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CORRELATION BETWEEN THE STRUCTURE AND FUNCTION OF THE RABBIT URINARY BLADDER FOLLOWING PARTIAL OUTLET OBSTRUCTION

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ABSTRACT

Purpose: To understand the relationship between contractile and structural changes in the obstructed bladder, rabbit bladder was partially obstructed for up to 70 days and alterations in tension response to field stimulation and carbachol were compared with alterations in ultrastructure and innervation of detrusor smooth muscle (SM). The effect of partial outlet obstruction on the physiological responses to field stimulation (FS) (nerve mediated contraction) and carbachol (receptor mediated contraction) were correlated with the structure and innervation of the detrusor smooth muscle (SM) of the same animal during a 70 day period.

Materials and Methods: 28 rabbits were subjected to 1 to 70 days of mild partial outlet obstruction. Sham operated rabbits were euthanized at 7, 14, 28, and 70 days post-obstruction. At each time period, isolated strips of bladder body were mounted in individual baths and the contractile response to FS and carbachol determined. Three additional strips from each bladder were fixed for electron microscopy.

Results: Bladder mass increased rapidly during the first 7 days after obstruction, was constant for the next 7 days, and then continued to increase gradually. Dysfunction of the contractile response to FS was noted as early as 3 days and progressively increased over the 70-day study period. The decrease in the response to FS increased at a significantly faster rate than the decrease in the contractile response to carbachol. In ultrastructure studies, at 3 and 7 days post-obstruction the majority of SM cells displayed the characteristics of hypertrophy. At 28 days some SM cells displayed loosely packed myofilaments and an irregular distribution of sarcoplasmic dense bodies. At 70 days swollen mitochondria were present in all cell types of the bladder wall. Evidence of axonal degeneration was first observed at 7 days post-obstruction and became more extensive thereafter. No evidence of mitotic figures, nerve growth cones or regenerating SM cells was observed.

Conclusions: Prolonged partial bladder outflow obstruction is accompanied by a progressive decrease in contractility of SM. The present study describes the structural damage that occurs in the bladder wall in response to partial outlet obstruction and correlates these observations with the contractile dysfunction with which it is associated. Furthermore, mitochondrial damage in vessels and fibroblasts is suggestive of bladder wall ischemia.

KEY WORDS: bladder, obstruction, nerve, mitochondria, sarcoplasmic reticulum

Bladder outlet obstruction, as seen commonly with disease of the prostate, causes characteristic changes including an increase in bladder mass, decreases in urinary flow and compliance and incomplete voiding.[1,2] In general, the responses of the bladder to prolonged partial outlet obstruction can be separated into two phases. Compensated bladder function (phase 1) is characterized by a relatively constant bladder mass and stable contractile responses to FS, carbachol and KCl stimulation. At the end of the compensated phase, the ability of the bladder to empty efficiently and completely is impaired, resulting in a shift to decompensated bladder function (phase 2).[3,4] An early sign of the shift to decompensation is a reduced contractile response to FS without a change in the response to direct muscarinic stimulation or to KCl.[3,4] This is characteristic of a selective loss in nerve mediated contraction, and is believed to be related to the observed denervation associated with partial outlet obstruction.[5,6] Decompensated bladder function is characterized by progressive deterioration in contractile and functional status (ability to empty), a further rapid increase in mass, and toward the end of phase 2, a progressive decrease in the volume fraction of SM elements within the bladder.[7] The end-stage of decompensation is characterized by, either an organ with a thick fibrous wall, low capacity, poor compliance and little or no contractile function, or a dilated bladder with a thin fibrous wall, high capacity and little or no contractile function.[1,2,7]

Previous morphological studies using a rabbit model of severe partial outlet obstruction demonstrated that decompensation can occur as early as 14 days following surgery, and is characterized by severe damage to SM and neuronal elements within the detrusor.[8–10] These previous EM studies looked specifically at the effect of short-term obstruction on SM, nerve, and interstitial components of the bladder, and

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did not directly correlate changes in morphology with the functional state of the bladder.[8–10]

Current studies using a rabbit model of mild obstruction demonstrate that decompensation occurs slowly over the period of several months, and is characterized by three specific cellular dysfunctions: progressive denervation,[5,6] selective decreases in mitochondrial function and enzyme activity,[11–13] and selective decreases in the enzyme activities of the sarcoplasmic reticulum (SR).[14,15] Thus, it is believed that the etiology for bladder dysfunction secondary to partial outlet obstruction is directly related to neuronal, mitochondrial, and SR damage.[7,16,17]

Our current theory is that the dysfunctions that mark the shift from compensation to decompensation are directly related to specific cellular and subcellular alterations in the neuronal, mitochondrial, and SR compartments. The purpose of the present study is to expand on previous studies by directly correlating the structure and function of the urinary bladder during the process of decompensation using a model of mild partial outlet obstruction and extending the period of study from 14 to 70 days after obstruction.

In view of the information linking mitochondrial and SR dysfunction to decompensation, the current work expands on the previous EM studies to include detailed analyses of these organelles in addition to the SM and neuronal elements.

MATERIALS AND METHODS

Surgical induction of partial outlet obstruction. 32 adult New Zealand White rabbits (6 months old, 4 to 4.5 kg.) were separated into 8 groups of four rabbits each. Twenty-eight rabbits were subjected to mild partial outlet obstruction and 4 to sham surgery. Four rabbits were evaluated at 1, 3, 7, 14, 28, 43, and 70 days following obstructive surgery. Sham rabbits were evaluated at 7, 28, and 70 days following sham surgery. Before surgery, each rabbit was sedated using an intramuscular injection of ketamine/xylazine mixture (25 mg./kg. ketamine, 10 ml./kg xylazine) and surgical anesthesia was maintained with intravenous infusion of nembutal (25 mg./kg.). The rabbit was shaved, painted with povidone-iodine solution and draped, maintaining sterility. The urethra was catheterized using an 8 Fr. feeding-tube and the bladder exposed through a mid-line incision. The bladder neck was cleared of surrounding connective and fatty tissues. A 3–0 silk ligature was passed around the proximal urethra-bladder neck area and tied loosely, such that a small hemostat could be placed through the ligature, alongside the urethra, without tension. This created a mild partial outlet obstruction. The abdominal wound was closed in two layers with 2–0 vicryl, using a running stitch on the inner layer and interrupted vertical mattress stitches on the skin. Sham operated rabbits underwent the same procedure except no ligature was placed around the urethra.

At the appropriate times following surgery, each rabbit was anesthetized, in the manner described above and the bladder excised. Three full thickness strips were fixed for electron microscopy (EM) studies. In addition, 4 full thickness strips were prepared from each bladder, mounted in individual 30 ml. baths, and evaluated for the physiological responses to FS and carbachol.

Contractility studies. Each bladder strip (5 mm. by 12 mm.) was mounted in a 30 ml. bath containing Tyrodes's solution + dextrose (1 mg./ml.) and gassed with 95% O_2, 5% CO_2 at 37C. Two grams of tension was applied to each strip and the strip equilibrated for 15 minutes. The maximal response to FS (2, 8, and 32 Hz, 80 V, 1 ms duration, 15 seconds train) and carbachol (100 μM) was determined. The mean of the 4 strips from one animal represented one individual preparation. The SEM for each experiment was based on the 4 individual preparations (animals). Tension was continually recorded using a Grass FT03 isometric tension transducer connected to a Grass polygraph. The analog signal from the polygraph was digitized using a Grass PolyView digital analytical system and the maximal rate of tension development determined. The contractile response to carbachol was completely inhibited by pre-incubation of the strips with 10 μM atropine, confirming that the response was cholinergic.

Electron microscopy. Each bladder strip was immersed in 4% glutaraldehyde in 0.1 M phosphate buffer and, following fixation, trimmed into 1 to 2 mm. small cubes and postfixed in 1% aqueous osmium tetroxide for 2 hours. Specimens were then dehydrated and embedded in Epon-812 resin. Semithin (1 μ thick) sections from each block were stained with toluidine blue and examined by light microscopy to select the most appropriate blocks for thin sectioning. Thin sections were cut, mounted on uncoated copper grids, double stained with uranyl acetate followed by lead citrate and examined in a Hitachi-7100 transmission electron microscope.

Statistical analysis. Significance was determined by one-way analysis of variance and the Newman Keuls test was used for group comparisons.

RESULTS

Bladder weight. Bladder weight increased progressively during the first week after obstruction (fig. 1) to 2.5 times control then remained stable from 7 to 14 days. By 28 days, the weight had doubled from that at 14 days; it was increased further by 70 days (fig. 1). Based on the bladder weights, the rabbit bladders remained in a compensated state until 14 days post-obstruction, at which time they progressed toward decompensation. By 70 days, the bladders were in a state of severe decompensation.

Bladder contractile responses. The maximal response to FS is presented in fig. 2. The response to 32 Hz was significantly lower than control at 14 days, whereas the response to 2 and 8 Hz was reduced by 7 days following obstruction. The response to all frequencies continued to decline throughout the study showing severe reductions by 70 days. The comparison between the maximal response and the rate of tension devel-

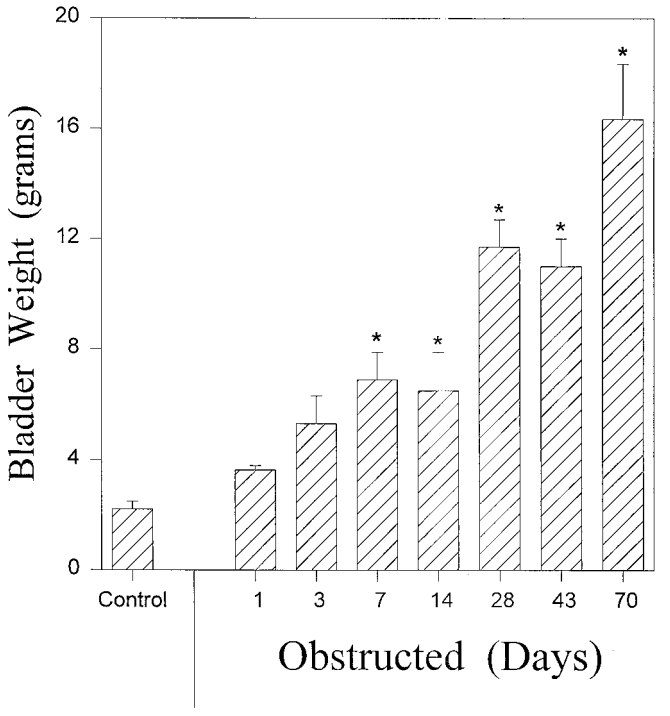

FIG. 1. Effect of partial outlet obstruction on bladder mass. Each bar is mean of 4 individual rabbits. * = significantly different from control ($p < 0.05$).

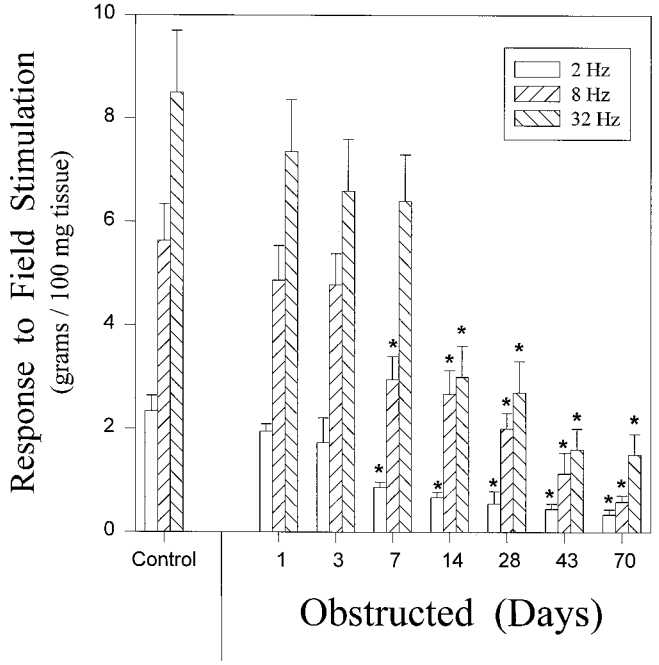

FIG. 2. Contractile responses to FS (maximal response) as function of duration of obstruction. Each bar is mean ± SEM of 4 individual preparations. * = significantly different from control ($p < 0.05$).

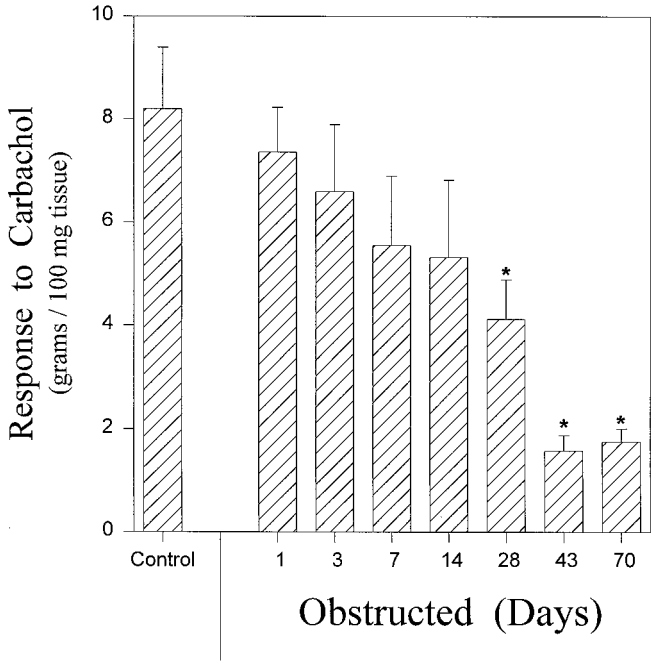

FIG. 4. Contractile responses to carbachol (maximal response) as function of duration of obstruction. Each bar is mean ± SEM of 4 individual preparations. * = significantly different from control ($p < 0.05$).

opment for 32 Hz is presented in fig. 3. Although the maximal response to FS was not reduced until 14 days following obstruction, the rate of tension development was significantly reduced by 3 days. Similarly, the rate of tension development for 2 and 8 Hz was also reduced by 3 days following obstruction (data not shown).

The response to carbachol (100 μM) is presented in figs. 4 and 5. The maximal response to carbachol remained at control levels until 28 days following obstruction (fig. 4) whereas the rate of tension development was reduced at 7 days after obstruction (fig. 5).

The response to FS was significantly reduced from control at times when the response to carbachol was normal. For both FS and carbachol, the rate of tension response showed

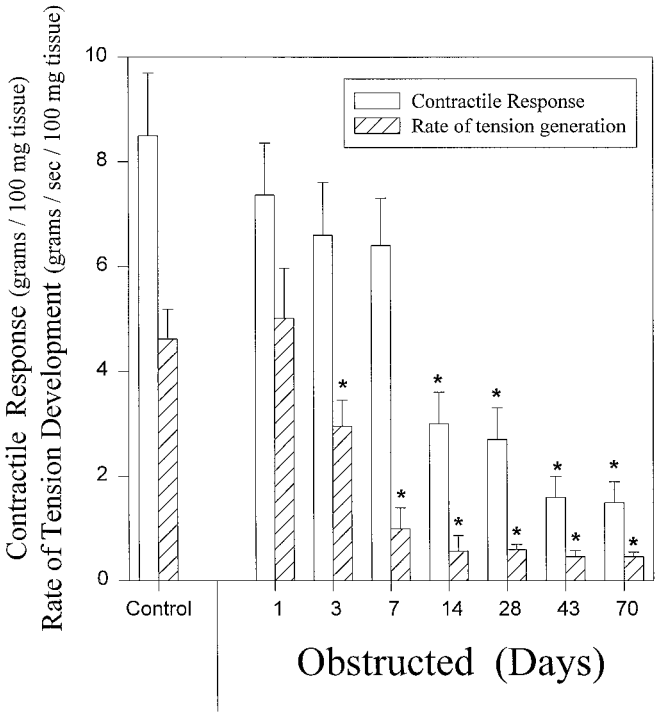

FIG. 3. Contractile responses to FS (maximal response and maximal rate of tension development) as function of duration of obstruction. Each bar is mean ± SEM of 4 individual preparations. * = significantly different from control ($p < 0.05$).

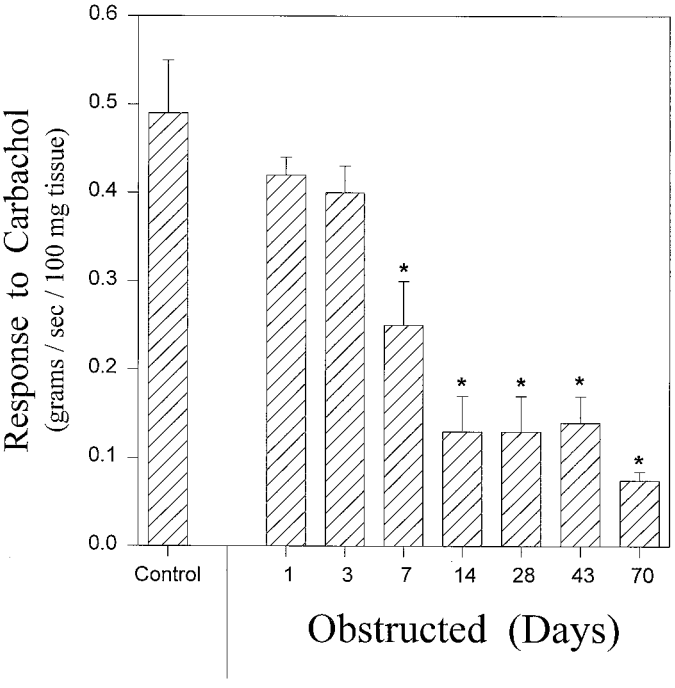

FIG. 5. Contractile responses to carbachol (maximal rate of tension development) as function of duration of obstruction. Each bar is mean ± SEM of 3 to 4 individual preparations. * = significantly different from control ($p < 0.05$).

significant dysfunction at times when the maximal response was equal to control.

Sham operated controls. The bladder weights and contractile responses of all shams were similar, and no different from non-operated controls.

Electron microscopy. At 1 day after partial obstruction the majority of detrusor SM cells had a normal fine structure although very occasional cells appeared vacuolated due to dilation and disruption of cisternae of SR in the perinuclear region. At 3 and 7 days after partial obstruction most SM cell profiles displayed an extensive corrugation of the cell membrane with sarcoplasmic projections from one cell interlocking with deep infoldings of neighboring cells (fig. 6). This appearance was associated with an increase in the length of subsarcolemmal dense bands with very few interposed caveolae. Myofilaments and sarcoplasmic dense bodies were evenly distributed throughout most SM cells. However small electron lucent areas in the sarcoplasm were sometimes observed (fig. 7) while the amount of perinuclear SR appeared greater than normal in some SM cells. Intercellular spaces were somewhat wider than normal at 7 days, being occupied by clusters of collagen fibrils and pale-stained elastic fibers. The majority of axon profiles and vesicle-filled varicosities were normal at this stage, although very occasionally an axon was observed to contain irregular masses of electron dense material.

At 14 days after partial obstruction SM cell profiles displaying dilated cisternae of perinuclear granular reticulum were frequently observed (fig. 8). Furthermore, many SM cells contained swollen rounded mitochondria lacking discernable cristae, while SM cell profiles containing small, elongated electron lucent spaces in the sarcoplasm were common. Irregular folding of the cell membrane, although not as prominent as that seen at 3 and 7 days after obstruction, was still evident in some SM cell profiles. Axon profiles containing irregular clumps of electron dense material were occasionally observed between the SM cells.

At 28 days after surgery grossly swollen mitochondria were present in most SM cells. In addition there was a non-uniform distribution of sarcoplasmic dense bodies in many SM cells such that large central areas of myofilament containing sarcoplasm were completely devoid of such bodies (fig. 9) while the myofilaments in such cells appeared more loosely arranged than normal. Where present, this alteration from normal fine structure was observed to affect most cells within a particular muscle bundle rather than occurring randomly. Some muscle cell profiles displayed large vacuolated areas containing amorphous electron dense material (fig. 10) while others appeared to be in an advanced stage of disintegration (fig. 11). The majority of muscle cell profiles had a relatively smooth outline at this stage although the numbers of surface caveolae were still reduced. The spaces between adjacent SM cells were judged to be slightly enlarged when compared with sham operated control specimens and contained strands of basal lamina-like material in addition to collagen and elastic fibers.

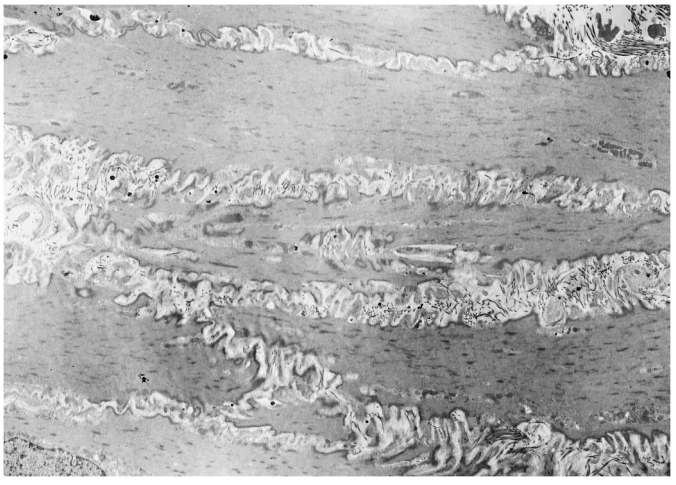

FIG. 6. Detrusor muscle cells 7 days after partial obstruction. Cell outlines are extremely irregular with cytoplasmic processes from one cell interlocking with those of its neighbors. Original magnification X 5,600.

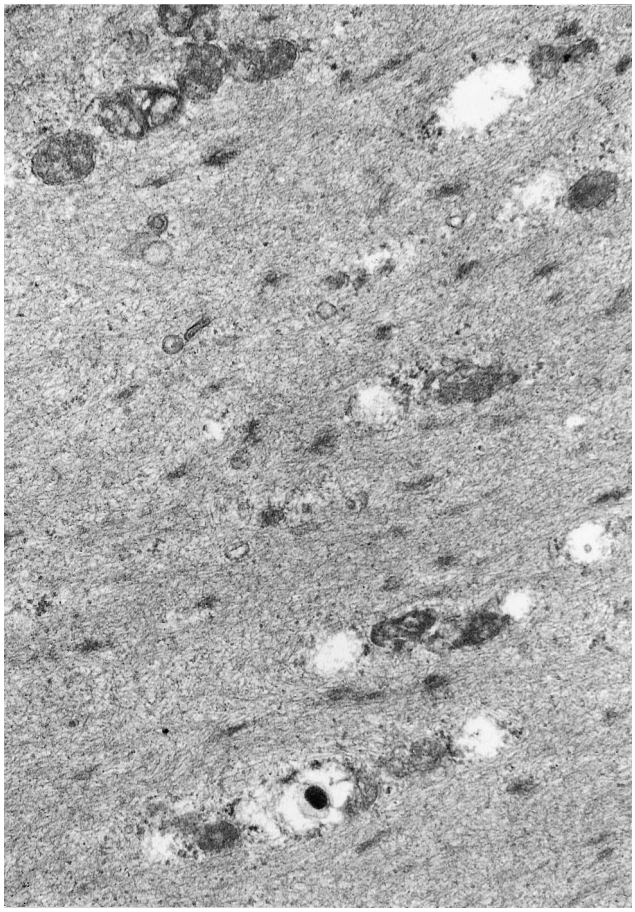

FIG. 7. SM sarcoplasm at 7 days showing numerous clear spaces among myofilaments. Original magnification X 25,500.

Similar but more extensive morphological alterations to the detrusor muscle were observed at 43 days (fig. 12) post-obstruction when abnormal axon profiles were more frequently encountered. At this stage swollen mitochondria were first observed in other cell types including fibroblasts, endothelial cells and perivascular SM. At 70 days after partial obstruction grossly swollen mitochondria were present in most detrusor muscle cell profiles (fig. 13) and were also observed in many other cell types within the bladder wall. Dilated SR and non-uniform distribution of sarcoplasmic dense bodies were observed in many detrusor muscle cell profiles while intercellular spaces were considerably enlarged with multiple strands of basal lamina-like material (fig. 13). Several large nerve bundles were encountered in which the majority of axon profiles were degenerating and contained amorphous electron dense material (fig. 14). Throughout the present study no evidence of mitotic nuclei, nerve growth cones or regenerating SM cells was obtained indicating a progressive deterioration of the structural elements within the bladder wall in relation to the effects of long-term outlet obstruction.

Sham operated controls. In all specimens, the morphology of the bladder wall failed to demonstrate any of the struc-

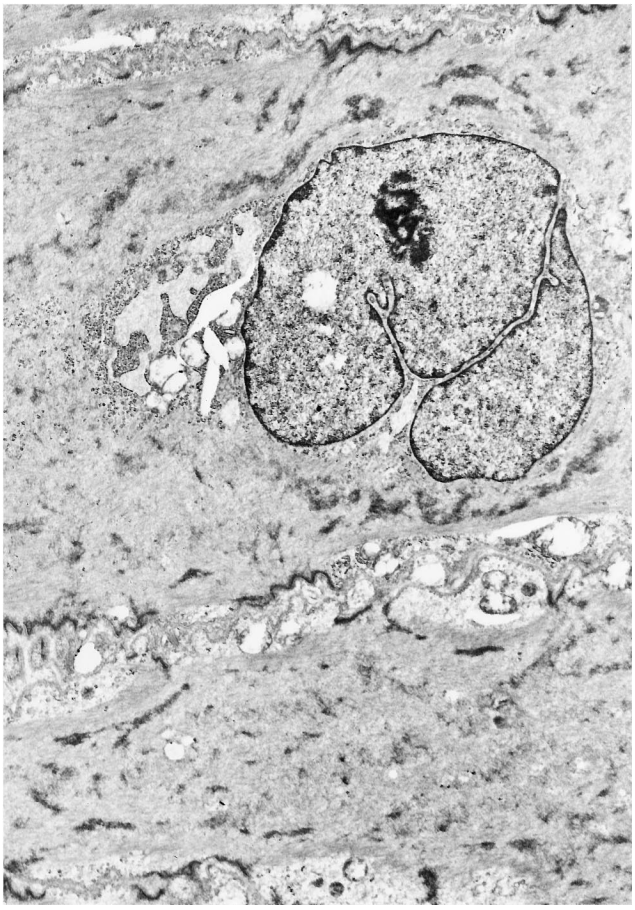

FIG. 8. Dilated cisternae of perinuclear granular reticulum in SM cell 14 days after partial obstruction. Original magnification X 6,600.

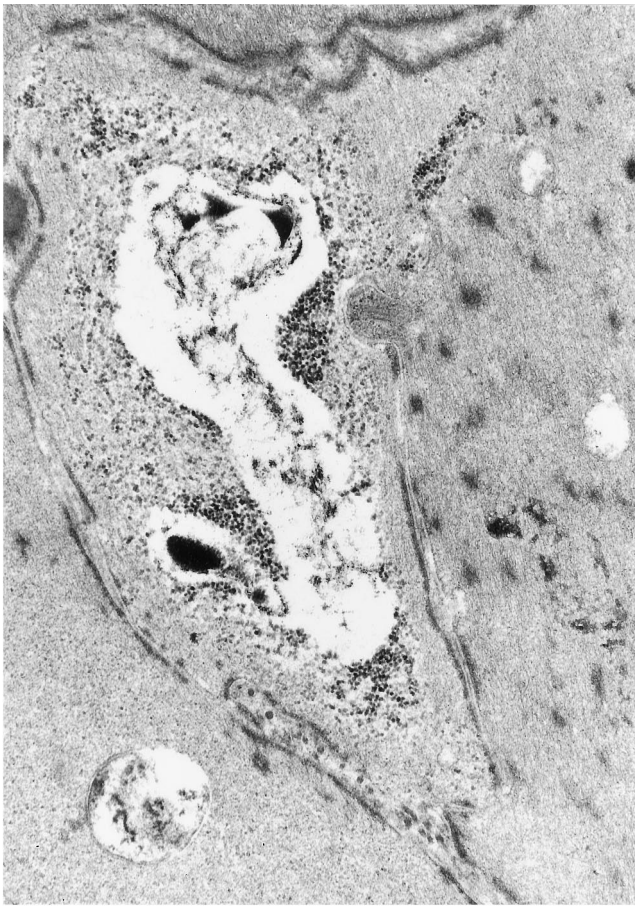

FIG. 10. Vacuolated SM cell profile 28 days after operation. Original magnification X 18,000.

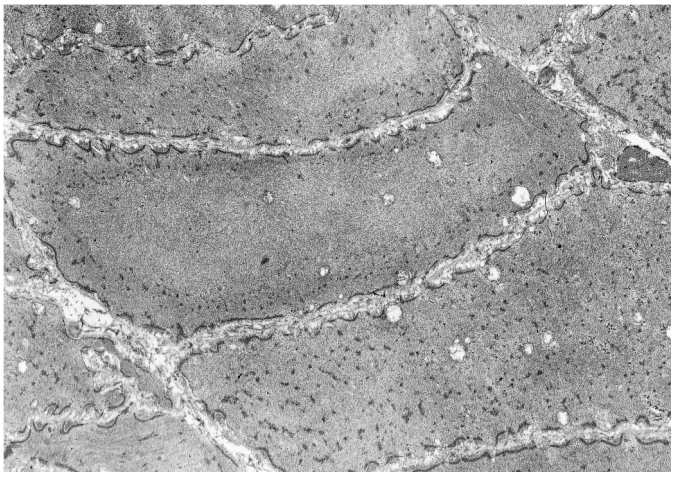

FIG. 9. SM cell profiles 28 days after partial obstruction. Mitochondria are dilated and central region of one cell displays loosely packed myofilaments and absence of sarcoplasmic dense bodies. Original magnification X 8,000.

tural alterations observed following partial outlet obstruction (figs. 15, 16).

DISCUSSION

Bladder outlet obstruction secondary to prostate disorders is a common problem for the aging male. It is well known that certain patients, despite relatively severe symptomatology, will remain in a compensated state for an indefinite period. Alternately, some progress at varying rates from a compensated to a decompensated state.[18–20] The response of the rabbit to partial outlet obstruction is similar. That is, the initial response is an increase in bladder mass while bladder function remains adequate for complete bladder emptying, indicating compensated bladder function. At some time thereafter, bladder function begins to deteriorate, and bladder mass continues to increase.[3,4]

Although differences exist in bladder size, structure, and autonomic receptor types and subtypes among different animal species, all species respond in a similar qualitative manner to FS and cholinergic stimulation in vitro.[21,22] Because of the similarity in the qualitative responses of the rabbit and human bladders to FS and carbachol, we believe that the data generated from studies on rabbits can be applied to humans. Additional similarities between the rabbit model of bladder outlet partial obstruction and obstructive dysfunction in humans include an increase in bladder mass in human males in association with obstructive dysfunction,[23–25] both rabbits and men show uninhibited bladder contractions during bladder filling,[2,3] and there is excellent evidence that partial denervation is characteristic of obstructive dysfunction in both rabbits and humans.[5,6,26–28] In a recently completed study, we compared the enzymatic activities of citrate synthase, malate dehydrogenase, and calcium ATPase in bladder samples obtained from men with symptomatic BPH (clinically obstructed), with the activities of bladder samples obtained from age-matched men with no symptomatic BPH.[29] The results clearly show that, similar to our rabbit data, the activities of the three enzymes are all significantly lower in the samples of bladder obtained from obstructed men than in the bladder samples obtained from men with no obstruction.[29] These studies confirm that the four cellular

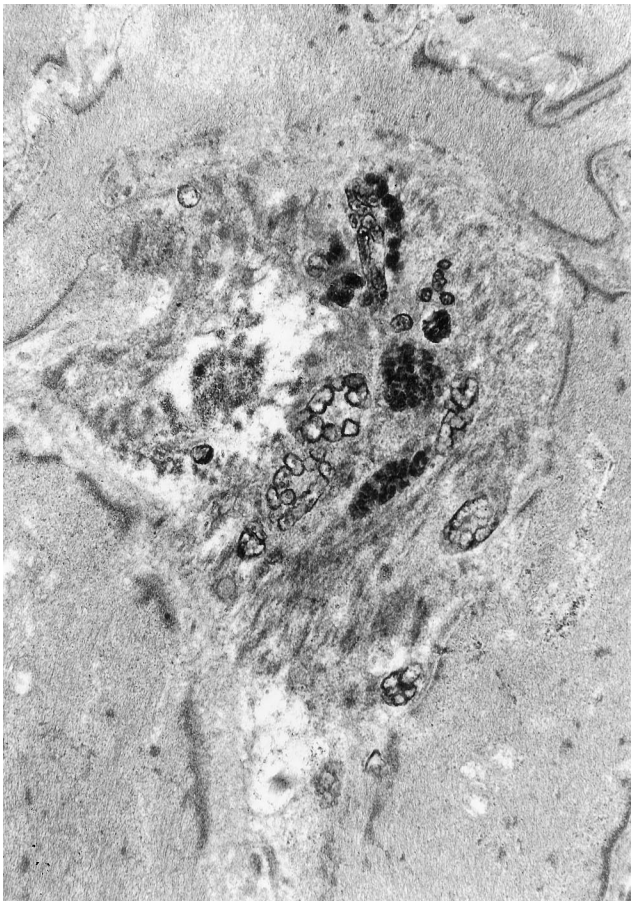

FIG. 11. Irregular electron dense material indicative of SM cell degeneration at 28 days. Original magnification X 15,000.

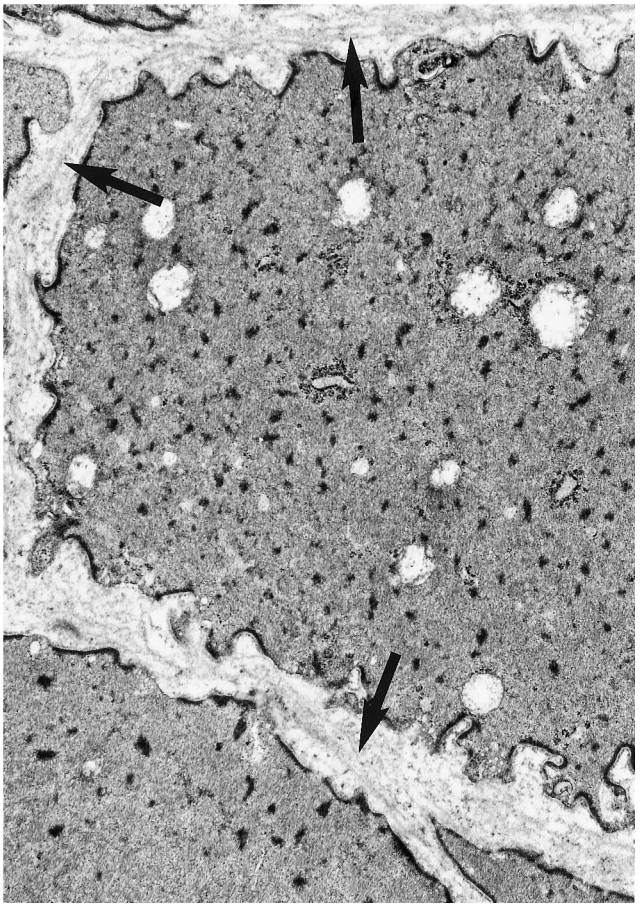

FIG. 13. Enlarged intercellular spaces containing strands of basal lamina-like material (arrows) are frequently observed at 70 days after partial obstruction. Original magnification X 10,000.

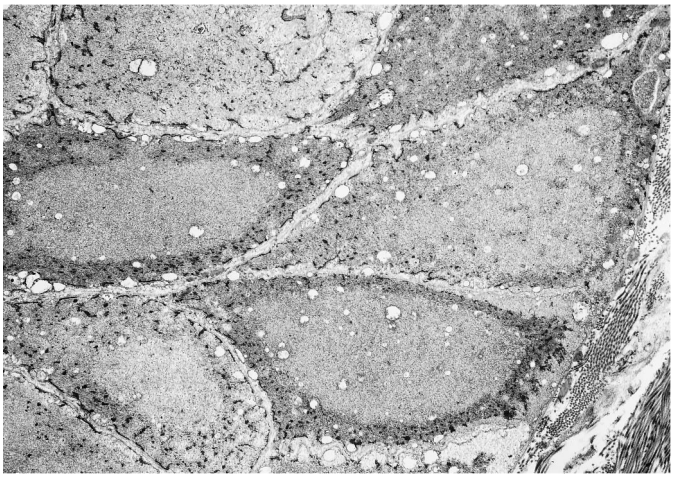

FIG. 12. SM cell profiles at 43 days after partial obstruction. Grossly dilated mitochondria and disorganised myofilaments are evident with sarcoplasmic dense bodies confined to periphery of each cell. Original magnification X 6,000.

characteristics of obstruction (increase in bladder weight, denervation, mitochondrial dysfunction, calcium disregulation) observed in rabbits are also found in humans.

A previous series of morphological studies on a rabbit model of severe partial outlet obstruction examined the ultrastructural changes that occurred from one day through two weeks of obstruction, looking primarily at changes to the SM, connective tissue, and neuronal elements.[8–10] Although no functional studies were correlated with these morphological studies, the results indicated that the rabbit progressed to decompensation within the two-week time period; decompensation was correlated with significant damage to the SM and neuronal membranes. Removal of the obstructions after 2 weeks resulted in only partial reversal of the morphological changes.[8–10]

Based on the severe nature of this model, we decided that it did not accurately represent the changes in bladder structure and function that occur secondary to BPH in men, and we developed a model of mild obstruction that did not progress to decompensation until 4 weeks to 6 months after surgery.[3,4,30] These long-term studies evidenced that the progression to decompensation related directly to changes in the mitochondria and SR, as well as the nerve and SM components.[3,4,30]

We used this model of mild obstruction for the present studies to directly compare duration of obstruction, contractile dysfunction, and ultrastructural changes in neuronal and SM cell and subcellular components.

Our current work confirms that decompensated bladders, whether 2 weeks post-obstruction as observed in the severe model or at 10 weeks after induction of mild obstruction as reported herein, are characterized by severe changes in their nerve and SM components. In addition, we examined the correlation between changes in detrusor ultrastructure and contractile dysfunction (level of decompensation), and the correlation between specific changes in neuronal, SR, and mitochondrial ultrastructure and the progression of decompensation, and whether the EM data supported the hypothesis that ischemia is a major factor in the etiology for obstructive bladder dysfunction.

The decrease in the contractile response to FS, as well as

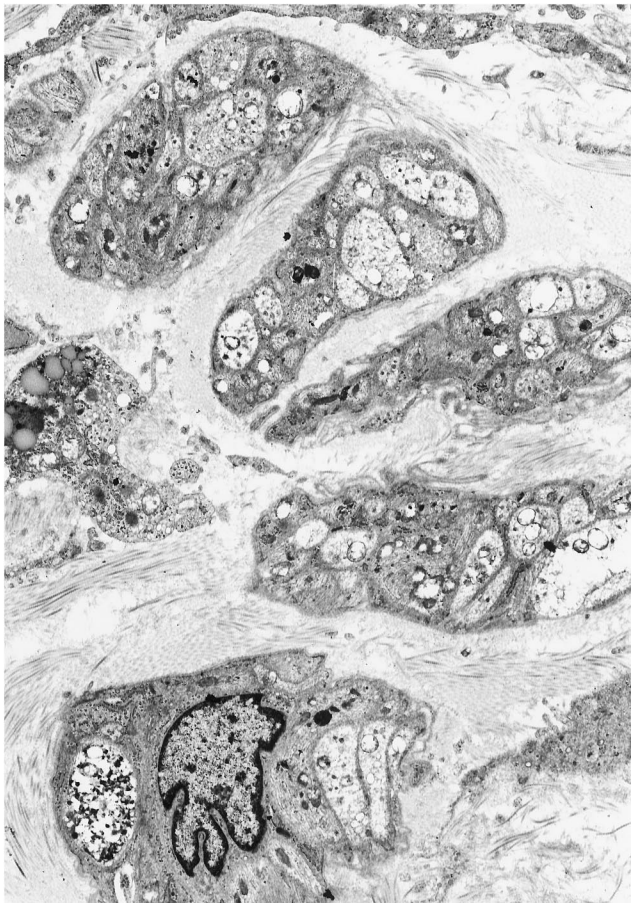

FIG. 14. Intramuscular nerve fiber at 70 days containing many degenerating axon profiles. Original magnification X 5,000.

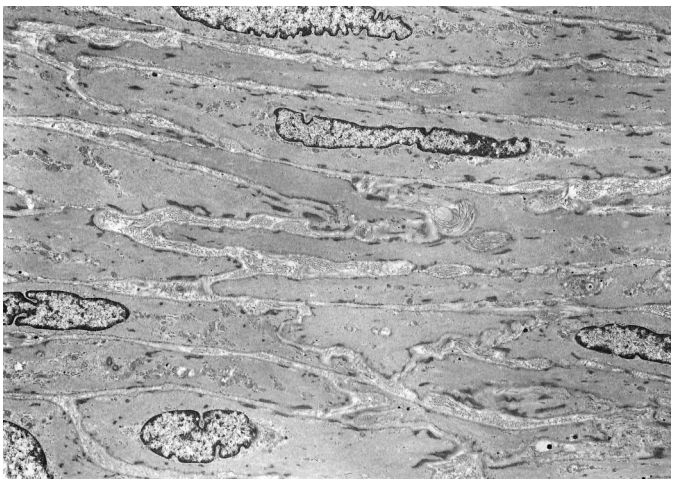

FIG. 15. Sham operated control at 7 days after surgery showing normal fine structure of detrusor SM. Original magnification X 5,000.

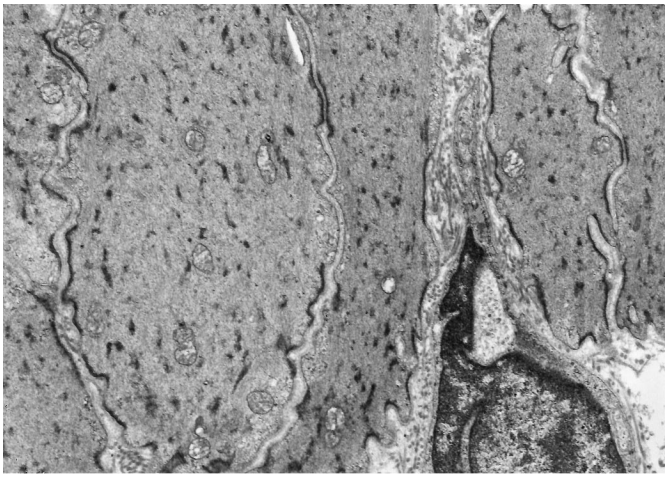

FIG. 16. Sham operated control at 28 days after surgery. Mitochondria retain normal fine structural appearance while myofilaments and dense bodies are evenly distributed throughout detrusor muscle cells. Original magnification X 18,000.

the decreased rate of tension development, occurs sooner, and is significantly greater than the decrease in the contractile response and rate of response to direct cholinergic stimulation.[3–5] In addition, the rate of tension development showed dysfunction significantly earlier than did the maximal response to stimulation for both FS and carbachol. The rate of tension development is dependent upon the integrity of the SR and SM membranes to a greater extent than is the magnitude of tension response, as described below. Thus, the rate of tension development in response to FS is sensitive to changes in neuronal as well as SM membranes, whereas the rate of response to carbachol is sensitive to changes in SM membranes only. Significant reductions in the rate of response to carbachol were seen at 7 and 14 days of obstruction, which correlate with changes observed in SM cell profiles. Nerve degeneration was one of the first degenerative changes noted, which correlates in time with the reduced rate of response to FS. Thus, in both cases, specific damage to SM and neuronal membranes can be correlated with specific decreases in the rate of response to carbachol and FS respectively. It is only when the damage becomes severe that the maximal response to the stimuli is significantly affected.

Based on bladder weight and their responses to FS and carbachol, we can conclude that the rabbit bladders obstructed for 1 to 7 days were compensated, while those that were obstructed for 14 days exhibited a minor shift from compensated to decompensated function. Decompensation proceeded progressively, as observed upon evaluation of the 28 and 43-day obstructed bladders; by 70 days post-obstruction all bladders were severely decompensated.

The present study has correlated these functional parameters with fine structural observations using tissues obtained from the same animals. Previous investigations[8–10] have described the initial effects of partial outflow obstruction using a severely obstructed model, and in general, the results of the current investigation have corroborated the morphological changes in SM and autonomic nerves which occur in response to outlet obstruction. However, the present study has been unable to confirm the occurrence of "regenerating" SM cells or autonomic nerves during the 70-day post-obstruction period. Interestingly, at 3 and 7 days after partial outflow obstruction the present investigation has shown that the majority of detrusor SM cells show morphologic evidence of hypertrophy and some cells possessed prominent perinuclear sarcoplasmic reticulum. Given the contractile responses of these bladders, this altered morphology is clearly compatible with relatively normal function, and as such must be considered to be of little physiological significance. At 7 days some axons showed evidence of structural change which progressed during the longer post obstructive periods. These findings correlate well with the decline in the response of detrusor SM to FS, especially in regard to the rate of tension development. Similarly the reduction in myofilaments and the presence of damaged mitochondria within the SM cells are compatible with the reduced response of the detrusor to carbachol stimulation. Indeed during the process of decompensation the structural damage observed at 14 days pro-

gressively increased up to 70 days after partial obstruction. Throughout this period mitotic nuclei or other signs of regeneration were absent indicating a continuous decline in bladder structure and function during decompensation. Of particular interest is the appearance at 43 days of morphological changes in tissue components (fibroblasts, vascular SM, endothelium) which are not directly involved in bladder contractility. These observations are compatible with the view that partial outlet obstruction induces hypoxia within the bladder wall. Both bladder SM and autonomic nerves appear to be the most vulnerable in that morphological changes are evident in the initial phase of outlet obstruction. Nevertheless by 43 days after obstruction other tissues within the bladder wall possess clear evidence of morphological damage.

In conclusion, the present study has demonstrated the structural damage that occurs in the bladder wall in response to partial outlet obstruction and has correlated these observations with the functional decompensation with which it is associated. Further studies are required to determine the stage during decompensation at which relief of the obstruction enables the bladder to regain its normal structural and functional characteristics. The present findings support the need for early treatment of partial outlet obstruction.

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