to be composed of five to six layers of cells. Cuboidal cells made up the basal layer, while those of the middle layer were pear-shaped, with the narrow end held between the cells of the basal layers. The surface of the bladder was covered with large, vesicular "covering" cells. They found the transitional epithelium to be situated on a thin basal membrane composed of reticular fibers. This observation confirmed the one made by Hanssens and Sebruyns (1957) on the existence of the basement membrane in the transitional epithelium in urinary tract of the dog, cat and rat.

Using electron microscopic techniques, Battifora, Eisenstein, and McDonald (1962, 1964) and Rosenquist (1969), studied the transitional epithelium of urinary bladders from humans and squirrels. They concluded that transitional epithelium is composed of many cell layers. However, these layers comprised three basic strata generally designated as superficial, intermediate, and basal. In addition, the epithelium was separated from the underlying lamina propria by a basement membrane. These studies were in agreement with the findings of Harvey (1909) and Petry and Amon (1966a).

Bizzozero (1894) noted that epithelial coverings, such as that covering

the luminal surface of urinary bladders, were tissues of transient elements and were capable of multiplying throughout the life of the individual. Since the time of Bizzozero, many investigators have been interested in the capability of transitional epithelial cells to reproduce themselves. Afterall, any epithelium is a protective barrier to the adverse external properties of the environment. The well-being of the entire organism is partly dependent upon the reparative ability of its epithelium.

Brauer (1926) reviewed the conclusions of earlier investigators. They held that new cells arise from the deepest cell layer of transitional epithelium and migrate to the surface. Brauer, on the other hand, found that mitotic activity occurred in the deep and intermediate strata with the greatest mitotic activity occurring in the intermediate stratum. He further noted that the superficial layer was composed of degenerate cells about to exfoliate.

Leblond, et al (1955) studied the mitotic activity in the urinary bladder epithelium of the albino rat. They not only confirmed the presence of mitotic activity in the basal and intermediate layer observed by previous workers, but found abundant mitoses in the surface layer which indicated to them that cellular replacement may also be provided by surface cells.

Leblond and Walker (1956) reported that mitosis is the only means of cell production in the renewal of cell populations. The number of mitoses occurring in a tissue represents the number of new cells formed in this tissue. Conclusions made by these workers implicitly associated renewal with irritation, assuming that mitotic activity was a regeneration in response to irritation.

The classification of adult organs in regard to mitotic activity was derived by Bertalanffy and Lau (1962). They classified adult organs as: (1) without mitotic activity, (2) with a small amount of mitotic activity daily, i.e., less than 0.8 to 1.5%, and (3) with mitotic activity greater than 0.8 to 1.5% daily. The rates of cell renewal determined by them for the urinary system was: (1) 3% daily renewal by mitoses for superficial transitional cells, and (2) 2% daily renewal by mitoses for the deep transitional cells. They arrived at a turnover time of 33 days for the superficial cells and 64 days for the deep cells. A final observation was that in tissues undergoing cell renewal there is a flow of cells from the basal layers, where cells arise by mitoses, to the superficial layers from whence older and often more differentiated cells desquamate.

Urinary bladder mucosa has been subjected to many experimental studies to determine its response to injury. Investigators have studied its cellular reaction to physical and chemical trauma. Alterations in the function of the entire bladder were also explored when the mucosa was subjected to various treatments.

The effect on micturition was studied by applying cocaine directly to the bladder mucosa (Barrington, 1921). In this study it was suggested that cocaine may prevent an emptying contraction of the bladder or may weaken or delay it. More recently Annis (1962) repeated the experiments of Barrington and concluded that cocaine applied to the mucosa did not have a direct effect on micturition. Annis suggested that the effects of cocaine on the bladder mucosa were not due to local action but were due to a central effect upon the spinal and cerebral centers concerned with the reflexes of micturition.

Carpenter (1951) examined the histologic changes in the feline bladder following bilateral parasympathetic denervation and prophylactic chemotherapy. He found the urine to be sterile, and microscopically there was no evidence of leucocyte infiltration, transitional epithelium denudation, nor cyst formation.

Mostofi (1954) made the observations that in humans transitional epithelium of the urinary bladder nevertheless was capable of: (1) non-neoplastic proliferation, (2) "metaplasia" into squamous, columnar, and cuboidal cells, and (3) neoplasia, not only to transitional, but also to squamous and glandular tumors.

Johnson (1964) reviewed the literature in regard to epithelium, including urothelium, and its reaction to injury. He concluded that three processes of repair occur, partly separately and partly simultaneously. The processes of repair described are migration, proliferation, and differentiation. He stated that these are also involved in regeneration of epithelium if regenerative activity is conceived as a separate subdivision of reparative activity.

The bladder mucosa of rats was found by Locher and Cooper (1970) to respond to chemical injury with cyclophosphamide by an initial period of necrosis, followed in 4 days by epithelial hyperplasia. By 2 weeks, the hyperplastic epithelium increased to a maximum thickness of 10 to 12 layers. The hyperplasia persisted until 10 weeks when most injured areas had returned to a normal thickness. Hyperplastic epithelium appeared to have a relatively slow turnover time. Earlier Santana (1963) studied the effect of a sulfonamide on the bladder mucosa of rats after oral administration.

After isolating bladder pouches from the main compartment of the bladder and administering the drug, he observed marked urothelial hyperplasia in the main compartment and no reaction in the isolated pouches. This substantiated that the hyperplastic reaction of urothelium to certain drugs was due to their execretion into the urine.

Another chemical often used to study wound healing is colchicine which stops mitotic activity in metaphase. Popovic and Unkovic (1965) studied the effects of colchicine on urinary bladder mucosa in rabbits which was already regenerating following injury. They found that colchicine initially delayed the process of regeneration of injured transitional epithelium but the epithelium finally compensated.

Rasmussen (1966) investigated the morphologic changes occurring during the healing of linear wounds in the urinary bladder and compared the observations with biochemical findings in the same wounds. He found that transitional epithelialization occurred to fill the linear defect in the original mucosa while the remaining bladder wall layers healed by formation of granulation tissue and collagen, whereafter smooth muscle fibers grew into the scar from both sides. Histochemically, there was a rapid increase of hexosamine uronic acid while simultaneously the hydroxproline content diminished. Rasmussen (1967) later studied changes in content of acid mucopolysaccharide and collagen during healing of urinary bladder wounds in rabbits. The changes were shown to correspond with alterations in cutaneous wound healing.

McMinn and Johnson (1955), and Aurora and Gupta (1967) found that transitional epithelium of urinary bladders in cats and rabbits, respectively, regenerated rapidly after first excising a small area from the original

luminal lining. Sanders, et al (1958) demonstrated the ability of bladder epithelium to fully regenerate in mongrel dogs following total excision.

The regenerative ability of urinary bladder mucosa was examined and observed in other instances. Neuhoff (1917) used fascial transplants to cover defects in the wall of urinary bladders or a defect over an entire apex in dogs. There was no interference with organ function and epithelium overgrew the transplants. Koontz (1929) used dead ox fascia lata that had been preserved in 70% alcohol as transplants to cover defects of hollow viscera in dogs. The mucosa of urinary bladders overgrew the transplants which covered experimentally induced defects in their walls within three months. DeMuth (1953) and Baret, et al (1953) used fascial grafts in dogs to cover visceral defects. By 52 days following operation, the grafts were covered with epithelium.

Joseph and Thomas (1958) transplanted ileal autografts with experiment-ally produced urinary bladder defects in rabbits. The graft formed part of the bladder wall. When the original blood supply to the autograft was preserved, the ileal mucosa retained its natural histological appearance. If the ileal mucosa was in any way disrupted, it was overgrown and replaced by adjacent transitional epithelium.

Tsuji, et al (1961, 1963) used alcohol or formalin-preserved bladder heterografts for experimental cystoplasty in dogs and found that although by two weeks the preserved graft would loosen, it served initially as a scaffold on which connective tissue developed. Urothelium had lined the connective tissue by 52 days. Goldstein and Dearden (1966) sutured a ped-iculated omental flap over experimentally prepared urinary bladder wall defects in rabbits. Complete regeneration of the bladder wall occurred by

44 days on the luminal side of the omentoplast with a transitional cell lining.

The regenerative ability of the epithelial lining of the urinary bladder of dogs was studied by Annis (1962). The regenerative power of urothelium was demonstrated and with complete epithelial regeneration, normal function was restored.

Schiller (1923) studied regeneration of the resected urinary bladder cranial to the trigone in rabbits and found regeneration of the bladder by 3 months with histologic similarity to the original bladder. Since previous investigators had only performed subtotal cystectomy, Bohne, et al (1955) studied bladder regeneration in dogs following total cystectomy. In 14 to 16 weeks the regenerated bladder had a wall comprised of transitional epithelium on a granulation base, a zone of fibroblastic giant cells, a zone of active fibroblasts, a zone of fibrous tissue with intermingled smooth muscle bundles, and an outer zone of fatty tissue. Than, et al (1956) examined the functional effects on the urinary bladder in dogs following total cystectomy in addition to the histologic response. In their work the bladders regenerated. They were formed of smooth muscle lined by transitional epithelium, and, although of limited capacity, were capable of contracting at intervals and emptying. Iida (1960) studied bladder regeneration in dogs with the purpose of making a bladder using the marked regenerative ability of the bladder epithelium as a stimulus. After subtotal cystectomy, the residual vesical stump was sutured to either the posterior surface of the rectus abdominus muscle or the dead space following rectal amputation. Regenerative activity was noticed only in association with the muscular tissue. Epithelium, resembling transitional epithelium

was noted 14 and 30 days postoperatively. Johnson, et al (1962) studied bladder regeneration in dogs over lucite molds. A fibrous pouch formed over the mold with a limited amount of regenerated epithelium after 42 to 92 days.

Richardson (1952) performed total cystectomy in a 66 year old man because of chronic ulcerative cystitis. A new bladder was regenerated but no postoperative histologic evaluation was made.

Baker, et al (1959),Sterrett (1962), and Tucci and Haralambidis (1963) reported subtotal or total cystectomy for urinary bladder carcinoma in human patients followed by bladder regeneration. McMallum (1965) reported on subsequent regrowth of a urinary bladder in a man which was resected due to gangrene.

EXPERIMENTAL PROCEDURE

I. Objectives:

    a. To determine whether urinary bladder transitional epithelium of the feline was capable of full regeneration after the mucosa was excised to the level of the trigone.

    b. To determine the length of time for total re-epithelialization.

    c. To gain insight into the nature or mechanism of transitional epithelial regeneration.

    d. To describe a surgical procedure that accomplished total urothelial excision.

II. Acquisition and Care of Experimental Animals:

Healthy adult, male and female, domestic cats were acquired from the Department of Surgery and Medicine at Kansas State University's College of Veterinary Medicine.

All cats were allowed a 5-day acclimation period before surgery. The experimental cats were kept in stainless steel cages prior to surgery and in stainless steel metabolism cages throughout the entire postoperative period. The housing area was in the experimental surgery facility of Dykstra Veterinary Hospital. A description of the physical appearance was recorded for each cat and included length and coloration of haircoat, sex, and weight when acquired. A complete physical examination was performed, a blood sample submitted for a complete blood count, and a urine sample was collected for routine urinalysis and aerobic bacterial culturing. All cats had been vaccinated against feline panleukopenia prior to acquisition. Throughout the acclimation period, a daily rectal temperature was recorded, and observations were made in

regard to physical health. At the end of the 5-day observation period, total excision of the transitional epithelium of the urinary bladder of each cat was accomplished, according to the surgical technique outlined below.

During the postoperative observation period of each cat, physical examinations were performed daily and included an assessment of physical status, attitude, approximate water intake and urine output, defecation, rectal temperature, hydration, and character of visible mucous membranes. Every 7 days a blood sample was collected for complete blood count, urine was collected for routine urinalysis and aerobic bacterial culture on blood and MacConkey's agar, and the cat was weighed.

The diet of each cat throughout the entire investigational period was Prescription Diet C/D$^{(R)}$ (Hill Packing Company, Topeka, Kansas). Water was available at all times and was supplied fresh each day.

No fluid or chemotherapy was administered at any time throughout the investigational period except that described for the surgical procedure.

III. Normal Feline Urinary Bladder Transitional Epithelium:

The bladder of one healthy adult male cat was utilized as a reference for normal feline transitional epithelium and lamina propria. This cat was subjected to all aspects of the surgical procedure except that the transitional epithelium of its urinary bladder was not excised. The urinary bladder was obtained while the abdominal cavity was open and after severing all tissues that would otherwise prevent its removal. The pelvic urethra was severed one centimeter distal to the neck of the bladder. The cat was euthanatized.

The bladder was fixed in 10% neutral buffered formalin and processed for histologic examination using the same technique described below for the experimental bladders.

Observations about the morphology of the cells that comprised the transitional epithelium, the lamina propria, the relationship of the transitional epithelium to the underlying lamina propria, and the absence or presence of mitotic figures were made.

IV. Surgical Technique:

Nineteen adult, domestic shorthair cats of both sexes were used in the investigation. The method of operation used to remove the entire transitional epithelial lining of the urinary bladder was as follows:

1. Anesthesia:

   a. Premedication:

   Atropine sulfate, 0.02 mg./lb., administered subcutaneously.

   b. Induction:

   Thiamylal sodium, 2.5% intravenously administered, followed by endotracheal intubation.

   c. Maintenance of anesthesia:

   Methoxyflurane plus O_2 or halothane plus O_2 to effect by inhalation through the endotracheal catheter.

2. Fluid administration:

   Lactated Ringer's Solution was administered intravenously at the rate of 30 to 45 drops per minute and was begun following induction of anesthesia and discontinued when the pre-determined volume of 10 ml. per pount of body weight had

been given. This fluid was given to assure urination
as soon after operation as possible so that the bladder
was flushed free of any accumulating blood clots.

3. Surgical preparation:

   The hair was clipped from the ventral abdominal skin with
   a No. 40 Oster Clipper blade and the skin scrubbed with
   surgical soap for 5 minutes. After scrubbing, the skin was
   rinsed with 70% isopropyl alcohol and allowed to dry. Prior
   to draping, an isopropyl alcohol-iodine antiseptic solution
   was applied and allowed to dry.

4. Surgical procedure:

   Four sterile towels were applied to cover the prepared ventral
   abdomen except for the immediate operative field. These were
   followed by placement of a laporotomy sheet.

   A midventral abdominal incision through the skin and subcutan-
   eous tissue was made extending caudally from 2 cm. posterior
   to the umbilicus to a point even with the brim of the pelvis.
   Skin drapes were applied. Hemostasis was accomplished by
   compression, forcipressure, or ligation as needed.

   The abdominal cavity was entered by incising through the linea
   alba. The urinary bladder was exteriorized and isolated from
   the abdominal cavity and remaining viscera by packing sterile
   saline-moistened 4 x 4 sponges around its neck (Figure 1).

   A 19-gauge needle on a 20 cc. syringe was used to puncture
   the urinary bladder on its mid-dorsal surface in order to
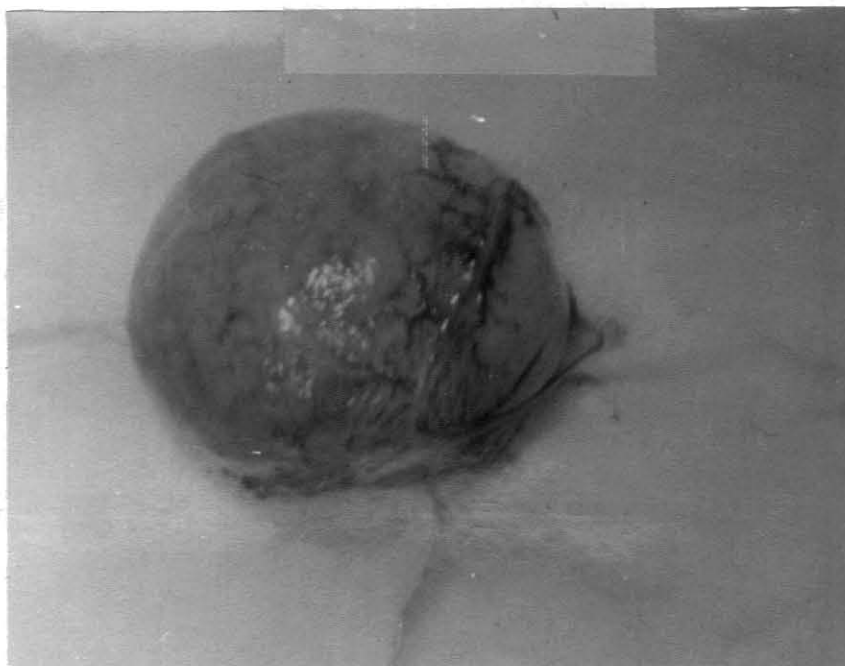
Fig. 1: Surgical procedure. The urinary bladder was
exteriorized and isolated from the abdominal
cavity with moistened sponges.

aspirate all urine from within. This aspirated sample in each case was submitted to the laboratory for routine urinalysis and aerobic culture.

The cystotomy incision was made on the mid-dorsal surface and extended the entire length of the vesicle ending immediately proximal to the trigonal area. Extending this incision distally into the trigonal area was avoided to assure that cystotomy closure did not interfere with the functional aspects of the trigone. This incision allowed the urinary bladder to be turned inside out without tearing the incised bladder margins. The ureteral orifices were visualized to facilitate the caudal extent of mucosal excision.

A 10 ml. syringe fitted with a 26-gauge needle and filled with sterile physiological saline was inserted beneath the transitional epithelium at the cranial edge of the opened bladder. Saline was injected to raise a small wheal of epithelium. The epithelial wheal was then converted into a large bulla by gently forcing a stream of saline under the epithelium in all directions from within the wheal (Figure 2 and Figure 3). A plane of cleavage was easily developed between the elevated epithelium and the submucosa. By blunt dissection the epithelium was completely lifted from the underlying submucosa over the entire luminal surface caudally to the level of the ureteral orifices. The epithelium was then sharply incised by a

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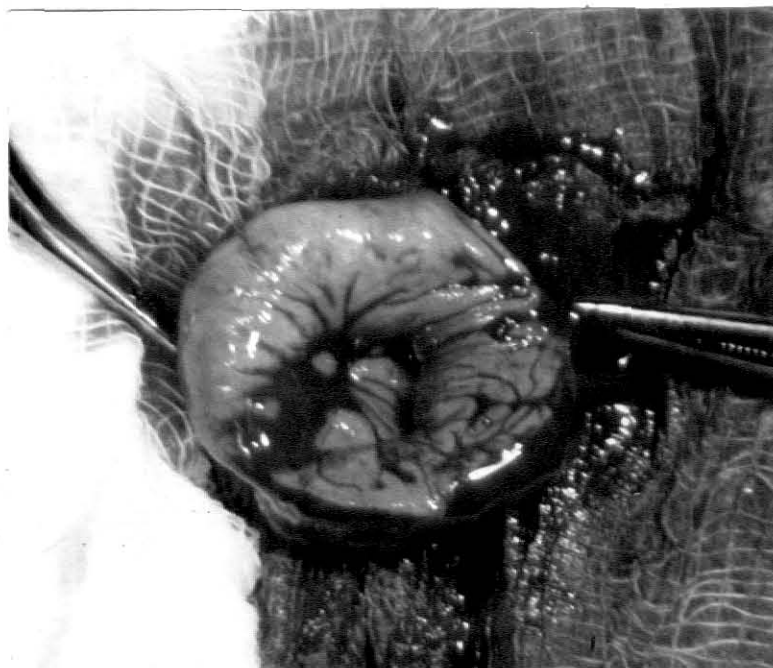
Fig. 2: Surgical procedure. Opened bladder with bulla developed by forcing saline beneath the epithelium into the submucosa.

Fig. 3: Surgical procedure. Opened bladder with bulla in the submucosa developed to a greater degree than seen in Fig. 2.

circular incision that passed tangentially to the ureteral orifices. Thus a circular incision of transitional epithelium at the cranial margin of the trigone allowed removal of the urothelium from the luminal surface. Hemorrhage of the underlying submucosa was controlled by firm application of a dry gauze sponge for one to two minutes (Figure 4).

The cystotomy incision was closed with one layer of continuous Connell sutures using 4/0 medium chromic surgical gut (Figure 5). Once closure was completed, the incision was checked for leakage by carefully distending the vesicle with saline injected through the closed cystotomy incision. Accidental laceration of the gut suture was avoided by careful insertion and withdrawal of the needle. Once the bladder was mildly distended, gentle pressure on the lateral bladder walls and close observation of the cystotomy incision line disclosed any gross leakage. Any area of leakage was satisfactorily sealed by placing a single Lembert suture with 4/0 medium chromic surgical gut.

Once the cystotomy incision was closed, the salinized sponge packing was removed from around the neck of the bladder and the bladder was returned to the abdominal cavity. The linea alba was closed with 3/0 medium chromic surgical gut sutures placed in a simple interrupted pattern after which the skin towels were removed and discarded. The subcutaneous tissue was closed with 3/0 medium chromic surgical gut sutures placed in a simple continuous pattern. The skin was closed with 3/0 monofilament
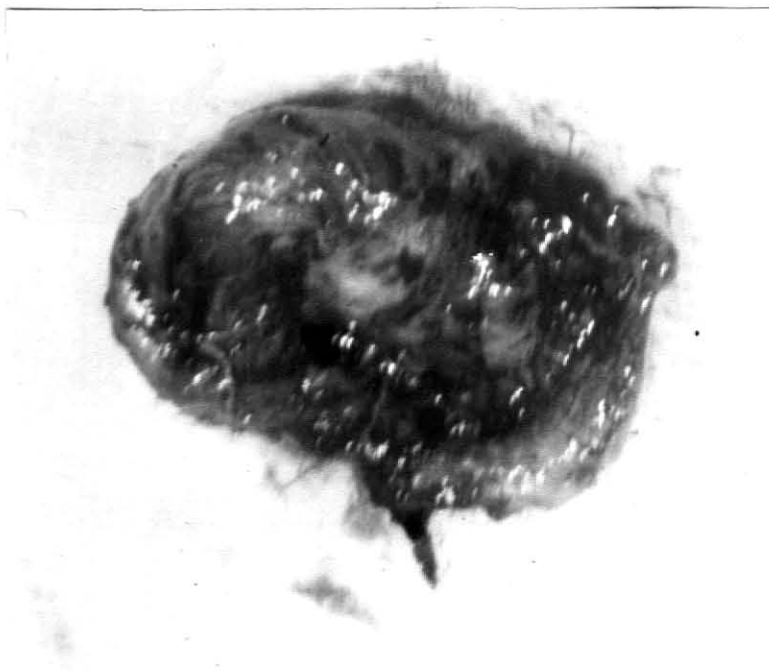
Fig. 4: Surgical procedure. Luminal surface after
total excision of epithelium. Hemorrhage
was controlled by firm applications of a
dry gauze sponge for one to two minutes.
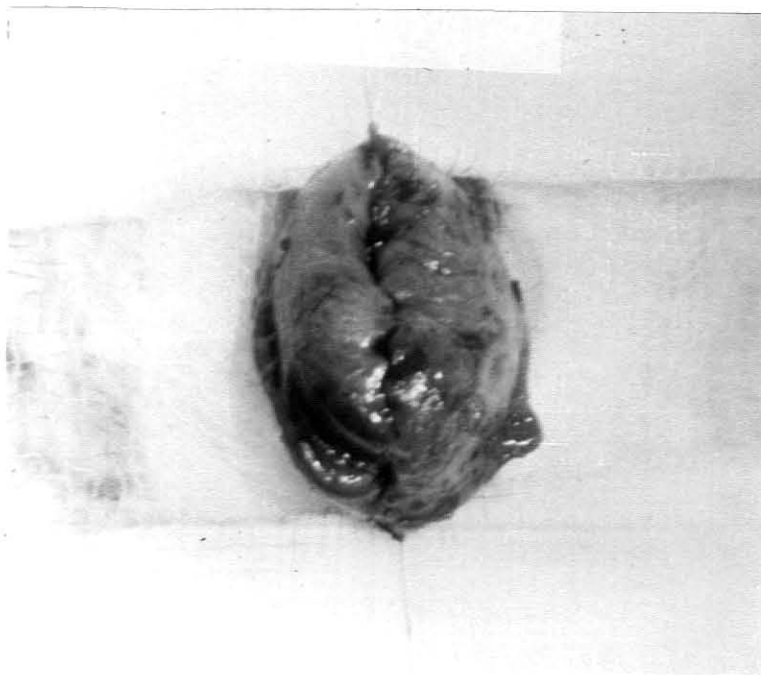
Fig. 5: Surgical procedure. The cystotomy incision
was closed with one row of a continous Connell
suture pattern. The suture line was checked
for leakage prior to returning the bladder to
the abdominal cavity.

nylon sutures placed in a continuous horizontal mattress pattern.

When the cat recovered from anesthesia, it was returned to the experimental ward and cared for and observed daily as described above.

V. Euthanasia of Experimental Cats:

On the 1st, 3rd, 7th, 10th, 14th, 21st, 28th, 63rd, 70th, 88th, and 112th day following the experimental cystotomy-urothelial denudation, one cat from the experimental group was euthanatized. On the 35th, 42nd, 49th, and 56th day following surgery, two cats were euthanatized. No attempt was made to alternate males and females in this study. Prior to euthanasia a complete physical examination was performed on each cat. Blood and urine samples were collected, rectal temperature was obtained, and the cat was weighed. A complete blood count, urinalysis, and aerobic bacterial urine culture were obtained. The cats were euthanatized with a lethal dose of concentrated pentabarbital sodium administered intravenously.

VI. Tissue Preparation and Histologic Evaluation:

The urinary bladder from each cat was obtained immediately following euthanasia. A midventral abdominal incision was made and the urinary bladder exteriorized. The middle and 2 lateral ligaments were severed along with the 2 ureters. Adipose tissue was stripped from the neck of the urinary bladder and pelvic urethra. The pelvic urethra was severed one centimeter distal to the bladder neck.

A small urinary catheter was introduced into the lumen at the cut end of the pelvic urethra and inserted into the urinary bladder lumen for

emptying. A ligature was then **tied around the urethra**. The urinary
bladder was infused with 10% **neutral buffered formalin** (10% BNF) through
the catheter until it was filled. When the bladder was full, the cathet-
er was withdrawn and the ligature was tied simultaneously. This left the
bladder moderately distended with intraluminal 10% BNF.

The urinary bladder was placed in a jar containing 10% BNF. The
ratio of urinary bladder tissue to 10% BNF was 1:10. The urinary bladders
were kept in this fixative solution for 3 days prior to cutting and pro-
cessing.

After 3 days each bladder was removed from the 10% BNF, dried with
paper toweling, and placed on white paper. With the bladder laying on
its ventral surface, an outline was traced to show the shape of the bladder.
The position of the ureteral orifices was indicated on the drawing. With
the bladder placed on its left lateral surface, a second tracing was made
and again the position of the ureteral orifices was designated. Finally,
a measurement in millimeters was made of the length from the ureteral
orifices to the apex, using the dorsoventral tracing, and another measure-
ment in millimeters was made at the widest point using the lateral tracing.

After tracing and measuring, each bladder was marked logitudinally
with India ink along its middorsal surface (Figure 6 and Figure 7). Each
bladder was cut transversely at intervals of approximately 3 millimeters.
The first cut was made approximately 3 mm. cranial to a transverse plane
through the ureteral orifices. The slicing was done in a free-hand fashion,
as near perfectly transverse as possible, for the entire length of the
bladder (Figure 8). This procedure made possible a uniform estimate of the
distance of a given section from a transverse plane through the ureteral
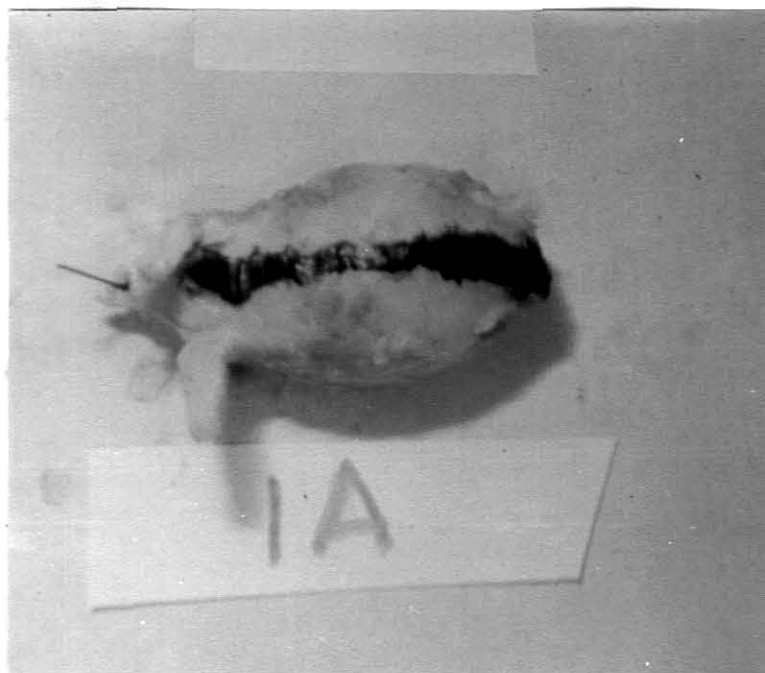
Fig. 6: Urinary bladder shown with its dorsal surface marked with India ink prior to cutting-in and processing.

Fig. 7: Urinary bladder shown in Figure 6 with its right lateral surface visible.

orifices. It also provided a means of determining the rate of re-epithelialization on histologic examination.

The middorsal point of each tissue slice was marked by either cutting through the entire wall or cutting a notch in the wall. Urinary bladder slices were dehydrated through graded ethanols, cleared in xylene, and embedded in Paraplast[R] (Sherwood Medical Industries, Inc.) with the cranial face of each transverse slice uppermost. Sections were cut at 6 microns and stained in racks with hematoxylin and eosin. Histopathologic examination of each stained tissue section was by light microscopy.
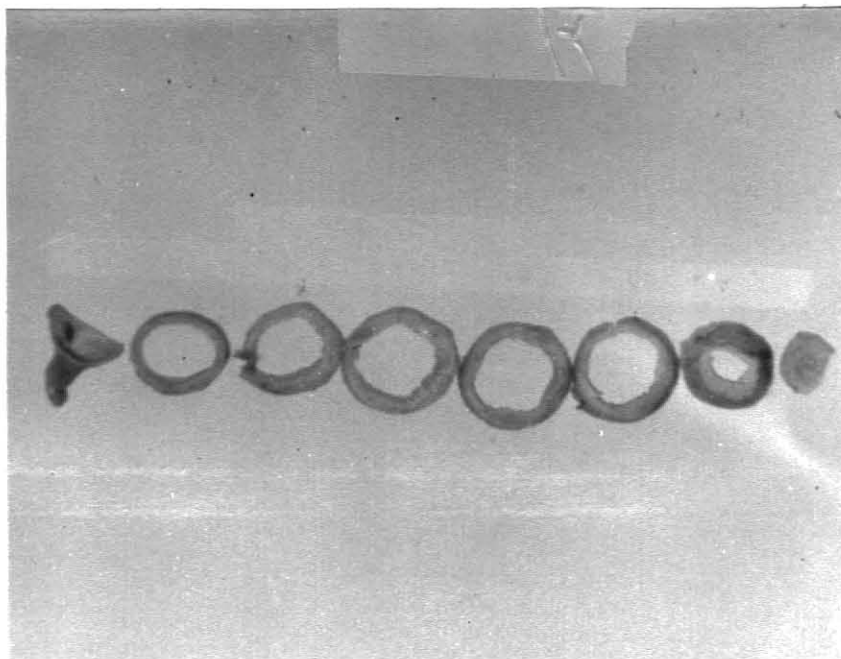
Fig. 8: Slices of urinary bladder cut transversely at distances of approximately three millimeters. The first slice was made approximately three millimeters cranial to a transverse plane through the ureteral orifices.

RESULTS

1. Clinical Studies:

All of the cats remained in good health throughout the 5-day long,
pre-operative, holding period. A survey of their clinical data, taken
as an average for each cat at a given point during the investigation, was
evaluated. The details of the results are given in figures and tables
designated below. The average water intake and urine output for 112 post-
operative days are given in Figure 9. The average rectal temperatures
for 112 postoperative days are given in Figure 10. Data on average body
weight for 112 postoperative days is presented in Figure 11. Average
hemogram and urine analysis are listed in Tables I and II, respectively.
Bacteriologic data is presented in Table III.

All of the cats survived the experimental period. Cats who were
euthanatized within the first 14 postoperative days were in various degrees
of physical embarrassment due to the stress of anesthesia and surgery, and
due to the physiologic changes brought about by urothelial denudation.
Those cats maintained longer than 14 days following surgery gradually re-
turned to a near-normal or normal physiologic status. These cats had un-
eventful convalescent periods throughout the remainder of the investigation.
The clinical signs and response to the operation followed a general trend
for each cat and resulted in a continuity which transcended the overall data
for the entire experimental group.

All of the cats were awake from anesthesia and in sternal recumbency
within one hour following the end of the anesthetic period. Urination occurred
within two hours. The volume of urine was usually small (less than 10 cc.)
and hemorrhagic. The cats, once fully recovered from anesthesia, spent the

majority of their wakeful hours either with a very quiet, depressed attitude, crouched with their legs drawn in tightly under their body, and very still, or they were crouched in a humped-up position for urination. They often remained in this latter position for several minutes because of an apparent urgency related to a desire to urinate. Frequently, these were unproductive attempts to urinate. Most cats remained in this depressed condition for three days, although a few cats were in a state of depression for as long as 5 days. Most cats were inappetant for 4 days. The first day after surgery, they experienced anorexia and gradually regained interest in food so that the most inappetant cat exhibited a normal appetite by the sixth postoperative day. All cats had a moderate to marked reduction in water intake and urine output for 2 to 10 days following surgery (Figure 9). This resulted in mild dehydration (4% or less) in more than half of the cats. Clinial dehydration, based on loss of skin tone and dry mucous membranes, was evident by the fourth postoperative day and was in remission by the tenth day.

All cats exhibited a postoperative febrile response that lasted between 4 and 10 days (Figure 10). A febrile response was regarded as approximately one degree Farenheit above the average temperature recorded during the 5 days prior to surgery. They were considered afebrile once the rectal temperature had returned to the pre-operative range.

Approximately one-third of the cats exhibited some degree of clinical anemia during the investigation period. This was determined by daily examination of the relative color of visible mucous membranes, weekly packed cell volume and hemoglobin determinations.

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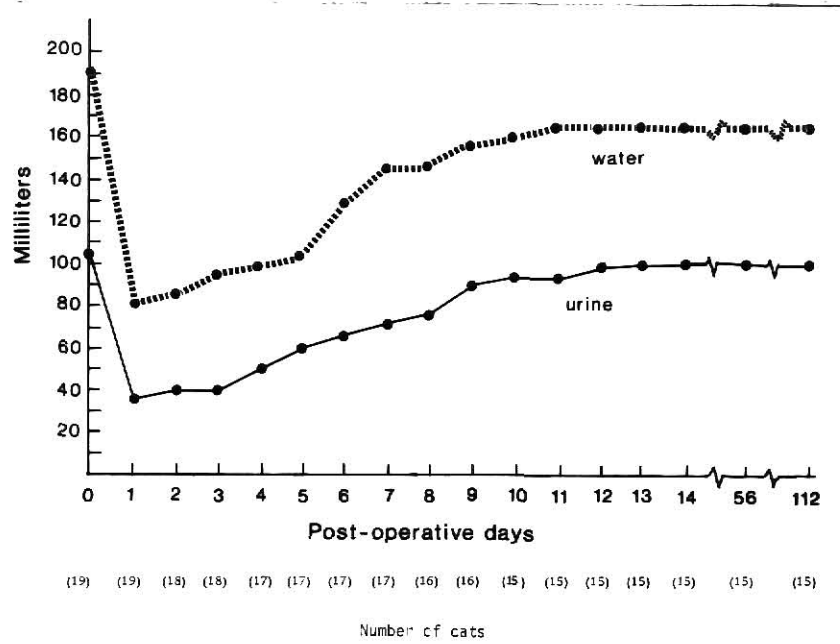
Fig. 9: Average water intake and urine output.
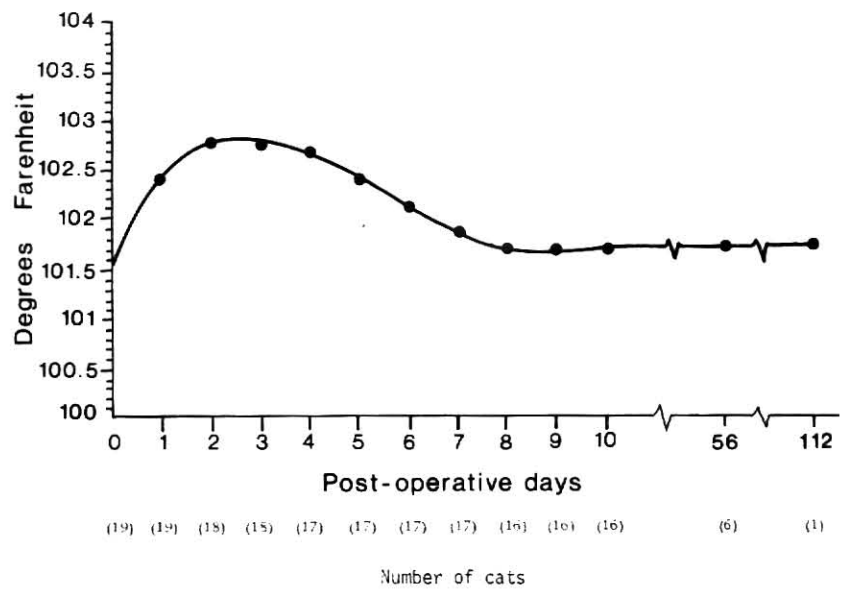
Fig. 10: Average daily rectal temperature.

The physical status recorded on each clinical data chart was based on criteria used in small animal anesthesia (Lumb, 1963). A status of 4 was assigned when an animal was depressed, inappetant, febrile, exhibited gross hematuria, and exhibited dysuria. A physical status of 3, 2 or 1 was made as these signs diminished or disappeared, and the cat was observed to be in a normal physiologic state.

An analysis of body weight revealed a mild loss for the first two weeks postoperatively, but a gradual return to the pre-operative weight was evident by the third week. By the end of the investigation, a moderate weight gain was recorded (Figure 11).

2. Clinicopathologic Data:

    a. Hematology:

    The hemograms were averaged together for the cats at each interval of the entire investigative period (Table I). Data from cats whose postoperative period was 1 day, 3 days, 10 days, 88 days, and 112 days were not averaged since they represented only one experimental animal respectively.

    Analysis of the average hemogram revealed a decrease in the packed cell volume and hemoglobin by the end of the first post-operative week. These parameters increased gradually to levels higher than the pre-operative values. The total leucocytes were within normal range as was the differential count. However, a mild regenerative left shift and increase in neutrophils occurred during the early post-operative period of approximately 7 days. Individually, a few cats had mild lymphocytic and eosinophilic depression during
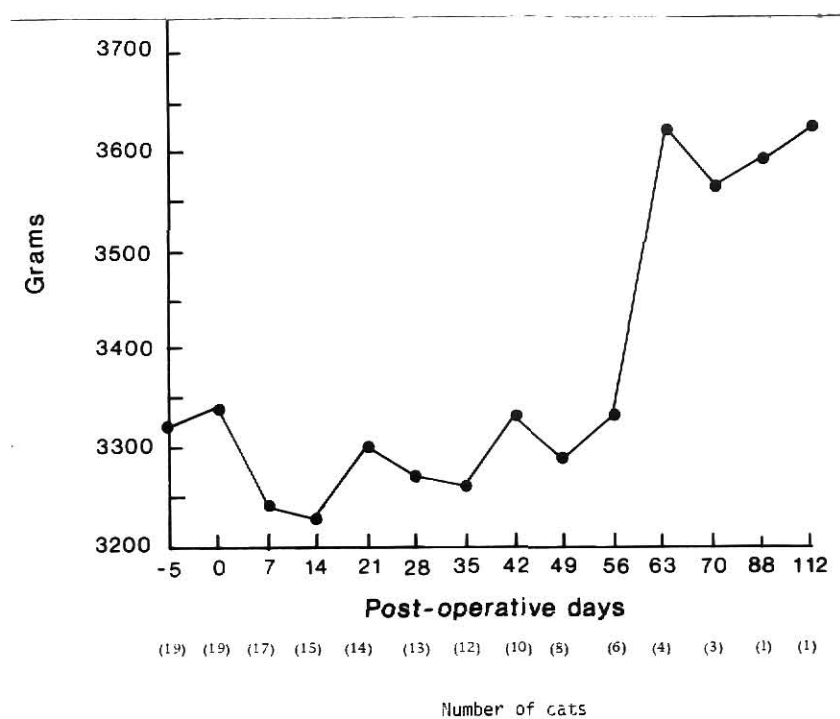
Fig. 11: Average body weight.

Table I: Average hemogram for the cats spanning the entire investigative period.

Day	No. of Cats	PCV (%)	Hb (Gm%)	Total Leucocytes/Cumm	Absolute Differential Leucocyte Count				
					Band	Neut.	Lymph.	Mono.	Eos.
-5	19	31.4	10.4	12,121	71	7,678	3,984	88	495
*0	19	32.3	10.9	12,654	64	8,023	4,166	63	910
1	1	26	8.6	22,600	226	16,950	5,198	0	226
3	1	28	9.6	11,500	0	8,510	2,415	115	460
7	17	30.2	10.0	15,333	351	10,294	4,117	193	278
10	1	29	9.5	21,100	422	15,192	4,220	0	1,266
14	15	30.4	10.1	14,200	299	9,464	3,823	98	349
21	14	30.4	10.2	13,963	205	8,908	4,379	155	389
28	13	31.9	10.3	13,659	134	8,902	4,502	126	382
35	12	32.8	10.9	12,775	73	7,860	4,412	255	340
42	10	32.3	11.0	12,870	102	7,906	4,781	228	388
49	8	33.1	11.2	13,750	74	7,781	5,346	181	381
56	6	34.5	11.2	13,000	91	7,135	4,621	265	362
63	4	33.0	11.3	14,025	61	8,783	4,806	156	219
70	3	33.6	11.4	16,300	0	10,835	5,461	155	415
88	1	34	11.2	12,200	0	6,710	5,124	0	366
112	1	37	12.3	13,600	136	7,480	5,440	136	408

*Day of surgery

the first 2 weeks following surgery which gradually returned to normal. But on the average for the experimental group, the hemograms remained within normal physiologic limits.

b.   Urine Analysis:

The data gathered by routine urinalyses were evaluated in composite form when averages were not possible. Other data were averaged (Table II). The urinalyses of all cats evaluated throughout their respective investigative periods revealed a fair degree of uniformity as to general trend. All urine samples were red in color due to hemorrhage for 2 weeks following urinary bladder mucosal denudation. Four cats had gross hematuria for 3 weeks following surgery. The urine of those cats whose postoperative period was longer than 3 weeks exhibited a change in color from blood red to straw or reddish-brown. Those urine specimens which were of a straw or reddish-brown color were cloudy. Urine sediment, in many instances, revealed "rbc/hpf: too numerous to count" for one to two weeks after the urine had changed from a red to a straw color. However, the presence of erythrocytes in the urine sediment declined steadily from the fourth or fifth week following surgery. The urine sediment contained a variable number of leucocytes. A pyuria paralleled the postoperative hemorrhagic cystitis. The urine revealed a marked to moderate proteinuria and occult blood which progressively declined from the immediate postoperative period to 5 or 6 weeks following surgery. The more persistent proteinurias, with or without occult blood, paralleled the presence of cells, cellular debris, bacteria, and spermatozoa in the

Table II: Average urinalysis for the cats spanning the entire investigative periods**

Day	No. of Cats	Specific Gravity	pH	Protein (1+ to 4+)	Occult Blood (1+ to 4+)	RBC/hpf	WBC/hpf
-5	19	1.034	6	0.5	0.1	3	2
*0	19	1.037	6	0.6	0.1	3	4
1	1	1.032	6	3.0	3.0	TNTC	0
3	1	1.040	6	3.0	3.0	TNTC	0
7	17	1.047	6	2.9	3.5	TNTC	27
10	1	1.040	6	3.0	3.0	TNTC	0
14	15	1.052	6	3.3	3.9	467	37
21	14	1.048	6	3.1	3.6	350	37
28	13	1.043	6	2.8	3.1	273	31
34	12	1.041	6	2.6	2.8	94	19
42	10	1.041	6	2.3	1.9	45	10
49	8	1.044	6	2.1	1.6	19	7
56	6	1.047	6	2.3	1.0	16	6
63	4	1.046	6	1.8	0.8	8	3
70	3	1.053	6	2.0	0.7	5	2
88	1	1.042	6	1.0	0	4	1
112	1	1.045	6	1.0	0	0	0

* Day of surgery
**See the text for other aspects of the urinalyses not adapted to average figures.

34

urine sediment.

c.   <u>Bacteriology</u>:

Samples of urine, cultured aerobically on blood and MacConkey's agar, at various times throughout the experimental period, grew bacteria (Table III). These bacteria, once isolated and identified, were considered to be either contaminants or non-pathogens of the feline urinary tract.   There was no correlation between positive urine cultures and other clinicopathologic data or clinical signs.

3.   <u>Gross Pathology</u>:

The bladder that was used as a reference for normal feline trans- itional epithelium and lamina propria was contracted following cystotomy. Infusion with 10% BNF for fixation was limited to a volume of less than 20 milliliters (Figure 12).   When this bladder was cut following the fixation period, its luminal surface was similar in appearance to that described for bladders in the postoperative period of more than 35 days (see below) (Figure 13).

At necropsy, no gross abnormalities were observed except that in a few cats some omentum had adhered to the middorsal cystotomy incision and apex of the bladder.   The omentum was easily freed from the surfaces of the bladder by blunt dissection.

All of the urinary bladders were distended with a small volume of urine.   When the urine was removed, they assumed a contracted state typical of an empty bladder.   It was often not possible to infuse a volume of formalin into the bladder as great as the volume of urine that was removed.   The middorsal cystotomy incision was visualized as a long- itudinal indentation in the tissue.   In some instances the urinary bladders

Table III: Bacteria cultured at various times during the experimental period.

| Bacteria	Time of Isolation	Comment
alpha hemolytic streptococcus	day of surgery	contaminant
Staphylococcus epidermia	3 weeks post-surgery, at time of euthanasia	contaminant
Moraxella sp.	day of surgery	contaminant
Staphylococcus sp.	2 weeks of post-surgery	contaminant
nonhemolytic E. coli	5 weeks post-surgery, at time of euthanasia	contaminant
nonhemolytic E. coli	4 and 5 weeks post-surgery	contaminant
beta-hemolytic E. coli	7 weeks post-surgery, at time of euthanasia	contaminant
Pasturella multocida	9 weeks post-surgery	not considered pathogenic to the feline urinary tract
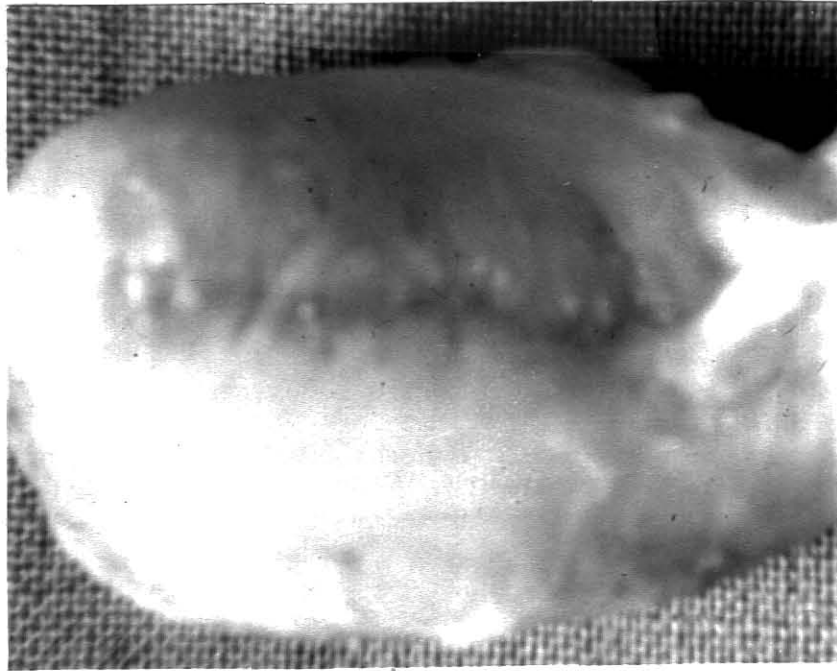
Fig. 12: Gross external appearance of urinary bladder used as a point of reference for normal feline transitional epithelium.

Fig. 13: Gross internal appearance of same bladder as that shown in Figure 12. Its luminal surface had an appearance similar to those bladders collected at periods greater than the thirty-fifth postoperative day.

were of different shapes than the physiologic pear-shape of normal
feline urinary bladders in situ distended with a small volume of urine.
Other bladders were more of an hour-glass shape with the waist of the
hour-glass usually occurring nearer the trigone than the apex. The
wall of some bladders at their apex were thickened and firm. When the
bladders were cut, the walls were thinner in the caudal half than in
the cranial half. They cut sometimes with a "gritty" feel. Small or-
ganized intraluminal blood clots were occasionally present while an
easily-detached, reddish-brown serofibrinous film partially or totally
covered the luminal surfaces of some bladders. The luminal surfaces
of bladders whose postoperative period was less than 14 days had a
reddish-brown, glistening, hemofibrinous film covering their entirety.
The luminal surfaces of bladders between 21 and 28 days postoperatively,
had this reddish-brown film over a small percentage in their cranial
half to one-third while a thin, glistening, granular or finely wrinkled,
yellowish membrane covered the more caudal surfaces. The luminal sur-
faces of all the bladders whose postoperative periods were longer than
28 days were covered with a thin, glistening, finely granular, yellowish
membrane.

4. Histopathology:

The epithelium and lamina propria of the bladder used as a reference
for the normal feline was examined microscopically. The lamina propria
was thrown into folds giving the appearance of multiple, uniform papillary
projections. The epithelium followed the papillary foldings with very
little alteration in its morphology or numbers of cells. (Figure 14)

The epithelium was arranged in 3 distinct layers. The superficial

layer was composed of large, round to polyhedral-shaped cells, with lightly basophilic staining cytoplasm and mild hydropic change. A thin film-like, acellular membrane covered the luminal surface of this cell layer and ran continuously without demarcation between cells. The cytoplasm of these cells was spacious and gave the impression of being in a ratio of approximately one superficial cell to every 2 to 3 under-lying intermediate cells (Figure 15).

The intermediate row of cells was composed of 2 to 3 cell layers, unevenly distributed. The cells were mostly two-thirds the size of a superficial cell, less round, and always with darker staining, basophil-ic nuclei. Their nuclear membrane was very dark and therefore distinct. Their cytoplasm was darker than that of the superficial cells and was slightly less foamy.

The basal layer was essentially comprised of one cell layer. How-ever, their palisade arrangement gave the false impression of more than one cell layer at this stratum. These basal cells differed markedly in shape from the cells of the intermediate and superficial layers. The basal cells were ovoid and plump. Their long axis was perpendicular to a plane tangential to the superjacent luminal surface. They appeared to partially intermingle with the intermediate cells. The nuclei of the basal cells generally had a darker basophilia than those of other cells (Figure 16).

The lamina propria subjacent to the epithelium was divided into 2 morphologically different areas. Immediately beneath the epithelium, the lamina propria was composed of a tightly-woven collagenous tissue. Fibro-cytes with elongated and diamond-shaped, darkly basophilic staining nuclei,

were moderately dispersed throughout this otherwise lightly acidophilic staining collagen. The external half of the lamina propria was composed of more loosely arranged collagenous fibers. These collagenous fibers were more intensely acidophilic than those of the superficial half and had relatively fewer mature fibrocytes scattered throughout (Figure 17).

Arterioles and venules were scattered throughout the lamina propria. Those in the external half of the lamina propria were many times larger than those in the internal half. Capillaries of various sizes were evenly and uniformly dispersed immediately adjacent to the epithelium in the internal-most aspect of the lamina propria.

A basement membrane relative to the epithelium was not visualized in these sections. The immediate subjacent morphology was essentially comprised of the above-described capillaries and strands or bundles of densely-packed collagen in the internal lamina propria. Collagen, in between the capillaries, gave the impression of sweeping into the deep portion of the epithelium, and seemed to completely disregard any barrier, such as a basement membrane, that was possibly there. The strands of collagen were intimate with the cytoplasm of the basal layer of epithelial cells (Figure 18).

A plump, ovoid, darkly basophilic cell-type was present in the superficial-most part of the lamina propria. This cell appeared to blend into the basal layer of the epithelium. The cell was oriented in an angle toward or was perpendicular to the epithelium and swept into the epithelium with the same consistency that was seen with the strands of collagen. These cells morphologically resembled pericapillary supporting cells or fibrocytes and appeared to originate from the area of pericapillary structures and collagenous tissue, respectively (Figure 19).
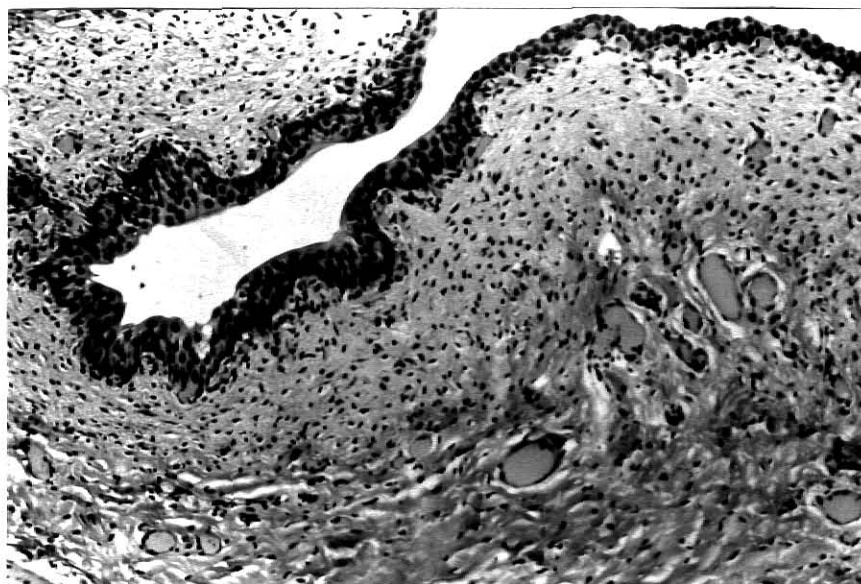
Fig. 14: Photomicrograph of normal feline urinary
bladder transitional epithelium and lamina
propria. Note the folding of the lamina
propria. H and E. 100X
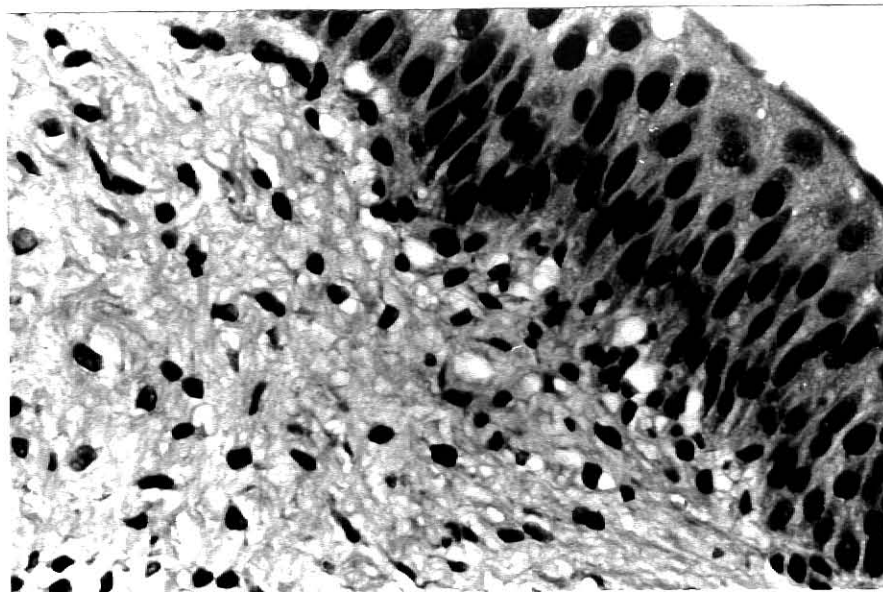
Fig. 15: Photomicrograph of normal feline urinary bladder
transitional epithelium. Note the vacuolated
appearance of the superficial cells. H and E. 400X
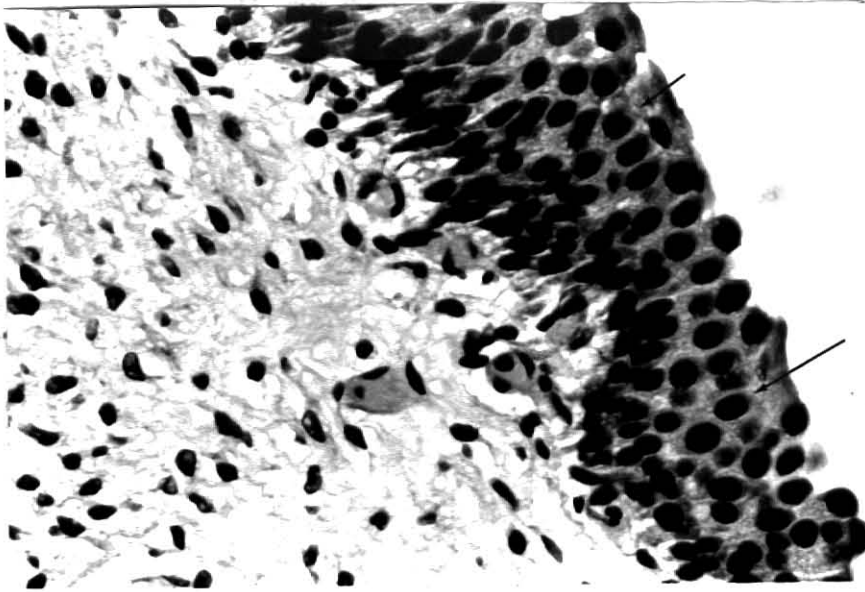
Fig. 16: Photomicrograph of normal feline urinary bladder
transitional epithelium. Note the intermediate
row of transitional cells (arrows). H and E. 400X
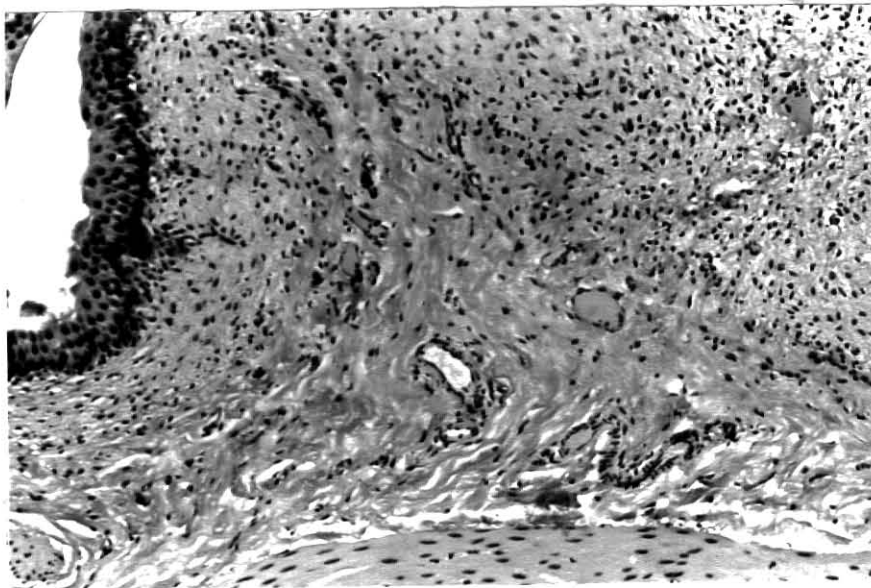
Fig. 17: Photomicrograph of normal feline urinary bladder
wall demonstrating the two zones of the lamina
propria. H and E. 100X
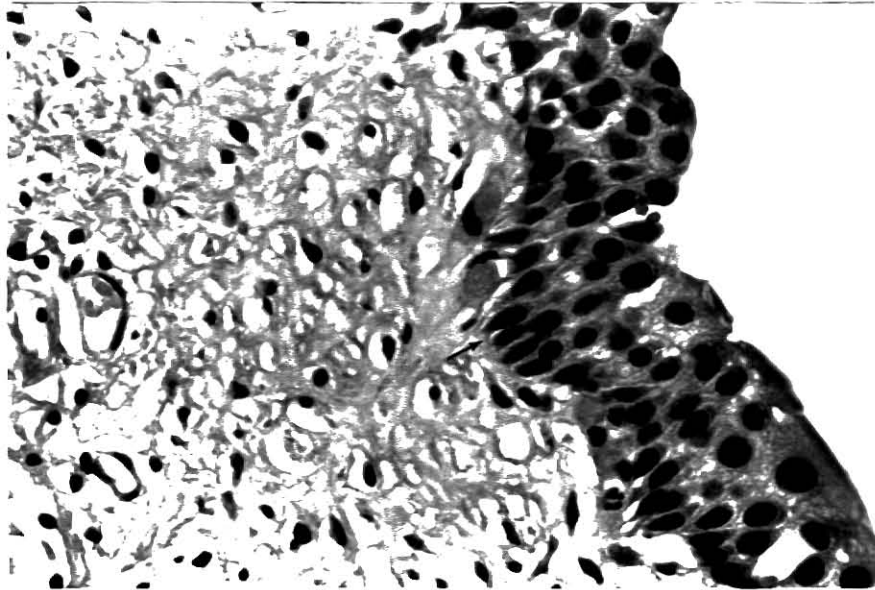
Fig. 18: Photomicrograph of normal feline urinary bladder. Note the intimate relationship of collagen from the lamina propria with the basal transitional cells (arrow). H and E. 400X
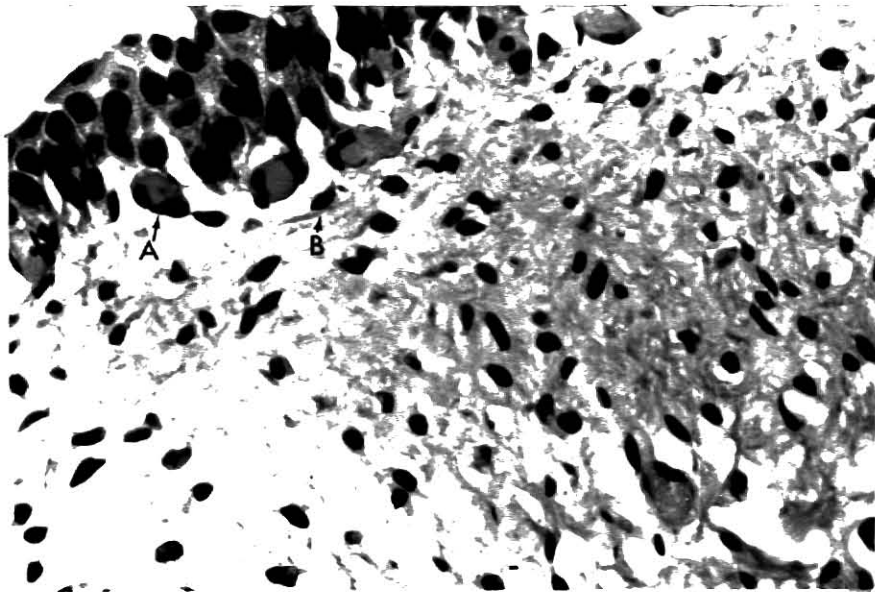
Fig. 19: Photomicrograph of normal transitional epithelium and lamina propria. Note pericapillary supporting cells (arrow A) and fibrocytes (arrow B). H and E. 400X

The experimental cats were euthanatized one to 112 days after surgical mucosal denudation of their urinary bladders. However, it was ascertained that regeneration of epithelium had totally resurfaced the lumen by the thirty-fifth postoperative day and the most striking regenerative activity occurred between the first and the twenty-first day. A histologic sequence covering the entire postoperative regeneration period was prepared.

a. Postoperative day 1:

At the end of 24 hours the luminal surface was devoid of transitional epithelial cells. An inflammatory exudate composed of serum (Figure 20), fibrin, and neutrophils covered the luminal side of this exudate. There was an occasional small blood clot with a few entrapped neutrophils. An inflammatory response characterized by hyperemia and a hypercellular reaction composed chiefly of neutrophils was present along the basement membrane and extended into the lamina propria. There was a different hypercellular response occurring deep in the lamina propria which was comprised of very young cells which had much the appearance of fibroblasts. These cells seemed to have evolved from the external half of the lamina propria and were directed en masse toward the internal half of the lamina propria and basement membrane.

b. Postoperative day 3:

Seventy-two hours following urothelial denudation there was no evidence of regenerated epithelium lining the bladder (Figure 21). However, at one point a small segment of original transitional epithelium was present along the right lateral surface. This segment was 5 cells in length and

between 1 and 2 cells in depth. There was a minimum of inflammatory response in the subjacent lamina propria.

The exudate covering the rest of the luminal surface was closely adherent to the lamina propria and a basement membrane was not visualized (Figure 22). The lamina propria was heavily infiltrated by neutrophils. The collagenous tissue in the external half of the lamina propria and between the muscle bundles revealed a proliferation of fibroblasts. These cells, especially near arterioles and venules situated between muscle bundles, were present in mesenchymal tissue comprising the supporting matrix in the external lamina propria and between the muscle bundles.

c. Postoperative day 7:

The first transverse section of this bladder was cut 4 millimeters cranial to the trigone. Four-fifths of its circumference had original transitional epithelium. The inclusion of original epithelium on this block of tissue was unintentional and resulted from inaccurately cutting in a true trans-verse plane. The midventral 20% of this section was devoid of transitional epithelium and likewise transitional epithelium was absent from the remain-ing luminal surface (Figure 23). An apparent basement membrane was present at variable foci of each tissue. In some areas it was quite thick. The lamina propria from which transitional epithelium was excised was covered by an inflammatory exudate. This exudate consisted of a serofibrinous material with an intermingling of erythrocytes and neutrophils. Small blood clots with entrapped neutrophils were present on the luminal side of the exudate in some areas (Figure 24).

The lamina propria beneath the retained original epithelium on either side

Inflammation

trigone

20

Distance from trigone

Fig. 20: Diagrammatic line drawing of feline bladder
on postoperative day one. Distance was
measured in millimeters

Inflammation

trigone

25

Distance from trigone

Fig. 21: Diagrammatic line drawing of feline bladder on
postoperative day three. Distance was measured
in millimeters.
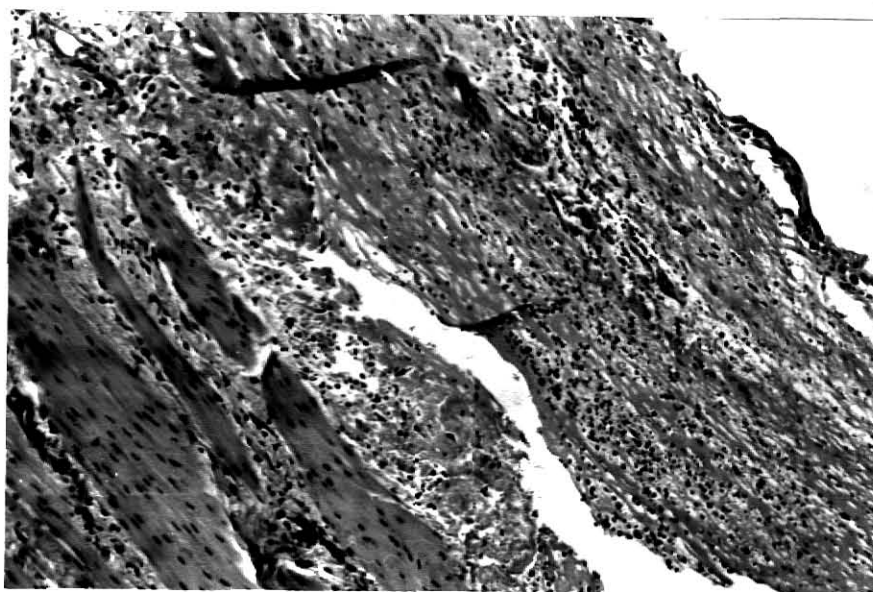
Fig. 22: Photomicrograph of feline bladder on
postoperative day three. Note the segment
of retained original urothelium and the
minimal inflammatory response in the
subjacent lamina propria. This section
is 3 millimeters from the trigone. H and E. 100X

of the one section of denudation (4 mm. cranial to the trigone) was in distinct contrast to the lamina propria beneath the denuded area. Beneath the area of denudation there was a marked neutrophilic infiltration with a different cellular response occurring in the deeper part of this area (Figure 25). This latter reaction was a proliferation of fibroblasts. In contradistinction, the other areas of lamina propria, including those adjacent to lamina propria beneath the denuded area, were devoid of either neutrophils or fibroblastic hypercellularity. In the lamina propria at greater distances more cranial to the trigone, there was a distinct zone of neutrophils above a zone of plump, densely basophilic fibroblasts (Figure 26). Also in these deep portions of lamina propria were other signs of inflammation; namely, hyperemia and edema.

d. Postoperative day 10:

Regenerated epithelium was present over the entire circumference from the trigone to 3 millimeters cranially. At 8 mm. cranial to the trigone, approximately 65% of the luminal surface was covered while the right ventro-lateral 35% was devoid of epithelium. At 13 mm. cranial to the trigone, the left dorsolateral 35% of the luminal surface was lined by regenerated epithelium while the remaining 65% was not yet covered (Figure 27).

An inflammatory exudate covered the denuded luminal surfaces and was characterized as an intense hemopurulent exudate with a concentration of fibrinous strands running through it. The erythrocytes and leucocytes appeared to be entrapped by the strands of fibrin. A basement membrane was not visualized over any of the denuded epithelial surface. The hemo-purulent, fibrinous exudate seemed to blend into the lamina propria. The

Fibrosis
Inflammation
Original epith.

trigone

4   32

Distance from trigone
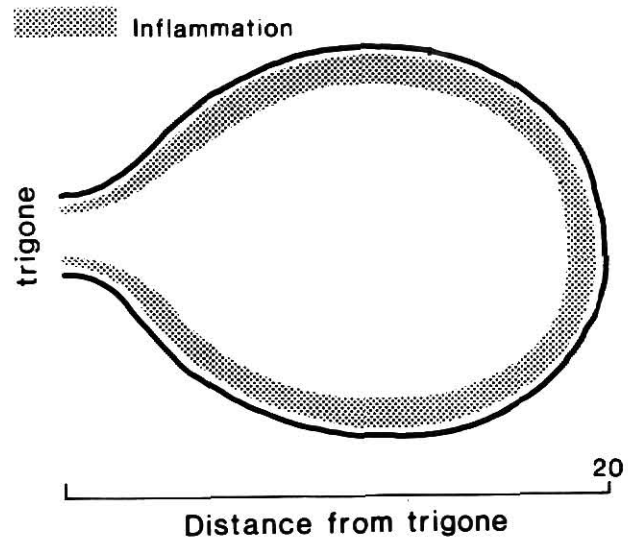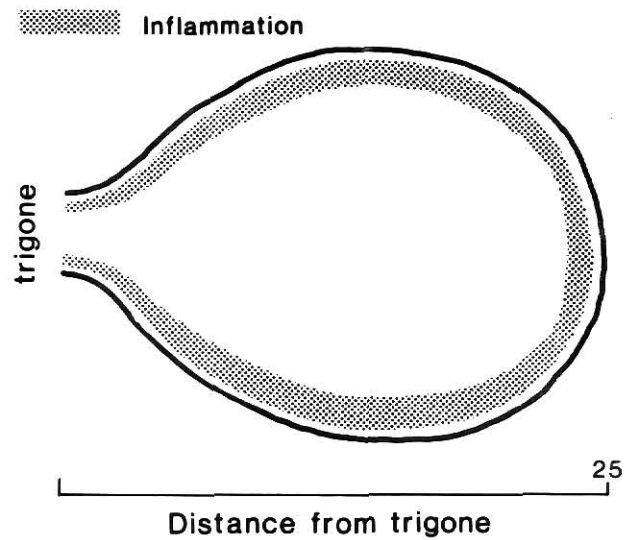
Fig. 23:   Diagrammatic line drawing of feline
           bladder on postoperative day seven.
   Distance was measured in millimeters.
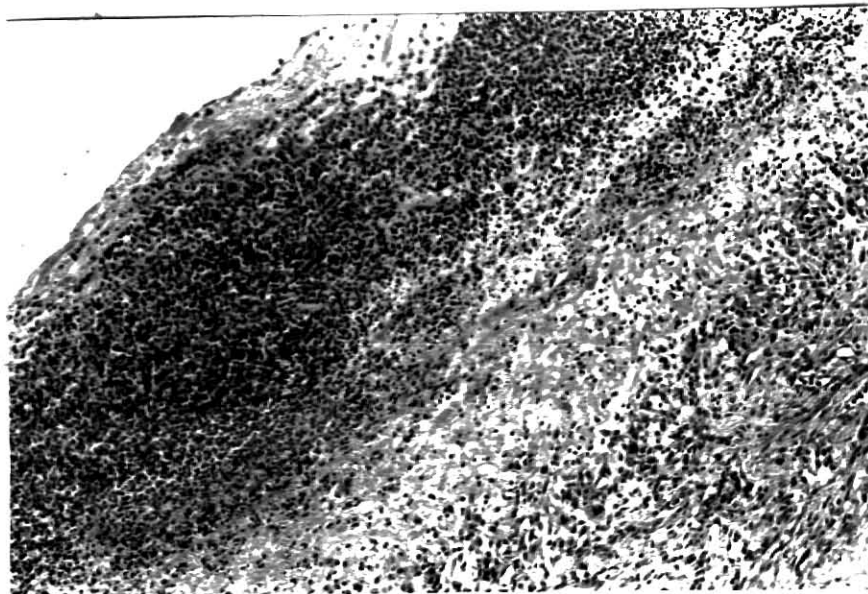
Fig. 24: Photomicrograph of feline bladder on postoperative
day seven. The lamina propria from which transition-
al epithelium was excised was covered by serofibrin-
ous exudate with an intermingling of erythrocytes
and neutrophils. H and E. 100X
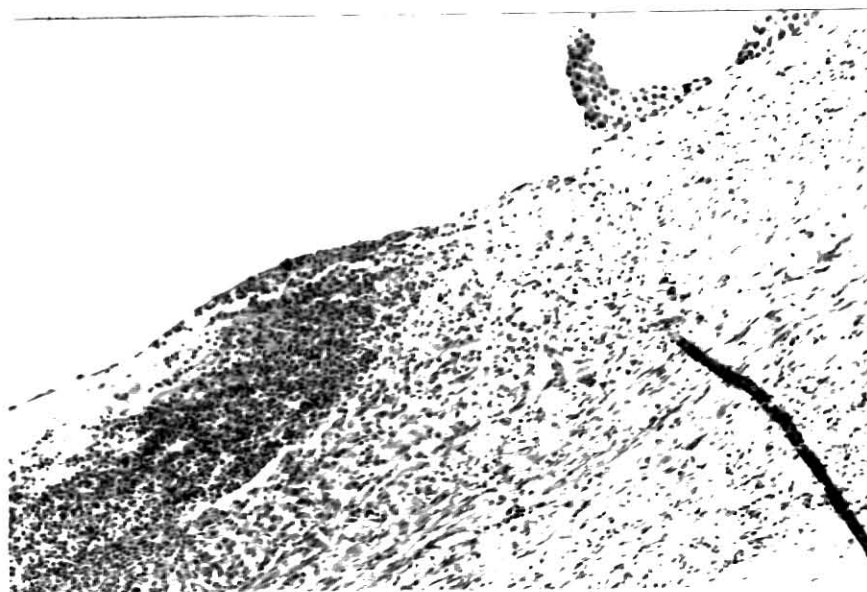
Fig. 25: Photomicrograph of feline bladder on postoperative
day seven. Note the inflammatory response in lamina
propria subjacent to denuded surface in contrast to
no inflammation beneath the epithelial covering.
H and E. 100X

The matrix of the lamina propria had a loose appearance with its internal half being hemorrhagic, edematous, and containing increased numbers of neutrophils. Deep in the lamina propria, an intense proliferative response occurred, characterized by a deeply basophilic, plump, fibroblast-like cellularity. Beneath the luminal surfaces which have been re-epithelialized, there was a continued intense proliferative activity and apparent migration of fibroblasts.

These fibroblasts proliferated in clumps in the superficial lamina propria. Many were directed at various angles toward the lumen, some being perpendicular. Other of these cells appeared to cross from the lamina propria into the epithelium. Mitosis was evident in these fibroblastic cells as well as in the regenerated epithelial cells (Figure 28).

e. Postoperative day 14:

Regenerated epithelium covered the entire circumference 3 mm. cranial to the trigone. The left dorso-lateral 20% of the circumference 7 mm. cranial to the trigone was devoid of epithelium while the remaining 80% had regenerated epithelium. The remaining luminal surface of the bladder was devoid of epithelium (Figure 29).

The epithelial covering was uniformly comprised of 2 to 4 cells. The cells are mostly positioned with the long axis of their nucleus parallel to the collagen fibers in the underlying lamina propria. Fibroblastic cells were visualized migrating into the epithelial layer in some areas. The lamina propria beneath this 14-day old epithelium appeared more mature than that seen underlying the 10-day old regenerated epithelium. An apparent basement membrane was prominent between the epithelium and lamina propria
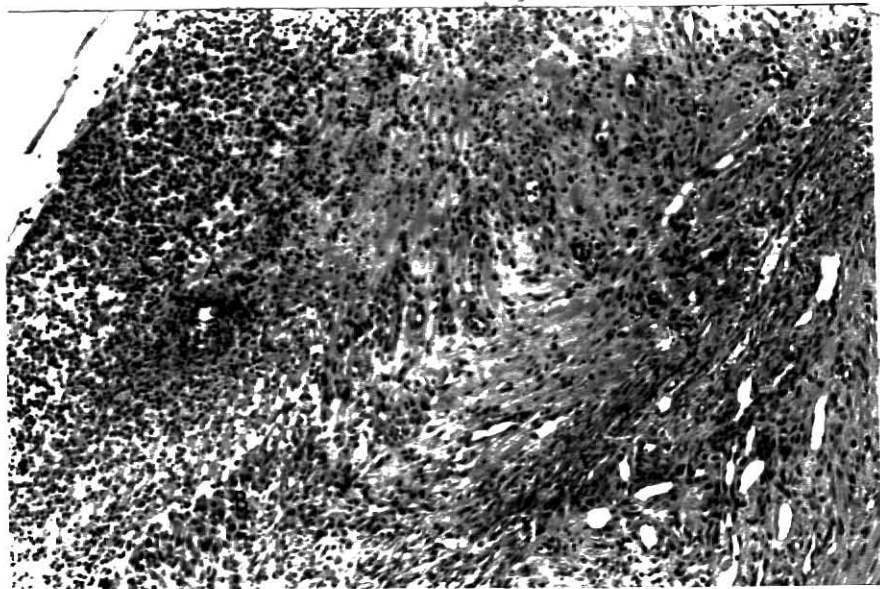
Fig. 26: Photomicrograph of feline bladder on postoperative day seven. This section was nearer the apex where the luminal surface was devoid of urothelium. Note the intense hypercellularity of neutrophils (A) and fibroblasts (B). H and E. 100X
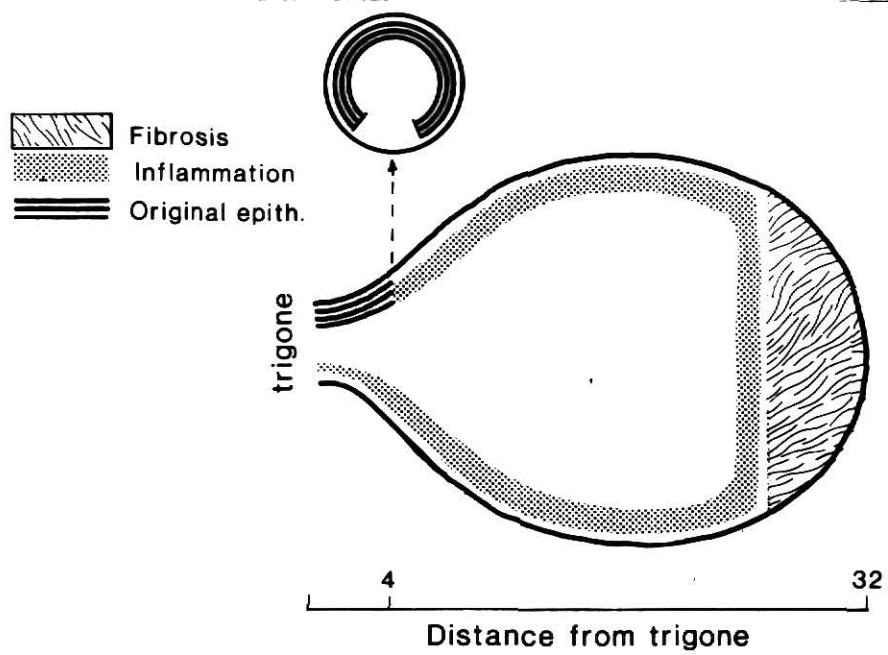

Fig. 27: Diagrammatic line drawing of feline bladder on postoperative day ten. Distance was measured in millimeters.

in some areas (Figure 30).

Capillaries of the lamina propria which were immediately subjacent to the basement membrane were filled with erythrocytes. The supporting peri-capillary cells had plump, darkly staining, basophilic nuclei. Throughout the lamina propria there was a proliferation of fibroblasts and the peri-capillary cells of the arterioles and venules. Edema and hyperemia completed the inflammatory features of the lamina propria.

f. Postoperative day 21:

The luminal surface was covered by regenerated epithelium from the trigone to a distance 6 mm. cranially. 66% of the luminal circumference at a distance 9 mm. cranial to the trigone was re-epithelialized. This extended over the dorsal half and the right and left ventrolateral areas. The dorsal 10% of the luminal surface was re-epithelialized 12 mm. cranial to the trigone. All remaining surfaces were devoid of epithelium (Figure 31).

Exudate, composed of serum, fibrin, erthrocytes and neutrophils, covered the luminal surface devoid of epithelium. At this stage of regeneration, the inflammatory exudate was not as profuse as it was at earlier periods. The underlying lamina propria over which epithelium was absent continued to show inflammatory activity. Edema and hyperemia was marked. In the internal half there was a dense infiltration of neutrophils while the external half of the lamina propria contained an intense proliferation of granulation tissue out of which fibroblasts appeared to develop and migrate outward to the luminal surface.

At the junction between the denuded surface and regenerated epithelium,

Fig. 28:   Photomicrograph of feline bladder on postoperative
           day ten.   Note dark-staining fibroblasts near the
           basal layer of epithelium (arrows). H and E. 400X

Fibrosis
Inflammation
Regenerated epith.

trigone

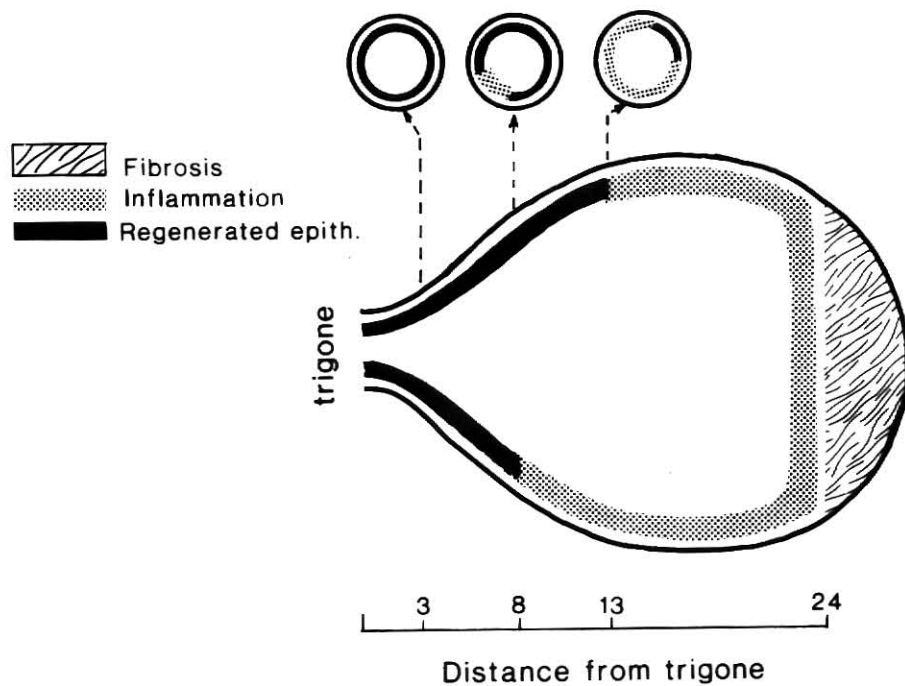3   7   20

Distance from trigone

Fig. 29: Diagrammatic line drawing of feline bladder
on postoperative day fourteen. Distance was
measured in millimeters.

Fig. 30: Photomicrograph of feline bladder on postoperative
day fourteen. Although the lamina propria presents
a separation artefact from processing, it neverthe-
less has a more mature appearance than the lamina
propria of earlier sections not covered by regener-
ated urothelium. H and E.   400 X

the following were noted: (1) A marked reduction of inflammatory
response in the lamina propria covered by regenerated epithelium as com-
pared to that under the denuded area; (2) An apparent migration of fibro-
blastic cells from the lamina propria into the luminal surface and their
apparent differentiation into transitional epithelium; and (3) A zone of
neutrophils at the surface of the lamina propria with the zone of totipotent
cells in the deep lamina propria (Figure 33) (See Discussion).

g. Postoperative day 28:

Re-epithelialization occurred 19 mm. cranial to the trigone for the entire
luminal surface except for one small defect approximately 5 mm. in length,
situated along the left ventrolateral circumference between 8 and 13 mill-
imeters cranial to the trigone. Hence, approximately 90% of this luminal
surface was re-epithelialized (Figure 34).

In the area not re-epithelialized, the typical inflammatory response was
present. This same inflammatory activity was also present at the apical
end of the bladder, 21 mm. from the trigone, at which point the internal
half of the lamina propria had a dense zone of neutrophils with a deeper
zone of actively proliferating and migrating fibroblastic cells in a rather
disorganized arrangement.

h. Postoperative day 35:

Both of these bladders show re-epithelialization of their luminal surfaces
(Figure 35). However, in one of the bladders, 10 millimeters from the trigone,
a defect was present wherein approximately 30% of the ventral luminal sur-
face was devoid of epithelium. The subjacent lamina propria showed the pre-

Fibrosis

Inflammation

Regenerated epith.

trigone
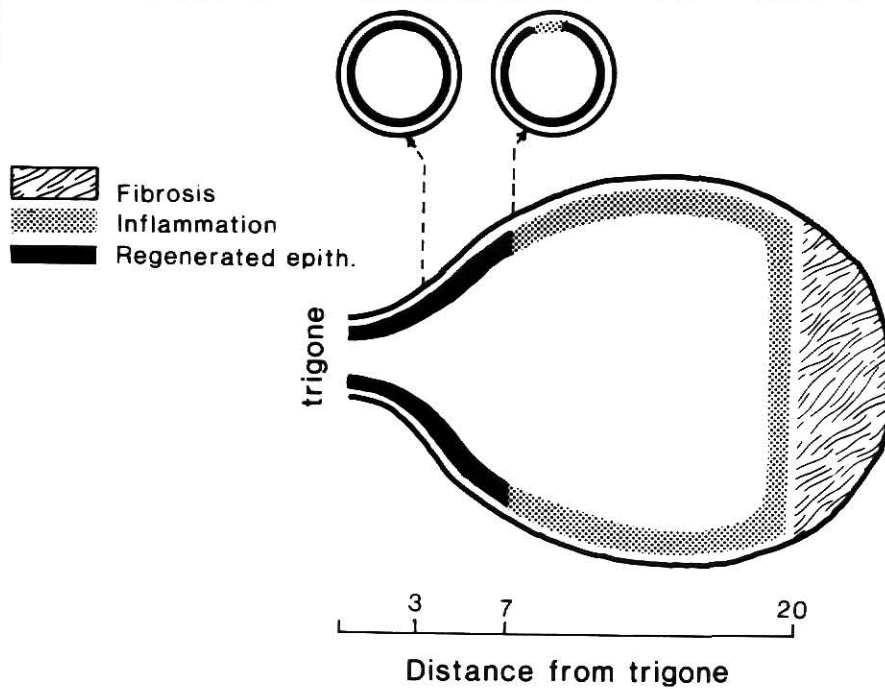
3 6 9 12 15

Distance from trigone

Fig. 31: Diagrammatic line drawing of feline bladder
on postoperative day twenty-one. Distance was
measured in millimeters.

Fig. 32: Photomicrograph of feline bladder on postoperative
day twenty-one. Note proliferative activity of fibro-
blast cells and their apparent migration toward the
luminal surface (arrow). H and E 100X
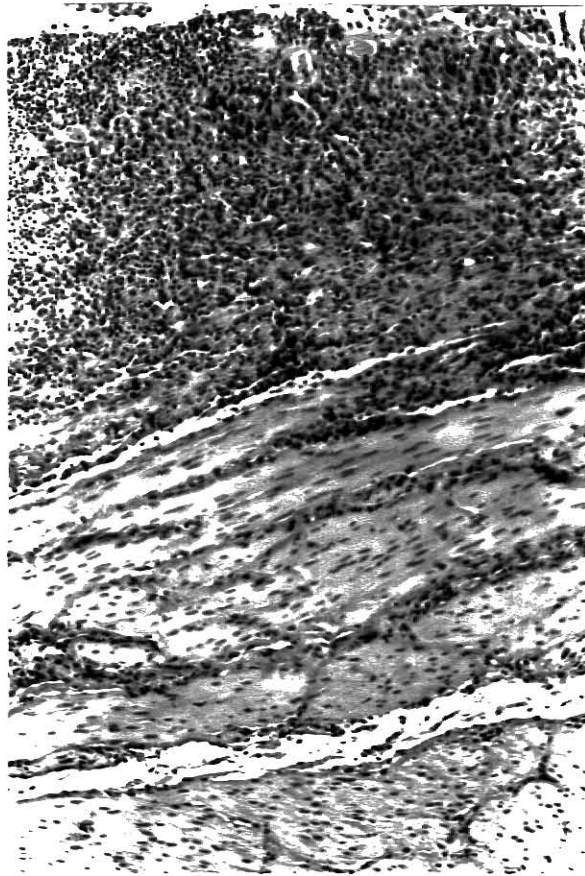
Fig. 33: Photomicrograph of feline bladder on postoperative
day twenty-one. Note how the fibroblasts were immed-
iately subjacent to the regenerated epithelium. To
the left where the luminal surface is devoid of
epithelium the superficial lamina propria was
heavily infiltrated with neutrophils. H and E. 100 X
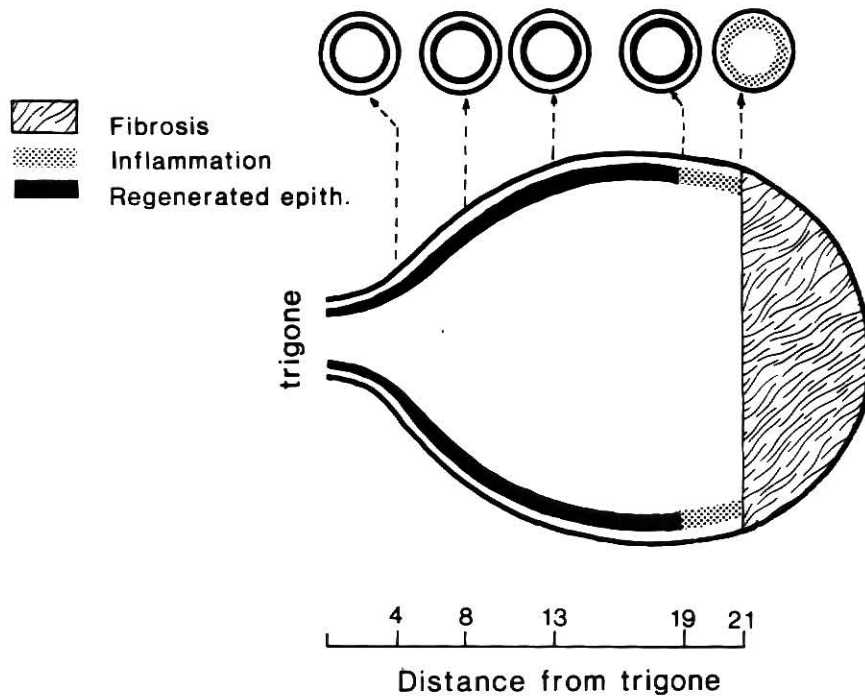
Fig. 34: Diagrammatic line drawing of feline bladder on postoperative day twenty-eight. Distance was measured in millimeters.
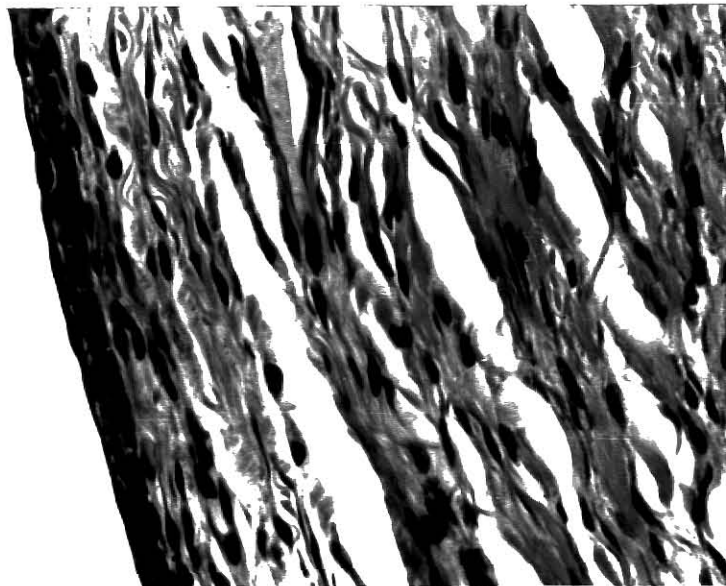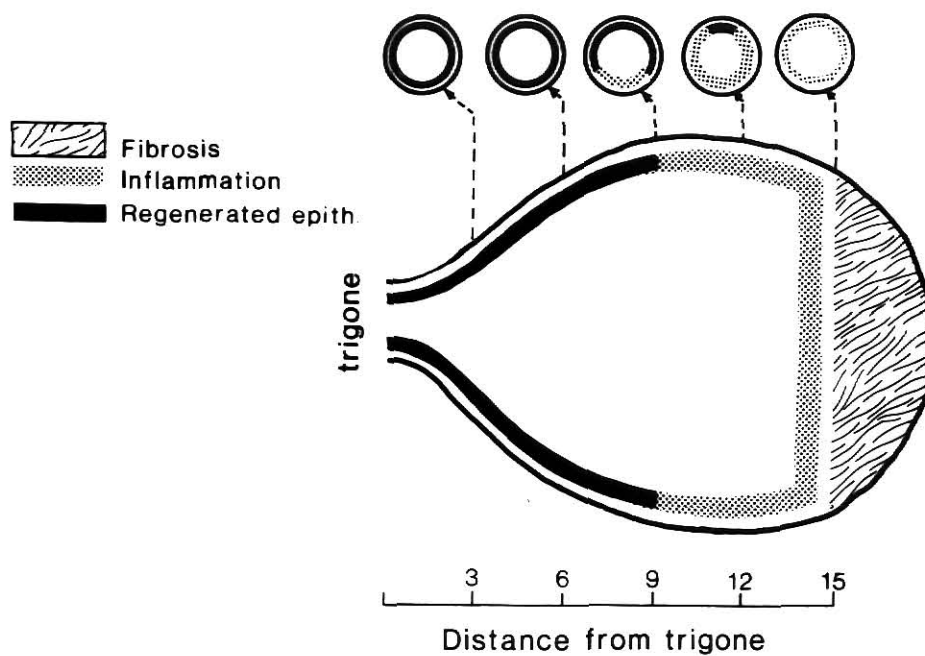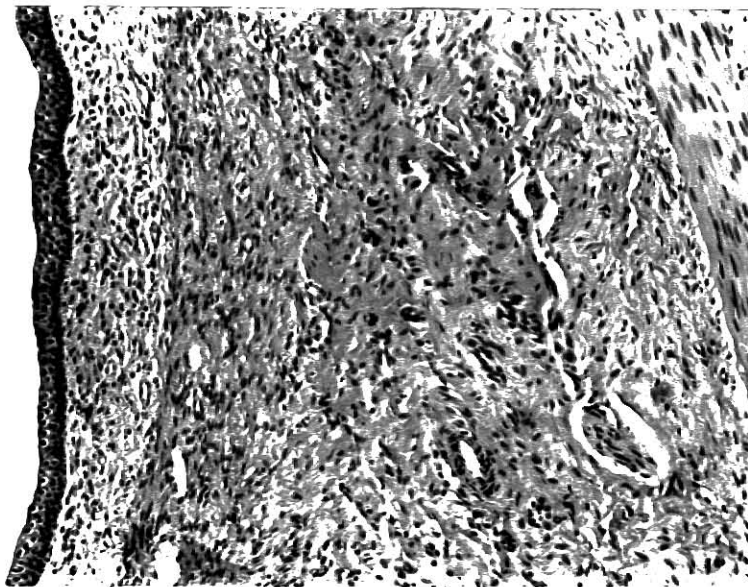

Fig. 35: Photomicrograph of feline bladder on postoperative day thirty-five. Note the uniform and organized appearance of the regenerated epithelium and subjacent lamina propria. H and E. 100X

viously described response to injury and the expected pre-regenerative changes (Figure 36). The lamina propria subjacent to the re-epithelialized luminal surface in an area nearer the apex had a mature and well-organized appearance. It was devoid of inflammatory changes such as those described for the pre-regenerative period (Figure 37).

i. Postoperative day 42:

Both of these bladders were re-epithelialized except for a small defect in one of them. This defect was 13 millimeters from the trigone and covered approximately 6% of the circumference along the ventral luminal surface. In this area the lamina propria was covered by some hemorrhage and a fibrinopurulent exudate and was itself infiltrated with neutrophils. Other signs of inflammation were hyperemia, edema, and a proliferation of fibroblastic cells in the external part of this area of the lamina propria (Figure 38). Along the remaining re-epithelialized luminal surface the lamina propria near the surface was heavily folded (Figure 39).

j. Postoperative day 49:

Both of these urinary bladders had complete re-epithelialization of their luminal surfaces. These two bladders, however, presented some other interesting phenomena. The epithelium lining the dorsal half of one bladder had more folding than that lining the ventral half. The basement membrane appeared quite distinct. The fibroblasts in the lamina propria were actively proliferating and emerging into the basal epithelial area (Figure 40).

At the apex of the other bladder there was an area of fibrosis that was beyond the storage portion of the bladder. This area comprised the cranial 4 millimeters and within it was a cavity which was lined by regenerated epithelium one to 2 cells thick (Figure 41).

61
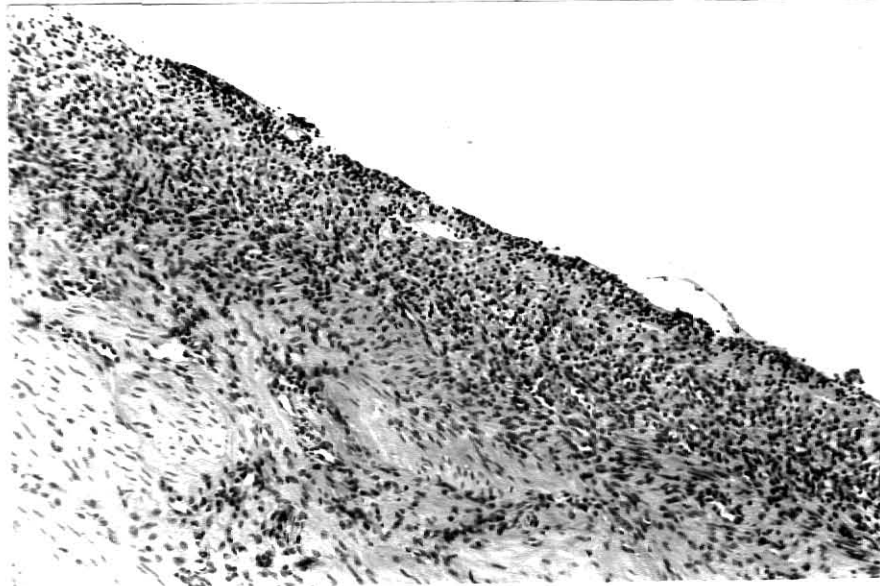
Fig. 36: Photomicrograph of feline bladder on postoperative
day thirty-five. Note the lamina propria subjacent
to an area devoid of epithelium. The response to
injury and expected pre-regenerative changes were
evident. H and E. 100X
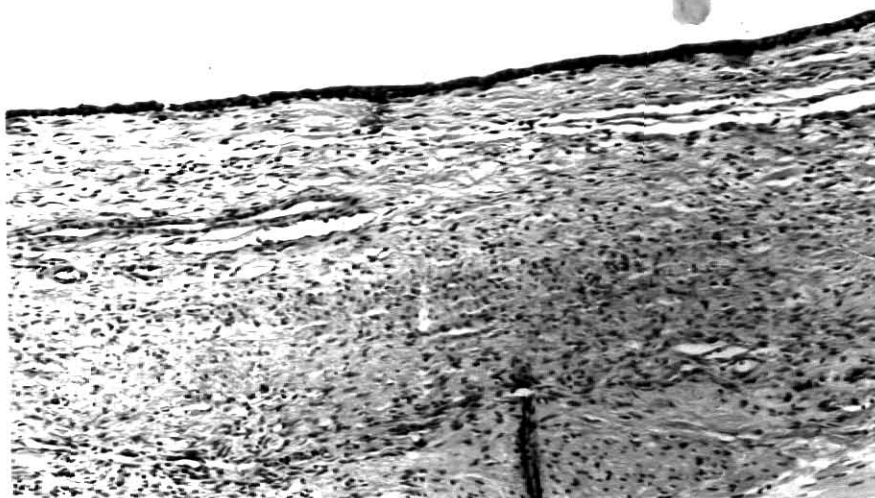
Fig. 37: Photomicrograph of feline bladder on postoperative day
thirty-five. Note the absence of inflammation in lamina
propria beneath regenerated epithelium. H and E. 100X
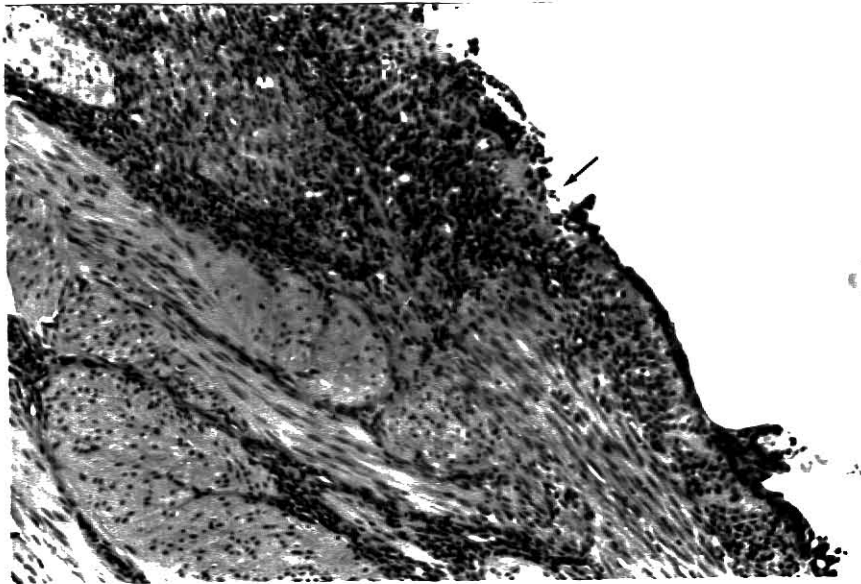
Fig. 38: Photomicrograph of feline bladder on postoperative day forty-two. Note inflammatory response in lamina propria subjacent to ulcerative defect (arrow). H and E. 100X
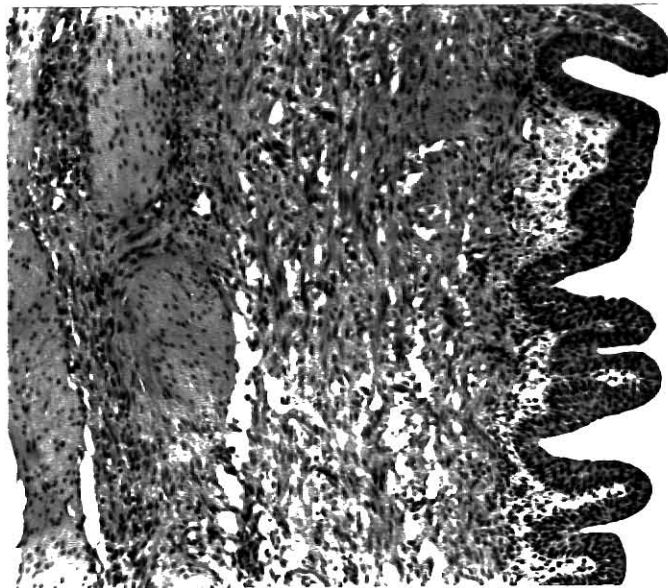
Fig. 39: Photomicrograph of feline bladder on postoperative day forty-two. The luminal surface was re-epithelialized and the superficial lamina propria was thrown into many folds. H and E. 100X
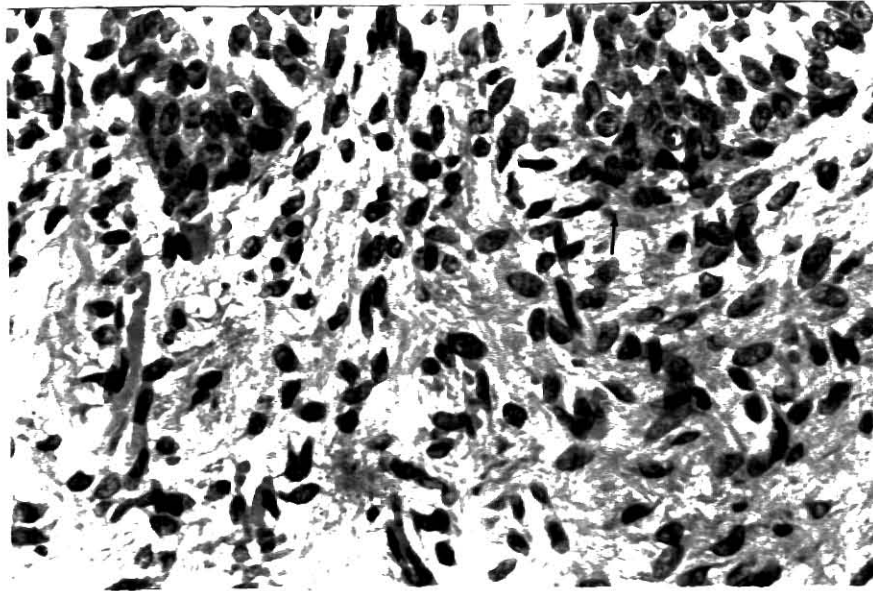
Fig. 40: Photomicrograph of internal half of the lamina propria
of bladder on postoperative day forty-nine. Note the
proliferative activity of fibroblastic cells and their
emergence toward the basal epithelial area (arrow).
H and E. 400X.
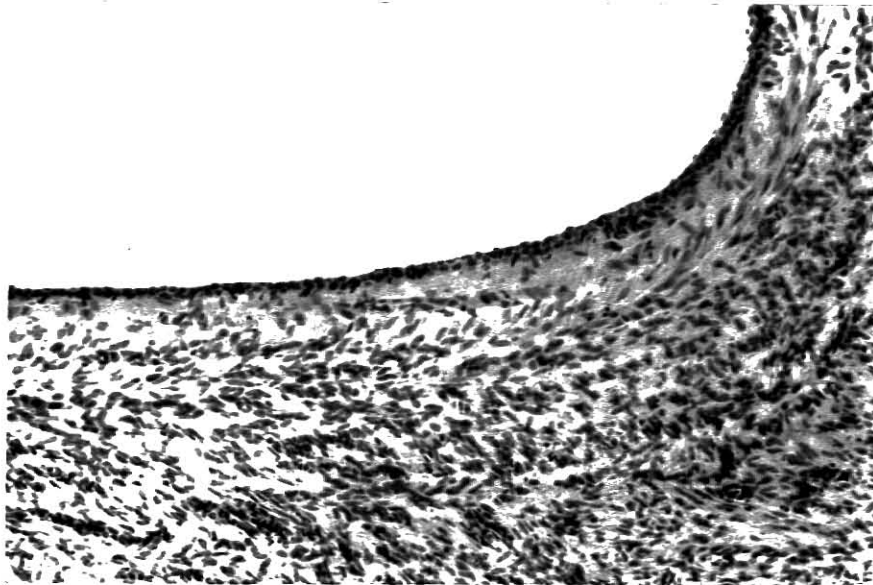
Fig. 41: Photomicrograph of cranial end of feline bladder on post-
operative day forty-nine. Note the regenerated epithelium
that had lined a cavity which was separate from the storage
portion of this bladder. H and E. 100X

k. <u>Postoperative days 56 to 112</u>:

These bladders, which represented postoperative periods of 56, 63, 70, 88, and 112 days, had complete regeneration of the mucosal covering of their luminal surfaces. All features described for the regenerated epithelium, corresponding subjacent basement membrane, and lamina propria of bladders of earlier postoperative periods were similar to these. The major difference was that the epithelium and lamina propria were more uniformly organized and mature in appearance (Figures 42 and 43).
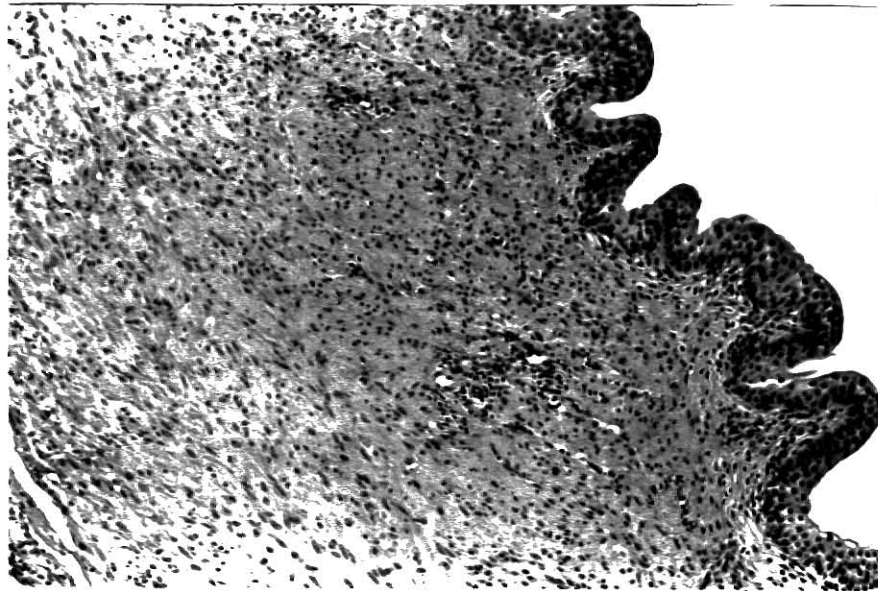
Fig. 42: Photomicrograph of feline bladder on postoperative
day eighty-eight. Note the intraluminal epithelial
projections with their core of lamina propria.
H and E. 100X
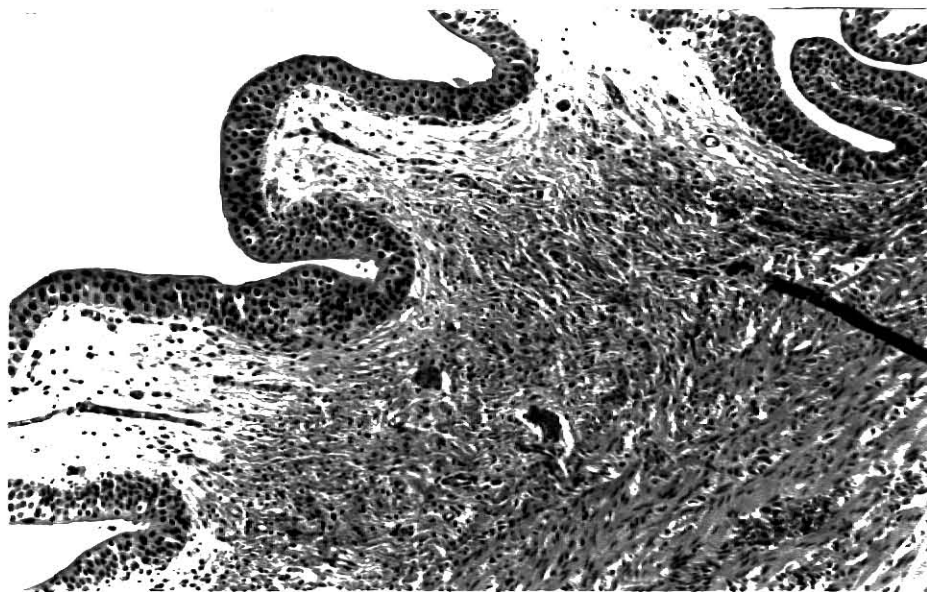
Fig. 43: Photomicrograph of feline bladder on postoperative day
one hundred and twelve. Note the regenerated epithelium
which had three distinct layers and was from four to
six cells thick. The lamina propria was heavily folded.
H and E. 100X

DISCUSSION

The cats in this study responded to surgical mucosal denudation of their urinary bladders in a clinical manner much the way any healthy cat is expected to respond to general surgery. The more prolonged post-surgical convalescence of these cats, however, was directly accounted for by the acutely induced hemorrhagic cystitis. The severe change in their habit brought on by such a large ulcerative lesion of the urinary bladder was no doubt an external manifestation of the discomfort and felling of urgency to urinate induced by the extensive mucosal excision. The removal of a relatively large amount of tissue, leaving essentially a raw bleeding surface, which was to be continuously bathed by an irritant substance, no doubt stimulated the release of endogenous pyrogens which resulted in the immediate post-operative febrile reaction. Hyperpyrexia and general malaise no doubt was manifested by the behavorial depression, loss of appetite, reduced water intake and urine output. The subsequent and somewhat transient dehydration and anemia resulted secondarily to decreased water and food consumption, and continuous blood loss from the urinary bladder.

The mucosal denudation in these cats confirmed the clinical results noted by Sanders (1958) and Annis (1962) in regard to micturition. They observed that this procedure in dogs had a profound and prolonged effect upon natural micturition. Their dogs were not incontinent. However, they exhibited an increase in the desire to urinate without always voiding urine. For the first few days the urine was only passed in drogs and the dogs remained in a position for urination several minutes. Gradually the dogs urinated or exhibited the desire to urinate less frequently and the urine was voided in a weak stream. Finally by the tenth week, the act of micturition had returned

to normal.

The hemograms were not remarkable. The expected response of anemia was indicated by the slight decrease in packed cell volume and hemoglobin. However, on the average, a severe state of anemia was not seen. In view of the neutrophilic response seen in the tissue sections, it was surprising that leucocytosis, neutrophilia, and a regenerative left shift was not more evident. For the entire experimental group, the hemograms stayed within normal physiologic limits (Schalm, 1965).

Urinalyses fell within the expectations dictated by the experiment. A gradual return to normal micturition, urine output, urine chemistry and sediment paralleled the disappearance of generalized malaise seen in the early post-operative days. Hematuria persisted no doubt because of the tendency for fragile new capillaries bursting as the bladder expanded and contracted, as it was filled or voided of urine. Normal transitional epithelium has a PAS positive material in the potential space between its cells (Aurora and Gupta, 1967). Histochemically, this suggested that a mucopolysaccharide is actively secreted by the surface cells as a protection against the changing micro-environment of the bladder lumen.

Bertalanffy and Lau (1962) commented on irritant agents, including urine, that confront the transitional epithelium and may have an adverse effect on the mitotic rate during physiologic cell renewal. In addition, the phagocytic and enzymatic activity of the neutrophils extruded might be in-criminated for prolonging some of the hemorrhagic aspects seen two to four weeks postoperatively.

Return of the urine to normal physiologic content also seemed to parallel the coverage of the luminal surface with epithelium. The urine appeared

to remain bacteriologically sterile except for the few contaminants. Some investigators who denuded the bladder mucosa used chemotherapy during their investigations (Sanders, 1958; Annis, 1962). The absence of bladder infection in this investigation was due to healthy experimental animals, aseptic operative procedure, frequent urination, and apparently reasonable tolerance by the cats.

Urinary bladders from all the cats were in a contracted state at necropsy. This was partly due to the acute inflammation in the early post-operative periods and partly due to the palpation and manipulation made in an effort to collect urine prior to euthanasia. The inflammatory reaction was considered responsible for the contracted state in the bladders up to twenty-eight days. Bladders of cats subjected to the creation of small artificial ulcers by McMinn and Johnson (1955) were contracted up to three days following surgery. Twenty-eight days later their distention varied within normal limits.

The results of this investigation demonstrated that transitional epithelium of the feline urinary bladder possesses the inherent ability to undergo regeneration in a reparative response to its complete removal. It was not surprising that this outcome occurred since it was shown by McMinn and Johnson (1955) that transitional epithelium of the feline urinary bladder had the ability to expand by mitotic proliferation and migration. They created ulcers, 0.5 to 1.0 cm. in diameter, in the epithelial lining of cats' bladders and made a sequential study of the healing process. Aurora and Gupta (1967) also showed healing of surgically produced mucosal ulcers, although in rabbits, by regenerative epithelialization. They noted complete healing by ten days. This remarkable proliferative capacity was

also consistent with the work of another group (Sanders, et al, 1958) who had long-recognized the regenerative potential of urothelium. They surgically denuded the entire mucosal lining of the bladder of dogs in much the same manner done in this experiment. They reported complete epithelialization of the denuded surface by sixteen weeks.

Iida (1960) had also noted the marked regenerative ability of bladder epithelium. He held it in such high regard that he set out to make an ideal artificial bladder within the peritoneal cavity of dogs using the retained transitional epithelium of the trigone following sub-total cystectomy as the stimulus.

Although the number of experimental animals in this experiment was kept to a minimum, the length of time for re-epithelialization to occur was determined with reasonable certainty. Statistical support, however, was unavailable because of the small number of experimental cats used. Nevertheless, there was a steady coverage of luminal surface from the 10th day to the 28th postoperative day. McMinn and Johnson (1955) noted coverage of relatively tiny defects by 10 days. The entire luminal surface of two feline bladders on the 35th postoperative day was covered and each period studied thereafter showed complete re-epithelialization. Although other investigators had shown the regenerative capacity of transitional epithelium, it had not been shown to be capable of covering an entire bladder luminal surface within 35 days. In fact, in this investigation, 90 per cent was covered in one bladder on the 28th postoperative day.

The initial tissue response to the surgical denudation was much like that described by Sanders, et al (1958) and Annis (1962) all of whom used very similar surgical techniques in dogs. The cystotomy incision appeared

to have little or no influence on subsequent epithelial regeneration.
By 24 hours after mucosal denudation a serofibrinous exudate covered
the entire luminal surface. The remaining layers through which the in-
cision was made had sealed so tightly that the incision line was not
readily discernable. Only a minimal, neutrophilic response was noted
which surrounded the chromic surgical gut sutures. This was consistent
with the work done by Rasmussen (1966) and Aurora and Gupta (1967) when
they studied the tissue reaction stimulated by incisions into the wall
of urinary bladders of rabbits.

The early gross and microscopic lesions resulting from surgical
denudation appeared similar to those described for acute ulcerative
cystitis in the cat (Bloom, 1954). This comparison raised an interesting
speculation. Healing of this latter disease may be by fibrosis and result
in a severely contracted vesical. Currettage or vigorous debridement
via cystotomy was described for treatment of various feline cystitides
(Fishler, 1967). The success of this no doubt depended on removal of nearly
all necrotic cellular debris and freshening the edges of the remaining epi-
thelium. In this experiment, there were no foci of necrosis and the epi-
thelial edges were viable. Unfortunately, in cases of currettage for
ulcerative hemorrhagic cystitis in cats, no thorough histologic study was
reported.

In this investigation, the denuded areas were always covered by a
hemopurulent exudate. The lamina propria, in its internal half, was heavily
infiltrated with neutrophils. An interesting observation was made when
there were areas of re-epithelialization and denudation adjacent to one
another. While this intense tissue neutrophilia characterized the lamina

propria subjacent to denuded areas, there was always an abrupt interruption
of this neutrophilia in lamina propria covered by epithelium. This seemed
to support the long-known importance of epithelium as a protective barrier.
This is not to suggest that the neutrophilic response was from the urine
or from within the lumen. Indeed, it was not. In fact, capillaries in the
lamina propria were often filled with neutrophils and neutrophils were noted
emerging from them into the lamina propria, presumably by diapedesis. What-
ever mucosal denudation did to stimulate the neutrophilic response re-epithel-
ialization appeared to depress it.

Sanders, et al (1958), and others who studied the ability of epithelium
to regenerate over the entire vesical surface, did not investigate the re-
generated epithelium histologically in the early periods of re-epithelial-
ization or at the time of re-epithelialization. In comparison to the orig-
inal feline transitional epithelium, which is not too dissimilar from other
species, some interesting differences can be noted.

The regenerated epithelial cells were not characteristically like the
original. Over any given re-epithelialized surface, sections examined more
distant from the trigone had epithelial cells that were flat and mostly
arranged with their long axis parallel to the luminal surface. These cells
were usually not arranged in cell layers traditionally described for normal
transitional epithelium. On the other hand, sections nearer the trigone
had epithelium with distinguishable strata; i.e., superficial, intermediate,
and basal layers. Hence, a pattern was established. The more recently re-
generated epithelium was uniform, only two to three cell layers deep, and
without distinguishable strata. Older regenerated epithelium, however,
was more nearly similar to original normal transitional epithelium. Annis
(1962) noted the establishment of superficial, intermediate, and basal

strata beginning near the trigone while it was thinner toward the apex and not stratified. This pattern disclosed the direction of regenerative activity which was from the trigonal region cranially to the apex.

Characteristically, in the contracted bladder, the lamina propria was described as thrown into folds, while the epithelium smoothly lined its surface as it sent papillary projections into the lumen. This can be seen in the photomicrographs of the normal feline bladder examined in this study (Figure 14). However, in bladders from the experimental cats, which were always contracted, the lamina propria was not arranged into folds until the sixth postoperative week, one week following full re-epithelialization. McMinn and Johnson (1955) noted that epithelium immediately surrounding the margin of the artificial ulcers they created had been reduced in thickness. This was surprising to them since these bladders were contracted, a state normally associated with epithelium many layers thick.

Although there are many descriptions in the literature for transitional epithelium there are no truly definitive descriptions for that of the cat. The investigators who have worked with cats, regardless of their experimental objectives, have not demonstrated nor described normal feline transitional epithelium. Brauer (1926) studied normal feline urinary bladder but was only concerned with the relationship between mitoses and cell layer. Carpenter (1951) presents merely a narrow section of partially distended bladder wall and describes it very briefly as he was interested in the changes that were to occur in the muscle layers of the feline bladder following bilateral parasympathetic denervation. Johnson and McMinn

(1955, 1956), and McMinn and Johnson (1955) made investigations using transitional epithelium of feline urinary bladders as their center of interest, but only reported histologic accounts of their results. In their firm affirmation on the existence of a basement membrane interposed between the transitional epithelium and the lamina propria in the urinary bladder of the dog, cat, and rat, Hanssens and Sebruyns (1957) do not focus full attention on the transitional epithelium per se. Trautman and Fiebiger (1957) described the histology of the urinary system of the domestic animals but fail to speak specifically about the cat. Interestingly, these latter authors state that, "a basement membrane is apparently lacking in this type of epithelium (transitional)". Interest developed in the presence of alkaline phosphatase and the activity of fibrogenesis in the cat, but the investigators (Johnson and McMinn, 1958, and McMinn, 1958), although part of the investigation involved the urinary bladder, did not concern themselves with the histologic aspects of this organ. Surgery of the feline urinary system was discussed by Archibald and Cawley (1967) but no mention was made of microscopic anatomy of the bladder as it might be involved with any of the described procedures. Bradley and Teague (1968) investigated the nervous innervation of the feline urinary bladder but found no necessity for histologic detail.

Nevertheless, interesting contrasts can be made between the normal feline mucosa and submucosa as described in this experiment and the regenerated epithelium and its underlying lamina propria.

In accordance with the above description, normal feline transitional epithelium lining a contracted bladder was four to six layers thick. These

layers were categorized into three distinct strata, characterized on the
basis of observation with the light microscope, as (1) The superficial
stratum, featuring the largest cells which were plump, round, lightly
basophilic, and only one cell layer thick. (2) The intermediate stratum
was composed of three to five cell layers. The cells of this intermed-
iate stratum were more ovoid in shape, directed with their long axes per-
pendicular to the luminal surface. They had darker staining basophilic
nuclei and were approximately one-half the size of the superficial cells.
(3) The basal stratum was composed of one cell layer. The transitional
cell of this stratum was more oval, darker basophilic, and directed per-
pendicular to the luminal surface.

The regenerated transitional epithelium was much more uniform. It
was composed of one, two, or three cell layers prior to thirty-five days
and was always composed of the least number of cell layers toward the
apex. After thirty-five days, the epithelium was more consistently com-
posed of three cell layers. However, it was not possible, even up to 112
days, to discern three strata on the basis of cell morphology. All of
the regenerated transitional cells had the same basic morphology regardless
of the cell layer in which they lay. Basically, they were more like the
transitional cell of the basal structure of original epithelium in appear-
ance. The cells, regardless of the row, were always directed with the long
axis of this nucleus parallel to the luminal surface. This gave the re-
generated transitional epithelium the overall appearance of being flat and
thin.

When McMinn and Johnson (1955) examined the regenerated epithelium

that had covered small artificial ulcers the cells were large, the cytoplasm stained less densely, and the nuclei were vesicular. This regenerated epithelium was different in appearance than that seen in this study but this may be due to the different circumstances of the investigation, i.e., this experiment observed the migration of regenerated transitional cells over a much greater area and the environment was of a more severely inflamed nature. Regenerated epithelium was observed by Sanders, et al (1958) at 112 days following total mucosal denudation in dogs. Transitional epithelium was found to be "normal in all respects and varied from three to twelve or more cells in thickness". The epithelium was noted to be thicker in the area of the trigone, particularly near the ureteral orifices.

Iida (1960) noted regenerated epithelium that closely resembled normal bladder epithelium in a series of experiments on dogs. This observation was made following subtotal cystectomy, and suturing of the residual stump to the posterior surface of the rectus abdominis muscle. Annis (1962) studied the regenerative ability of the epithelial lining of the canine urinary bladder. A detailed description of the regenerated epithelium was not presented but the epithelium in low and high power photomicrographs, said to be regenerated epithelium seven weeks postoperatively, had a very similar appearance to the regenerated epithelium seen at the same postoperative period in this experiment.

Five months following total surgical excision of human urinary bladder transitional epithelium, Baker, et al (1965) demonstrated a biopsy specimen of regenerated uroepithelium of only one to five cells in depth. By 11 months, however, another biopsy specimen revealed regeneration of a near

normal transitional epithelial lining.

Liang (1966) removed the fundus portion of the bladder in rats, sutured the opening to the abdominal wall to form a condition of exstrophy, and diverted the urine away from the area. Several times daily, distilled water was used to distend the bladder with the first objective being to study mechanical force (distention and contraction) as a factor in regeneration of the bladder. Bladder regeneration occurred and a normal transitional epithelium lined the regenerated portion. Neither a time-relationship nor a histologic description of the regenerated covering of epithelium were recounted.

Histology of an omentoplast formed from a pediculated flap of omentum and used to patch large urinary bladder wall defects was described by Goldstein and Dearden (1966). The luminal surface was covered by transitional epithelium which was two to four cell layers thick. The transitional cells were uniform in size and resembled the regenerated cells observed in this study. These flattened regenerated transitional cells were also observed by Rasmussen (1966) when the healing of linear incisions into the urinary bladder of rabbits was studied.

It was noted by Aurora and Gupta (1967) that in regard to studies involving regenerated urothelium, little attention had been given to describing the details of the regenerated urothelial cells. This was an outstanding observation made during the literature review for this investigation.

In rabbits, Aurora and Gupta (1967) removed a 4 to 5 millimeter piece of bladder mucosa. They described the normal urothelium as a three to four cell thick stratified epithelium with a basal layer of cuboidal cells. The intermediate cells were large and polyhedral in shape except for those of

the surface layer which were sometimes flattened out. The polyhedral cells were of two types, i.e., clear cells, which had thin, watery, poorly stainable cytoplasm, and compact cells with a dense, granular cytoplasm. Clear cells predominated in this normal epithelium.

By six to seven days postoperatively, the mucosal defect was noted by Aurora and Gupta (1967) to be covered by regenerated epithelium which was predominantly composed of compact cells. Complete re-epithelialization of the ulcer had not occurred. However, by the time the ulcer was completely re-epithelialized, 10 days postoperatively, the mucosa was composed of fewer compact cells in relation to clear cells. By the 45th postoperative day, the mucosa was 3-4 cells thick with a predominance of clear cells.

In this present study, the regenerated epithelial cells were more like the compact cells observed by Aurora and Gupta throughout the period of re-epithelialization which extended up to the 35th postoperative day. Past 35 days, some of the cells began to enlarge and the nuclei appeared more like their clear cells. The essential difference in response may be due to the greater surface over which the transitional cells in this study were required to migrate. These so-called compact cells seem to be regenerating cells in contrast to regenerated cells.

The regenerated urothelial cells seen in this study also resembled the normal transitional epithelial cells of urinary bladders subjected to various experiments that set out to elucidate changes in the urothelium when bladders were contracted and distended (Harvey, 1909; Vacek and Schück, 1960; Leeson, 1962; and Richter and Moize, 1963).

The sections studied in this experiment revealed a marked paucity of

mitotic figures. In spite of the tremendous ability of the transitional epithelium to regenerate and migrate to cover such a large area of mucosal excision, the relative absence of mitoses was not surprising.

It was shown that in normal transitional epithelium of urinary bladders of experimental animals, the rate of cell renewal or cell turnover is very low (Bertalanffy and Lau, 1962). It was reported that only 3% of the superficial cells and 2% of the deep cells were renewed daily by mitosis. This work was an extension of that done earlier on urinary bladder epithelial renewal (Vulpe, 1954, and LeBlond and Walker, 1956).

On the other hand, McMinn and Johnson (1955) and McMinn (1956) concluded that the rapid rate of repair seen in healing of small, 0.5 to 1.0 centimeter, diameter bladder mucosal ulcers in cats was due to mitosis and migration. The mitotic activity was increased over that expected for normal cellular turnover (LeBlond, et al, 1955, and LeBlond and Walker, 1956). It was observed that proliferative spreading occurs by the end of the second day and is then supplemented by increased mitotic activity. It was also noted that mitoses occurred in different layers of epithelial cells.

During the regenerative period in this study, some interesting changes occurred in the lamina propria. The earliest change observed was a marked infiltration of neutrophils into the entire lamina propria. These neutrophils were diffusely scattered throughout the lamina propria, traversed the luminal surface, and were entrapped by a serofibrinous exudate that covered the luminal surface. In the external portion of the lamina propria, a proliferative response composed of fibroblasts and pericapillary supporting cells were formed into two distinct zones situated, respectively, at

different depths in the lamina propria. These latter types of cells seemed to originate not just in the deep lamina propria but also in the connective tissue and supportive mesenchymal tissue between the muscle bundles.

At later stages in the regenerative period, the zone appeared to be situated in a more superficial location relative to the lumen. As regenerated epithelium covered the luminal surface, and hence, the subjacent lamina propria, the neutrophilic zone was no longer present. However, subjacent to the new epithelium, there was a zone of fibroblasts and pericapillary supporting cells. These cells were directed toward the basal layer of new epithelium. Some of these cells appeared to cross into the new epithelium and became indistinguishable from the newly regenerated basal transitional cell, the compact cell of Aurora and Gupta (1967).

This pattern was observed throughout the experiment. It was observed sequentially throughout the regenerative period and also seen at various levels of an individual bladder as the bladder was studied from the trigone area, which was covered with regenerated epithelium, cranially toward the apex, which was not yet re-epithelialized. Any area covered with epithelium was found to have an abrupt absence of neutrophils or any other cell associated with an acute inflammatory response. After the 35th postoperative day, when all bladders were re-epithelialized, the most notable feature of the lamina propria was a tendency to greater maturity of its collagenous tissue. However, there was still some activity associated with what appeared to be fibroblasts or pericapillary supporting cells arising from the walls of arterioles and venules. Some of these

cells were seen crossing or jutting into the basal area of the newly formed epithelium. This phenomenon was observed as late as 112 days postoperatively.

These observations aroused the interest shared by others in regard to the possible origin of the regenerated epithelium. deRouville (1897, quoted by Vulpe, 1955) stated that the subepithelial connective tissue cells changed into epithelial cells which subsequently were not seen to divide mitotically. The precise mechanism of epithelial regeneration is not clear. Regeneration of human and canine urinary bladders following total surgical excision indicated differentiation of a totipotent mesenchymal cell into a transitional epithelial cell which then underwent proliferation to ultimately re-epithelialize the entire intraluminal surface of the bladder (Baker, et al 1965). When Goldstein and Dearden (1966) experimentally repaired large urinary bladder defects in rabbits with omentoplasty, the grafted omentum was ultimately lined by regenerated transitional epithelium. They observed that the formation of a new lining epithelium strongly suggested that transitional epithelium may result, under cetain conditions from influences upon multipotent mesenchymal cells in connective tissue in the area of the trauma. They noted that twenty-four hours following surgery, at the margin of the defect opposite the muscular layer of the bladder, there were isolated areas of large cells seen to partially cover the interrupted bladder wall. Some of these cells were flattened as if tending to form a lining epithelium, whereas others were perpendicular to the surface with the major portion in the connective tissue of the muscular layer but with cytoplasm extending

to the surface. These cells appeared to be fibroblasts and were quite abundant in the connective tissue of the bladder wall at the margin of the defect. On the third and fourth postoperative day, fibroblasts were seen proliferating intensively in the connective tissue between muscle bundles of the bladder wall as well as in the omentoplast. Numerous mitotic figures were found in fibroblasts as well as in newly formed epithelium in the area of the bladder wall defect. Between the 6th and 8th day of repair, fibroblasts, singly or in groups, were noted to be oriented perpendicular to the luminal surface and in many instances in contact with the luminal surface. These cells appeared to be continuous with morphologically similar cells which had flattened and provided an epithelial lining for the defect. Mitoses were quite common among the cells forming the lining epithelium, as well as in fibroblasts immediately deep to the lining epithelium. Their final conclusions were that the process of epithelialization of the omentoplast and of the posterior bladder wall at the margins of the defect appeared to occur by extension of the existing bladder epithelium and by transition from fibroblasts in the connective tissue. It appeared that multipotent mesenchymal cells in the connective tissue between muscle bundles of the bladder and in the connective tissue layer external to the muscular wall are able to proliferate rapidly and differentiate into lining epithelial cells.

Many of these same observations were seen in this experiment. The process of re-epithelialization seen in this study was thought to have involved proliferation of cells from the remaining original epithelium of the trigone. Since this area was not examined, it was not possible to

know if the proliferative activity was due to mitosis. In this study, proliferation and migration was thought to be one phase of the process of re-epithelialization. The re-epithelializing migration apparently spread from the trigone to the apex. Implantations of epithelial nests from which epithelium regenerated were not seen.

The possibility of a second process of re-epithelialization was realistic in consideration of the zones of neutrophils and fibroblasts previously described. When regenerated epithelium was established, the neutrophilic response had "surfaced" and the fibroblastic reactivity was now in the superficial most aspect of the lamina propria. With these fibroblasts or fibroblast-like cells swirling toward and into the basal layer of the new lining epithelium and having a similar, if not identical, appearance, the hypothesis of first proliferation of a multipotential mesenchymal-fibroblast-like cell and subsequent differentiation into a basal transitional cell became very tenable.

SUMMARY AND CONCLUSIONS

Transitional epithelium lining the urinary bladder has been shown to have remarkable capabilities for repairing itself. Following primary wounding, such as incision through it or excision of a portion of its substance, the transitional epithelium has been able to repair the defect by regeneration of transitional cells.

A decription of normal feline urinary bladder transitional epithelium and lamina propria was prepared to serve as a source of reference for comparing the morphology of the regenerated epithelium and subjacent lamina propria.

Nineteen, adult, male and female, domestic shorthair cats were used in the experiment. Each cat underwent surgical denudation of the entire urinary bladder mucosa cranial to the trigone. The cats were euthanatized between one and one hundred and twelve days postoperatively. A histologic sequence covering the entire experimental period was prepared.

Following surgical excision of the urothelium, the urinary bladder transitional epithelium regenerated and covered the entire luminal surface of the bladder cranial to the trigone.

The total time for re-epithelialization was thirty-five days; however, ninety per cent of the luminal surface was relined in the cat whose bladder mucosal regeneration period was twenty-eight days.

The mechanism for re-epithelialization appeared to involve the processes of migration, proliferation, and differentiation. Migration of regenerated urothelium was in a cranial direction. There was a paucity of mitoses. A second possible mechanism was suggested by the presence of a multipotential

- mesenchymal-fibroblastic cell which appeared to migrate from the deep lamina propria to the basal layer of the epithelium and undergo transition into a basal transitional cell.

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RE-EPITHELIALIZATION OF THE FELINE URINARY
BLADDER FOLLOWING SURGICAL DE-EPITHELIALIZATION

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Approved by:

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ABSTRACT

RE-EPITHELIALIZATION OF THE FELINE URINARY
BLADDER FOLLOWING SURGICAL DE-EPITHELIALIZATION

by Jack D. Henry, Jr.

Transitional epithelium lining the urinary bladder has been shown
to have remarkable capabilities for repairing itself. Following primary
wounding, such as incision through it or excision of a portion of its
substance, the transitional epithelium of the urinary bladder has been
capable of repairing the defect by production of transitional epithelial
cells.

There were 4 objectives in this investigation: The first objective
was to prove that urinary bladder transitional epithelium of the feline
was capable of full regeneration after the bladder lining was excised to
the level of the trigone. The second objective was to determine the length
of time for total re-epithelialization. The third objective was to gain
insight into the nature or mechanism of transitional epithelial regenera-
tion. The fourth objective was to describe a surgical procedure that
accomplished total urothelial excision.

Nineteen, adult, male and female, domestic shorthair cats were used
in the experiment. Each cat underwent surgical denudation of the entire
urinary bladder mucosa cranial to the trigone. The cats were euthanatized
between one and 112 days postoperatively. A histologic sequence covering

the entire experimental period was prepared.

Following surgical excision of the urothelium, the urinary bladder transitional epithelium regenerated and covered the entire luminal surface of the bladder cranial to the trigone.

The total time for re-epithelialization was 35 days; however, 90% of the luminal surface was re-lined in one cat by the twenty-eighth postoperative day.

The mechanism for re-epithelialization appeared to involve the processes of migration, proliferation, and differentiation. Urothelium regenerated in a cranial direction. There was a paucity of mitoses. A second mechanism was suggested by the presence of a multipotential mesenchymal-fibroblastic cell which appeared to migrate from the deep lamina propria to the basal layer of the epithelium and undergo transition into a basal transitional cell.

A description of normal feline urinary bladder transitional epithelium and lamina propria was prepared to serve as a source of reference for comparing the morphology of the regenerated epithelium and subjacent lamina propria.

The surgical procedure developed in this study allowed separation of the transitional epithelium from the underlying lamina propria and its subsequent excision.