STUDIES ON THE PHYSIOLOGICAL AND PHARMACOLOGICAL ASPECTS OF THE LOWER URINARY TRACT IN NORMAL AND FOLLOWING SPINAL LESION

by

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PHYSIOLOGY AND PHARMACOLOGY OF THE BLADDER WITH SPINAL LESIONS

PREFACE

The results of the present work have been partly published in some journals during the period between 1983 and 1984 and the other part are submitted recently. (See Addendum)

Although the major experimental work was carried out by myself, an appreciable amount of help was kindly supplied by my supervisors and co-workers.

The processing and interpretation of the histologic sections throughout the work was done in the Pathology Department laboratories under the supervision of Dr. J. Lamarche in the University of Sherbrooke.

The in vivo procedure of the experimental set up in the cats and rabbits were carried out under the supervision of Dr. M. Elhilali and Dr. C. Galeano at McGill University and University of Sherbrooke respectively.

The supply of pharmacological agents, particularly the prostaglandins, was provided by the Pharmacology Department at the University of Sherbrooke.

The statistical interpretations of some of the results were achieved through the collaboration of personnel from the Department of Statistics at the University of Sherbrooke.

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I would like to extend my thanks to the colleagues with whom I worked in the laboratory throughout the years.

I am deeply indebted to Miss Gail MacLeod for the significant amount of time she devoted to the excellent word processing service. Her attention to detail and perfection is sincerely appreciated.

Finally, I would like to express my eternal gratitude to my parents and loving wife for their patience. without their support and understanding this work could not have been accomplished.

ABSTRACT

The mechanisms implicated in voiding and continence are far from being settled in the normal as well as in neurogenic bladder dysfunction. Understanding these mechanisms is an essential step in the management of the bladder dysfunction secondary to neurological lesions. In the course of our studies, we demonstrated the existence of a fine coordination "synergism" between the several components of the lower urinary tract i.e., detrusor, proximal urethral muscle and periurethral striated muscles. This synergism is found to be responsible for adequate bladder emptying during voiding in the animal model with intact neural axis. The synergism between the bladder and its outlet is under the control of a higher centre in the brain stem and mediated through the spinal cord and peripheral nerves. The cause of failure of bladder emptying following a spinal lesion was shown to be a lack of synergism between the bladder and its outlet. An animal model for chronic multiple sclerosis-like disease was developed and proved to show a good urodynamic and neurological correlation with that found in the human afflicted with multiple sclerosis.

The pharmcological investigation on the bladder and urethra shows that there is selective distribution of cholinergic, adrenergic and purinergic receptors along the individual layers of the smooth muscles of the urethra. On the other hand, the importance of the Calcium ions in the

contractility of the detrusor muscle was also studied. The effect of some Calcium ions antagonists on the detrusor contractility was evaluated. They show selective inhibition on the detrusor contraction both in vivo and in vitro. The calcium antagonists may present a new treatment modality for controlling bladder instability.

RESUME

Les mechanismes impliqués dans la continence et la miction sont loin d'être établis dans l'état normal ainsi que dans la dysfonction vesicale neurogène. Comprendre ces mechanismes représente une importante étape dans le traitement de la dysfonction vesicale suite à des lesions neurogènes. Pendant notre étude nous avons demontré la présence d'une coordination 'synergisme' délicate entre differentes composantes de l'appareil urinaire bas tel que le détruseur, la musculature de l'urètre proximale et la musculature striée periurèterale. Ce synergisme est indispensible pour une évacuation adéquate de la vessie pendant la miction chez le modèl-animal avec un système nerveux intact. Le synergisme entre la vessie et son col est controlé par un centre situé dans le mesencephale. La transmission se fait à travers la moelle epinière et les nerfs péripheriques. L'incapacité de vider la vessie après une lésion epinière est due à une absence de synergisme entre la vessie, son col et les muscles striés periuretéraux. Nous avons developé un modèl-animal pour la sclérose-en-plaques chez le lapin. Ce modèle-animal a montré une bonne correlation entre les changements urodynamiques et neurologiques semblable à la dysfonction vésicale chez les patients atteints de sclérose-en-plaques.

Les investigations pharmacologiques sur la vessie et

l'urètre ont montré une distribution selective dans les récepteurs cholinergiques, adrenergiques et purinergiques sur les differentes couches de muscle lisse de l'urètre. D'une autre part, l'importance du rôle des ions de Calcium dans la contractilité du muscle détruseur a été étudié. Les effects de quelques agents bloquers d'ions calciques ont été évalué sur la contractilité du détruseur. Ces agents montrent une inhibition sue la contractilité de la vessie in vivo et in vitro. Ces résultats montrent la possibilité d'utiliser une nouvelle modalité de traitement pour le control de la vessie instable.

LIST OF ABBREVIATIONS

Acetyl choline	A Ch
Circular smooth muscles of urethra	CIRC
Cystometrogram	CMG
Detrusor bladder muscle	DET
Electrocardiogram	EKG
Electromyogram	EMG
Experimental allergic encephalomyelitis	E.A.E.
Effective dose 50	ED50
Frequency in Hertz	Hz
French size	Fr
Hematoxilin and eosin stain	H&E
Intraurethral pressure	IUP
Intravesical pressure	IVP
Longitudinal smooth muscles of urethra	LONG
Multiple sclerosis	M.S.
Nifedipine	NFD
Noradrenaline	NA
Periurethral striated muscles	PSM
Parasympathetic	Parasymp
Prostaglandin	PG
Urethral pressure profile	UPP
Segontin	SGT
Sympathetic	Symp
Verapamil	VRP
Vertical bars ($\perp$) = standard deviation	STD

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CHAPTER II

INTRODUCTION

The present knowledge on the bladder began many centuries ago but it is only in the last fifty to sixty years that we are starting to understand its mechanism of function. Galen described the bladder as a hollow organ made of fibres with different directions and has a sphincter structure. The ancient Egyptians gave a fairly detailed description about the neurogenic bladder dysfunction in paraplegia in the Edwin Smith Surgical Papyrus (Elsberg, 1931). Griffiths in 1891 gave a detailed description of the anatomy of the bladder and the arrangement of the muscle fibres into different layers. Barrington was the first to describe the superior centres of micturition in the brain stem and their importance in regulating micturition.

Since then, the literature dealing with the lower urinary tract has expanded enormously year after year and many authors share the main concern of attempting to better understand the mechanism of bladder and urethral functions.

The neurogenic bladder dysfunction represents a continuous risk to the health and life of patients unfortunately affected. Besides being a social problem, the morbidity and mortality rises significantly in those patients with inappropriately managed bladder dysfunction. In the present work, I shall try to summarize the results of research conducted in the Urology Departments of the University of Sherbrooke and McGill University during the

last four years. Our approach to the problem of neurogenic bladder dysfunction was categorized under two main goals. The first goal was the development of an animal model for studying the mechanisms of continence and voiding in the animal with intact spinal cord. This entailed special emphasis on the role of the detrusor, the different components of the bladder outlet which are: the smooth muscle and striated muscle components of the urinary sphincteric mechanism. This work was followed by a study of the pharmacology of the different neurotransmitters and other agents and their effect on the urethral musculature. The other major goal was the study development of animal models for both acute and chronic spinal lesions for the study of theirsequelae on the dynamics of the bladder and its outlet. The possibility of the use of newer pharmacological agents to control the bladder irritability was also evaluated.

In the present thesis after a general review of the literature pertinent to the study, the results will be presented and discussed in detail in the chronological order of their achievement.

CHAPTER I

REVIEW OF LITERATURE

REVIEW OF THE LITERATURE

ANATOMY OF THE BLADDER AND URETHRA:

The bladder outlet and urethra are still the subject of many divergent opinions concerning both their structure and function.

The musculature of the urinary bladder or detrusor is classically described to be formed of three layers, an inner and outer longitudinal layer and a middle circular layer. These smooth muscle bundles intermingle with each other forming a broad or narrow meshwork with decussation all through the wall.

Woodburne (1968) and Gosling (1979) demonstrated that the musculature does not form separate layers.

At the level of the internal meatus, the detrusor bundles tend to converge (Bissada et al, 1977). However, Tanagho and Smith (1966) found that the inner longitudinal layer is missing posteriorly and that there is poor development of the middle circular layer. As the fibres of the inner longitudinal layer reach the bladder neck, they are arranged in a radial manner. They converge on the internal meatus where they meet the longitudinal fibres of the superficial trigone at the midline posteriorly. All these fibres sweep over the edges of the internal meatus to continue into the urethra as its inner longitudinal coat

(Hutch and Rambo 1967; Hutch, 1967 and Woodburne, 1968).

The middle circular fibres are well developed anteriorly in the midline above the internal meatus without encircling it. Posteriorly this layer is the least developed (Tanagho and Smith, 1966; Woodburne, 1968) forming thus an arc close to and just above the internal meatus. On both sides, the fibres fan postero-laterally to bind to the lateral edge of the deep trigone. Above the level of the ureteral hiatus, the bundles become more superficial (submucosal) to form a complete ring (Tanagho and Smith, 1966).

Hutch (1965) described this ring as the ventral segment of his "base plate". He counted them to be 15-18 individual string-like bands of circular smooth muscles. The most caudal ring passed around the bladder neck to insert into the apex of the trigone. The most cranial are inserted into the ureteral hiatus and the remaining rings are inserted into the trigone in corresponding position between these two points. (Figure 1)

The outer longitudinal layer form a well formed coat above the level of the internal meatus. As the bundles approach the bladder neck, the posterior fibres from both sides sweep posterolaterally and fuse with the anteerior wall of the prostate (Woodburne, 1968). The remaining fibres pass around the bladder neck to the opposite wall of the urethra thus forming the outer circular muscle coat of the urethra (Hutch and Rambo, 1967). (Figure 2)

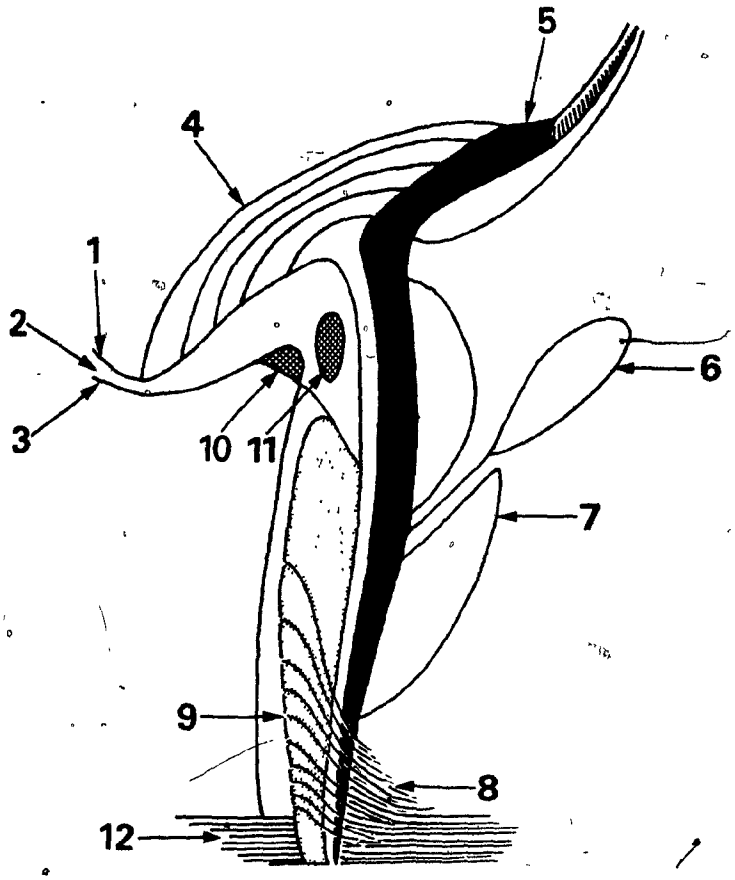


Figure 1. Sagittal section of the bladder base and male proximal urethra. (1) Mucosa and inner LONG muscle layer. (2) Middle CIRC layer. (3) Outer LONG layer. (4) Ring of middle LONG layer. (5) Deep trigone. (6) Median lobe of prostate. (7) Posterior lobe of prostate. (8) External urethral sphincter. (9) Periurethral striated muscle. (10) Transverse precervical arc. (11) Detrusor loop. (12) Smooth muscle sling from precervical arc to urethral inner LONG layer.

From Bissada et al (1977).

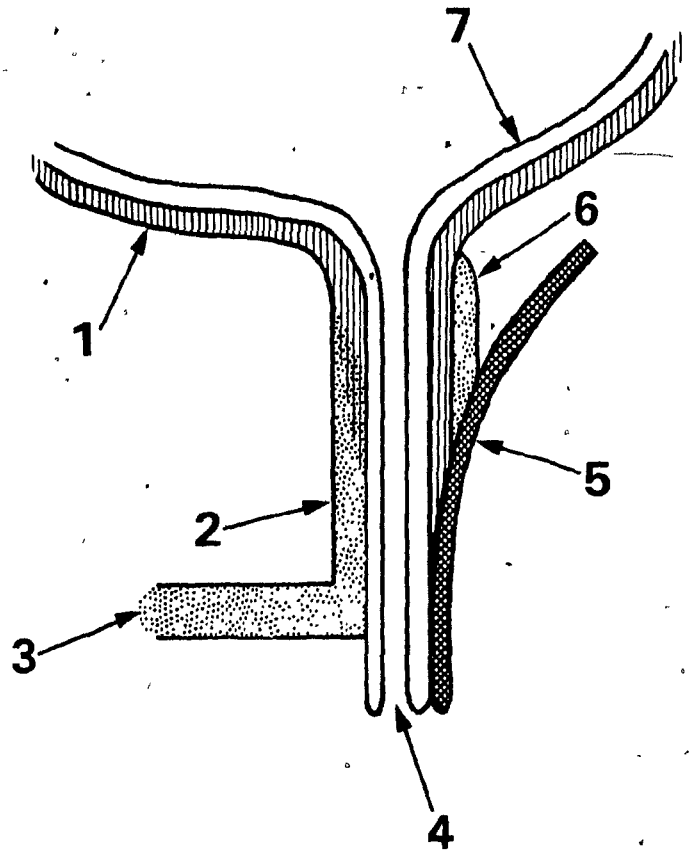


Figure 2. Sagittal section of the bladder base and urethra in the female. (1) External LONG layer. (2) Striated muscle. (3) Urogenital diaphragm. (4) Urethra. (5) Vaginal wall. (6) Striated muscle.

From Hutch and Rumbo (1967).

The trigonal musculature is the termination of the ureteric muscles in the bladder. Woodburne (1968) followed the ureteric muscles down to the bladder and noticed that they cross the midline to blend with those of the opposite side forming the interureteric fold. Moreover, there is a downward fanning of the ureteric muscle to form a dense lateral border of the trigone.

Bladder Trigone:

Hutch (1965) and Tanagho and Smith (1966) differentiated the trigone into superficial and deep strata. The superficial trigone is a direct continuation of the ureteric muscle. The longitudinal muscles of the ureter fan out uninterrupted down to the internal meatus. Passing over the posterior lip of the internal meatus, they unite with those of the opposite side. All of the fibres continue downward through almost the entire length of the female urethra whereas, in the male, most of them are inserted into the verumontanum where they fuse with the musculature of the ejaculatory ducts.

The deep trigone is a direct continuation of Waldeyer's sheath (Tanagho and Smith, 1966), and with its mate from the other side as well as variable contributions from the detrusor muscle, form a roughly triangular sheet of muscle that lies deep to the superficial trigone. The apex of this sheet lies at the internal meatus where most of the fibres are inserted. The Waldeyer's sheath is a fibromuscular

tubular structure that encircles the distal 3-4 cm of the juxtavesical ureter and follows the ureter through the ureteral canal. (Figure 3)

Woodburne (1968) denied the term "Waldeyer's sheath" and found that this investment is by the detrusor muscle and not as an organized connective tissue enclosure of the ureter. However, Woodburne believed that this "sheath" is a space that separates the ureter from the reflected detrusor muscle on its lowest part.

The Urethra:

The parts concerned in the mechanism of continence and voiding are the proximal urethra, in the male, down to the the membranous urethra and nearly the whole female urethra (Lapides, 1953, 1958).

The urethral musculature is formed of two coats: inner longitudinal and outer circular. The inner coat is a direct continuation of the inner longitudinal detrusor coat (Tanagho and Smith, 1966; Hutch, 1967 and Woodburne, 1968). It proceeds downwards and inserts into a dense collagenous tissue accumulated in the urethral wall a few millimeters proximal to the external meatus in the female. In the male subject, the lower end of the inner longitudinal coat is not sharply delineated and blends with the prostatic musculature and some fibres join the more distal urethral musculature. The crista urethralis represents a mucosal fold over the downward continuation of the superficial trigone (Hutch,

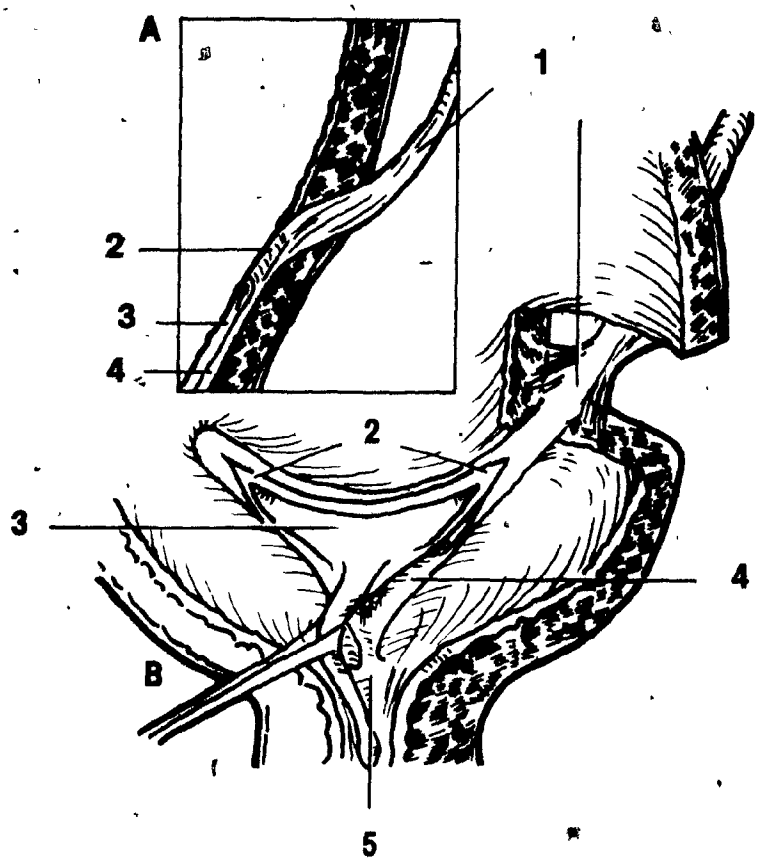


Figure 3. Schematic diagram of the region of bladder trigone. (1) Waldeyer's sheath. (2) Ureter. (3) Superficial trigone. (4) Deep trigone. (5) Bladder neck. From Tanagho and Smith (1966).

1967).

The outer circular muscle coat is probably derived from the outer longitudinal detrusor coat. As the latter fibres descend, they loop around the urethra and turn back (Tanagho and Smith, 1966; Hutch, 1967; Woodburne, 1968 and Droes, 1974). The inner longitudinal muscle layer is well developed in both sexes (Tanagho and Smith, 1966 and Woodburne, 1968). The circular muscle is absent or minimal in the female. This may be the anatomic basis of the greater vulnerability to incontinence in the female (Woodburne, 1968).

The Urethral Sphincteric Mechanisms:

There is still controversy about the urethral sphincteric mechanism. Most of the authors agree on the absence of an "anatomic" internal sphincter (Tanagho and Smith, 1966; Hutch, 1967; Woodburne, 1968 and Meunier, 1980).

Gosling (1979) described circularly arranged bundles of smooth muscle fibres encircling the bladder neck and the preprostatic portion of the urethra. The terms internal, proximal or pre-prostatic urethral sphincter provide an accurate description for this particular component of urinary tract smooth muscle. Consequently, the smooth muscle of the bladder neck, the pre-prostatic urethra and the prostatic capsule form a single morphological unit which is quite separate from the bladder detrusor. The bladder neck and urethral smooth muscle consists of small bundles which run an

oblique or longitudinal course in the urethral wall.

According to Hutch and Rambo (1967) the striated sphincter is mainly the "sphincter compressae urthrae" that lies between the two layers of the urogenital diaphragm. However some striated muscle fibres escape by bending sharply upward along the outer surface of the inner longitudinal layer of the urethra to form what he termed "the para-urethral striated muscle". This latter inserts into the inferior border of the outer circular smooth muscle coat at a point half way between the urogenital diaphragm and the bladder neck anteriorly in both sexes. In the male subject, the para-urethral muscle extends posteriorly for 1 cm above the urogenital diaphragm but the presence of the prostate seems to prevent its access to the superior half of the posterior urethral wall. In the female, striated muscle was abundant in the superior half of the posterior wall and was present up to the bladder neck posteriorly. (Figure 2).

Tanagho et al (1969) described the voluntary external sphincter as a group of striated muscle fibres encircling the membranous urethra in the male and usually the middle third of the urethra in the female or occasionally some fibres may reach up to the vesico-urethral junction. Throughout their serial histological studies done on human bladder and urethra, it was clear that the cranial extension of these striated muscle fibres is quite inconsistent.

Gosling (1979) gave another description for the striated

muscle components. In the male, a group of striated muscle fibres are found concentrated around the membranous urethra and extending up to the verumontanum. It constitutes the "distal intrinsic striated urethral sphincter". Its fibres may be traced inside the prostatic tissue and has a relatively minor contribution to the proximal urethra. This distal intrinsic sphincter consists of striated fibres that differ both anatomically and functionally from the extrinsic or periurethral striated musculature of the pelvic floor. The fibres of the intrinsic sphincter have a diameter of less than half the fibres constituting the periurethral levator ani muscle; furthermore they are functionally classified as "slow twitch" and they have a different innervation from the pelvic (splanchnic) nerve.

The fibres of the periurethral pelvic muscle belong to the "fast twitch" group and are innervated by the pudendal nerve (S2,3,&4).

In the female subject, the intrinsic sphincter is thickest along the middle third of the urethra except posteriorly where it is thin. This intrinsic sphincter is anatomically separate from the adjacent but somewhat remote peri-urethral striated muscle of the anterior pelvic floor. (Figure 4)

The "slow twitch" fibres are meant to ensure passive continence whereas the periurethral "fast twitch" fibres cannot maintain a long sustained contraction but they

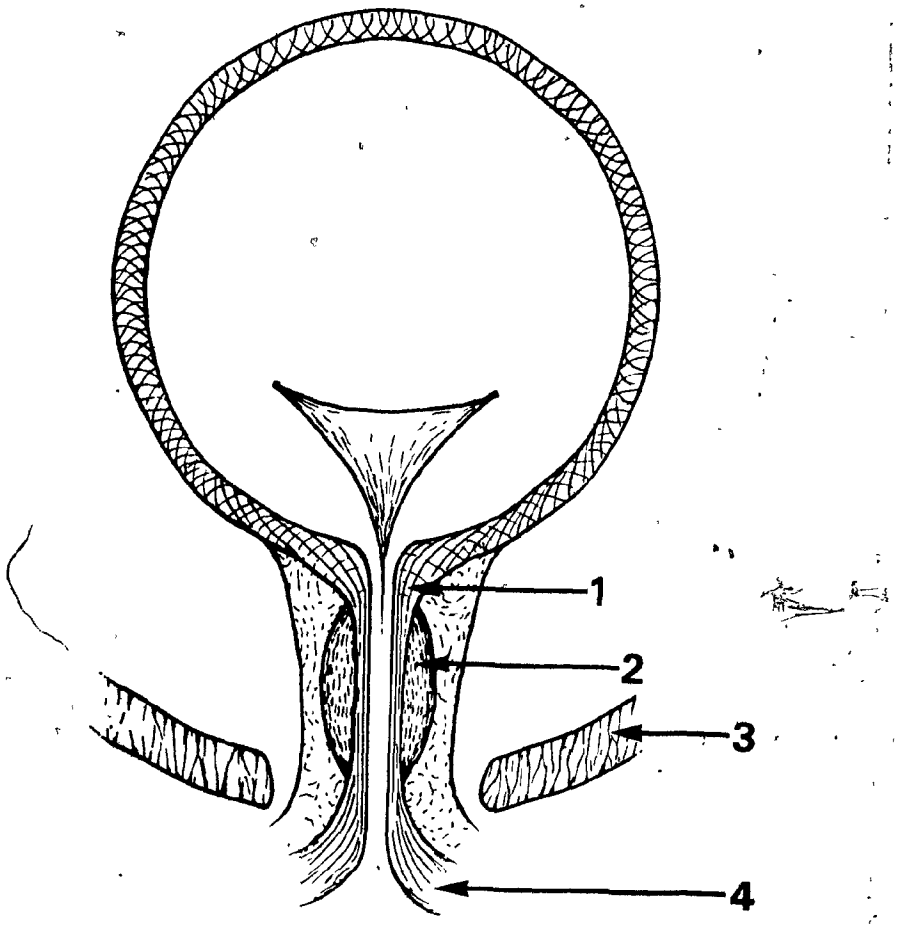


Figure 4. Schematic diagram of the female lower urinary tract shown in coronal section. (1) LONG urethral layer. (2) External striated sphincter. (3) Periurethral striated muscle. (4) LONG urethral layer.

From Gosling (1979).

increase the urethral resistance during coughing and straining.

CENTRAL NERVOUS REGULATION OF MICTURITION AND CONTINENCE

Great discrepancies still exist concerning the centres for the innervation of the bladder and urethra in the central nervous system. Most studies were performed on mammals (cats, rabbits, monkeys) and very few on man.

Nathan (1976) divided central bladder innervation into three levels: low, high and highest levels of autonomous bladder centre.

The low level autonomous bladder centre lies in the conus medullaris. The neurones concerned for detrusor contraction, i.e. parasympathetic lie in the intermediolateral grey matter of the 3rd sacral segment mainly and usually extend to the 2nd sacral segment and occasionally to the 4th and 5th segments. The cells of origin of the somatic neurones dedicated for the external striated urinary sphincter are in the anterior horn column of the 2nd to 4th sacral segments. The sympathetic efferent for the bladder and urethra arise from the intermediolateral grey matter of the spinal cord 11th thoracic segments to the 2nd lumbar segments.

The high level autonomous bladder centre lies in the junction of the telencephalon with the diencephalon. This

subcortical area consists of two adjacent parts: septal and pre-optic area. These areas are functionally and anatomically related to the hypothalamus below them and with the amygdalum and hippocampus above them. The role of these different areas on the bladder and pelvic muscles were studied in mammals: the parts of the hypothalamus concerned are the regions controlling the autonomic nervous system. The septum pallidum also is a contributor to the parasympathetic and sympathetic organized activities (Figure 5).

The highest levels influencing the high level autonomic centre lie in the frontal cortex (medial wall of the superior frontal gyrus), anterior part of the cingulate gyrus and the related part of the genu of C. callosum. Again, different areas could be assigned according to its effect on the bladder. In the cat, Gjone and Stelklew (1963) found excitatory areas on the 1st sensory-motor area, the anterior cingulate gyrus and the superior part of the pre-central gyrus. Inhibitory influence on bladder activity was obtained on stimulation of the same region of the pre- and post-central gyrus, and in the subcallosal part of the anterior cingulate gyrus, in the somatic sensorimotor area II and in the orbital gyrus.

In man, the paracentral lobule, i.e. the superior part of the pre- and post-central gyri, is concerned with the bladder evacuation, and control of the pelvic and perineal musculature. The anterior cingulate gyrus is also a region

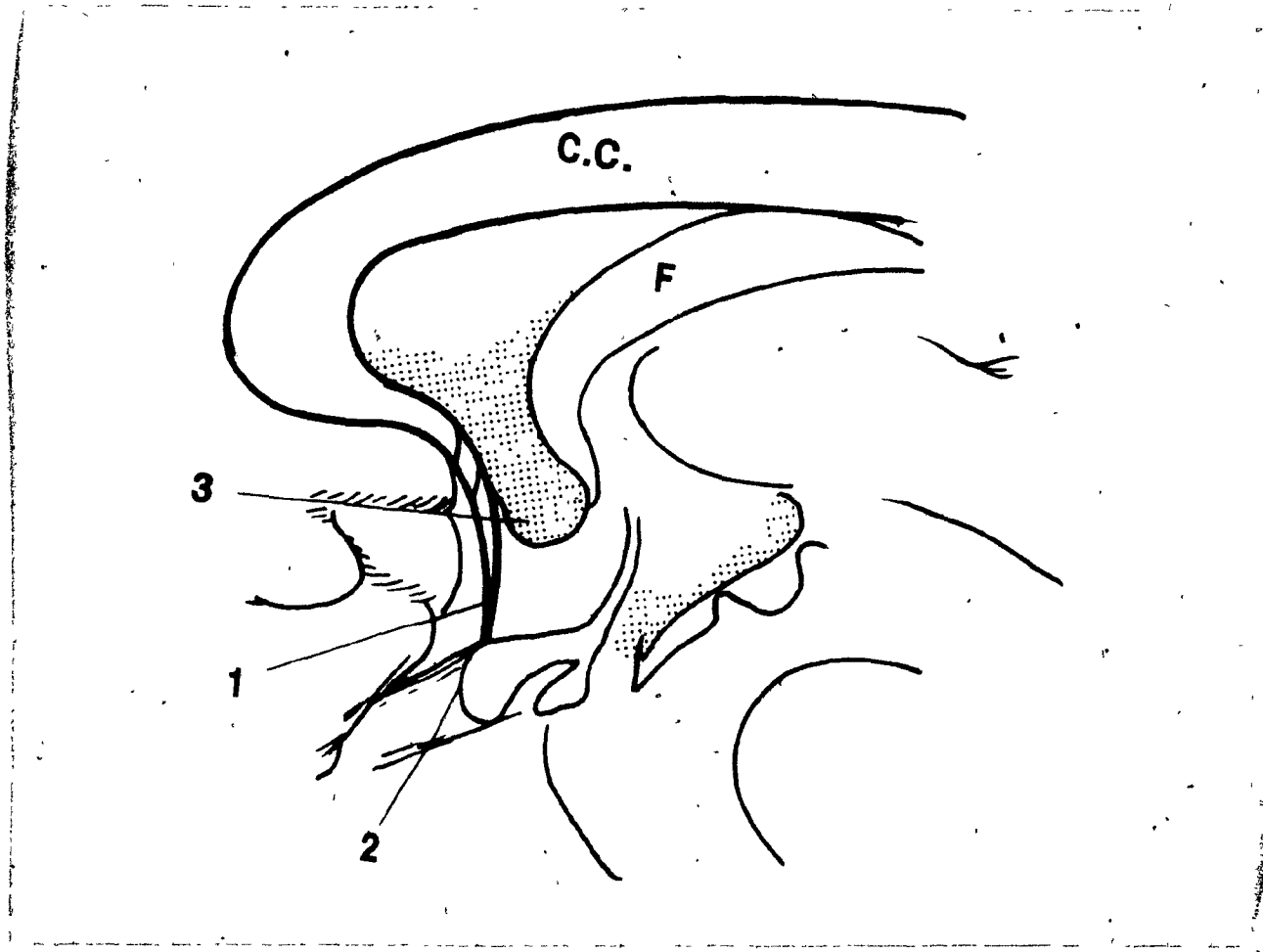


Figure 5. Sagittal section of the regions of the hypothalamus and septum pellucidum in the human brain. (1) Lamina terminalis. (2) Optic chiasmia. (3) Anterior commissure. The shading is over the regions constituting the high level of autonomous centre.

From Nathan (1976).

of bladder facilitation in man. No definite area concerned with bladder inhibition was found in or around the region of the orbital gyrus. (Nathan 1976).

Nerve fibres from the frontal and cingulate cortices project directly to reach the hypothalamus, the septum pellucidum and the pre-optic areas.

In man, pathways connecting high cortical and subcortical micturition centres are still debatable: after cingulotomies nothing is changed in micturition nor defecation. However, lesions in the paracentral lobule results in inability to micturate associated with strong spastic extension of the lower limbs; this is the paracentral lobule syndrome. Excessive spasticity of levatores ani and perineal muscles may be the cause of inability to initiate micturition.

The reticular formation lies in the brain stem. It is formed of short fibres connecting various nuclear groups concerned with the bladder. Both vesico-stimulant and vesico-relaxant tracts are closely related to each other, hence lesions in the brain stem in man almost always involve many tracts and structures.

Kuru (1965) found in the cat, fibres from the vesico-stimulant centre descend in the lateral reticulo-spinal tract and terminate in the intermediolateral region of the lumbosacral cord.

The nerve pathways from the vesico-relaxing centre descend in the ventral reticulo-spinal tract. Fibres related to the external sphincter of the urethra descend to the sacral segment along the ventral reticulo-spinal tract. However, Nathan (1976) found no disturbances of micturition in man with bilateral lesions of the ventral reticulo-spinal tracts.

The cerebellum also has some role in micturition. Stimulation of the fastigial nucleus depresses the pelvic nerve discharge and detrusor contraction. Ablation of the anterior lobe of the cerebellum in cats, dogs and monkeys resulted in a hyperactive bladder. The function of the cerebellum in innervation of the periurethral striated muscles remains undefined (Bradley and Teague, 1969).

The pathway that conveys information of bladder fullness to the cerebral cortex is a part of the spino-thalamic tract. The same tract conveys strangury, bladder pain, and pain related to the ureters, vagina and rectum (Nathan 1976).

Part of the sensation of bladder fullness is conveyed via the sacral part of the posterior columns. The spino-thalamic tract and the fibres of the posterior columns end in the vental posterior lateral nucleus of the thalamus (Nathan 1976).

Efferent Innervation:

The innervation of the smooth musculature of the bladder

and urethra is provided by both divisions of the autonomic nervous system. The external urethral sphincter receives somatic as well as autonomic innervation.

The parasympathetic efferent arises from the intermediolateral region of the gray matter of the sacral spinal cord. It travels for a distance with the sacral plexus then continues as the pelvic nerve in the cat which courses on each side of the rectum and joins the hypogastric nerve to form the vesical plexus at the bladder base. (Langley and Anderson 1896). These preganglionic parasympathetic fibres synapse with postganglionic fibres about the bladder and urethra (Wein and Raezer, 1979). In man, the parasympathetic efferent forms a pelvic plexus at the base of the bladder and not individual nerves (Walsh and Donker, 1982).

The sympathetic efferent originates from the intermediolateral cell columns of the spinal cord segments 11th thoracic through 2nd lumbar. The preganglionic fibres traverse the ventral roots and the trunk ganglion to extend distally as the hypogastric nerve to the vesical plexus. The majority of the sympathetic fibres synapse in the inferior mesenteric plexus and hypogastric plexuses and terminate as postganglionic adrenergic neurons on the smooth muscle fibres of the bladder and urethra (Figure 6).

The distribution of the cholinergic and adrenergic fibres in the bladder and urethra is still a matter of disagreement. El Badawi and Schenk (1966) found that the

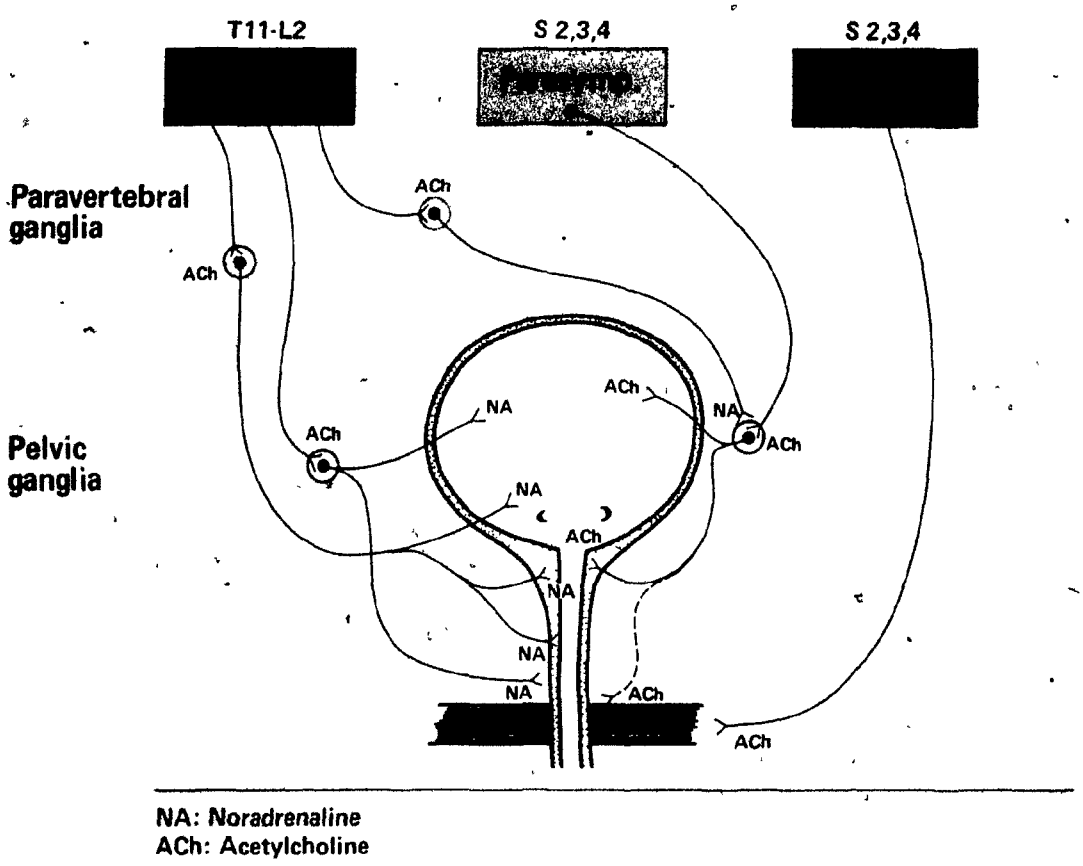


Figure 6. Diagram representing the efferent innervation to the lower urinary tract. Note the interconnection between both sympathetic and parasympathetic ganglia.

cholinergic fibres and ganglia are found in all layers of the bladder. Gosling (1979) agreed that the parasympathetic innervation is uniformly distributed throughout the detrusor. However, Razer et al (1973) found less neuronal cholinesterase in the body of the bladder than in the base. The density of the cholinergic fibres and their neuron-muscle ratio varies according to workers. El Badawi and Schenk (1968), Gosling (1979) and Schulman et al (1972) found a 1:1 neuron-muscle ratio of the parasympathetic components in the detrusor.

Razer et al (1973) stated that the nerve-muscle cell ratio is less than 1:1 in the bladder body. Ek et al (1977) found that the cholinergic nerve terminals in the human urethra is less dense than that in the detrusor.

The adrenergic innervation in the human detrusor and urethra are unequally distributed. Ek et al (1977) described numerous adrenergic nerve terminals in the smooth musculature of the trigone in both sexes. But, these nerve terminals are scanty in both urethra and bladder body. They surround both the smooth muscle bundles and the blood vessels. Gosling et al (1977) found that the adrenergic innervation in the bladder neck and the female urethra is scanty but it is densely arranged in the preprostatic urethra, hence, deduced that this part acts as a sphincter to prevent retrograde ejaculation.

Using specific histochemical techniques for the

selective demonstration of terminal cholinergic and postganglionic adrenergic (sympathetic) nerves, El Badawi and Schenk (1968) studied the intrinsic innervation of mammalian bladder. They demonstrated a pericellular plexus of sympathetic fibres around parasympathetic ganglion cells providing the first morphological evidence for an interaction of the two components of the autonomic nervous system peripherally in the bladder wall.

Innervation of the Striated Urethral Sphincter:

The external striated urethral sphincter receives somatic innervation via the pudendal nerve from the anterior horn cells in the grey matter of the 2nd, 3rd and 4th sacral segments of the spinal cord.

Wein et al (1979) found no evidence of the adrenergic innervation for the striated periurethral sphincter. However, Gosling (1979) found that the motor nerves to the male and female external intrinsic slow-twiched striated muscle fibres are branches of the pelvic (splanchnic) nerve and not the pudendal nerve. El Badawi and Schenk (1974) examined the innervation of the striated sphincter of different mammalian species and concluded that the urethral rhabdosphincter, particularly its upward paraurethral extensions has a triple innervation sympathetic, parasympathetic and somatic components. The sympathetic innervation is composed of the perivascular plexuses and numerous independant fibres running between the sphincter

muscle cells in close relationship to their surfaces. The parasympathetic innervation is represented by individually discernible nerve fibres forming intricate networks around skeletal muscle cells.

Afferent Innervation of the Bladder and Urethra:

Both parts of the autonomic nervous system convey afferent activity arising from the bladder to the central nervous system. Those afferent impulses which signal the bladder distension (propioception) travel via the pelvic nerve to the sacral cord. They ascend via the posterior columns. They are responsible for initiation of the detrusor reflex contraction in the cat. (DeGroat et al, 1975). Pain, touch and temperature impulses from the bladder reach the thoracic spinal cord segments via the hypogastric and inferior mesenteric plexus. These impulses travel via the spinothalamic tracts (Kuru, 1965 and Nathan, 1976).

Uemura et al (1973) using electron microscopy studied the afferent fibres of the bladder in cats after spinal ganglionectomies. They found that the sacral parasympathetic afferent innervation of the detrusor is equally distributed throughout the bladder. The afferent terminal axons within the muscle fascicles are in parallel with the smooth muscle cell, thus may act as stretch receptors for initiation of detrusor contraction.

The sympathetic afferent innervation of the detrusor was scarce throughout the bladder except in the trigone and

bladder neck where it was densely arranged. Uemura et al (1973) made an observation that the sacral afferent terminals were approximately twice as numerous as the lumbar afferents in the muscle layer. The sacral afferents were equally distributed in different region of the bladder but the lumbar afferents were concentrated in the bladder neck of the cat.

However, in the submucosa, the autonomic afferents showed a reversal ratio: the lumbar afferent outnumbered the sacral afferent terminals. Uemura et al (1973) could not find a functional role of the afferent endings in the submucosa but they suggested that pain is transmitted by these nerve endings to the hypogastric nerve.

Bradley et al (1976) had identified 2 types of sensory receptors in the mucosa and the muscle layer of the bladder of cat. Tension receptors were localized in the collagen layers surrounding the smooth muscle fascicles. Volume or length receptors were fewer than tension receptors and were located within the smooth muscle fibres.

In the urethra, the afferent innervation is brought by the branches of the pudendal nerve. Sensory receptors are found in the urethra and bladder. Both the free ending and the laminated types are present. Neither cholinergic nor adrenergic nerve fibres were traced to these sensory receptors (El Badawi and Schenk, 1974).

The peripheral innervation of the bladder and urethra

are summarized in Figure 6.

The thoracolumbar sympathetic outflow can be divided into three types:

1. Short preganglionic fibres (cholinergic) thaat relay in paravertebral ganglia. The postganglionic long fibres (adrenergic) supply the muscles of the bladder neck, posterior urethra, and the striated sphincter.

2. Long preganglionic fibres (cholinergic) that relay in the pelvic ganglia around the bladder base. From these, postganglionic short adrenergic fibres innervate the smooth musculature and the striated sphincter.

3. Postganglionic fibres (adrenergic) that are connected to the pelvic parasympathetic ganglia inhibiting the parasympathetic transmission.

The sacral parasympathetic outflow mainly consists of long preganglionic fibres synapsing in the pelvic parasympathetic ganglia. From the short postganglionic nerves, cholinergic fibres innervate the bladder, urethra and the striated sphincter.

Bradley, in 1978, gave a simpler description of bladder innervation. He assumed the presence of 4 reflexes or loops. In the cat, Bradley and Conway (1966) described an area in the rostral pons, i.e. junction between the mid-brain and pons in a dorsal position. This area is concerned with the bladder evacuation. It is termed the nucleus locus

coeruleus. Based on this brain stem detrusor nucleus, Bradley organized the four loops:

Loop I consists of axons connecting the frontal lobe cortex to the brain stem detrusor nucleus and vice versa. The latter receives inputs from the cerebral cortex, basal ganglia, cerebellum, as well as from the detrusor directly and indirectly. (Figure 7 & 8)

Loop II has a spinal and peripheral component. The peripheral component arises as afferent propioceptive axons whose sensory endings are in the detrusor muscle. These are conducted via the pelvic nerve to the sacral cord where they ascend as the posterior column to the brain stem detrusor nucleus. These axons do not synapse but rather convey long route to the brain stem. The descending or spinal component of Loop II originates from the brain stem detrusor nucleus and descends along the reticulospinal tract down to the intermedio-lateral neurones present in the gray matter of the sacral cord, 2nd, 3rd and 4th segments where they relay. The preganglionic parasympathetic and the postganglionic neurones form the rest of the peripheral component. (Bradley and Teague, 1968). (Figure 8)

The pudendal nucleus, present in the anterior horns of 2nd, 3rd, and 4th sacral segments, receives input from the sensory axons of the detrusor muscle and from the corticospinal tracts. Its output is directed to the periurethral striated muscles.

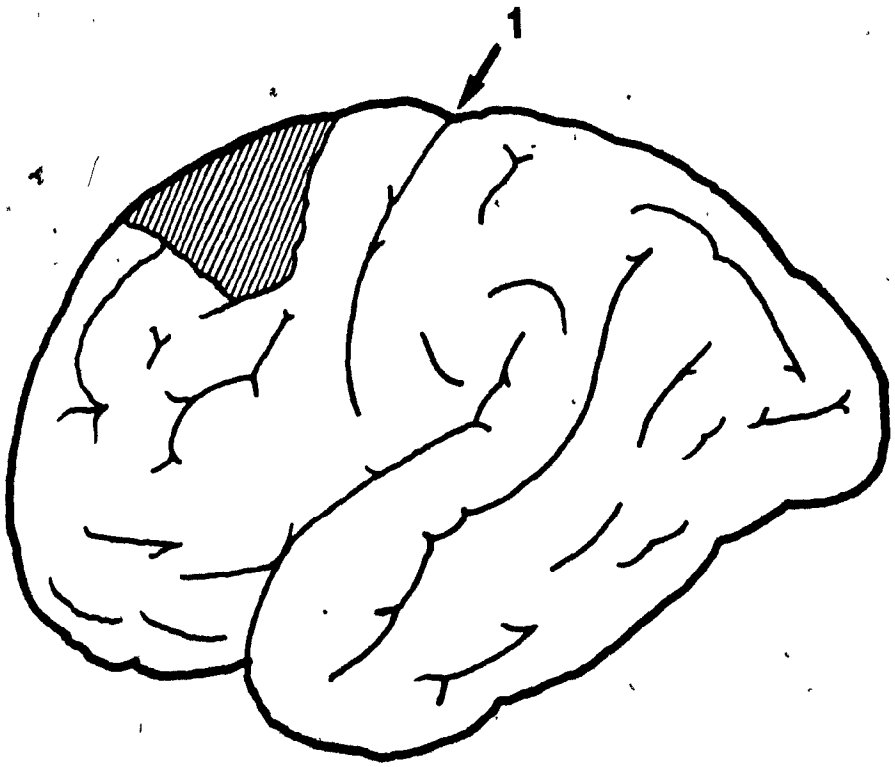


Figure 7. Diagram of the left human cerebrum showing the representation of the detrusor muscle control and the periurethral striated muscle in the cerebral cortex.

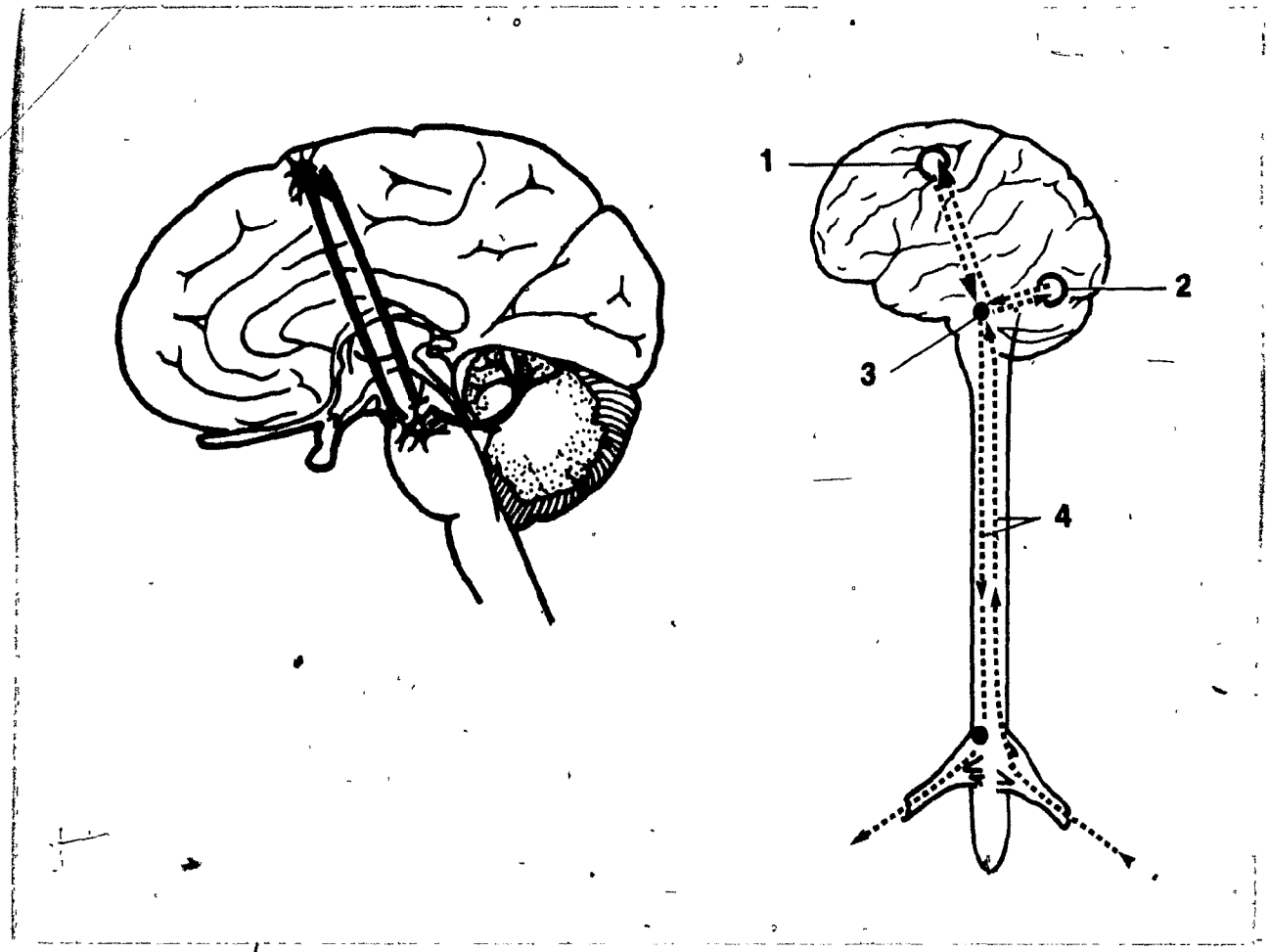


Figure 8. Diagram of loop I (left) and both loops I an II (right). (1) Para-central lobule. (2) Anterior vermis of cerebellum. (3) Pontine mesencephalic reticular formation. (4) Reticulo-spinal tracts.

From Bradley et al (1974).

Loop III consists of proprioceptive sensory axons from the detrusor muscle to the spinal cord to synapse on the motor neurones in the pudendal nucleus. These afferent impulses are inhibitory to the pudendal nucleus. The latter continually sends out motor impulses to maintain the pelvic floor musculature in tonic contraction during the storage phase of bladder filling. (Bradley, 1978). (Figure 9)

Loop IV consists of segmental and supraspinal innervation of periurethral striated muscles. (Figure 10)

The supraspinal innervation consists of sensory impulses from the muscle spindles passing cranially in the posterior columns. These axons send collaterals to the cerebellum and thalamus and terminate in the sensorimotor cortex of the frontal lobes.

The segmental innervation consists of axons arising from the muscle spindles in the anal sphincter, the pelvic floor musculature and to a lesser extent in the periurethral striated muscle. These muscle spindles are controlled by the contraction of intrafusal fibres supplied by gamma motor neurones. The afferent sensory axons reach the pudendal motor nucleus present in 2nd, 3rd and 4th sacral segments.

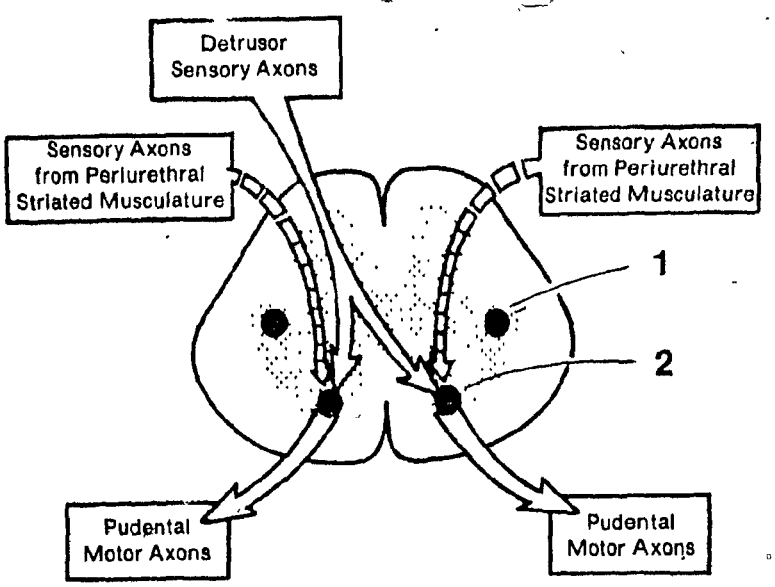


Figure 9. Diagram of loop III and segmental part of loop IV. (1) Detrusor nucleus. (2) Pudendal nucleus.
From Bradley et al (1974).

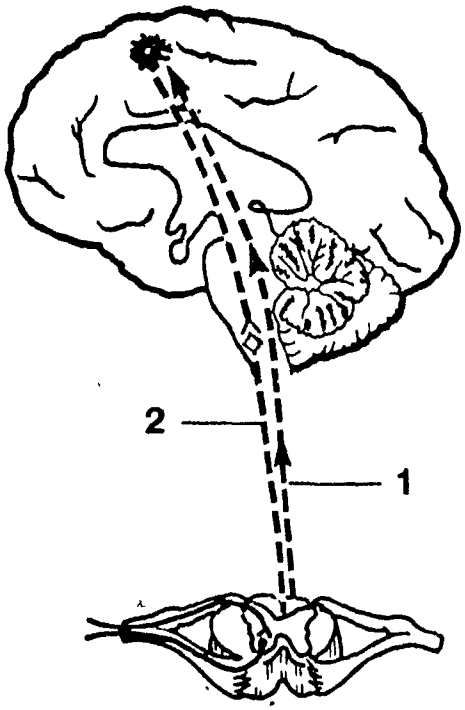


Figure 10. Diagram of supraspinal part of loop IV. (1) Sensory pathway. (2) Pyramidal tract.

NEUROPHARMACOLOGICAL RECEPTORS IN BLADDER AND URETHRA:

The effects of the sympathetic and parasympathetic innervation on lower urinary tract are mediated by special neurotransmitters acting on special pharmacological receptors. These receptors are components in the membrane that reacts with a specific neurotransmitter to produce a specific action (Raz, 1978).

A. Adrenergic function:

Norepinephrine is considered to be the chemical transmitter for the adrenergic receptors (Razer et al, 1973 and Wein et al, 1976). The adrenergic receptors are classified into alpha and beta subtypes. The beta-receptors predominates in the bladder dome. The alpha-receptors are numerous in the region of the bladder base, outlet and proximal urethra. (Razer et al, 1973; Edvardsen and Stekleiv, 1968 and Raz and Caine, 1972).

Stimulation of the alpha-receptors has been correlated with depolarization of the smooth muscle cells resulting in contraction; whereas beta-receptor stimulation results in cellular membrane hyperpolarization and inhibition with relaxation of the smooth muscle cells. (Bissada et al, 1977).

The alpha-adrenergic receptors present in the urethra are directly related to the smooth muscle fibres. (Raz and Caine, 1972). However, Tanagho et al (1969) suggested that the alpha-receptors are present in the blood vessels and not

the smooth muscles of the urethra. They affect the urethral resistance via the rich vascular bed of the urethra. Recently, there is evidence of two subtypes of alpha-adrenergic receptors, $alpha_1$ and $alpha_2$ (post and pre-synaptic respectively). They have been described in different tissues including the urethra. (Hoffman and Lefkowitz, 1980; MacGregor and Diokno, 1981 and Andresson et al, 1981).

B. Cholinergic Function:

Atropine-sensitive cholinergic receptors have been demonstrated in bladder base and body and urethra of mammals and man (Todd and Mack, 1969; Nergardh and Boreus, 1973 and Raezer et al, 1973) with variable concentrations.

Whether acetylcholine is the sole neurohumoral transmitter for the parasympathetic innervation is highly controversial. Both prostaglandins (Taira, 1974) and purine nucleotides such as ATP (Burnstock et al, 1972) are thought to be among the non-cholinergic transmitters.

Other authors (Ambache and Aboo Zar, 1970) presented experimental evidence against the concept of cholinergic motor neurones in the detrusor. They stimulated electrically the postganglionic motor neurones in the cat, rabbit and guinea pig bladder strips. They found little or no diminution in the contractions of the strips after prolonged exposure to high dose of atropine. Eserine failed to potentiate the atropine-resistant contractions.

Prostaglandins (PGs) are structurally derived from prostanoic acid and are 20-carbon polyunsaturated fatty acids, containing an internal cyclopentane ring from C_8 to C 12. The nine known PG families (PGA, B, C, D, E, F , F , G and I) are identified according to the substituents and their configuration on the 5-membered ring. The most abundant naturally occuring PG precursor is arachidonic acid which produces via an enzymatic system called PG-synthetase, PGs of the 2 series (PG_2). The initial step in the synthesis is the formation of PG endoperoxides (PGG_2 and PGH_2). Further transformation can lead to PGD_2, PGE_2, PGF_2 , Prostacyclin (PGI_2) and Thromboxane A_2. These compounds are widely distributed in tissues and act upon target cells in the immediate vicinity of their site of biosynthesis (Mathe et al, 1977). The role of PG's on the mammmalian urinary bladder was focused mainly on the PGE and F families. Ambache and Aboo Zar (1970) demonstrated sluggish contraction of detrusor strips under the effect of PGE_2 and PGF_2alpha. The ability of the detrusor muscle to synthetise PG was demonstrated by several authors. Bultitude et al (1976) presented evidence of PG-like activity in the bath fluid of rabbit detrusor strips using bioassay techniques. Using thin layer chromatography, these were shown to be of the PGE_2 type. Abrams et al (1979) noted that addition of arachidonic acid in crude human bladder homogenates promoted the biosynthesis of PGs and therefore they postulated the presence of PG-synthetase enzymatic system in human bladder.

Others demonstrated the release of PGs into the bladder lumen and pelvic venous blood in response to vesical distension and pelvic nerve stimulation. PGE_2 was the one mostly involved as shown by radio-immunoassay and bioassay techniques. (Ghoneim et al, 1976 and Khalaf et al, 1980).

The effect of PGs on the mammalian bladder muscle is in favour of direct rather than via a neurotransmitter. Abrams and Feneley (1976) noted significant contractions of human detrusor strips produced by the addition of PGE_1, PGE_2 and PGF_2. The action of PGs remained unchanged after the addition of antagonists to acetyl choline, 5-Hydroxytryptamine, histamine and epinephrine. Bultitude et al (1976) found PGE_2 and PGF_2 alpha to cause a dose related contraction of several mammalian detrusor strips. They observed that indomethacin caused a reduction in tone and loss of spontaneous activity. They concluded that PGE_2 is responsible for the tone and spontaneous activity in the bladder muscle. Khanna et al (1978) demonstrated differential action of PG on the bladder and urethra of the rabbit. Whereas PGF_2 alpha caused contraction of bladder base and the urethra, PGE_2 had no effect on these two structures. They showed that the action of these compounds is not mediated through muscarinic, nicotinic, adrenergic or histamine receptors. They proposed the concept of PG receptors. Evidence of different actions of PGF_2alpha and PGE_2 on human detrusor and urethral strips was also noted by Andersson and Persson (1977). They reported that both PGs

contracted detrusor strips but urethral strips responded by contraction to PGF_2alpha and were relaxed by PGE2. The addition of tetrodotoxin did not affect the PG induced contractions giving evidence against nerve transmission as part of the pharmacologic response.

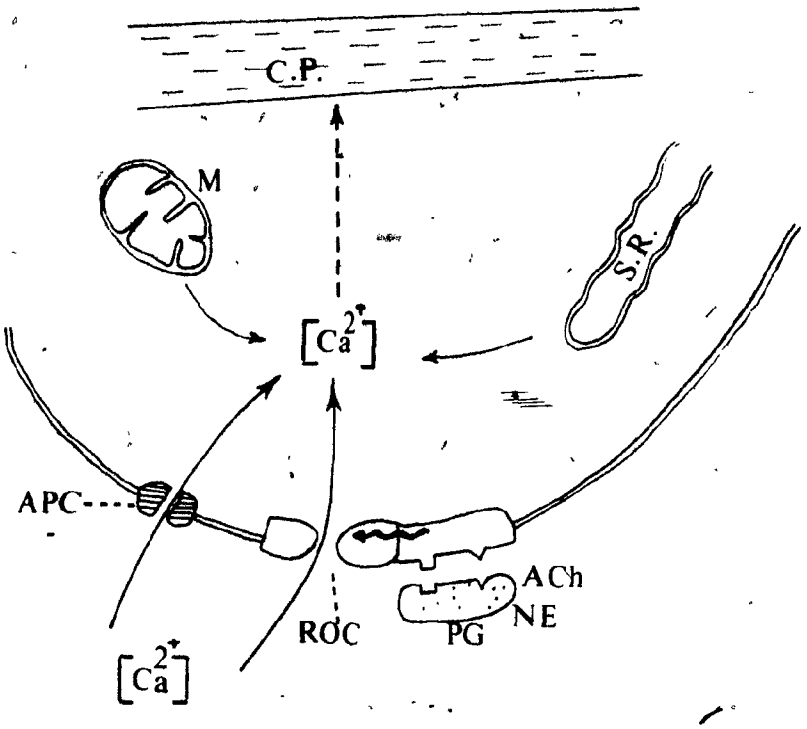
Although the effect of PGs on the activity of bladder and urethra seems to be independant of the cholinergic and adrenergic system, little is known regarding the exact mode of action of these agents. The presence of Calcium ions in the bathing medium is necessary for the contraction produced by PGs (Paton and Daniel, 1967) and elevating its concentration synergizes with or enhances their stimulant effect (Ealing et al, 1972).

The interaction between PGs and other "purinergic" substances had been raised as a possibility. Dean and Downie (1978a) and Burnstock et al (1978) separately showed evidence of ATP acting either as a neurotransmitter or a modulator of the postganglionic "purinergic" fibrres. The mediation of PG synthesis by the release of ATP is a distinct possibility and it seems that non-cholinergic, non-adrenergic neurotransmission requires the cooperation of ATP and PGs (Dean and Downie, 1978b).

Importance of Calcium Ions in Smooth Muscle Contractility:

The contractile protein system in the smooth muscle consists of a) thin filaments with a diameter of 5-8 nm called "Actin" and b) thick filaments 15-18 nm in diameter termed "myosin". In presence of ATP and Calcium ions (Ca^{++}), myosin reacts with the active site of the actin molecule. Beside the contractile protein system, there are two other regulatory proteins tropomyosin and troponin (Huxley, 1969). In the absence of Ca^{++} the troponin-tropomyosin system acts as an inhibitor which brings the adenosine triphosphatase (ATPase) activity to a low level associated with relaxed muscle. The addition of Ca^{++} merely removes this inhibition leading to a high level of ATPase activity and contraction (Perry and Grand, 1979).

The mechanism of action of the neurotransmitters on the smooth muscle fibres is probably mediated by 2 kinds of ion channels on the cell membrane that allow the entrance of Ca^{++} (and possible Na^{+}) inside the cell: potential-sensitive channel and receptor-operated channel (Bolton, 1979) (Figure 11). According to this theory supported by several evidences showing that various neurotransmitters (cholinergic, adrenergic, purinergic and Prostaglandins) activate or inhibit the smooth muscles by altering the level of intracellular Ca^{++} (Shibata et al, 1968, Paton and Daniel, 1967 and Bolton, 1979). Whether the Ca^{++} enter the cell by a potential sensitive channel or a receptor operating channel, the end result is an increase in the intracellular



Calcium-Channels Theory (Bolton 1979)

Figure 11. Schematic diagram of a smooth muscle cell showing calcium ions channels. (A Ch) Acetyl choline. (APC) Action potential channel. (C.P.) Contractile proteins. (PG) Prostaglandins. (M) Mitochondria. (NE) Norepinephrine. (ROC) Receptor operating channel. (SR) Sarcoplasmic reticulum.

From Bolton (1979).

Ca^{++} concentration that activates the coupling of actin and myosin. Preventing the Ca^{++} entry could be a logical way to control the myocardial and smooth muscle activity.

Calcium Ion Antagonists:

The term Ca^{++} antagonist was introduced by Fleckenstein (1971) to describe the action of those drugs that specifically block Ca^{++} dependant excitation-contraction coupling. A variety of names has been given to this group of drugs including Calcium antagonists, slow channel blocker, Calcium channel blocker and Calcium entry blocker (Zsoster and Church, 1983). The term "Calcium antagonists" is the most appropriate and will be used throughout the text.

Although the list of substances that interfere with Ca^{++} influx into the cell include cations (as Lanthanum, Manganese, Cobalt, Nickel), local anesthetics, nitrites (Rahwan et al, 1979), the true Calcium antagonists are organic compounds such as Verapamil, Nifedipine, Diltiazem and Prenylamine. The latter are the drugs mostly used therapeuticically for cardiac arrythmias, angina and hypertension (Zsoster and Church, 1983).

It is widely accepted that the predominant effect of Ca^{++} antagonists is on potential-dependant (AP) channel in smooth muscles (Bolton, 1979) and known as "slow channel" in myocardium (Zsoster and Church, 1983). The "fast channels through which Na^{+} enters cause the initial rise in the action potential (AP) in myocardium (Nayler and Grinwald, 1981).

The fast Na^+ channels are inactivated by K^+ depolarization or tetrodotoxin (Mascher, 1970).

Nayler and Grinwald (1981) showed that Verapamil, Diltiazem and Nifedipine act selectively on the slow channel through which Ca^{++} and possibly some Na^+ enter the myocardial cell. They suggested that Nifedipine may simply inactivate the calcium channels in a dose-dependent way, while Verapamil and Diltiazem in addition interfere with the kinetics of inactivation and recovery.

Some authors (Nayler and Grinwald, 1981 and Zsoster and Church, 1983) believed that these drugs interfere with the availability of Ca^{++} for its physiological role at intracellular site(s) as well. These sites are believed to be the sarcolemna, the sarcoplasmic reticulum and the mitochondria. Verapamil has been reported to interfere with Ca^{++}-ATP use in the sarcolemma. Nifedipine has a more selective action on the slow channels while Verapamil has a double inhibitory effect on the fast channel as well. The use of Ca^{++} antagonists on vesicourethral musculature had shown a dose-dependent depressing effect on smooth muscle contraction. Khanna et al (1983) demonstrated such effect of Verapamil on strips from the detrusor and urethra of the rabbit. Finkbeiner (1983) noted a diminution of in vitro spontaneous contractility of guinea pig detrusor after exposure to Diltiazem.

The clinical application of Ca^{++} antagonists in urologic

patients could be promising. Forman et al (1978) used Nifedipine on patients with detrusor irritability. They reported subjective and objective amelioration in their bladder capacity.

VISCO-ELASTIC PROPERTIES OF BLADDER AND URETHRA:

The creation of a mechanical model for the bladder and its mathematical analysis has contributed to the understanding of the physical behaviour of the detrusor.

The three basic mechanical elements known are briefly presented by Newton, Hooke and Maxwell elements (Figure 12).

Viscosity is expressed by the dashpot (Newton element) where the resultant force (F) is proportional to viscosity coefficient (n) and rate of deformation (X) according to the formula $F=nX$.

It is obvious that the dashpot responds only slowly to the action of the force regarding the resistance developed.

Elasticity is presented by a helical spring (Hooke element) where the force (F) is proportional to elasticity constant (E) and deformation (X)

$$F=EX$$

Maxwell element is represented by a Newton and a Hooke element in series. Both elements supply the same force on opposite direction (Kondo et al, 1972).

The behaviour of the bladder, being a biological system, depends in great part on the visco-elastic properties of its material.

Visco-elasticity is a "passive function". It is the tendency of the compliance of a material to increase with

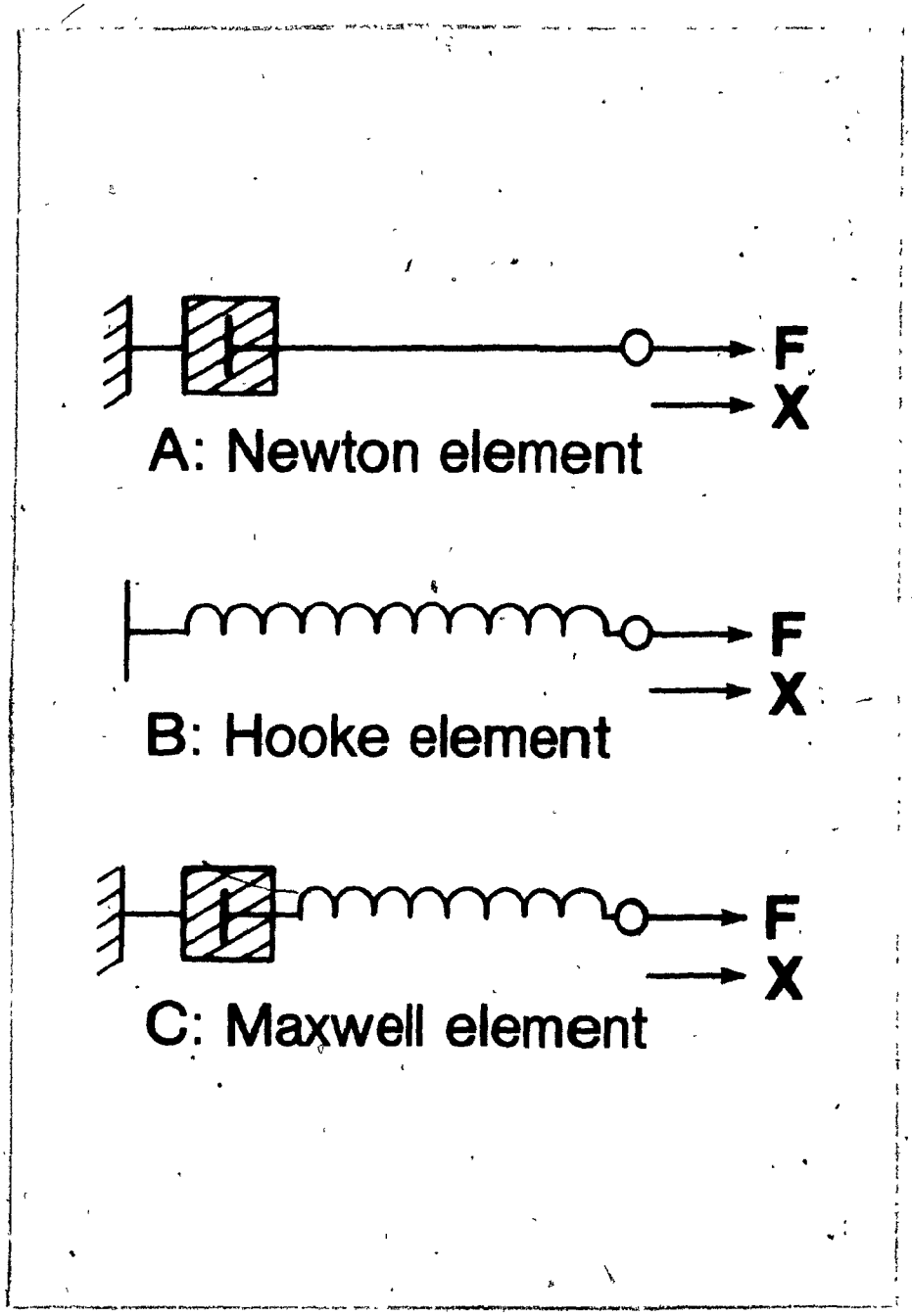


Figure 12. Diagram showing the 3 basic mechanical elements (F) force, (X) deformation.

From Kondo et al (1972).

time as it is subjected to stress (force). When a stress is applied steadily the tension which initially builds up, will gradually decrease (decay) with the passage of time.

Because the bladder wall contains collagen, elastin and smooth muscle, it was found that a combination of Maxwell and Hooke elements might represent a mechanical model for its viscoelastic properties. Kondo et al (1972) suggested a mechanical model of three Maxwell elements and one Hooke element for the bladder behaviour in response to filling (continence phase). They showed that rapid filling of the bladder (stress) was followed immediately by sudden rise in the intravesical pressure (tension). Analysis of this component fitted with a Hookian element. Then after stopping the filling, the intravesical pressure decreased rapidly (decay) then gradually to an asymptotic value which is greater than the intravesical pressure before the injection. They deduced that the pressure (tension) is separated into contribution from the collagen component (Hookian element) and the muscle component (Maxwell element). (Figure 13).

Coolsaet et al (1973) provided a mechanical model with two instead of three Maxwell elements to be more consistent with their measurements.

Law of Laplace:

The application of the law of Laplace has been used to explain urethral and vesical mechanisms of continence and voiding (Woodburne, 1960). This law expresses a relation

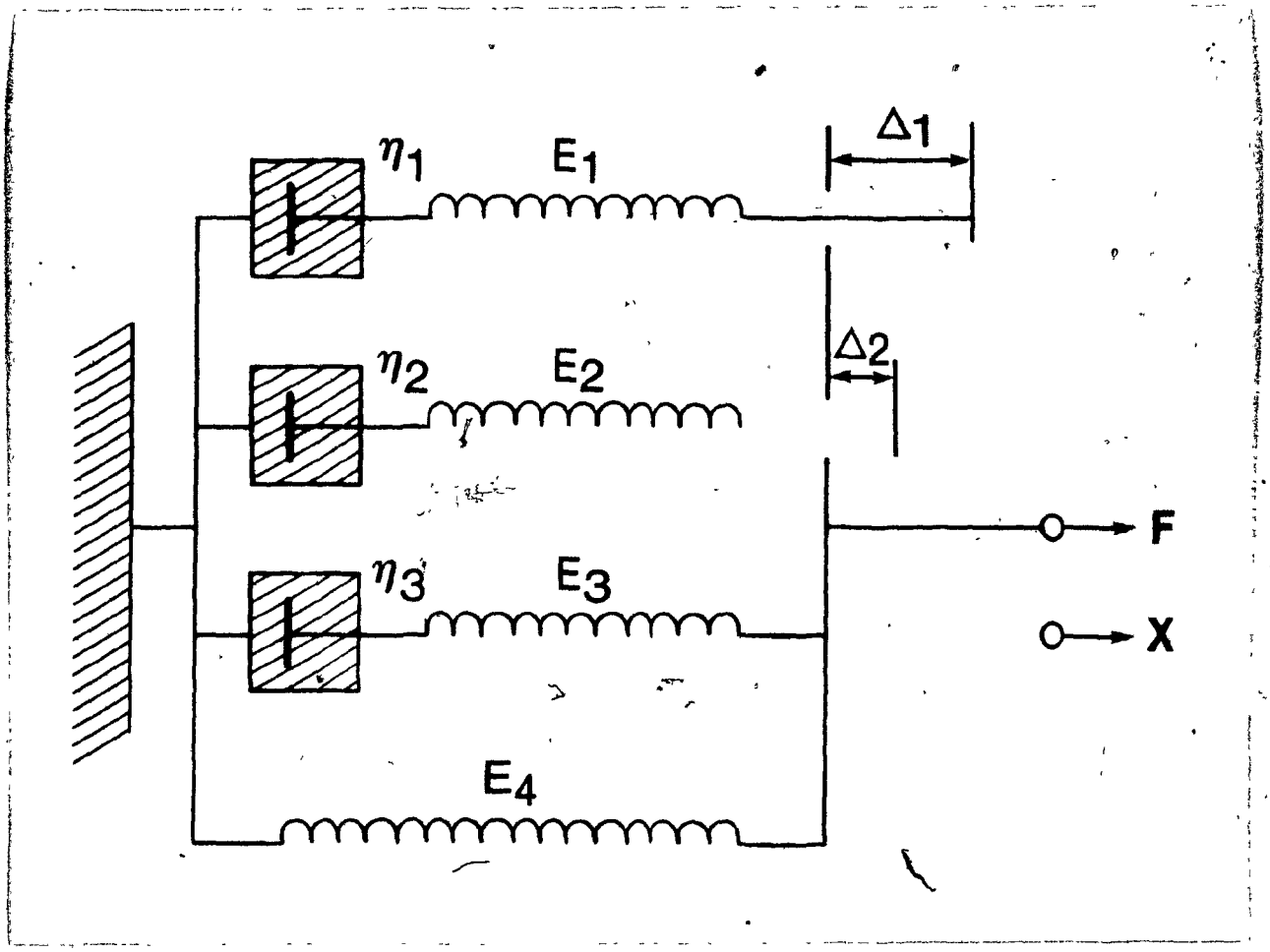


Figure 13. Diagram showing the mechanical model of the bladder (E_1, E_2, E_3 & E_4) constant of sping, (N_1, N_2, N_3) coefficient of dashpot.

between the pressure (P) inside a closed vessel and the forces arising from the tension (T) in the walls of the vessel. At equilibrium, the pressure is equal to the tension force. By introducing the dimensions of a sphere, the pressure (P) is equal to two times the wall tension (T) divided by the radius (R) of the sphere.

$$P = 2T/R$$

For a closed cylinder, Laplace's law becomes: pressure (P) is equal to the circumferential wall tension (T) divided by the radius of the cylinder (R), thus: $P = T/R$.

In case of the bladder and urethra, most authors (Zinner et al, 1977) assumed that the thickness of the wall is small compared to the radius. Thus for simplicity, Laplace's law could be expressed as the pressure (P) equal to the sum of tension (T) in any direction divided by the radius (R) of the curvature in that direction.

$$P = \frac{T_1}{R_1} + \frac{T_2}{R_2}$$

Bladder Behaviour During Continence (Accommodation):

Assuming that the bladder is a sphere, Laplace's law is $P = 2T/R$. At constant pressure, the wall tension is proportional to the radius and at constant radius, the pressure is proportional to wall tension. Zinner et al (1977) showed that the bladder behaves in a non-linear

(elastomer) fashion when wall tension is plotted against length (stretch). They showed that as the bladder is empty the radius is small and thus the pressure low. As the volume is increased inside the bladder there is initial rise in the wall tension and pressure. With further increase in the volume, the pressure rise is minimal, but the wall tension continues to rise until a point where some pressure is attained. At this point further increase in the volume (radius) will raise the wall tension and trigger the sensation of voiding. This represents the basis of the cystometric curve. Zinner et al (1977) showed that changing the degree of elasticity (degree of cross-linking) of the substance coincided with cystometrograms from cats after hypogastric nerve ablation.

Urethral Closure During Continence:

Application of Laplace's law on the urethra is not clearly justifiable because of changes in curvature of the urethra and thickness of its wall. Zinner et al (1976) stressed the importance of elasticity of the urethral wall. They proposed an "elastically-inert filler" that surrounds the urethral lumen. This filler transmits a pressure from surrounding elastic (muscle) sheath and does not exert any elastic forces; thus corresponding to a layer of spongy tissue.

THE MECHANISM OF VOIDING AND MICTURITION

The dual function of the bladder for storage and evacuation still requires explanation.

Several speculations have been put forward in order to explain both mechanisms of continence and voiding. As far as continence mechanism is concerned, several factors are involved. Lapides (1958) proposed two factors essential for the urethral resistance during the storage phase viz the inherent tension of the urethral wall and the length of the "functional" urethra. He described the internal vesical sphincter as a tubular structure composed of bladder smooth muscle and elastic tissue and that this sphincter represents the posterior male urethra and the upper 3/4 of the female urethra. Both segments represent the true bladder neck.

During storage, the internal sphincter is kept closed and thus maintains continence. This is carried out in an autonomous tireless fashion with a negligible expenditure of energy (Lapides, 1958). However, Hutch (1966) related the continence to two sphincters. The internal sphincter located in the base of the bladder "base plate" and the urethral sphincter located in the proximal 2-3 cm of the urethra. He believed that so long as the "base plate" is kept flat, continence is assured. Both sphincters help in this achievement.

Zinner et al (1976) described three major elements to ensure total continence: inner softness, inner compression

and outer tension of the urethral wall. They believed in the softness or plasticity of the inner urethral wall to seal the lumen. The inner urethral compression is a passive response to a squeeze from outside the region of compression.

The urethral wall tension is crucial and develops within the urethral wall and/or the surrounding structures and acts to compress the region of continence. Tension in the wall results in part from the passive characteristics of the elements forming the urethral walls which are collagen, elastin, smooth and striated musculature.

I Mechanisms of Bladder Outlet Opening:

Between continence and voiding, there is a transition period during which the bladder outlet opens. Several theories were proposed to explain the mechanisms of bladder outlet opening.

A. Theory of active relaxation:

According to this theory, the bladder outlet opens by an active (reflex) relaxation secondary to bladder contraction. This relaxation will result in a drop in the urethral resistance.

Evans (1936) presented evidence of reciprocal activity between the bladder fundus and neck. He postulated that once the bladder neck relaxed, the hydrodynamic squeezing of the detrusor allows the urethra to open and the bladder to empty.

Bradley et al (1974) measured electric activity in the hypogastric nerve and found a burst of activity at beginning and end of micturition. They concluded that the urethral opening and closure was a nerve mediated active function. Jonas and Tanagho (1975) showed a nerve-mediated spinal reflex that induced a drop in urethral resistance in response to detrusor contraction. They supported a role for the active urethral relaxation prior to voiding. On the other hand, Tanagho et al (1969) presented evidence of the role of the external sphincter relaxation during voiding.

From the above data it seems that for opening of the bladder outlet to occur, the theory of active urethral relaxation requires that both smooth and striated muscular components should relax since any of them can produce resistance if not relaxed.

B. Theory of active contraction:

The essence of this theory relied mainly on the anatomical differentiation of the smooth muscle layers of the proximal part of the urethra into longitudinal and circular layers. Lapides (1958) proposed that voiding occurs after the longitudinal urethral muscle layer contracted and forced the bladder neck into a funnel by a "bow string" action at the bladder neck.

Bro-Rasmussen et al (1965) after an exhaustive dissection of the outlet region held the trigone muscle responsible for bladder neck opening. They stated that by

contraction of the trigone muscle, it pulled the posterior part of the vesical orifice and the posterior upper part of the urethra upwards and backwards.

Tanagho et al (1969a) showed that the inner longitudinal urethral coat helps during voiding by reducing the urethral resistance.

In 1965, Hutch came with the theory of base plate that entailed an active contraction of the detrusor to open the bladder outlet. This theory held that the base of the non-voiding bladder is flat and disc-like. The internal urethral orifice penetrates this plate eccentrically. As the disc plate was flat, the bladder outlet was closed. During initiation of voiding, the contracting detrusor musculature pulled the lateral borders of the base-plate upwards converting it into a funnel and hence the bladder outlet was opened.

Briefly, the normal micturition can be summated as follows:

During the phase of urinary storage, the bladder accommodates urine without appreciable increase in the intravesical pressure. This is due in part to the visco-elastic properties of the bladder wall and in other part to neural control via the sympathetic outflow to the outlet and the dome. The net results will be closure of the internal sphincteric mechanism and inhibition of the bladder activity allowing more filling.

The micturition reflex is initiated mainly by the rise in intravesical pressure. It is the latter that provokes the sensation of bladder distension that is mediated through afferent impulses through pelvic nerves. Micturition is mediated via the parasympathetic outflow to the detrusor. Despite the fact that the sacral segments of the spinal cord harbour efferent parasympathetic neurones, it seems that the actual organization of the micturition centre lies in the brain stem. The final steps in micturition will be opening of the vesical outlet, synchronous contraction of detrusor with relaxation of the striated musculatrue of the pelvic floor and emptying of the bladder. The facilitatory and inhibitory impulses which originate at several levels of the nervous system including brain stem, cerebellum, and the cerebral cortex allow for full conscious control of micturition.

BLADDER AND URETHRAL BEHAVIOUR IN SPINAL CORD LESIONS:

The acute urinary retention following spinal cord injury has been described some several decades ago (Holmes, 1933). However the causes of failure of the bladder to evacuate during the spinal shock phase are still unknown. Some attributed it to bladder areflexia secondary to loss of nerve reflexes (DeGroat and Ryall, 1968 and Jonas et al, 1975) or to increase in the outlet resistance secondary to an increase in the sympathetic activity (Edvardsen, 1967; Awad et al, 1977 and Rossier et al, 1980).

The duration of "shock" phase in man is variable between a few weeks to several months depending on the management of the bladder and the extent and site of the lesion. Ultimately, the bladder will regain some activity though incomplete. The association of chronic spinal lesions with lower urinary tract dysfunction is particularly disabling in several diseases and particularly with multiple sclerosis (M.S.).

Multiple Sclerosis:

M.S. is the commonest demyelinating disorder characterized by a chronic and relapsing course of neurological disorder. The disease prevails in certain geographic distribution which is north to 40° latitudes.It affects equally males and females. About 67% of cases begin between the age of 20 to 40 years. There is no appreciable racial factor and a purely hereditary basis seems to be

excluded. Little is known about the etiology of the disease. Some workers claim to isolate a corona virus or a filterable agent that has a cytopathic effect (Mitchell et al, 1978). It appears that there is a correlation between measles and M.S. and other viruses (Poser, 1979). Immunologic studies to date have not yet established whether M.S. is the result of a hyperimmune state or a cellular immunodeficiency. Some factors are thought to be precipitating for the onset or aggravating for the course such as upper respiratory tract infection, immunization, pregnancy or excessive stress (Schoenberg et al, 1979).

Pathology of multiple sclerosis:

Macroscopic findings:

The disease is confined to the brain and spinal cord. Gross examination reveals circumscribed patches in the brain and spinal cord. The "old" lesions appear gray, gray-red or brown in colour. They are well circumscribed. The number, shape, size and location of the plaques vary in the nervous tissue. Although the plaques are distributed in random, in chronic cases, they are almost always paraventricular, i.e. between corpus callosum and caudate nucleus and around the posterior and inferior horns of the lateral ventricle. The plaques are mainly found in the white matter though the gray matter is sometimes involved. In chronic cases, various degres of atrophied plaques are found in the brain, spinal cord and the optic nerve. The leptomeninges are found to be thickened and firmly adherent to the brain (Peters, 1968).

Microscopic findings:

The essence of pathogenesis of M.S. is demyelination. The demyelination is found to start around a vessel then spreads irregularly towards the outer surface. It does not follow a particular tract. Contrary to the "fresh" lesions, the "old" patches are sharply demarcated from the surrounding normal nervous tissue. The foci of demyelination may coalesce and present larger plaques that in general are wedge-shaped or semicircular. They are different from the vascular necrosis of the nervous tissue.

The lesions in the gray matter are found to a variable extent in the cerebral cortex, basal ganglia and the adjacent white matter, eg the internal capsule. In the cerebellum, the deep white matter and the dentate nucleus are the predilliction areas for the plaques.

In the spinal cord, the lesions merge from white to gray matter. They are often symmetrical and located on both sides of the ventral fissure and the dorsal septum. Both the posterior and central parts of the lateral columns are sites for the plaques. The anterior columns are affected close to the ventral fissure. Occasionally, the lesions are concentrically found along the central spinal canal.

The optic nerve, chiasma and optic tracts are almost always involved. Sometimes they are completely demyelinated and due to the subsequent gliosis they become atrophic.

The myelin breakdown is a primary disorder leaving the axon more or less intact. In general the axons are resistant to the noxious agents in M.S. Only in longstanding cases, some foci may show a minority of degenerated axons. The nerve cells show higher resistance than the axons to the noxious agents. However, swelling of cells are sometimes found inside the plaques. The breakdown products of the myelin sheath are removed by phagocytes of glial origin. The presence of glial cells stuffed with fat droplets indicate a "fresh" lesion. In the centre of the "older" lesions, there are no fat-laden glia except perhaps around the vessels. The

"healed" lesions do not contain breakdown products.

The glial fibres form a thin network in the centre of a "fresh" lesion. As the lesion gets "older", the network of glial fibres becomes dense causing the gray-brown coloration and the firm consistancy of the lesions.

Variable amount of perivascular cellular infiltration are almost always found in the "fresh" lesions. This infiltration consists of plasma cells and lymphocytes. The blood vessels inside the plaques may show swollen endothelium or occasional thrombi particularly in "fresh" lesions (Peters, 1968).

Bladder and urethral dysfunction in M.S.:

Because of the involvement of the reticulospinal tract in the regulation of the lower urinary tract function, damage to its continuity by the demyelination process might explain the high incidence of bladder dysfunction in patients with M.S.

Bladder dysfunction is a common presentation in M.S. The incidence of occurance varies between 74-97% according to various authors (Miller et al, 1965; Piazza and Diokno, 1979; Blaivas et al, 1979 and Goldstein et al, 1982). The major symptoms are in the form of bladder irritability: urgency and frequency and urge-incontinence (Andersen and Bradley, 1976; Bradley et al, 1973; Summers, 1978 and Schoenberg et al, 1979) and represent 50-75% of the bladder symptoms. The presence of urinary infection is accelerated and perpetuated by residual urine and frequent instrumentations.

Urodynamic studies in the form of cystometry combined with EMG of the urinary sphincter revealed that detrusor hyperreflexia accounted for 50-75%, and bladder areflexia for 25-40% (Anderson and Bradley, 1976; Bradley et al, 1973 and Schoenberg et al 1979). The most common EMG finding was vesico-sphincteric dyssynergia (Goldstein et al, 1982) where there were persistant or augmented EMG activity during voiding. Uncontrolled sphincteric relaxation is a less common finding. During this phenomenon, there is an unvoluntary inhibition of the external sphincter represented by a period of reduced EMG activity associated with a bladder

contraction (Bradley et al, 1973). The incidence of urinary incontinence is more in women that in men. This is probably because of the better developed urinary sphincteric mechanism in men (Bradley et al, 1973).

The incidence of upper urinary tract involvement in M.S. has been reported varying from 21% and 55%. The cause of death in 55% of patients with M.S. was found to be due to urinary tract damage (Damanski and Keer, 1964).

In order to study the evolution of M.S., several attempts were done to induce a "demyelinating" disease of the central nervous system in several animal species by repeated injections of heterologous mammalian nervous tissue as a sensitizing antigen. The "experimental allergic encephalomyelitis" (E.A.E.) was proposed as a model for M.S. in rabbits and guinea pigs and appears to have had considerable support particularly after the close resemblance between clinical and pathological features of E.A.E. and those found in M.S. in humans (Lassman and Wisniewski, 1979). However the literature lacks, to our knowledge, the animal model for M.S. that allows adequate urodynamic studies during the various phases of the disease.

MANAGEMENT OF VESICAL DYSFUNCTION IN PATIENTS WITH SPINAL CORD LESIONS

Irrespective to the etiology of the spinal cord lesion the urologic treatment of the patients remains essentially the same. The primary objective in caring for these patients is to preserve the renal function. Donnelly et al (1972) and Hackler (1977) showed that between 40 and 43 percent of all spinal cord injury patients eventually will die of renal disease. Beside preservation of renal function the achievement of a socially acceptable means for handling both bladder functions is by itself an important goal.

Immediately following an acute spinal lesion (traumatic injury, acute exacerbation in M.S.) the state of spinal shock occurs and may last for a few weeks to a few months. The bladder activity is absent or severely depressed during this phase of spinal shock. The initial management that is agreed upon entails a short term (5-7 days) indwelling catheter followed, as soon as the condition of the patient allows, by an intermittent catheterization. Guttman and Frankel (1966) showed that intermittent catheterization during acute spinal shock reduced the incidence of infection and that 70% of the patients would become free of the catheter subsequently. If the intermittent catheterization program is not available in an institution, Graham (1981) suggested to move the patient to a centre where it is possible. The application of other modalities to evacuate the bladder during the shock phase showed no success. Yalla et al (1976) have demonstrated that

the use of Crede's manoeuvre to induce bladder emptying was associated with sphincteric contraction. Moreover, the same authors showed that injection of Bethanechol chloride caused a rise in the urethral resistance by contracting the urethral musculature as well as the detrusor.

After the spinal shock phase fades away, the bladder starts to regain some contractility. The urodynamic studies, and particularly, the cystogram will help in categorizing the nature of the established bladder and outlet dysfunction. Thus the follow-up of these patients with repeated cystogram had been advised on periodic basis of 8 weeks in order to decide on the therapy (Graham 1981). The stabilized lower urinary dysfunction can be managed by several protocols taking into consideration to start with the least invasive modalities and keep the irreversible drastic measure to the end.

I. Pharmacotherapy:

Pharmacotherapy has enjoyed immense popularity in the management of patients with urinary dysfunction. Unfortunately, there remain several impediments to the effective use of pharmaceutical agents. These obstacles include reliance upon the presence of a detrusor reflex and upon patient compliance. Moreover, the site of action of most pharmaceutical agents precisely on the lower urinary tract are not known precisely, furthermore, their central nervous system action is virtually unknown.

The choice of pharmacotherapeutic agents fall under the following categories of drugs:

1. Drugs that increase detrusor muscle contractility
2. Drugs that decrease detrusor muscle contractility
3. Drugs that increase urethral resistance
4. Drugs that decrease urethral resistance
5. Drugs for treatment of autonomic dysreflexia

Drugs increasing detrusor muscle contractility:

These drugs provide excitation of the neuromuscular junction. The most popular cholinergic agent used is Bethanechol (Diokno and Lapides, 1977 and Sonda et al, 1979). The indications for Bethanechol include detrusor areflexia, bladder denervation and increased residual urine volume.

Other drugs include Carbachol, Neostigmine and Mestinon. The side effects of these parasympathomimetics are nausea, cramping pain, diarrhea, bradycardia, postural hypotension and dizziness. The popularity of these agents has declined since the introduction of the intermittent self-catheterization (Lapides et al, 1974).

Drugs decreasing detrusor muscle contractility:

This group includes the anticholinergic drugs. The main indication is to suppress involuntary detrusor contraction. They include: Oxybutynin (Diokno and Lapides, 1972), Propantheline (Finkbeiner et al, 1977), Imipramine (Labay and Boyarsky, 1973), Methantheline (Finkbeiner et al, 1977),

Emepronium (Ulmsten and Anderson, 1977) and Dicyclomine (Awad et al, 1977). Their side effects vary in extent but mainly include: dryness of mouth, visual blurring, constipation and cardiac arrhythmias.

Drugs decreasing urethral resistance:

This group of drugs acts on smooth and/or skeletal muscle components of the urethral resistance. They include alpha-adrenergic blocking agents namely Phenoxybenzamine and Phentolamine (Krane and Olsson, 1973). Their main indications are to reduce residual urine volume. They are also used to prevent autonomic dysreflexia in patients with high spinal cord lesions (Scott and Morrow, 1978). Their side effects include postural hypotension, tachycardia and diaphoresis.

Drugs that reduce the striated muscle tone of the external sphincter include Diazepam, Dantrolene Sodium (Murdock et al, 1976) and Baclofen (Florante et al, 1980). The main indication for use of these agents is detrusor-sphincteric dyssynergia. Their side effects include weakness, drowsiness and hepatotoxicity (Dantrolene).

Drugs increasing urethral resistance:

These drugs act directly on the alpha-adrenergic receptors in the bladder outlet region. The result is a rise in the urethral pressure profile indicating a higher urethral resistance. This group of drugs include Ephedrine (Diokno

and Taub, 1975), and Phenylpropranolamine (Montague and Steward, 1979). Other drugs raise the urethral resistance by blocking the relaxing effects of beta-adrenergic receptors as Propranolol (Awad et al, 1974). The side effects include irritability, insomnia, elevation of blood pressure and cardiac arrhythmias.

Drugs for autonomic dysreflexia:

These include Phenoxybenzamine (Scott and Morrow, 1978) and the ganglion blocker agent Hexamethonium (Bors and Comarr, 1971). The main indication is autonomic dysreflexia in patients particularly with high spinal cord lesions, i.e. upper thoracic or cervical levels. The clinical presentation includes paroxysmal hypertension, headache and diaphoresis.

2. Surgical manipulation:

Several neurosurgical procedures were proposed to improve the impaired bladder function following spinal cord lesion. A limited number of patients may benefit from them. However, careful preoperative evaluation of the bladder and its outlet is important since most of these procedures are drastic and irreversible.

The surgical manipulation of the neurogenic bladder can be categorized under the following headlines:

2.A Surgery for improving detrusor force:

Several authors attempted several procedures with the objective being to reinforce a weak detrusor muscle. Some workers tried sympathetic denervation together with a pudendal neurectomy to leave the parasympathetic drive unopposed. The results were disappointing. Other authors favoured a partial bladder resection which may, providing that the detrusor reflex is preserved, re-establish satisfactory expulsive force in the remaining detrusor (Orr, 1937). These operations did not produce satisfactory results and thus were abandoned.

The addition of an intestinal segment to the bladder had been advocated to help increase the intravesical pressure. The bowel contraction was found insufficient for bladder emptying since the intestinal segment contraction does not generate enough tension to maintain a normal opening of the bladder outlet during voiding.

2.B Surgery to control bladder hyperactivity:

These procedures are reserved for persistant, crippling, uncontrollable detrusor hyperreflexia not responding to anticholinergic agents. The techniques aim at either the nerve roots concerned in bladder and outlet innervation or peripherally on the bladder. The preoperative evaluation

includes percutaneous anesthesia of the sacral roots that could be helpful in predicting the result of the operative procedure (Rockswold et al, 1974). By careful selective rhizotomy, the detrusor and pelvic floor spasticity were reduced as shown by Rockswold et al (1973).

Peripheral surgical denervation of the spastic bladder took several forms but the principle remains the same. A transvaginal approach was tried by Warrel (1977). Transection of the bladder above the trigone was carried out in order to interrupt the innervation (Turner-Warwick and Ashken, 1967). Complete perivesical dissection to denervate the bladder has also been tried (Raz et al, 1983).

2.C Surgery to reduce outlet resistance:

These procedures are reserved for decompensated bladders associated with high outlet resistance not responding to drugs. Pudendal neurectomy was tried but was found to be of little or no benefit in reducing the outlet resistance with the associated erectile impotence (Bors and Comarr, 1954). External sphincterotomy had replaced the need for pudendal neurectomy. Its main indication remains detrusor-sphincter dyssynergia with a decompensated bladder and associated vesico-ureteral reflux. Three types of incision have been used for achieving an external sphincterotomy; namely at 12 o'clock (Madersbacher and Scott, 1976), paramedian (Malament, 1972) and at 3 or 9 o'clock (Currie et al, 1970). If the external sphincterotomy is inadequate to relieve the

obstruction, the procedure could be repeated. External sphincterotomy can be associated with a transurethral prostatectomy if voiding is still impossible (Graham, 1981). Detrusor-sphincteric dyssynergia associated with some diseases particularly M.S. is much less severe and quite variable due to the evolutive process of the disease. Sphincterotomy should only be rarely indicated in M.S. (Blaivas et al, 1979).

3. Electronic control of micturition and continence:

The principle of electric stimulation for achieving bladder evacuation and storage appealed to several researchers particularly with the introduction of more sophisticated electronic devices.

For bladder emptying the early trials entailed direct bladder stimulation by application of electrodes on the bladder (Bradley et al, 1962). Later electrodes were put around the spinal cord for stimulation (Nashold et al, 1972). The problem of simultaneous pelvic floor contraction created a high intravesical pressure during voiding with high urethral resistance and poor emptying. By changing the modalities of stimulation, it was possible to induce fatigue of the external sphincter (Tanagho and Schmidt, 1982).

The use of electric stimulation to improve continence has been tried (Caldwell et al, 1965) with some promising results. Later, by the use of vaginal or anal devices,

electric stimulation improved urge incontinence particularly in women through a negative feed back to the detrusor (Godec et al, 1975).

Electric stimulation of skin dermatomes in the leg was shown to control urge incontinence though the mechanism is not understood (Khan, 1983).

4. Mechanical devices:

A multitude of devices had been proposed for reducing the social and hygenic problems associated with incontinence. They can be placed on a temporary or permanent basis. Some are very simple, others are more elaborate and sophisticated. All share the same disadvantage of carrying the risk of technical defect and embarrassment.

The male appliances are the condom urinal that fits snugly around the penis and has gained wide acceptance. The penile clamps carry the risk of penile and urethral erosion particularly in patients devoid of sensation in their genitalia.

The female appliances are more complicated and usually uncomfortable. The majority of the devices are applied intravaginally in an attempt t to compress the urethra against the symphysis pubis.

The artificial urinary sphincter had gained popularity particularly after improving the technical device. The

principle is to compress the bladder neck (or bulbar urethra) by a cuff containing fluid that can be shifted away (to a reservoir) prior to voiding. The main complications remain the risk of infection, erosion and mechanical failure.

5. Intermittent clean self-catheterization:

This method had gained wide popularity during the last decade for the management of patients with neurogenic bladder. The patient must have some dexterity with both hands. Teaching personnel are essential for guidance to the patients. Several reports showed remarkable decrease in urinary tract infection following the use of this technique (Naninga et al, 1982).

6. Indwelling catheter:

This solution becomes the last resort when intermittent catheterization is impractical or impossible either assisted or self-induced. However, the care of the indwelling catheter and the patient is important in order not to complicate matters further. Changing the catheter frequently, observation of closed irrigation drainage system and deblocking the catheter are among the basic rules that must be observed.

7. Urine diversion:

This represents the ultimate failure of all previous solutions be it pharmacological, pedagological or

conservative surgical. Urine diversion should not be the first choice and should be seriously reappraised because of the consequences.

CHAPTER II

OBJECTIVES

MATERIALS AND METHODS

OBJECTIVES OF THE STUDY

Throughout the present work we tried to shed some light on the following:

1. The motility of the bladder and its outlet during continence and voiding in cats with intact spinal cord.

2. In vitro pharmacological study of the proximal urethra and the detrusor exploring the effect of some neurotransmitters, Calcium ions and their antagonists.

3. The motility of the bladder and its outlet during acute spinal shock phase in cats.

4. The development of an animal model for M.S. to evaluate some urodynamic parameters during the different phases of the disease and to test the effect of pharmacological manipulations on these parameters.

MATERIAL AND METHODS

The handling and manipulation of the animals throughout the experiments were approved by the Animal Care Committee in both Sherbrooke and McGill Universities.

For the sake of convenience, the material and methodology will be categorized according to the four above-mentioned objectives.

Objective 1

Mechanism of continence and voiding in control cats: in vivo study:

A total of 86 adult male cats were included in this study. Their weight varied between 2.5 - 4 Kg. Sixty-one (61) were studied under general anesthesia (induction by ether inhalation then maintenance by alpha-chloralose* 50-60 mg/Kg of body weight dissolved in warm isotonic saline and injected I.V.). Twenty-five (25) cats were decerebrated under ether inhalation: once the craniotomy was done, the two occipital lobes were removed by suction revealing thus the corpora quadrigemina. A section in the brain stem was done by a blunt spatula between the superior and inferior colliculi. After proper hemostasis the scalp was sutured closed and ether was discontinued. The decerebrate rigidity soon appeared in the limbs.

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*alpha-chloralose: Abbott Laboratories

The experimental set up was the same for both the anesthetized and the decerebrate groups. The cat was put on its back near a heat lamp to keep the animal warm. The abdomen was incised along the mid-line from xiphoid process to the symphysis pubis. The intestines were packed away from the bladder after sectioning the rectum to prevent transmitted respiratory and intestinal movements from reaching the bladder and thus altering the recordings. The bladder, proximal (pre-prostatic) urethra and the periurethral skeletal muscles were exposed. Minimal dissection was carried out in the periurethral fat in order to preserve the innervation and vasculature as much as possible. The bladder was cannulated by a Size 8 Fr feeding tube for filling and pressure measuring. The filling medium was 0.9% saline warmed to 37°. Two fixed points were chosen on the anterior wall of the bladder and at the vesicourethral junction. One centimeter away from this fixed point, 2 fine black silk sutures were taken respectively on the bladder and along the longitudinal axis of the preprostatic urethra. A third suture was taken 0.5 cm lateral to the fixed point at the vesicourethral junction along the transverse axis of the preprostatic urethra. These silk threads were tied on to three force-displacement transducers (Grass ft 0.3) recording thus simultaneously the motility of the detrusor, longitudinal and circular urethral smooth muscles respectively. Upwards and downwards deflection of the writing pens coincided respectively with contraction and

relaxation. The vesical and urethral pressures were measured by two pressure transducers (Statham). The bladder and urethra were perfused by two Harvard continuous perfusion pumps at constant rates of 1.91 and 0.19 ml/min respectively.

The EMG (electromyographic) activity of the periurethral striated muscles was recorded by two stainless steel wire electrodes isolated except for 2 mm at the tip. These electrodes were inserted in the periurethral tissues at about 5 - 7 cm from the vesicourethral junction using two 21 gauge spinal needles (Figure 14). All tracings were recorded on a polygraph Grass EEG model 7.

In order to test the effect of anesthesia on the urethrovesical reflexes, we injected anesthetic agents systemically (alpha-chloralose 50-60 mg/Kg body weight I.V.) and locally around the urethra (2-5 ml of 1% Xylocaine) in 10 of the decerebrated cats that were kept until then without anesthesia.

By the end of the experiment, the cat was sacrificed by I.V. heavy dose of Nembutal. The decerebrated cats were perfused through the carotid arteries by 10% formalin solution in order to fix the brain in situ. The craniotomy was extended and the whole brain down to the cervical spinal cord was removed. Serial sagittal sections, at 0.5 mm intervals, of the brain stem were studied. The sagittal sections were stained by Luxol fast blue technique. The sections were magnified 5-7 times and projected on paper.

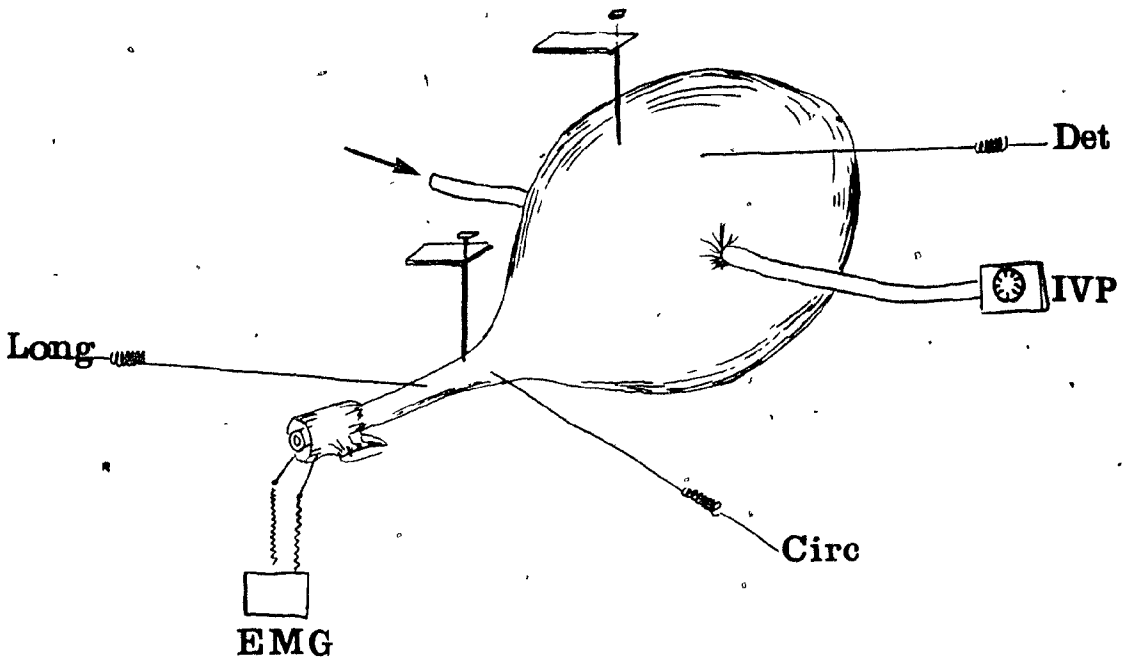

Figure 14. Diagram showing the in vivo experimental set up on the bladder of the cat. The arrow shows the direction of bladder infusion with warm saline.

Diagrams made of the sections were compared to those of Berman (1968). Special attention was focused on the relation of the brain stem lesion to the nucleus coeruleus and red nucleus. A total of 15 brains out of 25 decerebrated cats were studied. A correlation between the voiding pattern and the level of the lesion was then done.

In order to study the anatomy of the vesicourethral junction, the bladder, urethra and periurethral musculature were removed from 5 male cats, and fixed in 10% formalin solution. (Figure 15)

Under an operating microscope, a meticulous dissection for the bladder outlet was carried out to liberate the bladder neck, posterior urethra, prostate and periurethral striated muscles from surrounding tissues. Sagittal and transverse sections were then done and stained with Masson trichrome and examined with the light microscope.

Figure 15. Photograph of the bladder and urethra of the adult male cat. The arrow points to the area of prostate and periurethral striated muscles.

Objective 2

A. In vitro pharmacological study of the urethra:

Cats of either sex weighing 1-3 Kg were decapitated under light ether anesthesia. The proximal urethra was exposed and severed from the bladder and prostate (in male) down to the symphysis pubis. The urethra was then divided into two equal segments in length. One half was hooked through its lumen leaving it as an intact ring. The other half was split open along its longitudinal axis (Figure 16). Each segment was then suspended in a double jacketed tissue bath of 20 ml capacity. One end of the strip was tied to a fixed point in the bottom of the bath, the other end was connected to a force displacement Grass transducer (ft 0.3) by a 6-0 black silk thread. The temperature of the bath was kept constant at 37°C by a thermostatically controlled circulating pump (Haake). The bathing medium was Kreb's physiological solution prepared fresh daily and aerated with a mixture of 95% oxygen and 5% carbon dioxide. The constituents of the Krebs' solution were as follows: (gm/l) NaCl 5.54, KCl 0.35, $MgSO_4.7H_2O$ 0.29, $CaCl_2$ 0.28, KH_2PO_4 0.16, $NaHCO_3$ 2.1 and Glucose 2.1. The salts were dissolved in deionized water. Under a basal tension of 1 g, the strips were allowed to stabilize for 40-45 minutes before testing the drugs. The drugs were added directly to the bath and the response recorded as an isometric contraction (or relaxation) with a low level D.C. amplifier mounted on a Grass polygraph EEG model 7. The doses of the drugs were calculated as their

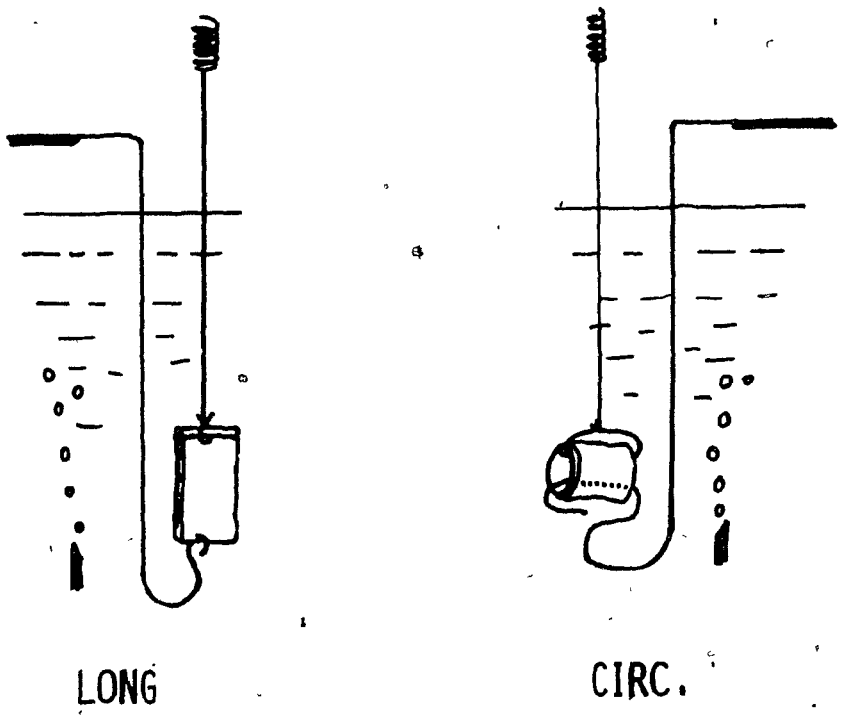

Figure 16. Schematic diagram showing the in vitro set up of the arrangement of the urethral strips in the cat.

final concentration per ml of the solution in the bath. The observations were plotted as dose-response curves. These data were analyzed using the Student's "t" test.

Drugs:

The following commercially available drugs were used: Bethanechol chloride, phenylephrine hydrochloride, atropine sulfate, isoproterenol, propranolol, phentolamine, prostaglandins (PG) F_2alpha and E_2, indomethacin, adenosine triphosphate (ATP), adenosine diphosphate sodium (ADP), cyclic 3,5 adenosine monophosphate (CAMP). Vasoactive intestinal peptide (VIP), substance P, bombesin, neurotensin and bradykinin were synthetized locally by solid phase methodology (St-Pierre et al, 1979; Fournier et al, 1980 and St-Pierre et al, 1981).

Stock solutions of PGs and peptides were prepared and stored at -20°C. PGs were dissolved in a small amount of absolute ethanol and diluted with Krebs' solution. All other drugs, except for indomethacin, were dissolved in Krebs' solution. Indomethacin, freshly prepared for each experiment, was dissolved in 0.2M Trizma HCl.

B. In vitro pharmacological study on the detrusor:

In order to test the importance of Calcium ions on the detrusor, the following experiments were designed. Adult male rabbits weighing 2-3 Kg were killed by direct blow on the neck then exsanguinated. The bladder was removed and

opened. The surrounding fat, serosa and mucosa were dissected off the muscle. The step of removing the mucosa was considered essential for improving the oxygenation of the preparation and for providing better drug contact with both surfaces of the thin muscle sheet. Several strips 10 x 4 mm were excised from the anterior wall along the vertical axis of the bladder. Each strip was fixed at one end to a Platinum electrode shaped like a hook and the other end tied to a force displacement transducer Grass (Ft.03) by 4-0 silk thread. Another Platinum ring shaped electrode surrounds the upper part of the strip. The strip and electrodes were bathed in Krebs' physiologic solution in tissue bath warmed to 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 as mentioned in the previous section. The strip is allowed a stabilization period of 40-45 minutes under a basal tension of 1 gm. The isometric contractions were recorded on a Grass polygraph similar to the one mentioned in the previous section (Figure 17). The electric stimulation of the strip was carried out by a Grass stimulator S88 connected to the electrodes. The parameters for electric stimualtion were 15-25 volts, 50-70 msec duration, applied as trains of rectangular pulses of 5 sec long and allowing 90 sec interval of rest between two consecutive stimulations. In some experiments, the frequency of stimulation was gradually increased from 5 to 60 Hz and the response noted. The same procedure was repeated 5-10 minutes after adding the Calcium antagonist.

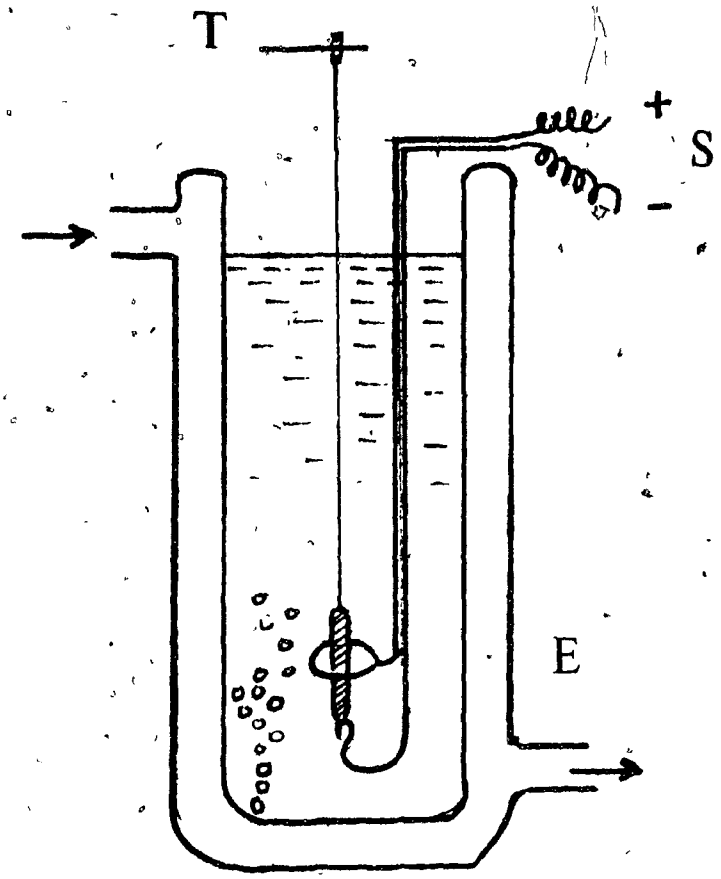

Figure 17. Schematic diagram of the set up used for in vitro study of the detrusor strips of the rabbit. (E) platinum electrodes, (S) electric stimulator and (T) force displacement transducer.

In the rest of the experiments, the frequency of electric stimulation was kept fixed between 10-15 Hz.

In order to check the effect of Ca^{++} on the detrusor, several strips were incubated in Ca^{++}-free Krebs' solution (from which $CaCl_2$ was intentionally removed) for a period of 60-90 minutes and stimulated electricallly every 10 minutes until the response disappeared.

Solution of $CaCl_2$ was added gradually in a cumulative manner to raise the final bath concentration up to 10 times the Ca^{++} concentration in the normal Krebs' solution. Five minutes after the addition of $CaCl_2$ solution, the stimulation is carried out. The strip was then washed with Ca^{++}-free Krebs' solution for 90 min until no response is obtained by stimulation. The Calcium antagonist was added to the solution. This was followed 5-10 minutes later, by $CaCl_2$ and the stimulation is repeated as before. Thus each strip served as its own control for the effect of Calcium antagonist.

In another part of the study, the detrusor strips were kept in normal Krebs' solution then subjected to increasing dose of the Ca^{++}-antagonists (Verapamil, Nifedipine or Segontin) 10-15 minutes prior to electric stimulation with fixed parameters. The response obtained was calculated as percentage of the response obtained by the strip at 0 concentration of Ca^{++} antagonists.

The amplitude of the responses (twitches) of the strips were calculated as the percentage of the maximal amplitude of the control strip. The data were analyzed by Student's "t" test and plotted on curves.

Drugs:

Verapamil (Searle), Tetrodotoxin (Sigma), Nifedipine (Miles), Prenylamine gluconate "Segontin" (Hoechst).

Objective 3

Study of the bladder and urethral mechanics in spinal shock phase:

This part of the work was conducted on 27 male and one female cat weighing between 3-4 Kg. The cats were grouped under 2 groups. Group I: 11 cats with high spinal lesion (between 5th and 6th cervical segments) that resulted in quadriplegic cats. Group II: 17 cats with lower spinal section (between 5th and 7th thoracic segments). The anesthesia used was ether inhalation during the laminectomy of Group I cats and ether then alpha-chloralose I.V. in Group II. Once the cat was anesthetized, the skin over the chosen vertebral spine was incised, the paravertebral muscles retracted and the vertebral laminae exposed. Laminectomy was then performed exposing the spinal cord and its meninges. The spinal cord was then sectioned and about 1 cm in length was removed by suction. The bleeding was controlled and the cat turned on its back. The abdomen was opened by midline incision, the bladder and its outlet were exposed. The set up was performed as mentioned above (in Objective 1). (Figure 14). The set up usually took 30-45 minutes to complete. The recordings of the motility of bladder, urethra and periurethral striated muscles were followed for periods of three to eight hours after the spinal section. The following components of bladder cycle were evaluated in 11 cats mostly of Group I: resting intravesical pressure (IVP) between two consecutive cycles, the maximum (IVP) at the highest point of

detrusor cycle and the frequency of detrusor cycles per minute. We compared these parameters with those of a control group of cats with intact spinal cord (decerebrated cats described in Objective 1). The data were analyzed using the Student's "t" test for comparing the significance of the results.

The pharmacological effects of Bethanechol chloride (0.05 mg/Kg of body weight I.V.) were studied on 10 cats and Phentolamine mesylate (1 ug/Kg of body weight) was used on six cats in attempt to induce voiding. Particular emphasis was directed toward the effect of these pharmacologic agents on the motility of the detrusor, the individual layers of the urethral musculature and the degree of bladder emptying.

Objective 4

Development of animal model for Multiple Sclerosis-like disease:

This part of the study was devoted to developing the experimental allergic encephalomyelitis (E.A.E.) in an animal that permits a convenient evaluation of the bladder and urethral behaviour under different phases of the disease.

A group of 9 adult male cats were immunized with mammalian nervous tissue in order to produce an E.A.E. in the cat. The nervous tissue utilized was spinal cord from sheep, human and guinea pig and injected in 4, 3, and 2 cats respectively. The immunizing mixture was prepared as follows: a homogenate of spinal cord mixed with equal amount of sterile physiological saline to which an equal volume of Freund's adjuvant R.A. (Difco Lab) was added. Several sites were chosen for inoculation: the back of neck, the feet pads in 4 and 5 cats respectively. The total dose inoculated varied between 2-4 ml. The procedure for sensitization was repeated three times at 4-6 week interval. The cats were observed weekly for appearance of signs suggesting neurologic lesions.

A group of 42 adult male New Zealand rabbits were segregated in pairs of equal body weights thus making 21 pairs. One rabbit of each pair was randomly chosen for immunization and the other member of the pair used as its control. The immunizing mixture contained 10 gm guinea pig

spinal cord homogenate mixed with 10 ml of isotonic saline and 20 ml Freund's R.A. Adjuvant. The spinal cords were removed aseptically from 30-35 guinea pigs after laminectomy. Care was taken to avoid as much as possible the nerve roots. The mixture was ground in a mortar under aseptic techniques then transferred into vacuum sterile test tubes of 10 ml capacity. The rabbit to be immunized was anesthetized by Nembutal 30 mg/Kg body weight intravenously. The paws were shaved with an animal hair clipper exposing the footpads. A tuberculin needle and syringe were used for the intradermal injection of 0.05 ml of the homogenate per foot pad. The injection site showed a slight bleb and the bleeding was negligible. Each rabbit had a total dose of 100 mg of homogenate intradermally.

The control member of the pair was anesthestized on the same day using Nembutal. The paws were shaved as in the immunized rabbit. Instead of the homogenate, 0.05 ml of sterile isotonic saline was injected in each foot pad using a similar 1 ml tuberculin syringe and needle. Each rabbit (control and immunized) was housed in a separate cage, fed a known amount of rabbit chow and kept in the same environmental conditions (room temperature, illumination and humidity). The rabbits were observed every 2nd day (even during the weekends and holidays) for the following data: body weight, amount of chow eaten, quantity of stools in the bottom of the cage, appearance of neurologic signs particularly in the gait, and the tail heralding the onset of

the disease.

On the day of the experiment, the immunized rabbit and its control underwent neurologic and urodynamic tests. The neurologic examination was for weight, gait on hard rough ground, eye pupils and reaction to light, eye nystagmus, respiratory rate, tone in limbs and tail, knee jerk and response to pin prick.

The urodynamic test was performed on both the immunized rabbit and his control the same day of the neurological examination. The urodynamic tests included measurement of urethral pressure profile and cystometry.

The catheter used was a locally made special double lumen size 8 Fr feeding tube graduated in centimeters. It consisted of a capillary tube lodged inside the 8 Fr feeding tube (Argyle). The tip of the capillary tube passed through a silicone plug held at 1 cm from the tip of the 8 Fr feeding tube. The capillary tube was connected to a chamber at the extreme end of the feeding tube, communicating with the exterior through two lateral holes. The chamber proximal to the silicone plug communicated with the exterior by two lateral holes (Figure 18). The capillary tube and the feeding tube were connected each to a pressure transducer (Statham or Trantec Bently 800) and to a perfusion pump (Harvard). The transducers were connected to D.C. low level amplifiers built in polygraph Grass 7D. This catheter allowed measuring the urethral pressure profile twice by a single withdrawal.

Figure 18. Photograph of the modified 2-lumen catheter size 8 Fr. used in the rabbits.

Measurement of the urethral pressure profile (UPP):

The UPP was done by the perfusion method described by Brown and Whickham (1969). It assumed that the intraurethral pressure at a point is proportional to the resistance offered to the continuous flow of liquid at this point. The catheter (8 Fr) was lubricated using a water soluble gel and introduced per-urethrum in the rabbit. The bladder was evacuated and flushed with isotonic saline to remove the characteristic urine sediments that might block the tube. Once trapped air bubbles were removed from the catheter, the pressure transducers and perfusion pumps were connected. The perfusion rate was adjusted at 0.38 ml/min with warm isotonic saline. This perfusion rate was found to cause a minimal rise in the base line of the pressure recording of 3-5 cmH_2O. The withdrawal of the catheter was done mechanically using a Masterflex speed controlled machine at a constant speed of 2 cm/min. The recording of UPP was done twice by a single withdrawal of the catheter. At least two reproducible UPP recordings were necessary for the data collection.

Measurement of the cystometrogram (CMG):

During CMG, the same catheter was used with one tube connected to the presure transducer and the other connected to the perfusion pump held at 1.91 ml/min of warm isotonic saline. The catheter was introduced to the bladder per urethrum and the bladder emptied before starting the procedure. In order to record the changes in the intra-

abdominal pressure that might affect the intravesical pressure, a simple 8 Fr feeding tube was inserted per anum, perfused with 0.38 ml/min and connected to the other pressure transducer. The bladder was filled until capacity at which spontaneous voiding occured.

Effect of the anesthesia on the parameters studied:

In order to obviate the effect of anesthesia, the above-mentioned urodynamic studies on each rabbit (immunized and control) were conducted under the immobilization reflex (I.R.) with some modification from that described previously (Rapson and Jone, 1964).

The rabbit was grasped firmly by its four limbs with both hands while it was in prone position. It was then suddenly turned 180° on its back and put in a plastic U-shaped trough (50 x 11 x 9 cm) and kept in this position with gentle hand pressure on for 5-10 seconds. The back and flanks of the rabbit were in contact with the floor and side walls of the trough respectively. When the rabbit relaxed, the hind limbs were gently strapped to the side walls of the trough. The size of pupils and respiratory rate were observed indicating the level of the immobilization reflex. Gentle strokes were applied on the neck and chest of the rabbit whenever needed to prolong the tonic immobility. All manipulations were done with the minimal possible noise and maximal gentleness as the animal was not anesthetized. The catheter was then passed per urethrum. The UPP followed by CMG were done as described above.

The whole procedure was repeated on the same rabbit after general anesthesia using alpha-chloralose (50-60 mg/Kg body weight) I.V. in 15 pairs and Nembutal (30 mg/Kg body weight) I.V. in 5 pairs. The level of anesthesia was kept close to the blink reflex on corneal touch.

Effect of Calcium Antagonists:

The effect of Verapamil was tested on 5 rabbits with developed E.A.E. CMG was done as mentioned above. Verapamil in the dose of 0.2 mg/Kg body weight was injected I.V.. The cystometry was repeated again 5 min after the injection. The changes in bladder capacity were recorded.

Histopathological studies:

At the end of each experiment, the immunized rabbit was sacrificed by heavy dose of Nembutal I.V. or 1 gm KCl I.V. The bladder and urethra were immediately removed. Four pieces of 10 x 4 mm were taken from the dome, anterior wall, trigone and proximal urethra and were immediately frozen under liquid nitrogen and stored there. In order to study the distribution and density of the cholinergic fibres, we used a specific acetyl cholinesterase staining technique described by Koelle (1955).

The principle for staining of specific acetyl cholinesterase (Ch.E)-containing fibres is based upon the enzymatic hydrolysis of thioanalogues of acetyl- and butyrylcholine by enzymes contained in the fresh frozen

section, with the precipitation of the liberated thiocholine as a mercaptide and its subsequent replacement by copper sulphide (CuS). The addition of diisopropyl fluorophosphate (DFP) inhibited the non-specific ChE.

The frozen sections, once cut, were placed on slides, allowed to thaw for one minute then put in the storage solution of DFP for 30 minutes. They were then transferred to the incubation solution for 5-60 minutes at 38°C.

The incubation solution contained Cu-glutamate 3.75 gm, glycine 250 gm, $CuSO_4.5H_2O$, q.S. 100.0 ml, Malic acid 9.6 gm, NaH maleate 52.2 ml, N NaOH q.S. 100.0 ml, Na_2SO_4 40% (W/V), the pH was adjusted to 6.00, $MgCl_2$ 9.52 g, acetylthiocholine iodide 23 mg and distilled H_2O 1000 ml.

The slides were then transferred to rinse solution 1 (20% Na_2SO_4 saturated with copper thiocholine "CuThCh") for 5 minutes then immersed for about one minute each in rinse solution 2 (10% Na_2SO_4), saturated with CuThCh and 3(CuThCh-saturated water), following which they were placed for 20 seconds in Cu S-saturated ammonium sulfide solution, rinsed rapidly in water, fixed in Cu S-saturated 10% formalin, dehydrated through Cu S-saturated alcohols and xylol, and mounted in balsam. Some preparations were counterstained with hematoxylin-eosin and others with the brilliant cresyl violet. Sections were also studied with phase-contrast microscopy.

In order to fix the brain and the spinal cord in situ, the ascending aorta was cannulated and perfused with 10% formalin solution. Through an extended craniotomy and laminectomy the brain and spinal cord including the nerve roots were removed en bloc and hung in a long cylinder with 10% formalin solution for further fixation. The brain and spinal cord were cut serially in transverse sections. A minimum of four transverse sections were chosen from each of the following levels: anterior and posterior hippocampus, midbrain, pons, medulla oblongata, cervical, dorsal, lumbar and sacral parts of the spinal cord. The sections were stained one with each of the following stains: hematoxilin and eosin for cellular infiltration, Luxol fast blue for the myelin substance, Bodian for the axons and Holzer for the glial cells. The sections were examined with light microscopy by an independant neuropathologist without knowing neither the neurologic nor the urodynamic findings of the rabbit. The lesions were classified according to the level within the central nervous system, site (gray or white matter) and degree. Grading the degree of lesions was from 1 to 3 according to the extent of cellular infiltration, and demyelination, grade one being the minimal damage.

CHAPTER III

RESULTS

RESULTS

The same sequential order will be followed in exposing the results of the present work.

Objective 1

Continence and voiding mechanics in the control cats:

Anatomy of the vesicourethral junction in the cat:

The gross dissection of the bladder and its outlet showed a preprostatic urethra of about 2 cm long extending from the bladder down to the prostate that lies deep behind the symphysis pubis. The fat surrounding the urethra showed a rich nerve plexus and blood vessels. At the level of the prostate, skeletal muscle fibres were found surrounding the prostate and fixed to the back of the symphysis pubis (Figure 19). The histologic examination showed that the preprostatic urethral musculature was totally smooth muscles. There were two distinctive separate layers: an inner longitudinal (Long) and outer circular (Circ) layers that mingled proximally with the detrusor fibres in continuity (Figure 20a). More distally, these two layers fused within the prostatic glands. The periurethral striated muscle fibres were found to be arranged mainly in circular fashion surrounding the urethra and mingling with the circular smooth muscle fibres (Figure 20b).

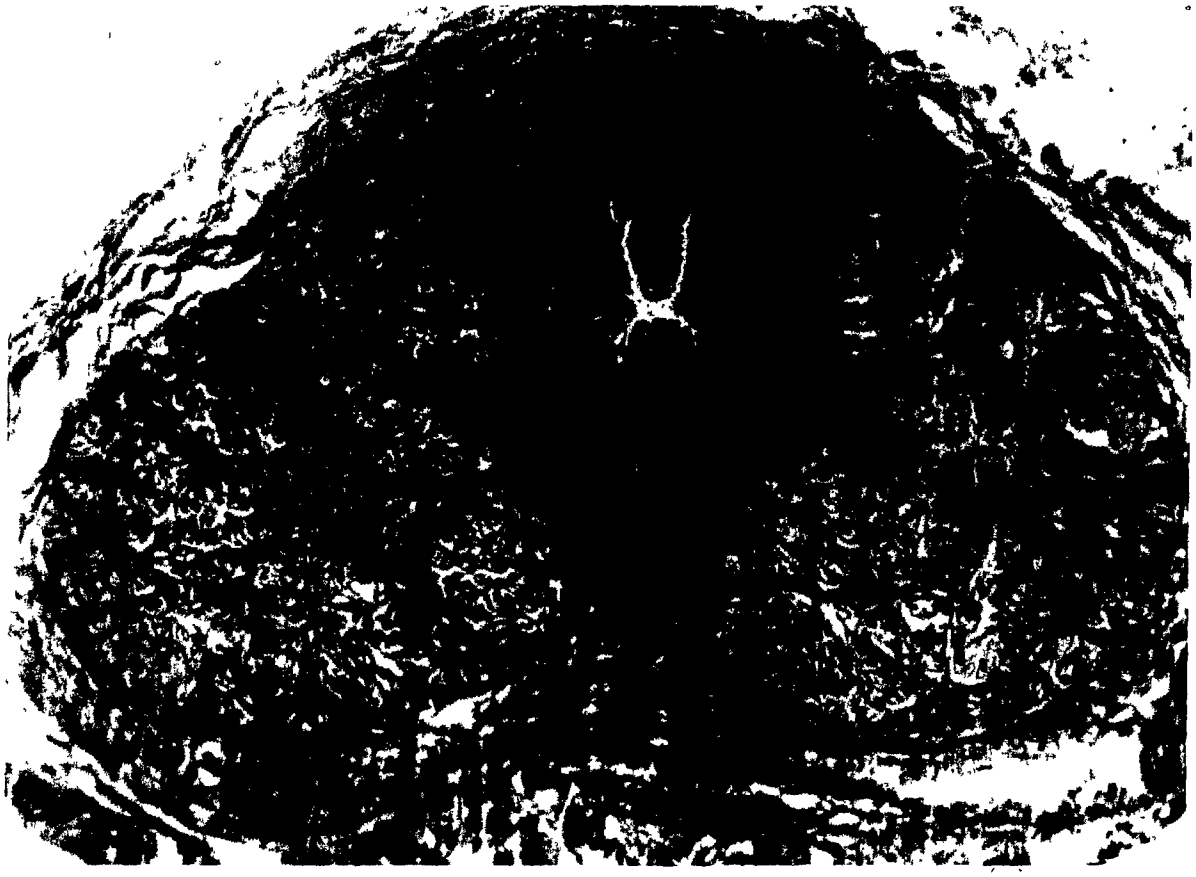

Figure 19. Microphotograph of a transverse section at the level of the prostate showing the striated muscle fibres surrounding the prostate. H&E x6.5. (U) urethral lumen.

Figure 20a. Microphotograph of a longitudinal section in the preprostatic urethra of the cat. Note the outer longitudinal (L) and inner circular (C) arrangement of the urethral smooth muscle fibres. H&E x20

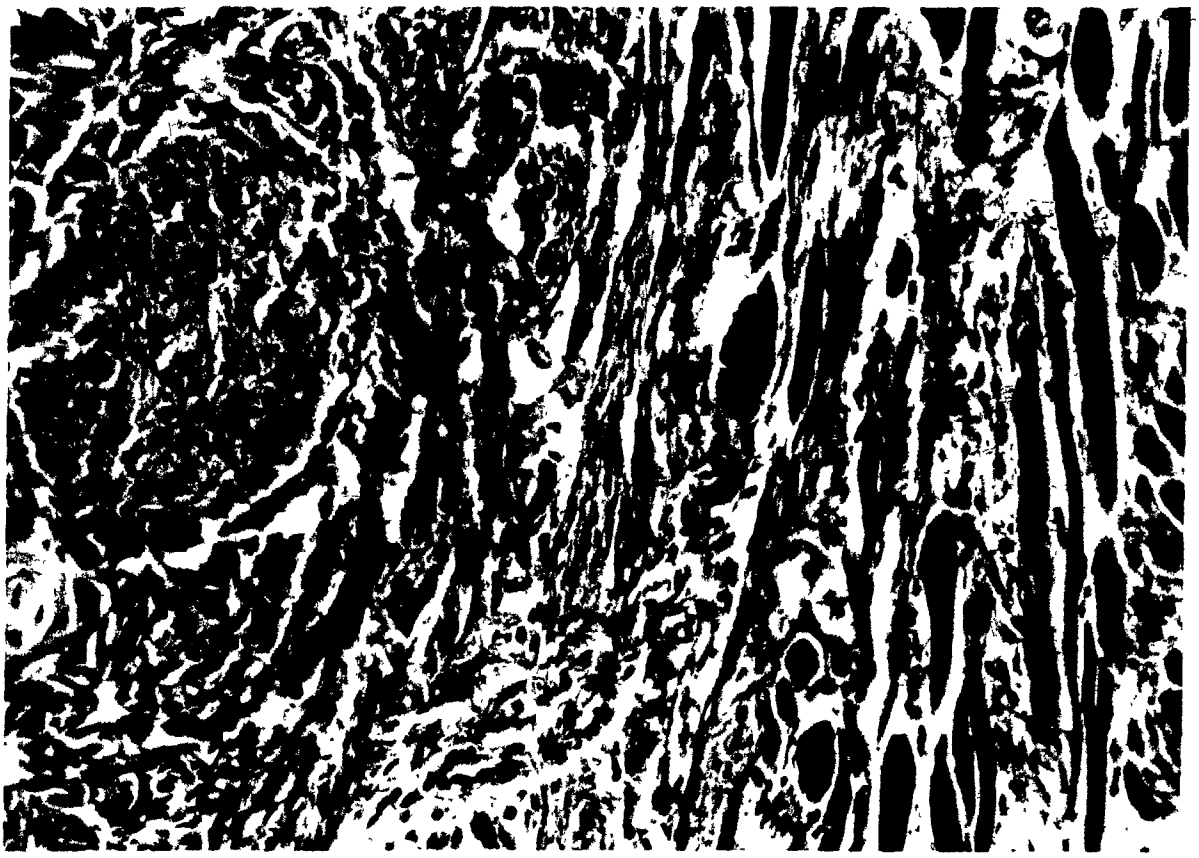

Figure 20b. Microphotograph showing the urethral smooth muscles intermingling with the periurethral striated muscles at the level of the prostate of the cat. H&E x20

Brain stem examination::

A total of 15 brains from decerebrated cats were available for study of the level of the lesion. We divided the lesions under three groups according to their sites in relation to the nucleus caeruleus at the upper part of the floor of the 4th ventricle (Figure 21a & b). Lesion type I was found in 7 cats, type II in 6 and type III in 2 cats.

Changes during continence and voiding:

During the storage phase, we found a minimal muscular motility of both Long and Circ layers of the urethral smooth muscle. The detrusor (Det) showed some oscillations. The frequency of detrusor contractions was 1.1/min $\pm$ 0.1 standard error (S.E.). The resting intravesical pressure (I.V.P.) was 5 mm Hg $\pm$ 0.9 (S.E.). The EMG activity of the periurethral striated muscles (PSM) was found stable with minimal activity. Just before voiding, we noticed a contraction of Det and 2 different movements of the urethral muscles: relaxation of Long and contractions of Circ. This "tightening" mechanism, described previously (Abdel Rahman et al, 1981), coincided with an additional burst of increased activity of the EMG of the PSM. This isovolumetric phase was associated with a rise in both the IVP and intraurethral pressure (IUP). The voiding phase started when the Long urethral layer contracted, the Circ layer relaxed, the DET contracted and the PSM showed reduced EMG activity. The IVP rose to 28.6 mm Hz $\pm$ 3.4 (S.E.) and the flow of urine appeared as a continuous stream. This continuous pattern of

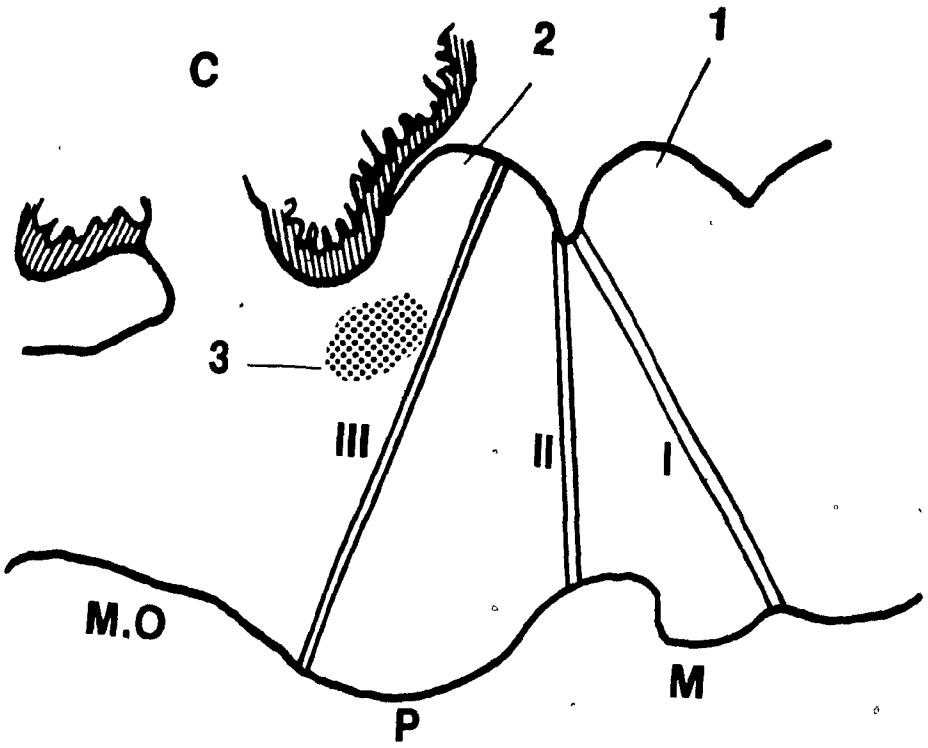

Figure 21a. Schematic diagram of the sagittal section in the brain stem of the cat showing the different levels of lesions (I-III). (1) Superior colliculus. (2) Inferior colliculus. (3) Locus cerulens. (C) Cerebellum. (M) Mid-brain. (M.O.) Medulla oblongata. (P) Pons.

Figure 21b. Photograph of the sagittal section in the brain stem of a decerebrated cat. Note the extent of the lesion. Masson x10.

voiding (first pattern) did not totally empty the bladder. As the urethra opened, IUP showed an initial drop then became equal to IVP throughout voiding since both bladder and urethral luminae are freely communicating. At the end of voiding, the urinary stream became interrupted in spurts and this 2nd pattern was associated with rhythmic bursts of EMG activities of the PSM (Figure 22). The bladder was then found totally emptied by the end of the 2nd pattern. This 2nd pattern was found in 22 out of 25 of the decerebrated cats and was abolished under anesthesia with alpha-chloralose I.V. and/or after local 2% Xylocaine infiltration around the urethra and bladder base. Under anesthesia (local and general) the voiding pattern was present only as a continuous stream (1st pattern) leaving the bladder half emptied.

The correlation between the brain stem lesions and the voiding pattern in 15 decerebrated cats, showed that when the lesion (type II) was closer to the rostral pons the 2nd pattern was lost. In 2 cats where the lesions (type III) were through the mid-pons (n. caeruleus), voiding reflexes were much inhibited (Table I).

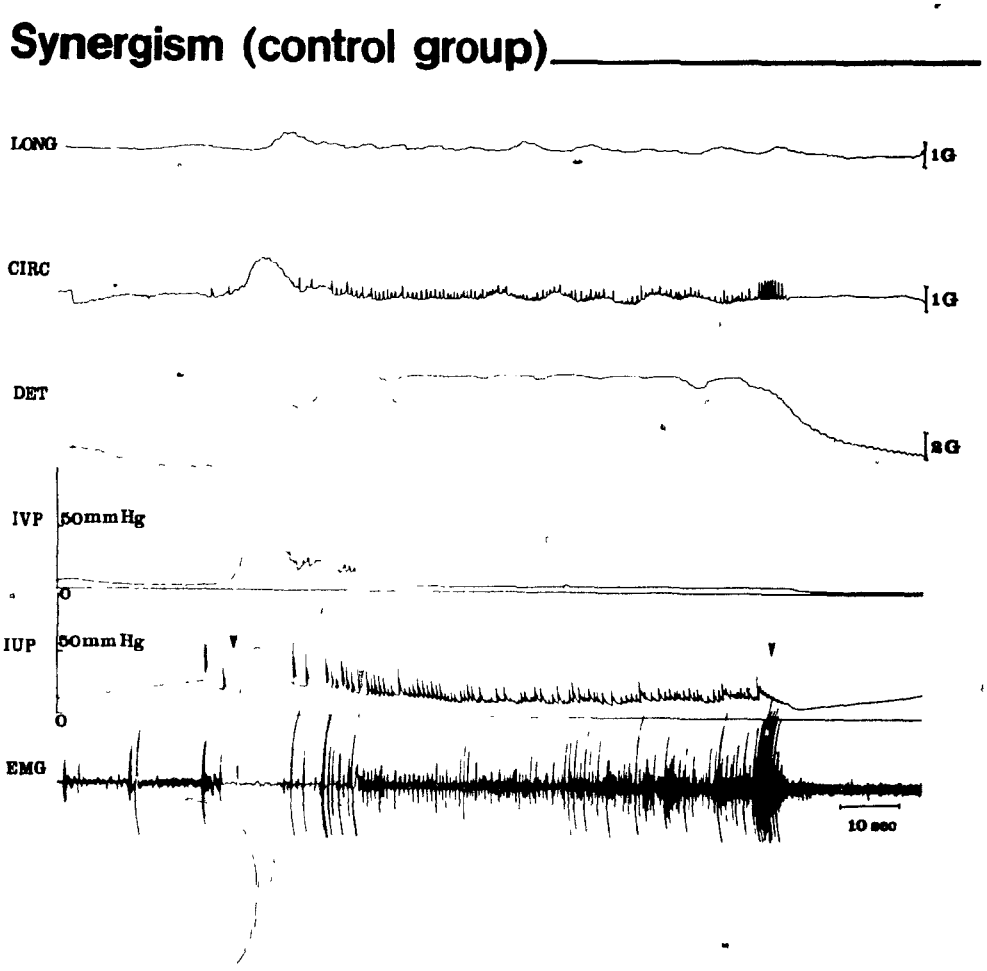

Figure 22. Recording of a complete voiding cycle (between two arrowheads) in the control group of cats. Note the period of EMG silence (1st pattern) followed by rhythmic contractions (2nd pattern). The intraurethral pressure (IUP) shows a short drop that coincides with relaxation of the CIRC and contraction of LONG urethral smooth muscle layers heralding the opening of the bladder neck.

TABLE I

CORRELATION BETWEEN THE LEVEL OF BRAIN STEM LESION

AND

VOIDING PATTERN IN 15 DECEREBRATED CATS

Lesion type	No. Cats	Synergism	(%)	2nd pattern voiding	(%)
I	7	7/7	(100)	7/7	(100)
II	6	4/6	(66.6)	5/6	(83.3)
III	2	0/2	(0)	0/2	(0)

Objective 2

A. In vitro study on the urethra:

Neither longitudinal nor circular urethral strips developed spontaneous rhythmic contractions.

Effect of cholinergic agonist (Bethanechol chloride) n=28 strips:

All the longitudinal strips responded to Bethanechol chloride by tonic contraction. Augmenting the dose resulted in an augmentation of the response. The response was immediate after adding the drug in the bath. Atropine (0.05 ug/ml) blocked the Bethanechol effect on the strips. The circular strips in their intact ring forms exhibited either minimal or no response to Bethanechol. The dose related tissue response (Figure 23) showed an overall higher response in the long strips. The differences between Long and Circ muscle response were highly significant particularly in high doses of Bethanechol chloride ($P<0.001$). The vertical bars represent the standard deviation.

Effect of alpha-adrenergic agonist (Phenylephrine hydorcholoride) n=28 strips:

Both Long and Circ strips responded by contraction to Phenylephrine. Figure 24 represents a dose-response curve that shows no significant differences in the response between both layers. The contracti l e effect of Phenylephrine was blocked by Phentolamine (0.01 ug/ml).

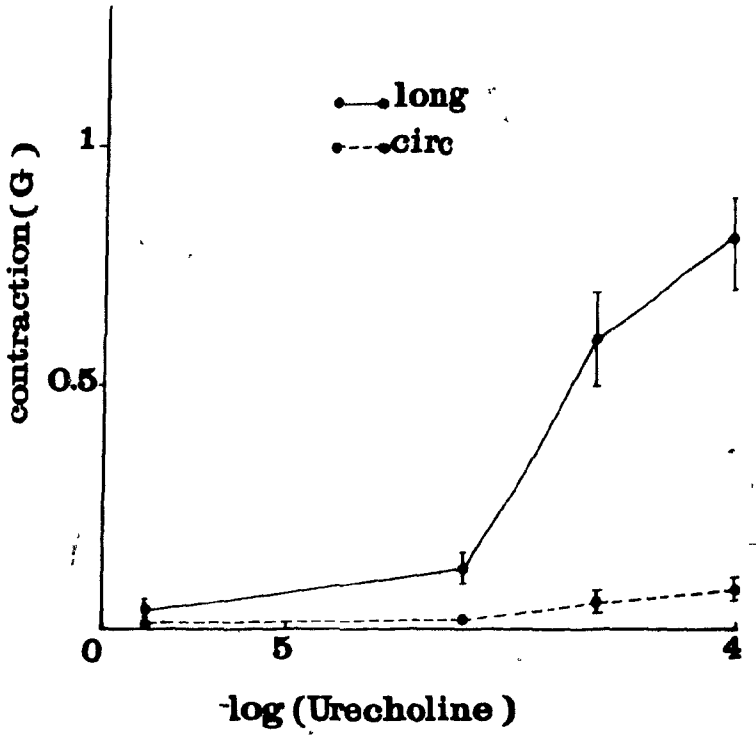

Figure 23. The dose-response curve of the effect of Urecholine on the urethra of the cat. Note the significant difference in the reaction of LONG and CIRC layers to the agent.

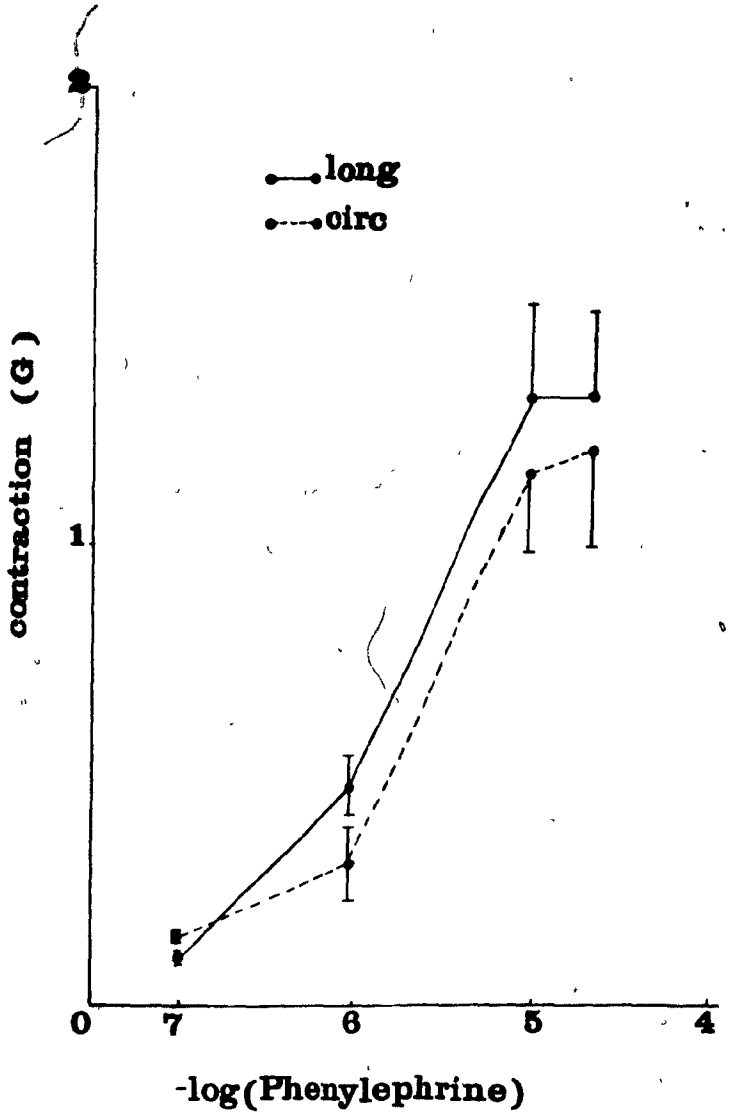

Figure 24. The dose-response curve of the effect of Phenylephrine on the urethra of the cat. Both layers (LONG & CIRC) reacted in similar fashion.

Effect of Beta-adrenergic stimulation (Isoproterenol) n=22 strips:

Isoproterenol in doses 0.01-20 ug/ml caused relaxation in both Long and Circ layers. Figure 25 shows the dose-response curve. The relaxing effect of Isoproterenol was more significant on the Circ layer ($P<0.001$). Propanolol in a dose of 5 ug/ml blocked the relaxing effect on both layers.

Prostaglandins F_2alpha and E_2 n=52 strips:

The effect of PGF_{2}alpha (3.5×10^{-6} - 3.5×10^{-5}M) was studied on 12 Long and 12 Circ strips. Both types of strips responded by contraction and the responses were dose-dependant. The difference in the contractile effect of PGF_{2}alpha on both Long and Circ strips was non-significant (Figure 26). The contractile effect of PGF_{2}alpha on the strips was not blocked by atropine (4.4×10^{-6}M), phentolamine (3.7×10^{-7}M) or by tetrodotoxin (1.5×10^{-6}M).

The effect of PGE_2 (3.5×10^{-6} - 3.5×10^{-5}M) was tested on 14 Long and 14 Circ strips. PGE_2 resulted in a contraction of the Long strips and a relaxation of the Circ oriented ones (Figure 27). The effects were dose-dependant. The contractile effect of PGE_2 on the Long strips was not blocked by atropine (4.4×10^{-6}M), Phentolamine 3.7×10^{-7}) or by tetrodotoxin (1.5×10^{-6}M). The relaxant effect on the Circ strips was neither affected by propanolol (1.9×10^{-5}M) nor tetrodotoxin (1.5×10^{-6}M).

The strips were desensitized to PGF_{2}alpha and E_2 by

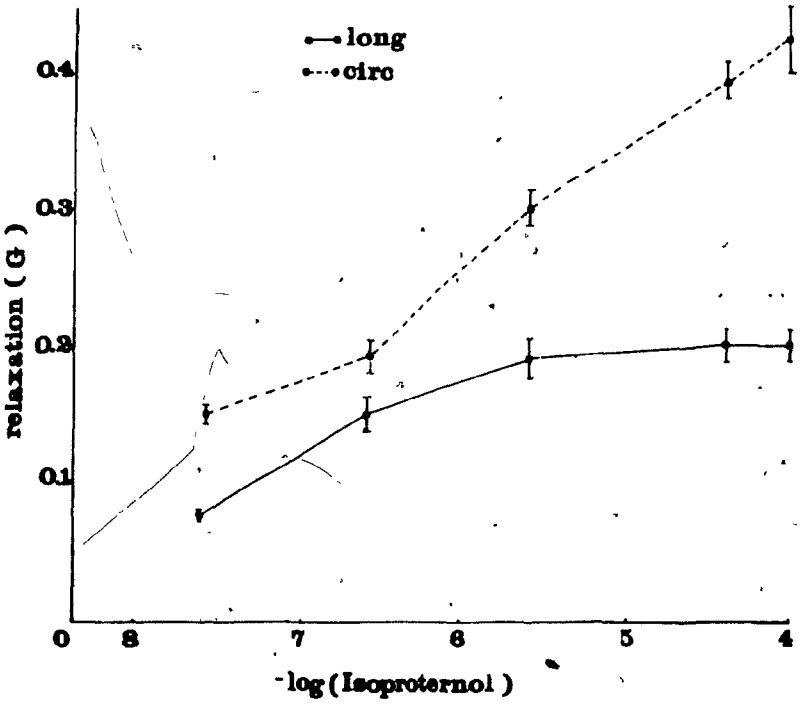

Figure 25. The dose-response curve of the effect of Isoproterenol on the urethra of the cat. CIRC layer shows a significantly higher response to the drug (Relaxation).

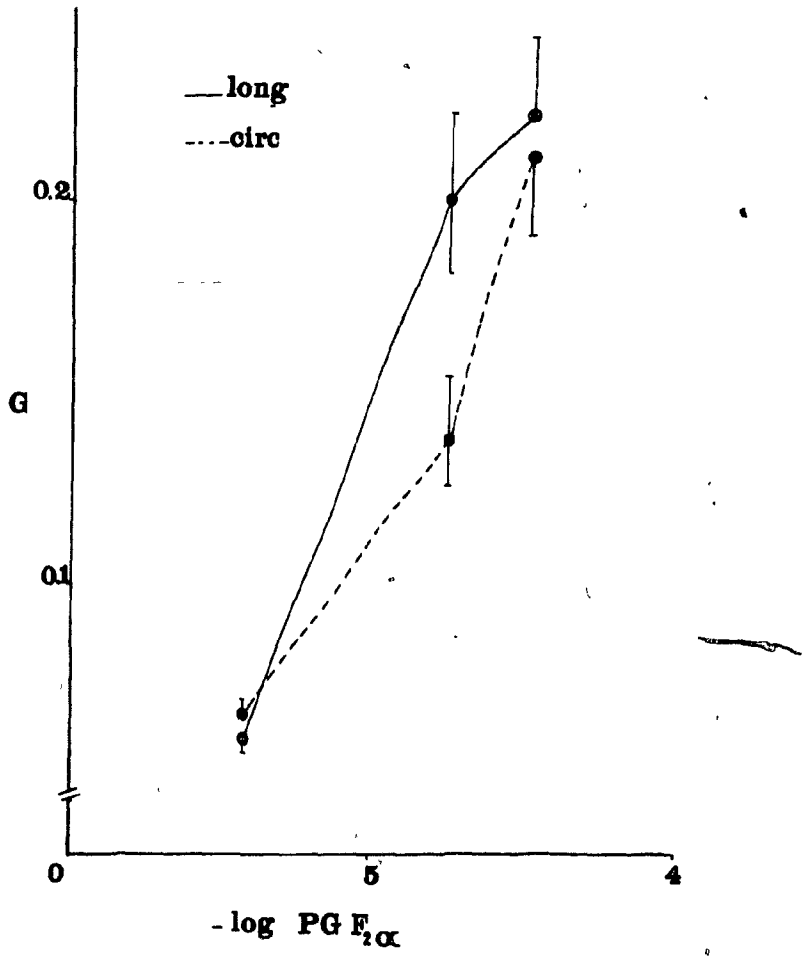

Figure 26. The dose-response curve of the effect of Prostaglandin F_2alpha on the urethra of the cat. Both LONG and CIRC react in similar fashion.

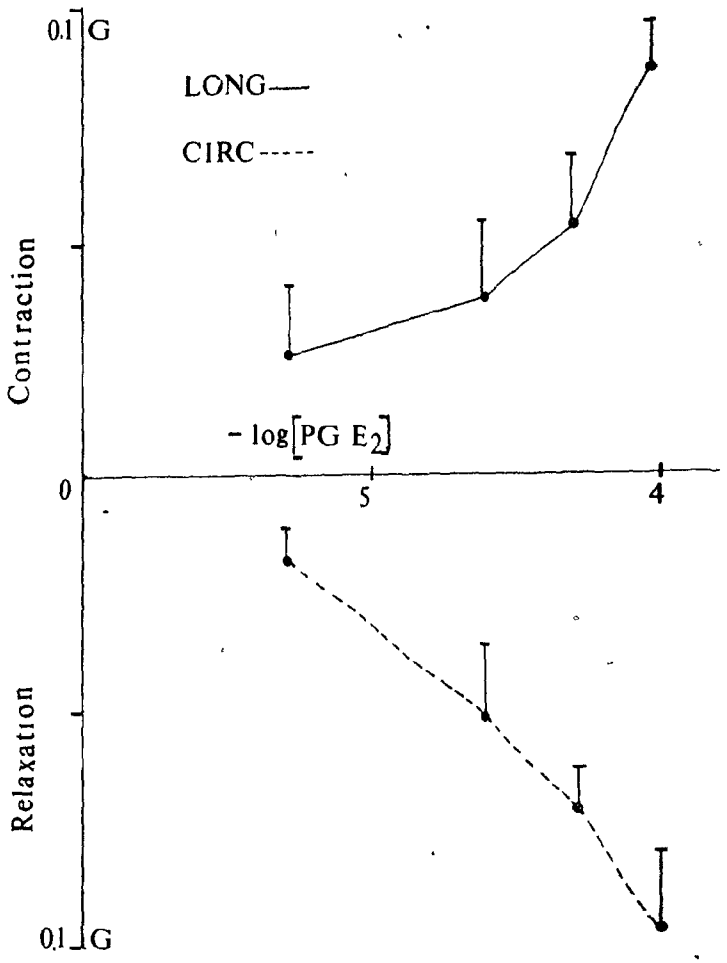

Figure 27. The dose-response curve of the effect of Prostaglandin E_2 on the urethra of the cat. Note the significant difference in contraction of LONG and relaxation of CIRC.

gradually increasing the dose until the effect was maximal and left in contact with the PGs until they returned to the base-line, usually within 10 to 20 minutes. The desensitized strips were then challenged with phenylephrine (1×10^{-5}M) and isoprenaline (2.1×10^{-6}M). The responses to these two agonists were similar in amplitude to those recorded in strips unexposed to PGs.

Effect of other agonists:

The myotropic effects of other agonists on the Long and Circ muscle of the urethra are summarized in Table II. Bradykinin contracted both urethral muscle layers and the effect was more pronounced on the Long strips. The contractile effect of substance P was almost equal on both muscle layers. All other tested drugs had no effect on the urethral muscles.

B. In vitro study on the detrusor:

In presence of normal Krebs' solution, 75-80% of the detrusor strips developed spontaneous rhythmic contractions and relaxations of average amplitude (about 0.5 - 1 gm force) and frequency (mean = 5.6/min). This spontaneous peristaltic movement disappeared in Ca^{++}-free Krebs' solution and reappeared by adding Ca^{++} to the bath to become less frequent by increasing $[Ca^{++}]$ concentration to 10 times the concentration present in normal Krebs' solution.

Response-frequency curve n= 16 strips:

The electric stimulation of individual detrusor strips (10-15 volts, 50-60 msec) resulted in muscle twitches whose amplitude increased gradually by increasing the frequency of stimulation until a plateau is reached at 55-60 Hz. Plotting the frequency of electric stimulation and the amplitude of the response, a constant frequency-response curve was obtained. The presence of Tetrodotoxin ($1 \times (10^{-6}M)$ in the bathing solution abolished the response of the strips to electric stimulation at 1-10 msec but demonstrated no or minimal effect at 50-60 msec duration.

After adding Verapamil ($1 \times 10^{-5}M$ and $2 \times 10^{-6}M$) to the bath, the frequency-response curve diminished to nearly 50% and 25% of the original control studied in the presence of normal Krebs' solution. All three Ca^{++} antagonists caused significant inhibition in the contractile response. This inhibition was shown to be dose-dependent with any of the Ca^{++}-antagonists used (Figure 28a, b & c).

After incubating the strip in Ca^{++}-free Krebs' solution for 60-90 min, no response was ellicited on electric stimulation using the same parameters that resulted in response when the stimulation was studied in the presence of normal Krebs' solution. By gradually adding the $CaCl_2$ to the CA^{++}-free Krebs' solution and by applying the same electric stimulation, 2 types of responses were noticed.

TABLE II

Agonist	Dose	Effect: Longitudinal	Effect: Circular	No. of Experiments
Bradykinin	0.025-5 g/ml	++	+•	8
Substance P	0.05-10 g/ml	+	+*	6
Neurotensin	1-5 g/ml	-	-	4
Vasoactive peptide	1-5 g/ml	-	-	4
Cyclic 3.5 adenosine monophosphate	1-5 g/ml	-	-	4
Cyclic 3.5 guanosine monophosphate	1-5 g/ml	-	-	4
Adenosine triphosphate	1-5 g/ml	-	-	4
Adenosine diphosphate	1-5 g/ml	-	-	4
Bombesin	1-5 g/ml	-	-	4

• Significant difference ($p<0.001$).
* No significant difference.
+ = contraction between 0-0.2 gm.
++ = contraction between 0.2-1 gm.

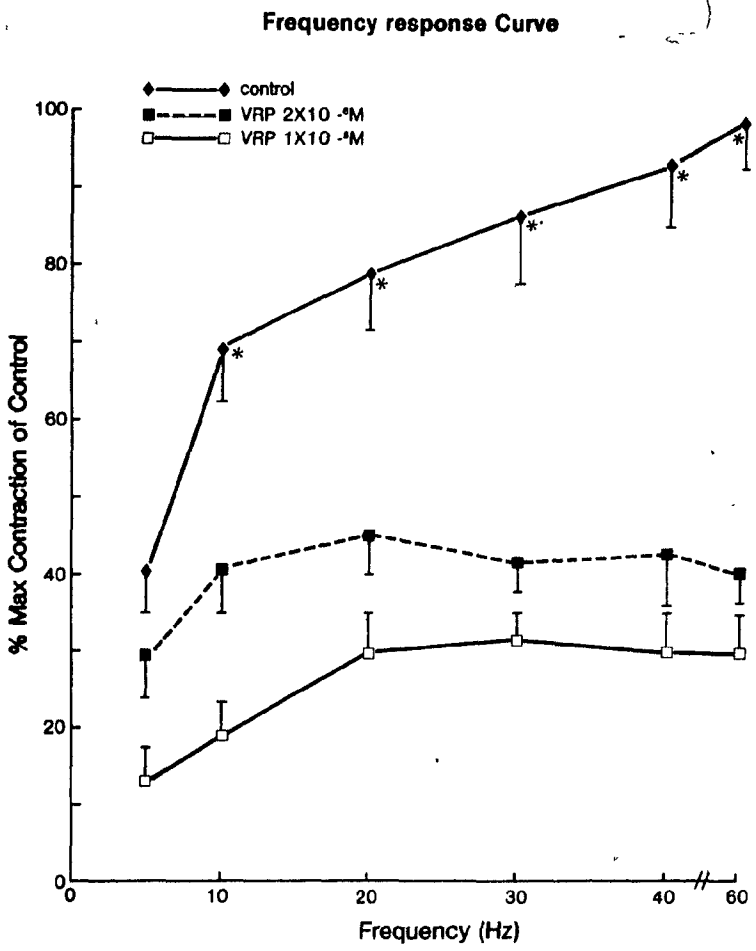

Figure 28a. Effect of Verapamil (VRP) on the frequency response curve of the rabbit detrusor. (*) means statistically significant ($P<0.05$).

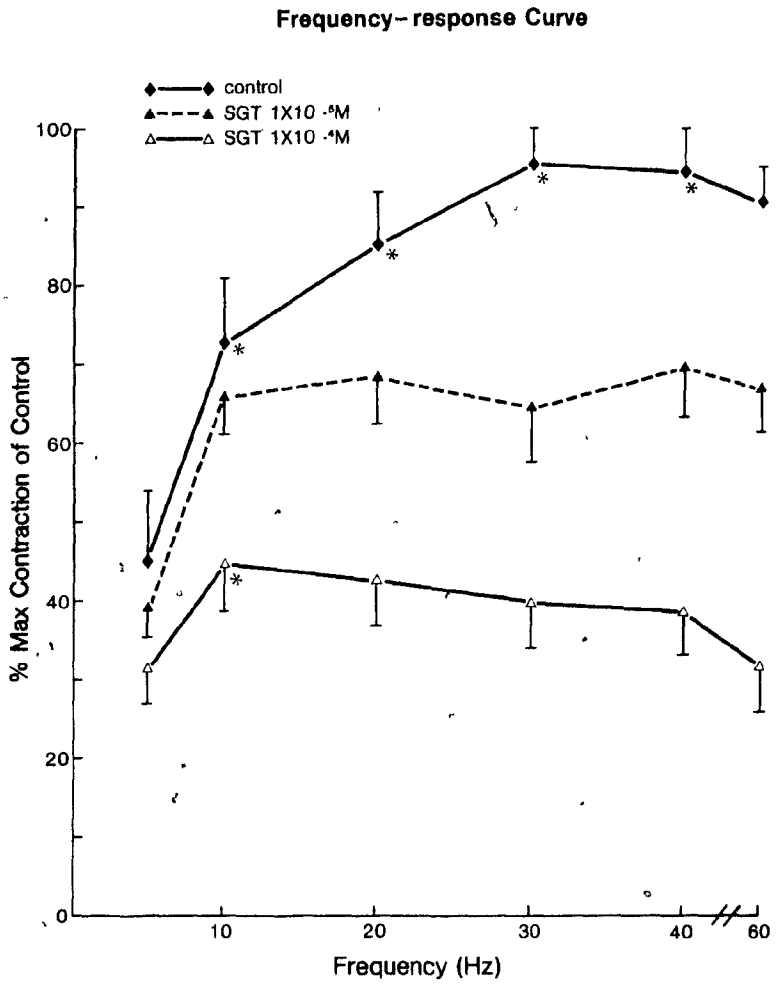

Figure 28b. Effect of Segontin (SGT) on the frequency response curve of the rabbit detrusor. (*) means statistically significant ($P<0.05$).

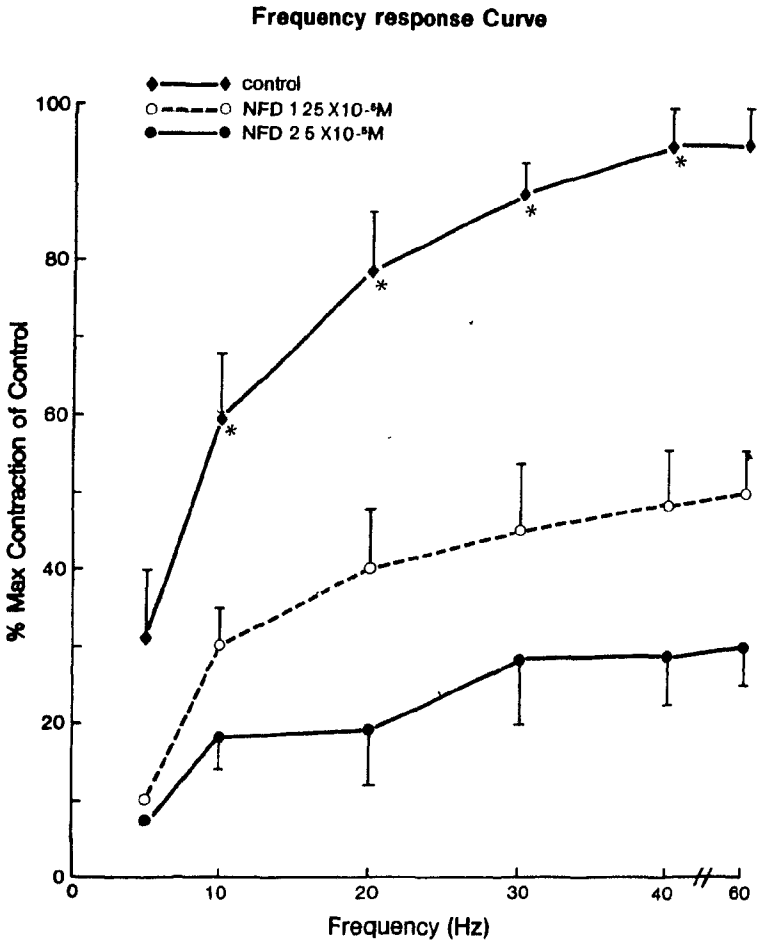

Figure 28c. Effect of Nifedipine (NFD) on teh frequency response curve of the rabbit detrusor. (*)means statistically significant ($P<0.05$).

a. A Phasic (P) response: a detrusor twitch that was almost coinciding with the application of the stimulation;

b. A Tonic (T) response: a gradual rise in the basal tension of the strip was noted irrespective of the electric stimulation.

By increasing the concentration of Ca^{++} in the medium, the phasic responses showed a progressive rise in the amplitude until a Ca^{++} concentration of 0.5 1M was reached and then gradually declined with further addition of Ca^{++}. (Figure 29).

In the presence of Verapamil (2×10^{-6}M) both the T and P contractions were significantly inhibited. It is interesting that Verapamil showed a non-competitive inhibition with Ca^{++} to suppress both types of detrusor contraction (Figure 30a & b). Nifedipine in concentration of 2.5×10^{-5}M showed non-competitive inhibition on the P contraction (Figure 31a). However, the T contraction was suppressed in a competitive manner under the effect of Nifedipine (Figure 31b). Segontin in concentration of 1×10^{-5}M depressed both the T and P contractions of the detrusor strips in a competitive inhibitory manner (Figure 32a & b). The inhibitory effects of the three Ca^{++}-antagonists were evaluated at ED50 and their respective concentration were compared. Verapamil and Segontin showed non statistical significance in their ED50. However, both were more potent in their inhibitory effect on detrusor contraction ($P<0.05$)

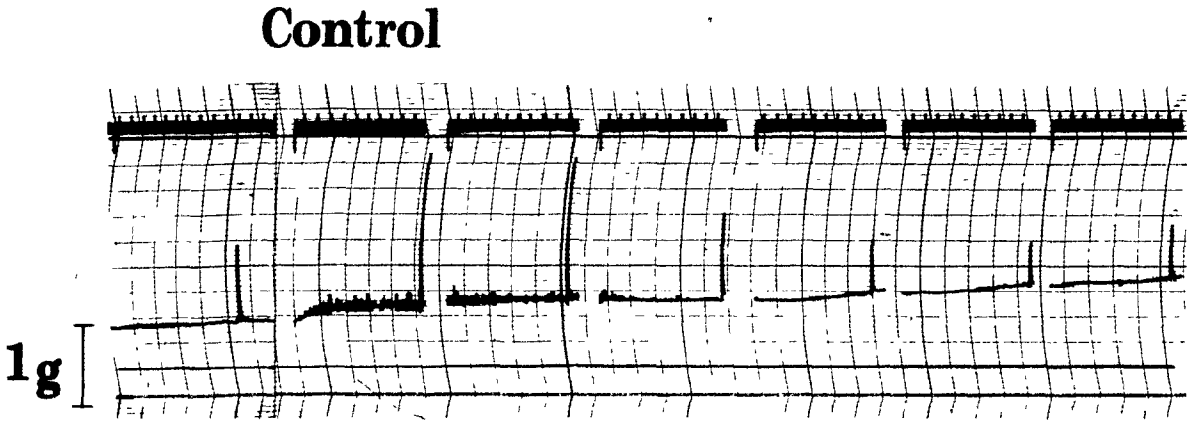

Figure 29. Effect of rising the $CaCl_2$ concentration on tonic and phasic contraction of the rabbit detrusor. Note the rise of tonic contraction and the diminution of the phasic contraction as the $CaCl_2$ concentration rises (each downward vertical dash).

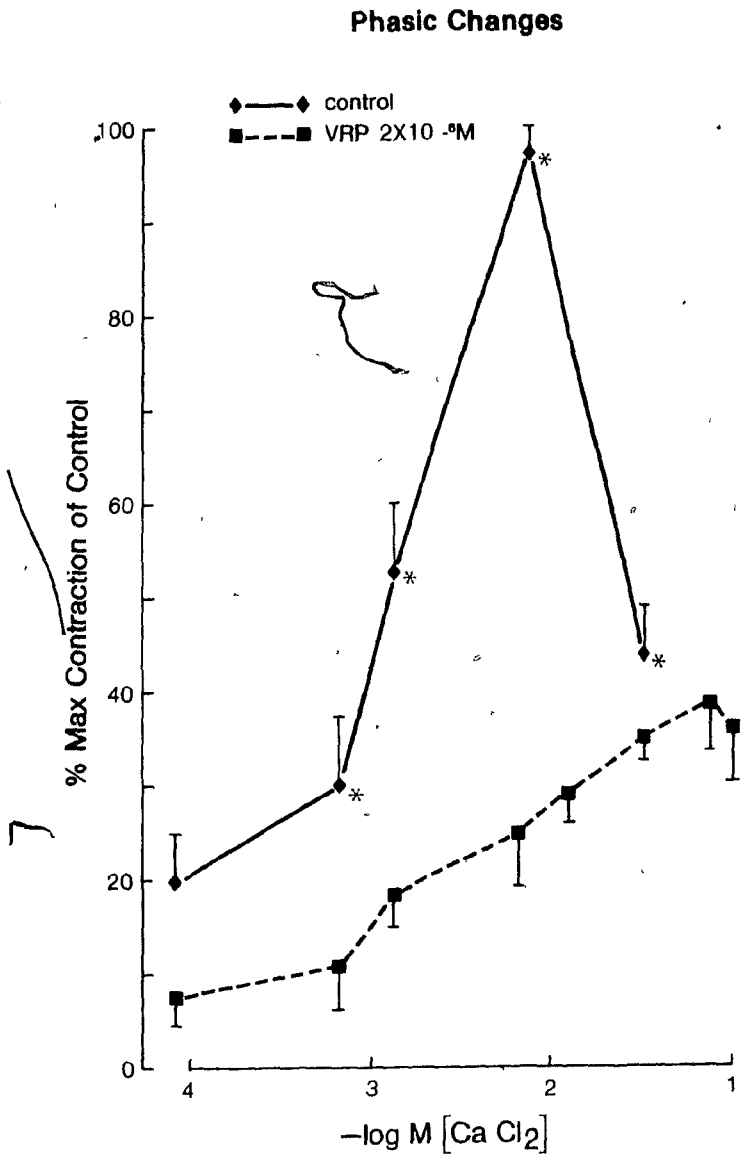

Figure 30a. Effect of Verapamil (VRP) on phasic contractions of the rabbit detrusor. (*) statistically significant ($P<0.05$).

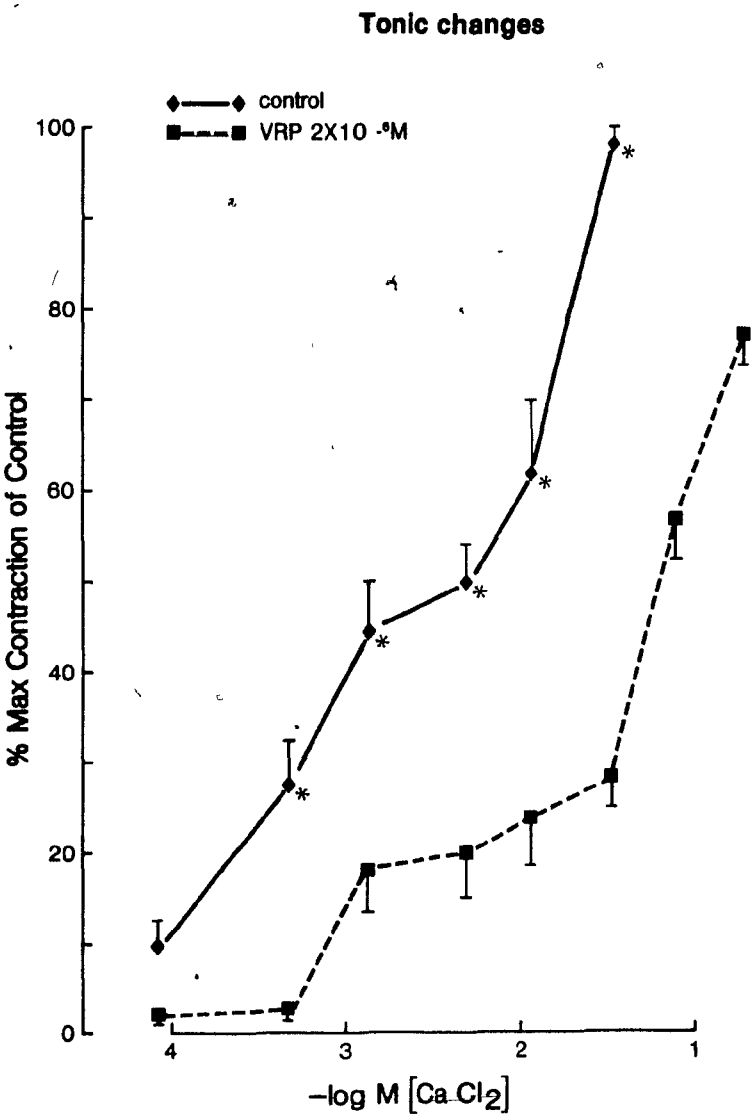

Figure 30b. Effect of Verapamil (VRP) on tonic contractions of the rabbit detrusor. (*) statistically significant ($P<0.05$).

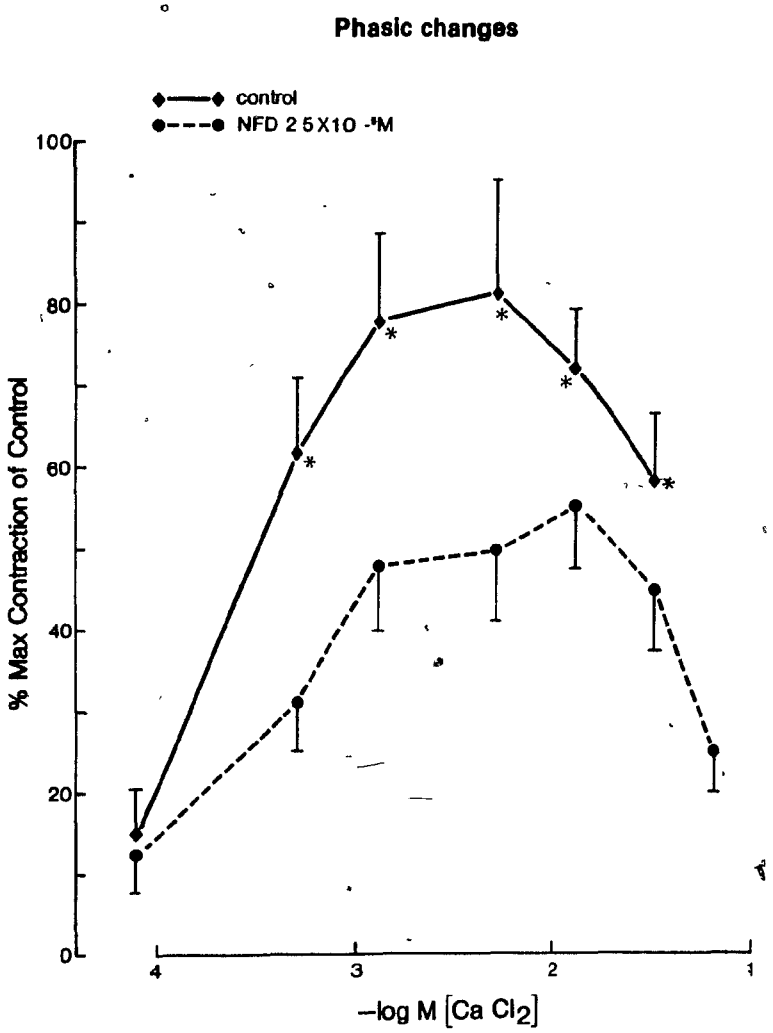

Figure 31a. Effect of Nifedipine (NFD) on phasic contractions of the rabbit detrusor. (*) statistically significant ($P<0.05$).

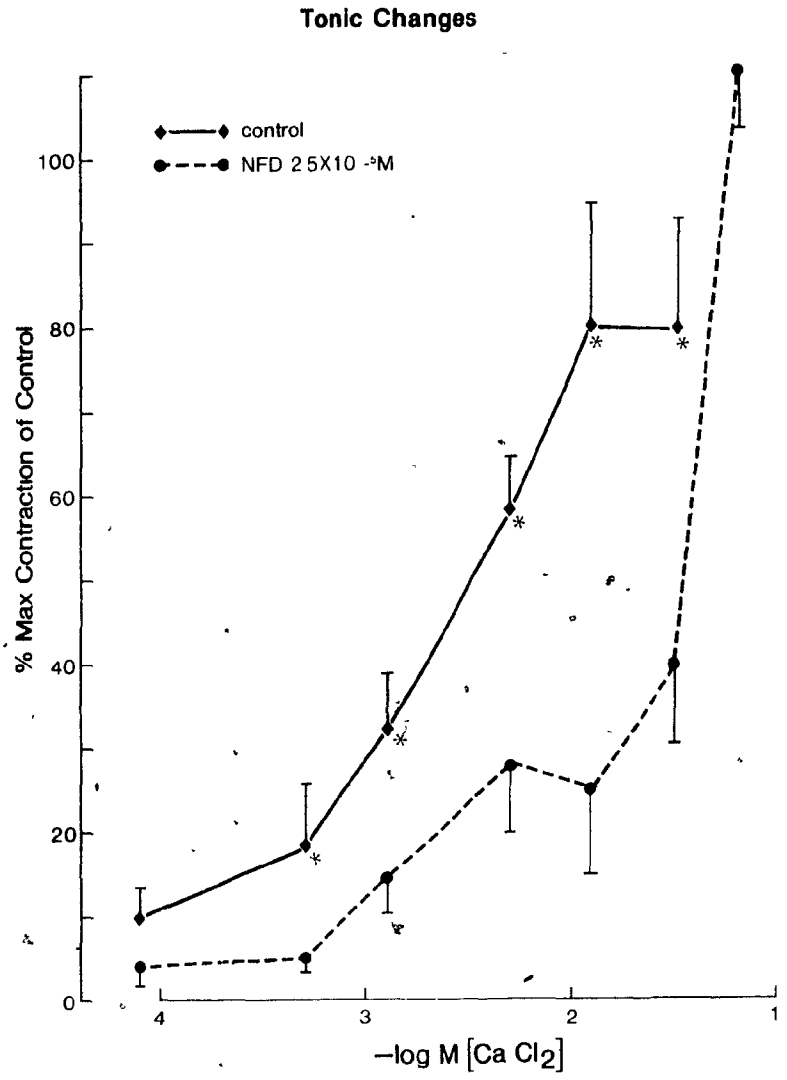

Figure 31b. Effect of Nifedipine (NFD) on tonic contractions of the rabbit detrusor. (*) statistically significant ($P<0.05$).

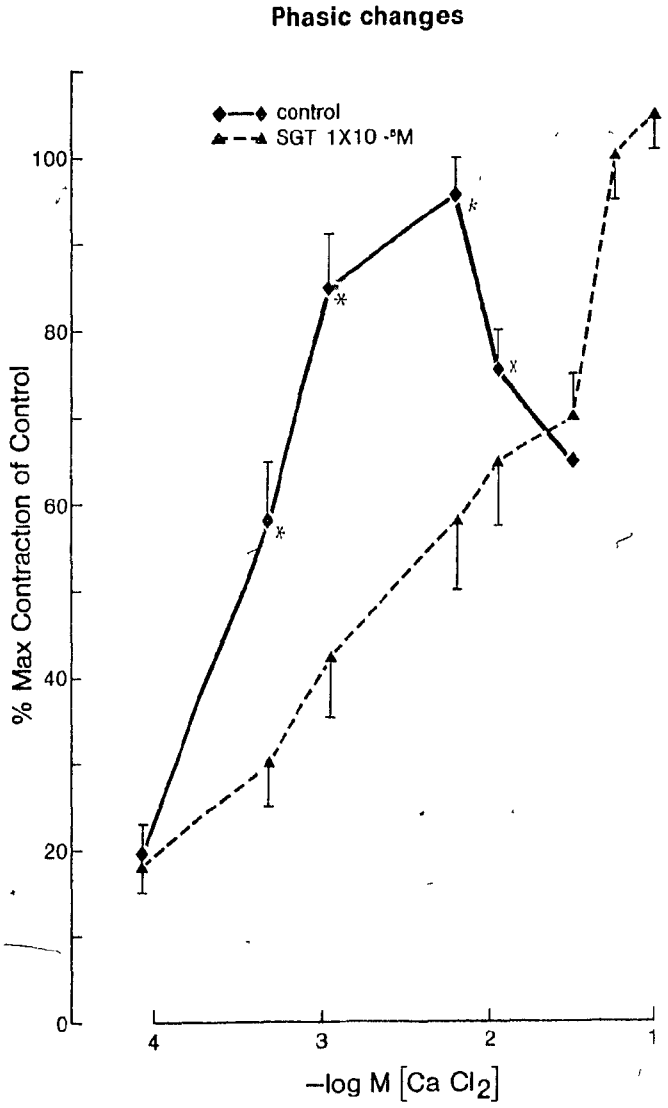

Figure 32a. Effect of Segontin (SGT) on phasic contractions of the rabbit detrusor. (*) statistically significant ($P<0.05$).

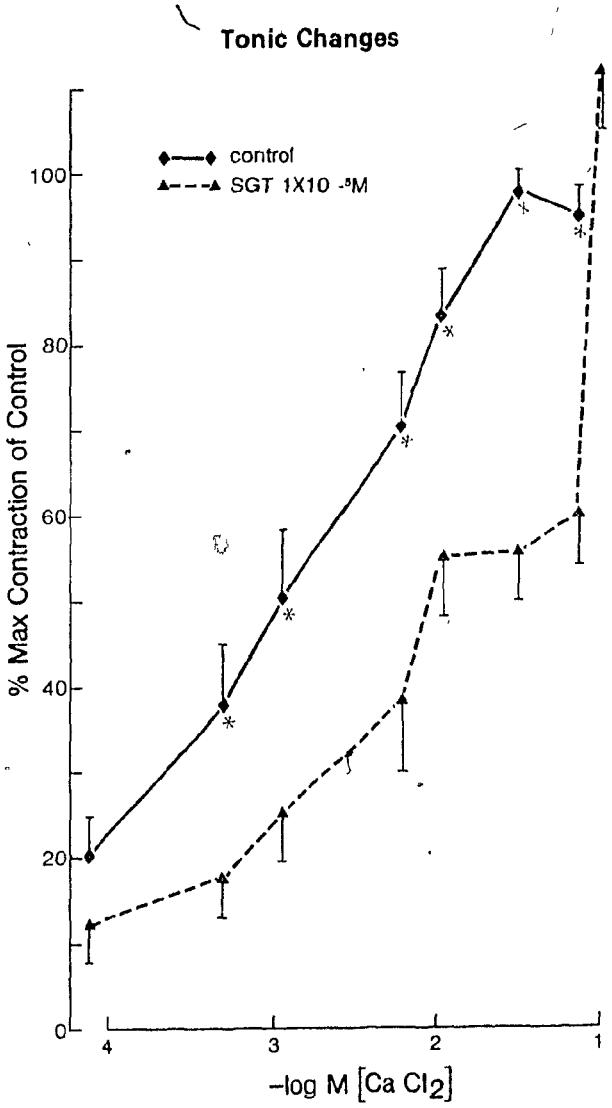

Figure 32b. Effect of Segontin (SGT) on tonic contractions of the rabbit detrusor.

than that of Nifedipine (Figure 33).

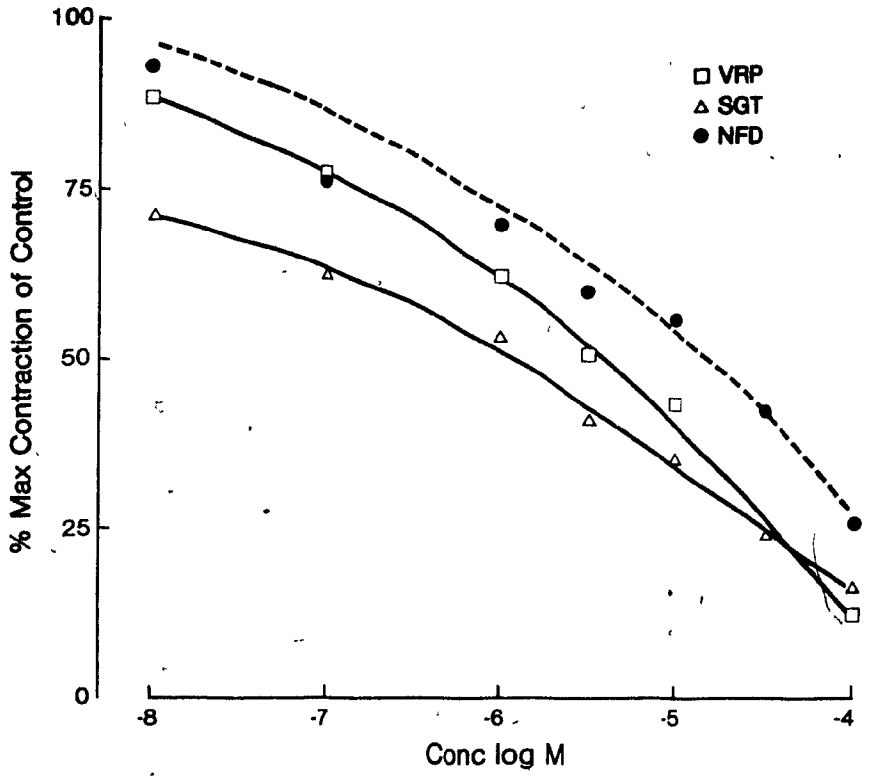

Figure 33. Conparative effect of the 3 Calcium antagonists on the rabbit detrusor contraction.

Objective 3

Bladder and urethral mechanics during spinal shock phase:

Twenty-six (26) out of 28 cats did not void during the experiment. The bladder capacity reached 40-50 ml without voiding even though it appeared to be severely distended. Urethral and detrusor muscle motility was demonstrated 30-45 minutes after the cord section (the period usually taken to set up the preparation). At a volume of 15-20 ml, the maximum intravesical pressure IVP in 11 cats varied between 15-28 mmHg with a mean of 20.1 mmHg $\pm$ 3.4 (S.E.); the mean resting IVP was 13.2 mmHg $\pm$ 0.7 (S.E.) and the mean frequency of detrusor contraction was 1.77/min $\pm$ 0.1 (S.E.). On comparing control and spinal section groups we found no significant difference between maximum IVP. However, the resting IVP and the frequency of detrusor contractions were significantly higher in the spinal section group.

A marked discoordination was found between Det, Long and Circ urethral muscle layers and sometimes the P.S.M. Vesicourethral dyssynergia was particularly evident, as well as a urethro-urethral dyssynergia between the Long and Circ layers of the urethra. This multiple dyssynergia was observed in 26 cats that did not void (Figure 34). In 2 cats, the vesico-urethral synergism returned after 6 hours and both voided spontaneously (Figure 35). In 13 cats where the intraurethral pressure (IUP) was recorded, it was found

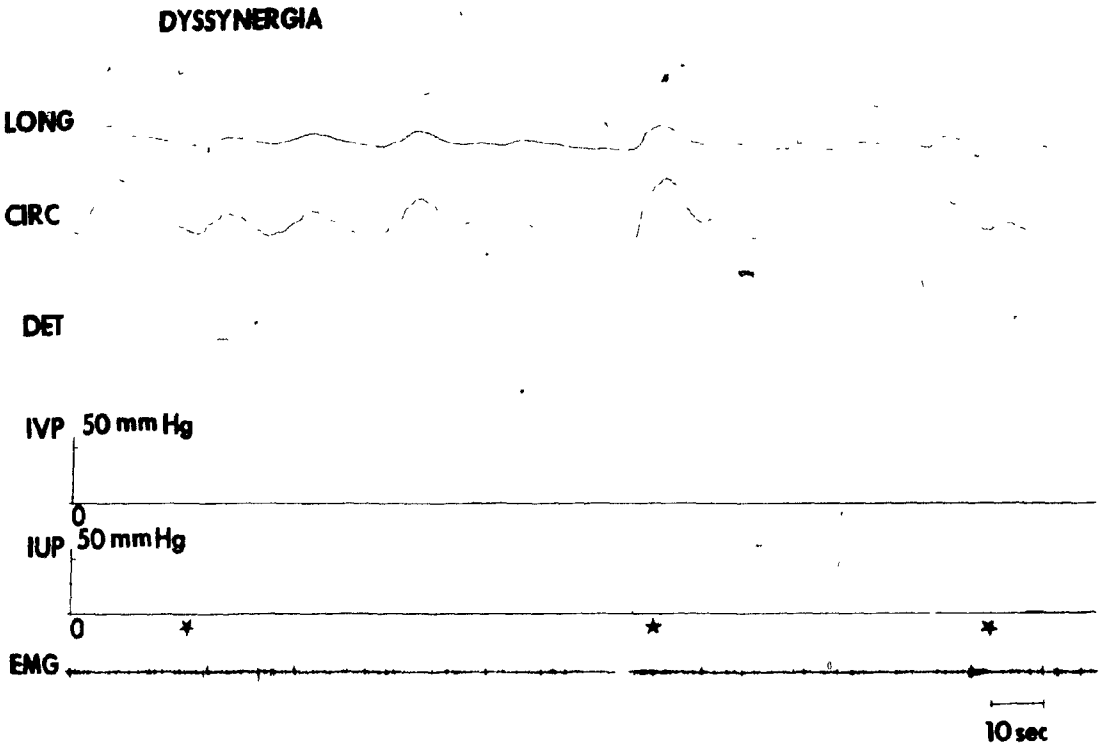

Figure 34. Recording of the lower urinary tract in the cat during spinal shock phase. Note the dyssynergia between different components of the urethral muscle and the detrusor. Note also the persistently higher intraurethral pressure (IUP) than the intravesical pressure (IVP). (*) represents the site of the tip of the urethral catheter measuring the IUP at a 1 cm difference between each 2(*).

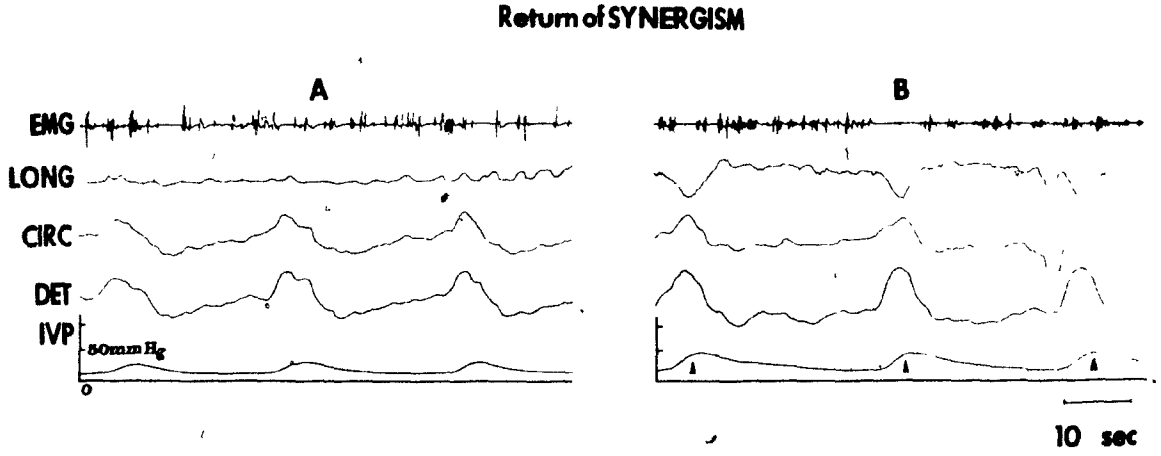

Figure 35. (A) Recording from a cat immediately after spinal lesion. Note the dyssynergia.

(B) Recording from the same cat 6 hours after showing return of synergism and voiding (arrows).

to be constantly higher than that of the bladder. The anesthesia did not seem to affect the vesico-urethral motility as the dyssynergic pattern was present in both high spinal unanesthetized and low spinal anesthetized groups.

Pharmacologic manipulations during spinal shock phase

Bethanechol chloride was injected I.V. 4-6 hours after the spinal section. In 7 out of 10 cats, there was a marked increase in the motility of the detrusor and urethral layers without voiding. The dyssynergic pattern between the bladder and urethral muscles persisted in all of the 10 cats (Figure 36).

Phentolamine mesylate (alpha-adrenergic blocker) was injected I.V. 4-6 hours after cord section. Voiding was not possible in 5 cats and the vesicourethral dyssynergia also persisted. The only cat that voided incompletely showed some relaxation in the circular muscle layer of the urethra.

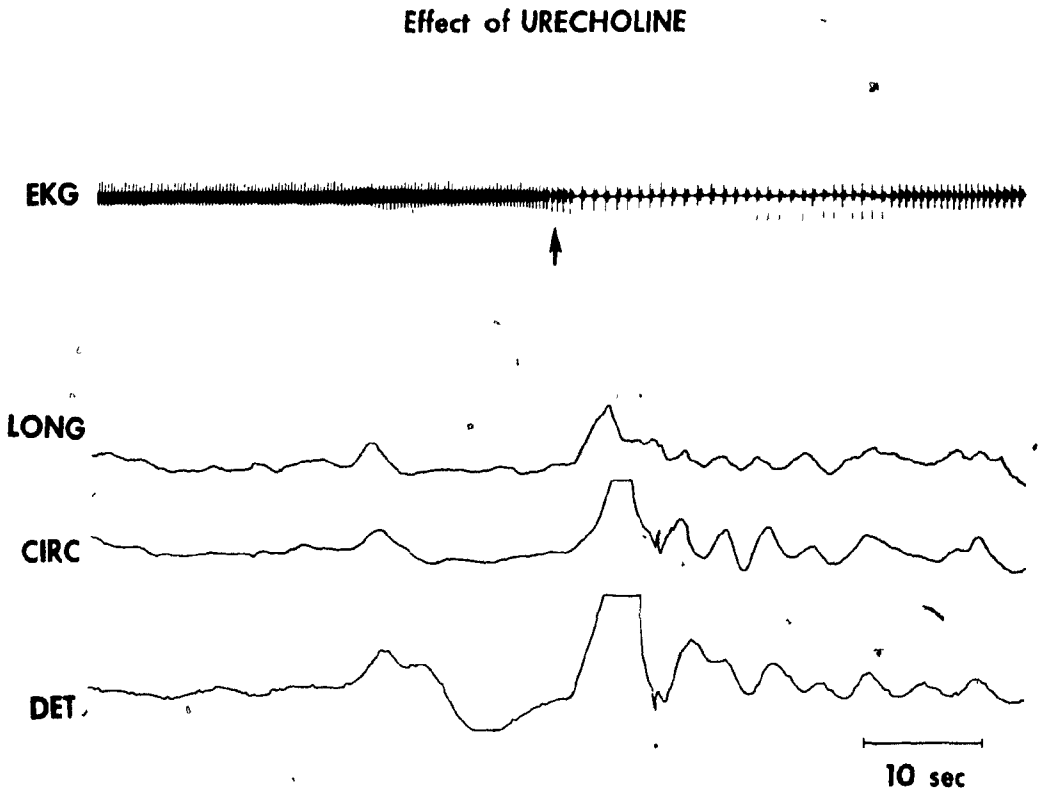

Figure 36. Effect of Urecholine injected (arrow) during spinal shock in cat. Note the marked dyssynergia between LONG and CIRC. In spite of strong DET contraction, voiding was minimal.

Objective 4

Animal model for multiple sclerosis-like disease:

The 9 cats that had been immunized with mammalian nervous tissue were followed up for 8-12 months with no evidence of neurologic abnormalities except in one cat that died after a short period of convulsions. The brain and spinal cord of this cat did not show any evidence of lesions when examined histologically.

Twenty-one (21) rabbits were immunized with the homogenate of mammalian tissue. The neurologic signs developed in 17 rabbits 10-21 days after the inoculation of the homogenate. Two rabbits died in a fulminating form of the disease. Of the diseased live rabbits, 10 showed paralysis in both hind limbs. Analgesia of 1 or both hind limbs was shown in 11, respiratory difficulty presenting with slowing and irregularity of the respiratory rate was noted in 9 and incontinence (urinary and fecal) seen in 13 rabbits. Nystagmus was noted in 2 rabbits. Mean weight loss was 400 gm. No symptoms or signs of neurologic deficit appeared in the respective controls.

The tonic immobility reflex was applied in 19 pairs of rabbits (immunized and control). It was accompanied by meiosis, normal breathing and reduced tone in 4 limbs. Arousal from the reflex was associated with the return of the pupil to normal size, sometimes with mydriasis, an increase in the respiratory rate and jerky movements. Tonic

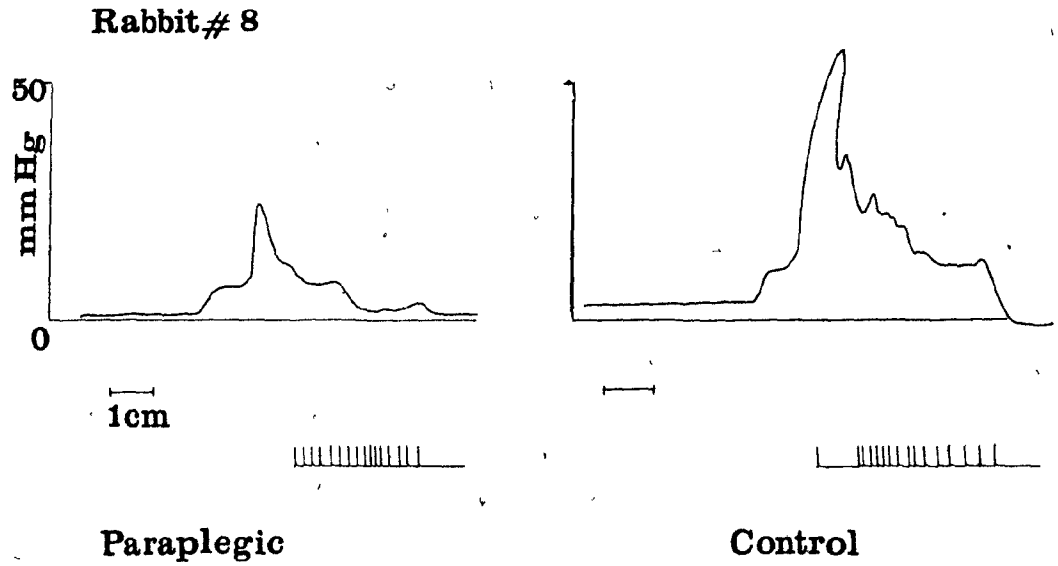

Figure 37. Recording of the urethral pressure profile (UPP) in a paraplegic rabbit. Note the lower maximum UPP as compared to the control.

immobility was maintained for periods of 1.5 to 2 hours throughout the experiment. The urodynamic studies were done successfully in 36 out of 38 rabbits under tonic immobility.

Urodynamic studies:

The maximum urethral pressure (UPP max) in the clinically diseased rabbits varied between 12 and 32 mmHg (mean 23.25 mmHg). In the normal control the UPP max varied between 48 and 55 mmHg. The UPP max in the paraplegic rabbits was significantly lower than that of their respective controls ($P<0.01$) using the Wilcoxon matched pairs test (Figure 37). The bladder volume at voiding (bladder capacity) in the clinically diseased rabbits varied between 10 and 55 ml (mean 28 ml). In the control group, the bladder capacity varied between 65 and 90 ml (mean 72 ml). The bladder capacity in the diseased rabbits was significantly lower than that of their respective controls ($P<0.01$) using the Wilcoxon matched pairs test (Figure 38).

The rabbits that showed no clinical evidence of disease on the day of the experiment (5 were in remission and 4 did not initially show any disease) had UPP max similar to their respective controls. The bladder capacity in the animals in remission was lower than that of their controls ($P=0.05$). The bladder capacity in animals with no evident disease was similar to controls.

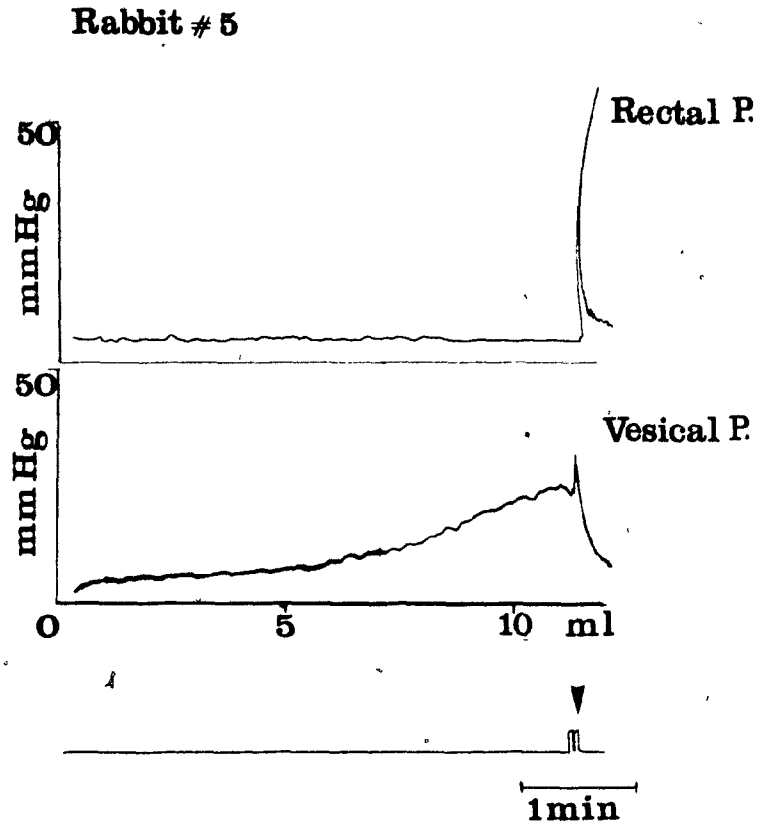

Figure 38. Recording of cystometrogram (CMG) in a paraplegic rabbit with E.A.E. Note the small bladder capacity. Voiding at arrow.

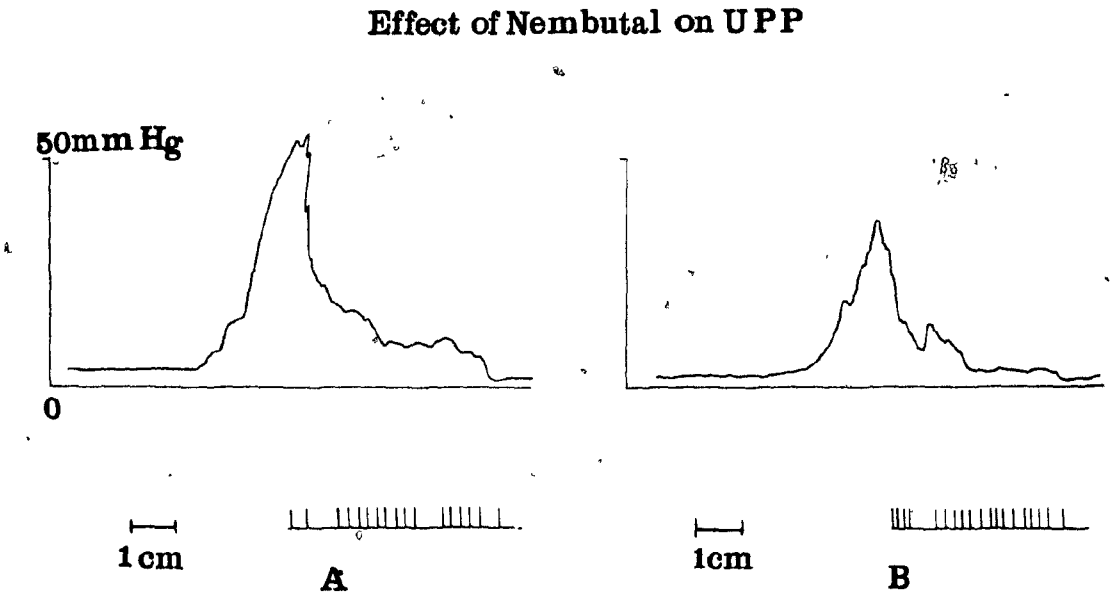

Figure 39. Recording of UPP before (A) and after administration of general anesthesia (B) in a normal control rabbit.

Effect of anesthesia on the urodynamic parameters:

In 19 normal rabbits from the control group, the UPP max was found to be significantly lower when measured under anaesthesia than that obtained using immobility reflex, ($P<0.02$) (Figure 39). The bladder capacity was found significantly higher in the anesthetized rabbits (using Mann-Whitney U test for independant samples).

Pharmacologic manipulation of the diseased rabbits:

Five diseased rabbits with evident small bladder capacity were given Verapamil 0.2 mg/Kg body weight intravenously. Repeating the cystometry 5-10 minutes later showed an increase in bladder capacity of 250-300% (Figure 40).

Histopathologic studies on the brain and spinal cord:

Microscopically, the typical lesions were characterized by perivascular cuffing of mononuclear cells with variable degree of infiltration of the surrounding parenchyma, concomitant with plaques of demyelinization of variable sizes (Figure 41). The axons were found swollen, partially or completely destroyed. The above-described lesions were found to be extensive in 8 of 10 paraplegic rabbits.

The topographic distribution of the lesions showed they are more extensive towards the lumbosacral cord segments, particularly the posterior and anterior columns. The anterior horns showed demyelinization in some cases (Figure 42).

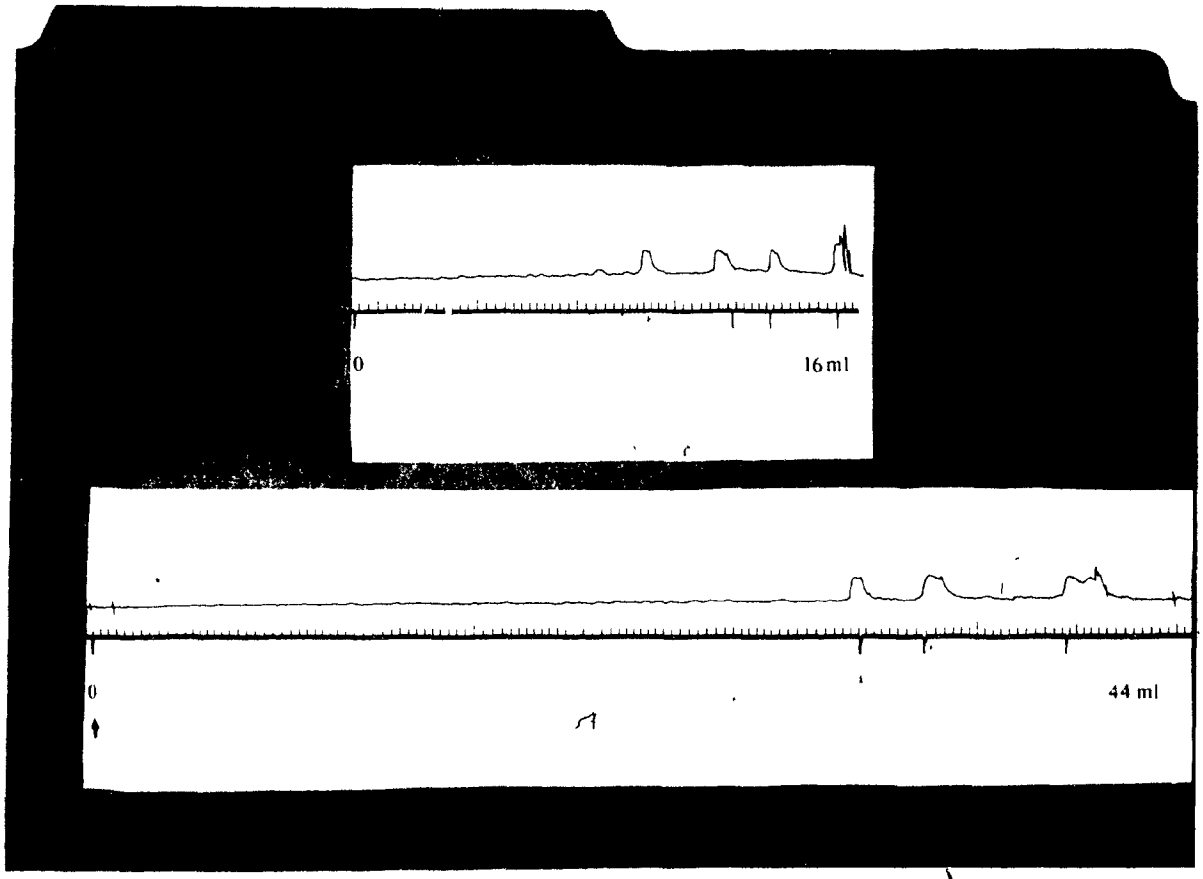

Figure 40. (A) CMG in a paraplegic rabbit. (B) After injection of Verapamil I.V. Note the augmentation in the bladder capacity.

Figure 41. Microphotograph showing anterior horn cells from lumbar level of spinal cord of rabbit. Note small perivascular cuff of chronic inflammatory cells without involvement of myelin. H&E x212

Figure 42. Microphotograph showing posterior column from lumbar level of spinal cord in a paraplegic rabbit. Note extensive focus of demyelinization. Luxol fast blue x33.

The histology of sections taken from the 5 rabbits that were in remission showed less extensive lesions. These lesions were characterized by sub-pial cellular infiltration, mainly monocytes and some lymphocytes. Very scarce demyelinization was found at the peripheries of the section. Neither cellular infiltration nor demyelinization was found in the parenchyma. Of the 4 rabbits that did not show any evidence of disease, 3 presented with very scarce lesions and one did not show any. The lesions were tiny sub-pial or perivascular cellular infiltrates (Figure 43).

Figure 43. Microphotograph showing the posterior column at the lumbar level of the spinal cord of a paraplegic rabbit. Note the perivascular chronic inflammatory infiltrate and demyelinization affecting the subpial zone. Luxol fast blue x212.

Histochemical studies of cholinergic fibres in the bladder and urethra:

We could not find a significant difference in the distribution of cholinergic fibres in the detrusor and urethral muscles between the control and the diseased rabbits.

A correlation between the neurologic lesions, clinical and the urodynamic findings are summarized in Table III. We found extensive demyelinizing lesions more localized in the lumbo-sacral segments of the spinal cord in those rabbits with paraplegia and low urethral resistance.

TABLE III

Correlation Between Urodynamic, Clinical and Pathological Findings in Immu

	Clinical Signs	Tonus H.L.	K.Jerk	Max. UPP (mm. Hg)	Bladder Cap. (ml.)	Ant.Hippo	Post.Hippo	Midbrain	Pons	M.Oblo
1.	P-plegic	↓R	N	26	45	++	++	++	+++	
2.	P-plegic	↓R & L	↑R & L	12	20	++	+++	++	++	++
3.	P-plegic	↓R & L	↓R & L	12	20	++++	+++	++++	+++	
4.	P-plegic	↓R & L	↓R & L	32	18	++	+++	+++	++++	++
5.	P-plegic	↓R & L	↓R & L	12	12	++	+++	++	+++	++
6.	P-plegic	↓R & L	↓R & L	15	25	++	++	++	+++	+++
7.	P-plegic	↓R & L	↑R & L	31	30	+++	+++	++	+++	++
8.	P-plegic	↓R & L	↓R & L	25	25	++	++	++	++	+++
9.	P-Plegic	↓L	↓R & L	15	55	++	++++	+++	++	++
10.	P-plegic	↓R & L	↓R	20	10		+	+++		++
11.	Remission	N	N	40	10			+	+	+
12.	Remission	N	↓L	51	20	+	+	+	+	+
13.	Remission	N	N	49	40					+
14.	Remission	↓R & L	↑R	42	20	+	+++	++	+	+
15.	Remission	N	N	40	10	+	+	+	+	+
16.	Normal	N	N	49	55					
17.	Normal	N	N	55	70			+		
18.	Normal	N	N	55	100			+		
19.	Normal	N	N	50	95	+		+		

Note the distribution of lesions and their extent: + minimal, ++ moderate, +++ severe, ++++ extensive. Sc
knee jerk (k.jerk) and tonus of hind limbs (h.l.) in right (R) and left (L) limbs. ↑ for augmented and ↓

1 of

TABLE III

ion Between Urodynamic, Clinical and Pathological Findings in Immunized Rabbits

ax. UPP . Hg)	Bladder Cap. (ml.)	Ant.Hippo	Post.Hippo	Midbrain	Pons	M.Oblong	Cervical	Thoracic	Lumbar	Sacral
26	45	++	++	++	+++		+++	++++	++++	++++
12	20	++	+++	++	++	++	+++	+++	++	++
12	20	++++	+++	++++	+++		++++	+++	++++	+++
32	18	++	+++	+++	++++	++	++	+++	++++	+++
12	12	++	+++	++	+++	++	++	++	++++	++++
15	25	++	++	++	+++	+++	+++	++++	++++	++++
31	30	+++	+++	++	+++	++	++++	+++	++++	++++
25	25	++	++	++	++	+++	+++	++++	++++	++++
15	55	++	++++	+++	++	++	++	+++	+++	++++
20	10		+	+++		++	++	++	+++	+++
40	10			+	+	+	+	+	+	
51	20	+	+	+	+	+	+	++		
49	40					+	+	+	+	+
42	20	+	+++	++	+	+	+		+	+
40	10	+	+	+	+	+	+	+	+	
49	55									
55	70			+					+	+
55	100			+					+	+
50	95	+		+				+		

eir extent: + minimal, ++ moderate, +++ severe, ++++ extensive. Some neurological tests were stressed: nbs (h.l.) in right (R) and left (L) limbs. ↑ for augmented and ↓ for diminished. N for normal.

CHAPTER IV

DISCUSSION

CONCLUSIONS

DISCUSSION

Lower urinary tract problems encountered in patients with lesions of the spinal cord vary according to the nature and location of the disease afflicting the nervous system. In order to individualize these problems, we followed a logic path along this study. Our primary concern was the study of the voiding continence mechanisms in an animal model (cat) with intact neuraxis and after producing lesions in the spinal cord. The pharmacologic studies on the bladder and its outlet confirmed the selective action of some drugs and neurotransmitters on the lower urinary tract. Finally, the development of an animal model for the multiple sclerosis-like disease helped us shed some light on the changes of the bladder and urethral dynamics during the evolution of the disease and presented an opportunity to evaluate its pharmacological manipulation in vivo.

The reason behind the choice of the cat as an animal model for studying the mechanics of voiding and continence is mainly because of the anatomical separation between the internal and external sphincteric mechanisms. In other mammals (dogs, rabbits) and in human the proximity of the prostate to the bladder neck makes it difficult to study the individual motility of the proximal urethra as an important entity in the internal sphincteric mechanism. Most authors negate the presence of an "anatomical" internal sphincter at the bladder neck (Hutch, 1966; Tanagho and Smith, 1966 and

Woodburne, 1968). Others emphasized the importance of the proximal urethra (Lapides, 1953 & 1958) for the sphincteric mechanism. In the adult cat, the pre-prostatic (proximal) urethra is about 2-2.5 cm long and this allows a closer study of its motility. The fact that the musculature of this urethra is distinguished into two layers, longitudinal and circular was, the focus of the experimental set-up used to clarify the mechanics of continence and voiding. During the bladder filling, continence is achieved by minimal or negligeable activity of the proximal urethral smooth muscles and the periurethral striated muscles (PSM). The mechanism of opening of the bladder outlet and voiding entails a synergism between the detrusor, the two urethral muscle layers (Long and Circ) and the periurethral striated muscles, the result of which is a free flow of urine out of the urethra. The reflexes involved in this mechanism are centrally controlled and resistant to the general anesthesia. Our brain stem lesions studies confirmed the importance of at least the locus caeruleus, the red nucleus and the adjacent reticular formation in central regulation of voiding that was described previously (Barrington, 1921).

The completion of the voiding cycle and emptying of the bladder completely necessitated the integrity of both central and local reflexes. We found that these reflexes disappear following central lesions in the upper part of the pons, under general and local anesthesia. Barrington (1921) described local urethro-sphincteric reflexes for the voiding

phase. He noted the depressing effect of anesthetic agents on them.

Bradley (1976) included the local reflex between the bladder and urethral sphincters in the 3rd loop. He proposed a central and peripheral control on the external sphincter in the 4th loop.

As the smooth muscle layers of the urethra showed individual synergistic actions during voiding, we found it necessary to study their individual response to neurotransmitters of different types. The in vitro urethral strips were taken from decapitated animals to avoid any interference of autonomic reflexes by the anesthetic agents should the study be conducted in vivo on anesthetized animal. The experimental design of splitting the urethra to study the longitudinal layer and keep the urethral ring intact to represent the circular layer, enabled us to evaluate the effects of pharmacologic agents separately on each layer in a practical manner. The closest work to ours was that of Nergaredh and Boreus (1973) in considering the urethral response of the 2 axes: longitudinal and transverse. However, in their preparation they included part of the detursor muscle in a more complicated manner. The longitudinal urethral muscle responded by contraction to the cholinergic agonist Bethanechol chloride. This corroborated the mechanical pattern which entailed a wave of contraction in the blader that propagates to the urethra to involve the longitudinal layer to initiate tthe opening of the bladder

outlet. The contractile power of the longitudinal urethral muscle is weaker than that of the detrusor under cholinergic stimulation (Khanna et al, 1981). This is acceptable since the longitudinal urethral layer is needed only to shorten the urethra and widen its lumen during opening of the bladder outlet unlike the propulsive strong contraction of the detrusor. The circular urethral muscle responded weakly or not at all under cholinergic stimulation. This finding suggests the presence of other non-cholinergic neurotransmitters involved in the relaxation of the circular urethral muscle layer during voiding. This hypothesis was confirmed by the selective relaxing effect of PGE_2 on the circular layer of the urethra.

Both the longitudinal and circular layers responded equally to adrenergic stimulation. This confirms the suggestion that the tonic closure of the urethra during continence is adrenergically mediated (Awad et al, 1974). The selective contraction of the circular layer during the isovolumetric phase may be mediated through adrenergic neurones connected to cholinergic nerves. The two muscle layers of the urethra might also function synergistically during the process of ejaculation: tight closure of the proximal urethra to prevent retrograde ejaculation by circular contraction while the urethra actively propagates the seminal bolus by longitudinal contraction.

Data about the effect of prostaglandins (PGs) on the

urethra are scarce. There is experimental evidence that PGE_2 had a relaxing effect on the urethra in the dog (Ghoneim et al, 1976) and in human (Andersson et al, 1977).

The present data demonstrated a selective relaxing effect of PGE_2 on the circular layer and contraction effect of PGF_2alpha on both longitudinal and circular urethral layers. These effects were shown to be unaltered by cholinergic antagonists (atropine), adrenergic antagonists (Phentolamine, Propanolol) and tetrodotoxin. This suggests a non-cholinergic, non-adrenergic, myotropic direct action of the PGE_2 and PGF_2alpha on the urethral muscles similar to those described for the action of PG on the detrusor (Andersson and Forman, 1978). The results from the present data showed that Ca^{++} are important in detrusor contractility both spontaneous and provoked. These data are in accordance with others (Rohner et al, 1976 and Khanna et al, 1983). We demonstrated that optimum Ca^{++} concentration in the extracellular fluid is mandatory for persistance of both spontaneous contraction (peristaltism) and provoked response (twitches). Raising Ca^{++} concentration 5 to 10 times the "physiologic" concentration might result in disturbance in the contractile protein system, though direct electron microscopic evidence is lacking. The difference in sensitivity between the phasic and the tonic components to Ca^{++} concentration is probably due to alterations in the channels of Ca^{++} influx into the cell. The difference in the tonic (T) and phasic (P) system had been discussed by

Golenhofen (1976). He showed that there are two chemically different systems for Ca^{++} activation on the membrane of some smooth muscle as aorta, portal vein and uterus. In the detrusor, we found that the Ca^{++} antagonists inhibit the P and T contractions in two ways: competitive and non-competitive. Both the P and T contractions were inhibited competively by Segontin and non-competitively by Verapamil. The latter finding was similar to those of Khanna et al (1983). Nifedipine was shown by others (Forman et al, 1978) to inhibit the chemically induced bladder contraction in human detrusor. Although we found a general inhibition on electrically-induced response of the detrusor, Nifedipine inhibited the P contraction in a non-competitive way whereas it inhibited the T contraction competitively. For the time being we do not have an explanation for this discrepancy in mode of inhibition of the T and P contractions. Perhaps a simultaneous recording of the electric membrane potential with the P and T contractions might elucidate their mechanism of action in the detrusor.

The data gathered from the work on the cat with intact spinal cord showed that voiding is a result of fine coordinated movements of the detrusor, both layers of the smooth muscles of the urethra and the periurethral striated muscles. The cause(s) of urinary retention and failure of bladder evacuation following acute spinal lesions are debatable: bladder areflexia (DeGroat and Ryall, 1969 and Jonas et al, 1975) or an increase in outlet resistance

(Edvardsen, 1967 and Awad et al, 1977). In our present work, the detrusor motility was recorded in less than an hour after the cord section. The changes in the maximum intravesical pressures (IVP) were similar to those of cats with intact spinal cord, but the resting IVP and frequency of detrusor contractions were significantly higher in the spinal section group. This suggests an incoordination in contractility of the detrusor and not areflexia. The failure of voiding in these cats cannot be explained solely by the effect of anesthesia used since both the paraplegic anesthetized and quadriplegic unanesthetized groups failed to void. The intraurethral pressure (IUP) was always higher than the IVP showing a higher urethral resistance. This resistance is persistant inspite of normal maximum IVP probably due to an incoordination between the bladder and urethra. In 2 cats, the return of vesico-urethral coordination (synergism) 6 hours after the spinal section was associated with reflex voiding. The cholinergic agonist Bethanechol chloride, and alpha-adrenergic blockers Phenoxybenzamine and Phentolamine, have been used either solely or in combination to help bladder emptying during the spinal shock stage. Failure of satisfactory bladder emptying was reported clinically (Yalla et al, 1976 and McGuire et al, 1976) and experimentally on dogs (Tullock and Rossier, 1977; Twiddy et al, 1980). The present data showed that these drugs were inefficient to induce voiding because they did not correct the vesicourethral dyssynergia.

We were unable to induce experimental allergic

encephalomyelitis in the cat. We chose the rabbit in our study because of the possibility to induce EAE and of the size of the animal that makes the urodynamic evaluation similar, in a way, to that used in humans. The rabbits were studied in pairs (immunized and controls) to eliminate the artefacts resulting from repeated manipulation, infection and possible trauma if the same rabbit was used as its own control. Moreover, the least number possible of manipulations of the bladder and urethra was carried out in each pair. Each member of the pair was studied on the same day to avoid any external environmental factor to alter our findings. The rabbits were sacrificed at the end of each experiment so as to get a reliable correlation between the urodynamic, clinical and histopathologic findings. In humans with multiple sclerosis, the cystometric findings were in favour of hyperreflexic bladder with small capacity in 60-70% of cases (Goldstein et al, 1982 and Sofras and Edwards, 1981). The results on the diseased rabbits showed a similar pattern of reduced bladder capacity that was significantly below that of respective controls. The histologic findings showed some damage (demyelinization) in the lateral columns of the spinal cord, site of the pathways of the reticulospinal tracts. Several authors (Miller et al, 1965 and Bradley et al, 1973) pointed to the relation of the hyperreflexic bladder with damage to the reticulospinal tract.

We could not find significant difference in the

cholinergic fibres density between the detrusor of diseased bladders and that of the controls. Assuming the staining technique is sensitive enough, the bladder dysfunction is probably due to impaired central neural control. Further electron microscopic studies on this issue might bring clarification on the intrinsic innervation of the detrusor in M.S.

Reducing the bladder irritability by Ca^{++} blockers showed significant increase in the bladder capacity of the diseased rabbits. This might open the door for the use of Ca^{++} blockers for diseases other than cardiovascular. Preliminary clinical applications had shown that Nifedipine can inhibit bladder contractility in patients with urgency and urge incontinence (Forman et al, 1978).

Contrary to the majority of patients with multiple sclerosis the maximum urethral pressure profile (UPP max) in the diseased rabbits was lower than that of the controls. The sites of lesions were confined to the lumbosacral segments that involved the anterior horn cells and lateral columns (pyramidal tracts) in these rabbits. In humans with multiple sclerosis, the sphincteric incompetence was attributed to damage to the pyramidal tract and pudendal nucleus (Bradley et al, 1973). In normal rabbits and those during remissions, the urodynamic data were similar to their controls in spite of the presence of minimal lesion in the nervous system. These lesions were probably insufficient to cause detectable clinical or urodynamic changes.

Anesthesia is frequently necessary for most of the experimental procedures. The depressing effect of anesthesia on the spinal reflexes is unpredictable and cannot be neglected (Barrington, 1921 and Tullock and Rossier, 1977). We conducted our experiments successfully under the tonic immobility reflex and shown the depressing effect of general anesthesia in changing the urodynamic parameters. In clinical situations the majority of urodynamic studies are done without anesthesia. Using some precautions, the tonic immobility reflex in the rabbit was shown to be reliable. Whether this reflex affects per se the voiding reflexes is unlikely since there is experimental proof that the rabbit is awake (Klemm, 1966).

We suggest that the rabbit is a good animal model to study the vesicourethral changes and pharmacological manipulation during the different phases of multiple sclerosis-like disease (EAE) without the effect of anesthesia.

CONCLUSIONS:

Several conclusions could be drawn from the present work that might shed some light on the neurogenic lower tract dysfunction.

1. The key for proper opening of bladder outlet is a synergism between different components of the lower urinary tract.

2. The lack of this synergism is responsible in great deal for the urinary dysfunction after spinal cord lesion. The conventional pharmacological manipulation fails to restore the normal synergism and subsequent adequate bladder emptying.

3. The highly selective pharmacologic manipulation on the individual layers of the urethra might be the solution in restoring normal voiding in lower urinary tract dysfunction.

4. The Ca^{++} blockers might have a role, at least partly, in correction of bladder instability.

5. The rabbit is a fair animal model for M.S. and could be exploited further to improve our understanding of the bladder dysfunction associated with M.S.

CHAPTER V

REFERENCES

REFERENCES

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ADDENDUM

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