## Committee 1

# Gross Anatomy and Cell Biology of the Lower Urinary Tract

**Chairmen**

*J. DeLancey (USA),*

*J. Gosling (USA)*

**Members**

*K. Creed (Austriala),*

*J. Dixon (U.K.),*

*V. Delmas (France),*

*D.Landon (U.K.),*

*P. Norton (USA)*

.

## CONTENTS



SUMMARY

I. MACROSCOPIC ANATOMY OF THE URINARY BLADDER

1. External Features
2. Internal Features
3. Relations of the female urinary bladder
4. Relations of the male urinary bladder

II. EXTRINSIC INNERVATION OF THE LOWER URINARY TRACT

III. THE BLADDER WALL

1. Smooth muscle
2. Stroma
3. Blood vessels
4. Urothelium
5. Intrinsic innervation of the urinary bladder

IV. BLADDER NECK

1. Female Bladder Neck
2. Male Bladder Neck

V. THE FEMALE URETHRA

VI. THE MALE URETHRA

1. Macroscopic anatomy
2. Prostatic urethra
3. Membranous urethra
4. Spongy urethra

VII. THE URETHRAL WALL

1. Smooth muscle
2. Striated muscle
3. Urethral lamina propria
4. Periurethral tissue

VIII. MODULATORY SYSTEMS

1. Nitric Oxide Synthase
2. Afferent innervation

IX. MACROSCOPIC ANATOMY OF THE MUSCULAR PELVIC FLOOR

1. Levator ani
2. Innervation of the levator ani

X. SUPPORT OF THE URETHRA AND PELVIC ORGANS

1. Support of the pelvic organs
2. Urethral and anterior vaginal wall support
3. Support of the posterior vaginal wall and rectum
4. Connective tissue of the pelvic floor

XI. EFFECT OF AGE AND PARTURITION ON PELVIC FLOOR MORPHOLOGY

XII. ANATOMY OF THE ANAL SPHINCTER COMPLEX

XIII. CONCLUDING REMARKS

# Gross Anatomy and Cell Biology of the Lower Urinary Tract

*J. DeLancey, J. Gosling,*

*K. Creed, J. Dixon, V. Delmas, D. Landon, P. Norton*

## SUMMARY

The structure and relationships of the male and female lower urinary tract have been considered with particular emphasis placed on those morphological components believed to be of importance in the maintenance of urinary continence. The autonomic innervation of the detrusor has been reviewed and the location of the vesical plexus and its constituent neurons has been noted. The location of this plexus close to the bladder neck renders the innervation of the detrusor vulnerable during surgical procedures in this region. The motor innervation of detrusor smooth muscle is considered to be mainly cholinergic in type and sympathetic influences are believed to act on autonomic neurons rather than directly on bladder smooth muscle. Encapsulated sensory nerve terminals are occasionally observed in the adventitia of the urinary bladder although most presumptive sensory nerves are thought to reside in the submucosa. The structure and innervation of the male bladder neck has been described and this region serves to prevent reflux of seminal fluid at the time of ejaculation. In both sexes the role of the bladder neck in maintaining urinary continence remains uncertain. The location and innervation of striated muscle within the wall of the urethra (rhabdosphincter, external urethral sphincter) in both sexes has been described. In the female this muscle forms an omega shaped sphincter which is thickest in the middle one third of the urethra and consists mainly of small diameter, slow twitch fibres. In the male, both large and small diameter fast and slow twitch fibres form the sphincter which lies at, and below, the apex of the prostate. In both sexes the sphincter is anatomically separate from the medial fibres forming the pelvic diaphragm. The structure and innervation of the levator ani have been described particularly with respect to the effects of parturition on the pelvic floor musculature. Finally the relevance of fascial thickenings in maintaining support for pelvic viscera has been considered. Whilst thickenings of the endopelvic fascia have been described macroscopically, their relative importance by comparison with the active muscle tone exerted by the pelvic floor remains to be determined. The anatomy of the anal sphincter mechanism has also been considered.

The upright posture of human beings means that gravitational forces and abdominal pressure generated by the striated muscle activity associated with normal posture both combine to exert continuous force on the outflow tract, and thus a continuous occlusive force has to be generated to maintain continence. This is achieved via both dynamic forces in the smooth and striated muscles of the urethral wall, and passive forces in the vascular filling of the lamina propria and the apposition of its surfaces. The dynamic occlusion forces need to be increased to prevent leakage with sudden rises in intrabdominal pressure, and decreased in synchrony with the elevation of the bladder pressure in micturition. Again a complex neuronal network is apparent in the urethral walls, with sensory fibres and several classes of motor nerve.

Throughout this report we have attempted to indicate those aspects of lower urinary tract morphology which require further investigation in order to clarify their role and relative significance in the prevention of urinary incontinence.

# I. MACROSCOPIC ANATOMY OF THE URINARY BLADDER

In both sexes, the urinary bladder lies in the anterior (ventral) part of the pelvic cavity. The proportion of the cavity that it occupies is dependent upon the volume of fluid contained within the vesical lumen. The full bladder is approximately spherical in shape, becoming more tetrahedral in form as emptying occurs. It is in the contracted state that the anatomical relationships of the urinary bladder are best considered. In the following account, the relations of the urinary bladder will be described separately for each sex.

## 1. External features

Although the urinary bladder is highly variable in shape it is convenient, if not strictly accurate, to consider the viscus as a tetrahedron, possessing an anterior, an inferior (caudal) and two posterolateral (dorsolateral) angles. The anterior angle is directed forwards and upwards and is attached to the urachus, a fibrous cord ascending in the extraperitoneal tissues of the abdominal wall as far as the umbilicus. The urachus is the embryological remnant of the allantois and occasionally remains patent throughout life. This condition may be unrecognized clinically until the bladder neck or urethra becomes obstructed, whereupon urine is discharged from the umbilicus. The two posterolateral angles are those regions in which the ureters pierce the bladder wall; the inferior angle corresponds to the bladder neck and associated internal urethral meatus. The distal 1-2 cm of each ureter is surrounded by an incomplete collar of detrusor smooth muscle which forms a sheath (of Waldeyer) separated from the ureteric muscle coat by a connective tissue sleeve. The ureters pierce the posterior aspect of the bladder (fig. 1) and run obliquely through its wall for a distance of 1.5-2.0 cm before terminating at the ureteric orifices. This arrangement is believed to assist in the prevention of ureteric reflux since the intramural ureters are thought to be occluded during increases in bladder pressure.

As a tetrahedron, the urinary bladder also possesses four surfaces which are readily discernible in the contracted organ. The two inferolateral surfaces conform to the pelvic walls and floor to which they become more closely related as bladder distension increases. The posterior surface, or base, of the bladder is small and varies in size to only a minor degree as the organ fills and empties. This surface extends between the entrances of the ureters into the bladder wall and the posterior aspect of the bladder neck. The superior surface (fundus) varies the most in shape and area, expanding upwards and forwards as the organ fills.

## 2. Internal features

When viewed from within, the mucosa lining the wall of the bladder presents three distinct apertures, namely, the ureteric orifices and the internal urethral meatus (fig. 2). These lie relatively close to one another and delimit the trigonal region of the bladder. The two lateral orifices appear slit-like and are formed by the internal openings of the ureters. Frequently, these two orifices are connected by a prominent ridge known as the interureteric bar. Extending inferomedially from the ureteric orifices, a pair of ridges corresponding to the lateral edges of the trigone extend as far as the internal urethral meatus. The latter lies in the midline and forms a circular aperture on the luminal aspect of the bladder neck region. With the exception of the trigone, the bladder mucosa is comparatively rugose in the undistended organ but becomes smoother as filling proceeds. The trigone is characterized by a relatively flattened appearance with a smooth urothelial covering and retains its appearance and size irrespective of the degree of distension of the bladder.

## 3. Relations of the female urinary bladder

In the female, the inferolateral surfaces of the bladder are closely applied to the fascia which covers the pelvic walls and floor (fig. 3). The lateral pelvic walls are formed by the obturator internus muscles, covered on their medial aspects by the obturator fasicae. The latter give attachment to the levator ani muscles which, together with the coccygeus, form the pelvic floor. The levator muscles are covered on their superior aspects by a further layer of fascia (the pelvic fascia).

Several nerves and blood vessels course in the fascia adjacent to the inferolateral surface of the bladder. The nerves include the obturator nerve and part of the pelvic plexus of autonomic nerves (the vesical plexus). The arteries related to these surfaces of the bladder are the superior vesical arteries (which continue anteriorly as the obliterated umbilical arteries), the obturator arteries and the inferior vesical and vaginal arteries. The obturator veins are discrete vessels which accompany the obturator arteries; the vesical veins form a network of venous channels, the vesical venous plexus, which lies adjacent to the bladder's inferolateral surface. The posterior relations are the cervix of the uterus and the vagina (fig. 4). The extravaginal portion of the cervix lies against the superior part of the base of the bladder while the anterior vaginal wall is in contact with most of the remaining areas of the bladder base. The superior relations of the female bladder are somewhat variable because the size, position and presence of the uterus are subject to alteration. The non-gravid uterus commonly inclines forwards

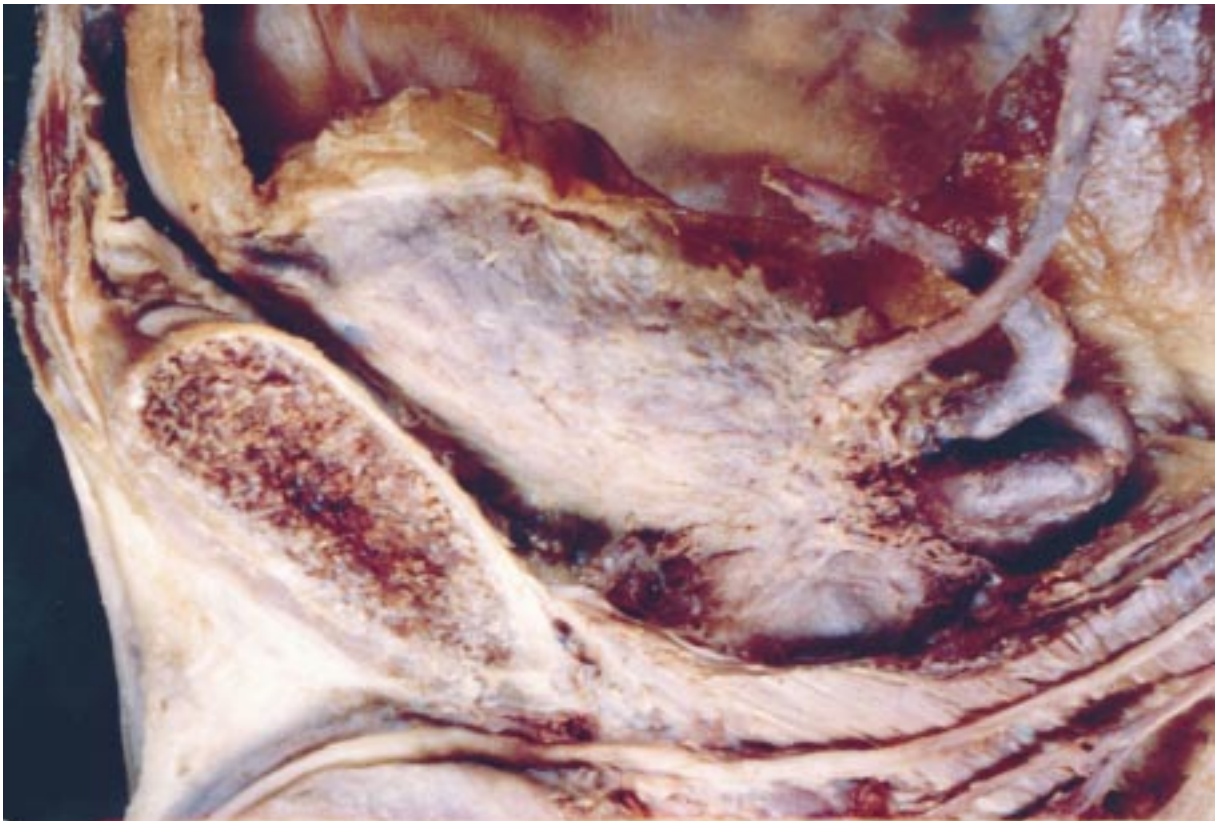


*Figure 1 : Lateral view of the male bladder after removal of the left pelvic wall. The left ureter enters the posterior surface of the bladder and is crossed by the terminal portion of the ductus deferens.*

*Figure 2 : Coronal section through the male pelvis to show the interior of the bladder and some of its relations.*

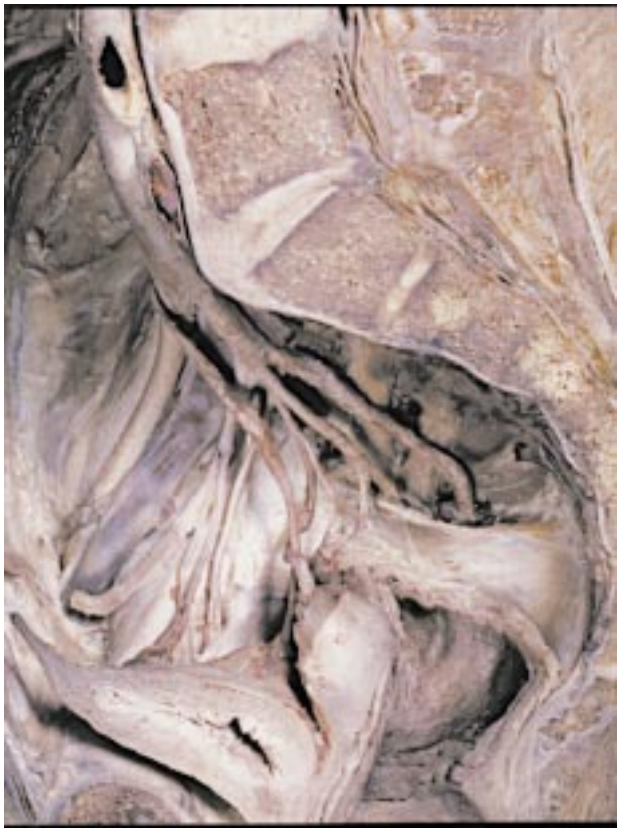


*Figure 3 : Female pelvis dissected to show some of the structures which relate to the infrolateral aspect of the bladder.*

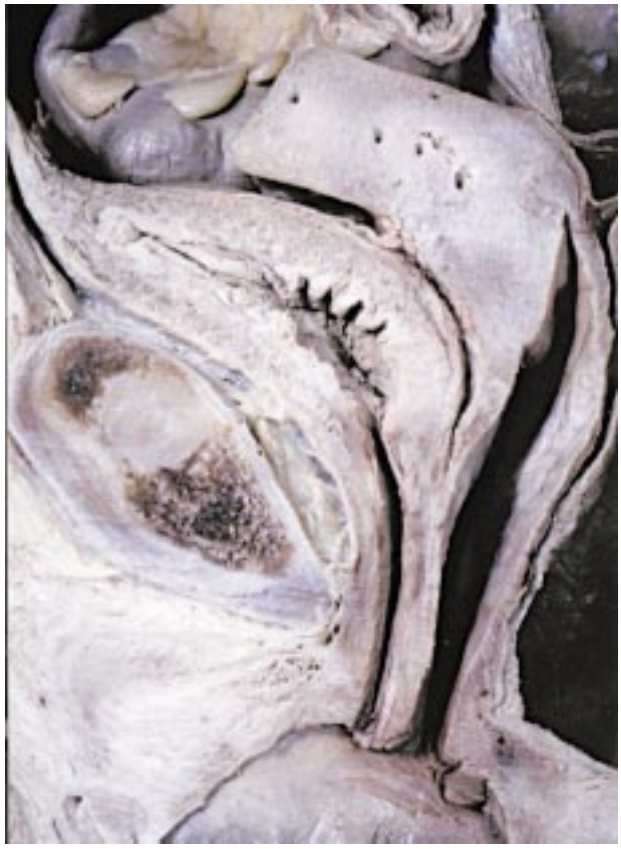


*Figure 4 : Median sagittal section through the female pelvis to show the bladder and some of its relations.*

and upwards and lies on the posterior part of the superior surface of the bladder (fig. 5). The remainder of the superior surface of the bladder is related to coils of intestine. However, a normal variant may occur whereby the body of the uterus is retroverted and directed towards the rectum and sacrum, thereby leaving the entire superior surface of the bladder in contact with intestines.

Between the bladder and the pubic bones lies adipose tissue, the terminations of the arcus tendineus fascia pelvis to the pubic bones, and numerous veins (see below). Vesical filling results in direct contact between the bladder and the anterior abdominal wall above the pubic symphysis. The peritoneum from the anterior abdominal wall continues onto the superior surface of the bladder (fig. 6) but extends posteriorly only as far as the isthmus of the uterus onto which it is reflected to form the uterovesical pouch.

## 4. Relations of the male urinary bladder

The inferolateral surfaces of the bladder are related to fascia and to the walls and floor of the pelvis (fig. 7) as described in the female. The base or posterior surface of the bladder is related to the seminal vesicles, the ampullae of the ducti deferentes, the rectum and the rectovesical pouch of peritoneum. The seminal vesicles and ampullae are applied to the lateral parts of the bladder base. These structures are partly covered by peritoneum which continues for a short distance from the superior surface of the bladder onto its base. This peritoneum caps the ampullae of the ducti deferentes and the seminal vesicles before being reflected at the rectovesical pouch onto the anterior surface of the rectum. When the rectum is distended its ampulla lies in close contact with the base of the bladder. However, when the rectum or bladder, or both, are relatively empty, coils of intestine frequently intervene and occupy the rectovesical pouch.

The prostate gland, surrounded by the prostatic venous plexus, lies inferior to the bladder neck (fig. 8). Between the bladder and the pubic bones lies the retropubic space containing adipose tissue and the puboprostatic and pubovesical ligaments. As bladder filling occurs the organ comes to occupy more of the retropubic space; further filling results in the superior surface of the bladder extending above the pubis, thereby displacing the peritoneum from the anterior abdominal wall. Thus, the anterior aspect of the distended bladder is in direct contact with the abdominal wall without the intervention of peritoneum. The whole of the superior surface of the bladder and the median portion of its base are covered by peritoneum and are related to intestines consisting of ileum, sigmoid colon and rectum.

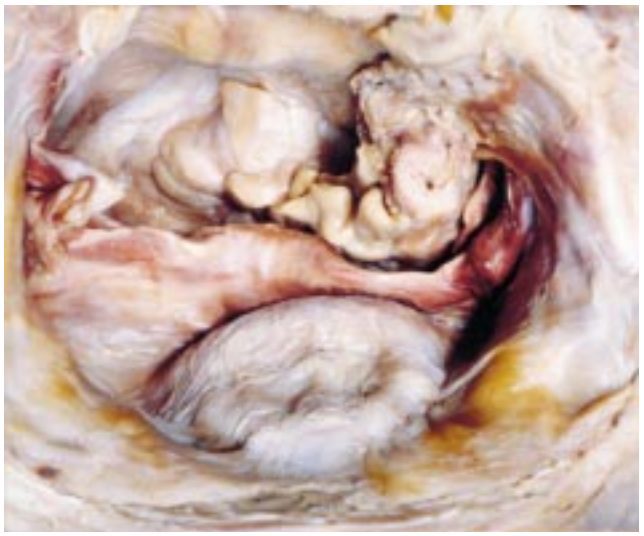


*Figure 5 : Superior view of peritoneum and organs within the female pelvis. The small intestine and most of the sigmoid colon have been removed.*

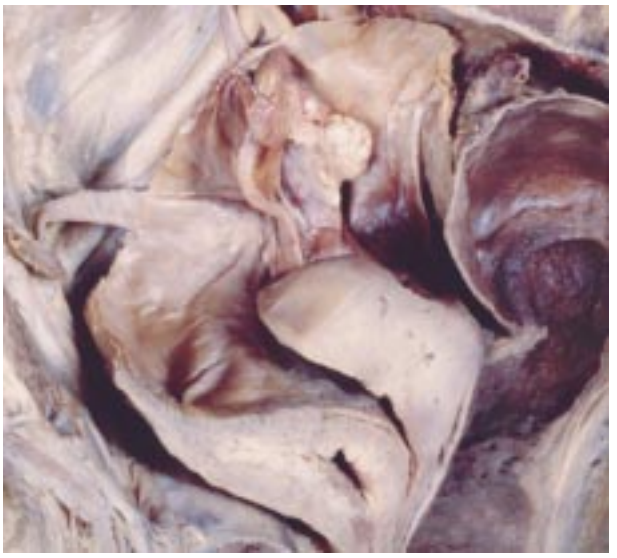


*Figure 6 : The superior surface of the bladder is covered by peritoneum. In this dissection the fat which occupied the retropubic space has been removed.*

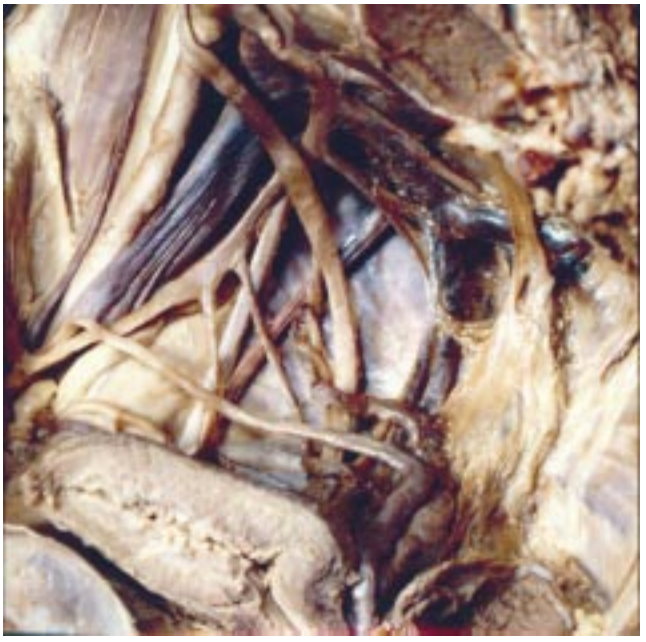


*Figure 7 : Some of the structures which relate to the infrolateral and posterior surfaces of the male bladder. The pelvic plexus forms a flat sheet which extends upto the seminal vesicle and ductus deferens.*

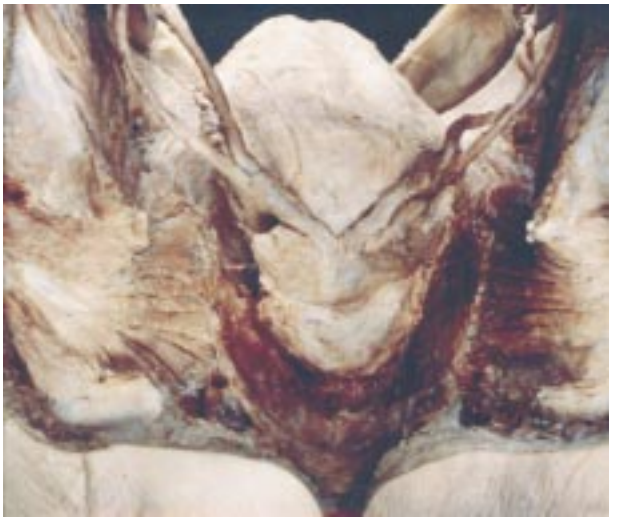


*Figure 8 : Removal of the rectum and the posterior wall of the pelvis exposes the bladder, ducti deferentes, seminal vesicles and prostate.*

## II. EXTRINSIC INNERVATION OF THE LOWER URINARY TRACT

Efferent sympathetic and parasympathetic fibres are conveyed to the bladder and urethra via the hypogastric and pelvic splanchnic nerves, respectively. These nerves as well as the pudendal nerves convey afferent (sensory) fibres to the spinal cord. The sympathetic fibres are derived from the lower two thoracic and upper two lumbar segments of the spinal cord. The parasympathetic fibres arise from the second to the fourth sacral segments of the spinal cord (the nervi erigentes or pelvic splanchnic nerves). The hypogastric and pelvic splanchnic nerves from each side unite to form the right or left pelvic plexus, which lies lateral to the rectum, internal genital organs, and bladder (fig. 9). That part of each pelvic plexus specifically related to the urinary bladder is referred to as the vesical plexus of autonomic nerves and contains both sympathetic and parasympathetic ganglion cells and nerve fibres together with occasional small intensely fluorescent (SIF) cells 1.

Afferent impulses arising from sensory nerve endings in the wall of the bladder and urethra pass to the spinal cord via the pudendal, pelvic splanchnic, and hypogastric nerves. The pudendal nerve transmits sensation mainly from the urethral mucosa (in addition to that from the skin of the genital area and the anal canal) and also transmits proprioceptive impulses from the striated muscle of the pelvic floor. The afferent pathway of the micturition reflex is carried in the pelvic splanchnic nerves, together with those afferents concerned with bladder mucosal pain and lower ureteric pain. The part played by the hypogastric nerves in relaying sensation from the lower urinary tract is not well defined. A detailed consideration of bladder afferent nerves is provided later in this report.

## III. THE BLADDER WALL

### 1. Smooth muscle

#### *a) Cellular morphology*

The individual smooth muscle cells in the bladder wall are typical smooth muscle cells – they are long spindle shaped cells with a central nucleus, and when fully relaxed are several hundred microns long, and five to six microns in diameter at their widest, where the nucleus is. As shown in figures 10 and 11, the cytoplasm is packed with the normal myofilaments, and the membranes contain regularly spaced dense bands, with membrane vesicles (caveoli) between them. There are also scattered dense bodies in the cytoplasm. Mitochondria and fairly sparse elements of sarcoplasmic reticulum (mostly near the nucleus) are also present. The smooth muscle cells are arranged in muscle bundles (fig. 12). These range extensively in size. In human detrusor the muscle bundles are large, often a few mm in diameter, and composed of several smaller sub-bundles 2. The bundles are not clearly arranged in distinct layers, but run in all directions in the detrusor. Amongst the smooth muscle cells can be found cells having long dendritic processes extending parallel to the smooth muscle fibres, which contain vimentin, an intermediate filament expressed by cells of mesenchymal origin (fig. 13) 3.

Within the main bundles the smooth muscle cells may exist in groups of small functional units, or fascicles 4. Intermediate junctions are seen, where adjacent cells have dense bands similarly aligned on their membranes, the gaps between the cells being quite narrow and filled with dense basement membrane. In the trigone the bundles are smaller and clearly differentiated into two layers 5. The orientation and interaction between the smooth muscle cells in the bladder are important, since this will determine how the bladder wall behaves and what effect activity in the cells will have on its shape and intraluminal pressure.

#### *b) Contractile machinery*

As far as is known, the contractile system in the bladder smooth muscle is similar to other smooth muscles, in that shortening occurs by interaction between thin and thick filaments. The thin actin filaments are anchored at the membranes on the dense bands, or in the cytoplasm on the dense bodies, and interact with the thick filaments through cross bridges formed by the heads of myosin molecules. Relatively few papers have been published which have specifically studied the contractile machinery in detrusor smooth muscle (but see below). From studies on other smooth muscles, it is clear that a spectrum exists from those specialised for producing phasic activity (such as the ureter) and those specialized for maintaining continuous tone (such as some vascular smooth muscles). In both cases the contractile system is a myosin-activated system. Cross bridge cycling is initiated when the ATPase activity of the myosin heads is switched on (fig. 14). This is achieved by phosphorylation of one of two light chains on the cross bridge, through a specific enzyme, myosin light chain kinase, which is activated by a rise in the intracellular calcium [$C^{a2+}$], with a half-maximal concentration at about 1$\mu$M. Tonically active smooth muscle may generate force with low expenditure of metabolic energy, through activation of a 'latch state' in the cross bridges, in which dephosphorylation of attached cross bridges occurs, allowing maintenance of force without energy expenditure. Information about

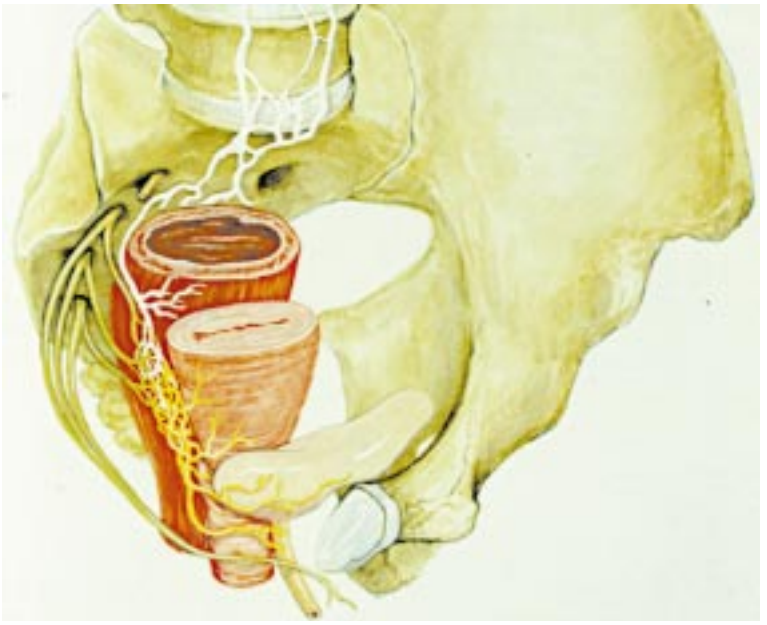
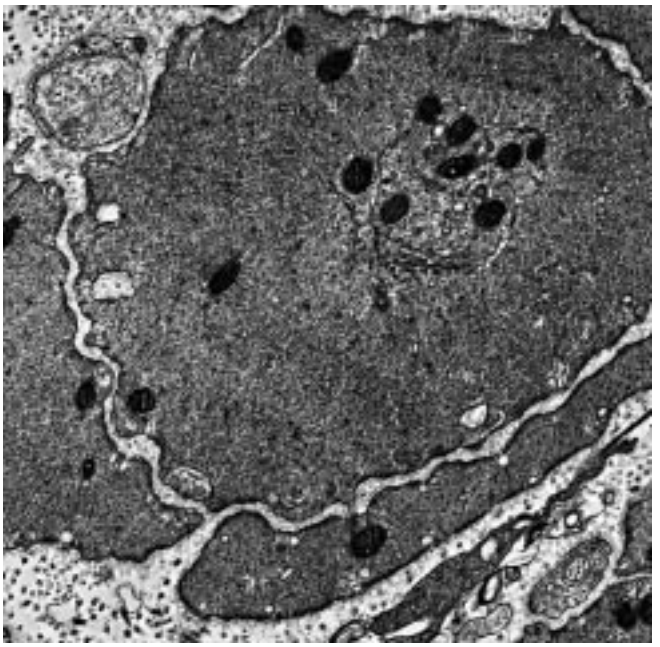
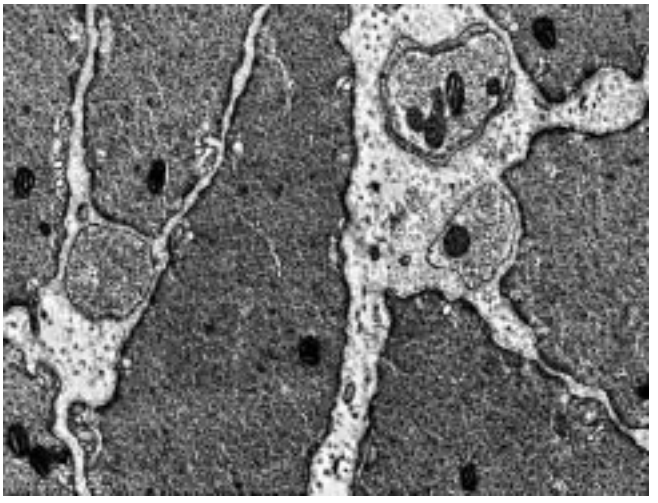


*Figure 9 : A diagram illustrating the autonomic nerves which form the pelvic plexus in the female.*



1$\mu$m

*Figure 10 : Transverse section of a muscle bundle in the bladder of a rat. In the centre a large muscle cell profile shows mitochondria, Golgi apparatus, sarcoplasmic reticulum, caveolae and other surface invaginations; most of the profile is occupied by myofilaments and dense bodies. To the top left is an axon packed with vesicles and only partly covered by a Schwann cell; the exposed axolemma lies very close to the surface of the muscle cell. To the bottom right a small axonal profile, which is the intervaricose portion of a terminal axon, displays only 6 microtubules and a vesicle and is fully wrapped by a Schwann cell. Source: Giorgio Gabella, unpublished.*



1$\mu$m

*Figure 11 : Transmission electron micrograph of a muscle bundle of the rat bladder showing partial profiles of muscle cells and three axons packed with vesicles; two of the axons are near to their end and are devoid of Schwann cell wrapping while lying very close to the surface of 1-3 muscle cells. The third axonal profile is wrapped by a Schwann cell except for a small region (a window) where the axolemma is directly exposed to the extracellular space and faces at close distance the surface of a muscle cells. All three axons are regarded as forming a neuro-muscular junction. Source: Giorgio Gabella, unpublished.*

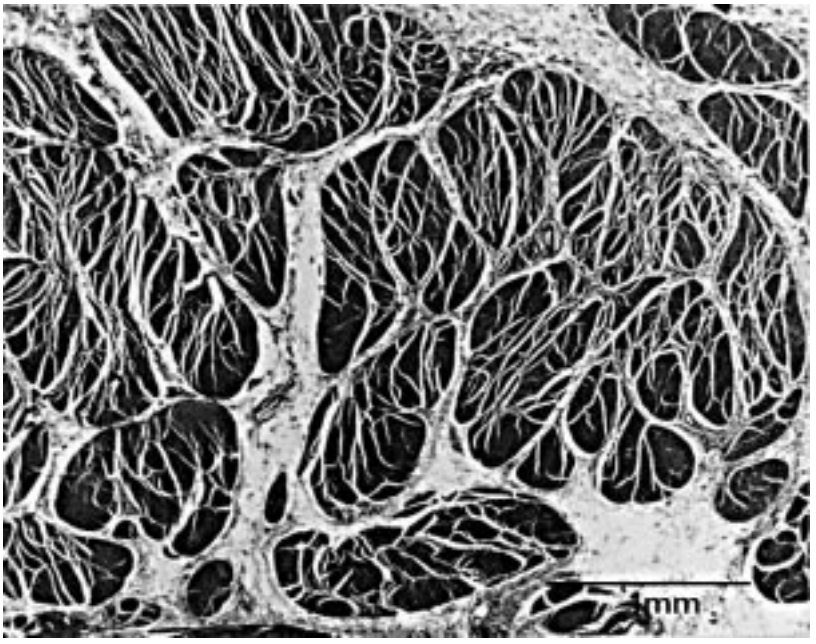



*Figure 12 : Low magnification cross section of muscle bundles from normal human detrusor. Note that the muscle cells are arranged into small bundles or fasicles that are grouped together into larger bundles divided from each other by connective tissue containing collagen.*

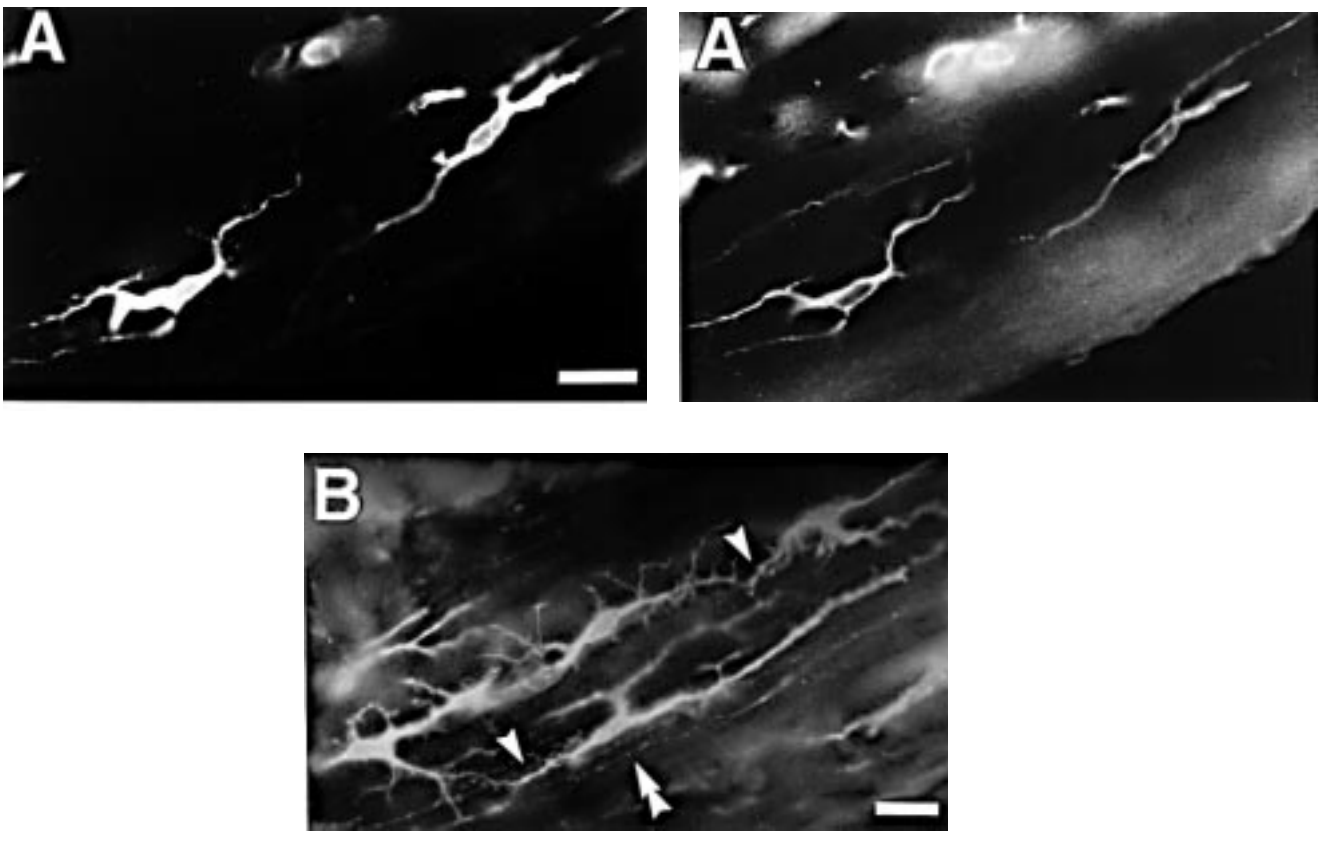



*Figure 13 : Interstitial cells in the guinea-pig bladder. In A and B the cells show immunofluorescence to cGMP after exposure to sodium nitroprusside. In A', the cells shown in A have also been labeled with a fluorescent antibody to vimentin. These interstitial cells have long dendritic processes that run in parallel to the smooth muscle cells. Scale bars 25 µm. From Smet et al., 1996 [3]*

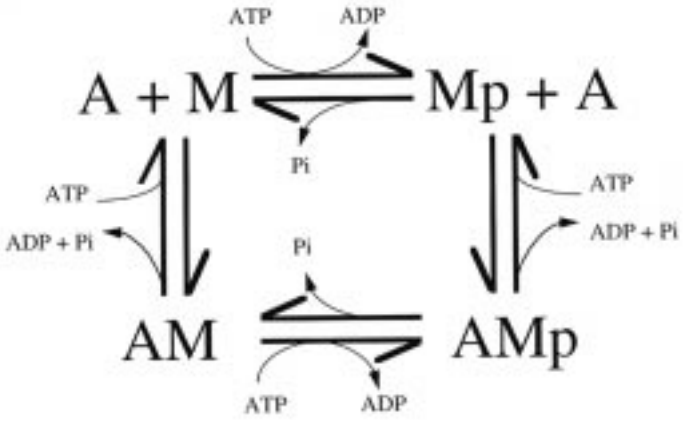



***Figure 14 : Activation of contractile machinery. Normal rapid cross bridge cycling on the right of the figure occurs between phosphorylated myosin heads (Mp) and actin (A), and involves the breakdown of one ATP molecule for each cycle. Cycling is switched on by phosphorylation of the regulatory light chains of the myosin head, catalyzed by the enzyme Myosin Light Chain Kinase (MLCK). Intracellular free calcium binds to calmodulin, which then activates MLCK. Dephosphorylation of the myosin heads (M) is achieved by phosphatases. Dephosphorylation of the attached cross bridges (AM) is thought to alter the kinetics of detachment, resulting in a «latch» bridge shown on the left of the figure, which detaches from the thin filament more slowly than in the phosphorylated state.***

smooth muscle contraction and the differences between this and striated muscle can be found in the following references [6 - 12].

Contractions of detrusor smooth muscle are more phasic in nature than seen in many other smooth muscles: during continuous excitation they show a transient phasic contraction which declines to a much smaller tonic component [13 - 14] that may be important for ensuring bladder emptying, but which tends towards the resting state. Changes in the contractile proteins occur in developing bladders, and during bladder hypertrophy, and several studies have mapped these changes [15 - 20]. Alterations in the intracellular environment may also affect contractility by altering myofibril $Ca^{2+}$- sensitivity [21].

### *c) Muscle mechanics*

The great compliance of the detrusor in normal bladders, and the possibility that changes in compliance occur in various forms of dysfunction, have stimulated considerable research into the mechanical properties of the detrusor. Detrusor smooth muscle exhibits gross muscle mechanical properties common to other smooth muscle. Thus it is possible to construct length-tension and force-velocity curves (figs. 15 & 16), although their interpretation is considerably more problematic than for striated muscles. The force-velocity relationship is variable, for example the maximum shortening velocity declines during the contraction and the velocity of shortening at any load is dependent on the previous contractile history of the muscle. These mechanical phenomena are believed to be dependent on the rate of cross-bridge cycling under these different conditions. An additional problem has been to correlate the contractile behaviour of the whole bladder to that occurring in the individual muscle cells of the detrusor mass. This has been attempted by correlating the in vivo and in vitro properties of the bladder and detrusor strip preparations as well as comparing the mechanical properties of multicellular detrusor preparations and isolated smooth muscle cells [22 - 24].

The detrusor shows some special mechanical features. As might be expected, the length-tension relationship is relatively broad, allowing tension to be developed over a large range of resting muscle lengths [25]. The tissue shows visco-elasticity, and the visco-elastic properties of the detrusor will influence the eventual translation of muscle tension into changes in bladder wall tension. The muscle is also known to exhibit significant stress-relaxation, whereby a rapid stretch of detrusor is followed by a partial visco-elastic relaxation of stress [26 - 27]. The rate of stress-relaxation is a function of the initial rate of stretch. The evaluation of those factors, whether passive or active, which determine the rate and extent of stress relaxation is important, since they will affect the measurements of bladder wall compliance [28 - 30].

### *d) Contractile characteristics of smooth muscle strips in vitro*

Isolated detrusor strips show spontaneous mechanical activity to a variable extent. It is more frequently seen in bladders from small mammals, [31] but can also be seen in strips from human detrusor, particularly if a strip is left unstimulated. Spontaneous contractions normally start from a baseline of zero tension, and are brief phasic contractions of variable height, but much smaller than the maximal force the strip can generate. Spontaneous fused tetanic contractions such as those commonly seen in smooth muscles from the gastro-intestinal tract and uterus, are almost never seen in normal bladders, although they are commonly seen in strips from unstable bladders (fig. 17). Evoked contractions often show two components, a rapid phasic contraction which with continuous activation relaxes into a smaller tonic response, which is also not well maintained.

### *e) Membrane electrical properties*

Early work from detrusor of small mammals has shown that the smooth muscle of the detrusor is able to support a regenerative action potential from a resting potential of -50 to -60 mV [32 - 34]. Similar action potentials can be recorded from strips of human detrusor muscle. The action potentials may have after hyperpolarizations (fig. 18) and several different K channels, such as

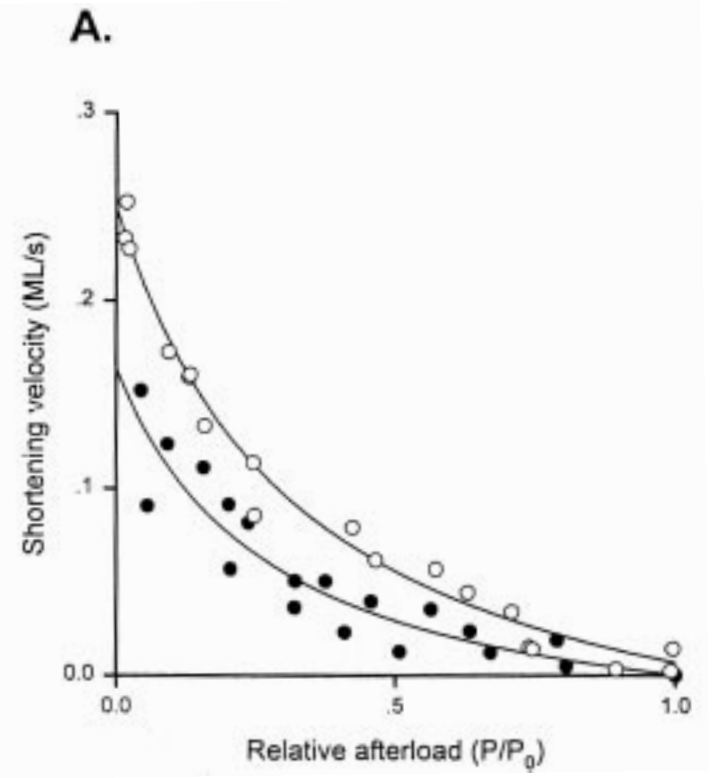



*Figure 15 : Force velocity relationship of smooth muscle strips dissected from normal (open circles) and hypertrophic (filled circles) rat bladders. The shortening velocity, in muscle lengths (ML) per second, is plotted against the relative afterload (P/Po). The lines are fits to the Hill equation (v = b(1-P/Po)/(P/Po + a/Po)) where Po is isometric force and a and b are constants. From Sjuve et al, 1996* [20].

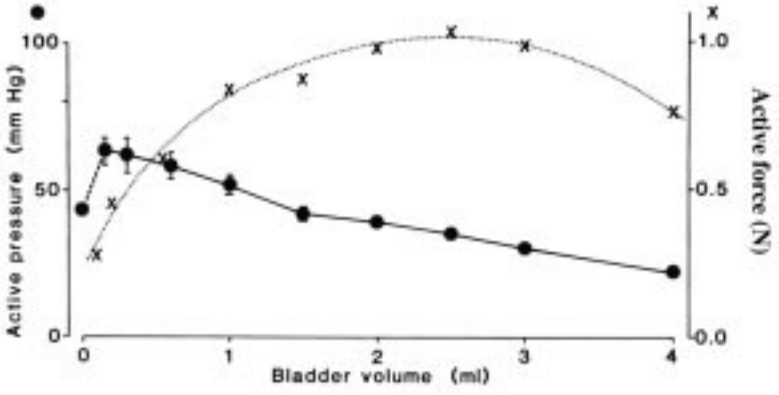



*Figure 16 : Relationship between bladder volume (and hence muscle length) active pressure (in response to supra-maximal stimulation of the pelvic nerves) and active force generated in the longitudinally oriented musculature of the guinea-pig bladder. Note how the active force remains nearly constant and maximal over a wide range of volumes, and hence cell lengths. From Uvelius & Gabella, 1980* [2].

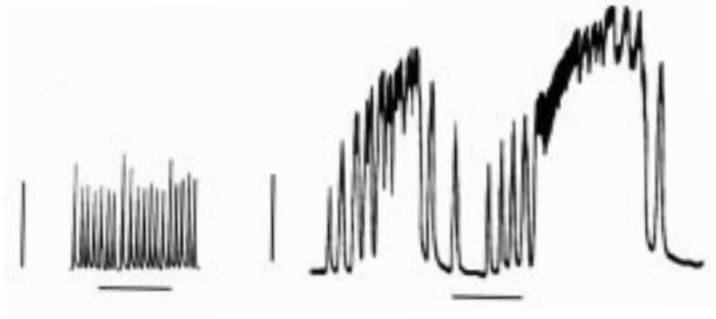


*Figure 17 : Spontaneous mechanical activity recorded from strips of pig detrusor. On the right is typical activity in a strip from a normal bladder. Note the contractions vary in height, but relax to the baseline and show no fused tetanus. On the left is an example of spontaneous activity from a strip dissected from an unstable bladder (instability induced by partial urethral obstruction). Note the spontaneous fused tetanic contractions. Vertical lines represent force (0.5 gms wt), and horizontal lines time (1 min).*

delayed rectifier and transient outward channels and both large and small $Ca^{2+}$-activated $K^+$ channels, appear to be involved in determining their shape [35 - 38]. The upstroke is supported by $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels. In addition a potassium ($K^+$) channel opened by reduced intracellular ATP has been demonstrated, [39 - 40] which on activation is profoundly inhibitory to the spontaneous activity [41]. Several other conductances have been demonstrated in detrusor smooth muscle which include a non-specific cation channel linked to the P2X receptor [42] and stretch-activated cation channels [43]. In human detrusor, electrical properties have been studied in isolated myocytes, and similar properties are seen to those in smaller mammals [44].

### *f) Propagation of electrical responses*

The lack of fused tetanic contractions in normal detrusor smooth muscle strips suggests that the electrical coupling between the cells may be relatively poorly developed, and this is supported by the absence of gap junctions between the cells [45] and by measurements of tissue impedance, [46 - 47] which suggest that detrusor is less well coupled electrically than other smooth muscles. In the guinea-pig detrusor, records of the effect of current injection into one cell on neighbouring cells (within 40 µm) showed only about 25% of the cells to be coupled electrically (fig. 19) [32] [48] Poor coupling could be a feature of normal detrusor to prevent synchronous activation of the smooth muscle cells during bladder filling. Some degree of coupling within a muscle bundle clearly does exist, since it is possible to measure the length constant of a bundle [48] but in the absence of morphologically demonstrable gap junctions there is little evidence as to what allows the coupling to occur. Such propagation requires the presence of either low-resistance gap junctions or mechano-receptive cell-to-cell junctions (the intermediate junctions or stretch activated channels could play a role here). It has been suggested that a change in the properties of the cell coupling may underlie the generation of the unsuppressible detrusor contractions occurring in unstable bladders, although the evidence is at present conflicting [49 - 50].

Some interesting observations have been made on the changing properties of the detrusor as it develops in rats from the new-born to adulthood. In the newborn detrusor spontaneous activity is absent, although low doses of excitatory agonists induce high amplitude phasic contractions that are almost maximal in amplitude. This type of activity then appears spontaneously during early post-natal development and as development continues, the adult pattern of low-amplitude phasic contractions develops. The implication of these findings is that the poor coupling between muscle cells observed in the adult detrusor may be preceded by a phase of good coupling. This phase co-incides with the somatovesical

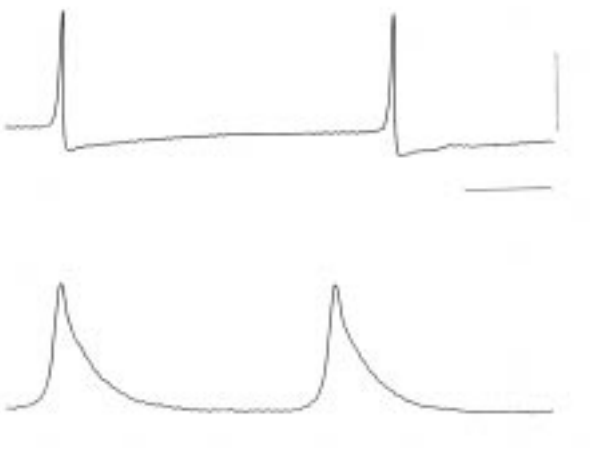


*Figure 18 : Action potentials recorded with a microelectrode from smooth muscle cells of guinea-pig detrusor. The top records are in normal conditions. Note the relatively rapid repolarization and pronounced after-hyperpolarization, both of which involve K channels. The bottom records are after application of 5 mM procaine, which has little effect on the upstroke, since this is caused by current flowing through calcium channels, but markedly prolongs the repolarization and abolished the after hyperpolarization. Procaine blocks K channels non-specifically. Horizontal bar 0.2 sec, vertical bar 50 mV.*

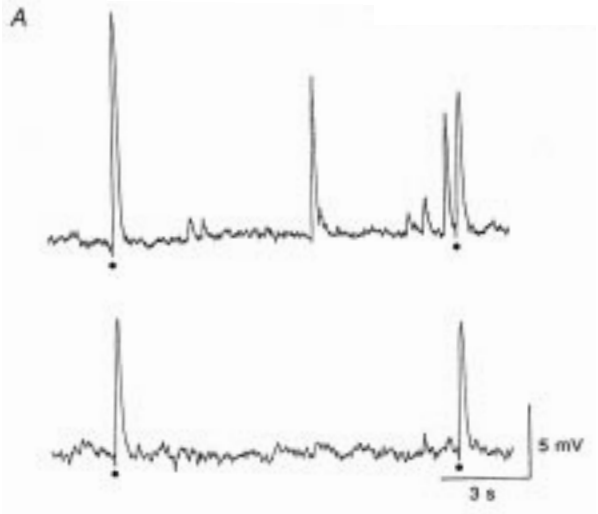



*Figure 19 : A. Excitatory junction potentials (EJPs) recorded with microelectrodes from two cells in guinea-pig bladder separated by a distance of 40 µm in the axial direction. Nifedipine (10 µM) present throughout to block action potentials. Transmural stimulation (at the dots) evoked EJPs simultaneously in both cells, because of the dense innervation. However spontaneous EJPs arising in the upper cell were not recorded in the lower cell, implying poor coupling between them. From Bramich, N. & Brading, AF. J. Physiol (Lond) 492, 185, 1996.*

reflex micturition that is seen in newborn rats, activated by the mother licking the perineal area. At this age the dense excitatory innervation has not fully developed, and good coupling may be necessary to ensure bladder emptying [51 - 54].

### *g) Neuronal control of contractile behaviour*

Normal bladder function requires co-ordinated detrusor relaxation and urethral sphincter contraction during the filling phase of the micturition cycle and the converse during micturition itself. Although to some extent the properties of the smooth muscles themselves help towards this end during the filling phase, control of the smooth muscle activity is achieved through the autonomic nervous system by the effects of excitatory and inhibitory transmitters, integration of which is brought about by the activity in sensory nerves and control centres in the spinal cord, pons, and forebrain [55].

***1) Structural aspects:*** In most species, including humans, ganglia are present in the bladder wall, although the rat detrusor does not contain them. Most of these are presumably ganglia in parasympathetic pathway's and will supply the smooth muscle, although there is good evidence that the ganglia are heterogeneous [56 - 57] and could be involved in local reflexes. Histochemical studies have shown that numerous guinea-pig intramural neurones stain with quinacrine, which labels ATP, providing support for the dual purinergic, cholinergic nature of the innervation (see below) [58]. Immunohistochemical studies [56] have looked at the distribution of peptides in the intramural ganglia of human detrusor. These contain immunoreactivity to vasoactive intestinal peptide, nitric oxide synthase, neuropeptide Y, and galanin, but are heterogeneous with regard to their content of these antigens, with the proportion of immunopositive cells ranging from 58-84%. Occasional neurones with immunoreactivity to the catecholamine-synthesising enzyme, tyrosine hydroxylase, are also observed. No cell somata, however, appear immunoreactive for enkephalin, substance P, calcitonin gene-related peptide or somatostatin.

The only detailed electron-microscopic study of the innervation of the detrusor using serial sectioning has been carried out in the rat [59]. This study has shown a uniform dense innervation with the penetrating nerves branching repeatedly into long varicose fibres largely encased in a Schwann cell sheath. The terminal varicosities become devoid of the Schwann cell sheath and lie close to the muscle cells forming a neuro-muscular junction with a gap normally of 30-50 nm (see fig. 11). The naked varicosities are packed with small agranular vesicles and a lesser number of dense-cored vesicles, although some large electron-lucent vesicles are occasionally seen. The density of the innervation is such that each smooth muscle cell receives at least one junction, and some may receive two. This study shows no evidence of separate purinergic and cholinergic nerves, although functionally purinergic innervation is important in the rat.

***2) Functional studies:*** Detrusor strips demonstrate phasic contractions to selective stimulation of their intrinsic nerves [60] [31] Selective stimulation can be achieved using short pulses (0.05 msec), and the selectivity confirmed by demonstrating that the contractions are blocked by tetrodotoxin. If the pulse width of the stimuli is increased, direct activation of the smooth muscle cells can occur, which is tetrodotoxin resistant. Most mammalian bladder strips contract to single stimuli of their intrinsic nerves. Maximum contractions with tetanic trains of 5 sec train length usually occur at frequencies of about 40 Hz (fig. 20). If trains are prolonged, a biphasic contraction with a rapid transient phase declining to a smaller more prolonged slow phase can be seen in most non-human bladders (fig. 21) [14]. The slow phase is abolished by atropine and enhanced in the presence of cholinesterase inhibitors, suggesting that the excitatory transmitter is acetylcholine, but the rapid phasic response shows considerable atropine resistance, and can be abolished by desensitisation of the P2x-purinoceptors with $\alpha$, $\beta$-methylene ATP, suggesting that ATP is normally a co-transmitter with acetylcholine [34] [61] [62] In contrast, detrusor strips from normal human bladders produce little response to single stimuli and require repetitive activation of the intrinsic nerves to induce a response, and the response can be completely abolished by atropine [31], suggesting that the response is purely cholinergic (fig. 22). Evidence suggests that the detrusor possesses both M2 and M3 cholinoreceptors [63 - 65]. Although M2 receptors predominate in receptor binding studies, it is the M3 receptor that is thought to mediate contraction.

Although purinergic innervation does not appear to be important in the normal human bladder, it seems probable that ATP is released with acetylcholine, and this innervation may become important in unstable bladders. The detrusors of mammalian bladders possess excitatory P2x receptors, [42] [66] inhibitory P2y receptors [67] and possibly other types of purinoceptor (fig. 23) [68 - 70]. Excitatory effects on the detrusor may also be mediated by local release of tachykinins (substance P, neurokinin A) and other peptides from sensory nerves in the bladder wall. These have been shown to produce diverse biological effects, such as smooth muscle contraction, facilitation of neurotransmitter release from nerves, vasodilatation, and increased plasma permeability [71 - 74]. The actions of the tachykinins are mediated by activation of three distinct receptor subtypes termed NK1, NK2, and NK3 [75].

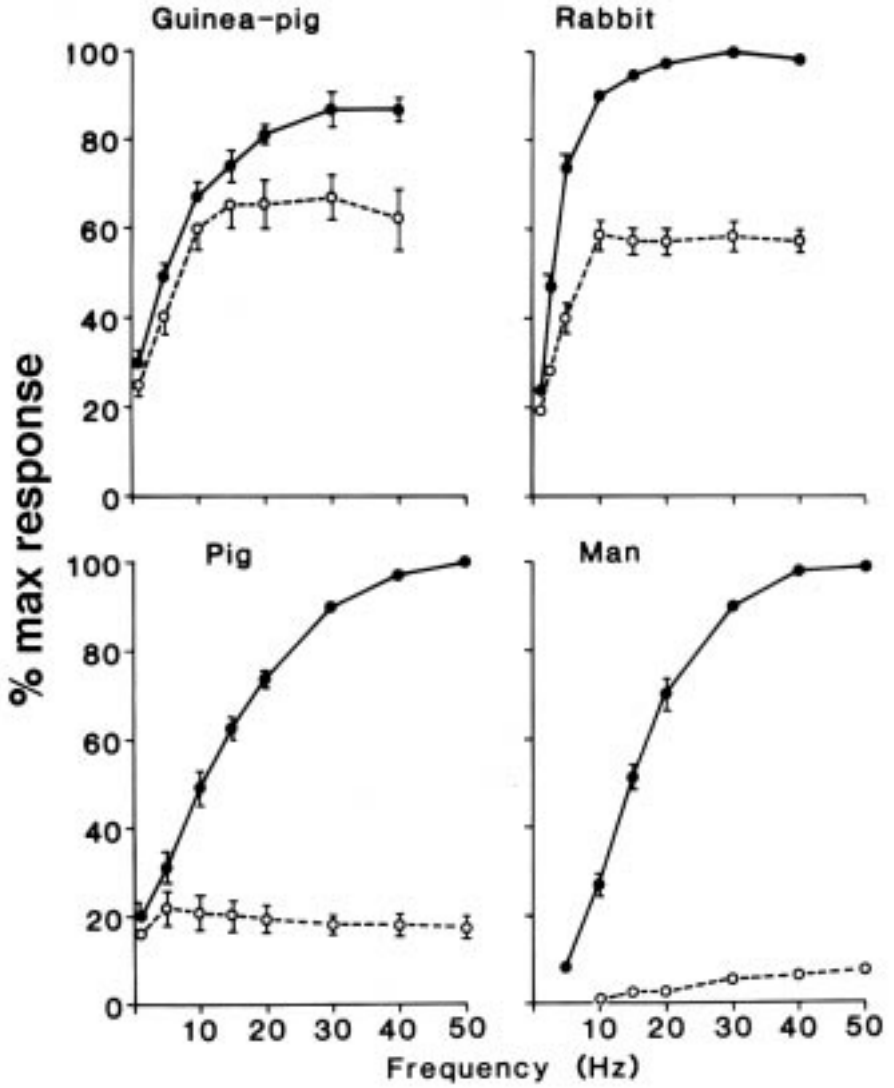



*Figure 20 : Responses of detrusor strips from varies species to electrical field stimulation of the intrinsic nerves, using 5 sec trains at different frequencies. The solid points are controls, and the open circles and dotted lines the response in the presence of muscarinic receptor blockade (atropine 5.10-7 - 10-6M). Note that in the guinea-pig, rabbit and pig, the tissues respond to stimuli at 1 Hz, with atropine resistant contractions. The size of the atropine resistant component at higher frequencies is species dependent, being large in the guinea-pig, and virtually absent in the human.*

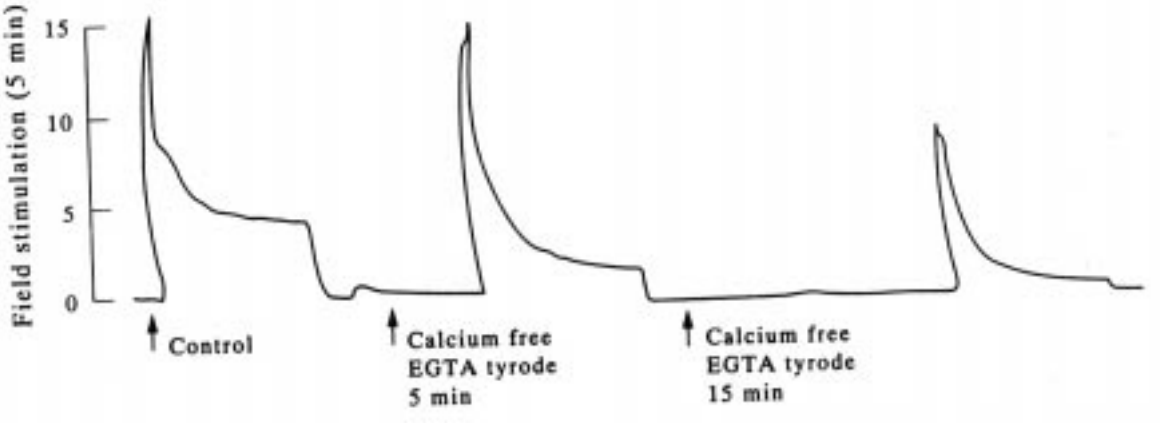



*Figure 21 : Responses of rabbit bladder smooth muscle to 5 min trains of electrical field stimulation (32 Hz, 80 V). Note transient peak followed by decline to a prolonged plateau. The peak component was initially less susceptible to calcium free solution than the plateau. From Zhao et al. 1993* 14

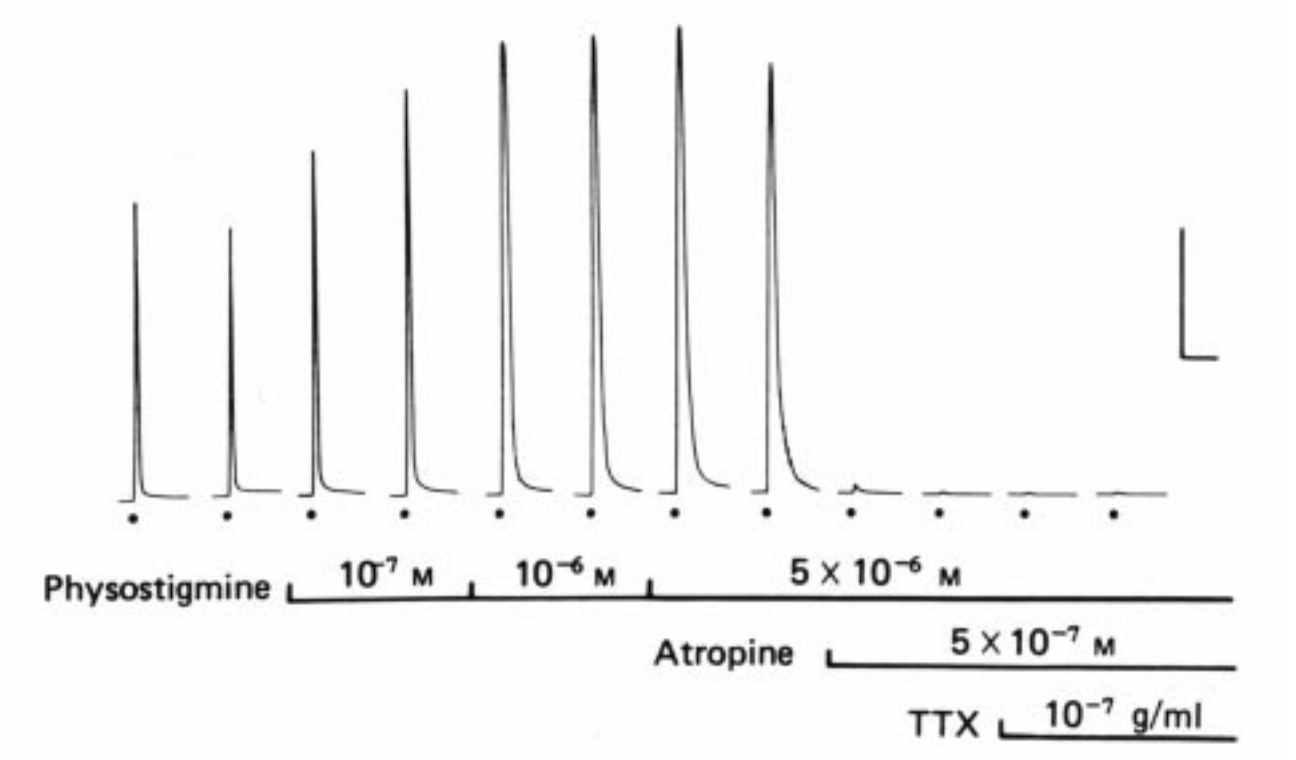



*Figure 22 : Responses of a strip of human detrusor to stimulation of its intrinsic nerves for 5 sec at 20 Hz. Note the size of the response is progressively enhanced as the cholinesterase is inhibited with physostigmine, and that the contractions are virtually abolished by 5.10-7M atropine. Horizontal bar represents 2 min, and vertical bar 2 gm wt.*

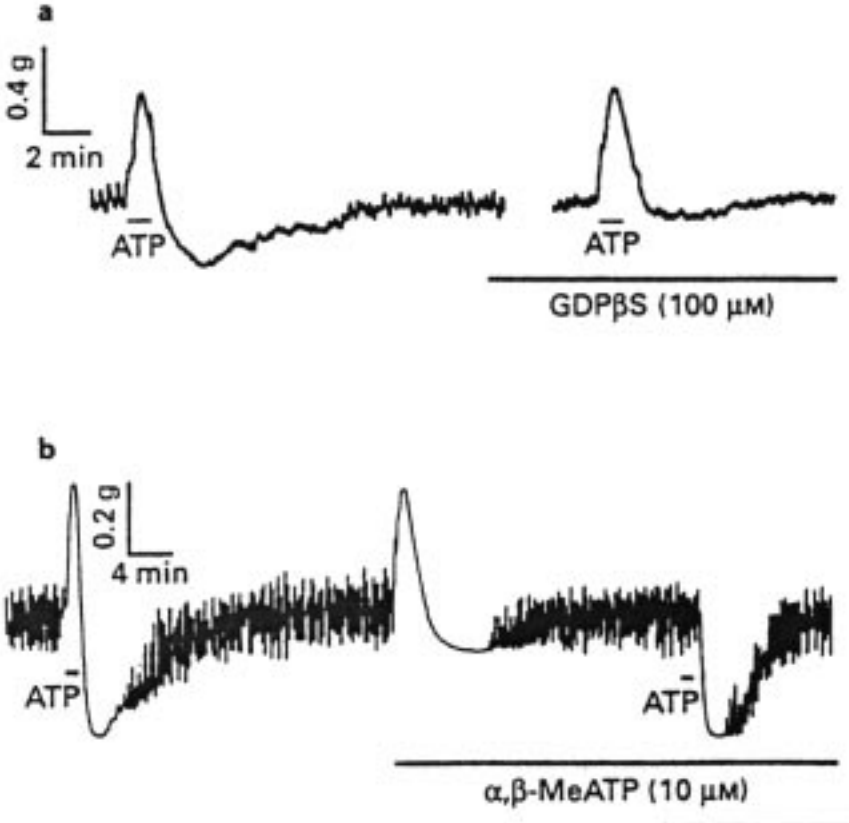



*Figure 23 : Responses of marmoset detrusor strip to ATP (1 mn, 60 sec application).*

*(a) Stimulation with ATP in the absence and presence of the G-protein inactivator, GDPβS which abolishes P2y mediated effects. Note that the relaxant phase is abolished, leaving a pure contractile response.*

*(b) Stimulation with ATP in the absence and presence of P2x receptor desensitization with, α, β-Me ATP. Desensitaization of the P2x receptors abolishes the contractile response, and leaves a pure relaxant response. From McMurray et al. 1998.* 67

There is normally no rise in bladder pressure during the filling phase until the bladder is near its full capacity, hence it has been suggested that activation of inhibitory nerves may be involved in keeping the smooth muscles quiescent. However morphological studies show a dense but uniform acetylcholinesterase positive innervation of the detrusor, and there is little evidence for direct innervation by adrenergic nerves [76 - 77] Electron microscope studies confirm the dense but uniform innervation by nerves that contain predominantly the small clear vesicles that are characteristic of cholinergic nerves (see fig. 11) [59]. The response of the detrusor to catecholamines varies between species, and depends on which area of the detrusor is being investigated. An α-receptor-mediated contraction of the trigone and bladder neck regions is commonly seen (fig. 24) and β-receptor mediated relaxant responses can also be found, and both β2 and β3 may be present (figures 25 & 26) [78 - 79].

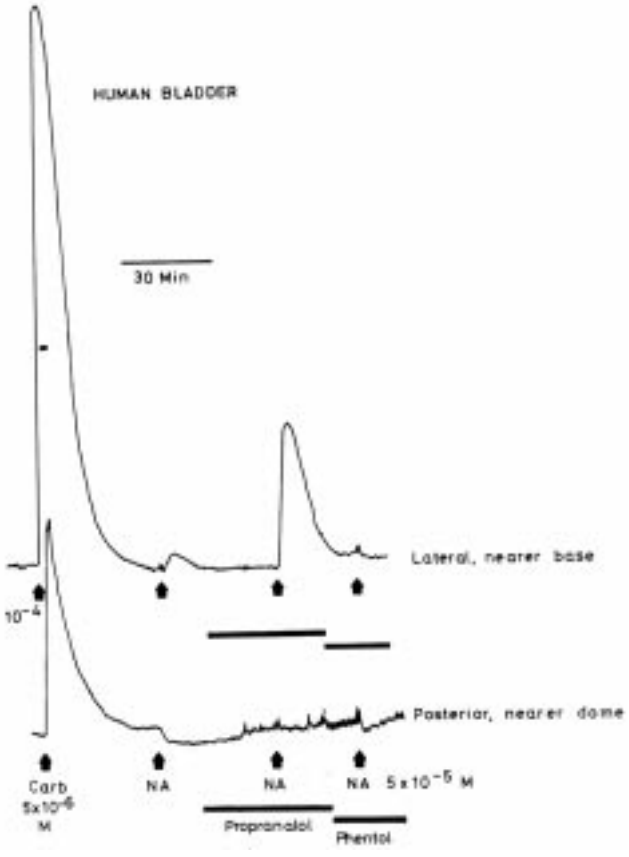



***Figure 24 : Responses of strips of smooth muscle from the human bladder, taken from the base (above) or the dome (below). Both strips respond to carbachol, but the response to noradrenaline varies. The basal strip contracts weakly, but the response is enhanced by blocking β-adrenoceptors with propranalol, and abolished by blocking the α-adrenoceptors with phentolamine. In contrast, the strip from the dome relaxes to noradrenaline , but the response is mediated by β-adrenoceptors since it is abolished by propranalol and unaffected by phentolamine.***

Although the evidence for anything other than a postganglionic parasympathetic input to the detrusor is poor, there is, however, evidence that inhibitory pathways to the bladder do exist, although it seems likely that these terminate at the level of the intramural ganglia. In the rabbit, many sympathetic fibres terminate on vesical parasympathetic ganglia and inhibit neurotransmission via α 2-adrenoceptors [80]. In the human bladder several types of presynaptic input onto intramural ganglia have been demonstrated [55]. It has also been shown that GABA inhibits bladder function in humans and other mammals through specific receptors both in the central nervous system and in the periphery [81 - 83].

In both humans and guinea pigs, varicose nitric oxide synthase-immunoreactive nerve terminals provided a moderate innervation to the detrusor muscle of the bladder body [3]. However, the smooth muscle cells do not react to stimulation with sodium nitroprusside either by relaxing or by expressing cGMP-immunoreactivity (the normal pathway for NO-mediated smooth muscle relaxation), although numerous interstitial cells throughout the bladder body demonstrated an intense induction of cGMP-immunoreactivity by sodium nitroprusside (see fig. 13). The function of these cells, and of the nitrergic nerves in the detrusor is unknown.

### *h) Excitation-contraction and receptor-effector coupling*

The trigger for contraction in all smooth muscles is a rise in intracellular free calcium concentration. Calcium may either enter down its electrochemical gradient from the extracellular medium, or be released from intracellular calcium stores. Excitation-contraction coupling describes the link between changes in the membrane potential and tension development, and receptor-effector coupling the pathways between receptor activation and alterations in contractile activity. The involvement of calcium entry through the membrane and store release vary markedly in different smooth muscles. The patterns in bladder and urethra are clearly different, although currently the details in both tissues are poorly understood.

The detrusor produces calcium-based action potentials, and it seems likely that the spontaneous contractile activity of strips is triggered by these. However, the frequency of spontaneous action potentials recorded with microelectrodes in single cells within a strip are usually higher than the frequency of the phasic contractions recorded from strips [84 - 85] This, and the lack of fused tetanic contractions which normally are seen in other smooth muscles with high frequency action potentials, suggests that the action potentials may not be well syn-

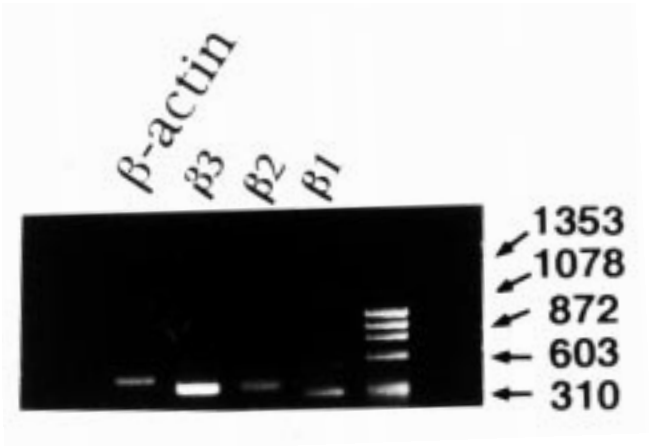



*Figure 25 : Demonstration of the presence of β1, β2 and β3-adrenoceptors in human detrusor. Total RNA was extracted from the detrusor, and amplification of the mRNA for β1, β2 and β3-adrenoceptors was carried out using the reverse transcription / polymerase chain reaction (RT/PCR). The figure shows the results of agarose gel electrophoresis and size fractionation of the products. From right to left, the first lane is a negative control (PCR product using primers specific for β3-adrenoceptors without RT), the next lane shows the presence of β-actin, a smooth muscle marker, the next three lanes show the presence of the three β-adrenoceptors and the last lane is a size marker.*

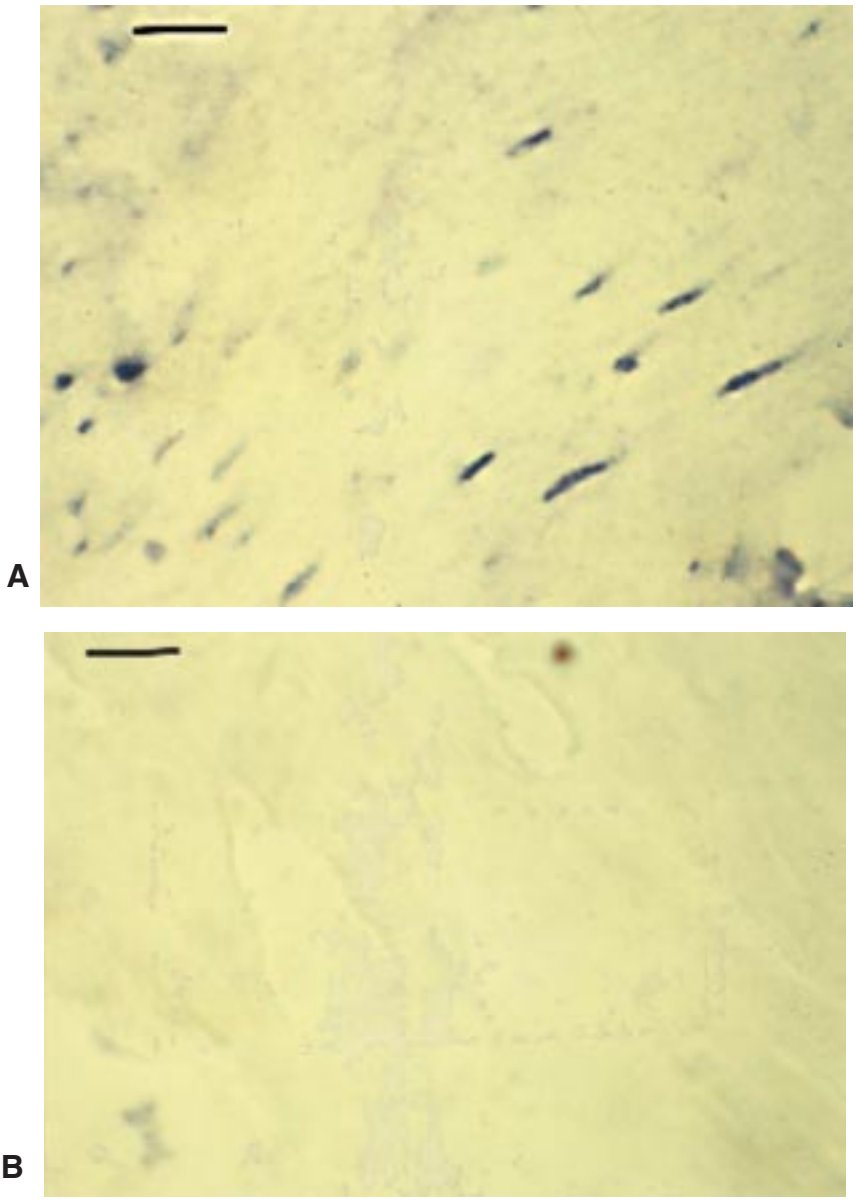



*Figure 26 : In situ hybndization of β3-adrenoceptors from human detrusor. (a) Blue stain indicates positive hybridization of a digoxtgenin-11-d-UTP labelled antisense oligonucleotide probe, suggesting the expression of β3-adrenoceptor mRNA. (b) Negative control, using a digoxtgenin-11-d-UTP labelled sense oligonucleotide probe. Bars denote 400 μM.*

chronized in detrusor strips. The spontaneous phasic contractions are usually much smaller than the maximal contraction that can be evoked by nerve stimulation, and thus it appears that $Ca^{2+}$ entering in the spikes does not elevate the free calcium levels sufficiently to cause much activation of the contractile machinery. In other smooth muscles, calcium entering in the spikes has been shown to activate release of calcium from internal stores by a calcium-activated calcium release channel. Evidence that such channels exist in the detrusor has been provided from studies on the effects of caffeine, a drug known to sensitise these channels and cause $Ca^{2+}$ release from stores (fig. 27) [86 - 87] However the response to caffeine is transient and much smaller than seen in other smooth muscles. The sarcoplasmic reticulum also acts as a powerful $Ca^{2+}$ accumulating organelle to terminate the contraction, via active transport using a magnesium ($Mg^{2+}$)-dependent ATPase [88].

The role of excitation contraction coupling in the response of the detrusor to transmitters is less clear. In detrusor muscle from non-human mammals, intrinsic nerve stimulation generates excitatory junction potentials and triggers synchronous action potentials in detrusor smooth muscle [46] [48]. The junction potentials are mediated by activation of P2x purinoceptors, which open non-selective cation channels, depolarising the membrane, and activating L-type $Ca^{2+}$ channels. The contractile response can be abolished by L-type Ca channel blockers, implicating $Ca^{2+}$ entry and possibly $Ca^{2+}$-induced $Ca^{2+}$ release from internal stores in the contractile response. This mechanism does not seem to be apparent in normal human detrusor, but is sometimes seen in unstable detrusor [89 - 90].

It is unclear what the role of electrophysiological changes are in the contractile response to acetylcholine. Activation of muscarinic receptors produces little change in the membrane potential, [84 - 85] but spike frequency is increased at concentrations able to elicit large contractions. Whole cell patch clamp studies show that acetylcholine causes an outward $K^+$ current, presumably mediated through calcium activated $K^+$ channels. M3 receptors are thought to act through increased polyphosphoinositide hydrolysis, inositol triphosphate (IP3) production (fig. 28) and release of intracellular calcium stores [63] [91] [92]. The rise in intracellular $Ca^{2+}$ will initiate contraction and may open $Ca^{2+}$-activated $K^+$ channels. This pathway also generates diacylglycerol in the membrane, which can activate protein kinase C which may be involved in generating the tonic element of the response through modulation of $Ca^{2+}$ and $K^+$ channels (fig. 29) [13 - 14]. M2 receptors mediate inhibition of adenylate cyclase, with the reduction of cAMP levels. Since elevated cAMP levels mediate smooth muscle relaxation, the role of the M2 receptors could be to render the smooth muscle more easily excited [93]

With reference to the release of calcium through activation of IP3 receptors, it is interesting that the response to muscarinic receptor activation can be blocked by L-type calcium channel blockers [94]. It is currently believed that $Ca^{2+}$ influx through $Ca^{2+}$-channels aids in filling intracellular stores and that open $K^+$-channels keep the membrane potential sufficiently negative to prevent too large a $Ca^{2+}$ influx through this route [39] [95].

### *i) The role of the smooth muscle in the bladder wall*

The role of the detrusor smooth muscle is to maintain the integrity of the bladder without generating significant intravesical pressure during filling, and to contract synchronously during micturition to elevate the intravesical pressure sufficiently and for long enough to empty the bladder. There is also smooth muscle in the trigone. This has different properties and innervation to the detrusor, [5] and may play a role in preventing reflux up the ureters during micturition. Changes in the properties of the detrusor could clearly result in major alterations in the behaviour of the bladder, and might result in significant dysfunction. Indeed such changes have been clearly demonstrated in patients and animals with unstable bladders, and there is currently argument as to whether such changes are a cause of unstable contractions or the result of other factors which may underlie instability. Those interested in pursuing this are referred to the proceedings of the consensus conference on the overactive bladder [95] [96].

## 2. Stroma

The bladder stroma could be defined as the bladder wall minus the smooth muscle and the urothelium. The main constituents are collagen and elastin in a matrix composed of proteoglycans. The main cells are fibroblasts. The passive mechanical properties of the bladder wall depends on the viscoelastic properties of the stroma and of the relaxed detrusor muscle (e.g. [97] [98]). Collagen and elastin are generally thought to be intimately related to bladder compliance.

### *a) Bladder wall collagen*

***Microanatomical organisation:*** The amount of collagen in the bladder wall is considerable; more than a third of the bladder dry weight is collagen [99] [100] A detailed morphological analysis of the collagen distribution in the human bladder [101] found differences in the collagen arrangement in the mucosal, muscular and serosal layers. The mucosal layer could be divided into 3 portions (fig. 30). Just under the urothelium there was a superficial portion interwoven densely by thin collagen fibrils running in all directions, forming a felt-like structure. Deeper to this there was a thicker intermedia-

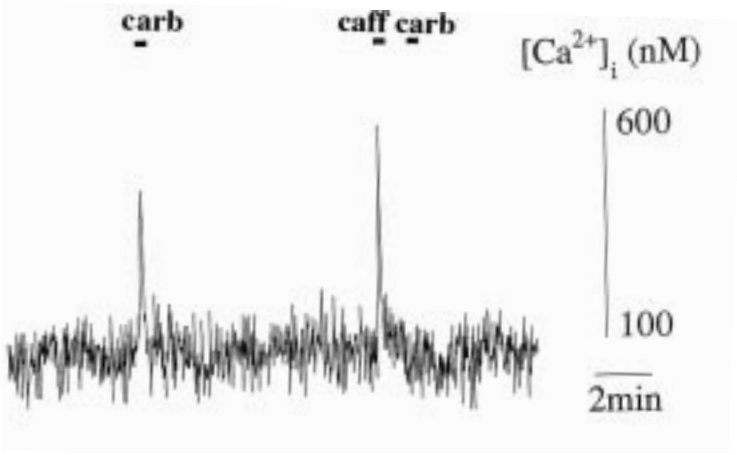



*Figure 27 : Intracellular calcium transients in a smooth muscle cell from guinea-pig detrusor. Carbachol (10-4 M) causes a transient elevation of intracellular calcium as does caffeine (10 mM). Caffeine appears to empty the intracellular stores completely, since carbachol applied immediately after caffeine no longer elicits a calcium transient.*

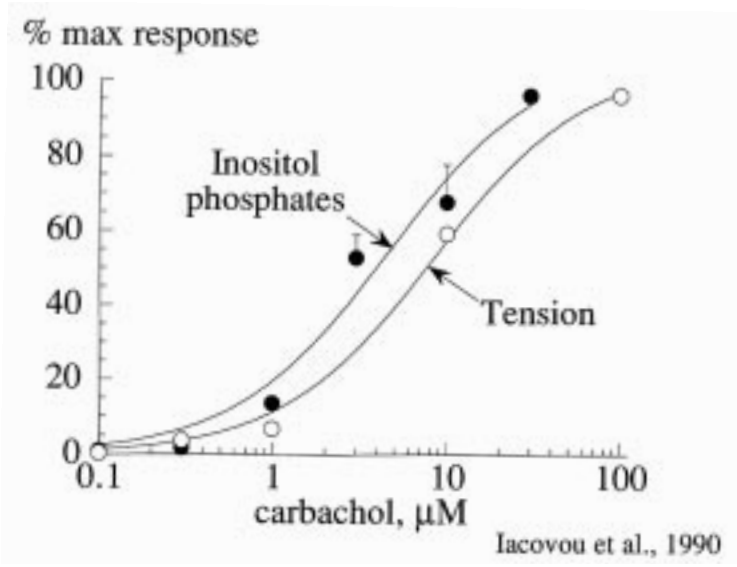



*Figure 28 : The effects of carbachol on intracellular inositol phosphates and tension in guinea-pig detrusor. Adapted from Iacovou et al., 1990* [91]

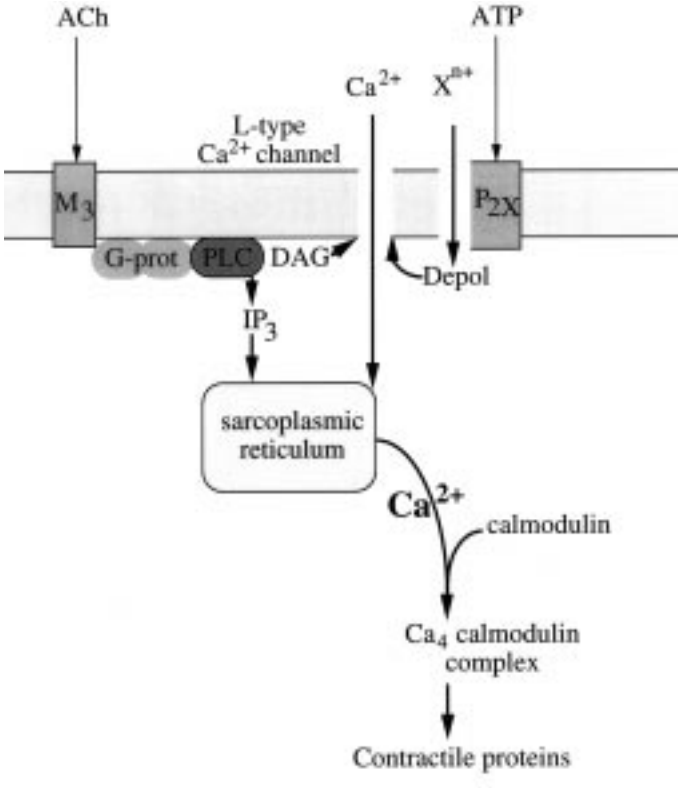



*Figure 29 : Diagram of pathways involved in the response of detrusor to acetylcholine (ACh) and ATP. ACh interacts with $M_3$-muscarinic receptors and activates phospholipase C (PLC) through a G-protein, leading to the production of inositol tris phosphate ($IP_3$) and diacylglycerol (DAG). IP3 elicits release of calcium from the sarcoplasmic reticulum through $IP_3$-receptors, and DAG may modulate voltage sensitive calcium channels in the plasma membrane. ATP acting through $P_{2x}$ purinoceptors opens non-selective cation channels in the membrane, leading to depolarization which opens voltage sensitive calcium channels. Both lead to entry of calcium. This triggers release of further calcium from the stores through ryanodine receptors. The rise in intracellular free calcium concentration triggers contraction, and may also open various calcium-activated channels in the membrane (such as calcium activated K channels), which can modulate the response.*

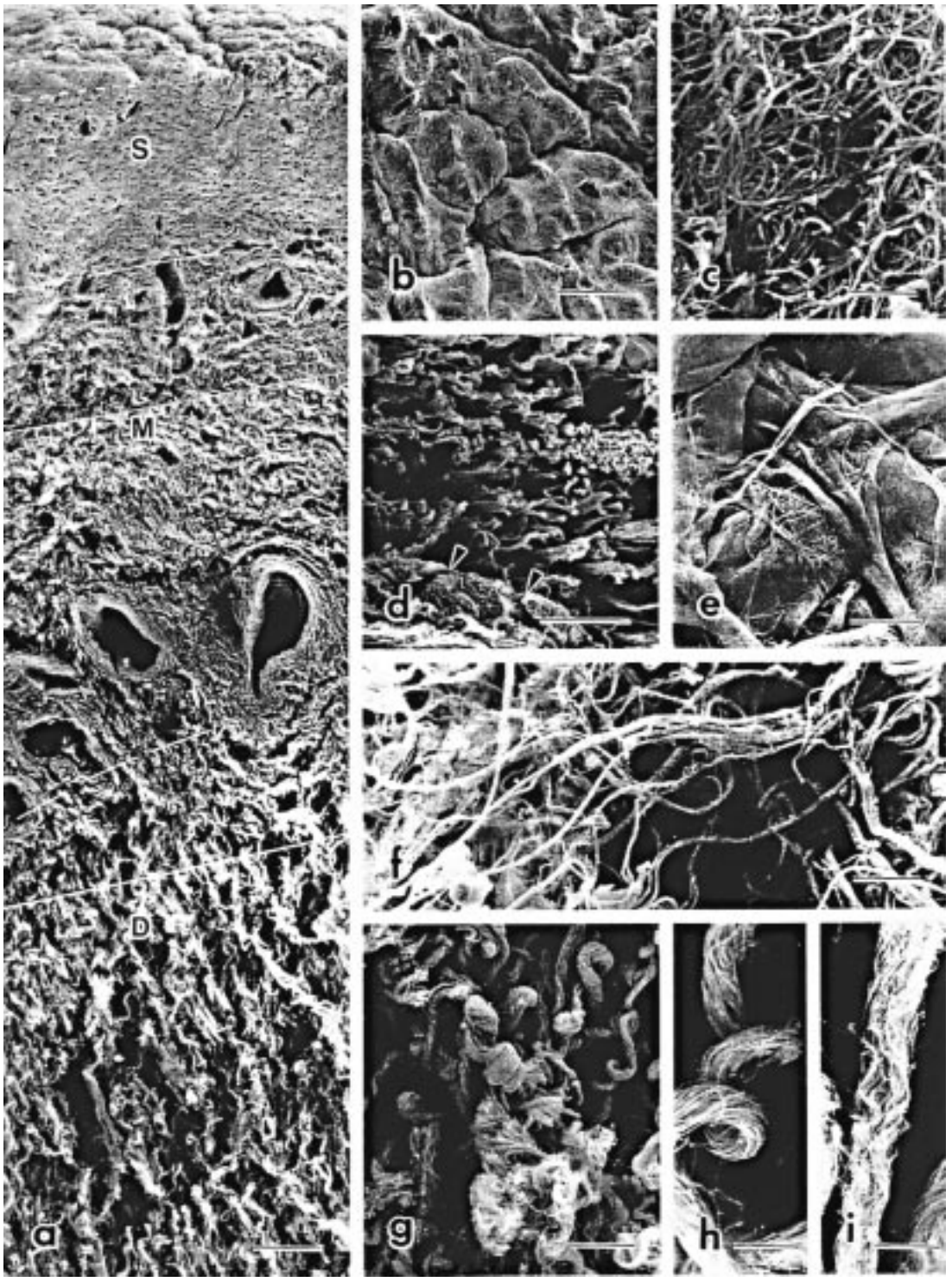



*Figure 30 : Collagen and elastin components of the mucosal layer of the urinary bladder of a 54-year-old male.(a) Low power view of the cut face of the mucosal layer. Bar 100 µM. The dotted line shows the innermost surface. Superficial (S), middle (M) and deep (D) portions are distinguishable according to the arrangement and density of the collagen fibrils. Numerous small holes for capillaries and nerves are seen in the superficial portion, and canals for larger vessels in the middle and deep portions. (b) and (c) lower and higher magnification of collagen fibres forming the innermost surface of the superficial portion just under the epithelium. Bars 20 µM and 2 µM. (d) stacks of collagen bundles in the middle portion running between the smooth muscle cells, the sheaths of which are indicated by arrowheads. Bar 50 µM. (e) flat tape-like collagen bundles arranged in two dimensions ruin parallel to the mucosal coat. Bar 5 µM. (f) loose network of elastin fibres in the middle portion. Bar 20 µM. (g) and (h) lower and higher magnification of loose network of curled collagen bundles in the deep portion from unstretched bladder. Bars 20 µM and 5 µM. (i) straight collagen bundle from deep part of stretched bladder. Bar 5 µM. From Murakumo et al., 1995* [101]

te portion with parallel bundles of thick collagen fibrils. A discontinuous muscularis mucosa separated this layer from the deep portion of the mucosa. This portion was the thickest, and contained a loose network of thick twisted strands of thin collagen bundles. These strands were strongly curled in the empty bladder, but straightened as the bladder was filled. The muscular layer consists mainly of muscle bundles, running in various directions. A rich amount of collagen fibres is found between the bundles. The collagen fibres are arranged in thick bundles often running transversely to the bundle they surround. Within the muscle bundles winding collagen fibrils form thin lace-like sheaths surrounding the individual smooth muscle cells. Outside the muscular layer there is a loose sub-serosal network of collagen bundles.

No such detailed morphological analysis exists for bladders from other species, but in the rat most of the collagen fibrils are found in the mucosa and between the muscle bundles [100]. Fibroblasts, which are common outside the muscle bundles are rarely seen within.

***Relative amount of interstitial tissue:*** Most of the bladder wall collagen is found in the connective tissue outside the muscle bundles. Changes in relation between the amount of muscle and non-muscle tissue in the bladder wall would therefore influence collagen concentration. In the rat infravesical obstruction or bladder denervation induce hypertrophy of the detrusor smooth muscle and the collagen concentration decreases [100] [102].

In man, the proportion of connective tissue relative to muscle in the bladder is lower in childhood than in the fetus [103]. Ageing is associated with a relative decrease in smooth muscle in both males and females [104]. Despite this, the collagen concentration in the adult male is not influenced by age [99]. This could perhaps be related to the decreased packing density of submucosal collagen during ageing [105].

***Collagen subtypes:*** Today a considerable number of collagen types are known. In the bladder types I, III and IV are the most important ones (see e.g. [106]). Types I and III form fibrils with a typical banding. There seems to be a correlation between the ratio of type III-to-type I collagen content and bladder compliance. In the foetal bovine bladder the increase in compliance during the second and third trimester is paralleled by a decreased ratio of type III:I collagen [107]. The adult bovine bladder has, on the other hand, a decreased compliance and an increased type III:I ratio. Also, the type III:I ratio is increased in low compliant dysfunctional human bladders [108] compared with age-matched controls. The increased amount of collagen type III is mainly between the cells in the smooth muscle bundles [109].

### *b) Bladder wall elastin*

Elastic fibres are amorphous structures composed of elastin and a microfibrillar component located mainly around the periphery of the amorphous component [110]. In the mature fibre, the amorphous component composes about 90%. The microfibrils contain a number of proteins; possibly the most important ones are the glycoproteins fibrillin and microfibril associated glycoproteins (MAGP).

Elastin fibres are sparse in the bladder, compared to collagen [101] but are found in all layers (see fig. 30). Anti-elastin immunohistochemistry [110] has shown that the elastin in the mucosa-submucosa appears to be arranged in fibres parallel to the urothelial lining. Elastin was also found between and in the smooth muscle bundles, although the organisation was less regular.

The elastin fibres can also be stained by antibodies against fibrillin and MAGP. These however, also cause an intense staining of the urothelium, suggesting the presence of microfibrillar protein in the absence of amorphous elastin. Most of these microfibrillar proteins seem to be localised together with collagen type IV in the basement membrane, [111] but the staining pattern also suggests that there is some fibrillin between the urothelial cells.

Elastin can be demonstrated histologically in increasing amounts from 20 weeks gestation in the human bladder [112]. The amount of elastin seems to have increased in the obstructed foetal bladder showing that the turnover of elastin can be influenced by pathophysiological conditions.

### *c) Bladder proteoglycans*

The non-fibrillar matrix in the stroma is largely composed of a gel of proteoglycans and water. Proteoglycans are glycoproteins with glycosaminoglycans covalently attached. The arrangement of the proteoglycans in the matrix creates a compartment of tissue water which has a viscous behaviour when subjected to deformation. The resistance to tissue deformation is further increased because the proteoglycans in the bladder wall are compressed as compared to their state in solution. The non-fibrillar matrix has not attracted the same interest in recent research as the fibrils embedded in the matrix, and consequently, the number of reports are limited.

The different proteoglycans do not have similar distribution in the bladder wall. Heparin sulphate is localised to the basement membrane under the urothelium [113] The mucosa contains the highest amount of chondroitin sulphate proteoglycan (CSPG) in the bladder, [114] while the submucosa is the richest site of decorin and hyaluronan, a glycosaminoglycan. Hyaluronan is most concentrated along the transition between the mucosa

and the submucosa. The uneven distribution of the proteoglycans would be expected to give different biomechanical properties to the different layers of the stroma; e.g. decorin, which is less hydrated than CSPG, would decrease submucosal compliance. Hyaluronan, which creates a more hydrated matrix than the other molecules, could create a loose matrix for movement of the mucosa over the submucosa during changes of bladder filling [114]

Pathophysiological processes can influence the proteoglycan distribution. In trabeculated bladders the high hyaluronan concentration has moved from the transition between mucosa and submucosa to the deep submucosa [114]. Apparently, the influence of possible changes in the non-fibrillar matrix has to be taken into account when explaining changes in bladder compliance secondary to neurogenic lesions or obstruction.

### *d) The role of the stroma*

In the past there has been an intense interest in bladder and urethral muscle. The stroma has, however, more or less been considered as a passive low metabolic tissue filling out the space between muscle bundles, vessels and nerves. In recent years the important role of the stroma in the adaptation of the bladder to pathophysiological conditions has been more appreciated. Collagen and elastin have been found to modify and better adapt to e.g. increased functional demands, but changes can also lead to decreased compliance. The cells in the stroma include myofibroblasts which under certain conditions can differentiate into new smooth muscle cells. In arteries, disruption of elastin in the stroma [115] can stimulate proliferation of smooth muscle. Although no such mechanisms are yet known in the bladder, it is possible that there could be a more intimate relation between changes in the composition of the stroma and muscle function and growth than is appreciated at present.

## 3. Blood vessels

### *a) Structure and properties*

The bladder vasculature is a multi-layer vascular plexus with morphological as well as functional implications. The vascular anatomy of the urinary bladder is not fully known, however the presence of an extramural as well as intramural and submucosal plexus has been shown [116]. The muscularis and submucosa contain relatively few vessels, and the arteries and veins run predominantly in the connective tissue between the muscle bundles, with mainly capillaries penetrating between the smooth muscle fascicles (fig. 31). In thick sections of the bladder the tortuous course of the blood vessels is clearly apparent (fig. 32). In contrast, the underside of the epithelium is scored by numerous grooves which are occupied by a dense network of blood capillaries. These vascular grooves allow a large number of capillaries to run at a distance of only a few microns from the epithelium (fig. 33) [117] These capillaries are partially fenestrated, with the fenestrated areas being predominantly located on the aspect of the capillary facing the urothelial basal lamina (fig.34). Fenestrated capillaries are a regular feature of many organs and tissues; they have been shown to be more permeable to both water and solutes than the more usual non-fenestrated capillaries in terms of both uptake (e.g. endocrine glands and the intestinal epithelium) and leakage (e.g. renal glomerulus and spinal and autonomic ganglia), and fenestrations are also transiently expressed in some developing tissues (e.g. dental pulp), and during regeneration following damage (e.g. peripheral nerve and striated muscle). The presence of fenestrae in the endothelial cells of the sub-urothelial capillary plexus may indicate that it has a role in the uptake of substances with an endocrine function, e.g. ATP, secreted by the urothelium, to be transported to more remote sites of action, e.g. the detrusor, autonomic ganglia and the CNS.

Because of the large increase in surface area of the bladder wall during filling, the blood vessels will need to be able to lengthen considerably, and to maintain a good blood flow, mechanisms may have to be present to ensure that the overall resistance of the vessels as they lengthen does not increase sufficiently to reduce the effective perfusion of the tissue. Several techniques have been used in order to study blood flow in the intact urinary bladder. However, almost no studies have been carried out on conscious subjects and most studies have been performed in various animal models [118]. Radioactive microspheres and radio-labelled tracer washout have been used to study the blood flow in rabbit, pig and dog bladders [119] [120] Recently laser Doppler probes placed in the bladder wall have been used to evaluate blood flow in the human bladder [121] [122] and pig bladder [123] and in vivo models for the study of the rat urinary bladder micro-circulation have also been developed to allow the evaluation of the microcirculatory response to vasoactive agents [124].

The effect of bladder filling on the blood flow has been investigated by several groups, with variable results. The majority of reports have shown that the blood flow is reduced by the distension during bladder filling [122], [118] In patients with a low compliant bladder there is a marked increase in the intravesical pressure and a more pronounced decrease in bladder blood flow as compared with normal controls [121]. The principal determinant of blood flow in the bladder wall seems to be the pressure within its lumen (see figs.35, 36). During normal filling the blood flow is able to adapt to the large increase in surface area until the pressure

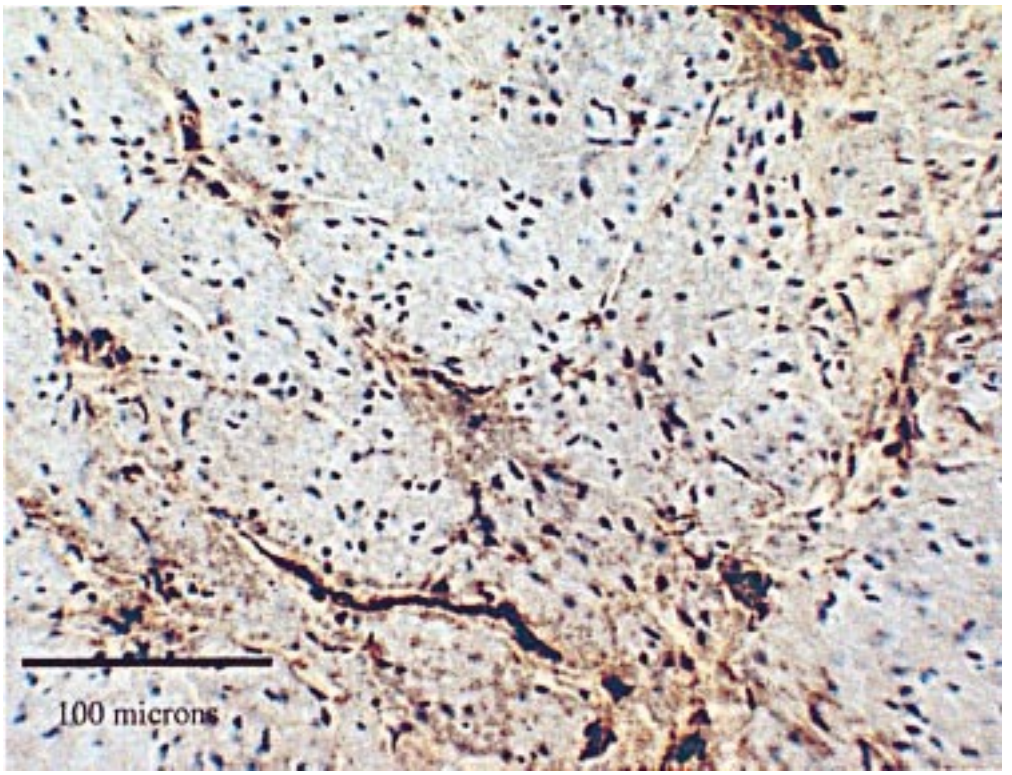



*Figure 31 : Section of pig detrusor through smooth muscle bundles, stained to reveal endothelial cells. Note that capillaries run between the smooth muscle bundles, but do not penetrate into them.*

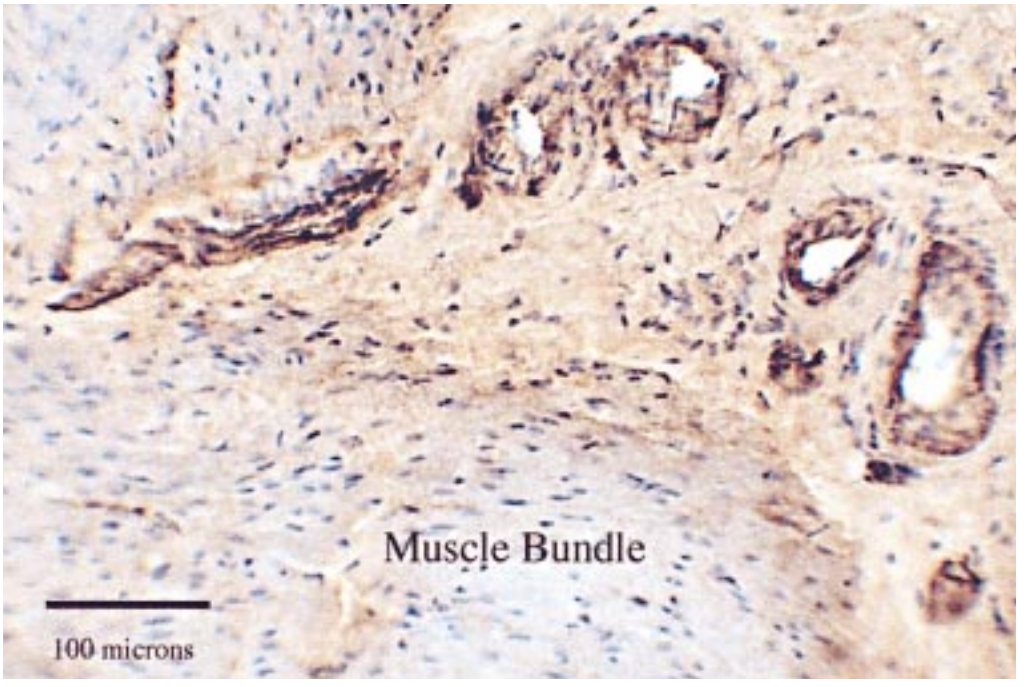



*Figure 32 : Tortuous course of small artery in the pig bladder wall.*

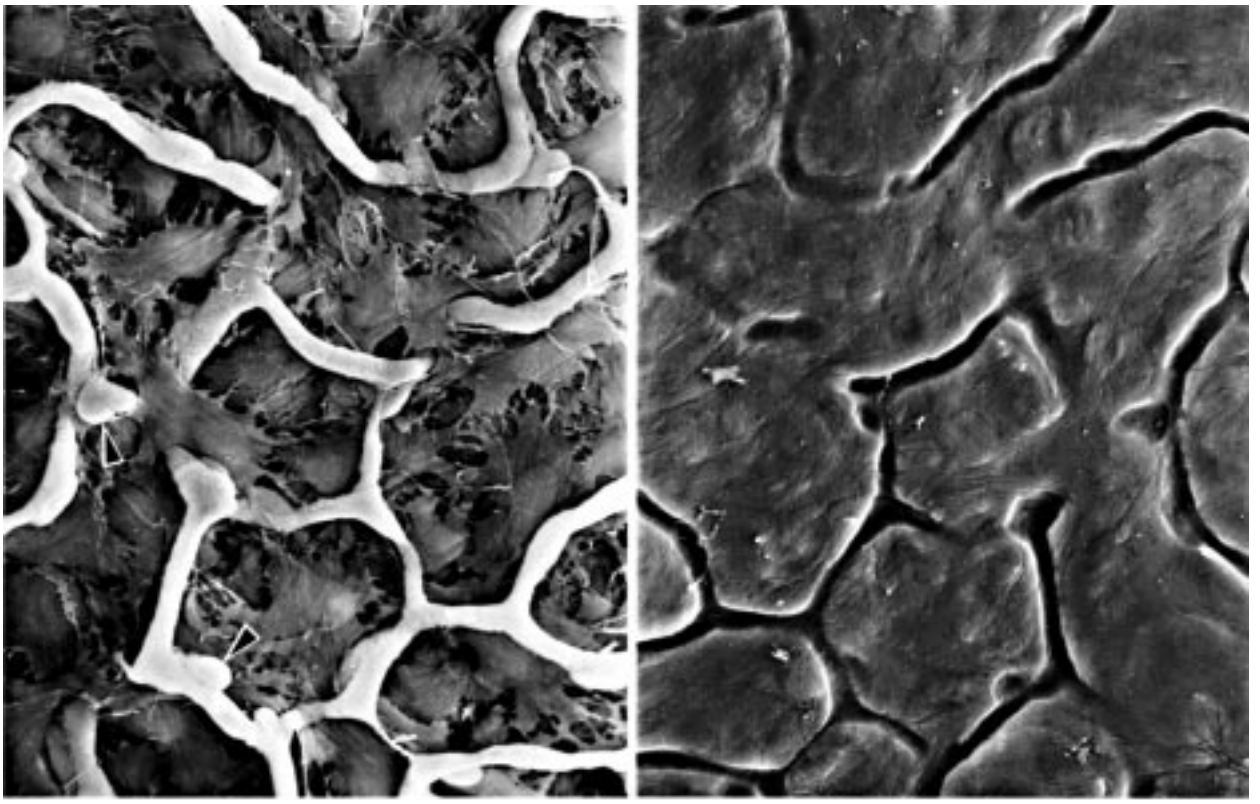



*Figure 33 : Scanning electron micrographs of a rat bladder mucosa; two complementary pairs show the interface between the lamina propria (left side) and the underside of the epithelium (right side). The capillaries form a network lying on a sheet of mucosal fibroblasts, and they correspond to the grooves seen on the underside of the epithelium. In the bottom pair of micrographs an axons with varicosities is visible. The arrowheads point to pericytes lying on the interstitial surface of the capillaries. (from Inoue and Gabella, 1992* **117**.

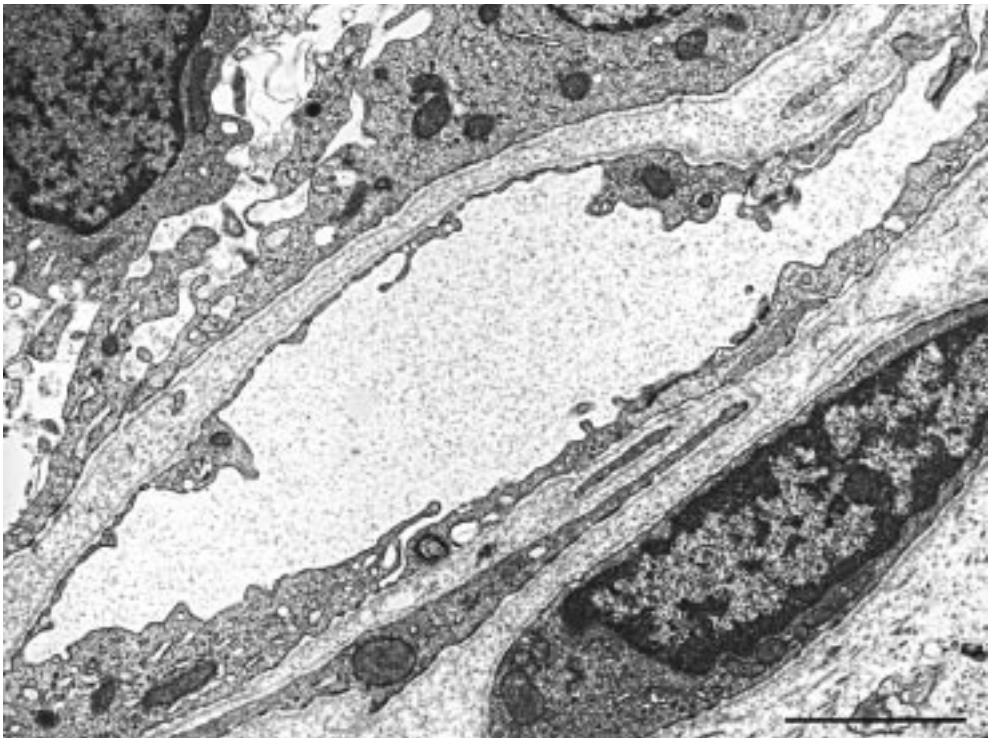


*Figure 34 : An example of an element of the suburothelial capillary plexus showing the fenestrated endothelial wall facing the deep surface of the urothelium. Scale = 2 μm.*

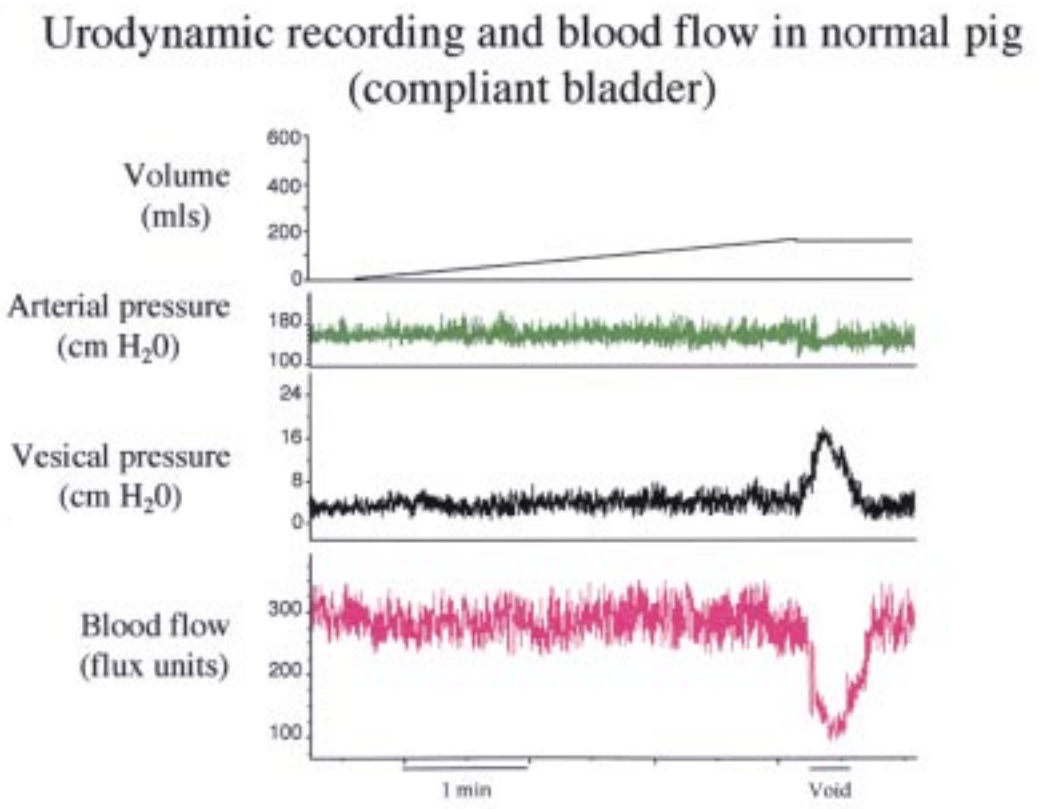



*Figure 35 : Cystometrogram, blood pressure and blood flow in the bladder wall during the micturition cycle in a female pig with a normally compliant bladder. Note little pressure rise in detrusor during filling, and no fall in blood flow until the voiding contraction raises the detrusor pressure.*

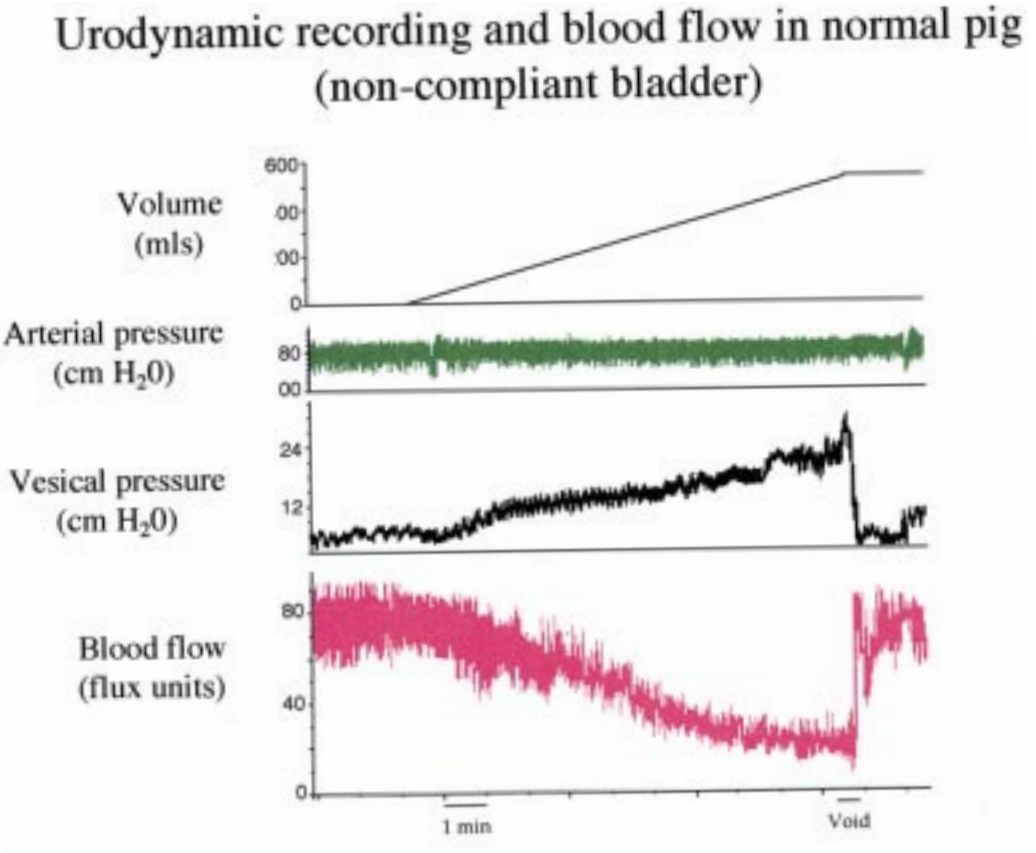



*Figure 36 : Cystometrogram, blood pressure and blood flow in the bladder wall during the micturition cycle in a female pig with a poorly compliant bladder. In this case the blood pressure in the bladder wall falls as the detrusor pressure rises.*

increases in the bladder (see 118). In the cat vasodilatation has been observed 125 during filling which suggests that local factors are involved in ensuring good perfusion of the bladder during the vascular rearrangement necessary with the increased surface area. Locally produced prostaglandins were implicated. The reflex cardiovascular responses to bladder distension in patients with spinal lesions 126 also suggest that neuronal pathways exist as well as local factors which could mediate control of blood vessels during filling. Bladder vasodilatation in response to pelvic nerve stimulation in the cat has been seen, 127 and pudendal nerve stimulation leads to an increased blood flow in the sphincteric regions of the dog urinary tract 128.

Histochemical and immunohistochemical studies have demonstrated the presence of catecholamine-containing, acetylcholinesterase-positive, vasoactive intestinal polypeptide-, substance P-, [Met] enkephalin- and gastrin-immunoreactive nerves on the adventitial-medial border of blood vessels in the pig urinary tract 129.

### *b) Roles of the blood vessels*

The urinary bladder requires a rich blood supply to maintain its functions, the storage and release of urine. The blood flow is important for tissue oxygenation and for the transport of nutrients and humoral factors as well as for the removal of metabolites. When the detrusor is deprived of oxygen and a metabolic substrate, as would occur in ischaemia, its contractile ability rapidly declines 130 131 and a rigor contraction develops 132. It has been suggested that ischaemia and reperfusion might lead to damage to intramural neurones and result in the patchy denervation and altered smooth muscle function seen in bladders of people with detrusor instability 50.

The subepithelial capillary plexus may be associated with maintenance of the barrier function of the urothelium, 133 reducing any exposure of the detrusor smooth muscle to substances diffusing from the urine, It may also play a role in epithelial transport function and be necessary for urothelial metabolism.

## 4. Urothelium

In the urinary tract, epithelia have continuously decreasing permeability from the extremely permeable kidney glomerular membrane down to the transitional epithelium of the ureter and bladder. The mammalian kidney produces urine which may vary considerably in ionic composition and tonicity, and one would expect that an important function of the bladder urothelium will be to prevent equilibration of the urine with the body fluids. The urinary epithelium is specialized, the cells form a continuous layer (fig. 37) and are joined with tight junctions. They have specialized cell-surface proteins, and ion pumps plus proteoglycans and glycoproteins, all of which function together to maintain the impermeability of the membrane 134 - 139 These same mechanisms also present an active defence against bacterial colonisation.

The urothelium, once categorized as “transitional” in type, is a stratified epithelium consisting of a minimum of two layers of cells; a superficial layer of capping or umbrella cells, and a basal layer separated from the submucosa/lamina propria by a continuous extracellular basal lamina. In histological preparations these two layers usually appear to be separated by one or more layers of “intermediate” cells. Evidence of epithelial cell division is rarely seen but can occur in superficial and deeper layers.

The superficial cells have large nuclei with prominent nucleoli and dispersed chromatin, are often multinucleate and are linked to their neighbours at their luminal aspect by tight junctions, zonulae adhaerentes and small desmosomes. They are highly pleomorphic, varying from flattened squamous to narrowly columnar in response to the degree of distention of the bladder lumen. Their cytoplasm contains dispersed small mitochondria, golgi complexes and rough endoplasmic reticulum, with varying numbers of large complex lysosomes. In the normal bladder their cell membranes are polarized, with the majority on their external surface in contact with the urine having a characteristic asymmetric trilaminar structure. They possess an irregular and angular profile due to their content of a family of particulate transmembrane proteins, the uroplakins, which stiffen the membrane to produce an assembly of semi-ridgid plaques. The immediately underlying cytoplasm contains numerous elliptical vesicles having the same trilaminar membrane structure and which can on occasions be seen to make direct contact with the surface membrane [fig. 37]. It has been claimed that these vesicles may contribute addition uroplakin-containing membrane to the cell surface when this is stretched by distention of the bladder wall. The lateral and basal surfaces of the umbrella cells lack the uroplakin component, are covered with fine folds and microvilli, and are linked to their lateral and deeper neighbouring cells by occasional small desmosomes (fig. 38, 39).

In many human bladder biopsies, however, surface cells with the usual angular, uroplakin-containing surface membranes are replaced, wholly or in part, by others lacking both these features and the sub-surface vacuoles, and having a flat surface mambrane bearing numerous well formed and regularly ordered microvilli covered by a conspicuous glycocalyx coat, and which also tend to contain larger and more numerous mitochondria (figs. 40, 41, 42). This change may represent a functional adaptation of the superficial cells to chan-

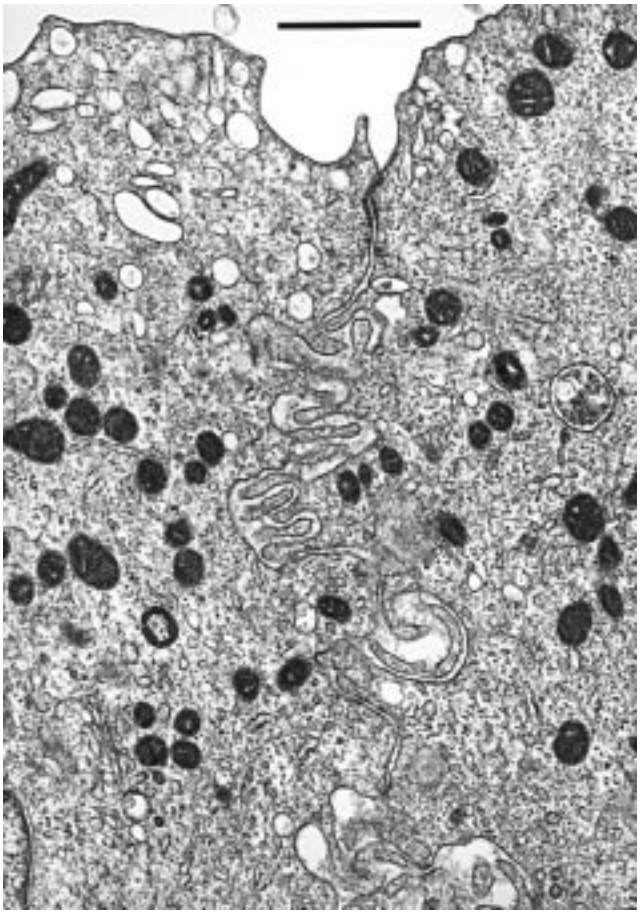


*Figure 37 : The interface between two superficial cells. Scale = 1 μm*

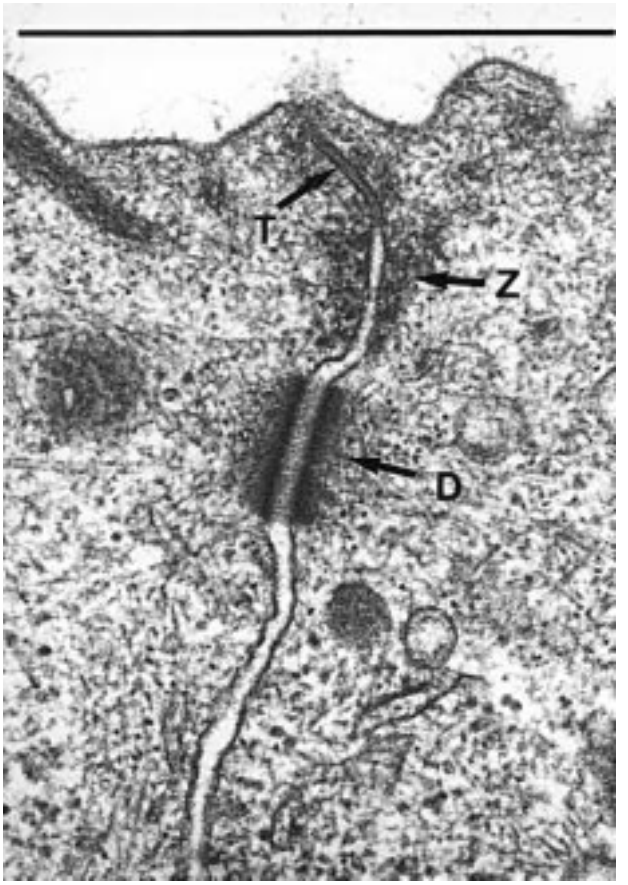



*Figure 38 : Fine structure of the intercellular attachments at the luminal surface of two superficial cells; the tight junction (T), the zonula adhaerens (z) and a desmosome (D). Scale = 1 μm.*

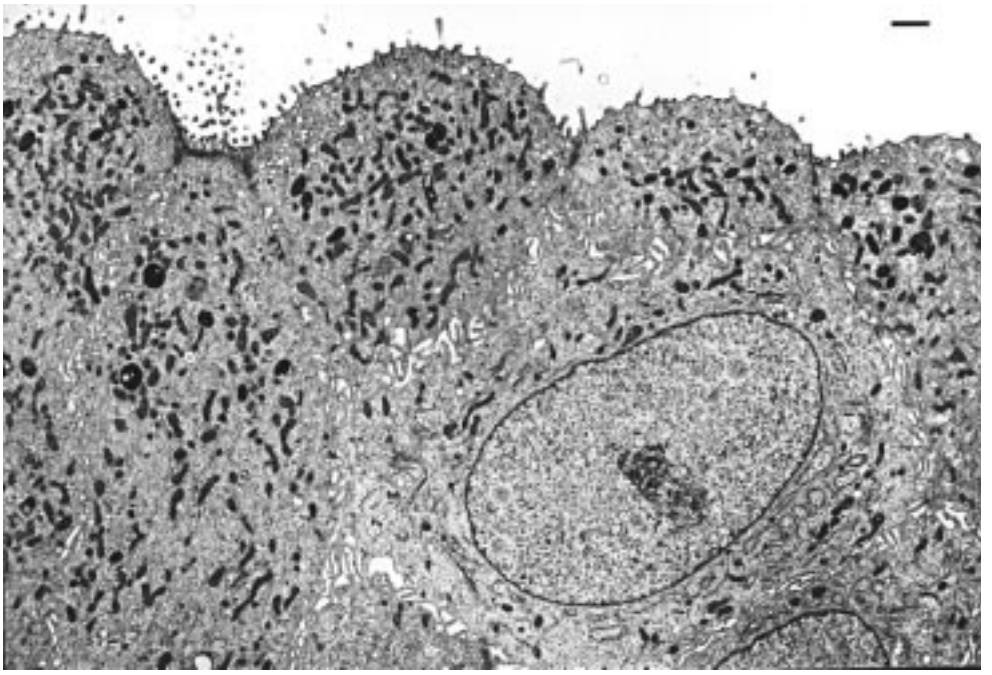


*Figure 39 : An example of urothelial surface cells lacking the usual angular surface profile and bearing numerous microvilli. Scale = 1 μm.*

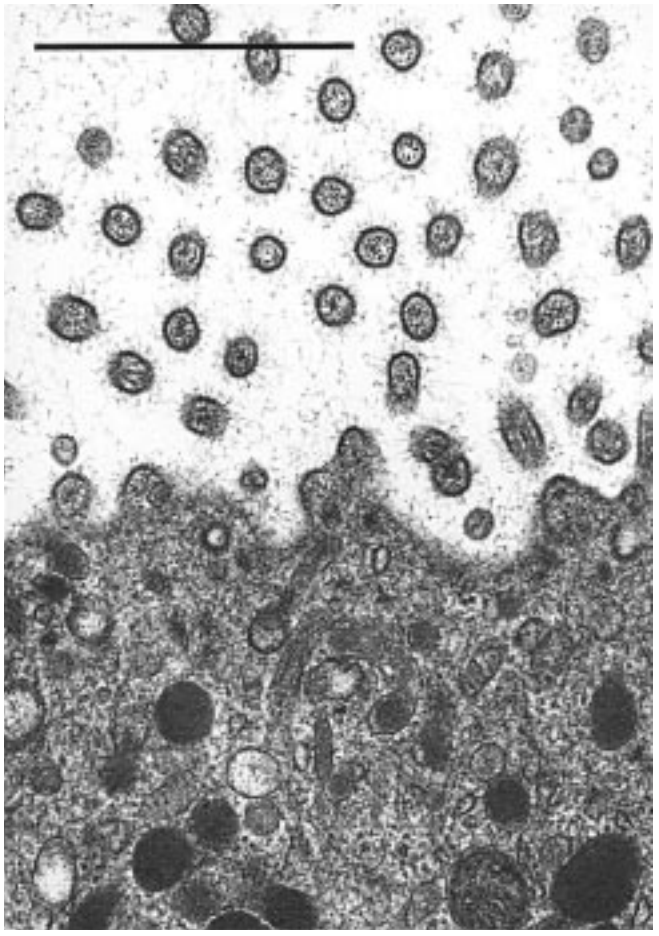


*Figure 40 : A detailed view of the microvilli on the surface of such a cell. Scale = 1μm.*

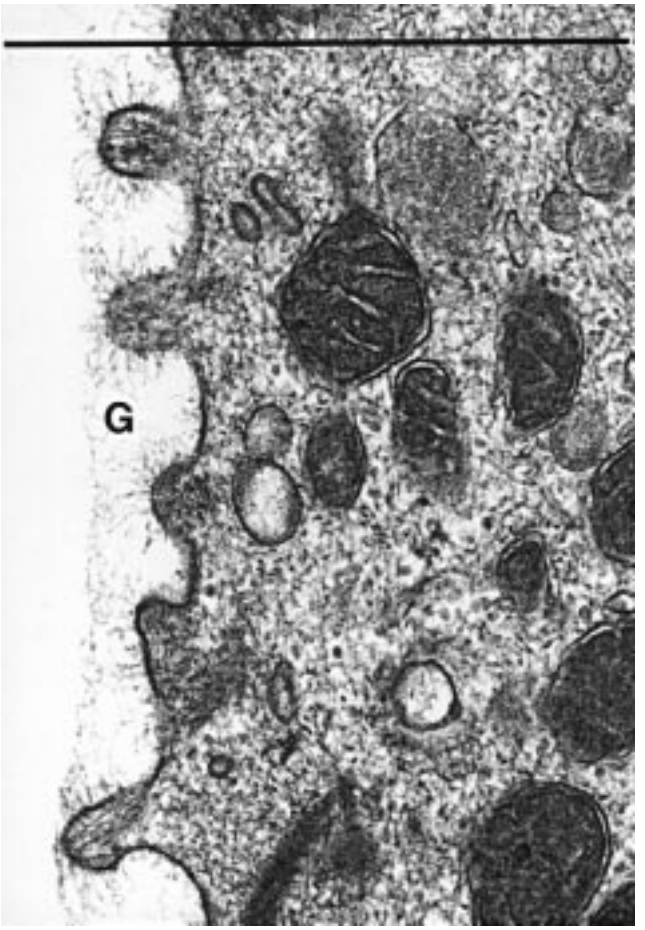



*Figure 41 : The surface glycocalyx on a microvillous urothelial surface cell. Scale = 1μ.*

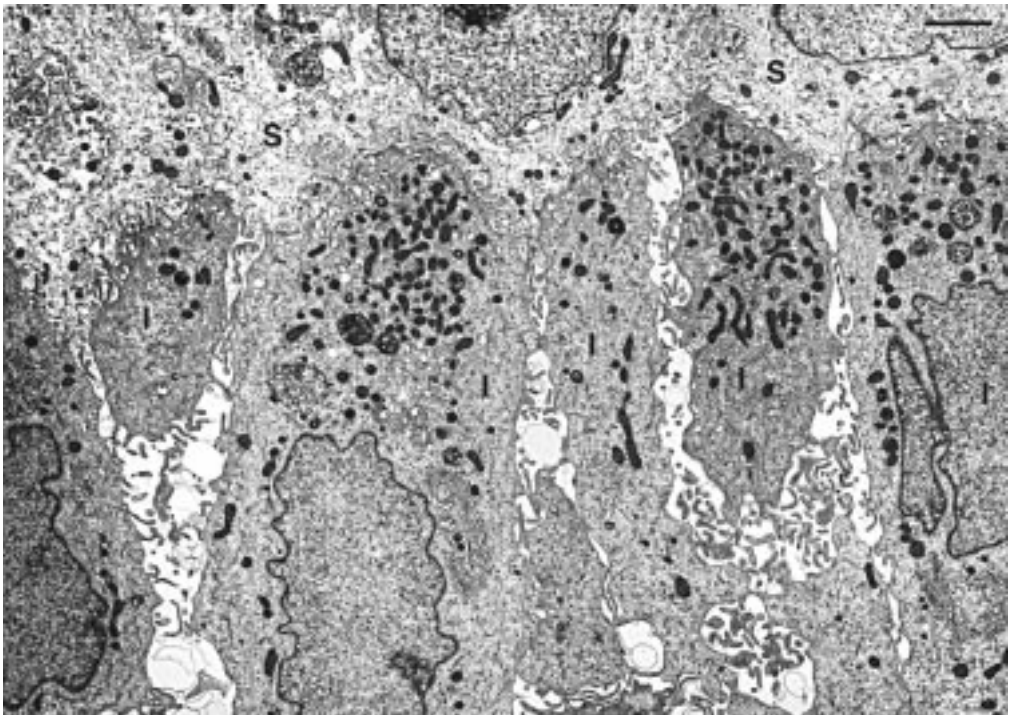



*Figure 42 : A row of intermediate urothelial cells (I) showing their relationship to the deep surface of the superficial cell (S), and the differences between these in respect of their cytoplasmic density and organelle content. Scale = 2μ.*

ge in their local environment, or their replacement by the insertion of deeper cells into the surface layer.

The deepest cells of the urothelium have basal processes which are closely adherent to the underlying basal lamina, with occasional hemidesmosomal attachments thereto, and abut those of their neighbours but without showing any specialized points of contact. Their cell bodies are generally columnar, having numerous folds and villi on their lateral and apical surfaces, and are adherent one to another only by means of scattered small desmosomes; they are thus well adapted to adjust their shape to the degree of stretch applied to the urothelium. Their nuclei are characteristically ellipsoid and indented, and are distinguishable from those of the umbrella cells by possession of a prominent rim of heterochromatin; their cytoplasm is polarized in that the majority of the cell organelles, including mitochondria, golgi complexes, rough endoplasmic reticulum and lysosomes are concentrated on the luminal aspect of the nucleus (fig. 43). The intermediate cells have similar cytological characteristics to the basal cells and can only be identified by their lack of contact with the basal lamina, a feature that is difficult to establish with certainty in tissue sections through such an irregularly convoluted structure.

In biopsy specimens of human bladder wall the intercellular space between the three classes of urothelial cells varies greatly in its extent, both between and within individual specimens, reflecting the degree of stretch of the membrane and other factors, such as the presence of underlying inflammation of the lamina propria; the only constraints on the relationships and separation of the urothelial cells being their apparently randomly scattered desmosomal contacts and the attachment of the basal cells to the urothelial basal lamina. This intercellular space appears to be freely accessible to leucocytes, mast cells and macrophages which can penetrate the basal lamina and migrate between the urothelial cells up to the level of the zonulae adhaerentes linking the margins of the umbrella cells. Hence the urothelial cells of all layers are open to continuous immunological surveillance.

Although structural features, i.e. the presence of tight junctions between adjacent apical cells, support the concept of an impermeable membrane, physiological experiments suggest otherwise. Although apical epithelial cells in the bladder are impermeable to water they actively transport sodium [140 - 142]. Sulphated polysaccharides, especially glycosaminoglycans, covering apical cells (fig. 41) act as an epithelial barrier to small molecules. Disruption of this polysaccharide layer increases permeability to urea and has been linked to inflammatory or hypersensitivity disorders of the bladder, such as interstitial cystitis [135].

A consensus has not been reached concerning the physiological role of active transmembrane transport mechanisms in the bladder. One function may be in epithelial cell volume regulation during changes accompanying distension [140]. Another role may be to actively maintain urine hypertonicity. A subepithelial vascular network could function as a countercurrent exchanger to maintain a hyperosmotic urine [143]. In addition, the microvasculature of the bladder can change with disease. Following obstruction, luminal diameters of intramural vessels increase and become more sensitive to alpha-adrenergic agonists [144]. Epithelial permeability could provide a mechanism for exposing smooth muscle to intravesical contents, thereby altering bladder contractility [143]. Investigators have demonstrated that intravesical installations of anti-neoplastic drugs, anticholinergic agents [145] and calcium channel antagonists, [146] influence detrusor function and access systemic circulation.

It is well recognised that the composition of the urine may have a marked effect on the volumes of urine voided. This could come about through penetration of small molecules through the urothelium having a direct sensitising effect on the dense meshwork of subepithelial sensory nerves, [147] or by production of substances by the urothelium that have this effect. ATP has recently been shown to be produced by epithelial cells in response to increased hydrostatic pressure changes in rabbit bladder, [142] and proposed to modulate sensory nerve activity. In the human urothelium iNOS activity has also been found after various treatments (e.g. cytokines or treatment with BCG) [148] [149] Recently it was found that patients with bladder inflammation showed a 30-60 fold increase in bladder NO levels [150]. Patients with interstitial cystitis or cystitis due to irradiation, BCG treatment or infection all showed a marked increase in the production of bladder NO. It is unclear whether the increased NO formation seen during inflammation has a role in the urgency symptoms found during such conditions. It is however likely that increased bladder NO levels can be used to distinguish between urgency caused by inflammation as compared to the urgency due to impaired function in the sensory afferent innervation. Thus, measurement of NO may be used to monitor inflammation in the urinary bladder.

## 5. Intrinsic innervation of the urinary bladder

### *a) Motor innervation*

The urinary bladder is profusely supplied with autonomic nerve fibres, which form a dense plexus among the detrusor smooth muscle cells. The majority of these nerves contain acetylcholinesterase (fig. 44), and while

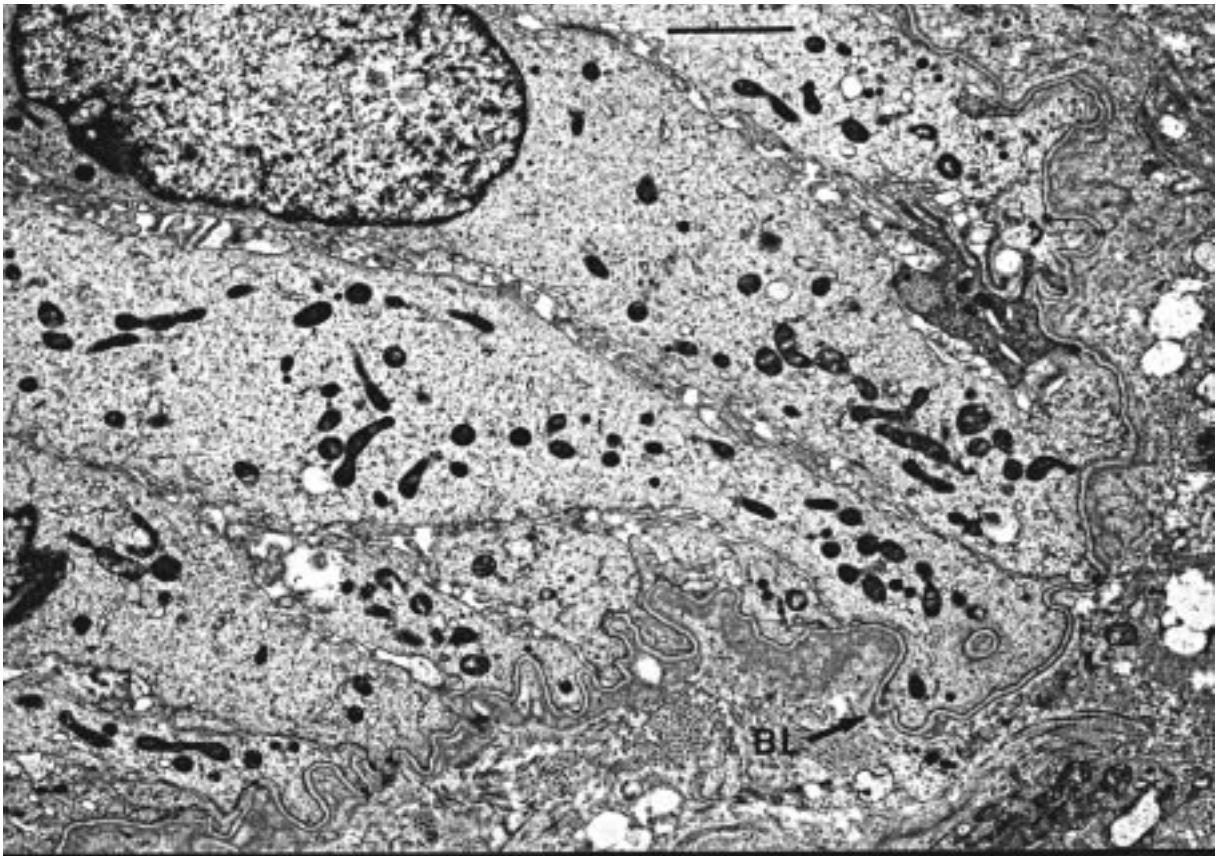



*Figure 43 : The basal processes of several basal urothelial cells showing their contact with the basal lamina (BL) and underlying lamina propria. Scale = 2μ.*

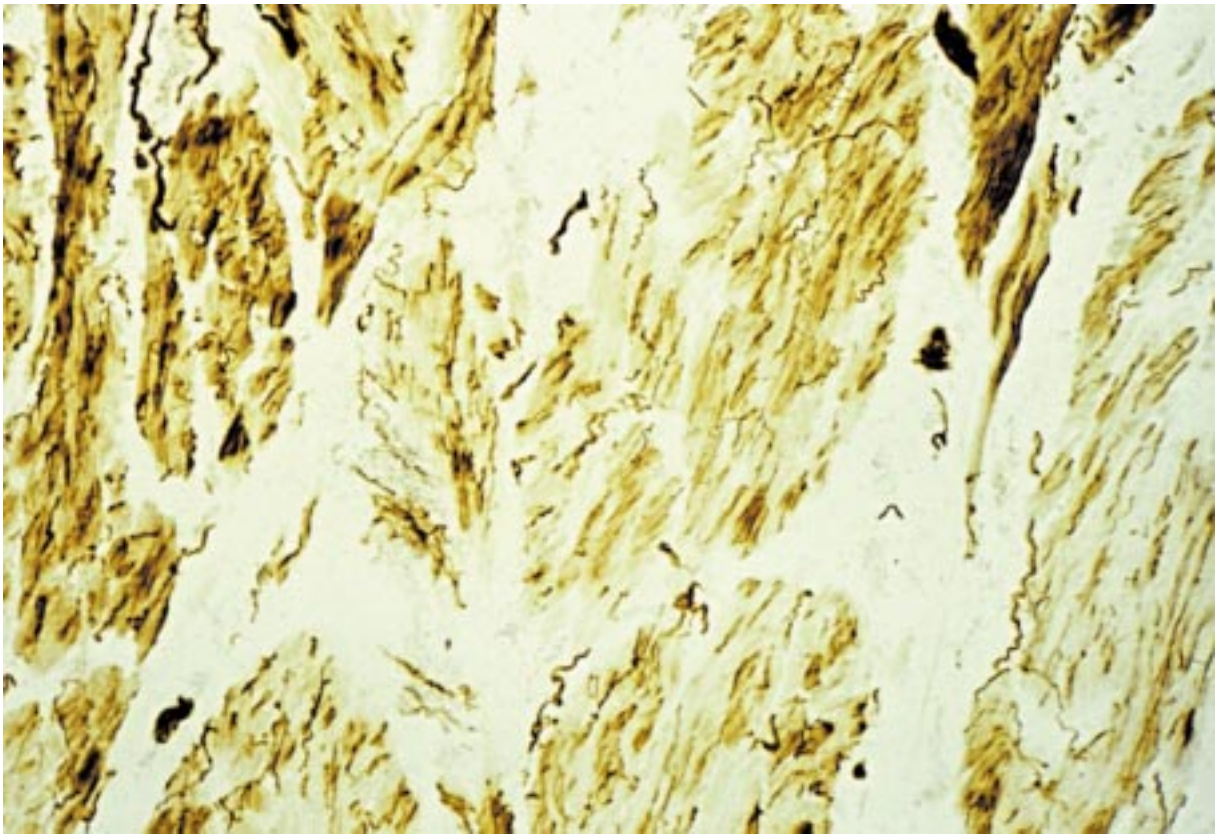


*Figure 44 : Numerous cholinesterase positive nerves supply the detrusor muscle. X100*

they occur in profusion throughout the muscle coat of the bladder, some muscle bundles appear to be more richly innervated than others. The majority of the autonomic nerves innervating the detrusor muscle are considered to be excitatory cholinergic in type [151], and contraction of the normal human detrusor is mediated almost exclusively through muscarinic receptor stimulation by released acetylcholine. Both nitric oxide synthase (NOS) and neuropeptide Y (NPY) are co-localised in some of these cholinergic nerves [3] [56] The human detrusor possesses an exceedingly sparse supply of sympathetic noradrenergic nerves [152 - 158]. Although nerves of this type generally accompany the vasculature to the bladder (fig. 45), they rarely extend among the smooth muscle cells of the detrusor. In addition, there is evidence for the occurrence of a nonadrenergic noncholinergic transmission in the human lower urinary tract, but so far the presence of such a transmitter, either excitatory or inhibitory, has not been definitely established. As indicated above, several neuropeptides, including vasoactive intestinal peptide (VIP) and NPY, have been demonstrated in a percentage of nerves within the detrusor muscle [56], [158], [159]. However, the precise function of these peptides as neurotransmitters and/or neuromodulators remains to be defined.

### *b) Sensory innervation*

The urinary bladder is supplied by afferent nerves travelling via the pelvic splanchnic and hypogastric nerves [160]. The cell bodies reside in dorsal root ganglia in the lumbosacral region, and no local afferent neurons are known to exist within the bladder itself [160]. A smaller group of sensory nerves extends to the striated urethral sphincter muscle via the pudendal nerve. The sensory nerves supplying the bladder are either thin myelinated A delta or unmyelinated fibres, and terminate as free nerve endings [160], [161]. A population of afferent nerves function as slowly adapting tension receptors, which respond to physiological distension in a graded manner. The same nerves also respond to bladder contraction, indicating that tension, rather than stretch, is the effective stimulus. In addition, the bladder is supplied by polymodal nociceptors. These fibres are typically unmyelinated and respond to stimuli associated with tissue damage, such as inflammatory mediators and ischaemia. The nocioeptors also respond to cold, but not to stretch within physiological parameters [160], [162].

In the human bladder, presumptive sensory nerves containing calcitonin gene-related peptide (CGRP) are rare, comprising less than 5% of the total bladder innervation, and are restricted in their distribution [56] [159]. CGRP nerves are typically found in the subepithelium, around blood vessels, and surrounding intramural ganglion cells [56] [159]. The fibres occasionally penetrate into the epithelium, particularly in the urethra. Unlike the situation in animals, CGRP fibres only very rarely project to the detrusor muscle in the human bladder [56] [159].

The projections of presumptive sensory fibres in the human bladder are consistent with their assumed role as tension receptors. In addition, the fibres may act as touch receptors if the walls of the bladder or urethra coapt, or as chemoreceptors of urine content. It has also been suggested that chemicals released by the bladder as a result of stretch, such as prostaglandins [163], nitric oxide, or ATP [164], may influence sensory transmission. The presence of presumptive sensory nerve terminals around local ganglion cells indicates that sensory collaterals may influence ganglionic neurotransmission [56]. Such transmission may become more prominent when the bladder functions abnormally [165]. Two neurochemically distinct subpopulations of presumptive sensory fibres have been identified in the human. One class contains CGRP and tachykinins such as substance P (SP) and neurokinin A, and the other class, representing 75% of the total afferent fibres, contains CGRP only [159]. Since these nerves account for such a small proportion of the total bladder innervation, it is possible that other populations of sensory nerves exist in human bladder which cannot be detected with these neurochemical markers. Electron microscope (EM) studies have identified SP-containing nerves in human bladder as being unmyelinated [166]. Myelinated nerves have also been detected in human bladder deep lamina propria by EM, and these fibres do not contain SP [166]. It is likely that these nerves are sensory, since the postganglionic parasympathetic and sympathetic nerves are unmyelinated. A detailed confocal/EM study examining the distribution and neurochemistry of myelinated nerves in human bladder would yield much valuable information.

Pacinian corpuscles have been identified in the subserosal region of the human bladder [167] These specialised sensory endings are extremely rare, and appear to be associated with noradrenergic fibres [168]. Pacinian corpuscles act as rapidly adapting pressure receptors – it is therefore unlikely that such fibres can transmit information related to slow bladder distension. The subserosal location of the receptors reinforces this argument. Pacinian corpuscles are most likely to be activated by rapid changes in bladder or abdominal pressure, as occurs during coughing or physical exertion. Such information may be used in “guarding reflexes” to close the striated urethral sphincter.

The sensory nerves that supply the bladder exhibit a remarkable plasticity. Collateral sprouting, and the formation of new and sometimes aberrant connections can occur as a result of spinal injury, inflammation [169],

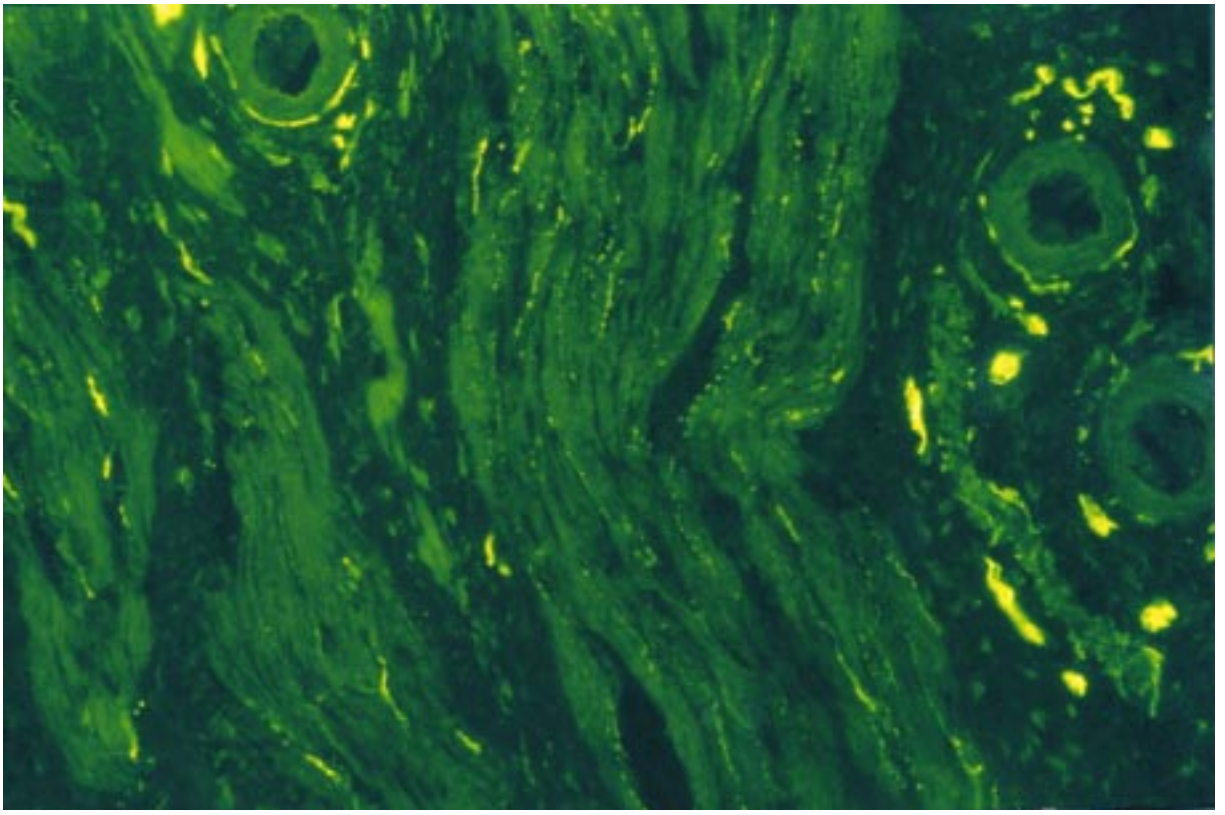


*Figure 45 A : The general nerve marker protein gene product 9.5 demonstrates the rich detrusor and perivascular innervation. X200*

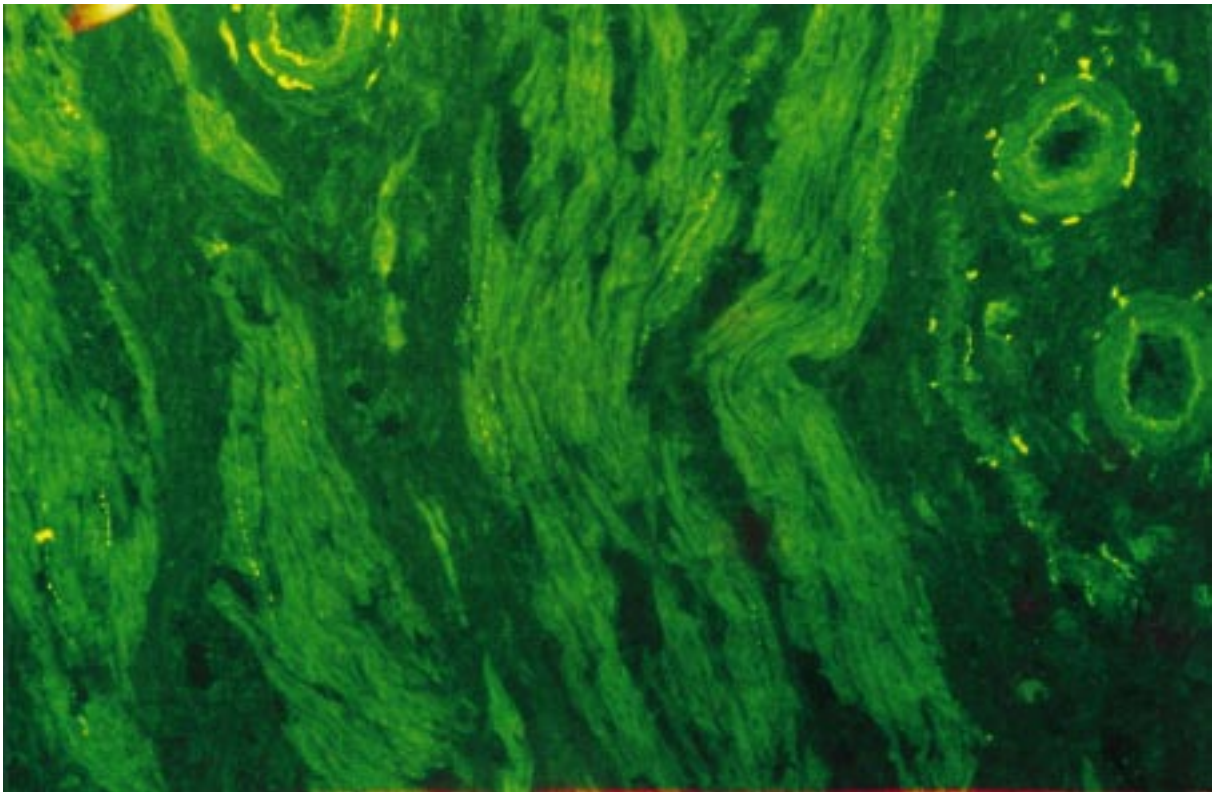


*Figure 45 B : An adjacent section to that in Figure 15.A showing perivascular dopamine β- hydroxylase positive nerves. Few such nerves are present in the detrusor muscle bundles. X200*

nerve injury [170] or bladder hypertrophy [171] in animals. This may occur at both the level of the spinal cord and within the periphery [171]. Recently it has been shown that the density of SP-containing presumptive sensory nerves is elevated in women suffering from bladder pain [173]. Similarly, the innervation of the bladder subepithelium by nerves containing SP and CGRP is increased in women with idiopathic detrusor instability [159]. This increase is relatively selective, and does not involve other populations of nerves. The role of sensory neuron plasticity in bladder malfunction, and the involvement of neuronal growth factors [170], [174] in this process clearly needs further investigation.

Although it has been assumed that the subepithelial plexus of nerves in human bladder is composed of sensory nerves, the evidence is as yet lacking. Many nerves in this region supply the rich vascular bed of the subepithelium, and need not, therefore, be afferent. While CGRP and SP have proved useful as markers, a definitive neurochemical marker for human afferent nerves is required. The finding that capsaicin-senitive sensory nerves express a vanilloid receptor [175] should aid in the future identification of sensory fibres in humans. Similarly, the trkA NGF receptor is expressed by many visceral sensory neurons [176], and may be useful in detecting putative sensory fibres. Myelin-specific antibodies, and the detection of myelinated fibres in the bladder by EM will also prove useful [166]. If the neurochemistry of myelinated fibres can be unravelled, it will go a long way towards establishing a marker for bladder sensory nerves. This work will form a solid foundation for examining the role of sensory nerve plasticity in the development of incontinence. Such research needs the development of a robust and reproducible test for assessing and quantifying lower urinary tract sensation [162], [177].

### *c) Autonomic ganglia*

Small clusters of autonomic ganglion cells occur throughout all regions of the human bladder wall, being especially numerous in the adventitia of the bladder base. These intramural ganglia together with those which populate the vesical plexus consist of different types of neurons on the basis of their histochemistry and immunochemistry [56]. One type contains noradrenaline and corresponds to the short noradrenergic neuron described in association with the genital tract of other species [178]. In addition to presumptive cholinergic (excitatory) preganglonic nerves, some ganglion cells receive noradrenergic (possibly inhibitory) input [56], [179] (fig. 46). Recent studies, however, have demonstrated that the types of neuron associated with the lower urinary tract are much more complex than was previously believed [56]

### *d) Paraganglia*

In addition to autonomic ganglia, numerous paraganglia [180] have been demonstrated in association with the human urinary bladder, being especially obvious in late fetal and early postnatal life. These paraganglia are located among the ramifications of the pelvic plexus, and the constituent cells receive a direct autonomic innervation. Many of the paraganglia that are present during fetal and early postnatal life contain specialized encapsulated sensory corpuscles (fig. 47) resembling small pacinian corpuscles [181], [182]. The functional significance of this developmental association remains obscure, as does the subsequent fate of these corpuscular nerve endings. However, occasional isolated corpuscles have been observed in the adventitia of the bladder in older children. The paraganglion cells are rich in catecholamines [183] and as a consequence contain large amounts of dopamine β-hydroxylase (DBH). However, the functional significance of the paraganglia and their relationship to developing autonomic ganglia remains to be determined.

## IV. BLADDER NECK

The smooth muscle of this region is histologically, histochemically and pharmacologically distinct from that which comprises the detrusor proper, hence, the bladder neck should be considered as a separate functional unit. The arrangement of smooth muscle in this region is quite different in males and females and consequently each sex will be described separately.

### 1. Female bladder neck

The female bladder neck consists of morphologically distinct smooth muscle, since the large diameter muscle bundles characteristic of the detrusor are replaced in the region of the bladder neck by those of small diameter (fig. 48). The majority of muscle bundles in the female bladder neck extend obliquely or longitudinally into the urethral wall. There is no anatomical smooth muscle sphincter at the bladder neck, and it remains to be determined whether this region plays a significant part in the maintenance of female urinary continence.

In contrast with the rich sympathetic innervation in the male, the smooth muscle of the female bladder neck possesses fewer noradrenergic nerves, but is well supplied with presumptive cholinergic fibres [152]. The role played by this type of nerve in the functional control of the bladder neck remains uncertain. In strips of bladder neck taken from female dogs stimulation invariably produces a large relaxation this is blocked by nitric oxide inhibitors to reveal a small tonic contraction (Creed and Van der Werf, personal communication).

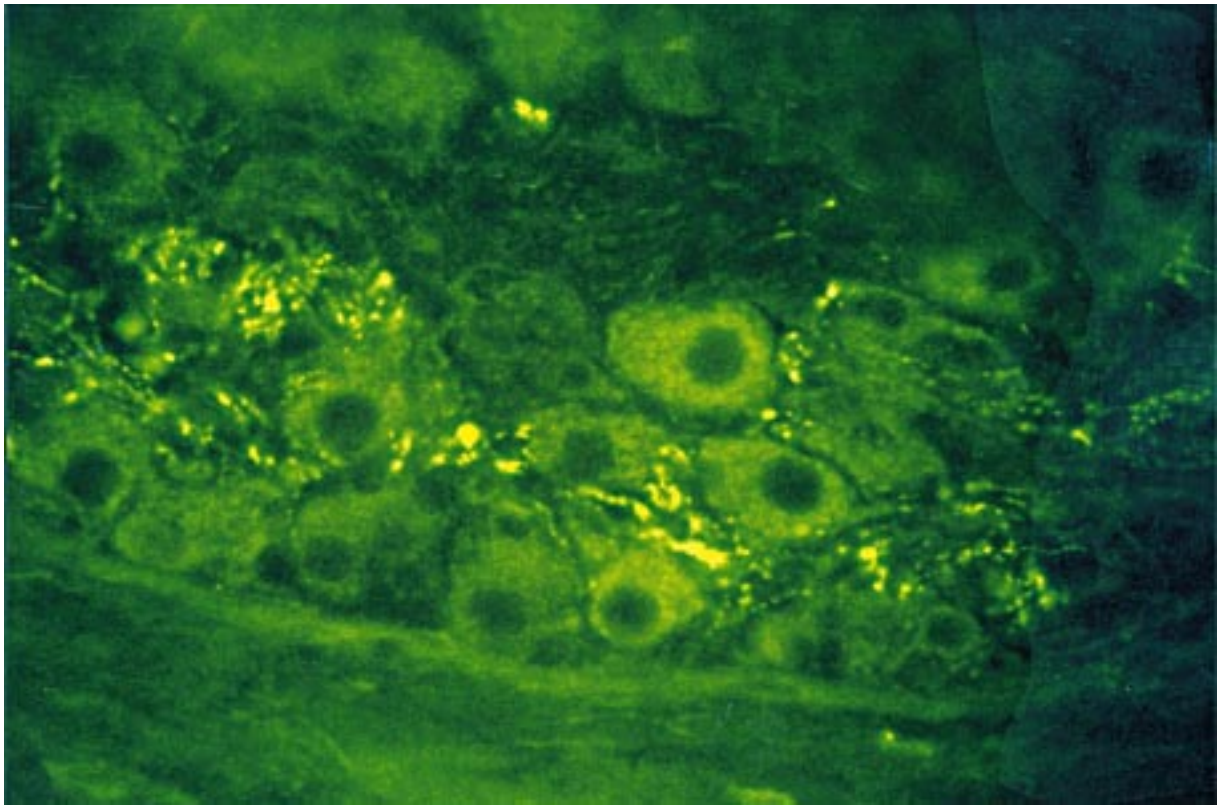


*Figure 46 : Numerous dopamine ß- hydroxylase-immunoreactive nerve fibres and terminals seen in close association with non-reactive (presumptive cholinergic) neurons in the vesicle plexus. X400*

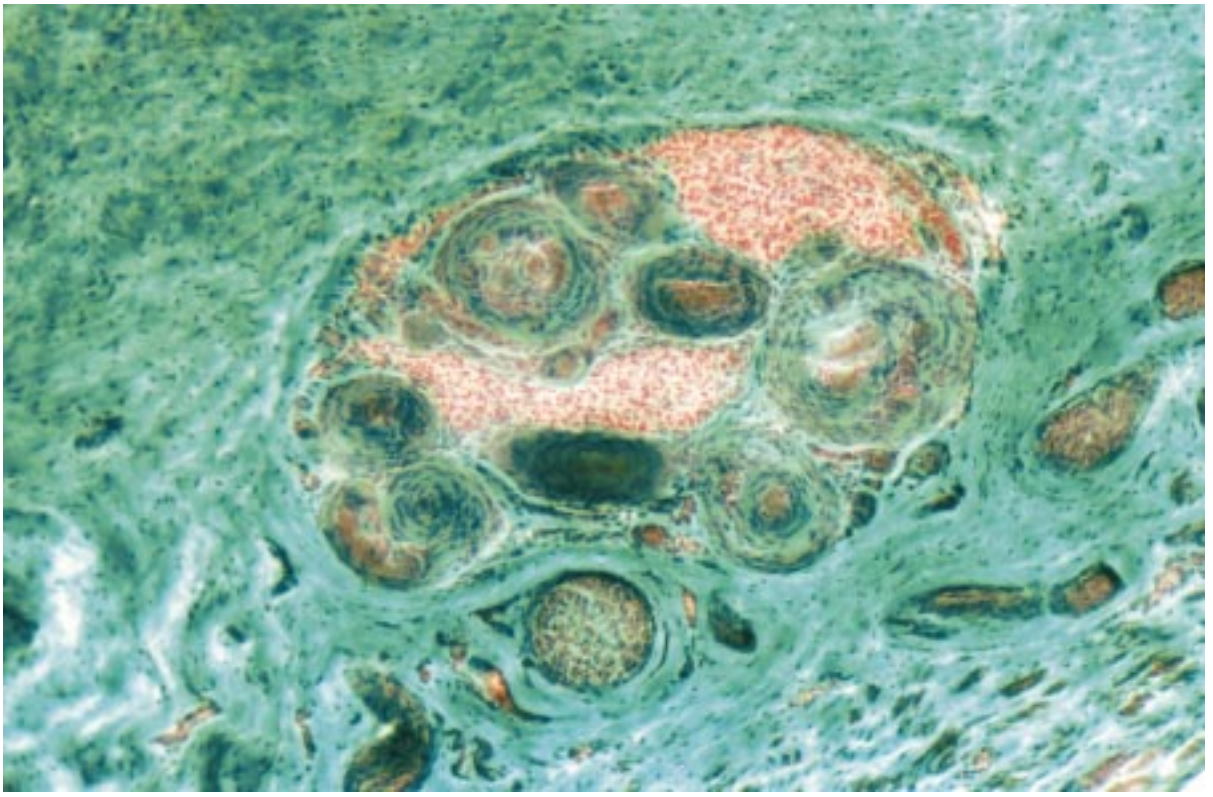


*Figure 47 : A paraganglion in the adventitia of the bladder of an 8 month old infant which contains several sensory corpuscles. Massons trichrome. X200*

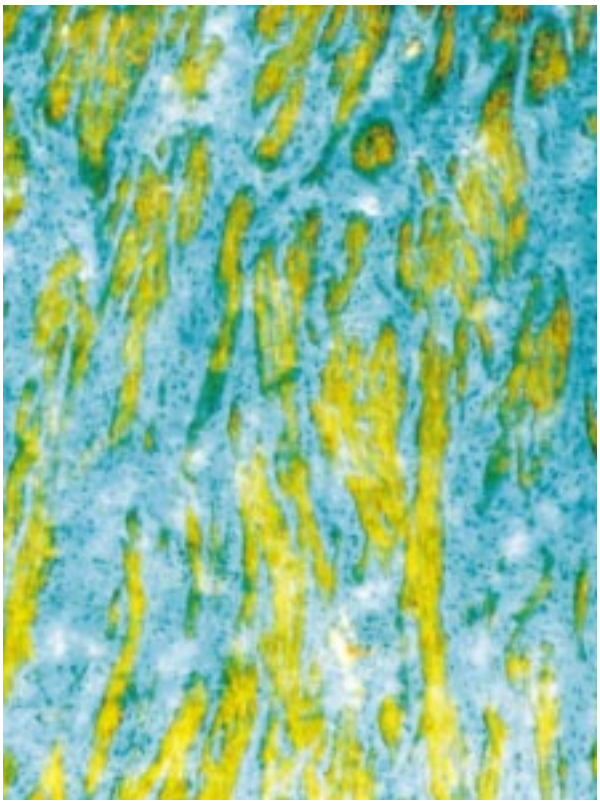


*Figure 48 : Small diameter muscle bundles in the female bladder neck. Massons trichrome. X200*

## 2. Male bladder neck

At the male bladder neck, the smooth muscle cells form a complete circular collar which extends distally to surround the proximal portion of the urethra. Because of the location and orientation of its constituent fibres, the terms internal, proximal or preprostatic urethral sphincter have been used as alternatives for this particular component of urinary tract smooth muscle. Distally, bladder neck muscle merges with, and become indistinguishable from, the musculature in the stroma and capsule of the prostate gland.

In the male, bladder neck smooth muscle is supplied with cholinergic (parasympathetic) nerves and also possesses a rich noradrenergic (sympathetic) innervation 153. A similar distribution of autonomic nerves occurs in the smooth muscle of the prostate gland, seminal vesicles and ducti deferentes. On stimulation the sympathetic nerves cause contraction of smooth muscle in the wall of the genital tract resulting in seminal emission. Concomitant sympathetic stimulation of bladder neck muscle causes sphincteric closure of the region, thereby preventing reflux of ejaculate into the bladder 184.

Although this genital function of the male bladder neck is well established it is not known whether the smooth muscle of this region normally plays an active role in maintaining urinary continence. Recently, NOS positive nerve fibres have been described amongst the smooth muscle of the region 185. Furthermore precontracted smooth muscle strips produce a relaxation response to nerve stimulation which is blocked by NO inhibitors. However the precise role of this type of nerve in the functional control of the bladder neck and urethra has yet to be established.

## V. THE FEMALE URETHRA

The female urethra (fig. 49) is a fibromuscular tube 3-4 cm long and begins at the internal urethral meatus of the bladder. Embedded in the anterior wall of the vagina it inclines downwards and forwards through a gap in the pelvic floor and terminates in the vestibule at the external meatus between the clitoris and the vaginal opening.

The female urethra is comprised of different regions along its length and can be understood by dividing the length of the urethral lumen into fifths, each 20 % of the total length 186. In the first quintile, the lumen of the urethra is surrounded by the vesical neck (0 – 20%). Next the sphincter urethrae and smooth muscle encircly the lumen from 20 – 60%. The arch shaped compressor urethrae and urethrovaginal sphincter are found from 60%-80% while the distal component includes only fibrous tissue and no significant contractile elements.

The striated urogenital sphincter muscle 187 has two components as this division implies (fig. 49). The urethral sphincter (rhabdosphincter) is circular in orientation and forms the outermost layer of the muscular wall. It is often deficient in the portion adjacent to the vagina, but can form a complete circle, especially in the young. The more distal portion that lies adjacent to the perineal membrane is comprised of two arch shaped straps of muscle. The compressor urethrae arises laterally near the ischiopubic rami while the urethrovaginal sphincter closely follow the wall of the vagina. Interestingly these muscles seem to be preserved with advancing age while the more proximal sphincter deteriorates, indicating the separate nature of these two elements.

The striated sphincter can be contracted to increase urethra closure during times of urgent need and micturition occurs when bladder pressure is higher than urethral pressure and is typically produced by contraction of the detrusor muscle of the bladder wall accompanied by relaxation of the intramural striated and smooth sphincters.

The wall of the female urethra comprises an outer muscle coat and an inner epithelial membrane which

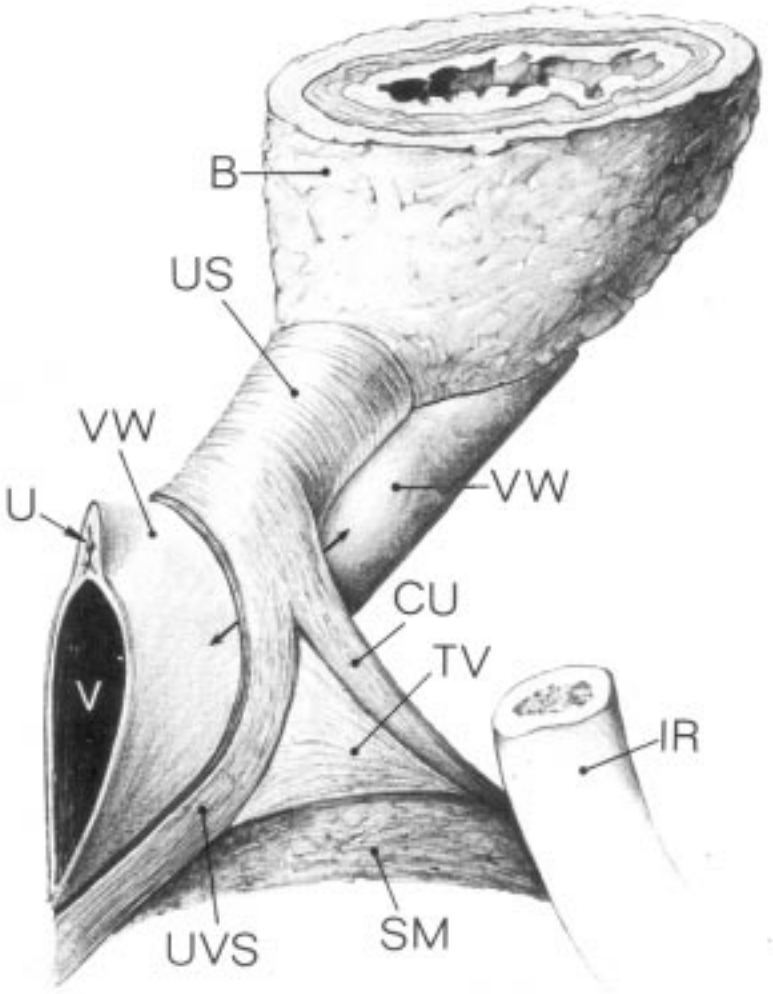



***Figure 49 : Striated urogenital sphincter muscle showing its urethral sphincter (US), compressor urethrae (CU) and urethrovaginal sphincter (UVS). Also shown, B, bladder; IR, ischiopubic ramus; SM, smooth muscle; TV transverse vaginal muscle; U, urethra; V, vagina; VW, vaginal wall. After Oelrich***

lines the lumen and is continuous with the epithelium of the bladder. The muscle coat consists of the previously described outer sleeve of striated muscle (striated urogenital sphincter or rhabdosphincter) together with an inner coat of smooth muscle fibres.

The striated muscle cells are of the slow twitch (type I) variety (fig. 50, 51) and are relatively small with diameters of 15-20 μm on average 188. These fibres exert tone upon the urethral lumen over prolonged periods, especially in relation to the middle third of its length. Periurethral striated muscle of the levator ani aids urethral closure during events which require rapid, albeit short-lived, increases in urethral resistance.

The smooth muscle coat extends throughout the length of the urethra and consists of slender muscle bundles, the majority of which are orientated obliquely or longitudinally and are associated with a considerable quantity of connective tissue. Of these layers, circular and longitudinal, the latter is by far the more prominent 189. The few circularly arranged muscle cells occur in the outer aspect of the smooth muscle layer and intermingle with the striated muscle forming the inner part of the striated sphincter. Proximally the urethral smooth muscle extends as far as the bladder neck. When traced distally, urethral smooth muscle bundles terminate in the subcutaneous tissue surrounding the external urethral meatus. The smooth muscle of the female urethra is associated with relatively few noradrenergic nerves but receives an extensive presumptive cholinergic parasympathetic nerve supply 152 identical in appearance to that which supplies the detrusor. From a functional point of view it seems unlikely that competence of the female bladder neck and proximal urethra is solely the result of smooth muscle activity in the absence of an anatomical sphincter. The innervation and longitudinal orientation of most of the muscle fibres suggest that urethral smooth muscle in the female is active during micturition, serving to shorten and widen the urethral lumen.

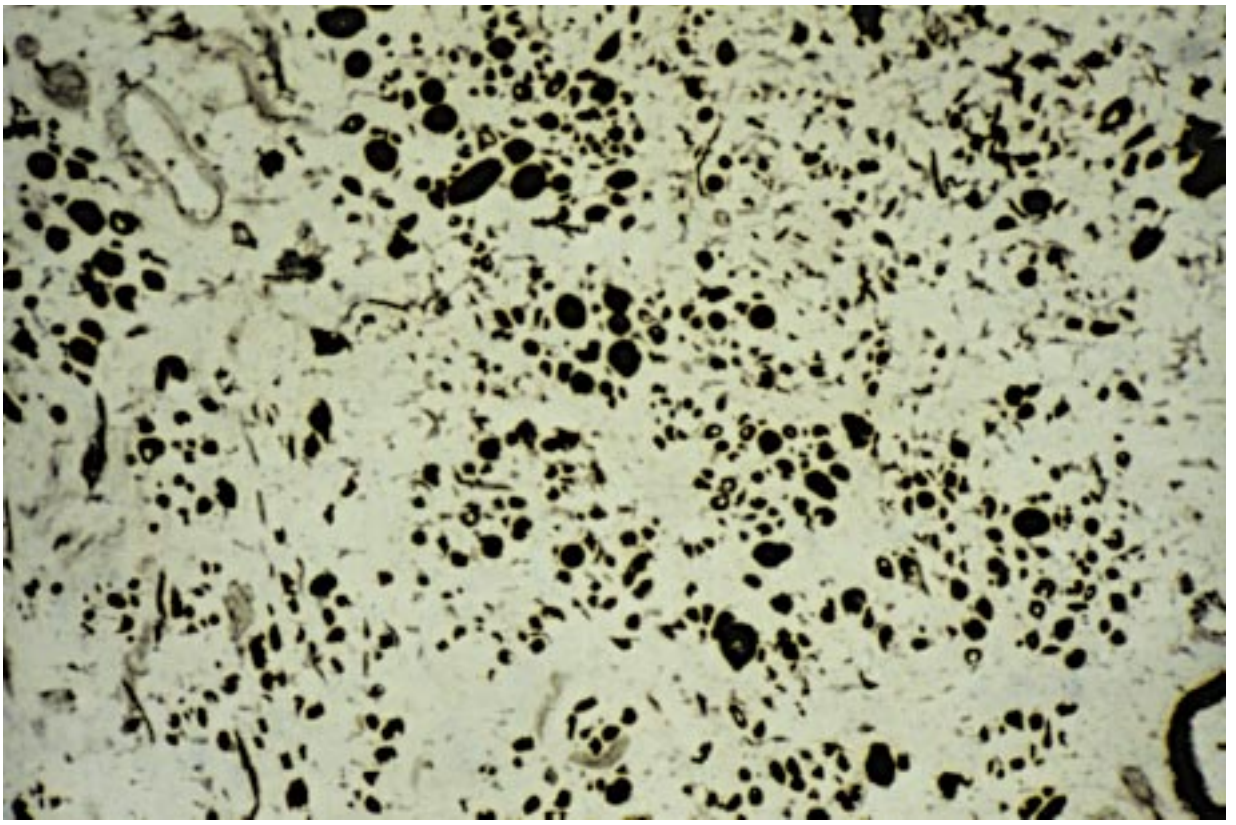


*Figure 50 : Female intramural urethral striated muscle showing darkly stained slow twitch (type I) fibres. X150*

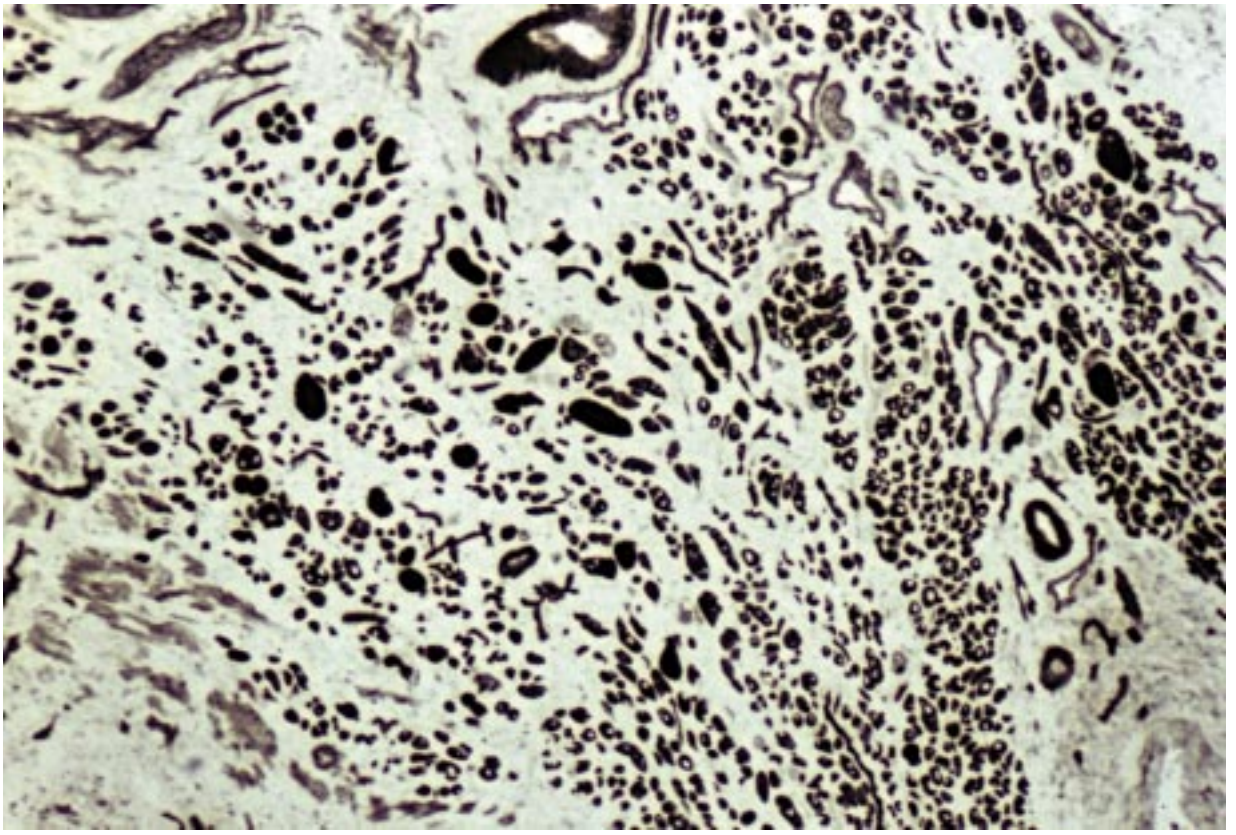


*Figure 51 : Inner aspect of the male intramural uretheral striated muscle coat consisting of darkly stained slow twitch (type I) fibres. X150*

# VI. THE MALE URETHRA

## 1. Macroscopic anatomy

The male urethra (fig. 52) is a fibromuscular tube approximately 20cm long and is usually described in three parts: prostatic, membranous and spongy. The prostatic and membranous parts pass downwards while the spongy part turns forwards in the bulb of the penis. This abrupt angulation is of considerable importance when catheters or cystoscopes are being introduced. Furthermore, although the spongy and prostatic parts can be readily dilated, the external meatus and the membranous urethra are comparatively narrow.

## 2. Prostatic urethra

The prostatic urethra is the widest and most dilatable part of the entire male urethra. It is about 3cm long and extends through the prostate from base to apex (fig. 53). The prostatic urethra is divided into proximal and distal segments of approximately equal length by an abrupt anterior angulation of its posterior wall at the midpoint between prostate apex and bladder neck. The angle of deviation is approximately 35°, but can be quite variable and tends to be greater in men with nodular hyperplasia. The prostatic urethra lies nearer the anterior than the posterior surface of the prostate. It is widest in the middle and narrowest below, adjoining the membranous part. In cross-section it appears crescentic in outline with the convex side facing ventrally.

The characteristic crescentic shape is due to the presence on the posterior wall of a narrow median longitudinal ridge formed by an elevation of the epithelial lining and its subjacent tissue, called the urethral crest (fig. 54). On each side of the crest lies a shallow depression termed the prostatic sinus, the floor of which is pierced by the openings of the prostatic ducts. About the middle of the length of the urethral crest, the colliculus seminalis (or verumontanum) forms an elevation on which the slit-like orifice of the prostatic utricle is situated. On each side of, or just within, this orifice are the openings of the two ejaculatory ducts. The prostatic utricle is a blind-ending diverticulum about 6mm long which extends upwards and backwards within the substance of the prostate. It develops from the paramesonephric ducts or urogenital sinus and as a consequence is a remnant of the system which forms the reproductive tract in the female.

## 3. Membranous urethra

Emerging from the anterior aspect of the apex of the prostate, the membranous urethra (fig. 55) descends in the midline and pierces the perineal membrane. It is approximately 2cm long and its mucosa is folded, giving the lumen a stellate appearance on cross-section. Within the wall of the membranous urethra is the intrinsic striated muscle which, as in the female, is often named the external urethral sphincter (or rhabdosphincter). Lateral to the sphincter are the medial borders of the levatores ani.

The intramural muscle of the wall of the membranous urethra consists of a relatively thin inner layer of smooth muscle bundles continuous proximally with those of the prostatic urethra, and an outer layer of circularly orientated striated muscle fibres. Most of the striated muscle fibres on the inner aspect of the sphincter are unusually small in cross-section with diameters of only 15-20 μm. The majority of these fibres are slow twitch in type (fig. 51) and, unlike the female merge peripherally with larger diameter fast (type II) and slow twitch fibres. Whether these large diameter fibres represent a proximal extension of the striated muscle surrounding the bulb of the penis remains to be determined. If established, this hypothesis offers an explanation for the apparent differences in intramural striated muscle fibre size and type between females and males.

## 4. Spongy urethra

The spongy (or penile) urethra is approximately 15cm in length, commencing in the bulb of the penis and traversing the erectile tissue of the corpus spongiosum and glans (fig. 52). The mucosa presents numerous small recesses or lacunae and most of its lumen forms a transverse slit. Within the bulb the urethra is wider, forming the intrabulbar fossa. The lumen is also expanded within the glans to form the navicular fossa which opens at the surface as a vertical slit, the external meatus.

# VII. COMPONENTS OF THE URETHRAL WALL

## 1. Smooth muscle

### *a) Basic properties*

The smooth muscle cells in the urethra are small, richly supplied with afferent and efferent nerve fibres, gathered into small bundles and linked to each other by many adherens type junctions but no gap junctions. The smooth muscle bundles in the urethral wall are thinner than in the detrusor and arranged in obvious layers (fig. 56). In humans and larger mammals there is a relatively thick inner layer that is predominantly longitudinally arranged and outside this, a thinner circular muscle layer (fig. 57). In the lamina propria of the urethra scattered small bundles of only a few cells are often found. Elastic fibres are well developed in the bladder neck and urethra.

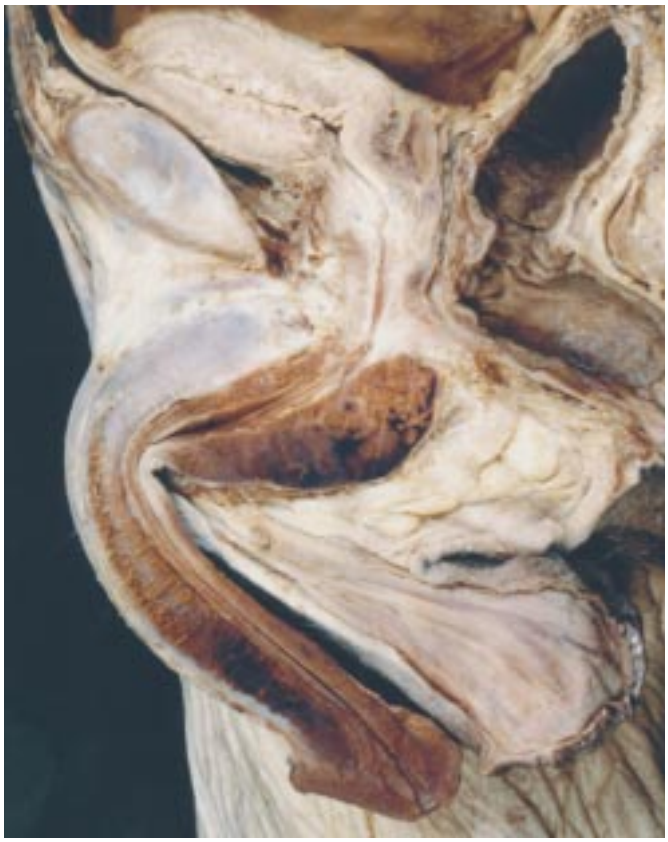


*Figure 52 : Sagittal section showing the parts of the male urethra.*

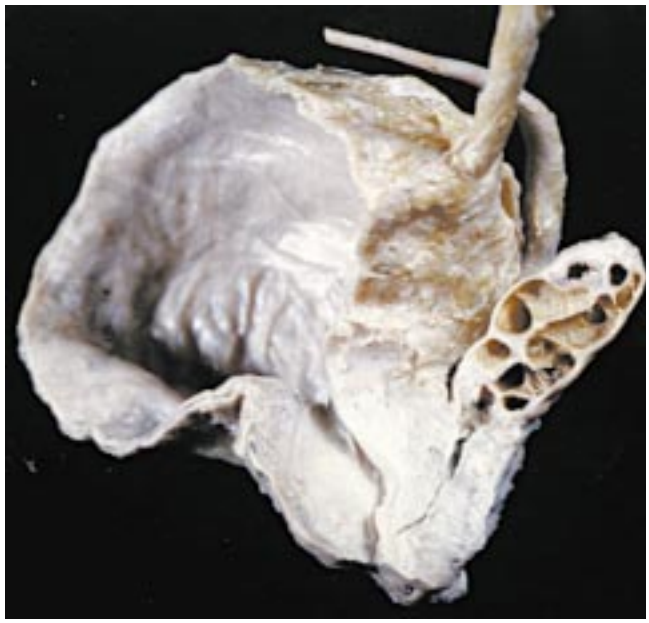


*Figure 53 : Dissection of the prostate and seminal vesicle to show the prostatic urethra and ejaculatory duct.*

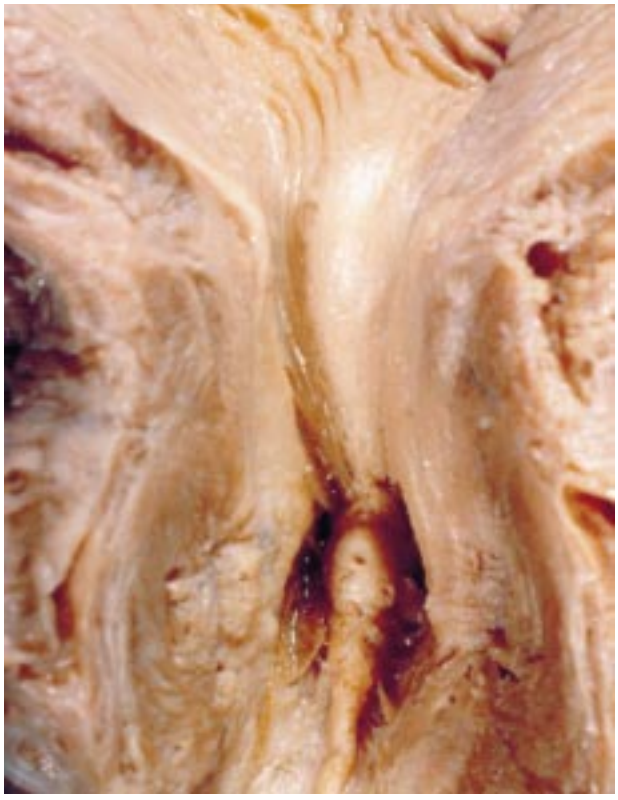


*Figure 54 : Sagittal section showing the parts of the male urethra.*

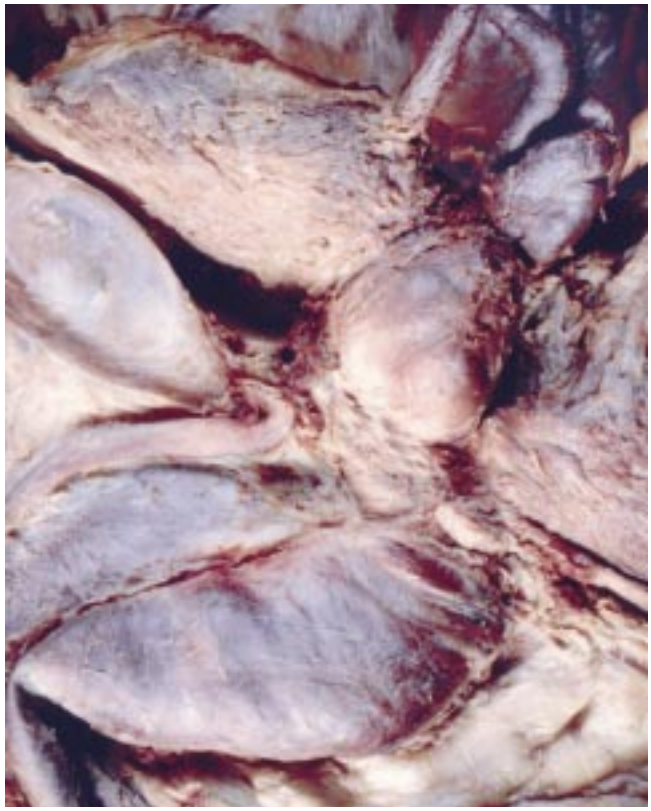


*Figure 55 : The left pelvic wall and levator ani have been removed to show the prostate, membranous urethra and bulb of the penis.*

In comparison with the detrusor, there is little published on the mechanical properties of the smooth muscle of the urethra. A recent study has, however, compared the force velocity relationship of circular and longitudinal smooth muscles from the rabbit urethra [190] and has shown that the maximum shortening velocity is three times higher in the longitudinal than in the circular smooth muscle. The authors conclude that the results are consistent with a tonic role for the circular smooth muscle in contracting the urethra during bladder filling, and a phasic role for the longitudinal smooth muscle in opening the urethra during micturition.

Behavioural studies demonstrate that the contractile properties of urethral smooth muscle are rather variable, depending on the region from which they are dissected, and possibly on age and hormonal status. In pig and man, smooth muscle dissected from the high pressure zone usually generates continuous sustained myogenic tone, sometimes interrupted by small oscillatory relaxations and contractions (fig. 58). A clear difference is thus seen in the contractile behaviour of smooth muscle from the detrusor and urethra. The ability of the circular elements to maintain continuous tone make it likely that there may be significant differences in their ability to develop latch bridges, or in the $Ca^{2+}$-sensitivity of the contractile proteins. The evoked responses are also sustained, in contrast to the detrusor, and in tissues generating tone, relaxations and contractions can be evoked by the correct stimuli.

Less work has been carried out on the electrical properties of urethral smooth muscle than on detrusor. In smaller mammals action potentials have been recorded, [191] but in the pig, which seems a better model for the human urethra, records from isolated urethral myocytes have membrane potentials of around –40 mV, and do not appear to support action potentials. They show spontaneous hyperpolarizations and possess large and small $Ca^{2+}$-activated K+ channels, and glibenclamide-sensitive ATP dependent K channels, but little evidence of voltage sensitive delayed rectifier or transient outward currents [37], [192]. There is currently no evidence about electrical coupling in urethral smooth muscle, but its in vivo ability to generate sustained myogenic tone suggests that the myocytes are well coupled.

### *b) Neuronal control of contractile behaviour*

The innervation of the urethral smooth muscle is more complicated than the detrusor, with both excitatory and inhibitory innervation. In the pig, intramural ganglia, composed of two to 30 neurones, are found in the bladder neck and middle and distal regions of the urethra. In the smooth muscle, and in the vicinity of the striated muscle regions of the intrinsic external urethral sphincter, there are small ganglia, containing two to three neurones, which are vasoactive intestinal polypeptide-, (met) enkephalin- and somatostatin-immunoreactive [129]. Similar ganglia have been demonstrated in the human urethra, and these ganglia also contain nitric oxide synthase, (NOS) and haemoxygenase (the enzyme synthesizing CO), with various degrees of co-localization (fig. 59) [193]. Histochemically evidence demonstrates the presence of cholinesterase-positive putative cholinergic nerves, and immunohistochemical techniques show the presence of tyrosine hydroxylase-containing putative adrenergic nerves [194].

Nerves staining for acetylcholinesterase, vasoactive intestinal polypeptide, catecholamines, substance P, (met) enkephalin, gastrin and somatostatin are also found in the urethral smooth muscle in the pig urethra [129] [194] In the rat urethra (NOS) nerves are very frequent in the smooth musculature together with cholinergic, adrenergic and neuropeptide Y (NPY)-immunoreactive (IR) nerves, whereas vasoactive intestinal peptide (VIP)-IR and calcitonin-gene-related peptide (CGRP)-IR nerves are much less abundant [195].

Acetylcholine and noradrenaline both mediate contraction in the urethra, [196] [197] and both appear to be involved in the excitatory response to intramural stimulation, although the noradrenergic component is the most significant (fig. 60). Both excitatory $\alpha 1$ and $\beta 2$ adrenoceptors are present, and there are distinct subtypes of $\alpha 1$ adrenoceptor subtypes in the human prostatic urethra (fig. 61) [198]. Nitric oxide synthase containing nerves also innervate the smooth muscle, and mediate a fast transient relaxation (fig. 62) [197 - 201]. In the pig, a systematic study has demonstrated that both the density of the nerves and the response to the various transmitters may vary along the length of the urethra [194].

Little information is available on the mechanisms coupling the membrane to the contractile machinery in urethral smooth muscle. In those preparations that show myogenic tone, this can be abolished by L-type Ca channel blockers, suggesting that continuous entry of calcium through these channels occurs in the resting state [197]. Depolarisation of the membrane by increasing extracellular K+ concentrations has variable results – small increases result in contraction, but if the concentration is increased it will often result in a biphasic response, or a pure relaxation. It has been suggested that sufficient depolarisation may result in voltage-dependent inactivation of the L-type calcium channels and relaxation [48]. Relaxation can also be mediated through activation of guanylate cyclase and elevation of intracellular cGMP, through nitrergic nerves or application of NO or NO donors (fig. 63) [3], [202]. An interesting field of research is opening up since it is likely that both the enzyme responsible for the synthesis of cGMP (guanylate cyclase) and the enzyme responsible for its

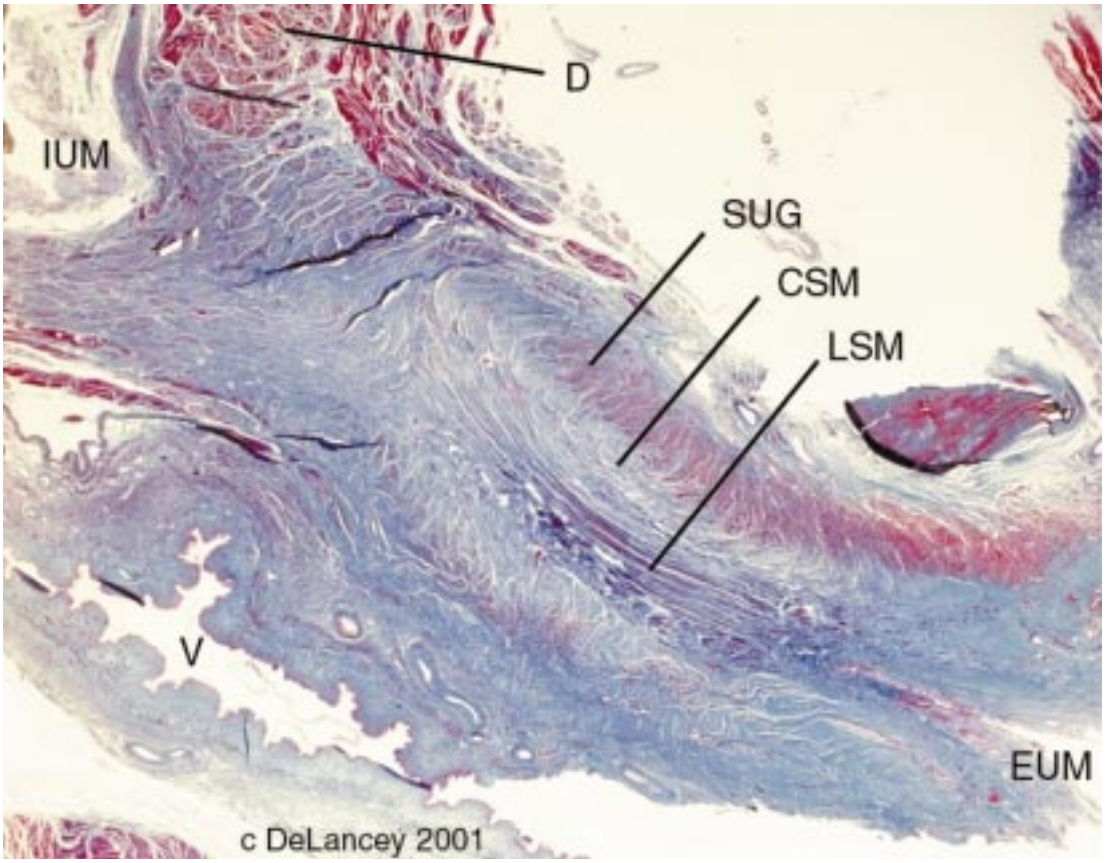



*Figure 56 : Transverse section of the mid-urethra with smooth muscle stain shown on the left and trichrome stain on the right. CSM, circular smooth muscle; LSM, longitudinal smooth muscle; SUG., striated urogenital muscle; V, vagina; EUM, external urinary meatus; IUM, internal urinary meatus*

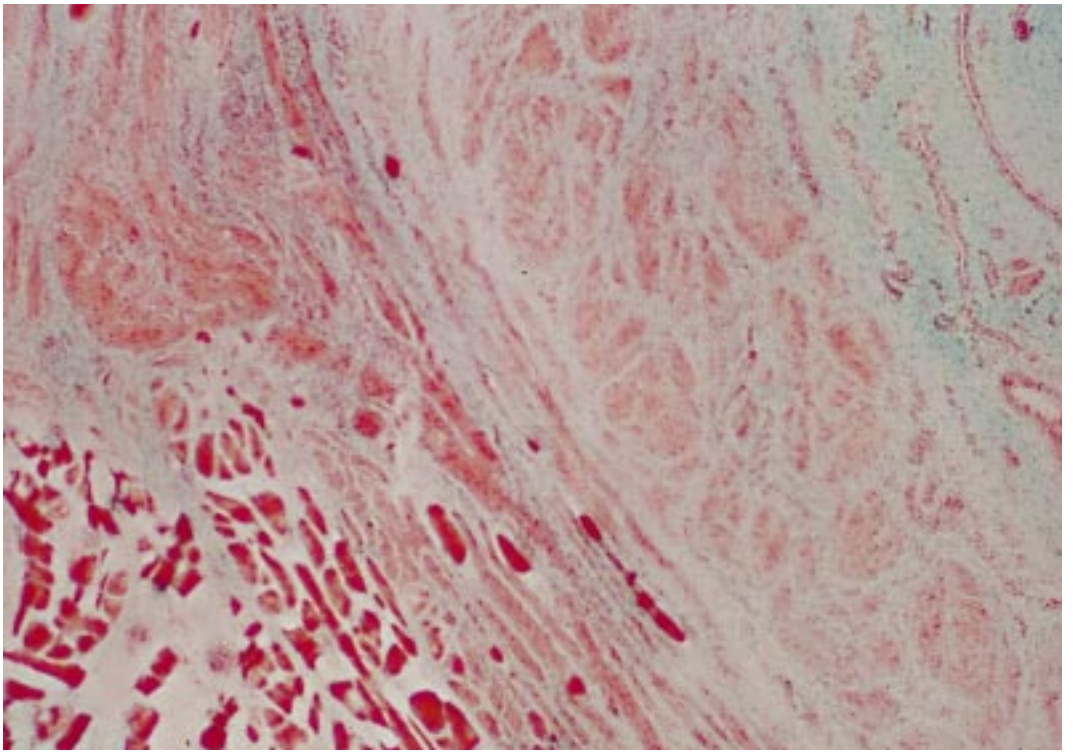


*Figure 57 : Cross section of human urethra. From the outside in you can see striated muscle fibres, then a circular smooth muscle coat in which some striated muscles are intermingled. Within this is a thicker layer of longitudinally oriented smooth muscle, then the lamina propria and the urothelium.*

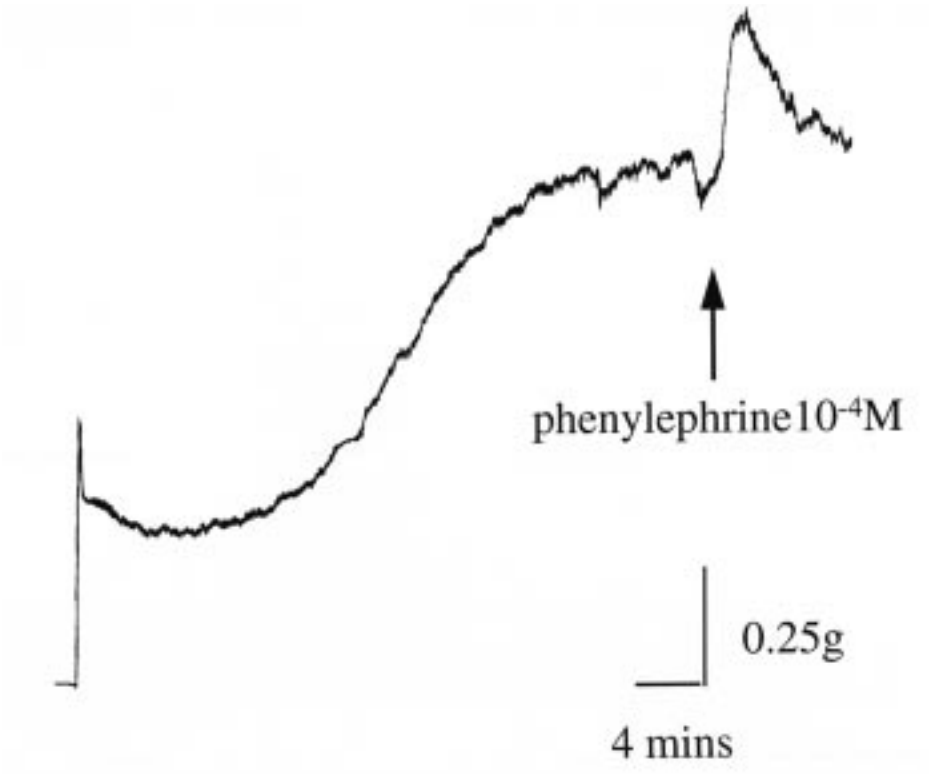
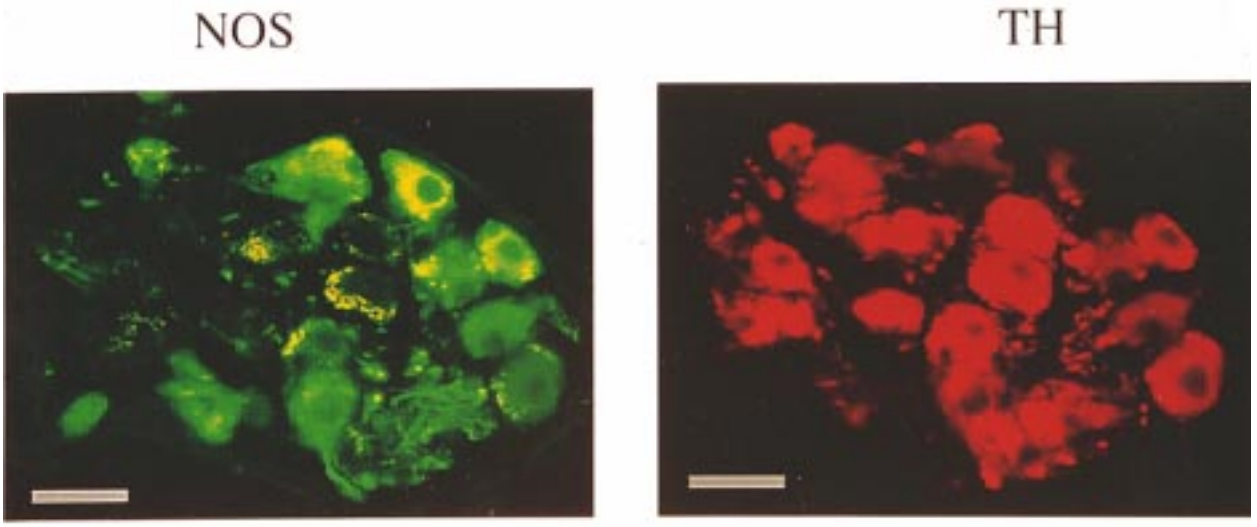



*Figure 58 : Contractile behaviour of a strip of longitudinal smooth muscle dissected from the proximal third of a human female urethra. Note the development of spontaneous myogenic tone, the spontaneous transient relaxations and the contractile response to the alpha agonist, phenylephrine.*




Human female urethra: proximal third, ganglion.
Bar = 100μm

*Figure 59 : Intramural ganglion in the proximal female human urethra showing neurones with positive fluorescence for nitric oxide synthase immunoreactivity (green) and tyrosine hydroxylase immunoreactivity (red). Note heterogeneity of neuronal population. Most neurones are immunoreactive to both enzymes, but others for only one or other. Micrograph kindly provided by Kossen Ho.*

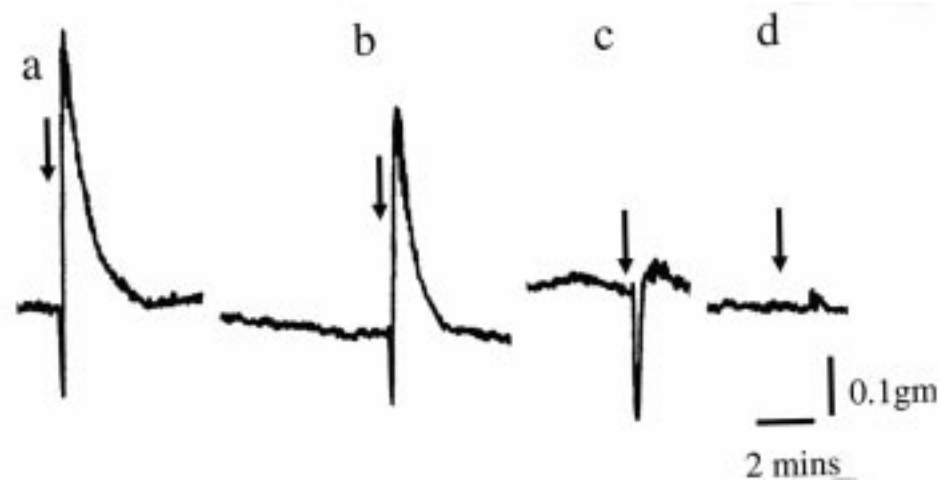
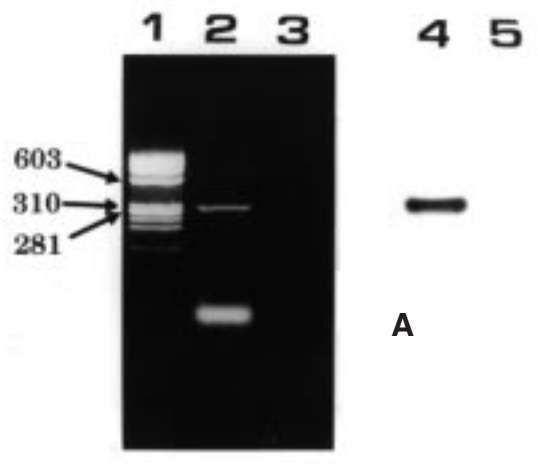
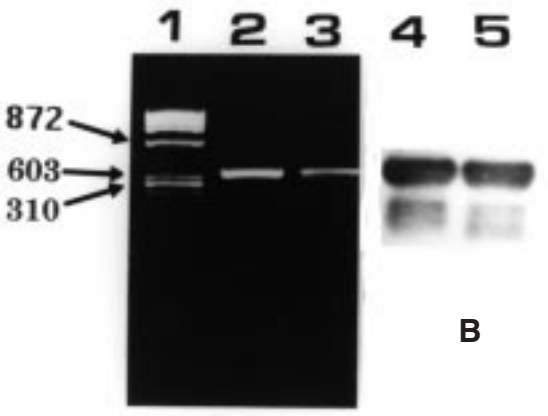



*Figure 60 : Response of human female circular smooth muscle from the proximal urethra to stimulation of the intrinsic nerves (30 V, 0.2 ms, 10 Hz, 5 s). (a) control, note transient relaxation followed by contraction (b) in the presence of atropine ($10^{-6}$ M) to block cholinergic transmission. Note reduced size of contractile response (c) in the presence of atropine and guanethidine ($3.10^{-6}$ M) to block cholinergic and adrenergic transmission. Contractile response abolished, leaving relaxation (d) in the presence of atropine, guanethidine and NOARG ($10^{-5}$ M) to additionally block nitrergic transmission.*



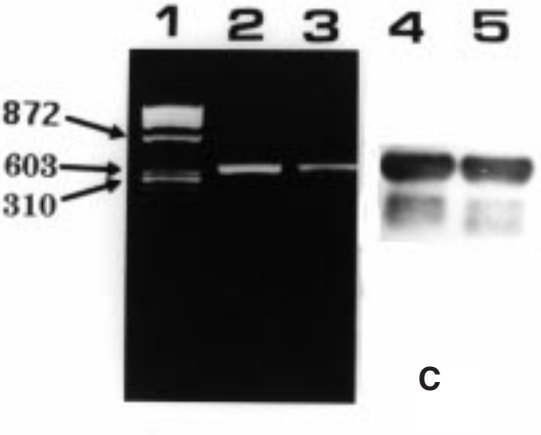




*Figure 61 : Demonstration of the presence of α1a, α1b but not 1d-adrenoceptors in human prostatic urethra. Total RNA was extracted from the prostatic urethra, and amplification of the mRNA for α1a, α1b and α1d-adrenoceptors was carried out using the reverse transcription / polymerase chain reaction (RT/PCR). The figures show the results of agarose gel electrophoresis and size fractionation of the products, with Southern hybridization. Also included are results from human internal iliac artery for comparison. (a) Primers specific for α1a-adrenoceptors were used. (b) Primers specific for α1b-adrenoceptors were used. (c) Primers specific for α1d-adrenoceptors were used. From right to left, the first lanes show a size marker, the second lanes shows RT/PCR products for primers specific for α1-adrenoceptors from the prostatic urethra, the third lanes show products for primers specific for α1-adrenoceptors from the iliac artery. Lanes 4 and 5 show Southern hybridization corresponding to lanes 2 and 3, using oligonucleotide probes for the α1-adrenoceptor sequences Note the presence of α1a and α1d-adrenoceptors but no α1b in the prostate, confirmed by the Southern blots; in contrast the iliac artery shows the presence of α1b and α1d-adrenoceptors but no α1a-adrenoceptors.*

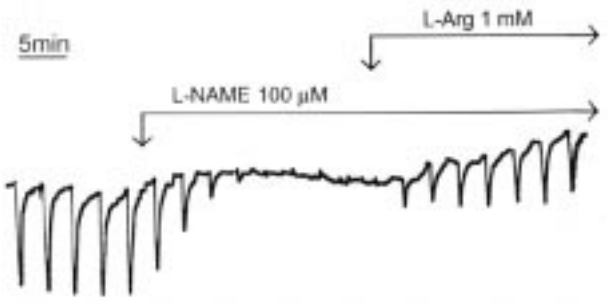



*Figure 62 : Responses of the smooth muscle of the urethra to transmural nerve stimulation. The transient relaxations are abolished by addition of the nitric oxide synthase inhibitor L-NAME (100 µM), and then restored by addition of the amino acid precursor L-arginine (1 mM), suggesting that the relaxations are produced by NO synthesis.*

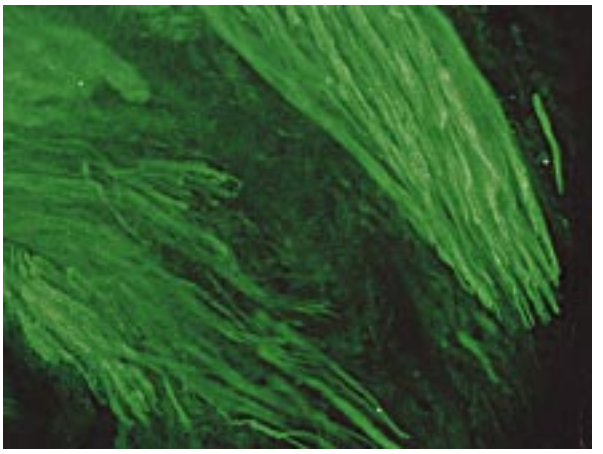


*Figure 63 : Smooth muscle cells from the human prostatic urethra showing immunofluorescence to cGMP after activation of guanylate cyclase by administration of sodium nitroprusside. From Smet et al., 1996* 3 .

breakdown (phosphodiesterase) may be useful targets for new drugs. Indeed inhibition of phosphodiesterase V has been shown to enhance erection in impotent men 203 and it is likely that effects on urinary outflow may be obtained by the use of similar drugs. Further studies will be necessary to investigate whether pharmacological alterations of cGMP levels may prove of importance for the development of new drugs in the treatment of voiding disorders.

### *c) The role of the smooth muscle*

The role of the smooth muscle in the urethra is more problematic than that of the detrusor, because of its orientation. Whereas some of it is circularly oriented, and could play a role in the generation of the resting urethral pressure profile particularly since the area generating the most myogenic tone coincides with the maximum urethral pressure 197 , the thickest layer is longitudinally arranged. Contraction of the longitudinal smooth muscle could play a role in stabilizing the urethra and allowing force generated by the circular muscular elements to occlude the lumen, or in aiding in the opening of the bladder neck during micturition. There is controversy about the relative roles of the urethral smooth and striated circular muscle and the lamina propria, in generating the urethral pressure profile, but it seems likely that both contribute 204 . Blocking striated sphincter activity with nicotinic neuro-muscular blocking agents has variable effects, and may reduce urethral tone, but rarely by more than 40%, suggesting that the smooth muscles are important. Blocking sympathetic tone with alpha-adrenoceptor blockers may also reduce urethral pressure by about a third 205 . There is little evidence for the involvement of the cholinergic innervation in generating urethral pressure: however, the continuous myogenic tone of the circular smooth muscle may contribute.

Damage to the innervation of the urethral smooth muscle, or changes in its properties would again be expected to significantly alter the behaviour of the urethra, and might result both in incontinence during the normal filling phase if the excitatory input is reduced, and detrusor sphincter dyssynergia if the mechanisms producing relaxation of the muscle are affected. However, the considerable difficulties in obtaining specimens of urethral smooth muscle from patients with known urethral dysfunction means that little if any work has been carried out in this area. The smooth muscle is an obvious target for pharmacological control of incontinence, as discussed in the report of the committee.

## 2. Striated muscle

Striated muscle occurs in the walls of the male and female urethra, where it forms a rhabdosphincter which is separate from the periurethral skeletal muscle of the pelvic floor. In the male, the striated muscle extends from the base of the bladder and the anterior aspect of the prostate to the full length of the membranous urethra. In the female, the striated muscle extends from the proximal urethra distally. The striated sphincter is horse-shoe shaped and the muscle cells are smaller than ordinary skeletal muscle, being 15 to 20 µm in diameter.

### *a) Fibre type*

Gosling et al [206] considered these striated muscle fibers to be slow twitch in nature. Others, however, believe that the striated sphincter is a heterogeneous population. Some [207], [208] have examined the fibre types in the striated muscle of the prostatic capsule, since this is easy to obtain, and derives from the rhabdosphincter. They have shown fast and slow fibres to be present. This has been confirmed more recently in rhabdosphincters taken from transplant donors [193]. In the male the rhabdosphincter consists of 35% fast and 65% slow twitch fibres, whereas in the female, 13% of the fibres are fast twitch (fig. 64). The slow twitch fibres could be important in developing sustained tone to occlude the urethra, whereas the fast twitch fibres could be involved in reflex contraction to elevate urethral tone when intraabdominal pressure rises. The majority of the fast twitch fibres and about a quarter of the slow twitch fibres in the intramural striated muscle of the human membranous urethral sphincter show positive staining for nitric oxide synthase in the sarcolemma [193].

### *b) Innervation*

The urethra has a complicated neuro-anatomy, receiving as it does input from both somatic and autonomic pathways. The rhabdosphincter receives somatic input from S2-S4 nerve roots via the pudendal nerve, and the pelvic nerves also enter the muscle [209], [210] In the cat, a triple innervation of the rhabdosphincter has been demonstrated histochemically (see [4], with a somatic, parasympathetic and sympathetic input. Evidence for a triple innervation in the human rhabdosphincter however is not strong, and it is likely that the functional innveration is somatic, although modulation through autonomic innervation cannot be ruled out. In the pig intrinsic external urethral sphincter, vasoactive intestinal polypeptide- and gastrin-immunoreactive nerve fibres have been found bordering a small number of individual striated muscle fibres, while catecholamine-containing nerves were found predominantly in the connective tissue surrounding the striated muscle fibres. Dense populations of acetylcholinesterase-positive nerve fibres are found associated with the striated muscle fibres, with end plates on some of them [129]. It is generally agreed that the motor cell bodies of the nerves supplying the urethral striated muscle lie in a discrete area of the anterior horns of the second, third and fourth sacral segments of the spinal cord collectively known as Onuf's nucleus [50 - 51].

Immunohistochemical studies have demonstrated the presence of nitric-oxide synthase containing nerve trunks and sparse fine nerve fibres running in the intramural striated muscle of the human membranous urethral sphincter, with the fine nerve fibres running parallel to and apparently innervating some striated muscle fibres [193]. Some nerve trunks containing tyrosine hydroxylase immunoreactivity course through the striated muscle, but do not seem to be specifically associated with the striated fibres, and may just been route to smooth muscle, or innervating blood vessels.

In vitro studies on the physiology of urethral striated muscles are rare. Recordings of contractile activity in rings of guinea-pig urethra have been made [211] but the results were difficult to interpret because the activity included contraction of both striated and smooth muscle activity. In vivo studies have investigated the effects of nicotinic receptor blockade on the urethral pressure in the pig [118], the dog [212] and in humans [204], demonstrating a role of the striated muscle in generating resting urethral pressure. In the dog pudendal but not pelvic nerve stimulation produces contraction of the striated muscle. Electromyography can be used to record activity in the human striated sphincter

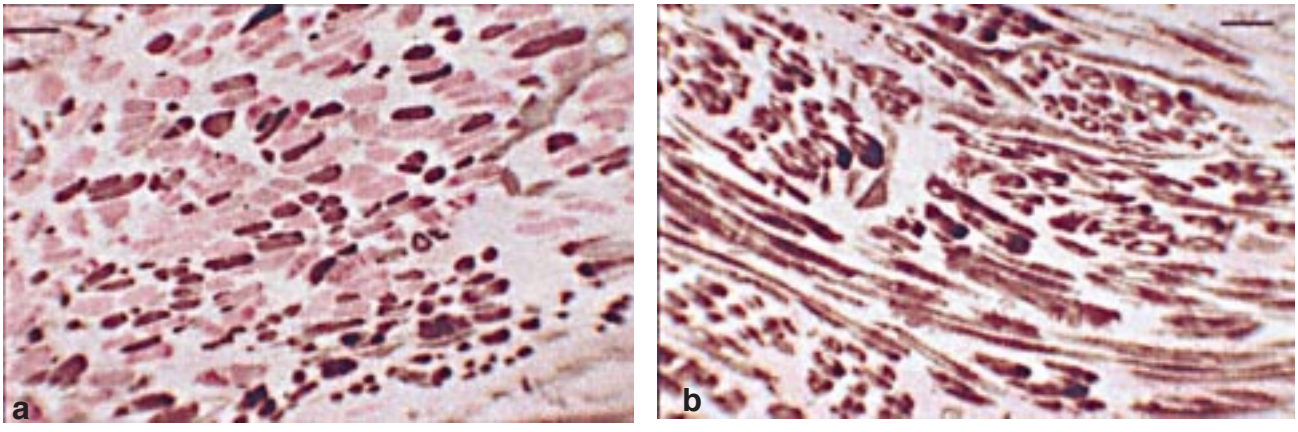



***Figure 64 : Sections from the proximal third of the human male urethra (a) and (b) the female membranous urethra stained for ATPase at pH 4.3, in which the slow twitch fibres appear darkly stained. Note that most of the female striated muscle is slow twitch, whereas a significant proportion of the male fibres are fast twitch.***

during filling, using concentric needle electrodes. Recordings show a steady firing of the muscle fibres at between 2-8Hz (fig. 65) [213]. Coughing, or other manoeuvres that increase intra-abdominal pressure produce a marked increase in the number of units firing. Abnormal activity can be recorded from patients with various disorders of urethral sphincter innervation [214].

### *c) The role of the striated muscle*

The main functions of the urethral striated muscle will be in helping to generate sustained urethral pressure during bladder filling, and providing reflex increases in urethral wall pressure to prevent leakage of urine when abdominal pressure rises. It seems likely that the slow twitch fibres will be more involved in the sustained generation of urethral pressure, and the larger component of these fibres in women may be a reflection of the shorter urethral length. As with the smooth muscle, it should be remembered that failure to switch off the tonic excitation of the striated muscle during micturition can result in detrusor sphincter dyssynergia, which can be a serious problem. The fast twitch fibres, with their greater fatigueability, have the properties necessary to mediate a rapid increase in urethral pressure to prevent stress incontinence.

## 3. URETHRAL LAMINA PROPRIA

### *a) Structure*

The urethral lamina propria extends from the longitudinal smooth muscle layer to the urothelium, and fills the lumen of the urethra. The lamina propria is lined proximally with a surface membrane consisting of transitional epithelium, and mucous glands have been described along its length in the female urethra [215]. Between this and the smooth muscle layer is an extensive stroma

***Figure 65 : Recording of the EMG of the urethral striated muscle in a normal woman during the filling phase of the micturition cycle. The recording was made with a concentric needle electrode. The electrode is picking up signals from at least four different motor units, distinguishable by the amplitude of the spikes. A large increase in the number of units firing is seen during manoeuvres that increase intra-abdominal pressure. From Fowler & Fowler, 1981 [73].***

surrounding a prominent vascular plexus. The urethral stroma has been less extensively studied than the bladder stroma, but it is known to contain primarily longitudinally arranged collagen fibres and elastin fibres [216 - 217]. More detailed studies have been made of the urethral stroma in female dogs, [218] and the female pig [219]. In the dog, the stroma comprises almost 80% of the total volume in the proximal urethra, and contains abundant collagen fibrils and longitudinally orientated elastic fibres. In the pig, the lamina propria is maximally developed near the vesico-urethral junction, where it occupies over 25% of the cross sectional area of the urethra. There is a dense capillary network under the mucosa in an areolar connective tissue of loose collagen fibres. The submucosa comprises a dense irregular connective tissue with collagen, elastin and fibrocytes, and an extensive vascular plexus. Small bundles of longitudinally arranged smooth muscle cells are also seen. The vascular plexus consists of predominantly longitudinally oriented blood vessels with abundant muscular arteries, large arterioles and thin-walled venules. The elastic fibres run mainly in a longitudinal direction near and amongst the longitudinal smooth muscle surrounding the mucosa. In the human female urethra the relative amount of blood vessels and striated muscle decreases with age [220]. The amount of smooth muscle remains unchanged, and the relative amount of connective tissue increases. Some stress-incontinent women seem to have a general decrease in tissue collagen concentration [221].

### *b) Vascular filling*

The vascular filling of the urethral lamina propria is thought to be of importance for urinary continence although the magnitude of its contribution to continence is still not understood [222]. Oestrogen is known to increase the urethral blood flow and it is likely that part of the possible benefit of oestrogen on stress urinary incontinence is due to an increase in the urethral blood flow resulting in an increased distension of the lamina propria blood vessels [223 - 224]. Impaired arterial blood supply to the urethra decreases the intraluminal pressure (fig. 66) [222] but it is presently not known whether it is the decrease in vascular filling or the urethral hypoxia which mediates the decrease in urethral pressure. Recently it was suggested that both these mechanisms may be involved, since it was shown that the initial drop in urethral pressure was mediated via decreased vascular filling whereas the later phase was due to an hypoxic effect on the urethral smooth muscle [224].

## 4. PARAURETHRAL TISSUE

Results are divergent regarding connective tissue outside the urethra. Paraurethral tissue biopsies from preme-

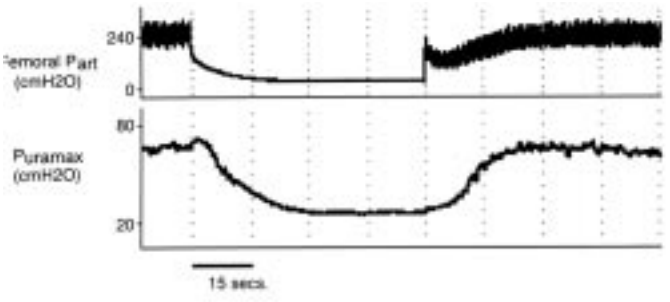



*Figure 66 : In vivo recording of the urethral pressure and the left femoral artery pressure on occlusion of the terminal aorta in the pig. Note that the urethral pressure falls as the arterial inflow is cut off.*

nopausal women with stress incontinence contained 30% more collagen and the diameter of the fibrils are 30% larger than in controls [225]. Postmenopausal stress incontinent women, on the other hand, have no difference in collagen concentration compared with their age controls [226]. Others have, however, found a decreased periurethral collagen concentration [227] and a decreased collagen type I to type III ratio in stress incontinent patients [228]. The macroscopic anatomy of these tissues is involved in overall support of the pelvic organs and will be discussed later in this chapter.

## VIII. MODULATORY SYSTEMS

### 1. Nitric Oxide Syntase

In recent years, a large number of studies have shown that the gaseous molecule nitric oxide (NO) is an important signalling molecule in the urogenital tract. NO is produced by a family of isoenzymes, NO synthases (NOS) and the amino acid L-arginine is the substrate for NOS forming NO and L-citrulline in equal amounts [229]. Several types of NOS have been found and cloned. Two types have been shown to be dependent on free calcium for their NO formation and they were named endothelial (eNOS) and neuronal (nNOS), respectively after the cell type in which they were first located [229]. The third isoform which has been cloned is iNOS. It differs from the previous isoforms in that its activity is not dependent on free calcium and that it is usually only expressed after stimulation by various cytokines [229], iNOS was first found in macrophages and is known to be activated during host defense reactions.

The enzyme forming NO from L-arginine has been found in various parts of the lower urinary tract. In the bladder nNOS has been found in perivascular nerves and in nerves innervating the detrusor muscle [230]. In the vascular endothelium eNOS has been found [231]. In the urinary tract iNOS is not expressed during normal conditions but may be found in the human bladder after treatment with BCG and various cytokines [148] [149]. Thus, NOS has been found in several different cell types in the lower urinary tract and NO may serve to mediate several different functions. NO has been suggested as a mediator of nonadrenergic non-cholinergic nerve-induced relaxation in the lower urinary tract in rabbit, [232] [233] pig, [234] sheep, [235] dog [201] and man (fig. 63) [236], [237]. The opening of the bladder neck and the dilatation of urethra during the micturition reflex is also likely to be NO mediated. Although the evidence for a role of NO in the urethra and bladder neck are compelling there is still only sparse evidence for a role by NO in the detrusor muscle. Inhibition of NOS only marginally affects nerve-induced smooth muscle activity in the human detrusor [238] [239] and the detrusor has a low sensitivity to NO, making it less likely that NO has a role as a relaxant neurotransmitter in this tissue. In spite of the above, several other studies have indicated a pathophysiological role for NO in the urinary 'urge syndrome'. Thus, in rats in vivo NOS inhibitors elicit bladder hyperactivity and decreased bladder capacity [234] and in nNOS knock-out mice hypertrophic and dilated bladders and dysfunctional urinary outlets are found [231]. Increased detrusor contractions may originate from the bladder outlet region rather than from the detrusor itself [240]. Such contractions can be observed in the majority of men with outflow obstruction [241]. The mechanism behind this overactivity is not known, but it is likely that lack of an inhibitory mediator in the detrusor or the outlet region, causing an increased afferent nerve activity, may be involved. Since studies on NOS activity and NOS histochemistry have revealed a marked increase in NOS localisation in the bladder neck and urethra as compared to the detrusor it is likely that impaired NOS activity in this region may result in the development of micturition disorders. This suggests that in the detrusor NO may modulate the effects of other transmitters or that it has an afferent function as has been shown in the central nervous system. Taken together the studies indicate that a decrease in NO production leads to an increase in urinary urge symptoms. In agreement with these studies daily treatment with the NO precursor L-arginine decreases urinary symptoms in patients with interstitial cystitis [242].

NO may also be involved in the increase in voiding disorders seen in menopausal women. It is known that oestrogen treatment significantly reduces the prevalence of voiding disorders in elderly women and it has been shown that oestrogen increases the expression of both eNOS and nNOS in the uterine artery, kidney, heart, oesophagus and cerebellum [243]. Subsequent studies have also shown that oestrogen may increase

the calcium-dependent NOS activity in the guinea pig urinary bladder, [244] but further studies are needed to evaluate the role of NO in voiding disorders in post-menopausal women.

In conclusion, accumulating evidence suggests that alterations in the L-arginine/NO pathway is involved in several pathophysiological mechanisms leading to voiding disorders. Further studies are needed to clarify all details of the involvement of NO in these voiding disorders but clearly the L-arginine-NO-cGMP pathway will be of great interest as a target for future drugs in this area.

## 2. Afferent innervation

### *a) Overview*

Afferent nerves provide background information to the central nervous system arising from the lower urinary tract which is required for the co-ordinated activation of all reflexes related to urine storage and voiding [245, 246]. As such, afferents have potential value as targets for drug therapy for urinary incontinence [72, 247, 248]. There is ample evidence indicating that afferents are heterogenous. This heterogeneity has been verified by neurophysiological techniques, documenting the variable conduction velocities of lower urinary tract afferents and establishing the relation between this parameter and their sensitivity to applied mechanical and chemical stimuli: [249 -253] these studies have revealed, among others, the existence of a population of 'silent' nociceptors which are mechanically insensitive in the normal bladder but display a novel mechanosensitivity after induction of inflammation [251]. This hereogeneity has also been documented by anatomical techniques, based on different diameters of afferent neurones and by demonstrating that primary sensory neurones innervating the lower urinary tract express a variety of transmitters/mediators in set combinations (chemical coding) [254 - 257].

### *b) Sensory-motor nerves*

One of the most intriguing criteria which detects the heterogeneity of lower urinary tract afferents is a pharmacological one, based on the selective stimulant and desensitizing actions of capsaicin and related vanilloid receptor agonists [247, 248, 258 - 260]. The capsaicin-sensitive primary afferent neurones (CSPANs) are present in the mammalian bladder, project to the spinal cord [261 - 262] and regulate the micturition threshold: [263 - 264] especially important is their ability to subserve the spinal vesico-vesical reflex which, [265 - 266] after chronic spinal cord transection, mediates the supraspinal vesico-vesical reflex underlying normal micturition. Moreover CSPANs are involved in signalling bladder pain [267] and activate cardiovascular reflexes arising from the lower urinary tract [268]. A further distinct feature of CSPANs is their ability to release mediators, notably peptides of the tachykinin family from their central and peripheral endings [74, 259]. Tachykinin release at spinal cord level activates micturition-related reflexes [269]. Tachykinin release from sensory nerves in the bladder wall can induce a prolonged spasm [73, 270] and inflammation (neurogenic inflammation) which is thought to be of pathogenic relevance for certain forms of cystitis [71, 271]. CSPANs are present in the human bladder [71] and their pharmacological manipulation promises to have useful diagnostic and therapeutic applications [272 - 274].

It appears that different subtypes of the capsaicin (vanilloid) receptor may exist, and evidence has been obtained for their expression by cells other than CSPANs, such as mast cells [275] and urothelial cells. Subtype 1 of the capsaicin receptor/ion channel has recently been cloned [276].

It should be noted that CSPANs, as identified pharmacologically [259, 277] do not overlap exactly with any of the sub-population of afferent nerves which can be distinguished on the basis of neurophysiological or anatomical criteria: although most CSPANs have axons conducting in the C-fibre range, yet not all C-fibres are capsaicin-sensitive and some CSPANs have conduction velocities in the Aδ range [278, 279]. Moreover although CSPANs have small dark, type B somata, not all primary sensory neurones of this type are capsaicin-sensitive [254].

# IX. MACROSCOPIC ANATOMY OF THE MUSCULAR PELVIC FLOOR

The bony pelvic ring lies inferior to the abdominal cavity and the levator ani muscles span this space. The borders of the opening spanned by the pelvic floor are the pubic bones anteriorly, the ischial spines laterally, and the sacrum posteriorly. Between the pubis and the spines lie the tendineus arches of the levator ani and the pelvic fascia. The sacrospinous ligament and its overlying coccygeus muscles lie between the spine and the sacrum. It is this polygonal opening that must be closed by the levator ani muscles.

From an organizational standpoint, the pelvic floor consists of two specific components, namely the levator ani and the coccygeus. The latter lies in the same morphological plane as the former and completes the pelvic floor posteriorly. The coccygeus forms a triangular structure the apex of which attaches to the spine of the ischium. From the ischial spine the coccygeus

forms a fibromuscular sheet which fans out medially and attaches to the lateral surface of the coccyx and the fifth segment of the sacrum. In actuality the coccygeus is nothing more than the musculotendinous internal surface of the sacrospinous ligament to which it is intimately attached. Unlike animals with mobile tails, the coccygeus is vestigial in humans and does not contribute to active movement of the pelvic floor.

### 1. Levator ani

In practical terms the pelvic floor is synonymous with the levator ani since this muscle forms the effective contractile support structure of the region. The muscle forms a broad thin sheet attaching anteriorly to the posterior surface of the body of the pubis and suspended laterally from the pelvic wall as far posteriorly as the ischial spine. Between the pubis and ischial spine the muscle is directly attached to (or sometimes slung from) the fascia covering the medial surface of the obturator internus. Anteriorly the levator ani is absent in the midline so that a fat-filled space containing numerous vessels lies immediately behind the pubic symphysis. That part of the muscle attaching to the pubis forms the medial component of the levator ani. In the male the most medial of these fibres from the pubis attach to the perineal body behind the prostate to form the levator prostate. In the female these medial fibres attach to the lateral vaginal wall (fig. 67) to form the so-called pubovaginalis. Other fibres from the pubis attach to the anorectal flexure where they fuse with the deep part of the external anal sphincter to form the puborectalis. More laterally placed fibres run from the pubis and the fascia covering obturator internus and are named pubococcygeus.

That part of the levator ani arising from the lateral wall of the pelvis posteriorly to the ischial spine is named the iliococcygeus. The distinction between the end of the pubococcygeus and the start of the iliococcygeus is arbitrary since one merges imperceptibly with the other as a continuous sheet of muscle. Nevertheless the fibres of iliococcygeus run medially at different angles of obliquity to merge with the component parts of the pubococcygeus.

It is generally recognized that parts of the levator ani collectively play an important role in maintaining the position of the pelvic viscera. In the female the attachments of the levator ani to the vagina and external anal sphincter are responsible, on contraction, for the anterior movement of these viscera towards the pubic symphysis [280 - 281]. On contraction of the pelvic floor, this anterior movement of the vagina produces occlusive forces on the urethra resulting in its forward displacement and compression against the posterior surface of the pubis. Contraction of the levator ani is responsible for the compression of the urethra at a site distal to the anatomic location of the intramural urethral striated sphincter. Thus, the levator ani assists this sphincter not only to ensure continence, but also to produce forceful occlusion of the urethra such as occurs during coughing [282].

### 2. Innervation of the levator ani

The levator ani is innervated by somatic nerve fibres which emanate primarily from sacral root S3, to a lesser extent from S4 and minimally from S2 to form the pudendal nerve [283]. The pudendal nerve is a mixed nerve carrying both motor and sensory fibres and is derived from the sacral plexus. Initially the pudendal nerve lies superior to the sacrospinous ligament lateral to the coccyx. The nerve leaves the pelvis, crossing the ischial spine to gain the ischiorectal fossa via the lesser sciatic foramen. It extends forward in a fibrous tunnel (Alcock's canal) on the medial side of the obturator internus muscle and distally gives rise to branches which supply the levator ani and the membranous urethra. Some variation occurs in the pudendal nerve peripheral anatomy.

## X. SUPPORT OF THE URETHRA AND PELVIC ORGANS

### 1. Support of the pelvic organs

In women, a special series of problems arise because the female pelvis and its supportive structures must accommodate vaginal birth. This results in a series of design compromises that must trade off the importance of pelvic organ support for the extra room needed to deliver the large-headed human fetus [284].

The pelvic organs are supported by a combination of muscle and connective tissue. The levator ani muscles have already been discussed and the connective tissue attachments will now be considered. An overview of this anatomy is shown in figure 68. The pelvic organs are attached to the pelvic walls. The connecting tissues are called the endopelvic fascia, a heterogeneous group of tissues including collagen, elastin, smooth muscle, blood vessels, and nerves [285 - 286]. It is common to speak of the fasciae and ligaments separately from the pelvic organs as if they had a discreet identity, yet unless these fibrous structures have something to attach to (the pelvic organs), they can have no suspensory effect.

The overall geometry of this tissue determines their mechanical function forming the endopelvic fascia attaches the uterus and vagina to the pelvic wall bilate-

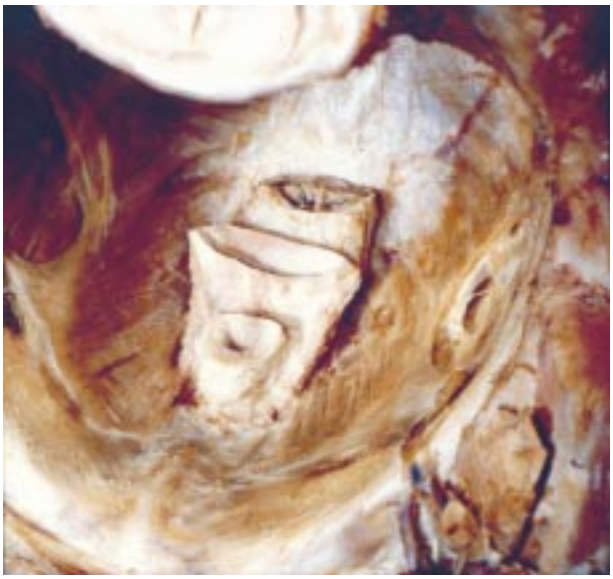


*Figure 67 : Female urethra and its relationship to the vagina and the levator ani muscles.*

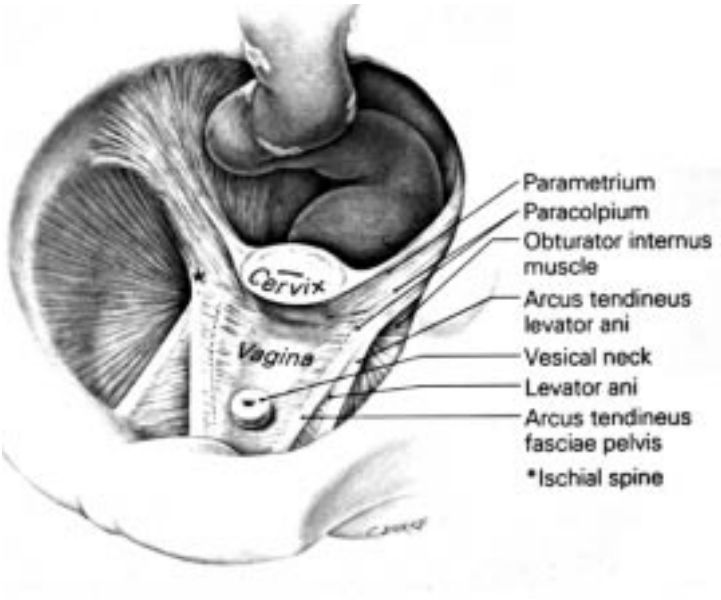



*Figure 68 : Anatomical supports of the cervix and vagina after removal of the bladder and uterine corpus. (copyright DeLancey 2000).*

rally. This fascia forms a continuous sheet - like mesentery extending from the uterine artery at its cephalic margin to the point at which the vagina fuses with the levator ani muscles below. The part that attaches to the uterus is called the parametrium and that which attaches to the vagina, the paracolpium.

The parametria referred to clinically the cardinal and uterosacral ligaments 285 - 287. These are two different parts of a single mass of tissue. The uterosacral ligaments are the visible and palpable medial margin of the cardinal-uterosacral ligament complex and is invested with a considerable amount of smooth muscle. The paracolpium, as we will see in subsequent paragraphs, attaches the vagina to the pelvic walls in a more direct manner.

Although we name these tissues “ligaments” and “fascia” they are not the same type of tissue seen in the “fascia” of the rectus abdominus muscle or the ligaments of the knee; both of which are made of dense regular connective tissue. These supportive tissues consist of blood vessels, nerves and fibrous connective tissue and can be thought of as mesenteries that supply the genital tract bilaterally. Their composition reflects their combined function as neurovascular conduits as well as supportive structures.

There are regional variations in these tissues that explain the differences between the types of pelvic support defects seen in women with pelvic organ prolapse (fig. 69). The upper third of the vagina has the same suspensory tissues as the uterus. These long fibres elevate the upper portion of the vagina after the uterus has been removed during hysterectomy. 288. In the middle third of the vagina the anterior and posterior vaginal walls are connected laterally to the pelvic walls. Continuous with the parametrium when the uterus remains in situ, this upper portion (Level I) consists of a relatively long sheet of tissue that suspends the vagina by attaching it to the pelvic wall. In the mid-portion of the vagina, the paracolpium attaches the vagina laterally and more directly to the pelvic walls (Level II). Ventrally, the vagina is attached to the arcus tendineus fascia pelvis. The combination of the vaginal wall and its attachments to the fascial arch comprise the structural layer that supports the bladder base and urethra. Dorsally, the vagina is attached to the inner surface of the levator ani muscles, a structural arrangement that helps to restrain the rectum from being displaced forward.

These attachments, that stretch the vagina transversely between the bladder and rectum, have functional significance. The structural layer that supports the bladder (pubocervical fascia) is composed of the anterior vaginal wall and its attachment through the endopelvic fascia to the pelvic wall. It is not a separate layer from the vagina as sometimes inferred, but is a combination of the anterior vaginal wall and its attachments to the pelvic wall. Similarly, the posterior vaginal wall and endopelvic fascia (rectovaginal fascia) form the restraining layer that prevents the rectum from protruding forward blocking formation of a rectocele as will be discussed in the next section.

In the distal vagina (Level III) the vaginal wall is directly attached to surrounding structures without any intervening paracolpium. Anteriorly the vaginal wall fuses with the urethra and is attached to the arcus tendineus,

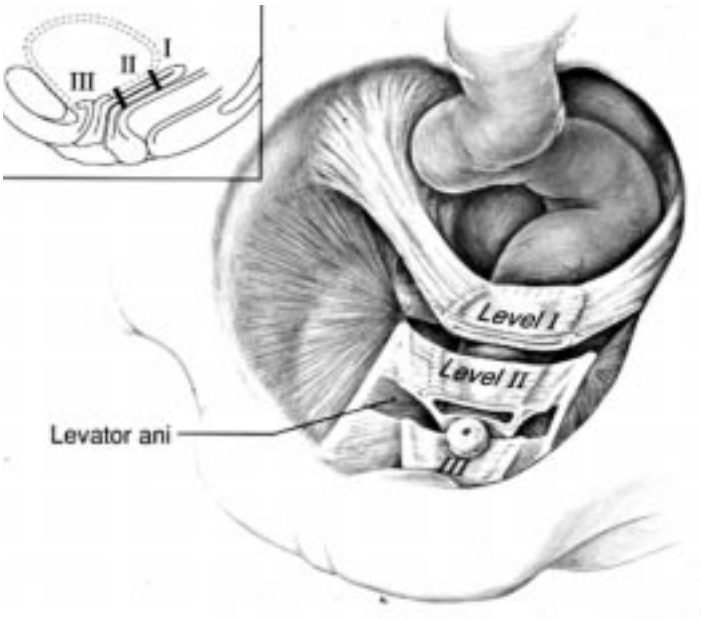



*Figure 69 : Different regions of vaginal support. Note this illustration shows anatomy after hysterectomy with hysterectomy scar shown at the vaginal apex. (from DeLancey 1992)*

posteriorly it fuses with the perineal body whose position is maintained through connections to the ischiopubic rami by the perineal membrane. Laterally it is attached directly to the levator ani muscles by the fibres of Luschka.

Damage to the upper suspensory fibres of the paracolpium causes a different type of prolapse from damage to the mid-level supports of the vagina. Defects in the support provided by the mid-level (pubocervical and rectovaginal fasciae result in anterior and posterior vaginal wall defects cystocele and rectocele) while loss of the upper suspensory fibres of the paracolpium and parametrium is responsible for development of vaginal and uterine prolapse. These defects occur in varying combinations and this variation is responsible for the diversity of clinical problems encountered within the overall spectrum of pelvic organ prolapse.

## 2. Urethral and anterior vaginal wall support

Urethral support is important to stress continence in women, as support in symptomatic women may be inadequate [289]. This support is supplied by a combination of connective tissue and muscle arranged to resist the downward force created by increases in abdominal pressure [290] The urethra lies adjacent to and is intimately connected with the anterior vaginal wall. The connections of the vagina and urethra to the levator ani muscles and the arcus tendineus fascia pelvis determine the structural stability of the urethra.

The arcus tendineus fascia pelvis is a fibrous band that is stretched between a fine tendon-like origin from the pubic bone anteriorly to an attachment to the ischial spine. The endopelvic fascia and anterior vaginal wall form a layer that supports the urethra and vesical neck by connecting to the arcus tendineus (fig. 70). In this region, the medial portion of the levator ani muscles has a direct connection to the endopelvic fascia and vaginal wall [291]. This muscular attachment permits contraction of the levator ani muscles to stabilize the urethra during a cough.

Urethral support, therefore depends on both connective tissue and muscle action. If the connective tissue fails then the urethral supports cannot stay in their normal alignment and stress incontinence is often the result. Conversely, if the muscles are damaged, as has been shown in MR images [292] their action in supporting the urethra may be lost. Recent evidence shows that in primigravid women with stress incontinence, urethral support is preferentially lost during a cough while mobility during valsalva is no different than in continent women. [293]

## 3. Support of the posterior vaginal wall and rectum

The distal rectum abuts with the dense connective tissue of the perineal body [294]. The perineal body represents the central connection between the two halves of the perineal membrane (urogenital diaphragm). When the distal rectum is subjected to increased force directed caudally, the fibres of the perineal membrane become tight and resist further displacement. These fibres derive their lateral support from their attachment to the pelvic bones at the ischiopubic rami (fig. 71). The ability of this layer to resist downward displacement depends on the structural continuity between the right and left sides of the perineal membrane.

The connection between the two halves of the perineal membrane extends cranially for a distance of approximately 2 to 3 centimeters above the hymeneal ring. It is thickest and densest in the distal perineal body becoming progressively thinner towards its cranial margin. The lateral margin of the perineal body contains the termination of the bulbocavernosus muscle and terminations of the medial fibres of the levator ani muscle.

The mid-portion of the posterior vaginal wall (Level II) is attached on either side of the rectum to the inner surface of the pelvic diaphragm by a sheet of endopelvic fascia. These fascial sheets attach to the posterior lateral vaginal wall where the dorsally directed tension results in a posterior vaginal sulcus on each side of the rectum (fig. 72). These endopelvic fascial sheets prevent the ventral movement of the posterior vaginal wall. The majority of the endopelvic fascia fibres atta-

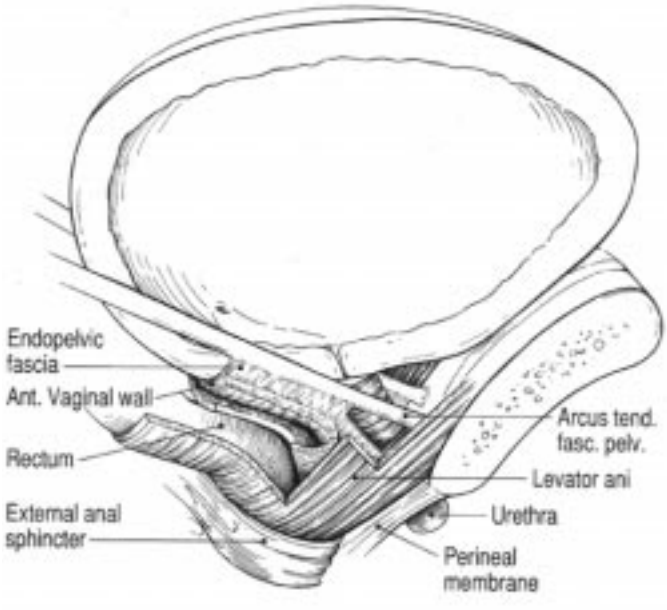



*Figure 70 : Lateral view of the structures supporting the urethra and vesical neck. Portions of the levator ani, lateral vaginal wall and endopelvic fascia have been removed to show midline structures (DeLancey 1994).*

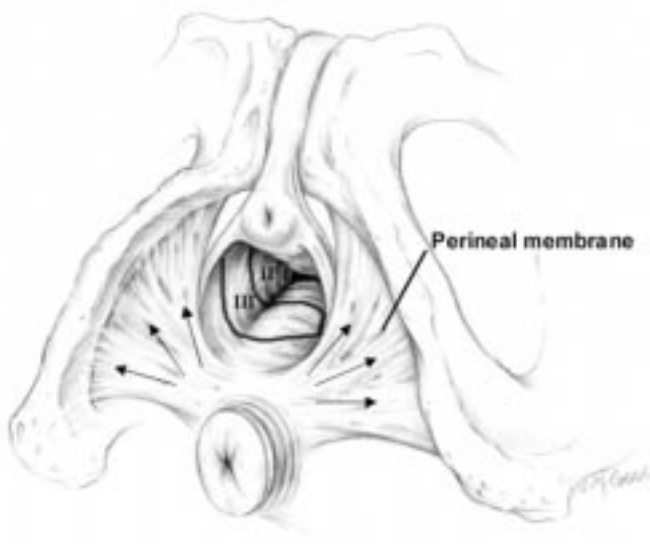



*Figure 71 : Support of the perineal body by the perineal membrane by its connection to the ischiopubic rami (DeLancey 1999)*

ch to the vaginal wall, with only a few fibres passing from one side to the other.

The Level II and Level III supports are continuous with one another. Force applied to the anterior rectal wall in Level II is resisted by the posterior vaginal wall and its attachments to the inner surface of the pelvic diaphragm. Pressure applied to the perineal body in a caudal direction in Level III is resisted, not only by the perineal membrane, but also by the connection of the upper vaginal wall to the Level II attachments that help hold the cranial end of the perineal body (Level III) in place.

## 4. Connective tissue of the pelvic floor

Considerable confusion persists with respect to the functional significance of the various fascial and ligamentous structures which have been described and implicated in the support of pelvic organs [291], [295 - 297]. Some authors have commented on the amount of connective tissue within human pelvic floor muscle compared with quadrapedal animals and have sought associations with the assumption of an upright posture [281]. However, the connective tissue component of the levator ani has not received the same attention as the striated muscle component, although several workers have suggested that deficient or abnormal collagen may be the cause of pelvic floor dysfunction in humans [298], [299]. A histological study of vaginal fascial connective tissue carried out in women with and without uterine prolapse reported abnormal histological changes in 7 out of 10 patients with uterine descent [300]. Other workers have shown a significantly higher incidence of pelvic organ prolapse in women with hypermobile joints compared with a control group with no clinical joint laxity, further implicating abnormalities of collagen in pelvic floor dysfunction [301]. Clearly, further studies are required in order to examine the role of contributing factors, such as age, oestrogen activity, obesity, parity and delivery, as well as sexual activity and physical work, on the structure and function of the connective tissue components of the levator ani. Data of this type may prove invaluable in furthering our understanding of the normal functioning of the pelvic floor.

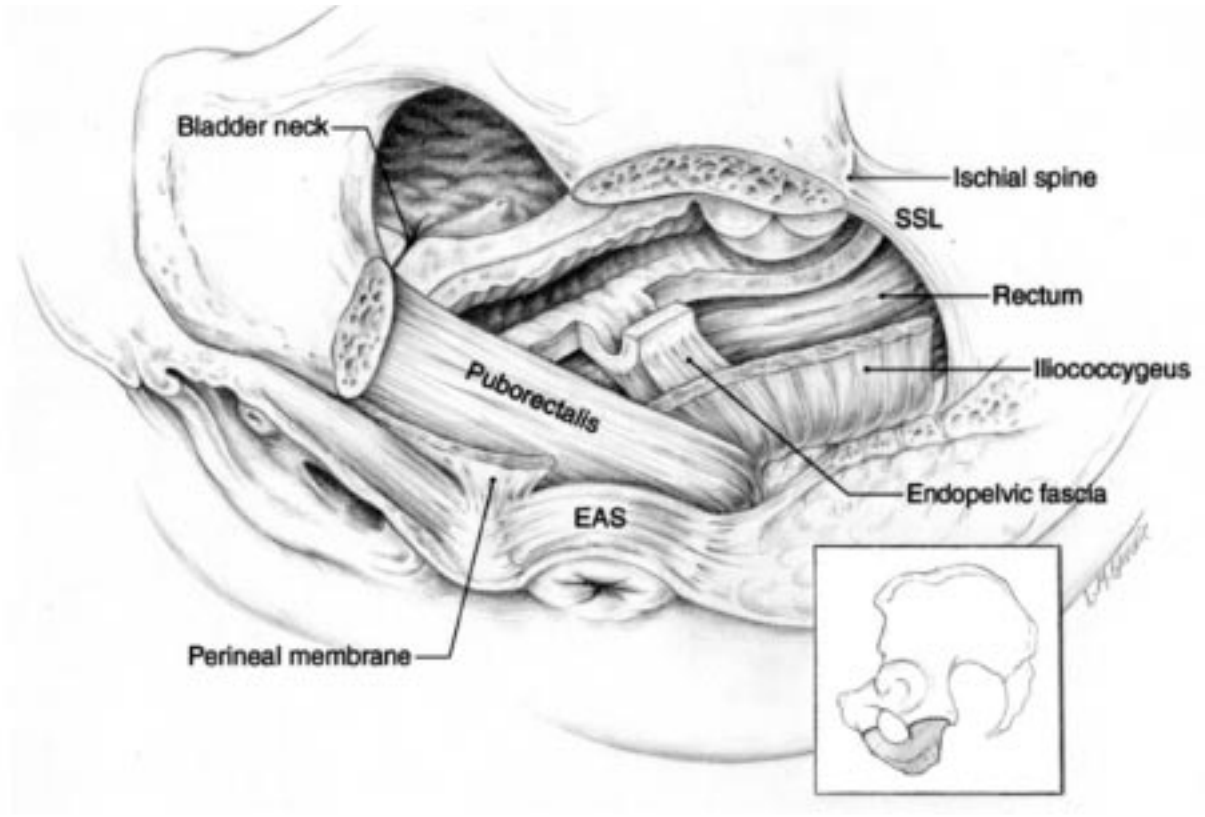



*Figure 72 : Lateral view of the pelvic floor structures. The ischium has been removed as have portions of the iliococcygeus muscle and lateral vaginal wall. Note attachment of the vaginal wall by the endopelvic fascia to the inner surface of the iliococcygeal muscle. (DeLancey 1999)*

## XI. EFFECTS OF AGE AND PARTURITION ON PELVIC FLOOR MORPHOLOGY

Both age and vaginal delivery have been shown to affect the morphology of the pelvic floor muscles. A study on biopsy samples from a group of women with genuine stress incontinence has shown that both the number and diameters of slow and fast twitch muscle fibres decrease with increasing age [302]. Using single fibre electromyography, it has also been proposed that partial denervation of the pelvic floor may occur as a consequence of vaginal delivery [303]. Furthermore, women with urinary stress incontinence or genitourinary prolapse (or both) are more likely to demonstrate electrophysiological evidence of partial denervation of the levator ani than are asymptomatic women. These findings imply that nerve damage is an important aetiological factor responsible for weakness of the pelvic floor. Delayed nerve conduction times are indicative of damage which has been localised to the terminal branches of the pudendal nerves supplying the pelvic floor in women with stress incontinence.

Histochemical evidence for the occurrence of partial denervation and subsequent reinnervation is provided by the clustering together of fibres that are all of the same type [304]. This fibre-type clustering has been observed in biopsy samples of levator ani removed from patients with rectal prolapse and faecal incontinence [305], [306] and in patients with urinary stress incontinence and genital tract prolapse [307]. However, similar fibre-type groupings have been observed in biopsy samples from nulliparous asymptomatic women. On the assumption that these results are representative of normal, it may be that the fibre-type grouping in the levator ani is normal and thus differs from that of a typical limb skeletal muscle. In the event that such an arrangement occurs in the undamaged levator ani, it is evident that fibre-type clustering demonstrated using histochemical methods cannot be regarded as indicative of partial denervation with reinnervation. In this context a recent study [308] has failed to demonstrate histochemical evidence for partial denervation of the levator ani although structural features of muscle cell damage are reported in association with aging and with vaginal childbirth. Morphological features such as centrally placed nuclei, fibre splitting and striated cell

necrosis are generally recognized as indicative of neuromuscular damage in limb skeletal muscle. Interestingly, all these features have been observed in biopsy samples of levator ani from women considered to be normal (i.e. asymptomatic and nulliparous). Thus the presence of such features in samples of levator ani cannot necessarily be interpreted as indicative of the presence of pathological damage. Nevertheless, vaginal delivery is associated with stress incontinence and urethral hypermobility, the etiology of which is probably multifactorial. Further detailed studies of the distribution of fibre types throughout the normal levator ani are essential before this uncertainty can be resolved.

## XII. ANATOMY OF THE ANAL SPHINCTER COMPLEX

Faecal incontinence is a devastating condition, often associated with childbirth [309]. Because of the frequent occurrence of both faecal and urinary incontinence, this issue is covered by the consultation and a brief overview of anal sphincter anatomy provided here. The anal sphincter mechanism contains both smooth and striated muscle. Although there has been considerable conflict about sphincter nomenclature [310] the actual anatomy of this region is relatively straight forward [311 - 313].

There is a tubular smooth muscle sphincter that encircles the anal canal over a distance of approximately 3-4 cm above the anal verge (fig.73). It is continuous with the circular muscle layer of the intestine. The transition to the internal sphincter is marked by a thickening of the circular fibres, by an increase in the amount of collage present in the muscle, and by a change in the shape of the muscle bundles encircling the lumen [313]

The external anal sphincter surrounds the internal sphincter in its lower 2 cm by a muscular component [314] that is tethered to the coccyx through the anococcygeal rapha. Just cephalic and anterior to the external sphincter, and blending with it on its dorsal side is the puborectalis muscle. This sling-like muscle originates from the inner surface of the pubic bones and passes dorsal to the anorectum just above the external anal sphincter. Its anterior traction produces the ano-rectal angle.

Between the internal and external anal sphincter is the inter-sphincteric groove. This space receives the downward extension of the conjoined fibres of the levator ani muscles and the longitudinal smooth muscle coat of the intestine [312 - 313]. These fibres suspend and elevate the anorectum preventing its downward prolapse. They terminate in the perianal skin. Their relaxation during defecation allows for eversion of the anal skin that is then pulled up into the anal canal at the end of defecation.

Much of the controversy in this anatomy has come from

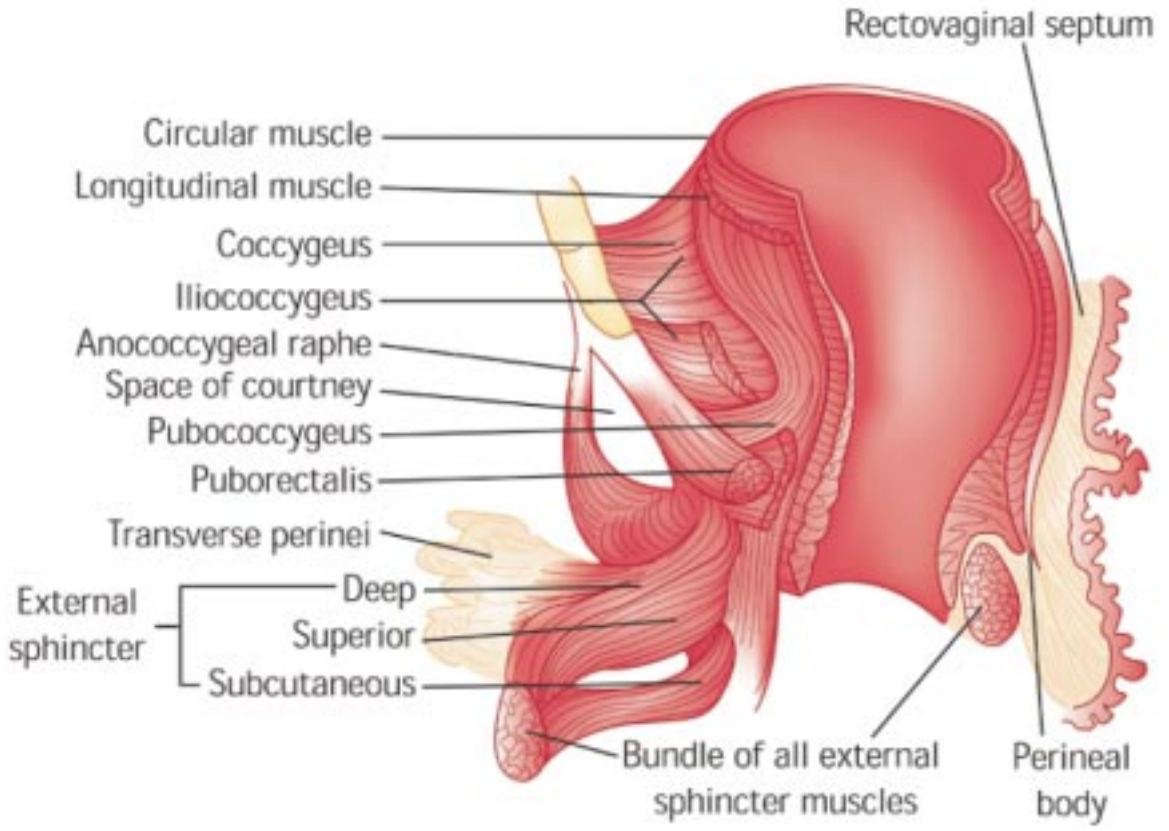



*Figure 73 : Lateral view of the anal sphincter muscles after reflection of the external anal sphincter muscles. (After Oh & Kark )*

differences of opinion concerning how many parts the external anal sphincter contains. It is beyond the scope of this chapter to cover this contentious issue, but may be visited in Dalley's interesting article on the subject [310]. There is little controversy about the existence of a superficial muscle associated with the perianal skin usually referred to as the subcutaneous sphincter. A larger and more robust part of the muscle lies above this platysma-like muscle. This is the external anal sphincter that encircles the anterior portion of the anal canal in the perineal body and that occasionally is lacerated during vaginal delivery. Further subdivision of this highly variable muscle into more portions has more academic than practical importance.

The external anal sphincter is innervated by S2-4 fibres that travel via the inferior hemorrhoidal portion of the pudendal nerve. While it is well accepted that childbirth leads to neurogenic damage to the innervation of the external anal sphincter [316] and to disruption of the internal and external sphincters [317] little is known on the impact of vaginal delivery on internal sphincter innervation and function even in the presence of an intact peroneum after delivery. Resting anal sphincter tonus is associated with normal internal sphincter function. Resting tone is known to be lower in women after vaginal birth complicated by a third-degree perineal tear compared to controls with rupture of the sphincters, but direct evaluation of the internal anal sphincter remains difficult.

## XIII. CONCLUDING REMARKS

Urinary bladder filling and emptying involve complex mechanisms at all levels from the central nervous system down to the individual muscle cells in the bladder and urethra. Disturbed function at any level might cause incontinence. The present review is concerned with bladder and urethral anatomy. The other levels of fuction and dysfuction will be dealt with later in this volume. The urinary bladder and the urethra contain an epithelial lining surrounded by densely innervated smooth muscle and a connective tissue stroma with blood vessels and sensory nerves. We have systematically described the normal function of these tissue elements and have also given some superficial information regarding their pathophysiology. We have chosen this approach in order to give a basic research background to the more clinically-oriented descriptions of pathophysiological processes leading to incontinence that will be presented in later chapters. The reader must keep in mind that the literature on structure and function of the normal bladder and urethra is overwhelming. The papers we refer to represent only a limited (and biased) selection, but will serve as key references into the literature about the various aspects of cellular mechanisms that are involved in continence and micturition in the healthy bladder.

## REFERENCES


1. Owman, C., Alm, P. And Sjöberg, N.-O.: Pelvic autonomic ganglia: Structure, transmitters, function and steroid influence. In: Autonomic Ganglia. Edited by L.-G. Elfvin. New York: John Wiley and Sons Ltd., p. 125, 1983.
2. Brading. A.F.: Physiology of bladder smooth muscle. In: The physiology of the lower urinary tract, edited by M.J. Torrens and J.F.B. Morrison, Springer Verlag: New York. p. 161,1987.
3. Smet. P.J., Jonavicius, J., Marshall, V.R. And De Vente, J.: Distribution of nitric oxide synthase-immunoreactive nerves and identification of the cellular targets of nitric oxide in guinea-pig and human urinary bladder by cGMP immunohistochemistry. Neuroscience. 71:337, 1996.
4. Elbadawi, A.: Comparative neuromorphology in animals. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. Springer-Verlag: Berlin. p. 23,1987.
5. Speakman. M.J., Walmsley. D. And Brading. A.F.: An in vitro pharmacological study of the human trigone - a site of non-adrenergic non-chloinergic transmission. Br. J. Urol., 61: 304, 1987.
6. Hai, C.M. And Murphy. R.A.: Ca2+, crossbridge phosphorylation and contraction, Ann. Rev. Physiol., 72:1334, 1989.
7. Stephens, N.L., Seow, C.Y., Halayko, A.J. And Jiang. H.: The biophysics and biochemistry of smooth muscle contraction. Canadian J. Physiol. Pharmacol., 70: 515, 1992.
8. Fuglsang, A., Khromov, A., Torok, K., Somlyo, A.V. And Somlyo, A.P.: Flash photolysis studies of relaxation and cross-bridge detachment: higher sensitivity of tonic than phasic smooth muscle to MgADP. J. Muscle Res. Cell Motility, 14: 666, 1993.
9. Harris. D.E., Work, S.S., Wright, R.K., Alpert, N.R. And Warshaw, D.M.: Smooth, cardiac and skeletal muscle myosin force and motion generation assessed by cross-bridge mechanical interactions in vitro. J. Muscle Res. Cell Motility, 15: 11, 1994.
10. Stephens, N. L.: Smooth muscle contraction: recent advances. Canadian J. Physiol. Pharmacol., 72: 1317, 1994.
11. Hellstrand. P.: Cross-bridge kinetics and shortening in smooth muscle Canadian J. Physiol. Pharmacol., 72: 1334, 1994.
12. Gunst, S.J., Wu, M.F. And Smith, D.D.: Contraction history modulates isotonic shortening velocity in smooth muscle. Am, J. Physiol, 265: C467, 1995.
13. Uchida, W., Masuda, N., Shirai, Y., Shibasaki, K., Saton, N. And Takenada, T.: The role of extra-cellular Ca2+ in

carbachol-induced tonic contraction of the pig derusor smooth muscle. Naunyn Schmiedebergs Arch. Pharmacol., 350: 398, 1994.

14. Zhao, Y., Wein, A. J. And Levin, R.M.: Role of calcium in mediating the biphasic contraction of the rabbit urinary bladder. Gen. Pharmacol., 24: 727, 1993.
15. Arner, A., Malmqvist, U. And Uvelius, B.: Cytoskeletal and contractile proteins in detrusor smooth muscle from patients with bladder outlet obstruction. Scand. J. Nephrol., 25: 262, 1991.
16. Malmqvist, U., Arner. A. And Uvelius, B.: Contractile and cytoskeletal proteins in smooth muscle during hypertrophy and its reversal. Am J Physiol., 260: C1085, 1991.
17. Malmqvist, U., Arner, A. And Uvelius, B.: Mechanics and Ca (2+) -sensitivity of human detrusor muscle bundles studied in vitro. Acta Physiol Scand., 143: 373, 1991.
18. Chiavegato, A., Scatena, M., Roelofs, M., Ferrarese, P., Pauletto, P., Passerini Glazel, G., Pagano, F. And Sartore, S.: Cytoskeletal and cytocontractile protein composition of smooth muscle cells in developing and obstructed rabbit bladder, Exp. Cell Res., 207: 310, 1993.
19. Wang, Z.E., Gopalakurup, S.K., Levin. R.M. And Chacko, S.: Expression of smooth myosin isoforms in urinary bladder smooth muscle during hypertrophy and regression, Lab. Invest., 73: 244, 1995.
20. Sjuve, R., Haase, H., Morano, I., Uvelius, B. And Arner, A.: Contraction kinetics and myosin isoform composition in smooth muscle from hypertrophied rat urinary bladder. J. Cell Biochem., 63: 86, 1996.
21. Wu, C., Kentish, J.G. And Fry, C.H.: Effect of pH on myofilament Ca 2+ - sensitivity I alpha-toxin permeabilized guinea pig detrusor muscle. J. Urol., 154: 1921, 1995.
22. Groen, J., Van Mastrigt, R. And Bosch, R.: Contractility of the guinea-pig bladder measured in situ and in vitro. Naunyn Schmiedebergs Arch. Pharmacol., 13:587, 1994.
23. Glerum, J.J., Van Mastrigt, R. And Shimada, Y.: Mechanical properties of mammalian single smooth muscle cells. III Passive properties of pig detrusor and human term uterus cells. J. Muscle Res. Cell Motility., 11:453, 1990.
24. Van Mastrigt, R., Koopal, J.W., Hak, J. And Van De Wetering, J.: Modeling the contractility of urinary bladder smooth muscle using isometric contractions. Am. J. Physiol., 251: R978, 1986.
25. Uvelius, B. And Gabella, G.: Relation between cell length and force production in urinary bladder smooth muscle. Acta Physiol. Scand., 110: 357, 1980.
26. Tammela, T. And Arjamaa, O.: Comparison of long-term and short-term stretch on rat urinary bladder in vitro. Urol. Res., 16: 277, 1988.
27. Venegas, J.G.: Viscoelastic properties of the contracting detrusor. I. Theoretical basis. Am. J. Physiol., 261: C255, 1991.
28. Finkbeiner, A.E. And O'donnell, P. D.: Responses of detrusor smooth muscle to stretch and relaxation: in vitro study. Urology., 36: 193, 1990.
29. Van Mastrigt, R.: The force recovery following repeated quick releases applied to pig urinary bladder smooth muscle, J. Muscle Res. Cell Motility., 12: 45, 1991.
30. Lin, A.T.-L., Yang, C.-H., Chen, C.-J., Chen, M.-T., Chiang, H. And Chang, I.S.: Correlation of contractile function and passive properties of rabbit urinary bladder subjected to outlet obstruction - an in vitro whole bladder study. J. Urol., 148: 944, 1992.
31. Sibley, G.N.A.: A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J. Physiol., 354: 431, 1984.
32. Creed, K.E.: Membrane properties of smooth muscle membrane of the guinea-pig urinary bladder. Pfl, ges Arch., 326: 115, 1971.
33. Klöckner, U. And Isenberg, G.: Action potentials and net membrane currents of isolated smooth muscle cells (urinary bladder of the guinea pig). Pfl, ges Archiv., 405: 329, 1985.
34. Fujii, K.: Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig rabbit and pig urinary bladder. J. Physiol., 404: 39, 1988.
35. Fujii, K., Foster, C.D., Brading, A.F. And Parekh, A.B.: Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. Br. J. Pharmacol., 99: 779, 1990.
36. Brading, A.F.: Ion channels and control of contractile activity in urinary bladder smooth muscle, Japanese J. Pharmacol., 58: 120, 1992.
37. Brading, A.F. And Turner, W.H.: Potassium channels and their modulation in the urogenital tract smooth muscles. In: Potassium channels and their modulators: from synthesis to clinical experience. Edited by J.M.Evans, et al., Taylor and Francis: London, p. 335, 1996.
38. Heppner, T.J., Bonev, A.D. And Nelson, M.T.: Ca2+-activated K+ channels regulate action potential repolarization in urinary bladder smooth muscle. Am. J. Physiol., 273: C110, 1997.
39. Bonev, A.D. And Nelson, M.T.: ATP-sensitive potassium channels in smooth muscle cells from guinea pig urinary bladder. Am. J. Physiol., 264: C190, 1993.
40. Green, M.E., G., E., Kirkup, A.J., Miller, M, And Weston, A.H.: Pharmacological characterization of the inwardly-rectifying current in the smooth muscles cells of the rat bladder. Br. J. Pharmacol., 119: 1509, 1996.
41. Foster, C.D., Speakman, M.J., Fujii, K. And Brading, A.F.: The effects of cromakalim on the detrusor muscle of human and pig urinary bladder. Br. J. Urol., 63: 284, 1989.
42. Inoue, R. And Brading, A.F.: The properties of the ATP-induced depolarization and current in single cells isolated from the guinea-pig urinary bladder. Br. J Pharmacol., 100:619, 1990.
43. Wellner, M.C. And Isenberg, G.: Properties of stretch-activated channels in myocytes from the guinea-pig urinary bladder. J. Physiol., 466: 213, 1993.
44. Montgomery, B.S.I. And Fry, C.H.: The action potential and net membrane currents in isolated human detrusor smooth muscle cells. J. Urol., 147: 176, 1992.
45. Gabella, G. And Uvelius, B.: Urinary bladder of rat: fine structure of normal and hypertrophic musculature. Cell Tissue Res., 262: 67, 1990.

46. Brading, A.F. And Mostwin, J.L.: Electrical and mechanical responses of guinea-pig bladder muscle to nerve stimulation. Br. J. Pharmacol., 98: 1083, 1989.

47. Pareka, A.H., Brading, A.F. And Tomita, T.: Studies of longitudinal tissue impedance in various smooth muscles. Prog. Clin. Biol. Res., 327: 375, 1990.

48. Brading, A.F. Teramoto, T., Nakayama, S., Bramich, N., Inoue, R., Fujii, K. And Mostwin, J.: The relationship between the electrophysiological properties of lower urinary tract smooth muscles and their function in vivo. In: Smooth Muscle Excitation, Edited by T. B. Bolton. Academic Press: London. p. 403, 1996.

49. Seki, N., Karim, O.M.A. And Mostwin, L.A.: Changes in action potential kinetics following experimental bladder outflow obstruction in the guinea-pig. Urol. Res., 20:387, 1992.

50. Brading, A.F.: A Myogenic Basis for the Overactive Bladder. Urology., 50: 57, 1997.

51. Maggi. C.A., Santicioli, P. And Meli, A.: Postnatal development of micturition reflex in rats. Am. J. Physiol., 250: R926, 1984.

52. Maggi, C.A., Santicioli, P. And Meli, A.: Postnatal development of myogenic contractile activity and excitatory innervation of rat urinary bladder. Am. J Physiol., 247: R972, 1984.

53. Maggi, C.A., Manzini, S., Santicioli, P. And Meli, A.: An atropine-like inhibitory effect of DMPP on rat isolated urinary bladder. J. Auton, Pharmacol., 6: 97, 1986.

54. Maggi, C.A., Santicioli, P., Geppetti, P., Frilli, S., Spillantini, M.G., Nediani, C., Hunt, S.P. And Meli, A.: Biochemical, anatomical and functional correlates of postnatal development of the capsaicin-sensitive innervation of the rat urinary bladder. Brain Res., 471: 183, 1988.

55. Blaivis, J.G.: The neurophysiology of micturition: a clinical study of 550 patients. J. Urol., 127: 958, 1992.

56. Smet, P.J., Edyvance, K.A., Jonavicius, J. And Marshall, V.R.: Neuropeptides and neurotransmitter-synthesizing enzymes in intrinsic neurons of the human urinary bladder. J. Neurocytol., 25:112, 1996.

57. Hanani, M. And Maudlej, N.: Intracellular recordings from intramural neurons in the guinea-pig urinary bladder. J. Neurophysiol., 74: 2358, 1995.

58. Crowe, R., Light, L., Chilton, C.P. And Burnstock, G.: Vasoactive intestinal polypeptide-, somatostatin- and substance P-immunoreactive nerves in the smooth and striated muscle of the intrinsic external urethral sphincter of patients with spinal cord injury. J. Urol., 136: 487, 1986.

59. Gabella, G.: The structural relations between nerve fibres and muscle cells in the urinary bladder of the rat. J. Neurocytol., 24: 159, 1995.

60. Palfrey, E.L.H., Fry, C.H. And Shuttleworth. K.E.D.: A new in vitro microsuperfusion technique for investigation of human detrusor muscle. Br. J. Urol., 56:635, 1984.

61. Burnstock, G., Cocks, T., Crowe, R. And Kasakov, L.: Purinergic innervation of the guinea-pig urinary bladder. Br. J. Pharmacol., 63: 125, 1978.

62. Brading, A.F. And Williams, J. H.: Contractile responses of smooth muscle strips from rat and guinea-pig urinary bladder to transmural stimulation: effects of atropine and a,b-methylene ATP. Br. J. Pharmacol., 99: 493, 1990.

63. Eglen, R.M., Reddy, H., Watson, N. And Challiss, R.A.: Muscarinic acetylcholine receptor subtypes in smooth muscle. Trends Pharmacol. Sci., 15:114, 1994.

64. Kondo, D., Morita T And Tashima, Y.: Muscarinic cholinergic receptor subtypes in human detrusor muscle studied by labeled and nonlabeled pirenzepine. AFDX-116 and 4DAM. P. Urol. International. 54: 150, 1995.

65. Yamaguchi, O., Shishido, K., Tamura, K., Ogawa, T. And Fujimura. T.: Evaluation of mRNA encoding muscarinic receptor subtypes in human detrusor muscle. Neurourol. Urodyn., 13: 464, 1994.

66. Inoue, R. And Brading, A. F.: Human, pig and guinea-pig bladder smooth muscle cells generate similar inward currents response to purinoceptor activation. Br. J Pharmacol., 103: 1840, 1991.

67. Mcmurray. G., Dass, N., Ho, K.M.T. And Brading, A.F.: Purinergic mechanisms in primate urinary bladder. Br. J. Urol., 80:182, 1997.

68. Palea, S., Corsi, M., Pietra, C., Artibani, W., Calpista, A., Gaviraghi, G. And Trist, D. G.:ADP beta S induces contraction of the human isolated urinary bladder through a purinoceptor subtype different from P2X and P2Y. J. Pharmacol. Exp. Ther., 269: 193, 1994.

69. Palea, S., Pietra, C., Trist, D.G., Artibani, W., Calpista, A. And Corsi, M.: Evidence for the presence of both pre-and postjunctional P2-purinoceptor subtypes in human isolated urinary bladder. Br. J. Pharmacol., 114: 35, 1995.

70. Wammack, R., Weihe, E., Dienes, H, -P, And Hohen-Fellner, R.: The neurogenic bladder in vitro. Akt. Urol., 26: 16, 1995.

71. Eglezos. A., Giuliani, S., Viti, G. And Maggi, C.A.: Direct evidence that capasaicin-induced plasma protein extravasation is mediated through tachykinin NK-1 receptors. Eur. J. Pharmacol., 209: 277, 1991.

72. Maggi, C.A.: Therapeutic potential of capsaicin-like molecules: studies in animals and human. Life Sci., 51: 1777, 1992.

73. Meinl, S. And Maggi, C.A.: Evidence for a capsaicin-sensitive, tachykinin-mediated, component in the NANC contraction of the rat urinary bladder to nerve stimulation. Br. J. Pharmacol., 112: 1123, 1994.

74. Maggi, C.: Tachykinins and calcitonin gene-related peptide (CGRP) as cotransmitters released from peripheral ending of sensory nerves. Progress in Neurobiology, 45:1, 1995.

75. Maggi, C.: The mammalian tachykinin receptors. General Pharmacology, 26:911, 1995.

76. Schulman, C.C., Duarte-Escalante, O. And Boyarsky, S.: The ureterovesical innervation. A new concept based on a histochemical study. Br. J. Urol., 44:698, 1972.

77. Elbadawi, A. And Schenk, E.A.: A new theory of the innervation of bladder musculature. 4. Innervation of the vesicourethral junction and external urethral sphincter. J. Urol., 111:613, 1974.

78. Khanna, O.P., Barbieri, E.J., Altamura, M. And Mcmichael, R.: Vesicourethral smooth muscle: function and relation to structure. Urology, 18: 211, 1981.

79. Takeda, M., Mitzusawa, T., Obara, K., Koizumi, T., Tsutsui, T.M Hatano, A., Takahashi, H., And Takahashi, K.: Adrenergic betal, beta2, and beta3 receptor subtypes in the detrusor of human urinary bladder - evaluation by mRNA expression and isometric contraction. J. Urol., 157 suppl 4: 82, 1997.

80. Tsurusaki, M., Yoshida, M., Akasu, T. And Nagatsu, I.S.O.: Alpha 2-adrenoceptors mediate the inhibition of cholinergic transmission in parasympathetic ganglia of the rabbit urinary bladder. Synapse. 5:233, 1990.

81. Kusonoki, M., Taniyama, K. And Tanaka, C.: Neuronal GABA release and GABA inhibition of ACh release in guinea-pig urinary bladder. Am. J. Physiol., 246: R502, 1984.

82. Santicioli, P., Maggi, C.A. And Meli, A.: GABAB receptor mediated inhibition of field stimulation-induced contractions of rabbit bladder musclin-vitro. J. Pharm. Pharmacol. 36: 378, 1984.

83. Chen, T. F., Doyle, P.T. And Ferguson, D.R.: Inhibitory role of gamma-amino-butyric acid in the rabbit urinary bladder. Br. J. Urol., 69: 12, 1992.

84. Fujii, K.: Electrophysiological evidence that adenosine triphosphate (ATP) is a co-transmitter with acetylcholine (Ach) in isolated guinea-pig, rabbit and pig urinary bladder. J. Physiol., 394: 26. 1987.

85. Hashitani, H. And Suzuki, H.: Electrical and mechanical responses produced by nerve stimulation in detrusor smooth muscle of the guinea pig. Eur. J. Pharmacol., 284: 177, 1995.

86. Ganitekevich. V. And Isenberg, G.: Caffeine-induced release and reuptake of Ca2+ by Ca2+ stores in myocytes from guinea-pig urinary bladder. J. Physiol., 458: 99, 1992.

87. Chambers, P., Neal, D.E. And Gillespie, J.I.: Ca2+ mobilization in cultured human bladder smooth muscle cells in response to hypotonic stimuli. Exp. Physiol., 82: 677, 1997.

88. Yoshikawa, A., Van Breemen, C. And Isenberg, G.: Buffering of plasmalemmal Ca2+ current by sarcoplasmic reticulum of guinea-pig urinary bladder myocytes. Am. J. Physiol., 271 (C40): C833, 1996.

89. Sjögren, C., Andersson, K.E., Husted, S., Mattiasson, A. And Moller-Madsen. B.: Atropine resistance of transmurally stimulated isolated human bladder muscle. J. Urol., 128: 1368, 1982.

90. Bayliss, M And Fry, C. H.: Cholinergic and purinergic mechanisms in human detrusor from normal and obstructed bladders. Br. J. Urol., 79: 57, 1997.

91. Iacovou, J.W., Hill, S.J. And Birmingham, A.T.: Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: evidence that muscarinic and purinergic receptors raise intracellular calcium by different mechanisms. J. Urol., 144: 775, 1990.

92. Harriss, D.R. Marsh. K.A. Birmingham, A.T. And Hill, S.J.: Expression of muscarinic M3-receptors coupled to inositol phospholipid hydrolysis: I. Human detrusor cultured smooth muscle cells. J. Urol., 154: 1241, 1995.

93. Hegda, S.S., Choppin, A., Bonhaus, D., Briaud, S., Loeb, M., Moy, T.M. Loury, D. And Eglen, R.M.: Functional role of M2 and M3 muscarinic receptors in the urinary bladder of rats: I. Vitro and in vivo. Br. J. Pharmacol., 120: 1409, 1997.

94. Rivera, L., Mcmurray, G. And Brading, A.F.: The role of calcium stores: I. The action of muscarinic agonists on mammalian urinary bladder smooth muscle. J. Physiol., 507P: 21P, 1998.

95. Abrams, P And Wein, A.J.: editor. The overactive bladder: from basic science to clinical management consensus conference. Elsevier: 1997.

96. Turner, W.H. And Brading, A.F.: Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. Pharmacol. Ther., 75: 77, 1997.

97. Kondo, A. And Susser, J.G.: Viscoelastic properties of bladder. II. Comparative studies in normal and pathologic dogs. Invest. Urol., 11:459, 1974.

98. Cortivo, R., Pagano, F., Passerini, G., Abatangelo, G. And Castellani, I.: Elastin and collagen in the normal and obstructed urinary bladder. Br. J. Urol., 53: 134, 1981.

99. Susset. J.G., Servot-Viguier, D., Lamy, F., Madernas, P. And Black, R.: Collagen in 155 human bladders. Invest. Urol., 16: 204, 1978.

100. Uvelius, B. And Mattiasson, A.: Collagen content in the rat urinary bladder subjected to infravesical outflow obstruction. J. Urol., 132: 587, 1984.

101. Murakumo, M., Ushiki, T., Abe, K., Matsumura, K., Shinno, Y. And Koyanagi, T.: Three-dimensional arrangement of collagen and elastin fibers in human urinary bladder: scanning electron microscopic study. J. Urol., 154: 251, 1995.

102. Uvelius, B. And Mattiasson, A.: Detrusor collagen content in the denervated rat urinary bladder. J. Urol., 136:110, 1986.

103. Shapiro, E., Becich, M.J., Perlman. E. And Lepor, H.: Bladder wall abnormalities in myelodys-plastic bladders: a computer assisted morphometric analysis. J. Urol., 145:1024, 1991.

104. Lepor, H., Sunaryadi. I., Hartanto, V. And Shapiro, E.: Quantitative morphometry of the adult human bladder. J. Urol., 148: 414, 1992.

105. Levy, B.J. And Wight, T.N.: Structural changes in the aging submucosa: new morphologic criteria for the evaluation of the unstable human bladder. J. Urol., 144: 1044, 1990.

106. Macarak, E.J., Ewalt, D., Baskin, L., Coplen, D., Koo, H., Levin, R.M., J.W., D., Snyder, H., Rosenbloom, J. And Howard, P. S., The collagens and their urologic implications. Advances Exp Med. Biol., 385: 173, 1995.

107. Baskin, L.S., Constantinescu, S., Duckett, J.W., Syder, H.M. And Macarac, E.; Type III collagen decrease in normal fetal bovine bladder development. J. Urol., 152: 688, 1994.

108. Landau, E.H., Jayanthi, V.R., Churchill, B.M. Shapiro, E., Gilmour, R.F., Khoury, A.E., Macarak, E.J., Mclorie, G.A., Steckler, R.E. And Kogan, B.A.: Loss of elasticity in dysfunctional bladders. J. Urol., 152:702, 1994.

109. Macarak, E.J. And Howard, P.S.: The collagens and their urologic significance. Scand. J. Urol. Nephrol., Supp. 184: 25, 1997.

110. Rosenbloom, J., Koo, H., Howard, P.S. Mecham, R. And Macarak, E.J.: Elastic fibers and their role in bladder extracellular matrix. Advances Exp Med. Biol., 385: 173, 1995.

111. Rosenbloom, J., Abrams, W.R., Rosenbloom, J., Kucich, U., Decker, S., Mecham, R., Macarak, E. And Howard, P.: Expression of microfibrillar proteins by bovine bladder urothelium. Urology., 49: 287, 1997.

112. Kim, K.M. Kogan, B.A., Massad, C.A. And Hung, Y, -C.: Collagen and elastin in obstructed fetal bladder. J. Urol., 146: 528, 1991.

113. Takamura, S., Hiyake, K. And Takeushi, J.: Immuno-histochemical localization of proteoglycans in interstitial elements of the human bladder. Hinyokika Kiyo., 37: 329, 1991.

114. Levy, B.J. And Wigh., T.N.: The role of proteglycans in bladder structure and function. Advances Exp med. Biol., 385: 191, 1995.

115. Li, D.Y., Brooke, B., David, E.C., Mecham, P.P., Sorensen, L.K., Boaqk, B.B., Eichwala, E. And Keating M.T.: Elastin is an essential determinant of arterial morphogenesis. Nature. 393: 276, 1998.

116. Sarma, K.P.: Microangiography of the bladder in health. Br. J. Urol., 53: 237, 1991.

117. Inoue, T. And Gabella, G.; A vascular network closely linked to the epithelium of the urinary bladder of the rat. Cell Tissue Res., 263: 137, 1991.

118. Greenland, J.E. And Brading, A.F.: Urinary bladder blood flow changes during the micturition cycle in a conscious pig model. J. Urol., 156: 1858, 1996.

119. Kroyer, K., Bulow, J., Nielsen, S.L. And Kromann-Andersen, B.: Urinary bladder blood flow I. Comparison of clearance of locally injected 99m -technetium pertechnate and radioactive microsphere technique in dogs. Urol. Res., 18: 223, 1990.

120. Nielsen, K.K., Nielsen, S.L., Nordling, J. And Kromann, A.B.: Rate of urinary bladder blood flow evaluated by 133Xe wash-out and radioactive microspheres in pigs. Urol. Res., 19: 387, 1991.

121. Ohnishi, N., Kishima, Y., Hashimoto, K., Kiwamoto, H., Esa, A., Sugiyama, T., Park, Y.C. And Kurita, T.: A new method of measurement of the urinary bladder blood flow in patients with low compliant bladder. Hiyokika Kiyo., 40: 663, 1994.

122. Batista, J.E., Wagner, J.R., Azadzoi, K.M., Karane, R.J. And Siroky, M.B.: Direct measurement of blood flow in the human bladder, J. Urol., 155: 630, 1996.

123. Brading, A.F.: Alterations in the physiological properties of urinary bladder smooth muscle caused by bladder emptying against an obstruction. Scand. J. Urol. Nephrol., Suppl., 184:51, 1997.

124. Schuschke, D.A., Reed, M.W., Wingren, U.F. And Miller, F.N.: The rat urinary bladder: vasoactivity and macromolecular leakage in a new model. Microvasc. Res., 38: 23, 1989.

125. Andersson, P.O., Bloom, S.R., Mattiasson, A. And Uvelius, B.: Changes in vascular resistance in the feline urinary bladder in response to bladder filling. J. Urol., 134: 1041, 1985.

126. Guttmann, L. And Whitteridge, D.: Effects of bladder distension on autonomic mechanisms after spinal cord injuries. Brain. 70: 361, 1947.

127. Andersson, P.O., Bloom, S.R., Mattiasson, A. And Uvelius, B.: Bladder vasodilatation and release of vasoactive intestinal polypeptide from the urinary bladder of the cat in response to pelvic nerve stimulation. J. Urol., 150: 1945, 1993.

128. Hofmann, R., Gomez, R., Schmidt, R. And Tanagho, E.A.: Effects of nerve stimulation on blood flow in the urinary bladder, urethra and pelvic floor in the dog. J. Urol., 150: 1945, 1993.

129. Crowe. R. And Burnstock, G.: A histochemical and immunohistochemical study of the autonomic innervation of the lower urinary tract of the female pig. Is the pig a good model for the human bladder and urethra? J. Urol., 141: 414, 1989.

130. Levin, R.M., Brendler, K., Van Arsdalen, K.N. And Wein, A.J.: Functional response of the rabbit urinary bladder to anoxia and ischaemia. Neurourol. Urodyn., 2: 233, 1983.

131. Zhao, Y., Wein, A.J., Bilgen, A. And Levin, R.M.: The effect of anoxia on in-vitro bladder function. Pharmcology. 43: 337, 1991.

132. Pessina, F., Mcmurray, G., Wiggin, A. And Brading, A.F.: The effect of anoxia and glucose-free solutions on the contractile response of guinea-pig detrusor strips to intrinsic nerve stimulation and the application of excitatory agonists. J. Urol., 157: 2375, 1997.

133. Hossler, F.E. And Monson, F.C.: Microvasculature of the rabbit urinary bladder, Anat. Rec., 243: 438, 1995.

134. Parsons, C.L., Stauffer, C. And Schmidt, J.D.: Bladder-surface glycosaminoglycans: an efficient mechanism of environmental adaptation. Science. 208: 605, 1980.

135. Parsons, C.C., Boychuk, D., Jones, S., Hurst, R. And Callahan, H.: Bladder surface glycosaminoglycans: an epithelial permeability barrier. J. Urol., 143: 139, 1990.

136. Hicks, R.M., Ketterer, B. And Warren, R.C.: The ultrastructure and chemistry of the luminal plasma membrane of the mammalian urinary bladder: a structure with low permeability to water and ions. Phil. Trans. R. Soc. Lond., 168: 23, 1974.

137. Jost, S.P., Gosling, J.A. And Dixon, J.S.: The morphology of normal human bladder urothelium. J. Anat., 167: 103, 1989.

138. Lewis, S.A., Berg, J.R. And Kleine, T.J.: Modulation of epithelia permeability by extracellular macromolecules. Physiol. Rev., 75: 561, 1995.

139. Hurst, R.E. And Zebrowski, R.: Identification of proteoglycans present at high density on bovine and human bladder luminal surface. J. Urol., 152: 1641, 1994.

140. Donaldson, P.J., Chen, L.K. And Lewis, S.A.: Effects of serosal anion composition on the permeability properties of rabbit urinary bladder. Am. J. Physiol., 256: 1125, 1989.

141. Lewis, S.A.: The mammalian urinary bladder: it's more accommodating. News Physiol. Sci., 1: 61, 1986.

142. Ferguson, D.R., Kennedy, I. And Burton, T.J.: ATP is released from rabbit bladder epithelial cells by hydrostatic pressure changes - a possible sensory mechanism? J. Physiol., 505: 503, 1997.

143. Hohlbrugger, G.: Vesical blood-urine barrier: relevant and dynamic interface between renal function and nervous bladder control. J. Urol., 154: 6, 1995.

144. Boels, P.J., Arner, A., Malmqvist, U. And Uvelius, B.: Structure and mechanics of growing arterial microvessels from hypertrophied urinary bladder in the rat. Pflügers Arch., 426: 506, 1994.

145. Brendler, C. B., Radebaugh, L.C. And Hohler, J.L.: Topical oxybutynin chloride for relaxation of dysfunctional bladders. J. Urol., 141: 1350, 1989.

146. Mattiasson, A., Ekström, B. And Andersson, K.E.: Effects of intravesical instillation of verapamil in patients with detrusor hyperactivity, J. Urol., 141: 174, 1989.

147. Gabella, G. And Davis, C.: Distribution of afferent axons in the bladder of rats. J. Neurocytol., 27: 141, 1998.

148. Olsson, L.E., Wheeler, M.A., Sessa, W.C. And Weiss, R.M.: Bladder instillation and intraperitoneal injection of Escherichia coli lipopolysaccharide up regulate cytokines and iNOS in rat urinary bladder. J. Pharmacol. Exp. Ther., 284: 1203, 1998.

149. Jansson, O., Morcos, E., Söderhäll, M., J., A., Lundberg, J.O.N. And Wiklund, N.P.: The role of nitric oxide in BCG medicated anti tumour effects. Br. J. Cancer (in press), 1998.

150. Lundberg, J.O., Ehren, I., Jansson, O., Adolfsson, J., Lundberg, J.M., Weitzberg, E., Alving, K. And Wiklund, N.T.: Elevated nitric oxide in the urinary bladder in infectious and noninfectious cystitis. Urology, 48: 700, 1996.

151. Dixon, J.S., Jen, P.Y.P., Gosling, J.A.: The distribution of vesicular acetylcholine transporter in the human male genitourinary organs and its co-localization with neuropeptide Y and nitric oxide synthase. Neurourol. Urodynam., 19: 185-94, 2000.

152. Ek, A., Alm, P., Andersson, K.-E. And Persson, C.G.A.: Adrenergic and cholingeric nerves of the human urethra and urinary bladder. A histochemical study. Acta Physiol. Scand., 99: 345, 1997.

153. Gosling, J.A., Dixon, J.S. And Lendon, R.G.: The autonomic innervation of the human male and female bladder neck and proximal urethra. J. Urol. 118 302, 1997.

154. Sundin, T., Dahlstrom, A., Norlen. L., And Svedmyr, .N.: The sympathetic innervation and adrenoreceptor function of the human lower urinary tract in the normal state and after parasympathetic denervation. Invest. Urol., 14: 322, 1977.

155. Benson, G. S., Mcconnell, J. A. And Wood, J. G.: Adrenergic innervation of the human bladder body. J. Urol., 122: 189, 1979.

156. Kluck. P.: The autonomic innervation of the human urinary bladder. Bladder neck and urethra. A histochemical study. Anat. Rec., 198: 439, 1980.

157. Mcconnell, J., Benson, G.S. And Wood, J. G.: Autonomic innervation of the urogenital system. Adrenergic and cholinergic elements. Brain Res. Bull., 9: 679, 1982.

158. Edyvane, K. A., Smet, P.J., Jonavicius, J., And Marshall, V.R.: Regional differences in the innervation of the human ureterovesical junction by tyrosine hydroxylase, vasoactive intestinal polypeptide-, and neuropeptide Y-like immunoreactive nerves. J. Urol., 154: 262, 1995.

159. Smet, P. J. Moore, Kh. And Joavicius, J.: Distribution and colocalization of calcitonin gene-related peptide, tachykinins, and vasoactive intestinal peptide in normal and idiopathic unstable human urinary bladder. Lab. Invest., 77: 37, 1997.

160. Vaughan, C.W. And Satchell, P.M.: Urine storage mechanisms. Prog. Neurobiol., 46: 215, 1995.

161. Janig, W. And Koltzenburg, M.: On the function of spinal primary afferent fibres supplying colon and urinary bladder. J. Auton. Nerv. Syst., 30: S89, 1990.

162. Fall. M., Lindstrom, S., And Mazieres, L.: A bladder-to-bladder cooling reflex in the cat. J. Physiol., 427: 281, 1990.

163. Maggi, C.A. And Meli, A.: The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen. Pharmacol., 19: 1, 1988.

164. Ferguson, D.R., Kennedy, I., And Burton, T.J.: ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes - a possible sensory mechanism? J. Physiol., 505: 503, 1997.

165. Kawatani, M., Whitney, T., Booth, A.M., And Degroat, W.C.: Excitatory effect of substance P I parasympathetic ganglia of cat urinary bladder. Am. J. physiol., 257: 1450, 1898.

166. Wakabayashi, Y., Tomoyoshi, T., Fujimiya, M., Arai, R., And Maeda, T.: Substance P-containing axon terminals in the mucosa of the human urinary bladder: pre-embedding immunohistochemistry using cryostat sections for electron microscopy. Histochem., 100: 401, 1993.

167. Kumamoto, K., Ebara, S., And Matsuura, T.: Noradrenergic fibers: I. The Pacinian corpuscles of the cat urinary bladder. Acta Anat., 146: 46, 1993.

168. Dupont, M., Steers.W., Miccarty.R., And Tuttle. J.: Neural plasticity and alterations in nerve growth factor and norepinephrine in response to bladder inflammation. J. Urol., 151:284, 1994.

169. Tuttle. J. B., Steers. W. D., Albo. M., And Nataluk. E.: Neural input regulates tissue NGF and growth of the adult rat urinary bladder. J. Auton. Nerv. Syst., 49: 147, 1994.

170. Steers.W.D., Ciambotti.J., Etzel. B., Berdman. S., And De Groat. W.C.: Alterations in afferent pathways form the urinary bladder of the rat I response to partial urethral obstruction. J. Comp. Neurol., 310: 401, 1991.

171. Steers W.D., Creedon D. J., And Tuttle J.: Immunity to nerve growth factor prevents afferent plasticity following urinary bladder hypertrophy. J. Urol., 155: 375, 1996.

172. Dixon J.S., Gosling. J.A., Canning. D. A. And Gearhart. J.P.: An immunohistochemical study of human postnatal paraganglia associated with the urinary bladder. J. Anat., 181: 431, 1992.

173. Pang X., Marchand. J., Sant. G.R., Kream. R., And Theoharides.T.C.: Increased number of substance P positive nerve fibres: I. Interstitial cystitis. Br. J. Urol., 75: 744, 1995.

174. Lowe R. M., Anand. P., Terenghi.G., Williams-Chestnuts. R., Sinicropi. D., And Osborne. J.: Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br. J. Urol., 79: 572, 1997.

175. Szallasi,. A., Conte, B., Goso, C., Blumberg, P. M., And Manzini, S.: Vanilloid receptors: I. The urinary bladder: regional distribution, localization on sensory nerves, and species-related differences. Naunyn Schmiedebergs Arch. Pharmacol., 347: 624, 1993.

176. Bennett, D., Dmietrieva, V., Priestley, J., Clary, D., And Mcmahon, S.: TrkA, CGRP and IB4 expression in retrogradely labelled cutaneous and visceral primary sensory neurones in the rat. Neurosci. Letts., 206: 33, 1996.

177. Yarnitsky, D. And Vardi, Y.: Bladder sensory power factor (BSPF) - a novel diagnostic tool. J. Neurol. Sci., 120: 137, 1993.

178. Sjostrand. N.O.: The adrenergic innervation of the vas deferens and accessory male genital glands. Acta Physiol. Scand., Suppl 257: 1, 1965.

179. De Groat. W. C. And Booth. A. M.: Inhibition and facilitation in parasympathetic ganglia of the urinary bladder. Fed. Proc., 39: 2990, 1980.

180. Gosling, J. A. And Thompson, S. A. Morphological and histochemical studies on developing human pelvic autonomic nerve cells and paraganglia. In: Peripheral Neuroendocrine Interaction. Edited by R.E. Coupland and W. G. Forsmann. London: Springer, pp. 37-47, 1978.

181. Thompson, S.A. And Gosling, J.A.: Histochemical light microscopic study of catecholamine-containing paraganglia in the human pelvis. Cell Tiss. Res., 170: 539, 1976.

182. Dixon, J. S., Gosling, J.A., Canning, D.A. And Gearhart, J.P.: An immunohistochemical study of human postnatal paraganglia associated with the urinary bladder. J. Anat., 181: 431, 1992.

183. Philips, M.: Fetal catecholamines. Am. J. Obstet. Gynecol., 146: 840, 1983.

184. Learmonth, J. R.: A contribution to the neurophysiology of the urinary bladder in man. Brain, 54: 147, 1931.

185. Andersson, K.-E. And Persson L.: Nitric oxide synthase and nitric oxide-mediated effects in lower urinary tract smooth muscles. World J. Urol., 12: 274, 1994.

186. DeLancey, J.O.L.: Correlative study of paraurethral anatomy. Obstet Gynecol, 68: 91-7, 1986.

187. Oelrich, T.M.: The striated urogenital sphincter muscle in the female. Anat. Rec., 205: 223-32, 1983.

188. Gosling, J.A., Dixon, J.S., Critchley, H.O.D. And Thompson, S.A.: A comparative study of the human external sphincter and periurethral levator ani muscles. Br. J. Urol., 53:35, 1981.

189. Huisman, A.B.: Aspects on the anatomy of the female urethra with special relation to urinary continence. Contrib. Gynecol. Obstet., 10: 1-31, 1983.

190. Arner.A., Mattiasson., A., Radzizewski, P. And Uvelius, B.: Shortening velocity is different in longitudinal and circular muscle layers of the rabbit urethra. Urol. Res., in press.

191. Creed, K.E., Oike, M. And Ito, Y.: The electrical properties and responses to nerve stimulation of the proximal urethra of the male rabbit. Br. J. Urol., 79: 543, 1997.

192. Teramoto, N., Creed, K.E. And Brading, A.F.: Activity of glibenclamide-sensitive K+ channels under unstimulated conditions in smooth muscle cells of pig proximal urethra. Naunyn Schmiedebergis Arch. Pharmacol., 356: 418, 1997.

193. Ho, K.M.T., Mcmurray, G., Brading, A.F., Noble. J.G., Ny, L. And Andersson, K.-E.: Nitric oxide synthase in heterogeneous population of intramural striated muscle fibres of human membranous urethral sphincter. J. Urol., 159: 1091, 1998.

194. Bridgewater, M., Davies, J.R. And Brading, A.F.: Regional variations in the neural control of the female pig urethra. Br. J. Urol., 76: 730, 1995.

195. Alm, P., Zygmunt, P.K., Iselin, C., Larsson, B., Uvelius, B., Werner, S. And Andersson, K.-E.: Nitric oxide synthase-immunoreactive, adrenergic, cholinergic and peptidergic nerves of the female rat urinary tract: a comparative study. J. Auton. Nerv. Syst. 56: 105, 1995.

196. Ek, A.: Innervation and receptor functions of the human urethra. Scand J. Urol. Nephrol. Suppl., 1997: 1, 1977.

197. Bridgewater, M., Macneil, H.F. And Brading, A.F.: Regulation of tone in pig urethral smooth muscle, J. Urol., 150: 223, 1993.

198. Hatano, A., Takahashi, H., Tamaki, M., Komeyanma, T., Koizumi, T. And Takeda, A.: Pharmacological evidence of distinct alpha I-adrenoceptor subtypes mediating the contraction of human prostatic urethra and peripheral artery. Br. J. Pharmacol., 113: 723, 1994.

199. Werkstr, M.V., Persson, K., Ny., L., Bridgewater, M., Brading, A.F. And Andersson. K.-E.: Factors involved in the relaxation of female pig urethra evoked by electrical stimulation. Br. J. Pharmacol., 116: 1599, 1995.

200. Andersson, K.-E, And Persson, K.: Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology. Scand. J. Urol. Nephrol., 175: 45, 1995.

201. Takeda, M. And Lepor, H.: Nitric oxide synthase in dog urethra: a pharmacological and histochemical analysis. Br. J. Pharmacol., 116: 2517, 1995.

202. Mcdonald, L.J. And Murad, F.: Nitric oxide and cyclic GMP signaling. Proc. Soc. Exp. Biol. Med, 211:1, 1996.

203. Boolell, M., Gepi-Attee, S., Gingell, J.C. And Allen, M. J.: Sildenafil. A novel effective oral therapy for male erectile dysfunction. Br. J. Urol., 78:257, 1996.

204. Thind, P.: The significance of smooth and striated muscles in sphincter function of the urethra in healthy women. Neurourol. Urodyn., 14: 585, 1995.

205. Torrens, M.J.: Human physiology. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F. B. Morrison. Springer-Verlag: Berlin. p. 333:1987.

206. Gosling, J.A., Dixon, J.S., Critchley, H.O. And Thomp-

son, S.A.: A comparative study of the human external sphincter and periurethral levator ani muscles. Br J. Urol., 53: 35, 1981.

207. Light, J.K., Rapoli, E. And Wheeler, T.M.: The striated urethral sphincter: muscle fibre types and distribution in the prostatic capsule. Br. J. Urol., 79: 539, 1997.

208. Elbadawi, A., Mathews, R., Light, J.K. And Wheeler, T.E.: Immunohistochemical and ultrastructural study of rhabdosphincter component of the prostatic capsule. J. Urol., 158: 1819, 1997.

209. Zvara, P., Carrier, S., Kour, N.W. And Tangho, E.A.: The detailed neuroanatomy of the human striated urethral sphincter. Br J. Urol., 74: 182, 1994.

210. Hollabaugh. R.S., Dmochowski, R.R. And Steiner, M.S.: Neuroanatomy of the male rhabdosphincter. Urology, 49: 426, 1997.

211. Von Heyden, B., Riemer, R.K., Nunes, L., Brock, G.B., Lue, T.F. And Tanagho, A.E.: Response of guinea-pig smooth and striated urethral sphincter to cromakalim, prazosin, nifedipine, nitroprusside and electrical stimulation. Neurourol. Urodyn., 14: 153, 1995.

212. Creed, K.E., Van Der Werf, B.A., Kaye, K.W.: Innervation of striated muscle of the membranous urethra of the male dog. J. Urol., 159: 1712, 1998.

213. Fowler, C.J. And Fowler, C.: Clinical neurophysiology, In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F.B. Morrison. Springer-Verlag: Berlin Heidelberg. p. 309, 1987.

214. Fowler, C.J., Kirby, R.S., Harrison, M.J., Milroy, E.J. And Turner Warwich, R.: Individual motor unit analysis in the diagnosis of disorders of urethral sphincter innervation. J. Neurourol. Neurosurg. Psychiatry. 47: 637, 1984.

215. Hutch, J.A.: A new theory of the anatomy of the internal urinary sphincter and the physiology of micturition. IV. The urinary sphincteric mechanism. J. Urol., 97: 705, 1967.

216. Hickey, D.S., Phillips, J.I. And Hukins, D.W.L.: Arrangements of collagen fibrils and muscle fibers in the female urethra and their implications for the control of micturition. Br. J. Urol., 54: 556, 1982.

217. Huisman, A.B.: Aspects on the anatomy of the female urethra with special relation to urinary continence. Contrib. Gynecol. Obstet., 10: 1, 1983.

218. Augsburger, H.R., Crus-Orive, L.M. And Arnold, S.: Morphology and stereology of the female canine urethra correlated with the urethral pressure profile. Acta Anat. (Basel), 148: 197, 1993.

219. Dass, N.: Studies of the anatomy and autonomic innervation of the vesicourethral junction and urethra. D. Phil thesis in Pharmacology. Oxford University, 1997.

220. Carlile, A., Davies, I., Rigby, A. And Brocklehurst, J.C.: Age changes in the human female urethra: a morphologic study. J. Urol., 139: 532, 1988.

221. Ulmsten, U., Ekman, G., Giertz, G. And Malmstrom, A.: Different biochemical composition of connective tissue in continent and stress incontinent women. Acta Obstet. Gynecol. Scand., 66: 455, 1987.

222. Rud, T., Andersson, K.E., Asmussen, M., Hunting, A. And Ulmsten, U.: Factors maintaining the intraurethral pressure in women. Invest. Urol., 17: 343, 1980.

223. Batra, S., Bjellin, L., Sjogren, C., Iosif, S. And Widmark, E.: Increases in blood flow of the female rabbit urethra following low dose estrogens. J. Urol., 136: 1360, 1986.

224. Greenland, J.E. And Brading, A.F.: The in vivo and in vitro effects of hypoxia on pig urethral smooth muscle. Br. J. Urol., 79: 525, 1997.

225. Falconer, C., Blomgren, B., Johansson, O., Ulmsten, U., Malmström, A., Westergrenthorsson, G. And Ekman-Ordeberg, G.: Different organization of collagen fibrils in stress-incontinent women of fertile age. Acta Obstet. Gynecol. Scan., 77: 87, 1998.

226. Falconer, C., Ekman-Ordeberg, G., Blomgren, B., Johansson, O., Ulmsten, U., Westergren-Thorsson, G. And Malmström, A.: Paraurethral connective tissue in stress-incontinent women after menopause. Acta Obstet. Gynecol. Scand., 77:95, 1998.

227. Rechberger, T., Donica, H., Baranowski, W. And Jakowicki, J.: Female urinary stress incontinence in terms of connective tissue biochemistry. Eur. J. Obstet. Gynecol. Reprod. Bio., 49: 187, 1993.

228. Keane, D.P., Sims, T.J., Abrams, P. And Bailey, J.: Analysis of collagen status in premenopausal nulliparous women with genuine stress incontinence. Br. J. Obstet. Gynaecol., 104: 994, 1997.

229. Knowles, R.G. And Moncada, S.: Nitric oxide synthases in mammals. Biochem. J., 298: 249, 1994.

230. Alm, P., Larsson, B., Ekblad, E., Sundler, F. And Andersson, K. E.: Immunohistochemical localization of peripheral nitric oxide synthase-containing nerves using antibodies raised against synthesized C- and N-terminal fragments of a cloned enzyme form rat brain. Acta Physiol. Scand., 148: 421, 1993.

231. Burnett, A.L., Calvin, D.C., Chamness, S.L. Liu, J.X., Nelson, R.J., Klein, S.L., Dawson, V.L., Dawson, T.M. And Snyder, S.H.: Urinary bladder-urethral sphincter dysfunction in mice with targeted disruption of neuronal nitric oxide synthase models idiopathic voiding disorders I humans. Nature Medicine, 3: 571, 1997.

232. Andersson, K.E., Garcia-Pascual, A., Forman, A. And Tottrup, A.: Non-adrenergic, non-cholinergic nerve-mediated relaxation of rabbit urethra is caused by nitric oxide. Acta Physiol. Scand., 141: 133, 1991.

233. Dokita, S., Morgan, W.R., Wheeler, M.A., Yoshida, M., Latifpour, J. And Weiss, R.M.: NG-nitro-L-arginine inhibits non-adrenergic, non-cholinergic relaxation in rabbit urethral smooth muscle. Life Sci., 48: 2429, 1991.

234. Persson, K. And Andersson, K.E.: Nitric oxide and relaxation of pig lower urinary tract. Br. J. Pharmacol., 106: 416, 1992.

235. Garcia-Pascula, A., Costa, G., Garciasacristan, A. And Andersson, K.-E.: Relaxation of sheep urethral muscle induced by electrical stimulation of nerves: involvement of nitric oxide. Acta Physiol. Scand., 141: 531, 1991.

236. Andersson, K.E., Garcia Pascual A., Persson, K., Forman, A. And Tottrup., A.: Electrically-induced, nerve-

mediated relaxation of rabbit urethra involves nitric oxide. J. Urol., 147: 253, 1992.

237. Leone, A.M., Wiklund, N.P., Hokfelt, T., Brundin, L. And Moncada, S.: Nerve-induced release of nitric oxide in the human urogenital tract. Neuroreport., 5: 733, 1994.

238. James, M. J., Birmingham, A.T. And Hill, S.J.: Partial mediation by nitric oxide of the relaxation of human isolated detrusor strips in response to electrical field stimulation. Br J. Clin. Pharmacol., 35: 366, 1993.

239. Ehren, I., Iversen, H., Jansson, O., Adolfsson, J. And Wiklund, N.P.: Localization of nitric oxide synthase activity in the human lower urinary tract and its correlation with neuroeffector responses. Urology., 44: 683, 1994.

240. Hindmarsh, J.R., Gosling, P.T. And Deane, A.M.: Bladder instability. Is the primary defect in the urethra? Br. J. Urol., 55: 648, 1983.

241. Mcguire, E.J.: Detrusor response to outlet obstruction. World J. Urol., 2: 208, 1984.

242. Smith, S.H., Wheeler, M.A., Foster, J.H.E. And Weiss, R. M.: Improvement in interstitial cystitis symptom scores during treatment with oral L-arginine. J. Urol., 158:703, 1997.

243. Weiner, C.P. Lizasoain, I., Baylis, S.A., Knowles, R.G., Charles, I.G. And Moncada, S.; Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc. Natl. Acad. Sci., 91:5212, 1994.

244. Ehren, I., Hammarstrom, M., Adolfesson, J. And Wiklund, N.P.: Induction of calcium-dependent nitric oxide synthase by sex hormones in the guinea-pig urinary bladder. Acta Physiol. Scan., 153: 393, 1995.

245. Morrison, J.F.B.: Sensations arising from the lower urinary tract. In: The physiology of the lower urinary tract. Edited by M. Torrens and J.F. B. Morrison. Springer-Verlag: Berlin. p. 89, 1987.

246. De Groat, W.C., Booth, A.M. And Yoshimura, N.: Neurophysiology of micturition and its modification in animal models of human disease. In: nervous control of the urogenital system. Edited by C.A. Maggi, Harwood Academic Publishers: Chur. p. 227, 1993.

247. Maggi, C.A.: The pharmacology of the efferent function of sensory nerves. Autonom. Pharmacol., 11:173, 1991.

248. Maggi, C.A.: The dual, sensory and efferent function of the capasaicin-sensitive primary sensory neurons in the urinary bladder and urethra. In: nervous control of the Urogenital System. Edited by C. A. Maggi, Harwood Academic Publisher: Chur. Switzerland, p. 383, 1993.

249. Bahns, E., Halsband, U. And Janig, W.: Responses of sacral visceral afferents from the lower urinary tract, colon and anus to mechanical stimulation. Pflügers Archiv., 410: 296, 1987.

250. Vera, P.L. And Nadelhaft, I.: Conduction velocity distribution of afferent fibres innervating the rat urinary bladder. Brain Res., 520: 83, 1990.

251. Habler, H.L., Janig, W. And Koltzenburg, M.: Activation of unmyelinated afferent fibres by mechanical stimulation and inflammation of the urinary bladder in the cat. J. Physiol., 425: 545, 1990.

252. Habler, H.L., Janig, W. And Koltzenburg, M.: Myelinated primary afferents of the sacral spinal cord responding to slow filling and distension of the cat urinary bladder. J. Physiol., 463: 449, 1993.

253. Sengupta, J. N. And Gebhart, G.F.: Mechanosensitive properties of pelvic nerve afferent fibres innervating the urinary bladder of the rat. J. Neurophysiology. 72:2420, 1994.

254. Lawson, S.N.: Morphology and biochemical cell types of sensory neurons. In: Sensory neurons, diversity development and plasticity. Edited by S. A. Scott, Oxford University Press: Oxford. P. 27, 1992.

255. Lawson, S.N. And Waddell, P.J.: Soma neurofilament immunoreactivity is related to cell size and fiber conduction velocity in rat primary sensory neurons. J. Physiol., 435: 41, 1991.

256. Kawatani, M., Lowe, I.P., Nadelhaft, I., Morgan, C. And De Groat, W.C.: VIP in visceral afferent pathways to the sacral spinal cord. Neuroscience. Letters. 41: 311, 1983.

257. Keast, J.R. And De Groat, W. C.: Segmental distribution and peptide content of primary afferent neuro-innervating the urogenital organs and colon of male rat. J. Comp. Neurol., 319: 615, 1992.

258. Maggi, C.A. And Meli, A.: The role of neuropetides in the regulation of the micturition reflex. J. Auto. Pharmacol., 6:133, 1986.

259. Maggi, C.A. And Meli, A.: The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen. Pharmacol., 19:1, 1988.

260. Szallasi, A. And Blumberg, P.M.: Resinifentoxin and its analogs provide novel insights into the pharmacology of the vanilloid (capsaicin) receptor. L. Sci., 47: 1399, 1990.

261. Sharkey, K.A., Williams., R.G., Schultz Berga, M. And Dockray, G.J.: Sensory substance P-innervation of the urinary bladder possible site action of capsicin in causing urine retention in rats. Neuroscience. 10: 861, 1983.

262. Jansco, G. And Maggi, C.A.: Distribution of capsaicin-sensitive urinary bladder afferents I the rat spinal cord. Brain Res., 418: 371, 1987.

263. Maggi, C.A., Santicioli, P. And Meli, A.: The effects of topical capsaicin on rat urinary bladder mobility in vivo. European J. Pharmacol., 103: 41. 1984.

264. Maggi, C.A., Saticioli, P., Giuliani, S., Furio, M. And Meli, A.: The capsaicin-sensitive innervation of the rat urinary bladder: further studies on mechanism regulating micturition threshold. J. Urol., 136: 697, 1986.

265. Maggi, C.A., Santicioli, P., Geppetti, S., Furio, M., Frilli, S., Conte, B., Fanciullacci, M., Giuliani, S. And Meli, A.: The contribution of capsaicin-sensitive innervation to activation of the spinal vesico-vesical reflex in rats: relationship between substance P levels in the urinary bladder and the sensory-efferent function of capsaicin-sensitive sensory neurons. Brain Res., 415: 1, 1987.

266. De Groat. W., Kawatani, T., Hisamitsu, T. Cheng, C. L., Ma, C. P . Thor, K., Steers, W And Roppolo, J.R.: Mechanisms underlying the recovery of urinary bladder

function following spinal cord injury. J. Autonom. Nerv. Syst., 30:S71, 1990.

267. Abelli, Li., Conte, B., Somma, V., Maggi, C.S., Giuliani, S., Geppetti, S., Asessandri, M., Theodorsson, E. And Meli, Al.: The contribution of capsaicin-sensitive sensory fibers to xylene-induced visceral pain in conscious, freely-moving rats. Naunyn Schmiedeberg's Arch. Pharmacol., 337: 545, 1988.

268. Giuliani, S., Maggi, C.A. And Meil, A.: Capsaicin-sensitive afferents I the rat urinary bladder activate a spinal sympathetic cardiovascular reflex. . Naunyn Schmiedeberg's Arch. Pharmacol., 338: 1988.

269. Lecci, A., Giuliani, S., Garret, C. And Maggi, C.A.: Evidence for a role tachykinins as sensory transmitters I the activation of micturition reflex neuroscience. 54: 827, 1993.

270. Maggi, C.A., Patacchini, R., Santicioli, P. And Giuliani, S.: Tachykinin antagonists and capsaicin-induced contraction of the rat isolated urinary bladder: evidence for tachykinin-mediated cotransmission. Br. J. Pharmacol., 103: 1535, 1991.

271. Maggi, C. A., Lecci, A., Santicioli, P., Del Bianco, E. And Giulian, S.: Cyclophosphamide cysititis in rats: m involvement of capsaicin sensitive primary afferents. J. Autonom. Nerv. Syst. 38: 201, 1992.

272. Barbanti, G., Maggi, C.A., Beneforti, P., Baroldi, P. And Turini, D.: Further studies on pain relief produced by intravesical capsaicin in patients suffering from hypersensitive disorders of the lower urinary tract. Br. J. Urol., 71: 1993.

273. Fowler, C.J., Jewkes, D. Mcdonald. W.I., Lynn. B. And De Groat, W.C.: Intravesical capsaicin for neurogenic bladder dysfunction. Lancet. 339: 239, 1992.

274. Flower, C.J., Beck, R.O., Gerrard, S., Betts, C.D. And Fowler, C. G.: intravesical capsaicin for treatment of detrusor hyperreflexia. J. Neurol. Neurosurg. Psychiatry. 57: 169, 1994.

275. Biro.T., Maurer. M., Modarres. S., Lewin, N.E., Brodie, C., Cas. C., Acs. P., Paus, R. And Blimberg. P.M.: Characterization of functional vanilloid receptors expressed by mast cells. Blood. 91:1332, 1998.

276. Caterina. M.J., Schumacher. M.A., Tominaga. M., Rosen. T.A., Levine.J.D. And Julius. D.: The capsicine receptor: a heat-activated ion channel in the pain pathway. Nature. 389: 816, 1997.

277. Maggi. C.A.: The role of peptides in the regulation of the micturition reflex: an update. Gen. Pharmacol., 22: 1, 1991.

278. Szolcasanye. J.: Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultraviolet irradiation. J. Physiol., 388: 9, 1987.

279. Jiang, W., Kibble, A. And Morrison. J.F.B.: The effect of capsaicin on pelvic afferent inputs from the urinary bladder of anaesthetized rats. J. Physiol., 487: 119P, 1995.

280. Wilson. P.M.: Understanding the pelvic floor. S. Afr. Med. J. 47:1150, 1973.

281. Wilson. P.M.: Some observations on pelvic floor evolution in primates. S. Afr. Med. J., 47: 1203, 1973.

282. Constantinou. C., Govan.D.: Spatial distribution and timing of transmitted and reflexly generated urethral pressures in healthy women. J. Urol., 127: 964, 1982.

283. Sato, K.: A morphological analysis of the nerve supply of the sphincter ani externus, levator ani and coccygeus. Acta Anat. Nippon, 44: 187, 1980.

284. Abitbol, M.M.: Evolution of ischial spine and of the pelvic floor in the hominoidea. Amer. J. Phys. Anthropol., 75: 53, 1988.

285. Campbell, R.M.: The anatomy and histology of the sacrouterine ligaments. Am. J. Obstet. Gynecol., 59: 1, 1950.

286. Range, R.L., Woodburne, R.T.: The gross and microscopic anatomy of the transverse cervical ligaments. Am. J. Obstet. Gynecol., 90: 460, 1964.

287. Blaisdell F.E.: The anatomy of the sacro-uterine ligaments. Anat. Record, 12: 1, 1917.

288. DeLancey, J.O.L.: Anatomic aspects of vaginal eversion after hysterectomy. Am. J. Obstet. Gynecol., 166: 1717, 1992.

289. Ala-Ketola, L.: Roentgen diagnosis of female stress urinary incontinence. Roentgenological and clinical study. Acta Obstet. Gynecol. Scand. Suppl., 23:1, 1973.

290. DeLancey, J.O.L.: Structural support of the urethra as it relates to stress urinary incontinence: The hammock hypothesis. Am J. Obstet. Gynecol., 170: 1713, 1994.

291. DeLancey., J. O. L., Starr. R. A.: Histology of the connection between the vagina and levator ani muscles. J. Reprod. Med., 35: 765, 1990.

292. Kirschner-Hermanns, R., Wein, B., Niehas, S., Schaefer, W., Jakse, G.: The contribution of magnetic resonance imaging of the pelvic floor to the understanding of urinary incontinence. Br. J. Urol., 72: 715, 1993.

293. Howard, D., Miller, J.M., DeLancey, J.O.L., Ashton-Miller, J.A.: Differential effects of cough, valsalva, and continence status on vesical neck movement. Obstet. Gynecol., 95: 535, 2000.

294. DeLancey, J.O.L. Structural anatomy of the posterior compartment as it relates to rectocele. Am. J. Obstet. Gynecol., 180: 815, 1999.

295. DeLancey. J. O.L: Correlative study of paraurethral anatomy. Obstet. Gynecol., 68: 91, 1986.

296. DeLancey. J. O.L.: Structural aspects of the extrinsic continence mechanism. Obstet. Gynecol., 72: 296, 1988.

297. DeLancey., J. O. L.: The pubovesical ligament. A separate structure from the urethral supports. (pubo-urethral ligaments). Neurourol. Urodyn., 8: 53, 1989.

298. Berglas. B. , Rubin, I. C.: Histologic study of the pelvic connective tissue. Surg. Gynecol. Obstet., 97: 277, 1953.

299. Ulmsten, U., Ekman, G., Giertz. G. , Malmstrom, A.: Different biochemical composition of connective tissue in continent and stress incontinent women. Acta Obstet. Gynecol. Scand., 66: 455, 1987.

300. Makinen, J. Sonderstrom, K. O., Kiilhoma, P., Hirvonen, T.: Histological changes in the vaginal connective tissue

of patients with and without uterine prolapse. Arch. Gynecol., 239: 17, 1986.

301. Norton, P., Baker, J., Sharp, H. , Warenski, J.: Genitourinary prolapse. Relationship with joint mobility. Neurourol. Urodyn., 9: 321, 1990.

302. Koelbl, H., Strassegger, H., Riss, P. A., Gruber, H.: Morphologic and functional aspects of pelvic floor muscles in patients with pelvic relaxation and genuine stress incontinence. Obstet. Gynecol., 74: 789, 1989.

303. Smith, A.R.B., Hosker, G. L. , Warrell, D. W.: The role of partial denervation of the pelvic floor in the aetiology of genitourinary proplapse and stress incontinence of urine. A neurophysiological study. Br. J. Obstet. Gynaecol., 96: 24, 1989.

304. Dubowitz, V.: The role of histochemistry in muscle disease. In: Modern trends in Neurology. Edited by D. Williams. London: Butterworth. Pp. 189-208, 1970.

305. Bearsiek, F. Parks, A. G. Swash. M.: Pathogenesis of anorectal incontinence. A histometric study of the anal sphincter musculature. J. Neurol. Sci., 342:111, 1979.

306. Parks, A. G., Swash. M. , Ulrich. H.: Sphincter denervation in ano-rectal incontinence and rectal prolapse. Gut 18: 656, 1977.

307. Gilpin, S. A., Gosling, J. A., Smith, A.R. B., Warrell, D.W.: The pathogenesis of genito-urinary prolapse and stress incontinence of urine. A histological and histochemical study. Br. J. Obstet. Gynaecol., 96: 15, 1989.

308. Dimpfl, T.H., Jaeger, C.H., Mueller-Felber. W., Anthuber. C., Hirsch, A., Brandmaier, R., Schuessler. B.: Myogenic changes of the levator ani muscle in premenopausal women: The impact of vaginal delivery and age. Neurourol. Urodyn., 17: 197, 1998.

309. Madoff, R. D., Williams, J.G., Caushaj, P.F.: Fecal incontinence. N. Engl. J. Med., 326: 1002, 1992.

310. Dalley, A. F.: The riddle of the sphincters. The morphophysiology of the anorectal mechanism reviewed. Amer. Surgeon, 53: 298, 1987.

311. Milligan, E.T.C., Morgan, C.N.: Surgical anatomy of the anal canal with special reference to anorectal fistulae. Lancet, ii: 1150, 1934.

312. Oh, C., Kark, A.E.: Anatomy of the external anal sphincter in men. Acta Anat., 105: 25, 1979.

313. Lawson, J.O.: Pelvic anatomy. II. Anal canal and associated sphincters. Ann. R. Coll. Surg. Engl., 54: 288, 1974.

314. DeLancey, J.O.L., Toglia, M.R., Perucchini, D.: Internal and external anal sphincter anatomy as it relates to midline obstetrical lacerations. Obstet. Gynecol., 90: 924, 1997.

315. Snooks, S.J., Setchell, M., Swash, M., Henry, M.M.: Injury to innervation of pelvic floor sphincter musculature in childbirth. Lancet, 2: 546-50, 1984.

316. Snooks, S.J., Henry, M.M., Swash, M. Faecal incontinence due to external sphincter division in childbirth is associated with damage to the innervation of the pelvic floor musculature: a double pathology. Br. J. Obstet. Gynaecol., 92: 824, 1985.

317. Sultan, A.H., Kamm, M.A., Hudson, C.N., Thomas, J.M., Bartram, C.I.: Anal-sphincter disruption during vaginal delivery. N. Engl. J. Med., 329: 1905, 1993.