An evaluation of appositional versus inverting suture patterns for cystotomy closure

by

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GENERAL INTRODUCTION

A cystotomy is a common surgical procedure performed in veterinary surgery. Its indications include calculi, polyps, diverticuli, trauma, and neoplasia. It can also be used as a diagnostic tool and for biopsy collection. Urogenital surgical techniques date back to Hippocrates (400 B.C.) who gave a specific injunction in his surgeons oath against "cutting for the stone" because mortality rates were excessively high.[1] Franco (1556 A. D.) emerged as the first surgeon to have consistant success with removal of cystic calculi via a suprapelvic cystotomy approach and is considered the father of urogenital surgery.[1,2] Very little progress was made in urogenital surgery until the nineteenth century. General anesthesia, aseptic technique and basic improvements in surgical techniques have allowed cystotomies to be performed on a routine basis in most surgical practices.

Most descriptions for a standard cystotomy closure recommend that a double inverting suture technique, such as a Cushing followed by a Lembert pattern, be used.[3-9] The philosophy of utilizing double layer inverting suture patterns in hollow organs originates from small intestinal surgery performed during the early nineteenth century. Lembert and Trevors early in the 1800s stated that the serosa had healing properties superior to other layers of the small intestine.[10,11] It was concluded that it was essential that an inverting suture pattern be used to create a tight serosa to serosa seal assuring a leakproof anastomosis and an adequate gain in wound tensile strength. This hypothesis remained unquestioned for nearly 150 years. Subsequently, it was shown that inverting suture patterns did not create a superior leakproof anastomoses as compared to appositional techniques. In contrast inverting patterns were found to cause anastomotic ischemia followed by tissue necrosis with a subsequent decline in wound tensile strength.[12-15] Subsequent clinical and research trials have demonstrated that single layer simple appositional suture techniques create an intestinal anastomosis which is stronger, produces less luminal stenosis and causes earlier mucosal regeneration than inverting suture patterns.[16-25]

Wound healing studies in the bladder have been limited to research in the areas of bladder regeneration following subtotal cystectomies and the effects of various suture materials on bladder healing. It is generally accepted that incised and sutured wounds in the urinary bladder heal with the same sequence of events as other tissues.[10,26-28] Numerous studies have demonstrated the remarkable regenerative ability of the bladder. For example, if the entire canine bladder is denuded of uroepithelium, regeneration from ureteral and urethral sources will occur within 30 days and be complete by 16 weeks.[10] In addition, the uroepithelial - submucosal complex has an intense inductive action on mesenchymal stem cells to differentiate and produce fibrous tissue in the surrounding bladder wall. Experimental subtotal cystectomies have resulted in a pseudocyst structure lined with uroepithelium surrounded by fibrous tissue and smooth muscle cells.[10,29,30]

A variety of different suture materials have been utilized for cystotomy closures. Because the suture material is a foreign body, special considerations in the choice of material to be used in the bladder has been necessary. Ideally the material should not serve as a nidus for stone formation, it should be noninflammatory and have a predictable absorption rate. Earlier descriptions of cystotomy closures often recommended suture patterns which penetrated the bladder lumen, leaving suture material exposed to urine.[9,10,26,28,31] Catgut was the suture of choice since nonabsorbable materials were found to have a high potential to stimulate stone formation.[9,10,31,32] However, catgut caused tissue inflammation and had an unpredictable rate of absorption. The development of the synthetic absorbable materials gave the surgeon the benefit of a suture with a low potential for stone formation, a minimal inflammatory response, a predictable absorption rate and greater tensile strength. It is generally accepted by most urogenital surgeons, that synthetic absorbable materials are the suture of choice for cystotomy closure because of their physical properties, and biological behavior.[26,28,31,33]

The following studies investigate the use of three different suture patterns; a single layer appositional, a double layer appositional and a double layer inverting suture pattern for cystotomy closure. The experiments were designed based on conclusions extrapolated from previous small intestinal anastomoses studies and due to a lack of previous research comparing different suture techniques for cystotomy closure. The effect of each suture pattern on physical alteration, mechanial strength and wound healing in the bladder were compared.

Explanation of Thesis Format

This thesis is prepared in the alternate format. A general introduction and literature review precede two manuscripts comparing inverting and appositional suture patterns for use in cystotomy closure. The second manuscript is followed by a general discussion and references.

Both sections of this thesis represent manuscripts which will be submitted for publication in Veterinary Surgery under the authorship of Robert M. Radasch, David F. Merkley, James W. Wilson, Yosiya Niyo and Robert D. Barstad. Robert M. Radasch was the principal investigator of each study. Each section was prepared according to the format for the journal, Veterinary Surgery.

SECTION I. APPOSITIONAL VERSUS AN INVERTING SUTURE PATTERN FOR CYSTOTOMY CLOSURE: A COMPARISON OF PHYSICAL AND MECHANICAL PROPERTIES

ABSTRACT

The current trend in closure of the urinary bladder advocates a time honored double layer inverting suture pattern. However, no scientific data supports the theory that inverting patterns create superior wound closure. The purpose of this investigation was to determine if there were any differences between appositional and inverting suture patterns used for cystotomy closure with regards to physical alteration of the bladder wall and wound strength. Sixty-four dogs were divided into three groups. Dorsal cystotomies were performed and closed using either a single layer appositional, a double layer appositional or a double layer inverting suture pattern. Four dogs from each group were euthanitized at 0, 3, 12, 18, and 24 hours postoperatively. Biomechanical strength of the cystotomies were evaluated using bursting wall tension. Cystotomy incisions were also evaluated for adhesion formation, mucosal edema and hematoma formation, amount of tissue inversion and the width of the mucosal defect created by each suture pattern. Both appositional patterns were found to provide similar mechanical strength as compared to the inverting suture pattern. The inverting suture pattern created less adhesions formation than either of the appositional closures. The amount of mucosal inflammation, inversion of tissue and the mucosal defect created was considerably

less with the appositional patterns than that seen with the inverting suture patterns. The results of this study found no reason why appositional suture patterns should not be used for closure of the urinary bladder.

INTRODUCTION

A strong water tight, noninflammatory closure of the urinary bladder is desirable. Physical properties offered by a suture pattern for cystotomy closure should include (1) ease of application, (2) minimal disruptance of the incisional blood supply (3) lack of tension at the incision, (4) no restrictive adhesion formation, (5) minimal compromise of luminal diameter, (6) anatomical re-alignment of tissue layers, (7) no mucosal penetration by the suture material and (8) a water tight seal protecting against urine leakage. Based on conclusions extrapolated from intestinal anastamosis studies, an appositional pattern theoretically should be superior to inverting patterns for cystotomy closure.[1-13] Controlled experimental studies comparing appositional patterns to the standard double layer inverting patterns for cystotomy closures have not been performed. Some surgeons advocate appositional suture patterns for cystotomy closure and have utilized the technique in clinical situations.[14-19] However, inverting suture patterns are still used by many surgeons, are routinely described in the literature, and are taught to veterinary surgical students as the suture pattern of choice for a cystotomy closure.[19-26]

The present study was designed to determine if there were any differences in physical properties and mechanical strength between a single layer interrupted appositional closure, a double layer interrupted appositional closure and a standard double layer continuous inverting closure for cystotomy closure in the dog.

MATERIALS AND METHODS

Sixty-four healthy adult female mongrel dogs, which were obtained from veterinary undergraduate teaching courses, were used. The animals weighed between 20 and 30 kg. Sixty dogs were randomly divided into three groups (designated S, SS and II) consisting of twenty dogs each. Four dogs served as a control group. During a three week preoperative quarantine period all animals were vaccinated and dewormed. The health status of the animals were verified by a physical exam, a PCV, TP, WBC determination and a Knott's heartworm test. In addition, urine samples were collected via catheterization prior to surgery to eliminate any animal from the study which did not have a normal urinalysis.

The dogs were fasted for twelve hours preoperatively. Antibiotics were not used in any dog. Anesthesia was induced with intravenous sodium thiopental and maintained with halothane and oxygen using a semiclosed circle rebreathing system. The dogs were placed in dorsal recumbancy and the ventral abdomen was routinely prepared for an aseptic posterior median celiotomy.

Surgical Technique

A ventral midline incision was made through the linea alba from the brim of the pubis cranial to the umbilicus. The bladder was gently exteriorized, retracted caudally and packed off from the abdominal cavity with laparotomy sponges. The apex of the bladder was held by a Babcock atraumatic tissue forcep in order to maintain the bladder's position during the remainder of the procedure. A full thickness 4 cm incision was made, avoiding major vessels, on the dorsum of the bladder in sixty dogs. In the remaining four control dogs a ventral midline celiotomy was performed, however, the bladders were not incised.

The dorsal cystotomy incisions were closed with one of three, suture patterns. To eliminate the influence of improving surgical skill the order of the suture patterns used were randomly varied. The cystotomy incisions were closed using either a single layer interrupted appositional pattern (Figure 1), a double layer interrupted appositional pattern (Figure 2), or a double layer continuous inverting pattern (Figure 3), designated as S, SS, and II respectively.

The suture material used was 3-0 coated polyglactin 910[a] swaged on a urogenital tapered needle. Nine sutures were placed in each layer for the simple interrupted patterns so that distance between sutures was 5 mm and the distance from the needle puncture site to the wound edge was also 5 mm. The sutures were tied with enough tension to gently appose the bladder layers without inversion or eversion of tissue. The continuous inverting patterns were placed to assure that the suture material penetrated the bladder at 5 mm intervals and that the distance from the suture puncture site to the wound edge was also 5 mm. Enough tension was applied to invert the bladder wall to assure

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[a] Vicryl, Ethicon Inc., Somerville, NJ.

Figure 1. Single layer interrupted appositional pattern, designated as S. The suture material penetrates the serosa, muscularis and submucosa of the bladder.

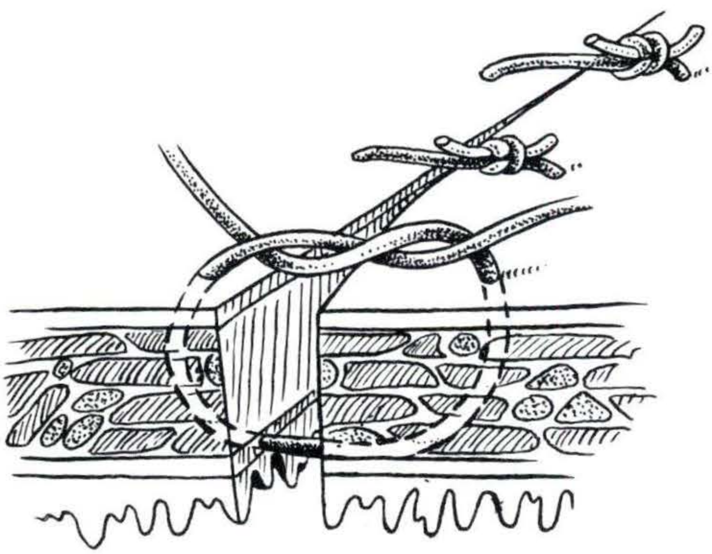


Figure 2. Double layer interrupted appositional pattern, designated as SS. The first layer of sutures are placed in the submucosa and muscularis. The second layer of sutures are placed through the muscularis and serosa of the bladder.

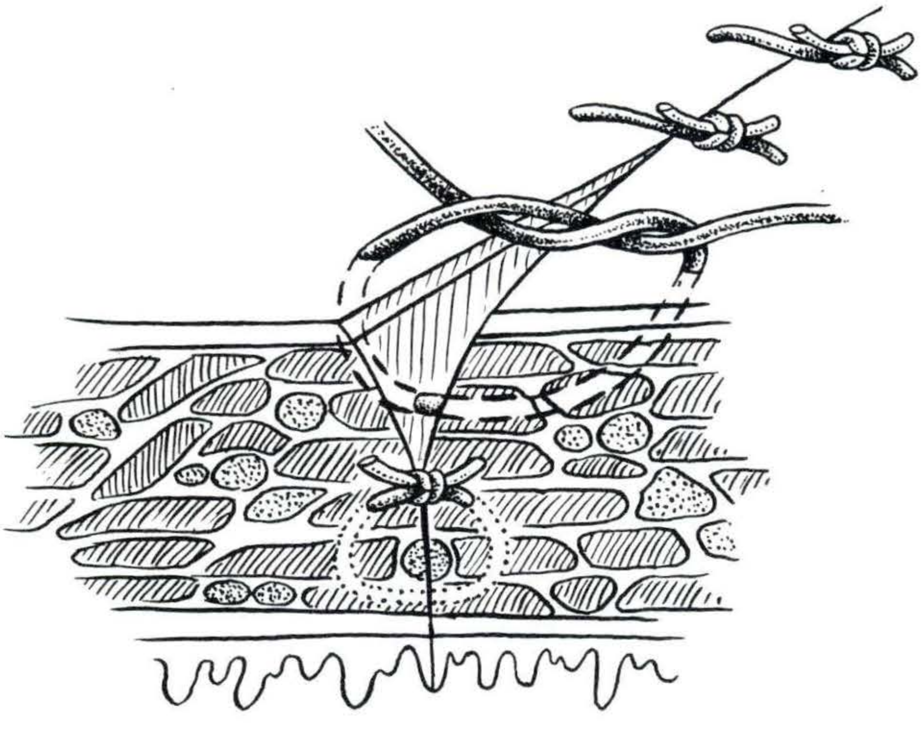
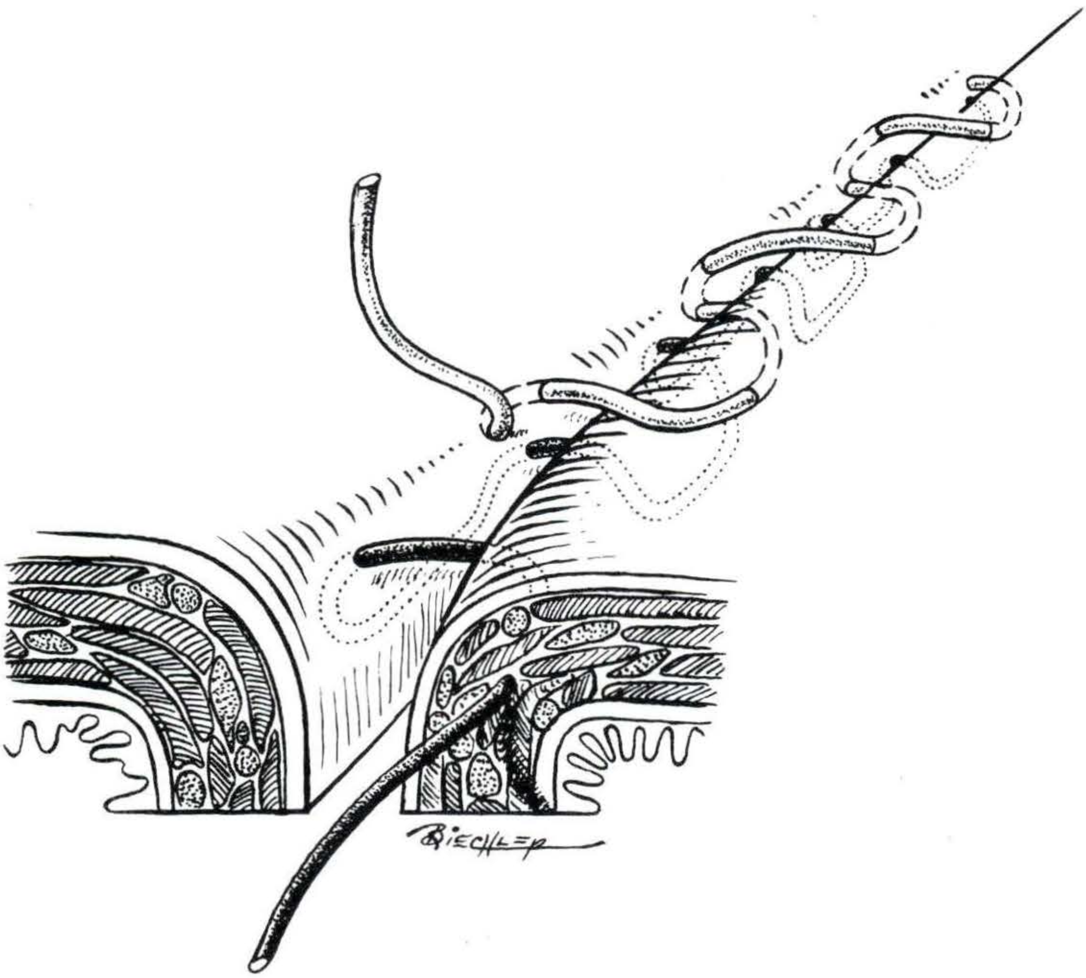


Figure 3. Double layer continuous inverting pattern, designated as II. A Cushing followed by a Lembert suture pattern was used. Both suture patterns were placed through the muscularis and serosa of the bladder.



a tight serosa to serosa contact between wound edges. All sutures were placed and knots tied so that no suture material penetrated the mucosa. All knots were tied with four throws of equal tension.

After the cystotomy incisions were closed, the bladders were returned to their normal anatomical location. A standard three layer abdominal closure was used, using chromic catgut for all buried sutures and nylon in the skin. Dogs were recovered and offered water as soon as they were ambulatory.

Postoperative Assessment of Incisions

Four dogs from each group were euthanitized at 0,3,12,18 and 24 hours postoperatively. The control dogs were euthanitized immediately postoperative. The abdomens were grossly examined for urine leakage and peritonitis. The cystotomy incisions were graded on a scale of 0 to 3 depending on the extent of adhesion formation (Table I).

The bladders were gently dissected free of adhesions without manipulation of the suture line, and were removed from the abdominal cavity. A 3 cm segment of urethra and a 2 cm segment of each ureter was left attached to the bladders. Any urine remaining in the lumen was drained. Each ureter was doubly ligated at the ureterovesicular junction. A 20 gauge French red rubber catheter was inserted into the urethra so that the tip of the catheter was at the urethralvesicular

Table 1. Adhesion grading system

Grade	Interpretation
0	No adhesion formation
1	Minimal adhesion formation - less than 50 percent of incision covered by an easily separated adhesion
2	Moderate adhesion formation - 50-100 percent of incision covered by an easily separated adhesion
3	Severe adhesion formation - incision covered by an extensive adhesion which does not easily separate

junction. Two cable ties[b] were positioned at the urethralvesicular junction and tightened to assure a water tight seal between the bladder and the catheter. The catheter was connected to a pressure transducer[c] which in turn was connected to a pressure monitor[d]. An infusion pump[e] was attached to the side arm of the pressure transducer (Figure 4). Saline with blue food coloring, to aid in the detection of leakage, was infused at a constant rate of 10 ml per minute.

The bursting pressure was indicated by the appearance of blue saline on the exterior of the bladder and by a drop in the intravesicular pressure recording. Sutures were not removed prior to bursting pressure determination. The pressure when leakage occurred (bursting pressure), volume of saline infused, and the site of rupture were recorded for statistical comparison between suture techniques.

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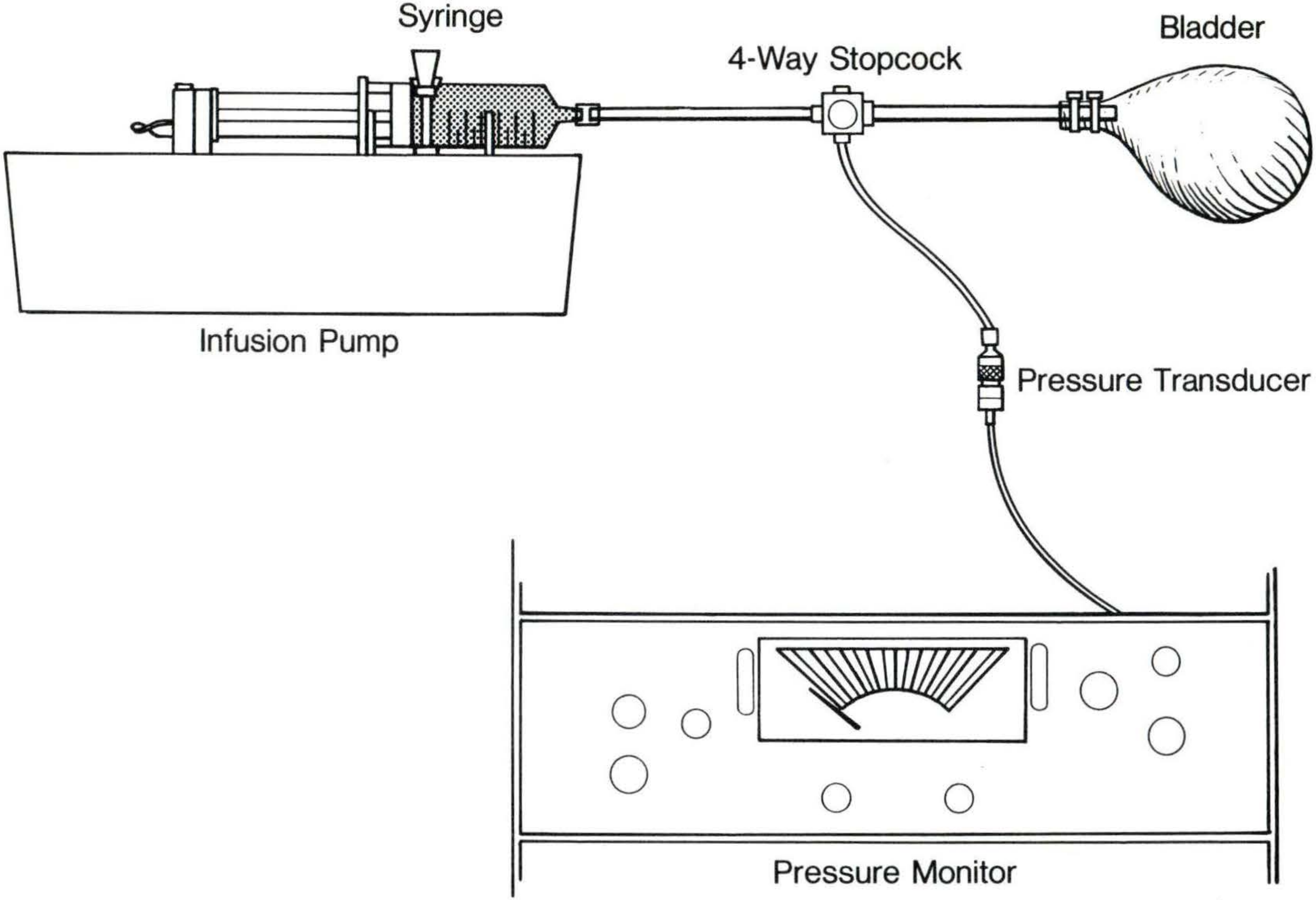
[b] Bar-Lok Cable Ties, Dennison Co., Framingham, MA.

[c] Physiological Pressure Transducer, Statham Lab Inc., Hato Rey, Puerto Rico.

[d] Simultrace Recorder VR-6, Electronics for Medicine Inc., White Plaines, NY.

[e] Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Millis, MA.

Figure 4. Diagram of the apparatus used to infuse bladders and measure bursting pressure for circular bursting wall tension (CBWT) determinations.


Syringe
4-Way Stopcock
Bladder
Infusion Pump
Pressure Transducer
Pressure Monitor

By regarding the bladder as a sphere, the radius at the time of rupture could be calculated from the formula:

$$r = \sqrt[3]{\frac{3\,V}{4\,\pi}}$$

r = principal radii of curvature (cm.)

V = volume of infused saline (ml.).

The circular bursting wall tension (CBWT) was then calculated based on Laplace's law which defines the pressure-wall tension relationship in hollow organs:

$$CBWT = \frac{Pr \times 1.33 \times 10^3}{2}$$

CBWT = circular bursting wall tension (dyne/cm.)

r = principal radii of curvature (cm.)

P = bursting pressure (mmHg)

1 mmHg = 1.33×10^3 dyne/cm.^{2} [6,27-30]

After CBWT determinations, the bladders were opened along their ventral surfaces. The amount and severity of mucosal edema and hematoma formation was subjectively graded as minimal, moderate, or severe. Calipers were used to measure the width of the mucosal defect as well as the amount of tissue inverted into the lumen.

Statistical comparisons were made between the three different suture patterns using a Chi-squared statistical analysis for the degree of adhesion formation ($P = .04$), and site of rupture ($P = .33$). The analysis of variance was used for CBWT determinations, width of the mucosal gap and amount of inverted tissue ($P = .05$).

RESULTS

There were no operative deaths. Clinically all dogs recovered well from surgery. All dogs exhibited some degree of hematuria during the first urination. By the second urination no dogs in any of the three groups showed visual evidence of hematuria.

Adhesion Formation

Gross evaluation of the abdominal cavities at the time of euthanasia revealed no evidence of peritonitis or urine leakage in any dog. No adhesions were detected in dogs at 0 and 3 hours. All adhesions formed were at 12, 18 and 24 hours and consisted of omental attachment to the incision. No adhesions to the abdominal wall or other organs were noted. Adhesion formation was graded, scores were cummulated and a mean cummulative adhesion score was determined for each group (Table 2). The double layer continuous inverting closure caused the least adhesion formation (mean cummulative score of 0.2) as compared to either of the interupted appositional closures (mean cummulative score of S = 1.0 and SS = 0.9). The adhesion formation differences were found to be statistically significant.

Table 2. Adhesion formation scores

Suture pattern group	Adhesion grade[a] 0	1	2	3	Mean adhesion score
S	9	3	7	1	1.0
SS	9	4	7	0	0.9
II	16	4	0	0	0.2

[a] Figures within the table represent the number of dogs in each group with the corresponding adhesion grade.

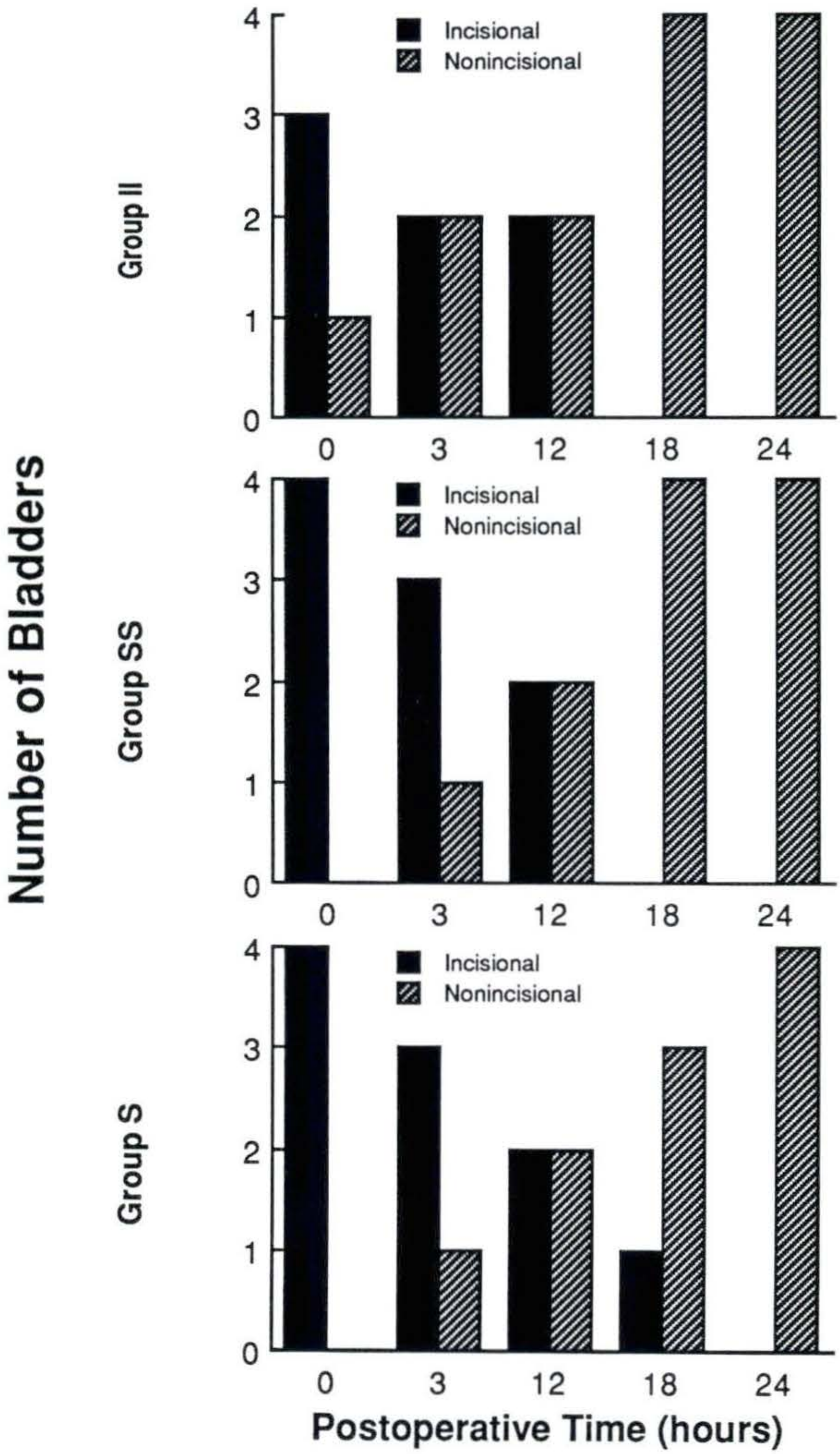
Rupture Location

Bladder rupture was classified as incisional when blue saline was observed leaking from the incision line or a suture hole. During bursting wall determination, no suture broke or untied in any bladder from any of the three groups. Occasionally as bladders expanded a suture partially tore the serosa and muscularis layer. The tear was never deep enough to cause leakage in any bladder. Whenever leakage was classified as incisional, regardless of the suture pattern or time studied, it always occurred from a suture hole and never from the incision line. Virtually all bladders ruptured at the incisional site at time 0 (92%). All three groups demonstrated a progressive decline in the number of bladders rupturing at the incisional site from 0 to 24 hours (Figure 5). No statistical differences between groups existed. No bladders in any group ruptured at the incision site at 24 hours. Leakage occurring at sites other than the incision were recorded as a rupture in either the dorsal or ventral half of the bladder. Nonincisional ruptures occurred in the ventral and dorsal half of the bladders with approximately equal frequency regardless of the suture pattern used.

Circular Bursting Wall Tension

The mean circular bursting wall tension (CBWT) for the three suture patterns at 0,3,12, 18 and 24 hours are graphically shown in Figure 6. All three groups exhibited the lowest mean CBWT at time 0.

Figure 5. Rupture location during infusion of the bladders after cystotomy closure.

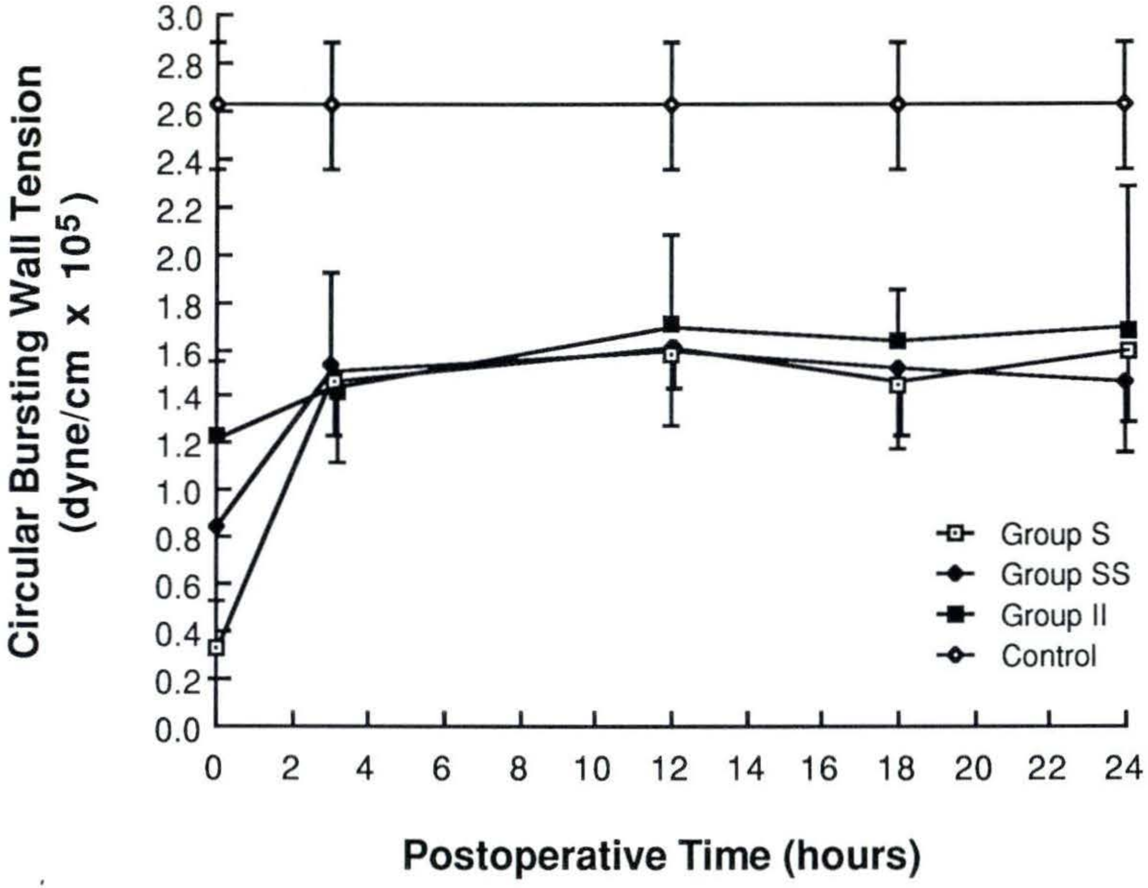

Number of Bladders
Group II
Group SS
Group S
Incisional
Nonincisional
0
1
2
3
4
0
3
12
18
24
Postoperative Time (hours)

Group S had the lowest mean CBWT (.31 $\pm$ .12 dyne/cm X 10^5) and group II demonstrated the highest mean CBWT (1.22 $\pm$ .32 dyne/cm X 10^5). Statistically groups SS and II had significantly higher mean CBWT than group S; however no differences between group SS and II existed. At 3, 12, 18 and 24 hours postoperatively, there were no significant differences in mean CBWT between any of the three groups. At the times studied, all three groups had a significantly lower mean CBWT than the control bladders (2.62$\pm$ .27 dyne/cm X 10^5).

The changes in mean CBWT within a group between time intervals were compared (i.e., from 0 hours to 3 hours, 3 hours to 12 hours etc.). Both group S and SS had a significant increase from time 0 to 3 hours. Group II did not demonstrate an increase in CBWT during this time period. After 3 hours, there were no significant increases in mean CBWT between consecutive time intervals for any of the three suture techniques studied. However, both groups S and SS had significant increases in the mean CBWT at each time studied as compared to the mean CBWT at time 0. Group II did not exhibit significant increase at any postoperative time as compared to time 0.

It should be noted that not all bladders ruptured at the incision. Mean CBWT were compared for bladders that ruptured only at the incision for each time and suture method. The same basic trends in bladder

Figure 6. Mean circular bursting wall tensions ($\pm$ SD) of the three suture patterns and controls.


Circular Bursting Wall Tension
(dyne/cm x 10^5)
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
22
24
Group S
Group SS
Group II
Control
Postoperative Time (hours)

strength were observed as in the previous comparison. However, statistical significance could not be determined due to an insufficient number of bladders rupturing at the incision in each group at 12, 18 and 24 hours.

Mucosal Integrity

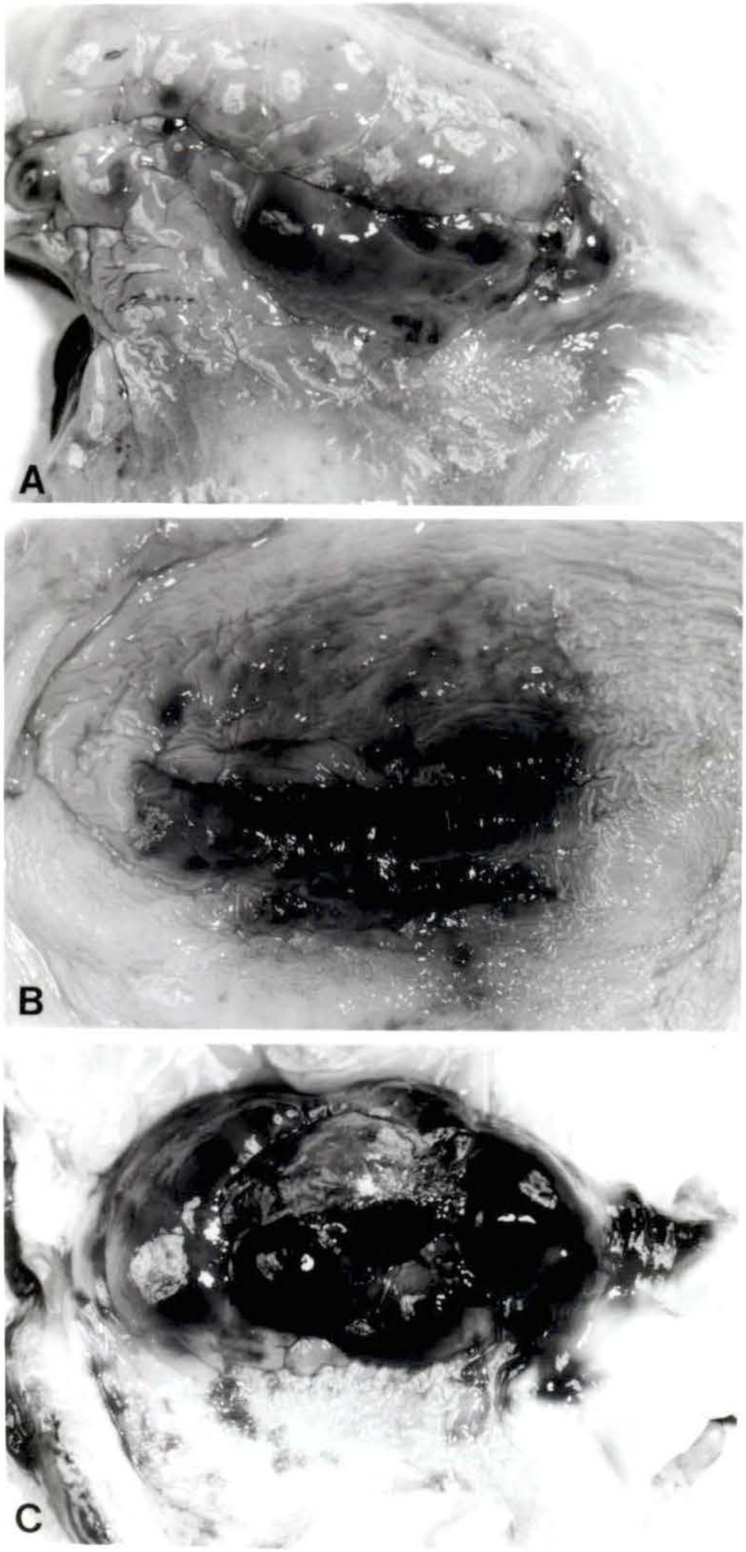
Gross visual examination of the mucosal surface of the bladders after completion of the CBWT determination, revealed that a difference between suture patterns existed with respect to edema and hematoma formation, width of the mucosal gap and the amount of tissue inverted at the incision (Figure 7). No group displayed any swelling of mucosa at the incision at time 0. At 3, 12, 18 and 24 hours the mucosa in the bladders of group II were severely swollen in the proximity of the incision. The mucosa in groups S and SS demonstrated minimal and moderate edema formation respectively, at the same time intervals. Hematoma formation was greatest in groups II at all times studied. Incisions in group II had large blood clots filling the mucosal defect and extending into the bladder lumen. In contrast, the incisions in group S and SS were seldom filled by a blood clot and if present it did not extend into the lumen. A dramatic difference in the width of the mucosal defect created by the different suture patterns was observed. Group S consistently had no defect with the mucosal edges in complete apposition. Incisional gaps in group SS were slightly greater than in group S with a mean gap of .85 $\pm$ .73 mm. However, the average defect created in group II was 14 ± 3.0 mm. with the mucosal edges inverted into the bladder lumen. The same trend was observed for the degree of tissue inverted into the lumen. No tissue was inverted in any bladder of group S or SS. In contrast, all incisions in group II demonstrated large amounts of inverted tissue (13 ± 5 mm.). Both appositional patterns had statistically smaller mucosal gaps and inversion of tissue than the inverting suture pattern.

Figure 7. Mucosal surfaces of the incisions after CBWT determination.

A. Single layer interrupted appositional pattern (S) at 12 hour postoperatively. The mucosal surface shows minimal edema, complete apposition and no inversion of tissue at the incision.

B. Double layer interrupted appositional pattern (SS) at 12 hours postoperatively. The mucosal surface shows moderate edema, a narrow gap and no inversion of tissue at the incision.

C. Double layer continuous inverting pattern (II) at 12 hours postoperatively. The mucosal surface shows severe edema, a wide gap and a large amount of inverted tissue at the incision.


A
B
C

DISCUSSION

Tissue hypoxia, serosal trauma and foreign material are the most common causes of adhesions in the abdominal cavity.[31,32] The results of this study show that all three suture patterns produce omental adhesions as early as 12 hours postoperatively. The suture material used for the cystotomy closures, polygalactin 910, is considered to cause a minimal inflammatory response.[33-35] However, since it is recognized as foreign material, it has the potential to cause irritation and create adhesions. The omental adhesions produced by the appositional patterns probably are a reflection of the amount of suture material exposed to the abdominal cavity. Nine knots and suture tags were exposed with both appositional patterns; however only the knots at the beginning and end of the suture line were exposed with the inverting pattern. The appositional patterns might have produced less adhesions if the knots had been buried. The minimal serosal edema and trauma produced by all three patterns further supports the theory that the difference in omental adhesions was due to the amount of suture material exposed to the abdominal cavity instead of one pattern causing more or less serosal irritation and trauma.

For a cystotomy adhesion to become a significant problem, it would have to form a fibrous attachment to the body wall or to an abdominal organ which would interfere with normal filling and emptying of the bladder. One study reported that a cystotomy incision was less likely to develop severe fibrous adhesions if covered by omentum.[36] The initial loose attachment of the omentum to the incisions is by fibrin. As the inflammatory reaction subsides, this fibrinous attachment may loosen freeing the incision of adhesions. Other possible beneficial effects of omental attachments would be to 1) increase the vascular supply at the incision, 2) seal small defects in the incision and 3) aid in the clearance of tissue debris and bacterial pathogens.[36] While speculative, omentum adhesions as seen in the two appositional suture patterns may prove to be beneficial to incisional healing.

The two basic methods to test anastomotic strength in hollow muscular organs are breaking strength and bursting strength.[12,28-30,37,38] Breaking strength measures the force applied in opposite direction that is required to disrupt an incision regardless of wound dimensions.[28-30] This measurement should be criticized for use in the bladder because the force is applied in a linear direction, perpendicular to the wound and does not take into account circular forces acting at the incision during filling.[29]

Bursting strength is the measurement of the resistance to an increase in intraluminal pressure.[12,28,30,37,38] It is considered a better indicator of wound integrity during the early stages of healing because it produces an evenly distributed transmural pressure at the incision edges.[8,39] The increasing transmural pressure reflects a physiological stress which can approximate the clinical situation since it is the force of distension which will rupture the incision line and cause leakage.[28,30] Bursting strength may be expressed as either the bursting pressure or the bursting wall tension.[28-30] Bursting pressure is the intraluminal pressure at which leakage occurs and is independant of the size of the structure being tested. It has been shown that bursting pressure gives no absolute value of the strength of the wound.[29] Bursting wall tension is defined as the tension present at any point along the bladder wall when leakage occurs.[28-30] It analyzes the force responsible for disruption of the wound by application of Laplace's law which defines the pressure-wall tension relationship in hollow organs.[6,27-30] Laplace's law takes into account the intraluminal pressure causing rupture in addition to the size and shape of the bladder when rupture occurs. During infusion of the bladder, circular tensions are acting at all points on the wall. By regarding the bladder as a sphere and using a constant infusion rate, this circular force can be calculated. Based on Laplace's law, it is obvious that for a given pressure the CBWT will be greater in a bladder with a larger radius. Because CBWT measurements correct for the size of the bladder and are a measure of true physiological stress placed on the wound when leakage occurs, it has been advocated as the most accurate measurement of incisional strength.[29,30]

The result of the CBWT measurements in this study clearly demonstrate that appositional suture patterns provide equal wound strength during the initial 24 hours when compared to the traditional double layer inverting pattern. In addition, the single layer appositional pattern exhibited strength comparable to both double layer suture patterns except at time 0. It is not surprising that all three groups demonstrated the lowest CBWT at time 0 since virtually all the strength in the wound is afforded by the suture material and the suture pattern. Group SS and group II suture patterns had mean CBWT at time 0, which were greater than group S. This can be attributed to more suture material holding the wound edges together. Since both double layer patterns were similar in strength it cannot be concluded that an inverting pattern is superior to an appositional pattern. It would be interesting to compare the strength of a single layer inverting pattern to the single layer appositional pattern.

The lower mean CBWT at time 0 for the simple appositional pattern did not prove to be of any clinical significance since urine leakage was not observed in any dog in group S. In addition, based on mean CBWT at 3,12,18 and 24 hours postoperative, the single layer pattern provided comparable strength as the double layer patterns. Similar results have been observed in small intestinal anastomosis studies.[9,13] An explanation for this observation may be that the single layer pattern causes less tissue trauma, less compromise of the incisional microvasculature and less anatomical alteration of tissue, all resulting in faster wound healing.

Examination of wound healing in the bladder has shown that it follows the same process of wound repair as other tissues. Multiple studies have also demonstrated that the bladder regains 100 percent of its normal tissue strength within 14 to 21 days post-wounding. Other hollow organs usually only reach 70 percent of their original strength even after several months of repair.[28,34,35] The original work of Hornes and his associates divided wound healing into three phases; the lag phase, the proliferative or fibroblastic phase and the maturation phase. Their work concluded that a gain in wound strength could only be observed during the later two phases of repair when fibrous tissue bridged the gap between the wound edges. In addition, any wound strength during the lag phase, which lasts between 3-5 days, was completely dependent upon the suture material.[28,37,40-43]

The significant increases in the mean CBWT during the initial postoperative hours would seem to refute the suggestion that no gain in wound strength can occur until the phase of proliferation has begun. Both appositional patterns had significant increases in mean CBWT at 3, 12, 18 and 24 hours post wounding as compared to their respective mean CBWT at time 0. However, the inverting pattern did not show this increase in mean CBWT at any postoperative time. If the wound strength is completely dependent upon the suture material then it would be logical that a double layer closure would have a higher mean CBWT than a single layer closure. This was not found to be true, as both appositional patterns produced insignificant differences in wound strength at 3, 12, 18 and 24 hours. This study clearly demonstrates that there is something present in the bladder wound during the initial hours of the lag phase which causes an increase in wound strength prior to fibrous tissue deposition in the wound.

The data substantiate that anatomical realignment of tissue planes is important for rapid gain in wound strength during the initial postoperative period. The inverting suture pattern causes a cuff of tissue to protrude into the bladder lumen so that serosa is in contact with serosa, disrupting the anatomic continuity of the tissue. Factors present in the wounds closed with appositional suture patterns may be mechanically blocked by inverted tissue, resulting in no early gain in wound strength. Therefore, any strength before fibroplasia begins would be dependent on the suture pattern used for the closure. The lack of gain in wound strength in the inverting pattern from time 0 to all other postoperative times studied could substantiate such a theory.

Several possible explanations which could cause an increase in wound strength during the lag period are 1) fibrin deposition in the wound, 2) capillary and vascular buds arising from the wound margins and 3) an adhesive force existing between wound edges. It is very likely that a multitude of factors are present in the wound. Each factor has a minimal contribution to strength by itself, but together they may have a profound effect on the strength and healing of the bladder wound during the lag phase of repair.

It has been documented that during the acute stages of inflammation the exudate contains a large amount of fibrinogen. The fibrinogen interacts with enzymes released from the blood as well as damaged tissue to form fibrin. If the mucosal continuity is re-established, as in the appositional closures, fibrin will seal the mucosal edges creating a water-tight anastamosis as well as contributing to wound strength.[28] An inverting suture pattern would create a larger gap between the mucosal edges delaying or minimizing the effects of a fibrin seal.

The process of acceptance of skin grafts ("graft take") has demonstrated that grafts are revascularized by the ingrowth of new vessels from the graft bed. The vessels may penetrate graft tissue or follow the path of old vessels.[44] As soon as vascular buds invade the graft, it is held stationary and an appreciable force is required to move the graft from its bed.[28] This revascularization process has been observed to occur as early as 6 hours post transplantation.[44] A similar process could occur between wound edges in the bladder if the tissue planes were in proper anatomical orientation. Vascular buds from one side of the incision could invade the opposite side and vice versa. If enough buds from each side of the incision invade the opposite wound edge, an appreciable increase in strength could be created during the early phases of inflammation. This theory would explain why there was no significant increase in strength for a bladder closed with the inverting suture pattern. The gap and discontinuity of wound edges created from the inversion of tissue would be too great for small vascular buds to cross and unite tissue during the initial post-wounding period.

A substance called fibronectin or "wound glue" could also be responsible for the rapid gain in strength during the initial lag period. Fibronectins are a family of high molecular weight glycoproteins which have been found to be secreted by multiple cell types in a wound.[28, 45-47] The substance has been isolated in wound plasma as early as 5 hours postwounding.[45] During the acute stages of wound healing, cells of inflammation (PMN and monocytes), dead tissue and exudate begin to coalesce forming islands of cell and debris which have been termed cell aggregation centers (CAC).[28,48] The CAC are located between wound edges and have been shown to secrete fibronectins. The fibronectin do not fill the gap between wound edges, but exist as cell surface proteins on the CAC. It is speculated that the cell surface fibronectins cause chains of CAC to be formed linking the edges of the wound together. Fibronectins have also been found to be bound to fibrin which may serve as the substatum for subsequent fibroblast migration.[45,46] Other investigators have indicated that fibronectins may have a profound effect on the early stages of wound healing, and due to their intercellular gluing properties, could create wound strength prior to fibrous tissue deposition.[48] Fibronectins present in the wounded bladder could account for the significant increase in wound strength during the initial postoperative period. If this wound glue does exist in bladder wounds, then it would seem reasonable that the maximum benefit to wound strength would occur if the wound gap was minimal with the edges anatomically aligned. This would explain why there was an increase in strength for both appositional patterns and not for the inverting one. The role of fibronectin causing an increase in wound strength during the initial hours of wound repair is only speculative at this time and would need to be substantiated by further studies.

It has been stated, that if rupture of a repaired bladder occurred, it would be at the incision site, since the strength of a scar does not reach that of normal uninjured bladder wall.[24,28] The results of this study demonstrated that the strength of the incision becomes as strong as the surrounding bladder wall in the early part of the lag phase of healing. Nonincisional ruptures could be detected as early as time 0 in group II and at 3 hours postoperative in groups S and SS. By 12 hours, 50 percent of the bladders in each group ruptured at a site other than the incision. Reasons for non-incisional rupture could be due to an extremely strong suture pattern which renders the incision stronger than normal bladder wall or some factor in the wound which causes an increase in the wound strength above that of untraumatized bladder wall. If the suture pattern were solely responsible for strength then it would be anticipated that the percentage of bladders rupturing at the incision would remain constant from one time period to the next. The data demonstrate that for each suture pattern studied there was a progressive decline in the percentage of bladders rupturing at the incision from time 0 to 24 hour postoperative. This strengthens the previous assumption that there is something occurring locally at the wound to increase its strength even during the lag phase of healing. The high percentage of bladders rupturing at time 0 reflects the inability of the suture patterns to make the incision as strong as unwounded tissue. Since the decline in the number of incisional ruptures between the three suture patterns is almost identical, no single suture pattern is superior to another based on its ability to prevent rupture at the incision.

No suture in this study broke during the bursting wall tension analysis. It would appear that 3-0 coated polygalctin 910 suture material is stronger than the bladder wall. In addition, regardless of the pattern, all incisional ruptures occurred where suture material penetrated the bladder wall instead of from the incision. Therefore, it would seem logical that the smallest diameter needle and suture material used, while still affording strength greater than the bladder wall, would be beneficial in preventing incisional ruptures. Perhaps 4-0 or smaller suture material would have provided adequate strength in addition to reducing the number of incisional ruptures as a result of a smaller puncture holes in the tissue.

The mucosal integrity was significantly altered by the inverting suture pattern as compared to the appositional patterns. Edema and hematoma formation, width of the mucosal gap and the amount of tissue inverted into the lumen were all greater in bladders closed with the inverting pattern. Tissue edema could have been caused by an increased amount of tissue trauma, hypoxia and damage to the blood supply by sutures inverting large pieces of tissue. In addition, the inversion of tissue would expose all layers of the wall to urine which could dissect between tissue planes resulting in a severe inflammatory response. The expansion of the bladders for CBWT determinations may have influenced the width of the mucosal gaps. However, a suture technique which limits mucosal separation with the subsequent exposure of underlying tissue to urine and possible pathogens is beneficial. Mucosal edges in close apposition should unite and seal faster than if a wide gap existed between them. This is beneficial since the mucosa acts as the initial barrier to pathogens in the urine.[28,49] The significance of the amount of tissue inverted into the bladder lumen is inversely proportional to the size of the patient. Smaller animals would have a relatively greater percentage of their luminal volume reduced with an inverting pattern, resulting in more frequent urinations which could be unacceptable or annoying to owners. In addition, the possible consequences of inverted tissue in the bladder lumen become greater the closer the incision is to the trigone. Excessive tissue inversion, edema or hematoma formation could create a functional obstruction near the trigone because of its narrow luminal diameter. The appositional patterns did not pose any of these problems because of proper anatomical realignment of tissue planes. Based on alterations of the normal mucosal integrity, the inverting pattern was inferior to the appositional techniques.

CONCLUSION

It is important to construct a cystotomy anastomosis in such a way as to make the incision resistant to circular wall tensions which could cause rupture. The suture pattern should also allow the incision to rapidly obtain strength comparable to unwounded tissue. Adhesion formation and mucosal integrity should be minimal. Based on the results of this study, appositional suture patterns provide equal mechanical strength and superior physical properties as compared to the standard inverting suture pattern. This study found no reasons why appositional suture patterns should not clinically be used for closure of a cystotomy incision.

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Section II. MICROVASCULAR AND CORRELATED HISTOLOGIC EVALUATION OF WOUND HEALING IN CYSTOTOMY CLOSURES: A COMPARISON OF APPOSITIONAL VERSUS AN INVERTING SUTURE PATTERN

ABSTRACT

The effects of three different suture patterns used for cystotomy closure; a single layer appositional, a double layer appositional and a double layer inverting pattern, were evaluated with regards to wound healing. Twelve dogs were divided into three groups. A dorsal cystotomy was performed on each dog and closed with one of the aforementioned suture patterns. One dog from each group was euthanatized at 24, 48, and 96 hours postoperatively. Wound healing was evaluated by utilization of microangiographic and correlated histology. The incisions were subjectively evaluated for vascular compromise, rate of revascularization, inflammation, mucosal regeneration, continuity of layers and fibrous tissue deposition. The inverting suture pattern retarded wound healing as compared to the appositional patterns. There was delayed formation of fibrous tissue and a substantially slower re-epithelialization at the incision. The inverting pattern also created pronounced tissue necrosis and ischemia during the first 96 hours postoperatively. Both appositional suture patterns allowed rapid mucosal regeneration, formation of mature connective tissue throughout the incision and minimal tissue ischemia adjacent to the wound edges. The results of this study suggest that the apposition of wound edges with precise anatomical alignment allows rapid wound healing which would warrent the clinical utilization of the appositional suture patterns for cystotomy closure.

INTRODUCTION

In addition to producing a strong watertight seal, a suture pattern utilized for cystotomy closure should allow for rapid mucosal regeneration, create minimal tissue ischemia and produce minimal fibrosis, edema and tissue necrosis at the incision. Any suture technique which compromises wound healing at the incision should be avoided, assuming that wound strength is unaltered. A previous study has demonstrated that appositional suture patterns provide equal mechanical strength as an inverting suture pattern for cystotomy closure.[1] However, experiments comparing appositional and inverting suture patterns with regards to the effects on wound healing are nonexistant.

The intent of this study was to compare a single layer appositional, a double layer appositional, and a double layer inverting suture pattern with respect to wound healing in cystotomy incisions. The three suture patterns were evaluated by comparing microvascular and correlated histologic changes directly at the incision.

MATERIALS AND METHODS

Nine healthy preconditioned female dogs (weight range 20 to 30 Kg) were randomly divided into one of three groups. Each group consisted of three dogs. After a twelve hour preoperative fast, each dog was induced with intravenous (IV)sodium thiopental (20 mg/Kg) and anesthesia was maintained with halothane and oxygen using a semiclosed rebreathing system. Dogs were placed in dorsal recumbancy and the ventral abdomens were prepared for routine aseptic posterior celiotomies.

After exteriorization, bladders were retracted and packed off from the abdominal cavity with laparotomy sponges. A full thickness 4 cm dorsal incision was made, avoiding the major vasculature. The cystotomy incisions were closed with one of three randomly varied suture patterns. Either a single layer simple interrupted pattern, a double layer simple interrupted pattern, or a double layer continuous inverting pattern were used. Descriptions of the suture patterns have been previously described.[1]

The suture material was 3-0 coated polyglactin 910[a] swaged on a urogenital tapered needle. Surgeries were performed by a single surgeon using previously described suturing techniques.[1] After cystotomy incisions were closed, the bladders were returned to the

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[a] Vicryl, Ethicon Inc., Somerville, NJ.

abdominal cavities. The abdomens were closed using a standard three layer closure technique. The dogs were recovered and offered food and water as soon as they were ambulatory.

Postoperative Assessment

One dog from each group was reanesthetized at 24, 48, and 96 hours postoperatively using IV sodium pentobarbital (30 mg/Kg). Dogs were perfused with barium sulfate for microangiographic and correlated histologic study of the cystotomy incisions according to previously published techniques used in other tissues.[2-7] Heparin was administered IV at a dose of 300 U/kg of body weight and allowed to circulate for five minutes. The animals were then euthanatized with an IV overdose of concentrated barbiturate anesthetic.[b]

A coronary perfusion catheter[c] was inserted into the abdominal aorta cranial to the umbilical artery, and the caudal vena cava. Umbilical tape was placed around each vessel to secure the catheter and occlude blood flow proximally. The microangiographic study was accomplished by connecting the arterial catheter to the infusion line and perfusing with 30% micropulverized barium sulfate[d] suspended in

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[b] Beuthanasia-D Special, Burns-Biotec, Omaha, NE.

[c] United States Catheter Inc., Billerica, MA.

[d] Micropaque, Picker x-ray Corp, Cleveland, OH.

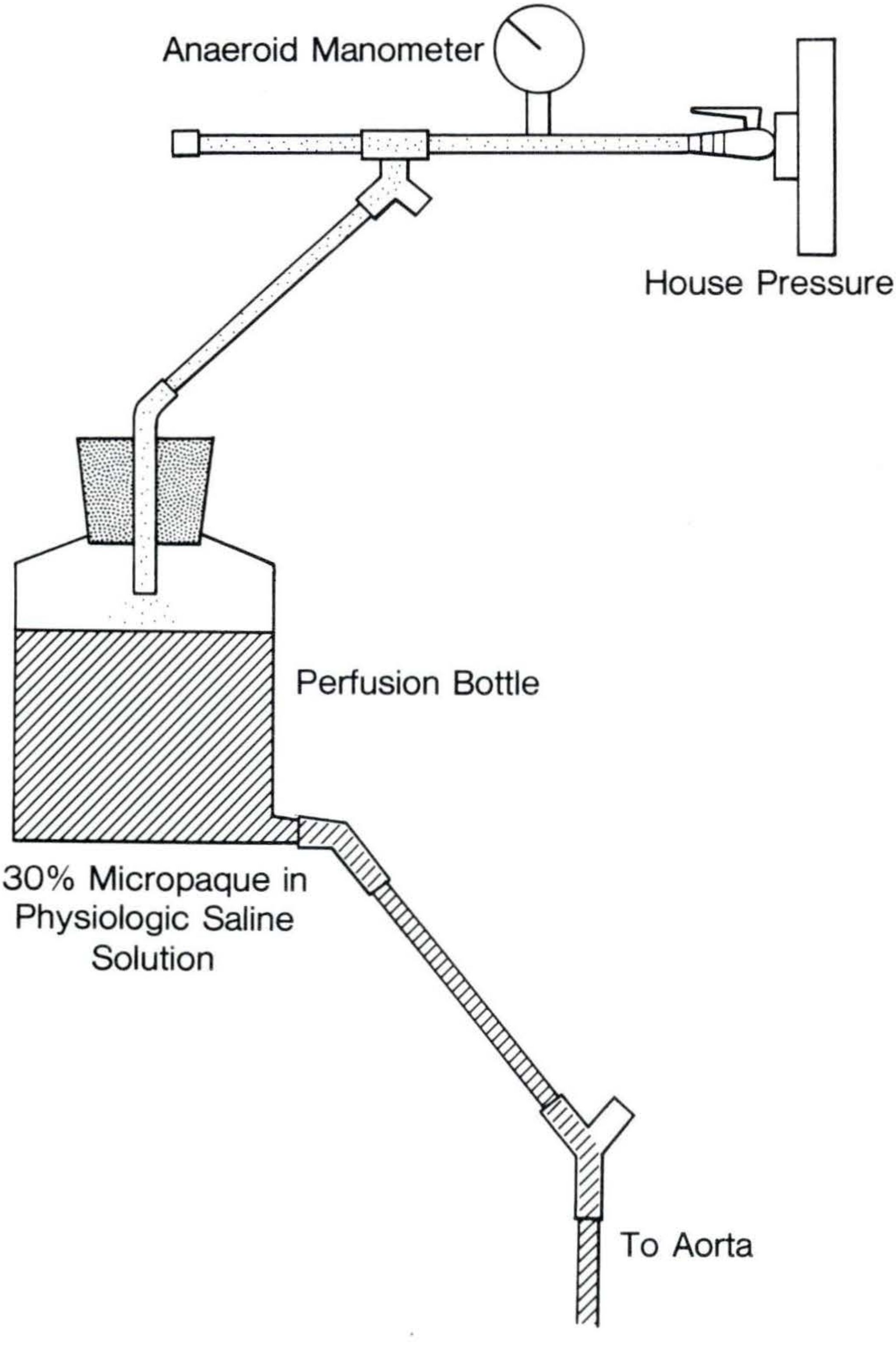
saline (Figure 1). Perfusion was at a constant normal mean systolic pressure of 120 mmHg to assure physiological perfusion. Perfusion was continued until white effluent was obtained from the caudal vena cava.

The bladders were removed en bloc from the abdominal cavities and immersed in 10% neutral buffered formalin for two weeks. Multiple 2 mm sections were made perpendicular to the cystotomy incision. Microangiograms were produced using spectroscopic plates and high resolution radiographic film according to previously published techniques.[2-7] The 2 mm slices were embedded in celloidin, cured, sectioned and stained for correlated histological analysis using trichrome, and hematoxylin-eosin stains.

The microangiograms of the three suture patterns were compared for relative vascular attenuation, general alteration of the vascular pattern and the overall process of revascularization at the incision. Histologic sections were subjectively evaluated for inflammation, mucosal regeneration, continuity of layers, and fibrous tissue deposition.

Figure 1. Diagram of the apparatus used to perfuse bladders with barium sulfate for microvascular evaluation.

Perfusion Apparatus



RESULTS

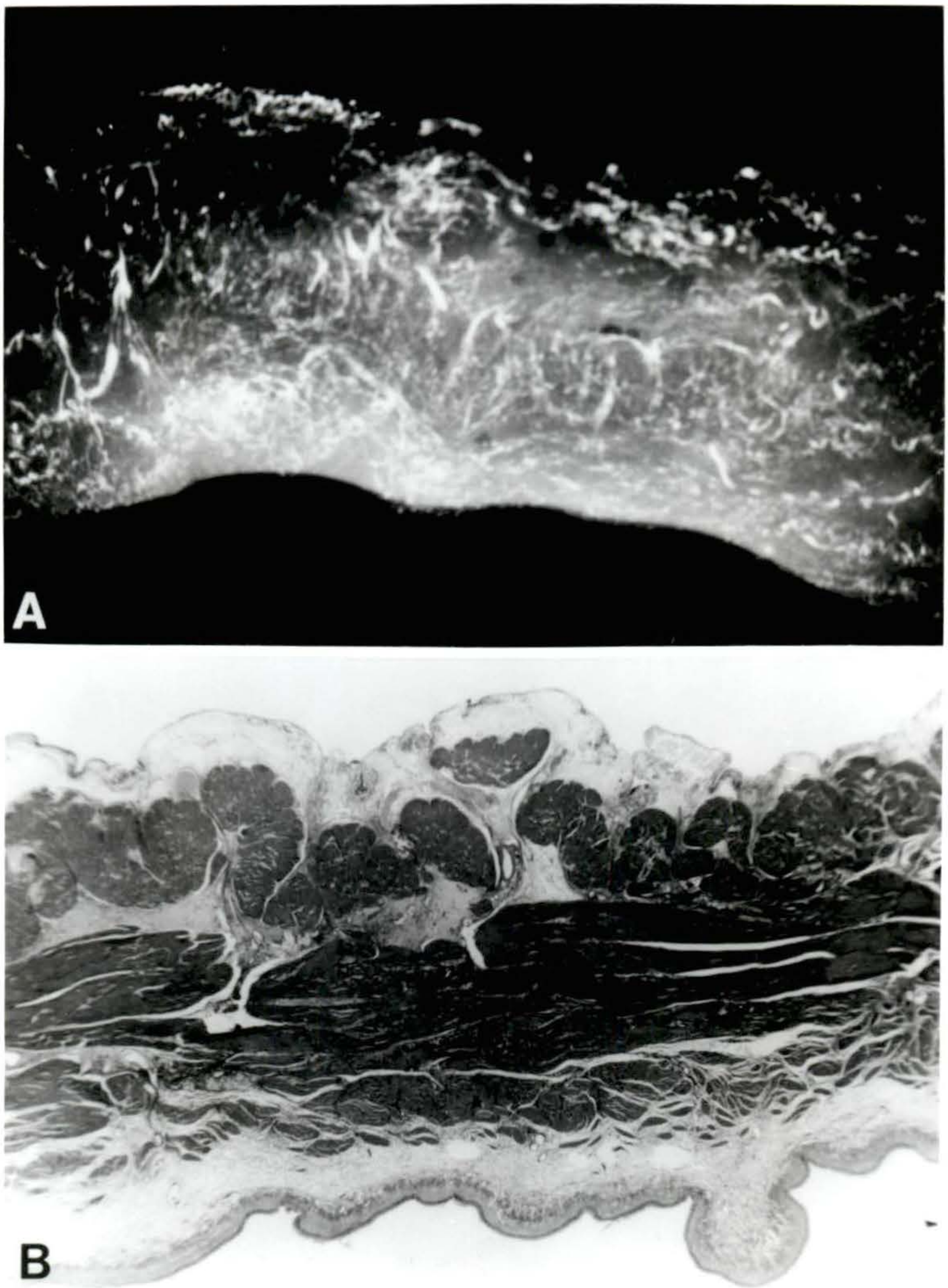
All dogs recovered well from surgery. No complications developed during either the surgical procedure or the profusion study. The subjective observations of microvascular and histologic changes seen in the wounds were compared to normal bladder wall (Figure 2).

24 Hour Postoperative Evaluation

The inverting suture pattern created extensive alteration of tissue planes. All layers were perpendicular to their normal anatomical orientation, and were exposed to the bladder lumen (Figure 3A). An extensive ulcerated mucosal gap was present at the incision which was filled with necrotic hemorrhagic tissue and a fibrin clot. The inflammatory reaction along the entire incision was severe. Extensive neutrophilic infiltration and tissue edema was present. The serosal edges were inverted into the incision with complete serosal contact. A large fibrin clot was present on the exterior serosal border of the incision. In contrast, both of the appositional patterns caused less alteration of the normal histologic anatomy. The double layer appositional pattern created some alteration of the muscularis layer which was slightly inverted. A small mucosal gap was present at the incision, and the mucosa adjacent was markedly edematous. However, the serosa, submucosa and mucosa layers were minimally disrupted. A fibrin clot filled the entire incision as well as the mucosal defect. The

Figure 2A. Microangiogram of normal bladder wall; X 3.75.

B. Histologic photomicrograph of normal bladder wall. Trichrome stain; X 2.


A
B

single layer appositional pattern resulted in close mucosal approximation with no alteration of any layer (Figure 3B). Only a small amount of fibrinous exudate was present throughout the incision. Tissue edema and neutrophilic infiltration was less as compared to the other two suture patterns. Both appositional patterns created complete serosal apposition with a fibrin clot present on the exterior of the incision.

The inverting suture pattern created large areas of avascularity in the mucosa, submucosa and the muscularis layers adjacent to the incision. In addition, the direction of the vasculature changed abruptly as it approached the incision. The vessels made a right angle turn and proceeded toward the bladder lumen (Figure 4A). In contrast, both appositional patterns caused less vascular compromise along all aspects of the incision (Figure 4B,C). The single layer appositional pattern resulted in less vascular attenuation than the double layer appositional pattern, which created a small zone of hypovascularity adjacent to the incision. Both appositional techniques allowed the vascular pattern to approach the incision perpendicularly with no deviation of vessel direction. All three suture patterns created greater vascular compromise in the mucosal-submucosal portion of the incision as compared to the muscularis or serosa. The initial postoperative evaluation showed that

Figure 3A. Histological photomicrograph through incision (arrows) of the double layer inverting suture pattern (II) at 24 hours. Extensive alteration of normal tissue orientation with all layers exposed to the bladder lumen. Trichrome stain; X 2.

B. Histologic photomicrograph through incision (arrows) of the single layer appositional suture pattern (S) at 24 hours. Note the close mucosal approximation with minimal alteration of tissue layers. Trichrome stain; X 3.

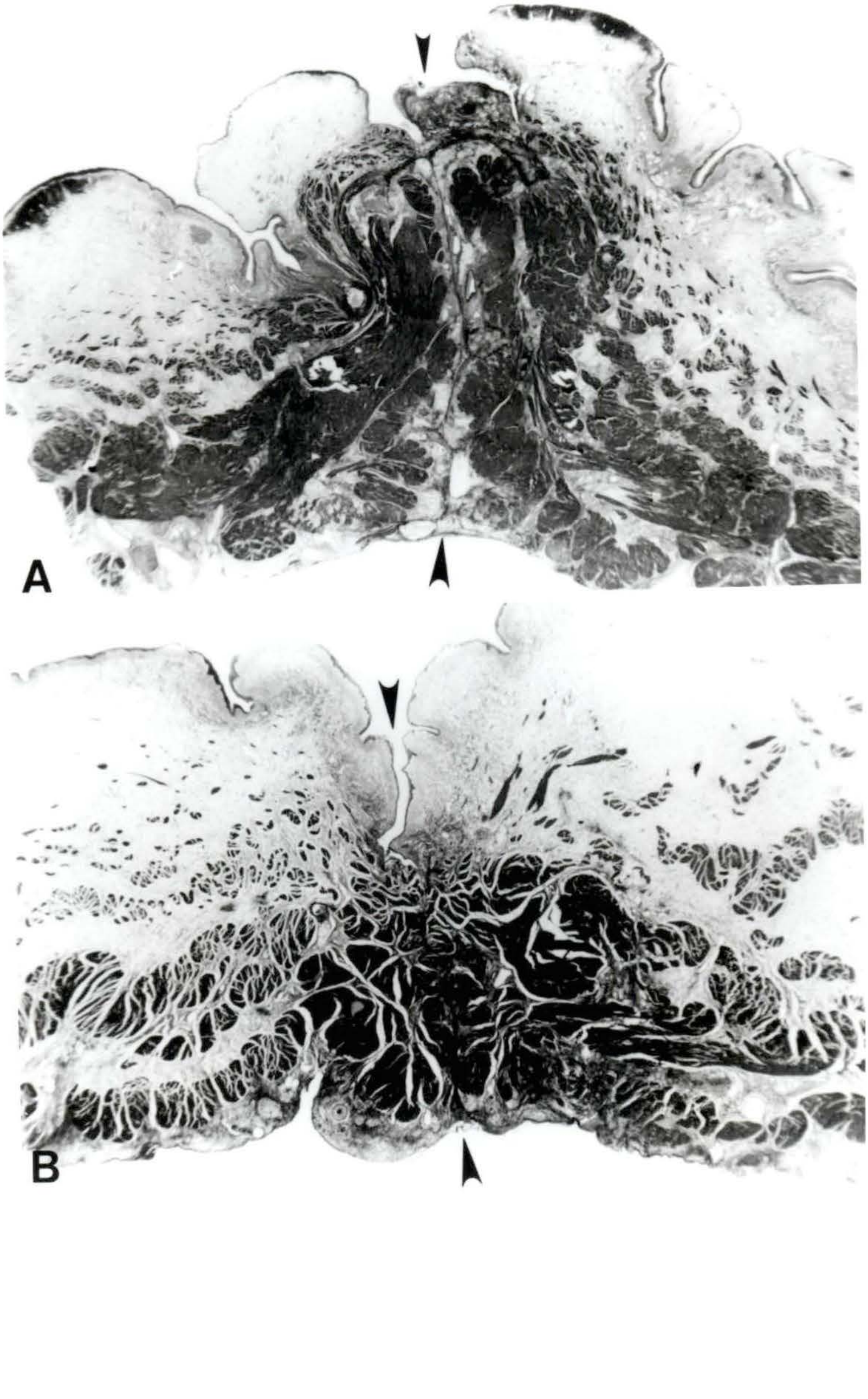

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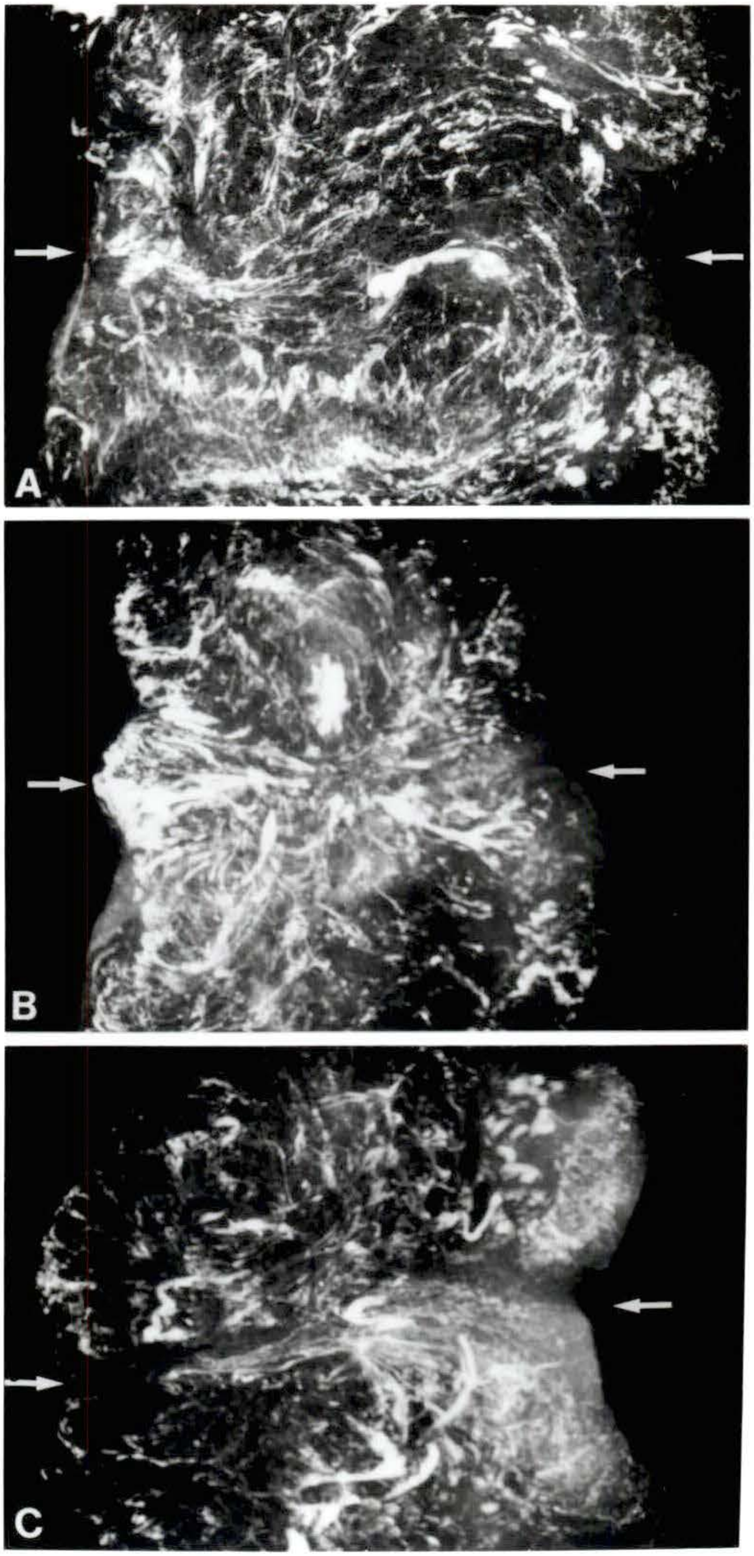
Figure 4. Microangiograms at 24 hours, arrows delineate incision.

A. Double layer inverting suture pattern (II). Note the avascularity adjacent to the incision and the abrupt change in vascular direction; X 2.5.

B. Single layer appositional suture pattern (S). Minimal vascular compromise was present along the incision. No deviation of vascular pattern was observed; X 5.

C. Double layer appositional suture pattern (SS). Note the slight zone of hypovascularity adjacent to the incision; X 5.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B
C

vascular compromise and alteration of the vascular direction at the incision, was greatest with the inverting pattern and least with the single layer appositional pattern.

48 Hour Postoperative Evaluation

Complete continuity of all tissue layers has been re-established with the single layer appositional pattern. Transitional epithelial cells along the margins of the incision have mobilized and migrated across the incision establishing a mucosal barrier (Figure 5). The incision was infiltrated by a large amount of fibrin and reactive fibroblast producing new collagen (Figure 6). The slight mucosal edema and inflammatory reaction along the incision seen at 24 hours had subsided. A small mucosal gap was present in the bladder closed with the double layer appositional pattern. However, the epithelial cells had begun to regenerate and migrate from the mucosal edges to cover the incision site. Fibrin and fibroblasts were present in the incision with new collagen formation (Figure 6). There was little change in the inflammatory reaction throughout the incision since the 24 hours evaluation. In contrast to the appositional patterns, the inverting suture pattern exhibited a wide, edematous mucosal gap. The gap was filled with necrotic tissue and an organized blood clot. There was no evidence of any mucosal regeneration or migration to cover the defect. An intense inflammatory response was still present along the entire

Figure 5. Histological photomicrograph of the single layer appositional closure (S) at 48 hours. At the mucosal border of the incision (arrow), transitional epithelial cells have migrated from the wound edge to establish mucosal continuity. Trichrome stain; X 51.

Figure 6. Histologic photomicrograph through an incision (arrows) representative of either a single or double layer appositional closure (S or SS) at 48 hours. Fibrin, reactive fibroblasts and new collagen were present throughout the incision. Trichrome stain; X 128.

incision, and only small amounts of fibrin with no fibroblasts were present. Extensive anatomical alteration of all tissue layers was still present. In addition, the inverting pattern showed fibroplasia of the inverted muscularis layer. Fibroblasts and new collagen were intermingled with the smooth muscle immediately adjacent to the incision (Figure 7). Fibroplasia of the muscularis layer was not observed with the appositional closures. Complete serosal contact, had occurred in all three suture patterns.

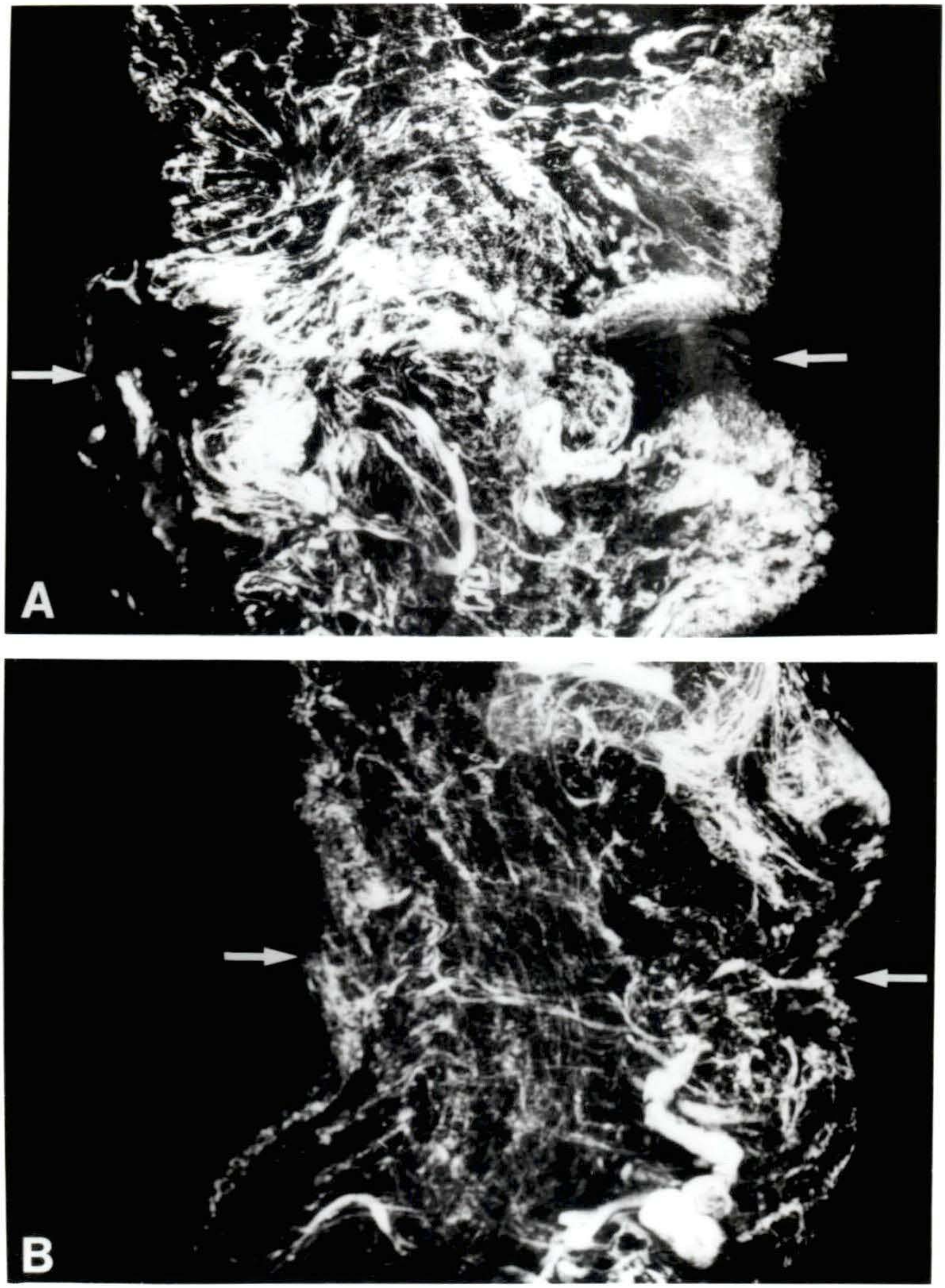
The inverting pattern continued to produce large areas of avascularity throughout the bladder wall at the incision. No changes in the overall vascular pattern or direction had occurred since the 24 hour evaluation. Both appositional patterns had new vascular ingrowth along the incision as compare to the 24 hour evaluation. The single layer appositional pattern demonstrated intense incisional revascularization (Figure 8A). Newly formed vessels were detected in all histological layers of the bladder. The vessels had larger diameter and were more numerous than vessels in adjacent uninjured tissue. The double layer appositional pattern also demonstrated new vascular formation. However, revascularization was not as intense as with the single layer appositional closure. The size and number of newly formed vessels was less, which left a narrow hypovascular zone along the incision as compared to normal bladder wall (Figure 8B).

Figure 7. Double layer inverting suture pattern (II) at 48 hours. Fibroplasia of the inverted muscularis layer has occurred. Fibroblast and collagen were intermingled with the smooth muscle adjacent to the incision (arrows). Trichrome stain; X 128.

Figure 8. Microangiograms at 48 hours, arrows delineate incision.

A. Single layer appositional suture pattern (S). Intense incisional revascularization had occurred in the wound. X 3.75.

B. Double layer appositional suture pattern (SS). New vascular ingrowth along incision. However a narrow hypovascular zone still existed adjacent to the incision. X 3.75.


A
B

96 Hour Postoperative Evaluation

The incision closed with the single layer appositional pattern had complete epithelial continuity. The transitional epithelium had proliferated and increased in thickness at the incision, making it indistinguishable from normal uninjured mucosa. A fibrovascular band extended along the entire incision, with increased numbers of active fibroblasts and new collagen deposition as compared to the 48 hour evaluation. Anatomical alignment of the histologic layers was comparable to normal bladder wall. Epithelial migration had successfully created mucosal continuity along the incision in the cystotomy closed with a double layer appositional pattern. In addition, the inflammatory reaction present at 24 and 48 hours had begun to subside. The entire incision was filled with active fibroblasts and new collagen deposition as seen with the single layer appositional closure. In contrast to the appositional patterns, the inverting pattern exhibited no evidence of any mucosal regeneration or migration at the incision. The mucosal gap was filled with an abundance of ulcerated fibrinonecrotic exudate (Figure 9). The inflammatory reaction along the serosal and muscularis portion of the incision at 24 and 48 hours had begun to subside. However, intense neutrophilic infiltration was still present at the base of the mucosal defect. Only small amounts of fibrin, with no active fibroblast or collagen synthesis could be detected along the incision up to the

Figure 9. Histologic photomicrograph of the double inverting suture pattern (II) at 96 hours. No mucosal regeneration or migration had occurred. The mucosal defect was filled with ulcerated fibrinonecrotic exudate (open arrows). Exuberant fibrous tissue had undermined the exudate present in the mucosal defect (closed arrows). Trichrome stain; X 8.

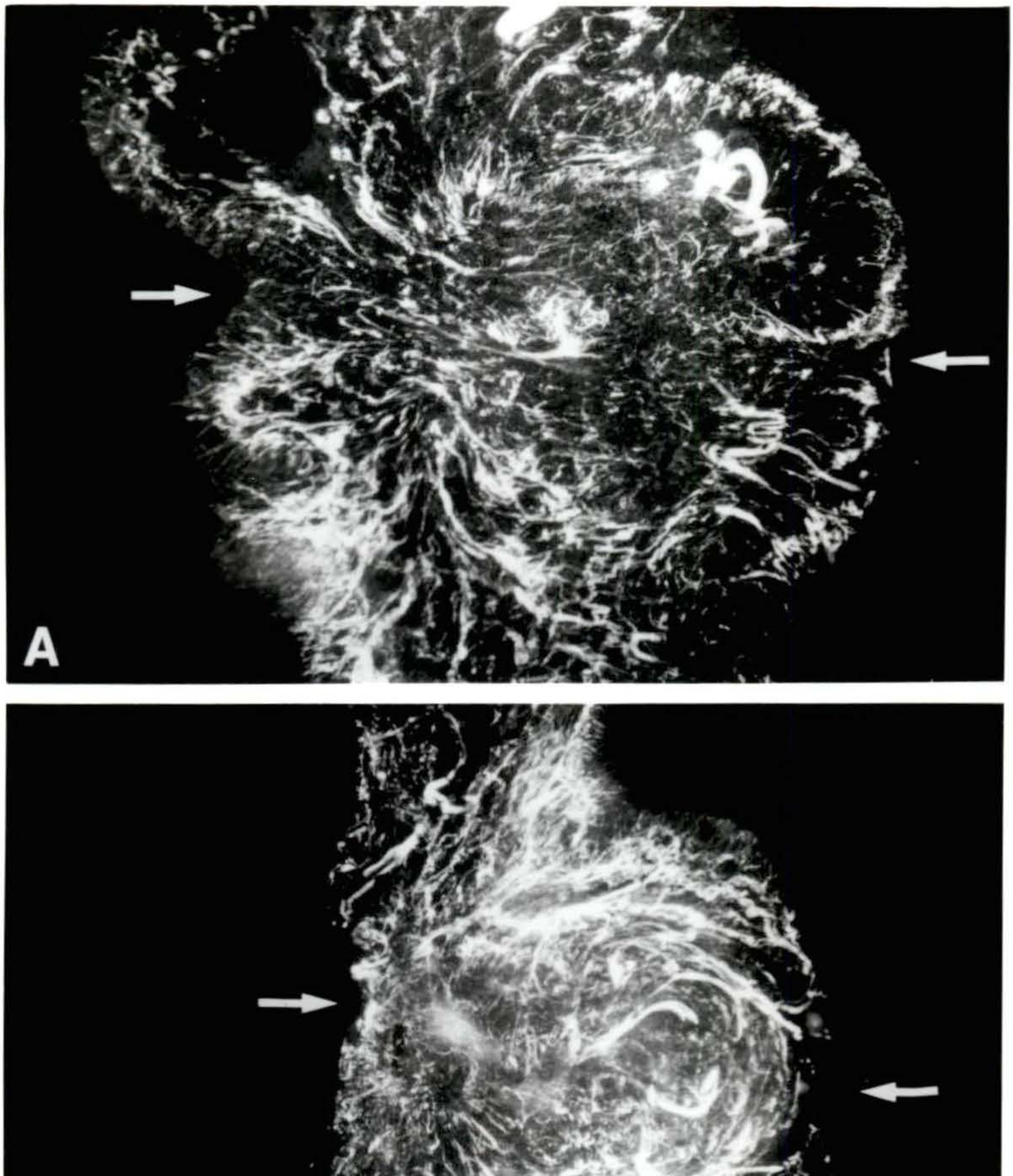
base of the mucosal defect. However, the base of the mucosal defect was infiltrated by an exuberant amount of fibroblasts and collagen. The fibrous deposition had completely undermined the exudate present in the mucosal defect. The inverting pattern exhibited continual fibroplasia of the muscularis layer adjacent to the incision. No evidence of muscular fibroplasia was present in either appositional closures. The fibrin clot present along the serosal border of the incision had continued to organize, with fibroblast and collagen deposition, in all three suture patterns.

The intensity of the revascularization response along the incision and surrounding tissue, observed at 48 hours in the appositional patterns had subsided. The vascular pattern and direction at the incision was comparable to normal uninjured bladder wall for both techniques (Figure 10A). However, the inverting suture pattern continued to create a wide area of hypovascularity throughout the incision and surrounding tissue. The direction of the vasculature remained altered, making an abrupt turn at the incisions and preceeding toward the lumen. There was no evidence of any new vascular ingrowth along the incision with the inverting suture pattern (Figure 10B).

Figure 10. Microangiograms at 96 hours, arrows delineate incision.

A. Representative microangiogram of either appositional suture pattern (S or SS). The vascular pattern was comparable to normal bladder wall. X 3.75.

B. Double layer inverting suture pattern (II). Continual avascularity adjacent to the incision, with altered vascular direction. There was no evidence of any revascularization at the incision as compared to either the 24 or 48 hour microangiograms. X 2.5.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B

DISCUSSION

Microangiography is a procedure where arteries and arterioles are visualized with the use of contrast media and special radiographic techniques.[2,4] The procedure allows for subjective comparison of the number, size and pattern of vessels in the tissue. Numerous studies have shown microangiography to be a reliable indicator of revascularization in wounds.[2,3,7,8,9] The results of this study demonstrate that the inverting suture pattern created a large zone of tissue avascularity along the incision at each time evaluated. The hypovascularity was probably due to the severe inversion of tissue at the incision which drastically altered the normal vascular direction causing compression of the microvasculature. Compression of the circulation may have been permanent since no subjective filling of the arterioles along the incision was observed between any postoperative evaluation.

Gentle apposition of the bladder wall by use of either appositional suture pattern created less alteration of the vascular direction and subjectively less incisional avascularity at the initial postoperative evaluation. The double layer appositional pattern resulted in more vascular compromise than the single layer pattern. The second layer of sutures in the muscularis and submucosa probably compressed vessels and would be responsible for the difference noted between the appositional closures. Histologicaly, this explanation was also substantiated since the double layer appositional pattern created some inversion of the muscularis layer, which could have slightly altered the direction of small vessels along the incision causing vascular compromise.

Both appositional patterns allowed for early revascularization of the incision, with a normal vascular pattern observed by 96 hours. Gentle apposition with minimal tissue alteration seemed to account for the rapid ingrowth of the vessels into the wound. Previous research has shown that necrotic tissue and hematoma formation will retard capillary ingrowth into a wound.[10] Except for the mucosa, no difference in the amount of necrotic tissue or hematoma along the incisions were observed between the appositional and the inverting suture patterns. However, the inverting pattern did create a large area of hemorrhagic necrotic exudate along the mucosal border of the incision at each time evaluated, which could have drastically compromised any revascularization. The avascularity and lack of incisional revascularization, as seen with the inverting suture pattern, was most likely due to a combination of mechanical inversion of tissue and the creation of necrotic exudate in the mucosal portion of the incision.

Sutured wounds of the urinary bladder heal with the same sequence of events as other tissues, namely scar formation.[10-12] Previous work has demonstrated that active collagen synthesis in the wounded bladder lasts for only 4-6 weeks as compared to other tissue such as the GI tract, were collegen synthesis is detected for over 17 weeks.[10] Wound repair begins as soon as the incision has been made. Undifferentiated mesenchymal cells differentiate into migratory fibroblasts. These fibroblasts use previously formed fibrin strands present in the wound clot as a scaffold to aid their migration. However, the fibrin strands are also believed to hinder the migration rate of fibroblasts. Therefore, fibrin is concurrently removed by enzymes released from endothelial cells which closely follow behind the migration of fibroblasts.[10] In essence, fibroblasts and newly formed capillaries are the hallmark of granulation tissue formation.

Both appositional patterns allowed extensive fibroblastic as well as vascular invasion of the incision as early as 48 hours. In addition, new collagen deposition throughout the incision could be detected in both suture patterns at the same postoperative time. In contrast, even at the 96 hour evaluation, only occasional fibroblasts were present along the incision closed with the inverting suture pattern. It seemed that the inversion of tissue not only hindered vascular ingrowth, but also delayed the formation of fibrous tissue in the wound.

The inverting suture pattern consistently produced a mucosal defect filled with fibrinonecrotic exudate. At 48 hours, an intense inflammatory reaction was present at the base of the defect. By 96 hours an excessive amount of fibroblasts and newly formed collegen was undermining the nerotic tissue. In addition, at both 48 and 96 hours, a portion of the muscularis layer that was inverted showed evidence of fibroplasia with collegen deposition intermingled between smooth muscle cells. Excessive fibroplasia was not observed with either appositional pattern.

Research in the area of total bladder wall regeneration after subtotal or total cystectomy, has suggested that more fibrous tissue depostion occurs in wounds that are exposed to urine as compared to similar wounds where urine has been diverted.[10,13,14] Exposure of large amounts of tissue to urine could explain the fibroplasia of the muscularis layer and fibrous infiltration under the mucosal defect, as seen with the inverting suture pattern. This theory is further substantiated since realignment of all tissue layers with subsequent protection of tissues from urine, as produced with both appositional suture patterns did not allow excessive fibroplasia of the tissue under the mucosal border of the incision.

It is generally accepted that epithelium is the body's first line of defense against the environment.[10,15] The uroepithelium acts as a selective barrier against toxic materials, bacterial and viral pathogens from gaining entrance into the body. Other specialized functions of uroepithelium include prevention of fluid and electrolyte loss from the bladder into hypertonic urine, and structural adaptation during expansion and contraction of the bladder.[15] Ideally, a suture pattern used for cystotomy closure should allow for rapid mucosal regeneration in order to establish complete continuity at the incision.

This study clearly demonstrated that the appositional suture patterns created mucosal continuity quicker than the inverting pattern. Mucosal re-epithelialization occurred faster with the single layer appositional closures. This pattern allowed close mucosal apposition even at 24 hours, with mucosal continuity established by 48 hours. The slightly slower mucosal regeneration seen with the double layer appositional closure was probably a result of the alteration of the muscularis layer and the subsequent narrow mucosal gap created. However, this pattern did allow for epithelial proliferation and migration, along the wound at 48 hours, with complete regeneration by 96 hours. Both appositional patterns allowed close histological tissue apposition and narrow mucosal defects with direct bridging of the defect by regenerated mucosal cells. This type of healing is classified as primary wound healing.

In contrast, the inverting pattern consistantly created a wide mucosal defect which was filled with necrotic exudate and surrounded by an intense neutrophilic inflammatory reaction. Healing in such a wound requires the formation of a granulation bed, wound contraction in addition to re-epithelialization.[10] No mucosal regeneration could be observed at any postoperative time. However, a granulation bed had formed beneath the mucosal defect by 96 hours. For healing to be completed, the uroepithelial cells at the wound margins would have to migrate across the previously formed granulation bed under the necrotic exudate. The sequence of healing created by the inverted suture pattern is classified as secondary wound healing which takes considerably longer for completion than healing as observed with the appositional closures.

CONCLUSION

Inversion of tissue at the incision, as created with a standard double layer inverting suture pattern, retarded the healing process. There was delayed formation of vascularilzed fibrous tissue with substantially slower re-epithelialization at the incision. This pattern also created pronounced tissue necrosis and ischemia along the incision during the first 96 hours postoperatively.

Gentle apposition of the wound edges with precise anatomical alignment of the tissue layers, as produced with both appositional suture patterns, consistently allowed rapid mucosal re-epithelialization, and formation of well vascularized connective tissue throughout the incision. Tissue necrosis and ischemia were minimal with both appositional techniques. The results of this study, indicate that wound healing was drastically hindered by the use of an inverting suture pattern. The rapid healing at the incision, as observed with either a single or double layer appositional closure warrant the clinical utilization of such techniques for cystotomy closure.

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GENERAL DISCUSSION AND SUMMARY

The use of inverting suture patterns for cystotomy closure is a time honored method which has been proven successful by years of clinical experience. However these studies have shown that the inversion of tissue does not result in superior wound closure. Wound strength, as evaluated by the use of circular bursting wall tension, was found to be similar in incisions closed with either appositional or inverting suture patterns. In addition, the gentle apposition of the wound edges with precise anatomical alignment of tissue, as produced by both appositional patterns allowed for less mucosal inflammation, less inverted tissue and narrow mucosal gaps as compared to the inverting suture pattern. The appositional patterns also resulted in wounds which underwent rapid mucosal epithelialization and fibrous tissue deposition with minimal vascular compromise of the incision. In contrast, the inverting suture pattern drastically hindered wound healing and resulted in severe tissue ischemia adjacent to the incision.

By virtue of these results, appositional closure of a cystotomy incision has been shown to be a viable technique and warrants the utilization of such suture patterns in clinical situations.

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APPENDIX: INFORMATION ON THE USE OF ANIMALS IN RESEARCH

This reserach was conducted according to the rules and regulation of the Animal Welfare Act.