INTRODUCTION

Normal bladder storage and voiding involve low pressure and adequate bladder volume filling followed by complete detrusor contraction associated with adequate relaxation of the sphincter complex. This process requires normal sensation and adequate bladder outlet resistance. Achievement of urinary control is equally complex and as yet not fully understood. Urinary control develops during the first four to six years of life and is a highly complex phenomenon [61].

Besides a normal anatomy of the lower urinary tract, a diffuse neuronal network has to be present and be centrally connected and controlled. Bladder emptying in infants gradually changes to voluntary voiding as the central nervous system gains control of the micturition impulse during the first four years of life[82].

It is understandable that this series of complex events is highly susceptible to the development of various types of dysfunctions. Various functional disorders of the bladder sphincter perineal complex may occur during this sophisticated course of early development of normal micturation control mechanisms. These acquired functional disorders overlap with other types of bladder functional disturbances that may have a more organic but congenital underlying pathophysiological basis[23].

Overactive bladder (OAB) can be defined as a symptom complex of urinary urgency with or without urge

incontinence, urinary frequency (voiding eight or more times in a 24-hour period), and nocturia (awakening two or more times at night to void). The ICS (International Continence Society) has classified overactive bladder as a syndrome for which no precise cause has been identified, with local abnormalities ruled out by diagnostic evaluation[56].

The prevalence of the symptoms of frequency, urgency, and urge urinary incontinence individually was low, as was the joint occurrence of all 3 symptoms. The prevalence of urge urinary incontinence, either alone or combined with another symptom, was 9%. Thus, over half of all cases of OAB are *wet*[57].

Botulinum toxins have been used for years as a first line of treatment for various clinical conditions. Local reduction of strength in the injected muscle indicates the expected result. Side effects reported in nonurologic applications generally are limited to local effects and transient[53].

Botulinum toxins exert their paralyzing effects by inhibiting the release of acetylcholine from the motor nerve into the neuromuscular junction[42]. Without acetylcholine release, muscles are unable to contract. After an intramuscular injection of botulinum toxin, temporary chemodenervation and muscle relaxation can be achieved. There is also evidence that BTX-A decreases the afferent signals from the muscle spindles, thereby directly reducing the neural activity and reflex arc that results in spasticity[43].

AIM OF THE WORK

Injection of botulinum toxin in the detrusor muscle and evaluation of the detrusor muscle to find out a scoring system for detrusor muscle structure (number of junction present per field, gaps between cells in different field, contents of gaps, state of the muscle cell and spectrum of cellular injury present mild, moderate or severe) based on electron microscopic examination of the bladder biopsies.

STRUCTURE OF SMOOTH MUSCLE CELL

Sarcolemma of smooth muscle cells:

The sarcolemma of smooth muscle cells consists of a regular arrayment of firm regions in which the cytoskeletal proteins take root. These are interspersed with abundant vesicular regions, "hinge" domains with plasmalemmal reserve folds for facile movement of the sarcolemma[(1)].

Apart from these so-called macrodomains, the sarcolemma contains "microdomains". These assemblies of cholesterol and glycosphingolipids form dynamic structures termed lipid "rafts" or detergent-insoluble glycosphingolopid complexes (DIGs). Membrane rafts are associated with characteristic sets of proteins, self-associate to form higher order structures with higher physical stability and constitute hubs of signaling activity[(2)].

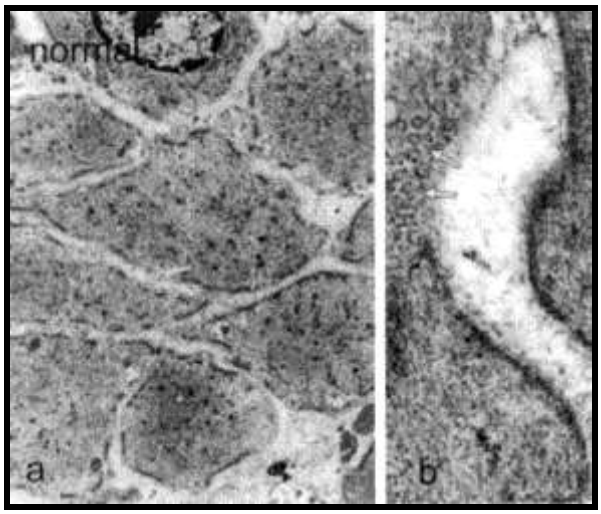


Figure (1): Normal smooth muscle cells are packed with myofilaments[(2)].

Annexins belong to a family of Ca^{2+} dependent, lipid-binding proteins and have been assigned roles in the regulation of Ca^{2+} homeostasis and signal transduction. Annexins 2 and 6, which are particularly abundant in smooth muscle cells, are associated with lipid microdomains[3]

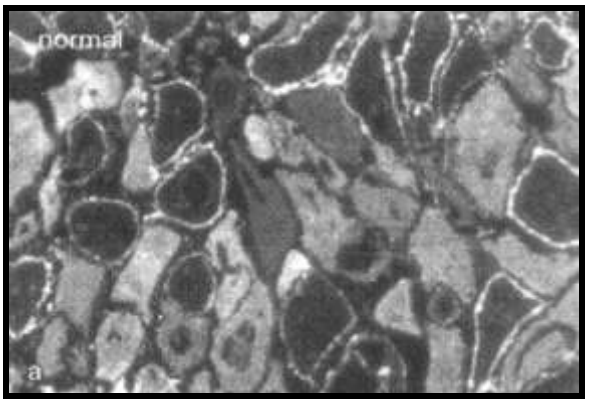


Figure (2): Annexin 6 expression in smooth muscle cells from normal bladder [3].

Myofibroblasts:

Myofibroblasts are crucial to smooth muscle function in many organs and their presence is now recognized through- out the urinary tract. Fibroblastic cells in the human detrusor are predominantly fibroblasts. Vimentin-like immunoreactivity is widely expressed by fibroblastic cells. The c-kit-like immunoreactivity observed was weak and localized to the cell somata, and cannot on its own be considered a reliable indicator of a myfibroblast phenotype. The lack of α-smooth muscle actin and desmin-like immunoreactivity was also consistent with fibroblasts. Ultrstructural features of typical fibroblasts in vivo include

prominent rough endoplasmic reticulum and Golgi apparatus, and cytoplasm containing vesicles, vacuoles and mitochondria with relatively few microfilaments or intermediate filaments and rare intercellular contacts[4].

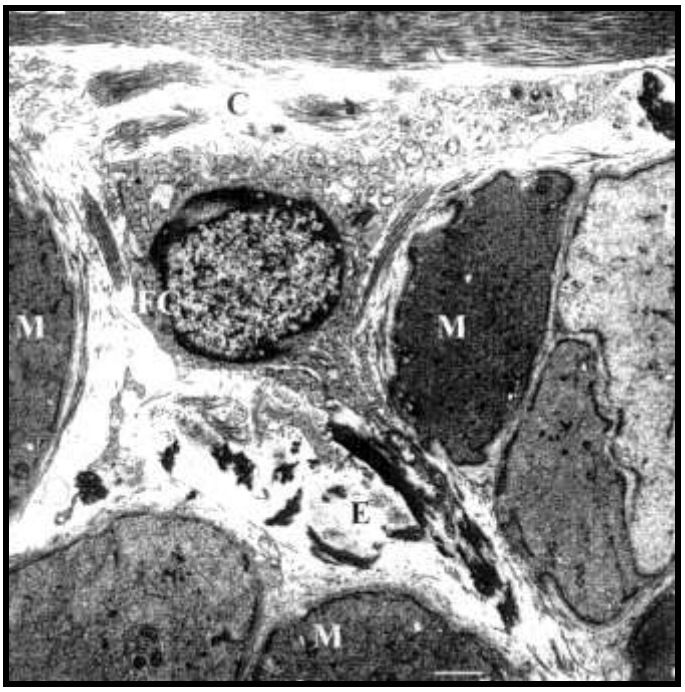


Figure (3): Fibroblastic cell ultrastructure[4].

Myofibroblasts usually possess an electron dense nucleus with peripherally distributed heterochromatin, well developed rough endoplasmic reticulum, incomplete basal lamina, abundant mitochondria, membrane caveolae, dense bodies, gap junctions and bundles of filaments running parallel to the long axis of the cell[5].

The distribution of fibroblasts cells and their intimate relationship with connective tissue components

ultrastructurally suggests that they are important for the secretion and maintenance of extracellular material, like fibroblasts else where. As such, they are the probable source of increased connective tissue seen in detrusor overactivity[5].

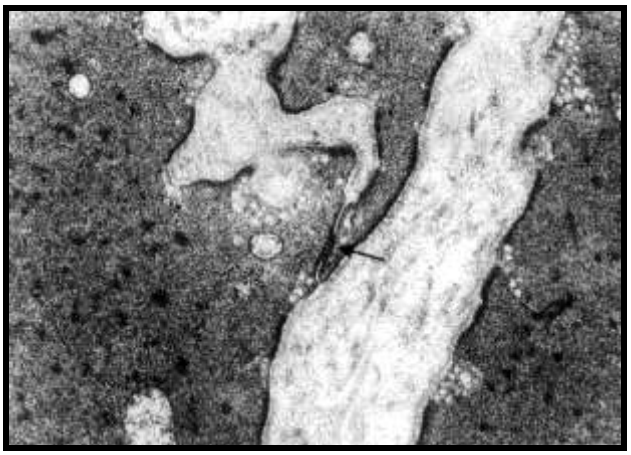
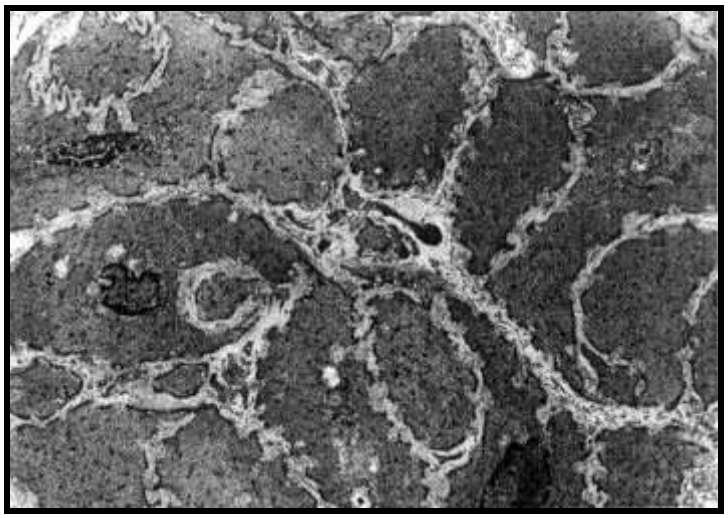


Figure (4): Muscle cell junction. Protrusion junctions (black arrow).

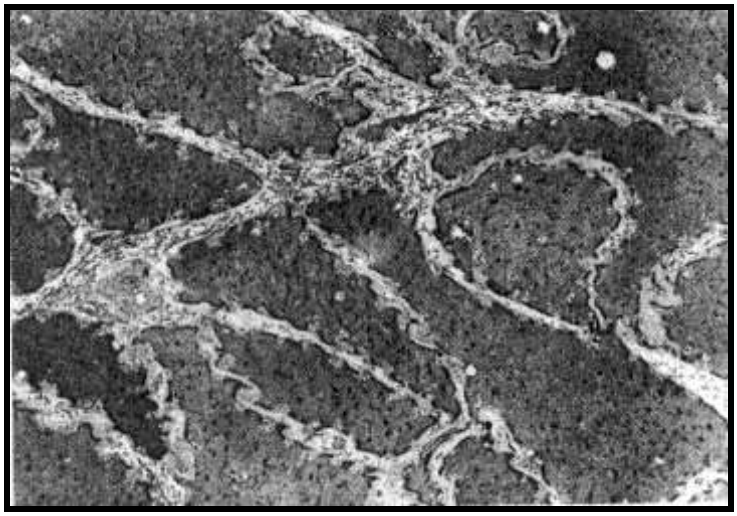
Ultrastructural studies of the overactive detrusor, that has not been treated with Botulinum toxin type A, have demonstrated changes in the intrinsic innervation including a widespread axonal degeneration and a limit axonal regeneration combined with Schwann cells activation independent of the duration of the neurogenic disorder[5].

Myogenic changes included the presence of different extents of muscle cell degeneration, a reduction of intermediate cell junctions of muscle cells and a dominance of intimate cell appositions with much narrow junctional gaps forming chains ≥ 5 muscle cells[6].

Repeated injections of Botulinum toxin type A represent microtrumas to the detrusor and may result in microscaring with increased production of collagen and subsequent reduction of bladder compliance[6].



[A]



[B]

Figure (5): Muscle cell fascicle structure. Detrusor biopsies from the same patient before (A) and after botulinum toxin type A injection (B) with compact/intermediate muscle cell fascicles[6].

Individual non striated myocytes when seen with the light microscope show a centrally placed nucleus, which may be highly elongated in stretched muscle, or ovoid with a crenated surface in contracted muscle. The cells are weakly birefringent, indicating some degree of longitudinal orientation of the cell components[7].

Ultrastructurally the cytoplasm of the non striated myocyte is seen to consist mainly of closely packed fine filaments lying parallel to the long axis of the cell. Most of the usual cellular organelles including Golgi complexes, sarcoplasmic reticulum, free ribosomes, and lysosomes are positioned near both ends of the nucleus (the nuclear poles), very rarely, irregularly shaped lipid droplets and small dense glycogen granules may be observed but clusters of oval mitochondria are also found elsewhere in the cytoplasm, either peripherally or in deeply placed scattered groups, occasional oval are randomly scattered among the myofilaments, the site varying with different types of non striated muscle[7].

Mitochondria

Mitochondria are membrane bound organelles of great metabolic significance. Under light microscope were, first observed as thread like, spherical, or ellipsoidal bodies present in the cytoplasm of most cells particularly those with a high metabolic rate. In size most mitochondria range from 0.5-2 urn wide by 3-10 μm long. Their distribution in

cell varies. They tend to accumulate in parts of the cytoplasm where metabolic activity is more intense e.g., along the long axis of smooth muscles of the bladder. Under the electron microscope, each mitochondrion is composed of an outer and an inner membrane, the inner membrane project folds, termed cristae (creating a relatively large surface area), into the interior of the mitochondrion, these membranes enclose two spaces. The cristae also are numerous and complex in smooth muscles. Also within the matrix are a variety of inclusions including calcium salts, organic crystals and glycogen. There are also ribosomes and the nucleic acids RNA and DNA. The functions of mitocondria are mainly concerned with oxidative phosphorylation associated with kreb's cycle and the cytochrome electrontransport sequences of respiration. The end result of these reactions is production of CO_2., water, and heat and high energy organic phosphate compounds particularly ATP and GTP. These compounds pass to other parts of the cell, where they take part in energy consuming reactions [8].

The cytoplasm (sarcoplasm) of each smooth muscle cell contains innumerable myofilaments, most of which are oriented parallel to the long axis of the cell. Many of the myofilaments are apparently attached to dense bodies (focal densities) which occur throughout the sarcoplasm and consist of condensations of electron-dense material, each is about 0.5 urn in diameter. Similar dense bodies are

situated immediately beneath the plasma membrane and believed to represent the sites of anchorage of the myofilaments to this membrane[8].

The plasma membranes of smooth muscle cells possess numerous small flask shaped infoldings called caveolae (Fig. 6)[7], group of these caveolae occur in rows arranged parallel to the long axis of the cell, separated from one another by dense filament attachment zones. The function of these caveolae is unknown, but they evidently increase the surface of each cell and may serve to sequestrate part of the extracellular fluid for utilization by the muscle.

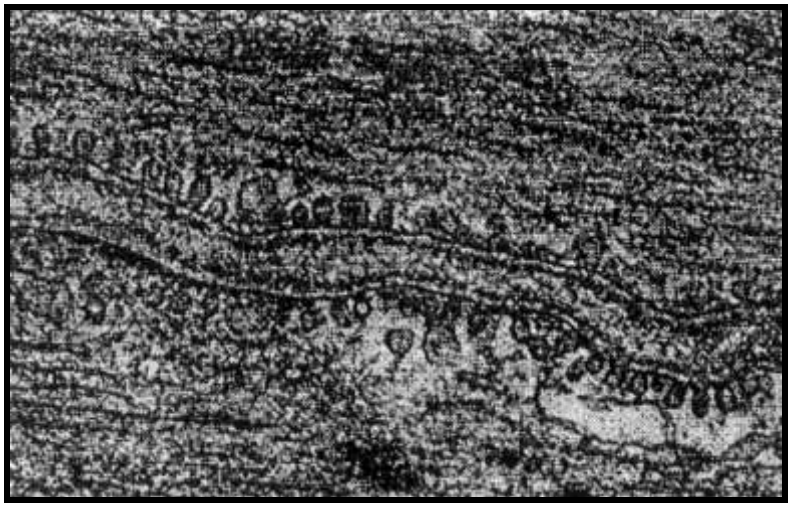


Figure (6): Shows sacs of sarcoplasmic reticulum with caveolae[7].

Smooth muscle cell interrelationships:

Within any one muscle bundle the plasma membranes of adjacent cells are frequently observed in

mutual apposition to form an intercellular junction of which four different types have been observed. These are, regions of close approach, peg- and socket junctions, intermediate junctions and gap junctions [9].

Regions of close approach:

This is the most common form of junctions between neighboring muscle cells in which the apposed plasma membranes converge to within 10-20 nm of each other. Basal lamina material is reflected from one cell to another at the margins of the junction and does not extend into the narrow intercellular cleft. However, neither the plasma membranes nor the adjacent subsarcolemmal cytoplasm show any form of specialization. Intercellular junctions of this type often occur between the tips of narrow cytoplasmic protrusions from adjacent smooth muscle cells. This type of junction is responsible for the electronic propagation of contraction waves from one smooth muscle cell to its neighbours[9].

Peg and socket junctions:

Another common type of association between smooth muscle cells called peg and socket junction (Fig. 8). This type consists of an elongated or bulbous projection from one cell which fits snugly into a depression in an adjacent cell. At the interdigitation zone basal lamina material is absent from the 10 nm gap separating the membranes of the

two adjacent muscle cells. Occasionally, the apposing cell membranes are more closely related in the narrow stalk of the projection. It has been suggested that these interdigitations provide a mechanical linkage between neighbouring cells[7]. Alternatively, they may represent a special type of close approach and permit myogenic conduction.

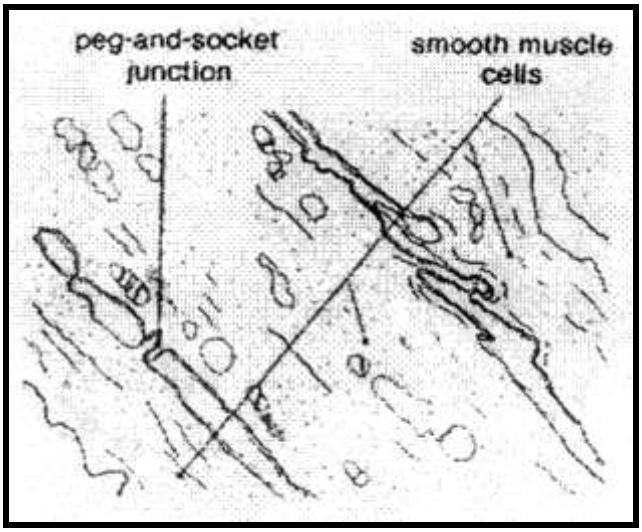


Figure (7): Peg and socket junctions [7].

Intermediate junctions

These junctions are frequently observed between adjacent smooth muscle cells (Fig. 8) and consist of regions where apposing plasma membranes lie parallel to each other for distances up to 2.5 mm. The membranes are separated by a gap of about 50 nm and the intercellular space contains a single basal lamina. In addition, the sarcoplasm underlying the apposing membranes has an increase electron density identical to the plaque-like

condensation which occur at other regions of the musle cell membrane. It is not known whether the intermediate junctions have any functional significance in smooth muscle; they may simply represent regions where dense filament attachment zones of adjacent cells are, by chance, in register. Nevertheless, in these regions the close proximity of adjacent cells narrows the extracellular space such as the basal laminae become confluent [7].

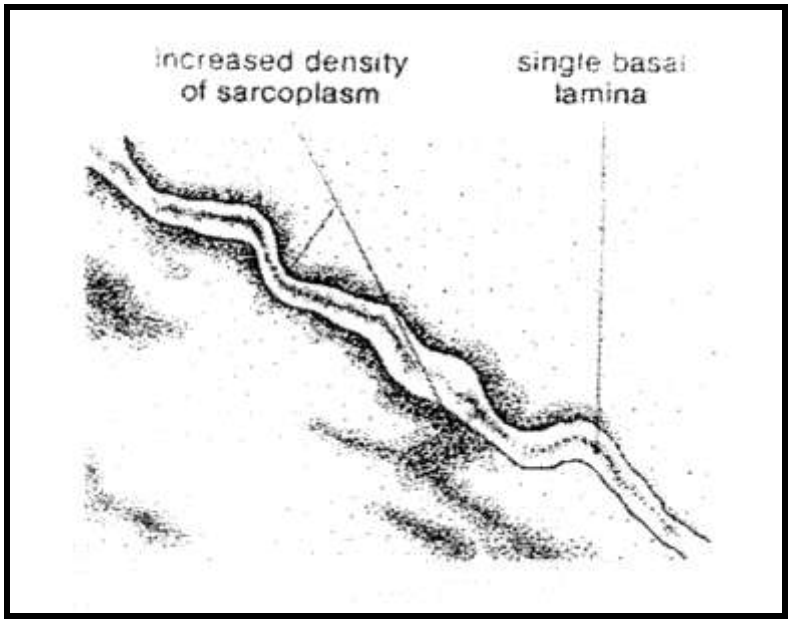


Figure (8): Intermediate junctions [7].

Gap junctions (Nexus):

This type of junction is exceedingly, rarely observed between smooth muscle cells, and as previously noted it seems likely that one or more of the other types of intercellular junction is responsible for cell to cell (myogenic spread) of electrical activity in the muscle (Fig. 9). The structural appearance of gap junctions varies with the type of fixation employed during processing for

electron microscopy. After routine fixation, this type of junction appears as a five layered structure in which the outer lamellae of apposing muscle cell membranes are fused together to form a central dense line. The intermediate clear zones and inner leaflets of the two membranes are situated on either side of the central line. Recently, the use of improved fixation procedures and of freeze-fracture techniques has revealed that a narrow gap about 2 nm wide separates the two outer apposing leaflets. Hence, the term gap junction rather than nexus is a more accurate description of these regions. The junction is usually a circular zone of cell to muscle cell is reflected to become continuous with that of its neighbour. Consequently, basal lamina material does not extend between the cells at the junction, the intercellular gap being occupied by discrete bridges between the apposing membranes. It is now believed that a narrow channel occurs within each of these bridges, allowing ions and small molecules to pass from one cell to the next [(7)].

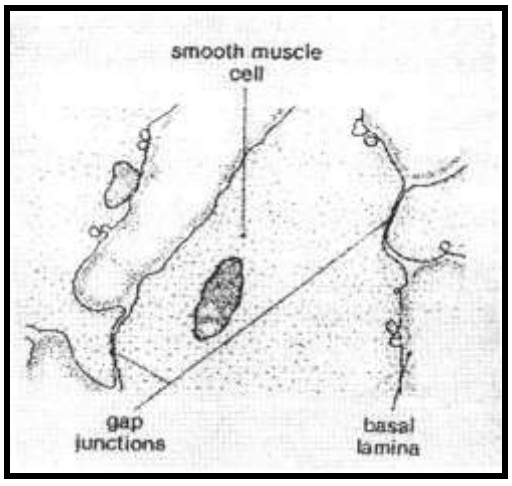


Figure (9): Gap junctions (Nexus) [(7)].