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Smooth-Muscle Regeneration after Electrosurgical Endopyelotomy in a Porcine Model as Confirmed by Electron Microscopy

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ABSTRACT

Background and Purpose: Endopyelotomy is the preferred treatment for ureteropelvic junction (UPJ) obstruction because of its short operating time, limited morbidity, fast recovery, and reasonable efficacy. We used tissue and immunohistochemistry staining and electron microscopy to look at the muscle regeneration following an endopyelotomy incision in a porcine model.

Materials and Methods: Bilateral electrosurgical endopyelotomy was performed in six domestic pigs with placement of 7F 20-cm Percuflex[®] double-J stents for up to 4 weeks, and urinary tracts were harvested at 3 or 5 months. Specimen evaluation included tissue staining with hematoxylin-eosin, Masson's trichrome, and Verhoeff's iodine and Van Gieson solution; histochemical staining for smooth-muscle actin, desmin and myosin staining, and electron microscopy. Each specimen was assigned a "healing" score of 0 (normal) 1 (slight changes), 2 (mild changes), or 3 (severe changes). The fibrosis score was based on six factors: muscle layer fibrosis, lamina propria fibrosis, amount of granulation tissue present, new deposits of collagen, fibrosis in the periureteral fat, and presence of myofibroblasts. The muscles were characterized with immunohistochemistry and electron microscopy.

Results: At both 3 and 5 months, the urothelium was healed, and the lamina propria was healed with focal loss. By 3 months, smooth-muscle bundles bridged the defect, and by 5 months, the whole defect was covered. Smooth muscle cells were evident by electron microscopy by 3 months, and actin and myosin could be detected by immunohistochemistry. Desmin-positive cells accounted for 50% of the population at 3 months and 40% at 5 months. The regenerated smooth-muscle bundles were oriented in different directions and intermingled with fibrous tissue. They could be distinguished easily from normal ureter under the microscope.

Conclusion: Verifiable, functional smooth-muscle bundles bridge the endopyelotomy defect by 3 months, as confirmed by immunohistochemistry staining and electron microscopy.

INTRODUCTION

ENDOPYELOTOMY is a preferred treatment for ureteropelvic junction (UPJ) obstruction because of the short operating time, reduced morbidity, and fast recovery. Despite the

widespread application of the technique, however, the mechanism of smooth-muscle regeneration in endopyelotomy incisions has not been well characterized. Healing is by secondary intention, but its exact manner has been the focus of much controversy. The major questions have been: does the muscle of

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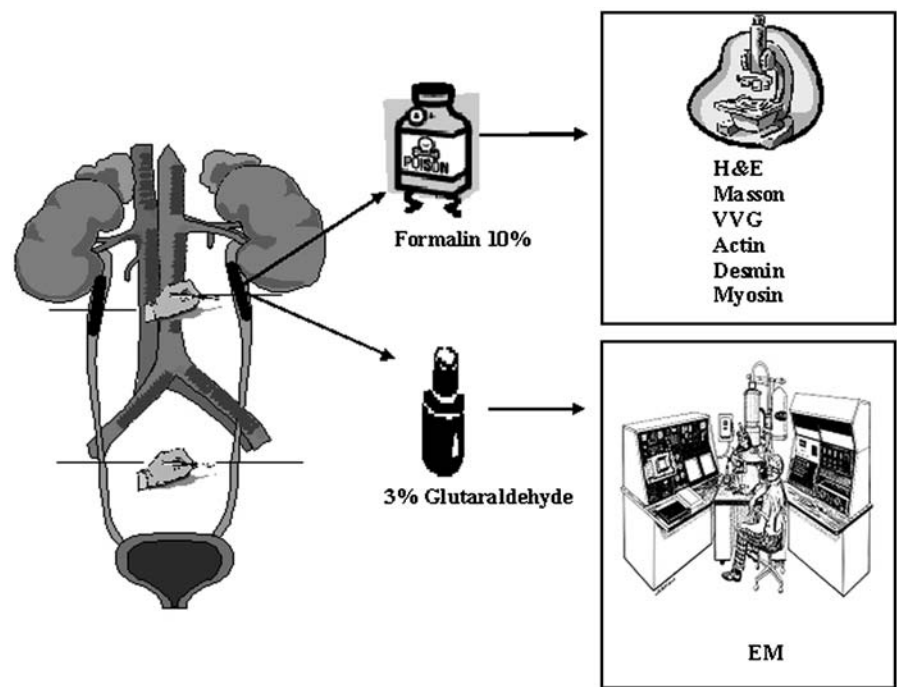


FIG. 1. Endopyelotomy site and schema of tissue retrieval and preparation.

the ureter regenerate or merely contract around the ureterotomy? and how rapidly do the muscular layers re-form? We studied muscle regeneration following an electrosurgical endopyelotomy incision using immunohistochemistry staining and electron microscopy.

MATERIALS AND METHODS

Surgical technique

After approval of the protocol by the Animal Studies Committee, six domestic pigs weighing 34 to 47 kg (average 39 kg) were selected. All urine cultures were negative preoperatively. After induction of general anesthesia with endotracheal intubation, the animal was placed in a dorsal lithotomy position. A cystogram was done to rule out vesicoureteral reflux and hydronephrosis, flexible cystoscopy was performed, and the ureteral orifices were identified. Under fluoroscopic guidance, a 5F angiographic catheter was passed to the midureteral bilaterally, and retrograde pyelograms were performed. Provided the collecting system appeared normal, a 0.035-inch Bentson guidewire was passed up each ureter through the angiographic catheter to the level of the renal pelvis. The angiographic catheters were then removed. An Acucise device was advanced over the guidewire to the level of the UPJ. With the cutting wire facing laterally on fluoroscopy, Acucise endopyelotomies were performed bilaterally by activating the device at 75 W of pure cutting current for 5 seconds. After 10 minutes, the Acu-

TABLE 1. HISTOLOGIC FEATURES ANALYZED DURING HEALING

<i>Inflammatory reaction</i>
Mural
Lamina propria
Edema
Periureteral fat
<i>Fibrotic reaction</i>
Muscle layer
Lamina propria
Granulation tissue
New deposit of collagen
Periureteral fat
Myofibroblasts/smooth-muscle cells
<i>Regeneration</i>
Percentage of mucosa healed
Presence of tunica propria
Presence of smooth muscle
Muscular layer: inner longitudinal (present or disorganized)
Muscular layer: outer circular (present or disorganized)
Percentage of smooth-muscle bridging
<i>Others</i>
Focal foreign-body reaction
Squamous metaplasia
Mucinous metaplasia
Thrombosis
Elastin
Hemosiderin deposits
Periureteral hemorrhage
Necrotic debris/bacteria/infection/periureteral fat necrosis

TABLE 2. HEALING AS ASSESSED BY IMMUNOHISTOCHEMISTRY STAINING AND ELECTRON MICROSCOPY

	3 Months (N = 4)	5 Months (N = 8)
Urothelium	Totally healed	Totally healed
Lamina propria	Healed with focal loss	Healed with focal loss
Smooth muscle cell	Muscle bundles bridge defect	Muscle bundles cover defect
Electron microscopy	Smooth-muscle cells present	Smooth-muscle cells present
Actin ⁺ cells (%)	70%	70%
Desmin ⁺ cells (%)	50%	40%
Myosin	Positive	Positive

cise balloon was deflated and withdrawn to the midureter; a retrograde pyelogram was performed through the catheter bilaterally to document extravasation of contrast medium. The catheters were then removed, and 7F 20-cm Percuflex[®] stents (Microvasive, Natick, MA) were passed bilaterally over the guidewire under fluoroscopic control. After confirmation of the correct position of the stents by fluoroscopic imaging, the guidewire were withdrawn, and a 14F Foley catheter was left in the bladder for 1 day. The indwelling ureteral stents were removed at 4 weeks.

Tissue retrieval

Animals were sacrificed and urinary tracts harvested at 3 months (two animals) and 5 months (four animals) (Fig. 1). At each time point, a midline laparotomy was performed, and the kidneys, ureters, and bladders were harvested *en bloc*. Gross findings, such as the location, volume of any periureteral fluid collection, degree of hydronephrosis, ureteral thickness, and the characteristics of the endopyelotomy defect (i.e., length and extent of healing) were assessed. The endopyelotomy area was

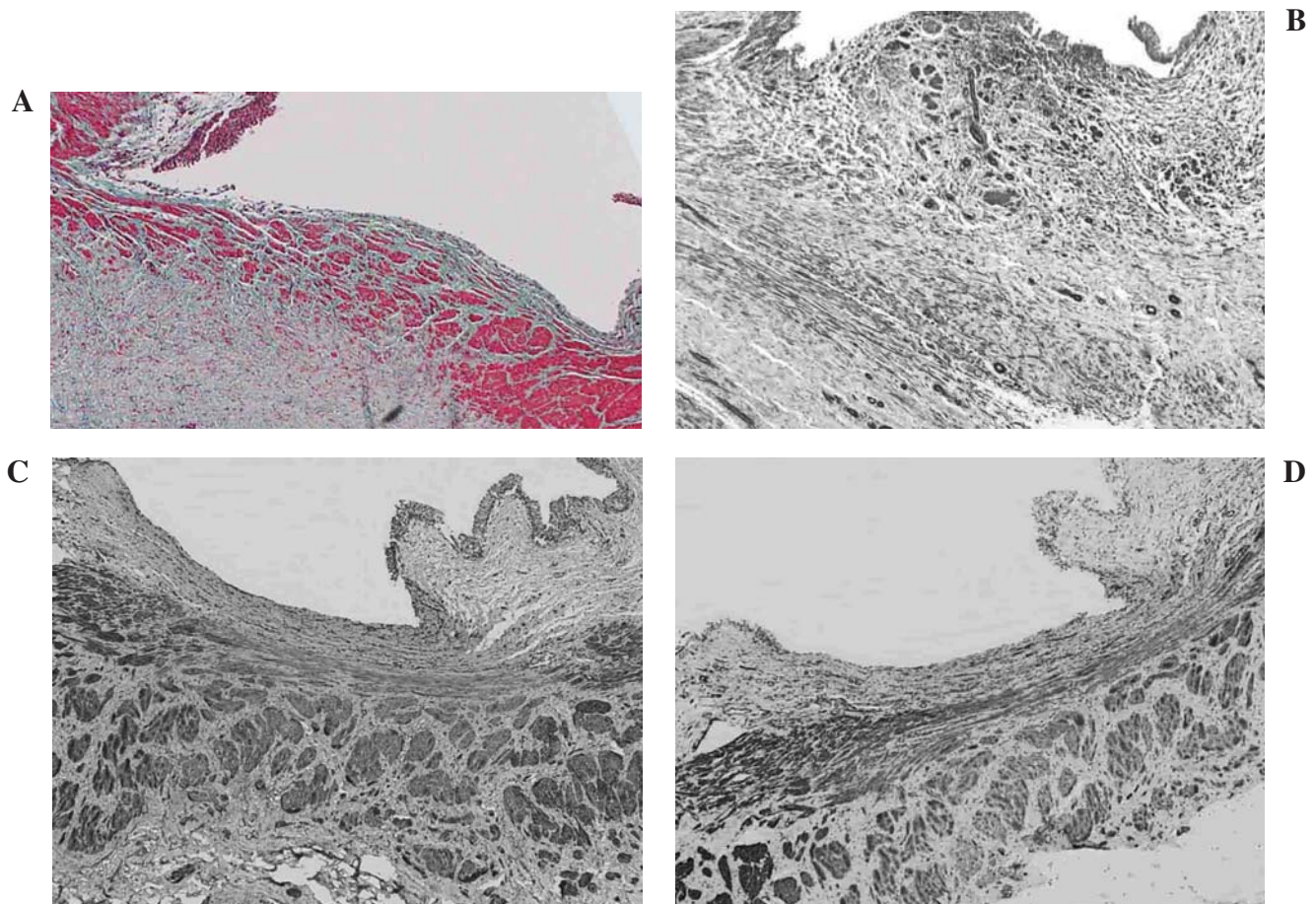


FIG. 2. Endopyelotomy site at 3 months. (A) Hematoxylin-eosin stain. (B) Actin stain. (C) Desmin stain. (D) Myosin stain.

carefully dissected, and the proximal half was sectioned and fixed in 10% Formalin. The other half was put in 3% glutaraldehyde for electron microscopy.

Histopathologic evaluation

Evaluation of the endopyelotomy sites included staining with hematoxylin-eosin, Masson's trichrome, Verhoeff's iodine and Van Gieson solution (VVG); immunohistochemistry staining for smooth-muscle actin, smooth-muscle desmin, and smooth-muscle myosin; and electron microscopy. The histopathologic changes were analyzed for several factors, each of which was scored from 0 to 3 (0 = normal, 1 = slight changes, 2 = mild changes, 3 = severe changes). The pathologist estimated the

percentage of mucosa that was healed, as well as the percentage of smooth-muscle layer bridging the defect. The treated section of the UPJ was further subdivided into the edge of the incision and the incisional site or area of the defect. The defect site was the site of the incision and the tissues therein. The edge site was the area along the incised edge of the ureter when an edge could not be clearly identified. The slide section of the midureter was analyzed and scored in its entirety as a control section of the ureter. The features analyzed are listed in Table 1.

Immunohistochemistry studies

Tissue sections were fixed in 10% buffered Formalin and processed for routine paraffin embedding. Sections were cut at

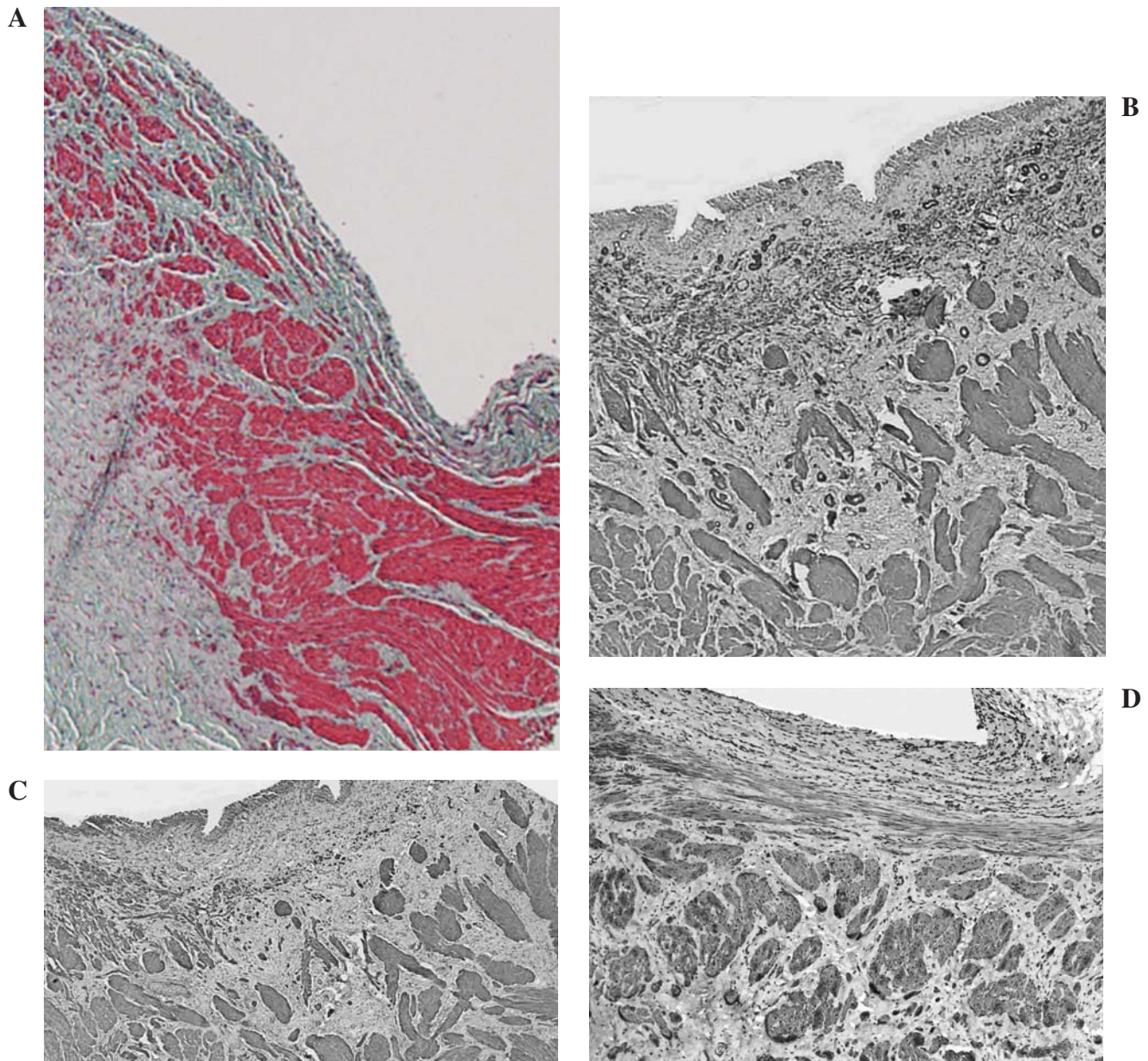


FIG. 3. Endopyelotomy site at 5 months. (A) Hematoxylin-eosin stain. (B) Actin stain. (C) Desmin stain. (D) Myosin stain.

5 μ m, deparaffinized, rehydrated through a graded series of ethanol, and processed for immunohistochemistry studies. Endogenous peroxidase activity was blocked using 0.3% H_2O_2 in phosphate-buffered saline for 10 minutes at room temperature. Nonspecific immunoglobulin-binding sites were blocked using 2% bovine serum albumin (Sigma Chemicals, St. Louis, MO). Nonspecific affinity-purified antibodies from Dako were diluted and incubated overnight at 4°C as follows: anti-desmin was diluted 1:10, anti-smooth-muscle actin was diluted 1:200, and anti-smooth-muscle myosin was diluted 1:100. Bond antibody was detected using reagents from Biocare Medical Corp. (Walnut Creek, CA) following the manufacturer's instructions and 3,3'-diaminobenzidine tetrahydrochloride as the chromogenic substrate. Sections were counterstained with hematoxylin and eosin. Negative controls were obtained by incubating sections with mouse IgG serum at a comparable dilution.

Electron microscopy

For electron microscopy, tissues were fixed in 3% glutaraldehyde at room temperature. Postfixation in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 60 minutes at room temperature was followed by dehydration in ethanol (25 minutes in 50%, 70%, and 95%, 100% ethanol) and propylene oxide. The detailed steps were as follows. Step 1 was removal of fixative and addition of buffer, after which the specimen was allowed to stand for 25 minutes. Step 2 was removal of buffer and addition of 1% OsO_4 for 60 minutes. Step 3 was removal of the OsO_4 and addition of buffer, which was allowed to stand for 25 minutes. Step 4 was removal of the buffer and addition of 50% ethanol for 25 minutes. Step 5 was removal of the 50% ethanol and addition of 3% uranyl acetate for 25 minutes. For Step 6, the uranyl acetate was removed and 70% ethanol was

added for 25 minutes. For Steps 7 and 8, the 70% ethanol was removed, and 95% and then 100% ethanol were added for 25 minutes each. In Step 9, the 100% ethanol was removed, and a 50/50 mixture of spurr plastic (see below) and 100% ethanol was added for 2 hours. For Step 10, this mixture was removed, and fresh spurr plastic was added for 60 minutes. In Step 11, the tissue was put into capsules filled with fresh spurr plastic and labeled with the EM number. Finally, the capsules were placed in a vacuum oven timed to expose them to 80°C from 20:00 to 08:00.

The spurr plastic was obtained from an embedding kit from EMS. For each 100 mL, the solution contained ERL (vinyl cyclohexene), 12 g of DER-736 resin, 52 g of nonenyl succinic anhydride, and 0.6 g of DMAE-2-(dimethylamino).

RESULTS

Extravasation was observed in all cases after creation of the endopyelotomy incision. No immediate postoperative complications were encountered.

Grossly, the healed tissue was slightly less pink than uninjured tissue. On magnification, it was likewise easily distinguished from normal surrounding ureter tissue by its color. Smooth muscle had completely bridged the defect at 12 weeks and was intermingled with fibrous tissue (Table 2). These cells were uniformly positive for actin, desmin, and smooth-muscle myosin (Figs. 2 and 3).

Electron microscopy confirmed smooth-muscle cells in both the 3-month and the 5-month group (Fig. 4). Muscles were present in bundles but distributed haphazardly among fibrous tissue. The ratios of muscle were more in both groups as compared to fibrosis. The section of healed tissue could be identified easily.

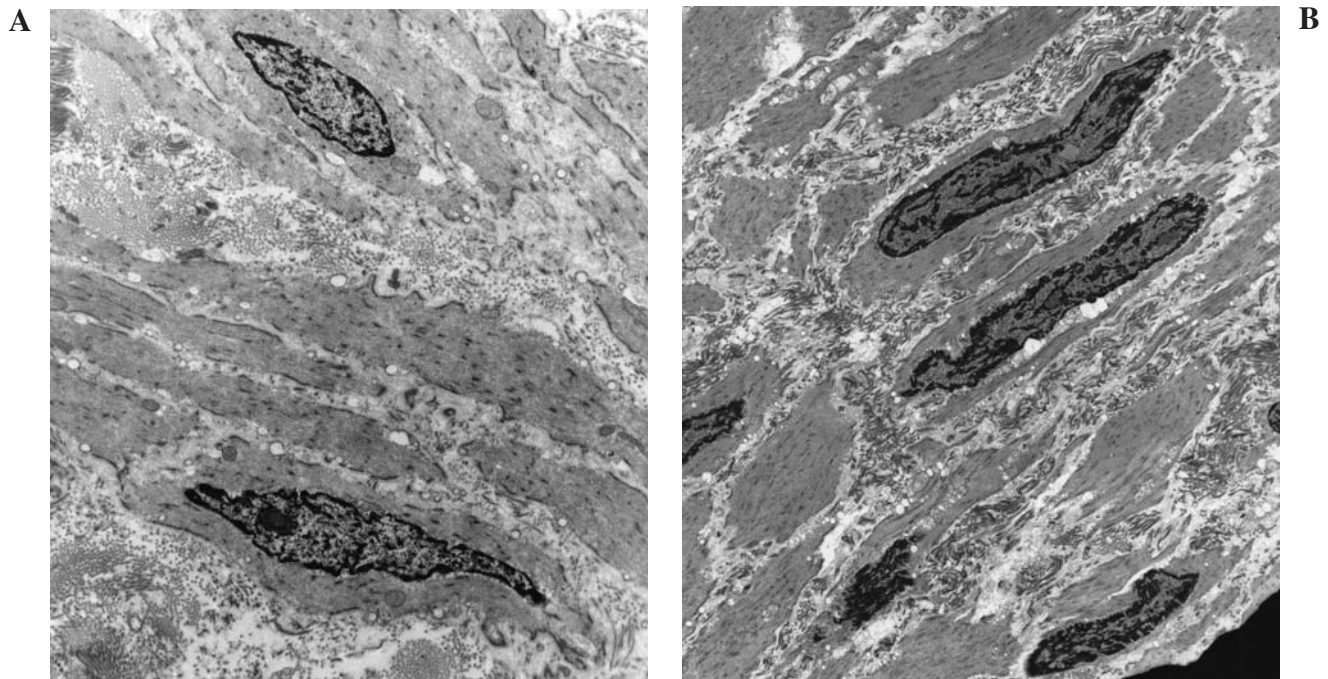


FIG. 4. Electron microscopy showing smooth-muscle cells. (A) At 3 months. (B) At 5 months.

DISCUSSION

Endopyelotomy heals by secondary intention, with granulation tissue, wound contraction, and epithelial as well as smooth-muscle regeneration from the edges. Smooth-muscle regeneration has been the topic of much controversy over the last few decades, the chief points in dispute being whether the muscle of the ureter regenerates (smooth-muscle cells) or merely contracts around the ureterotomy (myofibroblasts). Another question is how long it takes the urothelial and muscular layers to re-form completely. Recent studies by Andreoni and associates¹ addressed this question in detail but did not include electron microscopy.

Since Dr. Davis described intubated ureterotomy in 1943 for ureteral stricture disease,^{2,3} considerable research work has been done in the area.⁴⁻²⁵ The Davis operation was based on a study in dogs that confirmed that smooth muscle occupied 90% of the ureteral circumference at 6 weeks.²⁴ Lapidus and Caffery²⁵ excised a 2 cm × 4-mm window of ureter, which was wrapped with periureteral fat. Similar to the findings of Davis, the resected portion was almost completely bridged by muscle in the animals that had been intubated for 6 weeks, whereas the ureters not covered with fat were angulated and rigidly fixed to the underlying tissue. In 1955, Oppenheimer and Hinman⁵ excised one half of the circumference of the ureter in 12 dogs and placed a polyethylene splint, which was fitted with either a barrier of tantalum mesh or a grid of stainless steel wire to prevent contraction. This allowed healing only if epithelial and smooth-muscle cells could grow through the mesh. By 6 weeks, in most animals, the unbarricaded ureter showed almost complete reconstitution of smooth muscle, while in the barricaded area, the regeneration was slower and less complete. Those authors concluded that although contraction is important in the repair of ureteral defect, actual smooth-muscle regeneration appears to be a major factor in ureteral healing. In contrast, Boyarsky and Duque,⁴ who carried out Davis intubated ureterotomy with 4F to 7F stents in animals, found the healed area to consist more of fibrous tissue than of muscle, which were haphazardly arranged. Hamm and Weinberg⁶ were unable to determine whether true regeneration of the muscle layer occurred. Similarly, Baker and colleagues⁷ concluded that healing could occur even without ureteral intubation.

In order to better understand ureteral healing, we elected to largely duplicate the earlier canine studies, albeit in an animal model more similar to the human condition, namely, the pig. The most startling finding from our study is the appearance of smooth muscle that was strongly positive for smooth-muscle actin, desmin, and myosin. This assessment of the type of cell was further confirmed by two pathologists. This controversy over whether, after endopyelotomy, the healed area has smooth-muscle cells or myofibroblast was not settled by the recent study by Andreoni and associates.¹ They encountered different types of myofibroblasts; all of them staining strongly for vimentin and actin, while a minority stained for desmin and none for myosin.

CONCLUSION

Smooth-muscle bundles bridge the endopyelotomy defect by 12 weeks, as confirmed by immunohistochemistry stud-

ies and electron microscopy. However, this smooth muscle is oriented haphazardly and is intermingled with fibrous tissue.

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