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Update on bladder smooth-muscle physiology*

R. M. Levin[1, 2], A. J. Wein[1], R. Buttyan[3], F. C. Monson[1], and P. A. Longhurst[1]

[1] Division of Urology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA
[2] Division of Urology, Philadelphia Veterans Administration Medical Center, Philadelphia, Pennsylvania, USA
[3] Department of Urology, Columbia Medical School, New York, New York, USA

Summary. The urinary bladder responds to distension induced by a number of different stresses with rapid and substantial increases in bladder mass and concomitant alterations in the contractile responses to neuronal stimulation, pharmacological simulation by autonomic agonists, and membrane depolarization. Furosemide, sucrose, or diabetes-induced diuresis, as well as outlet obstruction and overdistension all produce similar effects on the bladder. Accompanying the increases in bladder mass and contractile changes are increases in DNA synthesis and [^{3}H]-thymidine uptake. Autoradiographic studies have localized the increased DNA synthesis following bladder distension initially to the urothelium, followed by slower increases in labelling of the lamina propria and extramural connective tissue. The net result of these compartmental differences in DNA synthesis is a reorganization of the structural relationships between smooth-muscle cells, the connective-tissue matrix, and the extrinsic connective-tissue lamina. This may contribute to the functional changes which occur after severe overdistension. Increases in the expression of heat-shock protein-70, basic fibroblast growth factor, *N-ras*, and *c-myc*, and decreases in transforming growth factor-beta occurred acutely after obstruction, suggesting that these changes may play a role in obstruction-induced bladder hypertrophy. Removal of the obstruction induces apoptosis of urothelial and connective tissue elements in the bladder, accompanied by increases in transforming growth factor-beta and decreases in basic fibroblast growth factor genes, and a reversal of the bladder dysfunction. Therefore the bladder hyperplasia after outlet obstruction and the regression following removal of the obstruction seem to be directly opposing processes governed by gene expression.

The urinary bladder is a smooth-muscle organ whose function is to collect and store urine at low intravesical pressure and then to expel the urine periodically by means of a highly coordinated sustained contraction [1–3]. Efficient emptying requires contraction of the bladder body smooth-muscle elements in coordination with relaxation of the bladder neck and urethra [1–5]. To a large extent, urinary bladder function depends upon the underlying structure of the organ as a whole, particularly on the interrelationships among the smooth-muscle, connective-tissue, and epithelial elements. An alteration in the ratio of connective tissue to smooth muscle, for example, can significantly alter compliance and functional capacity, structurally impairing the bladder's ability to empty efficiently and fully [5–7]. Thus, a change in structural compartmentation can affect bladder function independently of the autonomic receptor density, the response to receptor stimulation, and the contractile capabilities of the smooth-muscle elements.

On an intracellular basis, detrusor contraction, similar to that of all smooth muscles, depends upon the interaction of the contractile proteins actin and myosin and phosphorylation of myosin light chains by myosin light-chain kinase [8, 9]. In turn, phosphorylation of the myosin filaments depends upon a phasic increase in the intracellular free calcium concentration as a result of translocation from the extracellular space and release of intracellularly bound calcium [10, 11]. The rate and magnitude of pressure generation requires the active interaction of actin and myosin with calcium, mediated by myosin light-chain kinase and the net breakdown of cytosolic adenosine triphosphate (ATP). Bladder emptying requires a sustained increase in intravesical pressure during urine expulsion [2, 7, 12–14]. A defect in the bladder's ability to sustain a contraction can seriously reduce its ability to empty while not affecting its ability to generate phasic pressure [7, 15, 16]. Energetically, the phasic generation of pressure utilizes cytosolic ATP. In the presence of anoxia, the rate of decay of the phasic response to stimulation (field stimulation and bethanechol) is similar to the rate of ATP hydrolysis [17–19]. However, the ability of the bladder to sustain tension and empty is lost at a significantly greater rate (Fig. 1). Substrate depletion results in similar reductions in the phasic response to stimulation and in the concentration of cytosolic ATP [20]. Similarly, in the absence of glycolytic substrates, the sustained pressure response to field stimulation and bethanechol declines at a significantly greater rate than the phasic response to stimulation [17–20]. These experiments indicate that the ability of the bladder to sustain pressure in response to receptor-mediated stimulation (but not to general depolarization by KCl) utilizes oxidative energy, whereas the phasic response to stimulation utilizes cytosolic ATP.

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Correspondence to: Dr. R. M. Levin

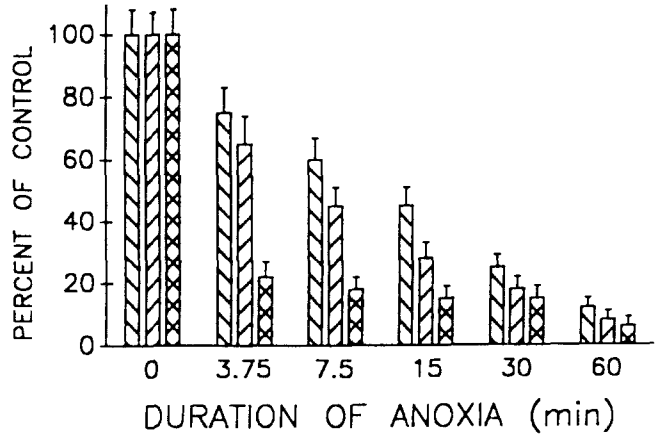

Fig. 1. Time course of the effects of anoxia on ATP concentration and peak plateau contraction in response to field stimulation (80 V, 32 Hz, 1 ms) of isolated rabbit-bladder body strips. Anoxia was induced by changing from aeration with 95% O_2/5% CO_2 to a mixture of 95% N and 5% CO_2. Each bar is the mean value ± SEM for 4–6 individual preparations. ▨ ATP concentration; ▨ peak tension; ▨ plateau tension

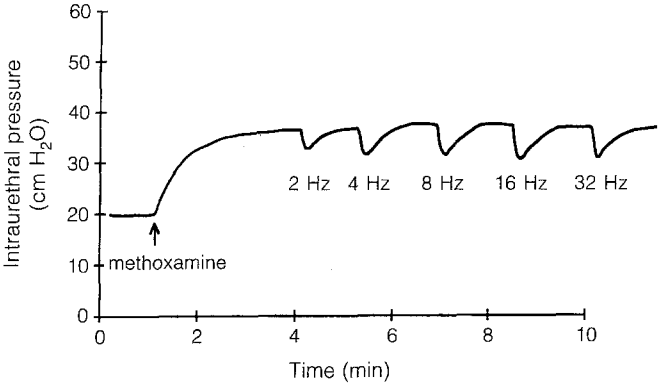

Fig. 2. Representative tracing of the effect of field stimulation (32 Hz, 80 V, 1 ms) on an isolated whole-cat urethra preparation prestimulated with methoxamine (1 m*M*)

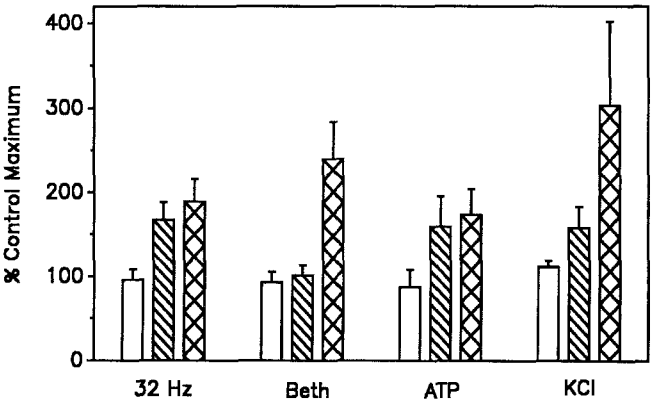

Fig. 3. Response of rabbit bladder body strips to 32-Hz field stimulation, 0.1 m*M* bethanechol *(Beth)*, 10 m*M* ATP, and 124 m*M* KCl after 3, 7, and 14 days of furosemide treatment. Data are expressed as percentages of the age-matched control rabbit-strip maximum. □ 3 Days; ▨ 7 days; ▨ 14 days

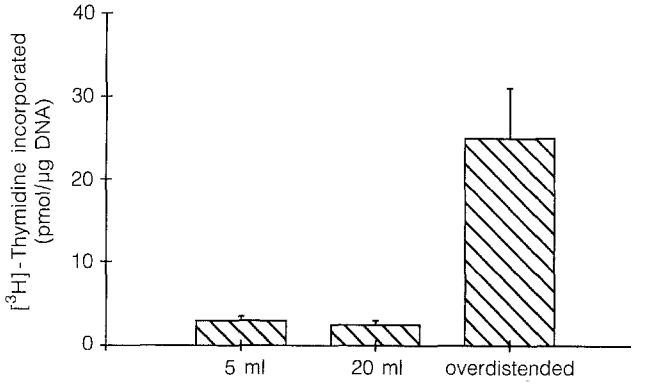

Fig. 4. Effects of in vitro overdistension on thymidine incorporation into DNA. Individual isolated whole bladders were distended to 5 or 20 ml or overdistended to 120% of the cystometric capacity for 7 h. Then the incubation and intravesical solutions were replaced with equal volumes containing [^{3}H]-thymidine (0.5 µCi/ml). Each bladder was incubated for 1 h with labeled thymidine. The tissue was then washed free of labeled thymidine and the specific activity of the extracted DNA was determined. Each bar represents the mean value ± SEM for 4–6 individual preparations

In summary, bladder function depends upon a number of factors, including (a), the state of neuronal innervation, (b) the structure of the organ as a whole, (c) the contractile response of the smooth-muscle elements, and (d) the availability of metabolic energy (in the form of cytosolic ATP and oxidative metabolism). These four factors are intimately associated with each other, and an alteration in one can induce substantial adaptive changes in the others.

Efficient bladder emptying is associated with urethral opening as a result of inhibition of alpha-adrenergic tone (i.e., urethral relaxation) [1–3]. There is increasing evidence that efficient bladder emptying is associated with an active, nitric oxide-stimulated urethral relaxation [2, 21–25]. Experimentally, field stimulation induces a rapid and substantial relaxation of the precontracted urethra, mediated locally by nitric oxide release (Fig. 2) [2, 21–25]. Recent studies also indicate that the nitric oxide synthase inhibitor L-NAME alters micturition parameters, suggesting that the nitric oxide pathway participates in normal micturition. Although nitric oxide-stimulated relaxation of the bladder body has not been demonstrated, there is evidence that nitric oxide may play an important role in fetal bladder development [26, 27].

One of the most striking characteristics of the urinary bladder is its ability to respond to various forms of stimuli and stress with rapid and substantial changes in bladder mass and concomitant alterations in the contractile responses to neuronal stimulation, pharmacological stimulation by autonomic agonists, and general membrane depolarization (see reviews in [1–3, 7]). Just as striking is the ability of the bladder to recover following the removal of the stress [28–31].

Chronic diuresis in rabbits induced by furosemide caused a progressive increase in bladder mass and an increase in compliance [32]. In addition, diuresis caused a significant increase in the response of bladder body strips to field stimulation, bethanechol, ATP, and KCl (Fig. 3) [32]. Even when the contractile response was normalized to tissue mass, the contractile responses were significantly

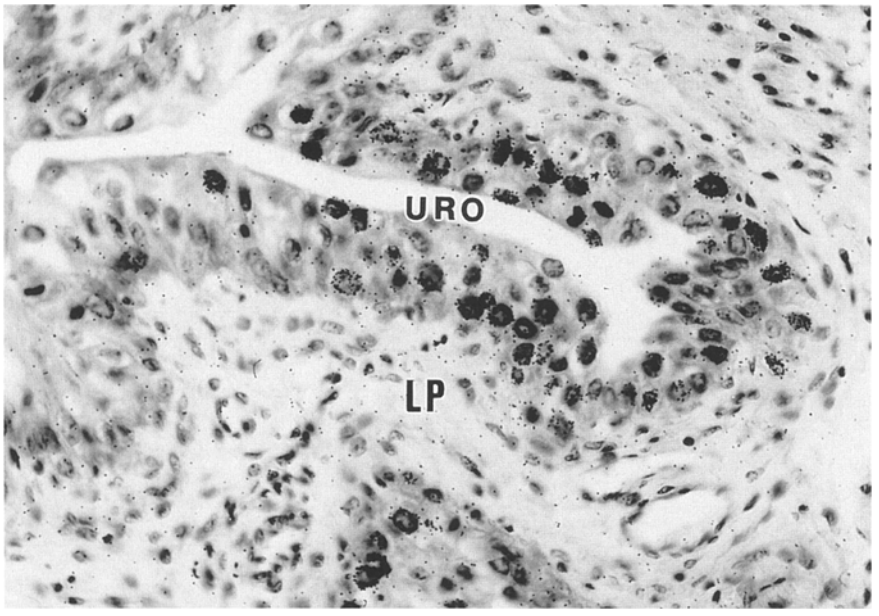

Fig. 5. Representative example of an autoradiogram showing the effects of partial outlet obstruction on [^{3}H]-thymidine incorporation at 1 day following obstruction. Note the evidence of substantial labeling of the urothelium. × 25

increased. In parallel experiments, the urinary bladder DNA concentration and [^{3}H]-thymidine uptake were significantly increased at 3 days following the initiation of furosemide diuresis and returned to control levels by 7 days [33]. Autoradiography demonstrated significant increases in [^{3}H]-thymidine incorporation into both connective-tissue and smooth-muscle cells [33]. Similar changes are seen after the induction of streptozotocin-induced diabetes in rats. Contractile responses of bladder strips to field stimulation and agonists are increased [34], the bladder mass increases, and bladder [^{3}H]-thymidine incorporation increases within 7 days [35]. Thus, the diuresis-stimulated increase in bladder mass is characterized by an increased smooth-muscle mass, increased compliance, and increased contractile responses to all forms of contractile stimulation, but no change in extrinsic connective tissue occurs [32, 33]. Conversely, diversion causes a rapid decrease in bladder mass, decreased capacity and compliance, and decreased contractile responses to all forms of stimulation [36]. Virtually all changes induced by alterations in urine formation and excretion are reversible [34].

Acute overdistension of the rabbit bladder induced an immediate decrease in the contractile response to bethanechol-induced receptor-mediated contraction, whereas the response to depolarization elicited by KCl did not change [37]. Simultaneous with the decreased contractile response, there was a rapid increase in DNA synthesis as measured by [^{3}H]-thymidine incorporation [38]. Autoradiography demonstrated that the increased DNA synthesis was limited almost exclusively to the basal cells and lamina propria of the urothelium [38]. In separate experiments, in vitro overdistension of the bladder induced DNA synthesis that was also limited to the basal cells of the urothelium (Fig. 4) [39]. Thus, the increased DNA synthesis stimulated by acute overdistension was not secondary to either neuronal reflexes or penetration of irritative substances found in the urine. This finding is further emphasized by the studies of Karim et al. [40], who showed that in vitro stretching of young guinea-pig bladder strips stimulated DNA synthesis. Autoradiography after 12 h of stretching localized labeling to the myocyte nuclei. These authors concluded that smooth-muscle growth could be regulated by physical factors such as stretching in the absence of additional trophic factors.

Partial outlet obstruction in rabbits resulted in a marked increase in bladder mass, a decrease in compliance, a decreased response to field stimulation relative to the response to bethanechol and KCl, and a significantly greater decrease in the ability of the in vitro whole-bladder preparation to empty in comparison with the ability to generate pressure [6, 7]. Obstruction stimulates an immediate and rapid activation of DNA synthesis in the urothelium (similar to stimulation by acute overdistension; Fig. 5, Table 1) followed by activation of cellular elements in the intrinsic and extrinsic connective-tissue compartments (Fig. 6, Table 1) [38, 41]. [^{3}H]-Thymidine incorporation into DNA and its autoradiographic localization was determined in rabbit bladders at 1, 3, 5, 7, 14, and 21 days following partial outlet obstruction. [^{3}H]-Thymidine incorporation into DNA increased by a factor of 7 at day 1, reached a maximum at day 3, and decreased to stable levels at between 14 and 21 days (5 orders of magnitude greater than control levels). The urothelium showed intensive DNA synthesis at day 1, moderate incorporation at day 3, and no further incorporation thereafter. The lamina propria and connective tissue elements showed increased activity at 3 days, reached a maximum at 5 days, and returned to stable levels (above control levels) thereafter. No significant labeling of smooth muscle was observed (Table 1) [38, 41]. Similar changes have been observed in guinea pigs with urethral obstruction [42]. Increases in the peak voiding pressure, decreases in the mean flow rate, and decreases in the percentage of void developed during the first few weeks after obstruction, accompanied by increases in detrusor mass and DNA synthesis. Autoradiography localized DNA synthesis to the smooth-muscle nuclei, with an absence of labeling of non-myocyte nuclei,

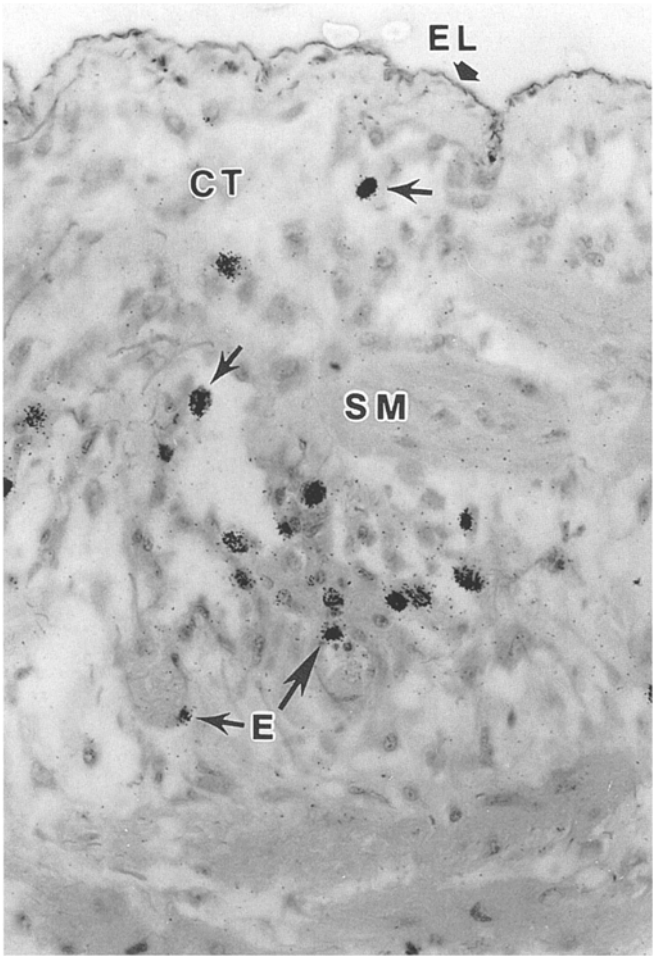

Fig. 6. Representative example of an autoradiogram showing the effects of partial outlet obstruction on [^{3}H]-thymidine incorporation at 3 days following obstruction. Note that by day 3, connective tissue *(CT)* is labeled *(plain arrows)*, whereas smooth muscle *(SM)* is not, and that at least some of the CT cells are endothelial (E, labeled arrows). It is not unusual for clusters of labeled cells to be found in CT, as can be seen in the center of this photomicrograph. × 25

indicative of myocyte hyperplasia. Removal of the obstruction reversed all changes.

The net result of the dynamic DNA synthesis described above is a reorganization of the structural relationships among the smooth-muscle cells, the surrounding connective-tissue matrix, and the extrinsic connective-tissue lamina. In response to partial obstruction, this structural rearrangement increases the bladder's tensile strength or "stiffness" and is responsible for its decreased compliance, which may help explain the obstructed bladder's markedly decreased ability to empty through the structural impairment of its ability to change shape during micturition.

The rapid nature of the changes described above for acute obstruction and partial outlet obstruction indicates that the bladder can adapt very quickly to stress situations. A series of studies were completed by Buttyan et al. [43] that characterized the effect of these two specific pathological models on a series of early-response genes that control cellular growth and division. In the first study, bladder tissues were obtained from untreated control rabbits and from a series of rabbits at progressive intervals (2, 6, 10, 12, 24, and 48 h) after the creation of a partial outlet obstruction. Simple analysis of the bladder tissues recovered over this period showed a reproducible doubling of the wet weight, demonstrating the onset of hypertrophic changes [43].

Poly(A)+ RNA was extracted from individual specimens and these RNAs were comparatively electrophoresed (5 µg/lane) on formaldehyde-agarose gels and then transferred to nylon filters for Northern-blot analysis. Several blots were generated, each containing RNAs from the full complement of time points, and each blot was hybridized successively to three or four different ^{32}P-labeled gene probes. The autoradiographs derived from each probe hybridization were analyzed by laser densitometry to quantitate the autoradiographic band density of the appropriate transcript in bladder RNA at each time point following obstruction.

The activity of several genes changed very abruptly early after obstruction (Table 2). The transcripts encoding the mammalian 70-kDa heat-shock protein (hsp-70; 2.5 and 3.2 kb), while showing some slight elevation as soon

Table 1. Effects of chronic obstruction and acute in vivo overdistension on DNA synthesis by the rabbit bladder

Experiment	Mucosa		Submucosa			fmol [^{3}H]-TdR/µg DNA ± SE
	URO	LP	SM	Extramural CT		
				ICT	ECT	
Normal	±	–	±	±	NP	0.64 ± 0.10
1 day[a]	4+	–	–	2+	NP	4.74 ± 0.00
3 days	2+	4+	±	3+	3+	18.03 ± 2.75
5 days	1+	3+	–	2+	2+	5.95 ± 0.53
7 days	±	2+	–	2+	1+	7.20 ± 0.23
14 days	±	–	–	1+	1+	4.91 ± 2.03
21 days	±	1+	–	1+	1+	3.44 ± 0.67
OD	3+	2+	–	1+	NP	1.84 ± 0.17

±, 1–3/section (25–50 fields at 250 ×); –, none observed; 1+, 1–3/field; 2+, 3–5/field; 3+, 5–10/field for some fields; 4+, > 10/field for many fields; NP, tissue not present; *OD,* overdistension; *URO,* urothelium; *LP,* lamina propria; *SM,* smooth muscle; *CT,* connective tissue; *ICT,* intrinsic CT; *ECT,* extrinsic CT

[a] After partial outlet obstruction

Table 2. Northern-blot assay of rabbit-bladder RNA

Probe	Control	Obstruction	Overdistension
hsp-70	1.00[a]	23 (10 h)	10 (1 h)
bFGF	ND	400 (10 h)[b]	1.00 (1 h)[a]
TGF β_1	1.0[a]	0.4 (24 h)	0.5 (1 h)
c-fos	ND	ND	1.00 (1 h)[a]
c-jun	ND	ND	1.00 (1 h)[a]
N-ras	1.0[a]	900 (48 h)	ND
cHa-ras	ND	600 (24 h)[b]	ND
c-myc	ND	450 (24 h)[b]	ND
c-sis	ND	ND	ND

Values in parentheses represent the time after obstruction or overdistension where the signal change is maximal
ND, Not detected
[a] Assigned an arbitrary value of 1.00. Used for relative comparison with obstructed and overdistension values for the same probe. This value is not necessarily the minimal detectable level
[b] Greater than the minimal detectable level

as 2 h after the obstruction, exhibited a 20-fold elevation in the 10-h-obstructed rabbit bladder. mRNA encoding beta-actin (1.7 kb), a cytostructural element, showed an almost 10-fold elevation at 24 h after obstruction. On the basis of their intensity, both of these changes are indicative of a period of rapidly enhanced cellular growth and are consistent with the weight changes observed in the rabbit bladder after obstruction.

A survey for the expression of protooncogenes during bladder obstruction has provided some intriguing data (Table 2). There was a dramatic elevation in the expression of the 2.4-kb *c-myc* transcripts, which peaked in the 10-h-postobstruction specimens. The induction of this protooncogene is consistent with the rapid stimulation of [^{3}H]-thymidine incorporation into DNA observed. A survey for the expression of three different *ras* genes also provided interesting data. Transcripts encoding the *cKi-ras* gene were undetectable in RNA from normal rabbit bladder and from all specimens subjected to partial obstruction, whereas 1.2-kb *cHa-ras* transcripts were detected in control bladder specimens and showed slight (from 3- to 5-fold) elevation in the later stages of obstruction. The transcripts for *N-ras*, however, although undetectable in normal rabbit-bladder RNA, showed remarkable elevation, especially in the 24-h obstructed specimen. Thus, there is a clear discrimination in this gene family of the activity of individual genes during early bladder hypertrophy. These results suggest that the product of the *N-ras* gene might be the one most involved in this process. In guinea-pig bladders, changes in protooncogenes are also evident after obstruction. Transient increases in *c-fos* and *c-myc* expression were found at 1 and 2 weeks after obstruction, but these values returned to control levels with increasing duration of obstruction, despite continued urodynamic changes [42].

Studies of growth factor expression during this early period of bladder hypertrophy have also proven to be most interesting (Table 2). Partial outlet obstruction caused an early and substantial induction of the mRNA encoding basic fibroblast growth factor (bFGF) along with a simultaneous decrease in transforming growth factor beta (TGF-b). Increases in bFGF and decreases in TGF-b are consistent with the stimulated [^{3}H]-thymidine incorporation and probable hyperplastic response [43]. Urethral obstruction in rats and humans is associated with increases in nerve growth factor (NGF) concentration [44]. In rats, these changes were coincident with increases in bladder mass, voiding frequency, and pelvic ganglion-cell somata cross-sectional area. Induction of autoimmunity to NGF in rats prevented the increase in neuronal size but did not completely prevent the obstruction-induced increases in voiding frequency and bladder mass.

In comparison, acute overdistension stimulates an increase in the expression of hsp-70, bFGF, *c-fos*, and *c-jun* and a decrease in TGF-b. Time-course studies demonstrate that the stimulated expression of all these genes (and the decreased expression of TGF-b) reaches a maximum with a few hours following acute overdistension and returns to control levels by 24 h [45]. Thus, acute overdistension results in a much shorter and less pronounced alteration in gene expression than does partial outlet obstruction [45].

If the partial outlet obstruction is removed, these structural and functional changes are readily and rapidly reversed, and the enlarged bladder regresses in size and weight to normal values [28–31, 42, 44]. The cellular mechanisms that mediate the decrease in mass are not known at the present, but they would involve both a decrease in the volume of the hypertrophied smooth-muscle cells and a decrease in the cellular structure and content of the urothelial and connective-tissue elements. In a recent study, Santarosa et al. [29] demonstrated that apoptosis is a key element in the regression of the bladder following reversal of the partial outlet obstruction.

Apoptosis, also referred to as "programmed cell death," was originally described as a cell-elimination process that is extensively invoked during embryogensis [46]. More recently, it has become apparent that apoptosis is an important mechanism for the maintenance of adult tissue homeostasis. It plays a complementary and opposing role to mitosis in regulating populations of cells [46–48]. Apoptosis can be identifed by certain characteristic features, including distinct morphologic changes in the cell nucleus and cytoplasm and cleavage of chromatin at regularly spaced sites [49]. These changes are readily identifiable and easily distinguishable from cell death by necrosis. Through the appropriate analysis of these markers, one can measure apoptosis even when the dying cells are only a small proportion of the cells in the tissue [49].

In the study of Santarosa et al. [29], rabbits were obstructed for 7 days, at which time the partial outlet obstruction was removed. Bladders were removed at times ranging between 12 and 72 h following the reversal. The presence of apoptosis was both determined quantitatively by studies on the bladder tissue and visualized qualitatively by in situ histological analysis. Quantitatively, no apotosis was present in the control bladders. Apoptosis was minimally present in tissue obtained from the 7-day-obstructed rabbits. The level of apoptosis present increased substantially at 12 h following the reversal, reached a maximum at 24 h, and declined at the 48- and

72-h time points. Qualitatively, the apoptotic cells were confined to the urothelial as well as intrinsic and extrinsic connective-tissue elements. No apoptotic smooth-muscle cell was observed. These are the same cellular elements that displayed hyperplasia during bladder growth following partial outlet obstruction [29].

Further studies demonstrated that reversal of the partial outlet obstruction induced a marked increase in the expression of genes coding for TGF-b and a simultaneous decrease in the expression of bFGF. These alterations in gene expression are exactly the opposite of those observed during bladder growth induced by partial outlet obstruction. These results indicate that bladder hyperplasia induced by partial outlet obstruction and regression (apoptosis) following the reversal of obstruction are directly opposing processes that are controlled by opposite effects on gene expression [29].

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