EFFECTS OF REPRODUCTIVE HORMONES, STRAIN, AGE AND HYPOTHERMIA ON CONTRACTILE RESPONSES OF ISOLATED RAT URINARY BLADDER TO CARBACHOL

By

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EFFECTS OF REPRODUCTIVE HORMONES, STRAIN, AGE AND HYPOTHERMIA ON CONTRACTILE RESPONSES OF ISOLATED RAT URINARY BLADDER TO CARBACHOL

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PREFACE

Modulatory effects of reproductive hormones, strain, age and hypothermia on the contractile responses of isolated rat urinary bladder strips to carbachol were studied.

I wish to express my sincere gratitude to Dr. Subbiah Sangiah, my major professor, without whose continuous support and encouragement this work would have not been possible. I have benefited from his insight and knowledge greatly. I am also indebted to him for his trust, guidance and friendship.

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My parents deserve my deepest appreciation for their continuous moral and financial support, especially my mother for being the source of my inspirations.

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LIST OF SYMBOLS

mm	millimeter
cm	centimeter
mg	milligram
g	gram
kg	kilogram
ml	milliliter
mM	millimolar
M	molar
s.c.	subcutaneous
NaCl	sodium chloride
$CaCl_2$	calcium chloride
KCl	potassium chloride
$NaH_2PO_4.H_2O$	sodium phosphate monobasic
$NaHCo_3$	sodium bicarbonate
EC50	effective concentration
S.E.	standard error
Ach	acetylcholine
NE	norepinephrine
α	alpha-adrenergic receptor
β	beta-adrenergic receptor
i.p.	intraperitoneal
min.	minutes

CHAPTER I

INTRODUCTION

Biological responses of human or animlas to various drugs either *in vivo* or in isolated tissues from different organs are greately influenced by many factors. Age, sex, body weight, species and strain variations, physiological conditions and environment are among factors which are most important in experimental pharmacological studies and in therapeutic uses.

There is accumulating evidence indicating that reproductive hormones such as estrogen and progesterone can influence the function of organs other than reproductive organs. Modulating effects of reproductive hormones was first noticed when Salmon et al. (1941) reported that a number of women with disorders of micturition, including urinary frequency, urgency and incontinence, experienced relief from these symptoms while being treated with estrogen for the usual menopausal complaints. Since then, estrogens have been widely used for treatment of stress urinary incontinence in post-menopausal women (Salmon et al., 1941) and Eckerling et al., 1972) and "hypoestrogenic urinary incontinence" in dogs (Osborne et al., 1980). It has recently been shown that urinary incontinence following castration of male

dogs responds to testosterone treatment (Barsanti et al., 1981). Urinary incontinence in the aged pony also responds to estrogen therapy (Madison, 1984). Although the etiology and pathophysiology of hypoestrogenic urinary incontinence have not yet been determined, administration of relatively low doses of estrogen to affected dogs is frequently associated with remission of clinical symptoms. However, recurrence of incontinence associated with the withdrawal of estrogen therapy has been interpreted by some to indicate that the disorder is caused by hypoestrogenism.

Recent experimental studies indicate that reproductive hormones can affect both electrical and mechanical properties of various smooth muscles including those of the urinary bladder. Findings of Schreiter et al. (1976) indicate that contractile responses of urethral smooth muscle mediated by α-adrenoceptors are increased after estrogen treatment. Pretreatment of the guinea-pig with estrogen has been shown to increase the contractlie responses of gallbladder smooth muscle to acetylcholine whereas progesterone pretreatment has been shown to have the opposite effect (Ryan and Pellecchia, 1982). A significant reduction in the contractlie activity of gastrointestinal smooth muscle of the rat after pretreatment with progesterone was also observed (Bruce and Behsudi, 1979 and 1980). Levin et al. (1980) demonstrated that estrogen induces a marked increase in the response of the rabbit bladder body and mid-bladder to α-adrenergic, muscarinic cholinergic and purinergic agonists.

Creed (1983) concluded that pretreatment with stilbestrol increases the sensitivity of urethral smooth muscle of the dog to phenylephrine whereas progesterone pretreatment has no effect. In female rabbit urethra, estrogen pretreatment increases the contractile responses to norepinephrine (Larson et al., 1984). Estrogen also increases the sensitivity of the rabbit urinary bladder and urethra to phenylephrine (Hodgson, 1978). In their study Raz et al. (1973) reported that progesterone enhances β-adrenergic responses in the urethra of the dog. Bruce and Behsudi (1979 and 1980) have shown that progesterone pretreatment in the rat reduces the contractile activities of gastrointestinal tissues such as the esophagus, antrum and colon.

Effects of reproductive hormones on tissues besides the smooth muscle have also been investigated. It has been shown that secretion of β-endorphins in the women treated with estrogen and progestin is increased (Shoupe et al., 1985). Estrogen and progesterone also increase the number of the GABA receptors of the central nervous system (Maggi et al., 1984). Joyce et al. (1984) have reported that dopamine-mediated behaviours such as contralateral postural deviation and rotation are suppressed by estradiol benzoate.

Differences in the strain of an animal species can have a profound effect on its growth rate, size, physiology and biochemistry. Boyd et al. (1982) have shown that Fischer-344 rats have a smaller body size and a slower growth rate than those of Sprague-Dawley rats. The pulmonary function of

these two different strains is also reported to be different. Body weight and hematological traits of inbred rats of different strains have been shown to be different (Hackbarth et al., 1983). In their study of strain differences in kidney function of inbred rats, Hackbarth et al. (1981) reported that body weight, kidney weight and glomerular filteration rate were significantly different between strains.

Although the basic physiology remains the same throughout the life-span of an individual animal or human being, aging has pronounced effects on various parts of the body. Many of these effects are manifested and recognized during the normal process of aging. However, the molecular basis of numerous physiological changes that occur with age are not well understood (Masoro, 1980). Biochemical changes ocurring with aging has been studied extensively in various tissues. Some of these changes are the result of variation in hormonal balance with increasing age. In the submandibular gland of mice, β-receptor activity decreases with advancing age. This age-related decrease has been found to be partially corrected in thymus-grafted old animals. Hence, it is suggested that thyroid hormones are involved in aging and thymus-dependent regulation of β-receptors of submandibular glands in mice (Fattoretti et al., 1982). The natural aging process in the female urethra has also been shown to be related to physiological variations in estrogen activity (Smith, 1972). It is also known that the concentration of certain hormone receptors such as androgen receptors of

prostate are reduced during aging in several animal species (Roth et al., 1976; Shain et al., 1973 and Singer et al., 1973). Aging also affects other tissues. In rat urinary bladder, aging is associated with an increase in the response to cholinergic muscarinic stimulation mediated by an increase in the number of the receptors. There is, however, no change in α-adrenergic stimulation with increasing age (Kolta et al. 1984). Contractile responses of tracheal smooth muscle from rabbit to acetylcholine, histamine and KCl (Hayashi et al., 1980) and guinea-pig to carbachol (Duncan et al., 1985) deteriorate with age. Activity of β-adrenergic receptors of aortic strips from rabbit, rat, cat and guinea-pig (Fleisch, 1970; Fleisch et al., 1981 and Tuttle, 1966), mononuclear cells of man (Schocken and Roth, 1977) and submandibular glands of mice (Piantanelli et al., 1980) decrease with increasing age. In venous smooth muscle of the rat, in contrast to arteries, β-adrenergic relaxation is well maintained throughout senesence (Duckles, 1985). Docherty and O'Malley (1985) have shown that peripheral α-adrenoceptor responsiveness has generally been found to be either unchanged (human blood vessels) or reduced (rat vas deferens, aorta and ventricle) with increasing age. However, responsiveness of α-adrenoceptors of the eye in man apparently increases with age. It has also been suggested that there is a reduced responsiveness of α-adrenoceptors of the vas deferens in old rats (Hyland and Docherty, 1985). Binding of ligand to the adrenergic and dopaminergic receptors in the cortex and

striatum of senescent rat brain decreases substantially with age (Misra et al., 1980). Severson et al. (1980) also showed a progressive age-related decrease in dopaminergic binding sites in the striatal membrane of mice brain. These reports provide substantial evidence to support that aging exerts its influence at the level of the receptor. There is also an apparent interaction between hormonal balance and aging.

Studies dealing with the effects of temperature on pharmacological responses of various isolated tissue prepe-rations are very limited. Wöppel and Trendelenburg (1973) have shown that reduction of temperature from 37°C to 25°C produced supersensitivity to catecholamines in isolated guinea-pig atria. A similar effect has also been demonstrated in isolated mouse atria (Monuz-Ramirez et al., 1973). Broadley et al. (1977 and 1985) have reported that cooling of isolated cardiac tissue preperation of guinea-pig induces supersensitivity to isoprenaline and carbachol. It appears that the hypothermia-induced supersensitivity is not present in all the tissues of the body. Studies of Chess-Williams and Broadley (1985) indicated that while tissues from the atria, trachea and small intestine of guinea-pig show supersensitivity to catecholamines at 30°C, uterine, lung and vas deferens tissues are not affected.

The purpose of this study was to investigate the effects of reproductive hormones, strain, age and hypothermia on contractile responses of isolated rat urinary bladder to carbachol, a parasympathomimetic drug.

CHAPTER II

URINARY BLADDER

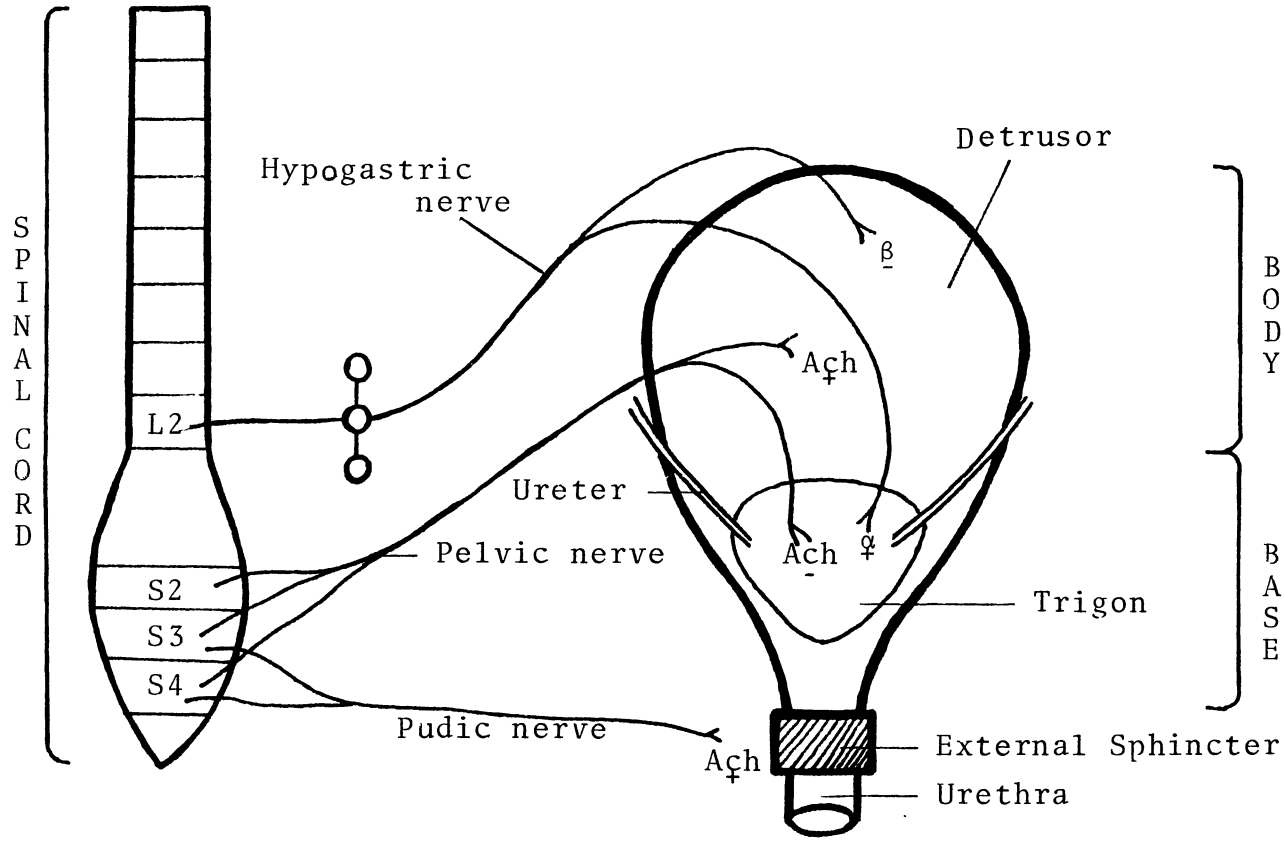
Anatomy

The urinary bladder is a collapsable bag of smooth muscle located behind the pubic symphysis and the peritoneum. Detrusor muscle forms the body of the bladder and provides a source for storage and forceful contraction during voiding. The bladder base connects the body to the proximal urethra. It includes a triangular area at the internal floor of the urinary bladder, called the "trigon" . Ureters and urethra are connected to the bladder at each of the three angles of the trigon. The internal sphincter includes the distal part of the bladder base and the proximal part of urethra. It is composed of smooth muscle and is under autonomic nervous system control. The external sphincter is located around the distal part of the urethra and is composed of skeletal muscle which is under somatic nervous system control (Figure 1).

Innervations

The urinary bladder is innervated by both sympathetic and parasympathetic divisions of the autonomic nervous system. Sympathetic innervation originates from the lumbar (L2) region of spinal cord. After passing through paravertebral

Figure 1. Anatomical Divisions and Innervations of the Urinary Bladder


SPINAL CORD
L2
S2
S3
S4
Hypogastric nerve
Pelvic nerve
Pudic nerve
Detrusor
β
Ach
Ureter
Ach
α
Trigon
Ach
External Sphincter
Urethra
BODY
BASE

ganglia, it innervates the bladder through hypogastric nerve. Preganglionic sympathetic nerves are cholinergic and their neurotransmitter is acetylcholine which acts on nicotinic cholinergic receptors of ganglia. Postganglionic sympathetic nerves are adrenergic and their neurotransmitter is norepinephrine which acts on both α- and β-adrenoceptors. Its activity on α-receptors of the bladder base and β-receptors of the bladder body leads to closure of the bladder outlet and relaxation of the bladder body respectively.

Parasympathetic innervation originates from the sacral region of the spinal cord (S2, S3 and S4). After synapsing with intramural ganglia in the bladder wall through the pelvic nerve, it innervates both the body and the base of the bladder. Parasympathetic nerves are cholinergic at both pre- and postganglionic levels. Their neurotransmitter is acetylcholine which acts on both nicotinic receptors of ganglia and muscarinic receptors of the neuroeffector junction. Activity of acetylcholine on muscarinic receptors of the body and the base of the bladder leads to contraction of detrusor muscle and relaxation of the bladder base.

The external sphincter is innervated by the somatic nervous system and is under voluntary control. The pudic nerve originates from the sacral (S3 and S4) regions of the spinal cord and innervates the external sphincter. The neurotransmitter at the skeletal neuromuscular junction is acetylcholine which acts on the nicotinic receptors of the external sphincter to keep the outlet closed (Figure 1 and Table I).

TABLE I

NEURAL ACTIVITIES DURING MICTURITION

Innervations	Body	Base	Ext. Sphincter
Parasympathetic pelvic (mixed) muscarinic — Ach	Contraction (voiding)	Relaxation?	None
Sympathetic hypogastric (mixed)			
β ——— NE	Relaxation (filling)	None	None
α ——— NE	None	Contraction	None
Somatic pudic nicotinic — Ach	None	None	Contraction (tonic)

Physiology of Micturition

Micturition is the process of slow storage of urine in the urinary bldder and its discharge in a quick and complete manner upon reaching the functional capacity of the bladder. It is a complex phenomenon and it involves neural integration at the level of the spinal cord as well as higher levels of the central nervous system.

As urine accumulates in the urinary bladder, outlet resistance increases due to the activity of α-adrenoceptors. A rise in the urine volume causes the tension in the bladder wall to increase which in turn leads to an increase in intravesicular pressure. Intravesicular pressure continues to increase until a threshold is reached. This phase of the collection of urine is called "storage phase" and it is dominated by the excitatory activity of α-adrenoceptors of the bladder base and inhibitory activity of β-adrenoceptors of the bladder body. During this phase, parasympathetic activity is inhibited. Once the threshold is reached, impulses from stretch receptors of the bladder wall trigger spinal reflexes which lead to activity at muscarinic receptors of the body and consequent contraction of the detrusor muscle. At the same time, muscarinic receptor activity at the bladder base is inhibited and the muscles relax and allow for the complete discharge of urine. This phase is called the "voiding phase" during which sympathetic activity is reduced (Table I).

Although much of the neural control of the urinary

bladder occur at the level of the spinal cord through various reflexes, the higher centers of nervous system have a great influence on the integration of responses at lower levels. Micturition urgency can be subjugated by voluntary control of the external sphincter through the central nervous system activity.

CHAPTER III

MATERIALS AND METHODS

Animals

Male, three to six month old Sprague-Dawley rats (321-580g) were purchased from local sources and were used in hormone experiments. Male, 3 month (237-265g) and 12 month (387-456g) old Fischer-344 rats from Hilltop Laboratories were used in the rest of the experiments. The animals were housed in specially designed living quarters with temperature, humidity and air circulation maintained at optimal level. They were allowed free access to food and water and were kept on a 12-hour light-dark cycle.

Drugs and Chemicals

Carbachol (carbamyl choline chloride), 17 β-estradiol and progesterone were purchased from Sigma Chemical Co., St. Louis, Mo. Reagent grade potassium chloride, sodium chloride, calcium chloride, sodium bicarbonate, sodium phosphate monobasic, dextrose and sucrose purchased from Fisher Scientific Company were used in making modified McEwen physiological solution. Carbachol and modified McEwen physiological solutions were prepared fresh in distilled water every day prior to experiments.

Experimental Design

The animals were divided into various groups depending on the objective of each experiment. Group I (n=15) served as control. Rats in this group received injections of peanut oil (0.3-0.5ml) for 5 to 9 days. Animals in group II (n=10) received daily injections of 17 β-estradiol (0.4mg/kg, s.c.) in peanut oil for 9 days. Group III animal (n=6) received daily injections of progesterone (2mg/kg, s.c.) in peanut oil for 5 days. Three month old Sprague-Dawley rats (n=5) and three month old Fischer-344 rats (n=6) were designated as group IV and group V respectively and were used in strain experiments. Group VI (n=6), 12 month old Fischer-344 rats, and group V rats were used in age experiments. Animals in groups IV, V and VI were also used to study the effects of hypothermia.

Physiological Solution

Modified McEwen physiological solution was used in all experiments. Solutions were prepared fresh daily in distilled water. The millimolar composition is as follows: NaCl, 130.0; KCl, 5.6; $CaCl_2$, 2.16; $NaHCO_3$, 25.0; $NaH_2PO_4.H_2O$, 1.0; dextrose, 11.1 and sucrose, 13.15.

Tissue Preparation

Animals were lightly anesthetized (sodium pentobarbital, 20.0mg/kg, i.p.). The urinary bladder from each animal was removed and was placed in a petri dish of warm (37°C) modi-

fied McEwen solution aerated with 95% O_2 and 5% CO_2. A 6mm x 2mm strip of tissue from the mid-dorsal region of the body was removed. The strip was placed in an isolated tissue bath immediately. It was suspended with a 1g tension for 30 minutes prior to attachment to a transducer. The tissue was washed every 5 minutes during this period.

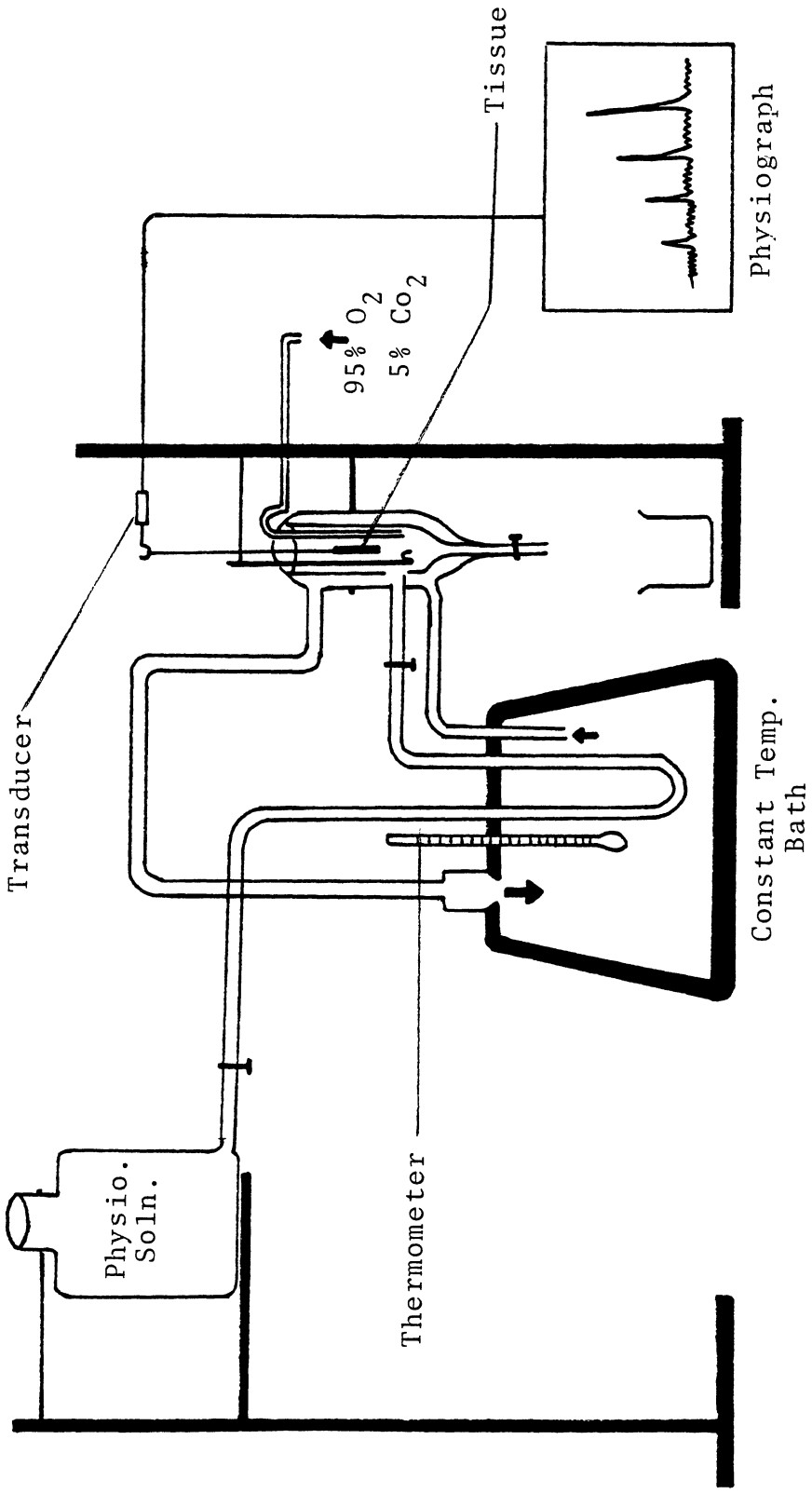
Isolated Tissue Bath

A constant temperature circulator-bath (HAAKE FE2, HAAKE Inc., Saddle Brook, NJ) was used to keep the temperature of the bath constant at 37°C and 25°C. The volume of the bath was kept constant at 20ml throughout the experiments. The physiological solution in the bath was continuously aerated with a mixture of 95% O_2 and 5% CO_2 gases (Figure 2). The pH of the solution measured periodically at either temperature was found to be 7.4 to 7.6.

Determination of Concentration-Response at 37°C

Following 30 minutes suspension with 1g tension, the individual urinary bladder strips were connected to a physiograph (Model MK-IV-P, Narco Bio-Systems, Houston, TX) by a force displacement transducer. The physiograph was calibrated (1g tension=5 or 10mm in height of contraction) before the begining of each experiment. A resting tension of 1g was maintained throughout the experiments. Contractile responses of urinary bladder strips of individual rats within groups I, II and III to various non-cumulative concentrations of carba-

Figure 2. Illustration of the Experimental Set-up


Tissue
Physiograph
95% O_2
5% Co_2
Transducer
Constant Temp.
Bath
Thermometer
Physio.
Soln.

chol ($0.5x10^{-6}$ M to $4.0x10^{-6}$ M) were recorded. Each concentration of carbachol was added to the bath in a constant volume of 0.1ml. When the contractile response reached the maximum at a given concentration of carbachol, the tissue was washed with three volumes (20ml) of physiological solution and was allowed to return to the baseline resting tension prior to addition of the next higher concentration of the drug.

A cumulative concentration (1/2 log concentration from $1.0x10^{-7}$ M to $1.0x10^{-4}$ M) of carbachol was used to record the contractile responses of urinary bladder strips from individual rats in groups IV, V and VI.

Determination of Concentration-Response at 25°C

The temperature was lowered gradually. Following 30 minutes at 37°C, temperature was reduced by 2°C every 10 minutes after a wash. Once the temperature of the bath reached 25°C, 30 minutes was allowed for equilibration. The procedure, as described previously, was used in determining the concentration-response of urinary bladder strips from groups IV, V and VI.

Measurement of Urinary Bladder Weight

Each urinary bladder was dissected free of fat and serosa. A transverse cut was made above the level of ureteral insertion. Then the bladder was blot-dried with gauze and its weight was measured.

Determination of EC50 values

Effective concentration (EC50) values were determined graphically. The percent maximal contractile response of each urinary bladder strip to carbachol was calculated and was plotted as the function of the concentration of carbachol. The EC50 value for each strip was read off the percent maximal contraction curves. The mean ± S.E. EC50 values for each group was then calculated.

Statistical Analysis

The mean ± S.E. of contractlie responses (g tension) of each group at each concentration were compared by method of either paired or unpaired Student t-test where applicable.

CHAPTER IV

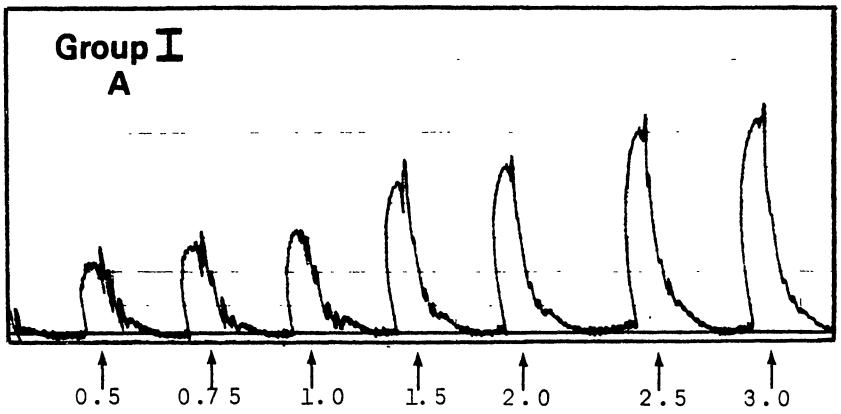
RESULTS

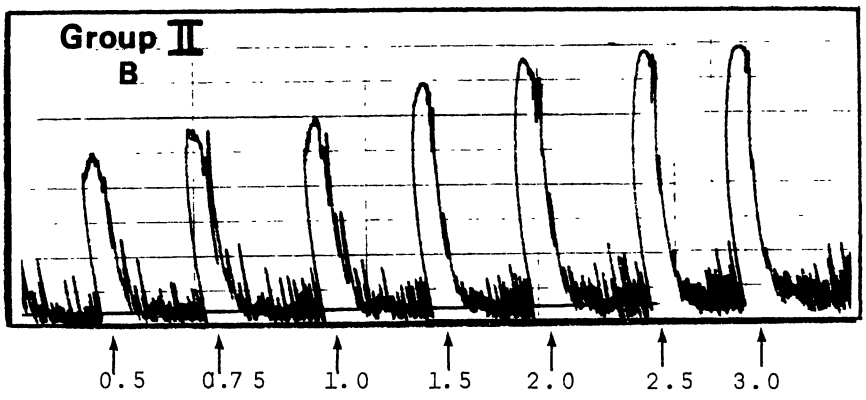
Non-cumulative and cumulative concentrations of carbachol consistently produced a concentration-dependent increase in the contractile responses of urinary bladder strips from all the rats tested (Figure 4, 6, 8, 11, 13, and 15). In groups I, II, and III, the maximal contractile response was developed in response to 4.0×10^{-6} M carbachol (Table II). In groups IV, V and VI, the maximal contractile response was developed in response to 3.0×10^{-5} M carbachol at 37°C (Table IV and VI) and 3.0×10^{-6} M carbachol at 25°C (Table VII, VIII and IX).

Effects of 17 β-Estradiol and Progesterone

Pretreatment with 17 β-estradiol potentiated the contractile responses of urinary bladder strips to each non-cumulative concentrations of carbachol (Figure 3B) as compared to those of the control group (Figure 3A). The increase in response was significant ($p<0.05$) at concentrations of 0.5×10^{-6} M to 2.5×10^{-6} M carbachol (Table II). Concentration-response curves of strips from the 17 β-estradiol pretreated group showed a shift to the left from that of the control group, indicating a potentiation of response to car-

Figure 3. Recordings of the Contractile Responses of the Isolated Rat Urinary Bladder Strips to Non-cumulative Concentrations of Carbachol. (A). Control Group (peanut oil, s.c., 5 to 9 days), (B). 17 β-Eestradiol (0.4mg/kg, s.c., 9 days) Pretreated Group and (C). Progesterone (2mg/kg, s.c., 5 days) Pretreated Group.


Group I
A
0.5
0.75
1.0
1.5
2.0
2.5
3.0
Concentrations (x10-6 M)


Group II
B
0.5
0.75
1.0
1.5
2.0
2.5
3.0
Concentrations (x10-6 M)

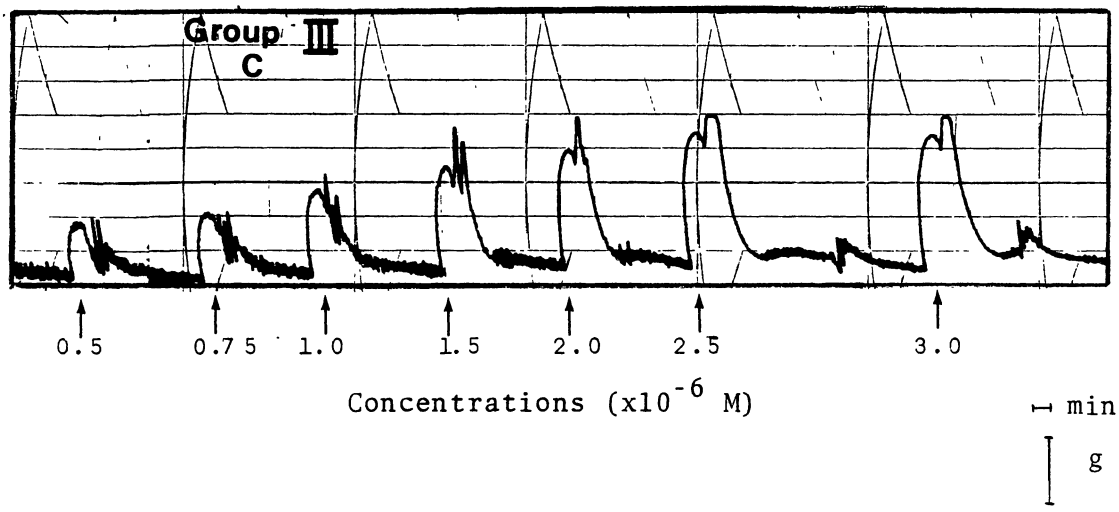

Group III
C
0.5
0.75
1.0
1.5
2.0
2.5
3.0
Concentrations (x10-6 M)
min.
g

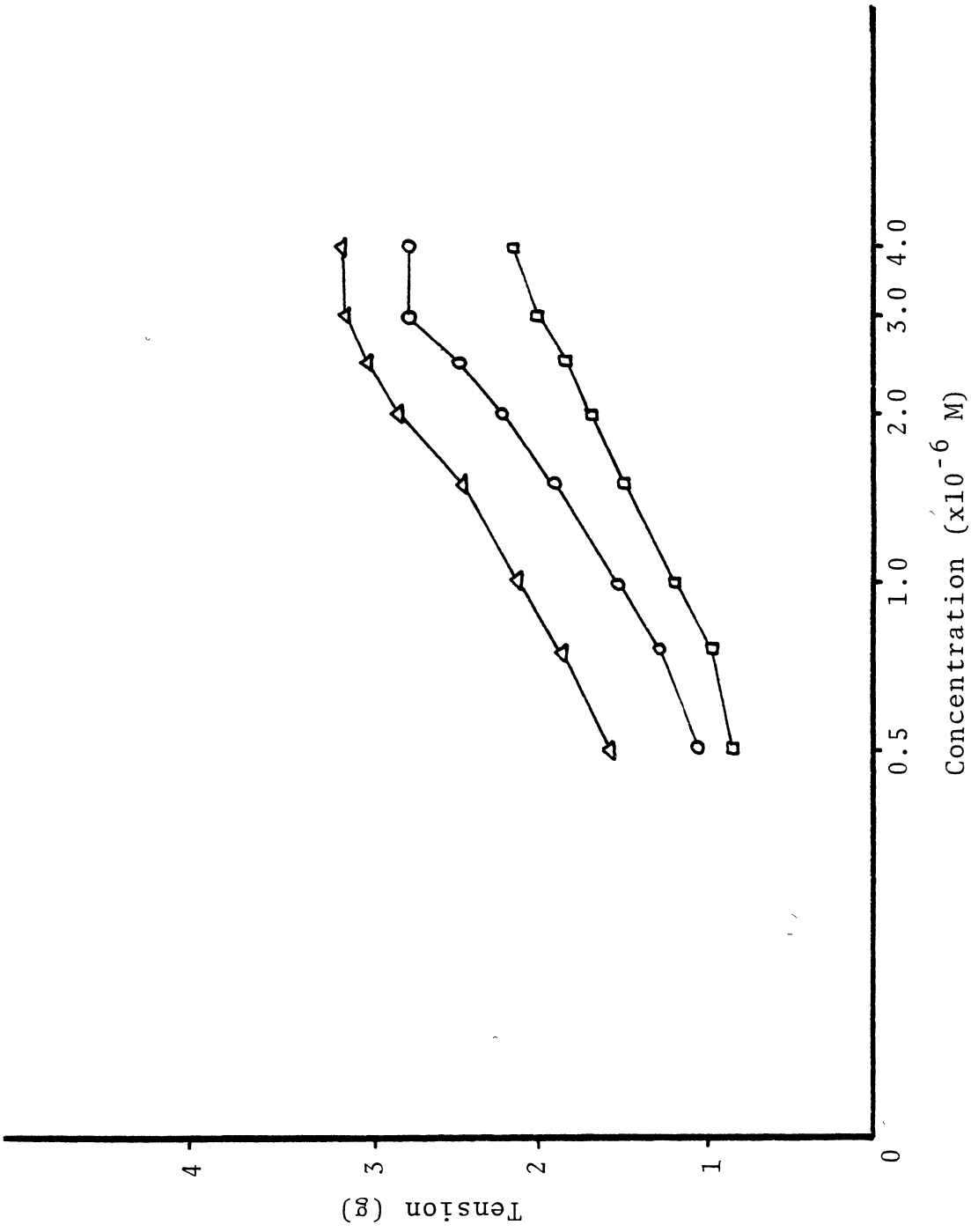
TABLE II

MEAN ± S.E. OF THE CONTRACTILE RESPONSES OF CONTROL 17 β-ESTRADIOL PRETREATED AND PROGESTERONE PRETREATED RAT ISOLATED URINARY BLADDER STRIPS TO NON-CUMULATIVE CONCENTRATIONS OF CARBACHOL

Concentration (M)	Tension (g)					
	Control	n	17 β-estradiol	n	Progesterone	n
0.50×10^{-6}	1.05±0.07	15	1.56±0.15*	10	0.83±0.11	6
0.75×10^{-6}	1.31±0.09	15	1.85±0.17*	10	0.95±0.13*	6
1.00×10^{-6}	1.55±0.09	15	2.11±0.16*	10	1.20±0.13*	6
1.50×10^{-6}	1.90±0.09	15	2.44±0.18*	10	1.48±0.15*	6
2.00×10^{-6}	2.22±0.10	15	2.75±0.18*	10	1.68±0.15*	6
2.50×10^{-6}	2.51±0.12	15	3.02±0.18*	10	1.85±0.16*	6
3.00×10^{-6}	2.77±0.12	15	3.13±0.11	10	2.02±0.15*	6
4.00×10^{-6}	2.76±0.18	9	3.17±0.21	6	2.23±0.11*	6

* Significantly different from control group ($p<0.05$)

Figure 4. Non-cumulative Concentration-response Curves of Isolated Rat Urinary Bladder Strips to Carbachol. (O) Control Group (peanut oil, s.c., 5 to 9 days), (△) 17 β-Estradiol (0.4mg/kg, s.c., 9 days) Pretreated Group and (□) Progesterone (2mg/kg, s.c., 5 days) Pretreated Group. Each Point Represents the Mean ± S.E. of 6 to 15 Experiments.


Tension (g)
4
3
2
1
0
0.5
1.0
2.0
3.0
4.0
Concentration (x10^{-6} M)

bachol (Figure 4). Progesterone pretreatment reduced the contractile responses of urinary bladder strips to each non-cumulative concentration of carbachol (Figure 3C) as compared to the responses of the control group (Figure 3A). These reductions in response were significant ($p<0.05$) at concentrations of $0.75x10^{-6}$ M to $4.0x10^{-6}$ M carbachol (Table II). Concentration-response curve of strips from the progesterone pretrated group showed a shift to the right indicating an attenuation of response to carbachol (Figure 4). Mean EC50 values of carbachol for 17 β-estradiol and progesterone pretrated groups were lower and higher than mean EC50 values of control group respectively (Table X). However, only the EC50 value of 17 β-estradiol pretreated group was significantly ($p<0.05$) different from that of control group.

Effects of Strain

Body weights and wet weight of urinary bladder of 3 month old Sprague-Dawley rats were significantly ($p<0.05$) higher than those of 3 month old Fischer-344 rats (Table III). Contractile responses to cumulative concentrations of carbachol were higher in Sprague-Dawley rats (Figure 5). These increased response were significant ($p<0.05$) at $1.0x10^{-6}$ M to $3.0x10^{-5}$ M carbachol (Table IV). Concentration-response curve of Sprague-Dawley rats showed a shift to the left of the curve from Fischer-344 rats, indicating an increased responsiveness of the urinary bldders from Sprague-Dawley rats to carbachol (Figure 6). Mean EC50 values of

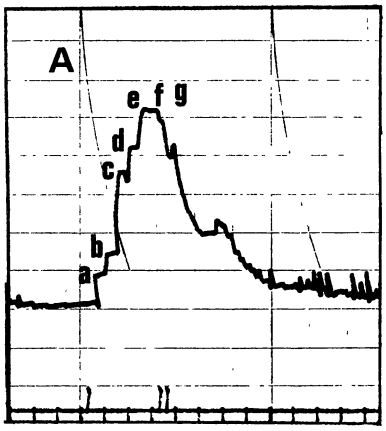
TABLE III

EFFECTS OF STRAIN ON BODY WEIGHT AND WET WEIGHT OF URINARY BLADDER IN THREE MONTH OLD RATS

Strain	Body weight (g)	Bladder weight (mg)
Sprague-Dawley	375.6±14.8*	80.30±3.73*
Fischer-344	253.8±4.50*	62.47±1.35*

* Significant difference ($p<0.05$)

Figure 5. Recordings of the Contractile Responses of Isolated Rat Urinary Bladder Strips of Different Strains to Cumulative Concentrations of Carbachol at 37°C. A. Fischer-344. B. Sprague-Dawley. ($a=1x10^{-7}$ M; $b=3x10^{-7}$ M; $c=1x10^{-6}$ M; $d=3x10^{-6}$ M; $e=1x10^{-5}$ M; $f=3x10^{-5}$ M and $g=1x10^{-4}$ M)



⟷ min.

I g

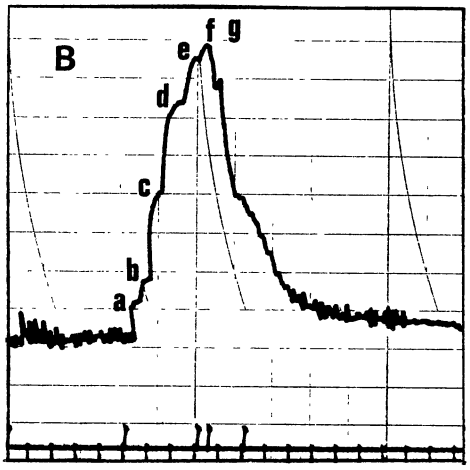


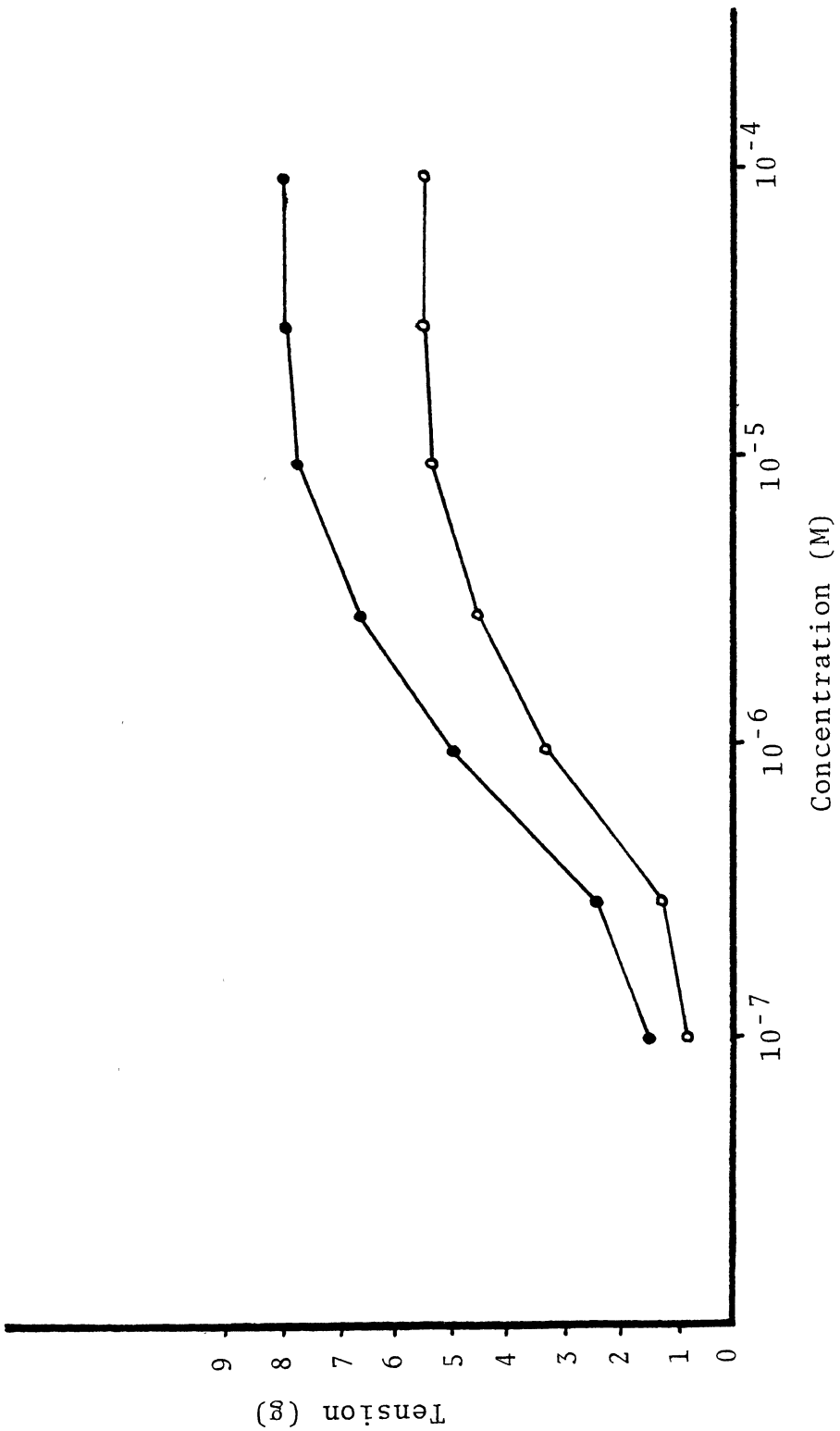
TABLE IV

EFFECTS OF STRAIN ON CONTRACTILE RESPONSES OF ISOLATED URINARY BLADDER STRIPS OF THREE MONTH OLD RATS TO CARBACHOL AT 37°C

Concentration (M)	Tension (g)	
	Sprague-Dawley (n=5)	Fischer-344 (n=6)
$1.0x10^{-7}$	1.40±0.48	0.62±0.08
$3.0x10^{-7}$	2.22±0.56	1.10±0.11
$1.0x10^{-6}$	4.74±0.73*	3.12±0.21*
$3.0x10^{-6}$	6.38±0.46*	4.33±0.18*
$1.0x10^{-5}$	7.44±0.35*	5.08±0.26*
$3.0x10^{-5}$	7.62±0.34*	5.20±0.20*
$1.0x10^{-4}$	7.62±0.34*	5.20±0.20*

* Significant difference ($p<0.05$) between the strains.

Figure 6. Cumulative Concentrations-response Curves of Isolated Rat Urinary Bladder Strips to Carbachol at 37°C. O Fischer-344. ● Sprague-Dawley. Each Point Represents the Mean ± S.E. of 5 to 6 Experiments.


Tension (g)
0
1
2
3
4
5
6
7
8
9
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration (M)

these strains were not significantly ($p<0.05$) different (Table X).

Effects of Age

Body weight and wet weight of urinary bladders of 12 month old Fischer-344 rats were significantly ($p<0.05$) higher than those of 3 month old Fischer-344 rats (Table V). Contractile responses of strips from 3 month old rats to cumulative concentrations of carbachol were lower than the responses of 12 month old rats (Figure 7). Strips from 12 month old rats showed an increased response to every concentration of carbachol (Table VI). These increases are only significant ($p<0.05$) at $1.0x10^{-6}$ M and $3.0x10^{-6}$ M carbachol. Concentration-response curve of strips from 12 month old rats showed a shift to the left of the curve from 3 month old rats, indicating an increased responsiveness of the urinary bladder to carbachol with increasing age (Figure 8). Mean EC50 values for these two groups were not significantly ($p<0.05$) different (Table X).

Effects of Temperature

While the amplitude of spontaneous contraction of urinary bladder strips increased as temperature was lowered from 37°C to 25°C, the frequency of contraction was reduced (Figure 9). Contractlie responses of urinary bladder strips from groups IV, V and VI to cumulative concentrations of carbachol were increased at 25°C. This increase was signifi-

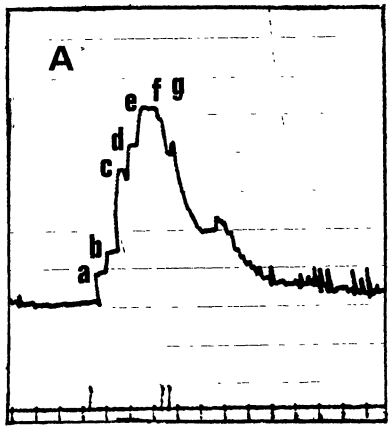
TABLE V

EFFECTS OF AGE ON BODY WEIGHT AND WET WEIGHT OF URINARY BLADDER FROM FISCHER-344 RATS

Age	Body weight (g)	Bladder weight (mg)
3 months	253.8±4.48*	62.47±1.35*
12 months	426.0±11.5*	86.67±3.14*

* Significant difference ($p<0.05$)

Figure 7. Recordings of the Contractile Responses of Isolated Urinary Bladder Strips from 3 Month (A) and 12 Month (B) Old Fischer-344 Rats to Cumulative Concentrations of Carbachol at 37°C. ($a=1x10^{-7}M$; $b=3x10^{-7}M$; $c=1x10^{-6}M$; $d=3x10^{-6}M$; $e=1x10^{-5}M$; $f=3x10^{-5}M$ and $g=1x10^{-4}M$).



↔ min.

I g

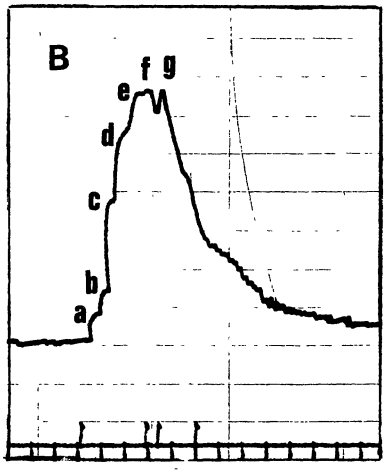


TABLE VI

EFFECTS OF AGE ON CONTRACTILE RESPONSES OF ISOLATED URINARY BLADDER STRIPS FROM FISCHER-344 RATS TO CUMULATIVE CONCENTRATIONS OF CARBACHOL AT 37°C

Concentration (M)	Age	
	3 months (n=6)	12 months (n=6)
	Tension (g)	
1.0×10^{-7}	0.62±0.08	0.87±0.12
3.0×10^{-7}	1.10±0.11	1.52±0.18
1.0×10^{-6}	3.12±0.21*	3.78±0.19*
3.0×10^{-6}	4.33±0.18*	5.02±0.24*
1.0×10^{-5}	5.08±0.26	5.53±0.29
3.0×10^{-5}	5.20±0.20	5.55±0.30
1.0×10^{-4}	5.20±0.20	5.55±0.30

* Significant difference ($p<0.05$)

Figure 8. Cumulative Concentration-response Curves of Isolated Urinary Bladder Strips from Fischer-344 Rats to Carbachol at 37°C. O 3 Month Old. ● 12 Month Old. Each Point Represents the Mean ± S.E. of 6 Experiments.

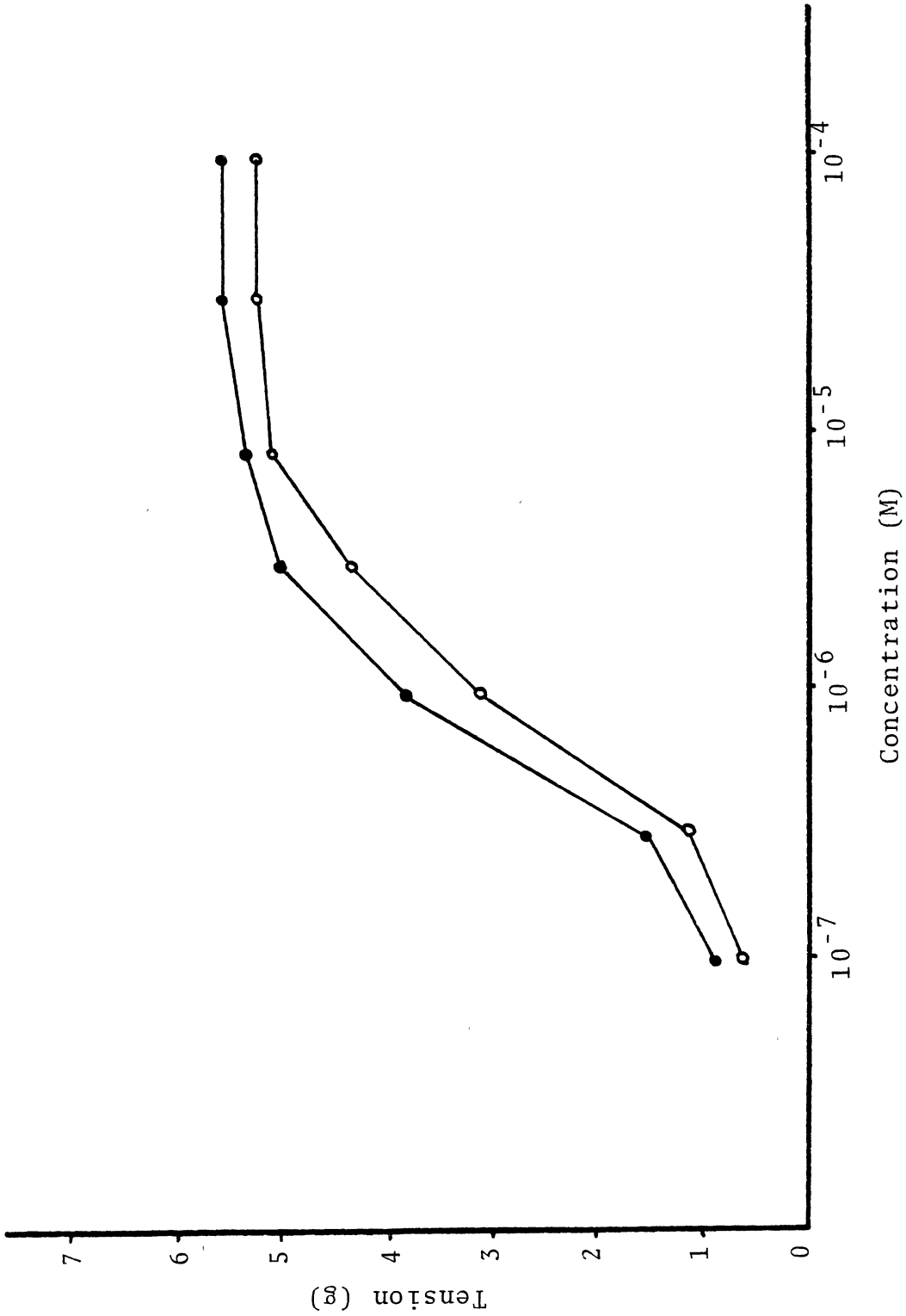

Tension (g)
0
1
2
3
4
5
6
7
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration (M)

Figure 9. Recordings of Changes in the Amplitude and Frequency of Spontaneous Contractions of Isolated Rat Urinary Bladder Strips due to a Gradual Reduction of the Temperature.

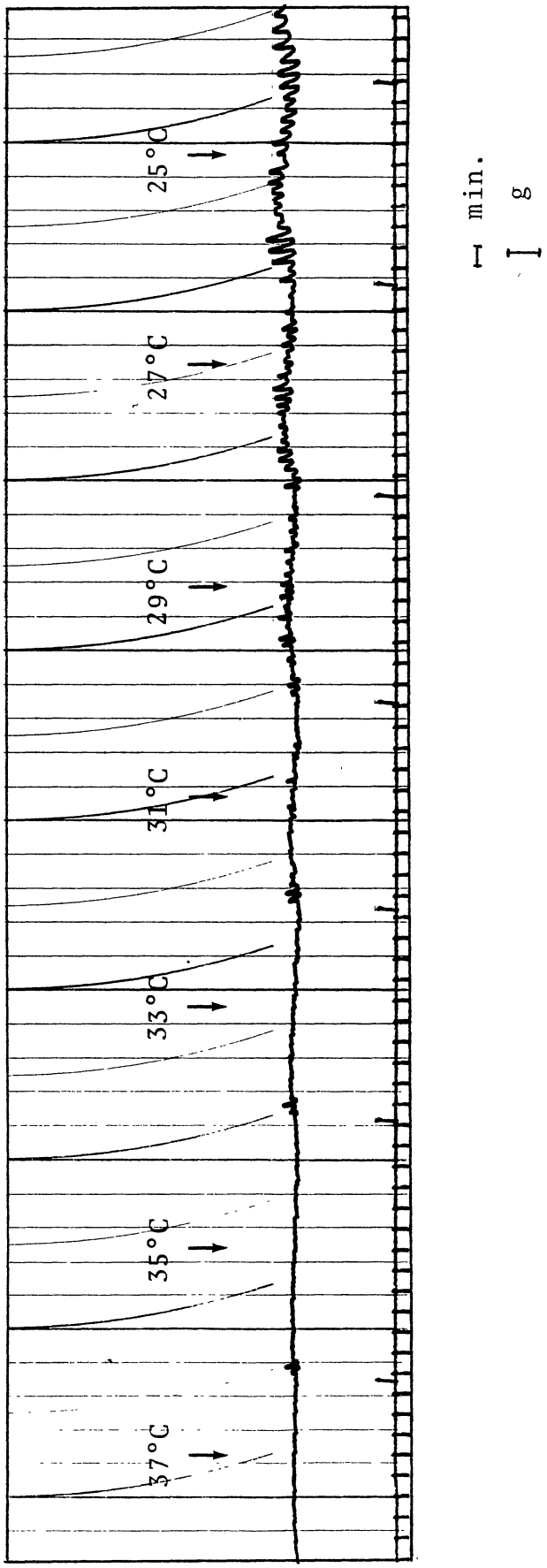
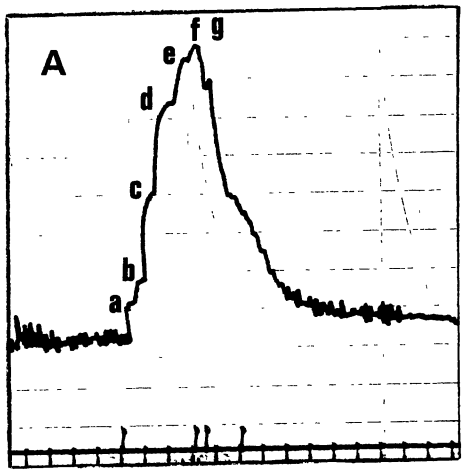

25°C
27°C
29°C
31°C
33°C
35°C
37°C
min.
g

Figure 10. Recordings of the Contractile Responses of Isolated Urinary Bladder Strips from 3 Month Old Sprague-Dawley Rats to Cumulative Concentrations of Carbachol at 37°C (A) and 25°C (B). (a=$1x10^{-7}$M; b=$3x10^{-7}$M; c=$1x10^{-6}$M; d=$3x10^{-6}$M; e=$1x10^{-5}$M; f=$3x10^{-5}$M and g=$1x10^{-4}$M).



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I g

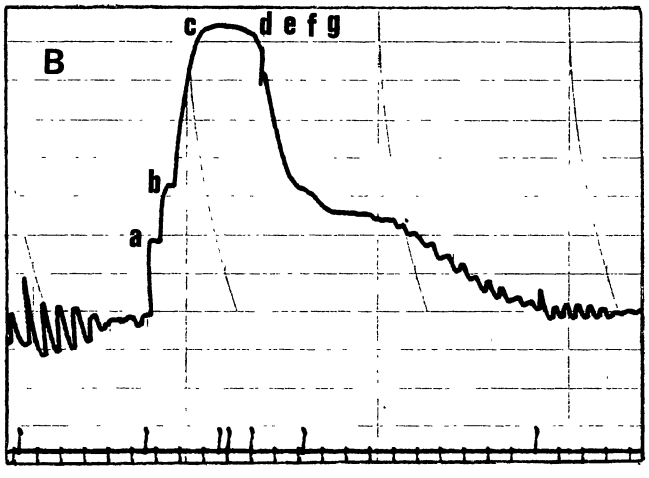


TABLE VII

EFFECTS OF TEMPERATURE ON CONTRACTILE RESPONSES OF ISOLATED URINARY BLADDER STRIPS FROM THREE MONTH OLD SPRAGUE-DAWLEY RATS TO CUMULATIVE CONCENTRATIONS OF CARBACHOL

Concentration (M)	Temperature (°C)	
	37 (n=5)	25 (n=5)
	Tension (g)	
1.0×10^{-7}	1.40 ± 0.48*	2.58 ± 0.44*
3.0×10^{-7}	2.22 ± 0.56*	4.10 ± 0.56*
1.0×10^{-6}	4.74 ± 0.73*	8.14 ± 0.42*
3.0×10^{-6}	6.38 ± 0.46*	8.24 ± 0.39*
1.0×10^{-5}	7.44 ± 0.35	8.24 ± 0.39
3.0×10^{-5}	7.62 ± 0.34	8.24 ± 0.39
1.0×10^{-4}	7.62 ± 0.34	8.24 ± 0.39

* Significant difference ($p<0.05$)

Figure 11. Cumulative Concentration-response Curves of Isolated Urinary Bladder Strips from 3 Month Old Sprague-Dawley Rats to Carbachol at 37°C (O) and 25°C (●). Each Point Represents the Mean ± S.E. of 5 Experiments.

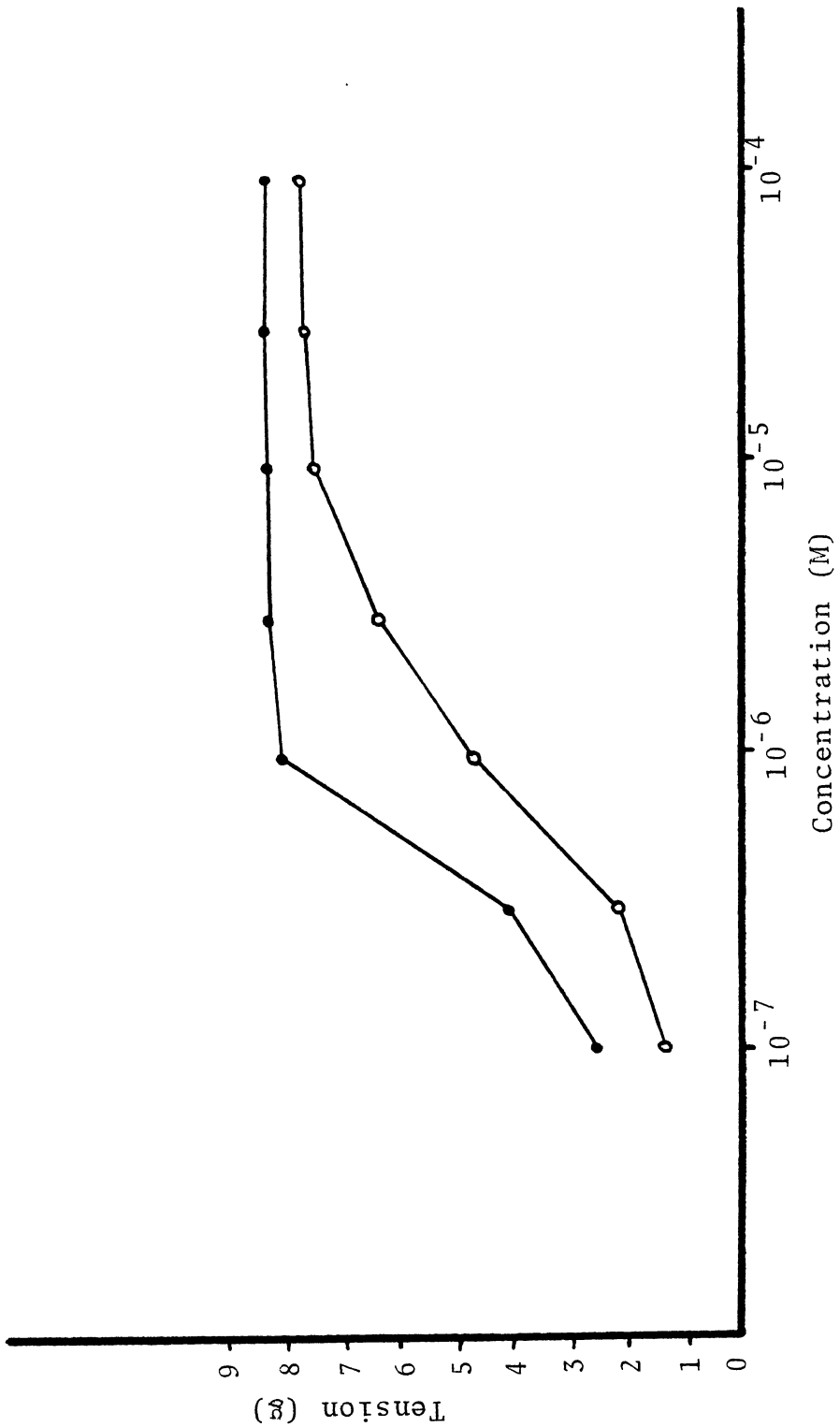
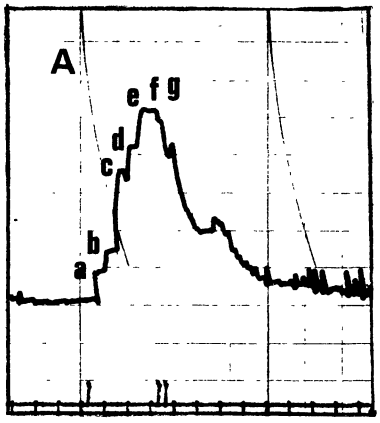

Tension (g)
0
1
2
3
4
5
6
7
8
9
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration (M)

Figure 12. Recordings of the Contractile Responses of Isolated Urinary Bladder Strips from 3 Month Old Fischer-344 Rats to Cumulative Concentrations of Carbachol at 37°C (A) and 25°C (B). (a=$1x10^{-7}$M; b=$3x10^{-7}$M; c=$1x10^{-6}$M; d=$3x10^{-6}$M; e=$1x10^{-5}$M; f=$3x10^{-5}$M and g=$1x10^{-4}$M)



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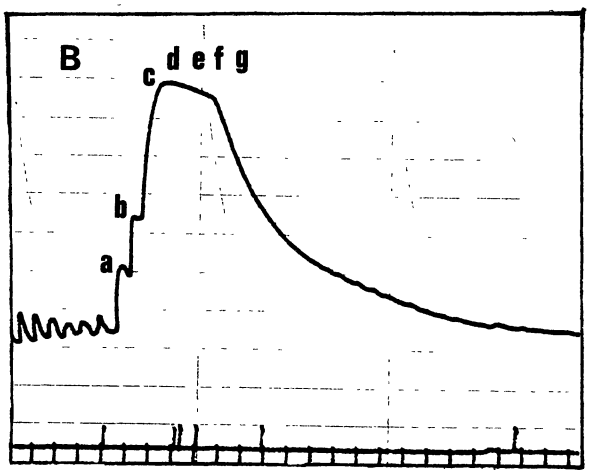


TABLE VIII

EFFECTS OF TEMPERATURE ON CONTRACTILE RESPONSES OF ISOLATED URINARY BLADDER STRIPS FROM THREE MONTH OLD FISCHER-344 RATS TO CUMULATIVE CONCENTRATIONS OF CARBACHOL

Concentration (M)	Temperature (°C)	
	37 (n=5)	25 (n=5)
	Tension (g)	
1.0×10^{-7}	0.62 ± 0.08*	1.70 ± 0.15*
3.0×10^{-7}	1.10 ± 0.11*	3.05 ± 0.20*
1.0×10^{-6}	3.12 ± 0.21*	5.85 ± 0.41*
3.0×10^{-6}	4.33 ± 0.18*	5.88 ± 0.43*
1.0×10^{-5}	5.08 ± 0.26*	5.88 ± 0.43*
3.0×10^{-5}	5.20 ± 0.20*	5.88 ± 0.43*
1.0×10^{-4}	5.20 ± 0.20*	5.88 ± 0.43*

* Significant difference ($p<0.05$)

Figure 13. Cumulative Concentration-response Curves of Isolated Urinary Bladder Strips from 3 Month Old Fischer-344 Rats to Carbachol at 37°C (O) and 25°C (●). Each Point Represents the Mean ± S.E. of 6 Experiments.

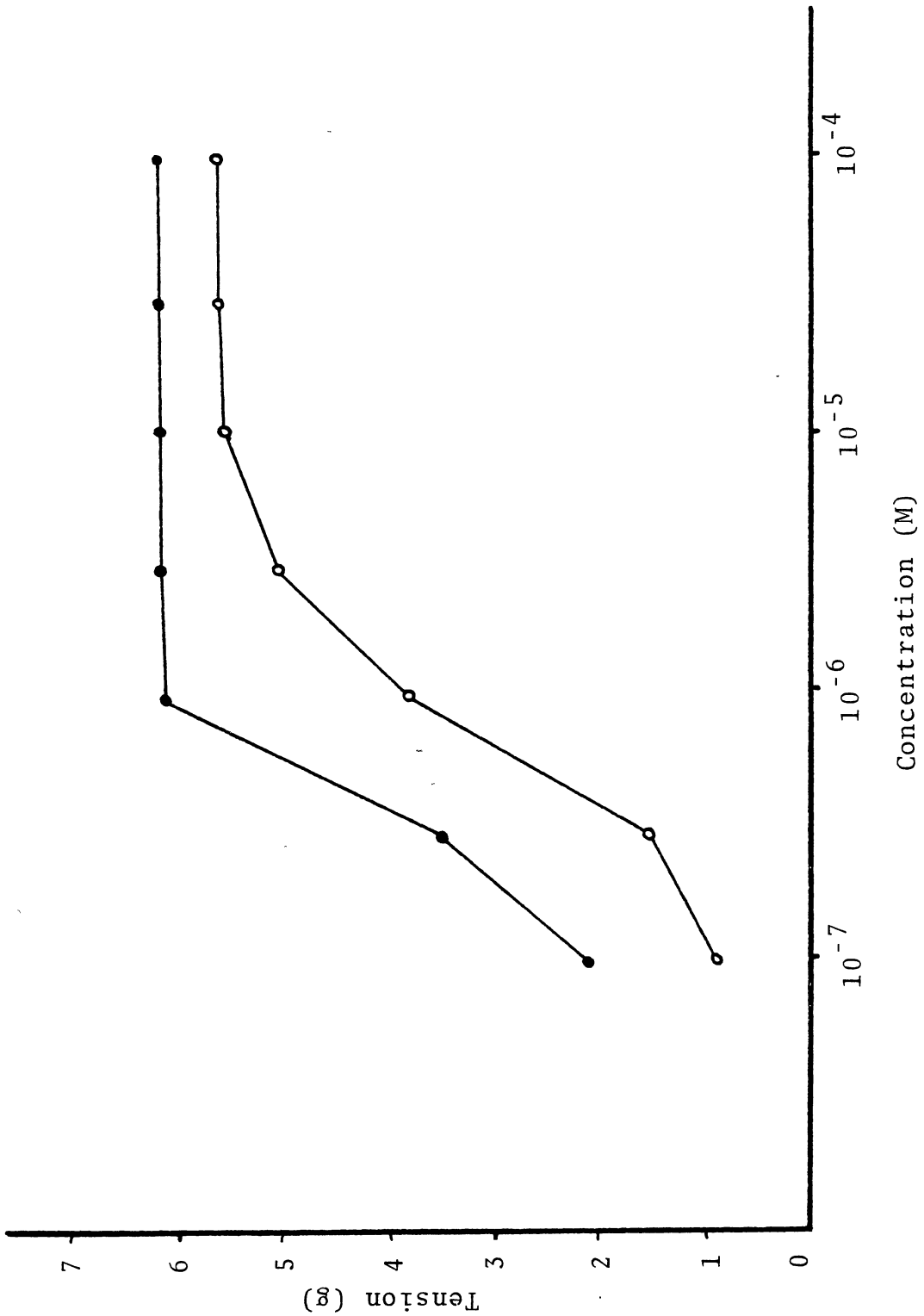
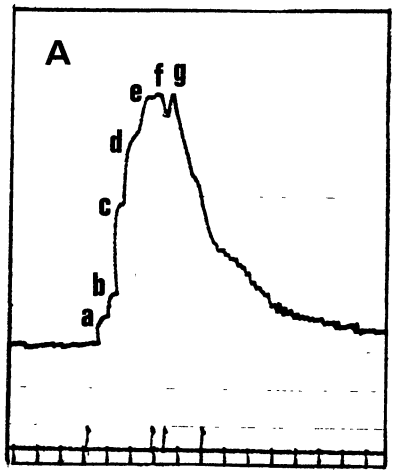

Tension (g)
0
1
2
3
4
5
6
7
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration (M)

Figure 14. Recordings of the Contractile responses of Isolated Urinary Bladder Strips from 12 Month Old Fischer-344 Rats to Cumulative Concentrations of Carbachol at 37°C (A) and 25°C (B). (a=$1x10^{-7}$M; b=$3x10^{-7}$M; c=$1x10^{-6}$M; d=$3x10^{-6}$M; e=$1x10^{-5}$M; f=$3x10^{-5}$M and g=$1x10^{-4}$M).



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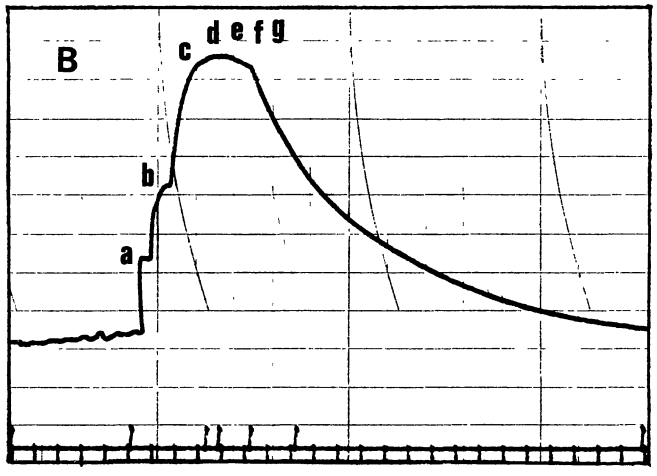

TABLE IX

EFFECTS OF TEMPERATURE ON CONTRACTILE RESPONSES OF ISOLATED URINARY BLADDER STRIPS FROM TWELVE MONTH OLD FISCHER-344 RATS TO CUMULATIVE CONCENTRATIONS OF CARBACHOL

Concentration (M)	Temperature (°C)	
	37 (n=5)	25 (n=5)
	Tension (g)	
1.0×10^{-7}	0.87 ± 0.12*	2.12 ± 0.18*
3.0×10^{-7}	1.52 ± 0.18*	3.50 ± 0.32*
1.0×10^{-6}	3.78 ± 0.19*	6.12 ± 0.39*
3.0×10^{-6}	5.02 ± 0.24*	6.12 ± 0.39*
1.0×10^{-5}	5.53 ± 0.29	6.12 ± 0.39
3.0×10^{-5}	5.55 ± 0.30	6.12 ± 0.39
1.0×10^{-4}	5.55 ± 0.30	6.12 ± 0.39

* Significant difference ($p<0.05$)

Figure 15. Cumulative Concentration-response Curves of Isolated Urinary Bladder Strips from 12 Month Old Fischer-344 Rats to Carbachol at 37°C (O) and 25°C (●). Each Point Represents the Mean ± S.E. of 6 Experiments.

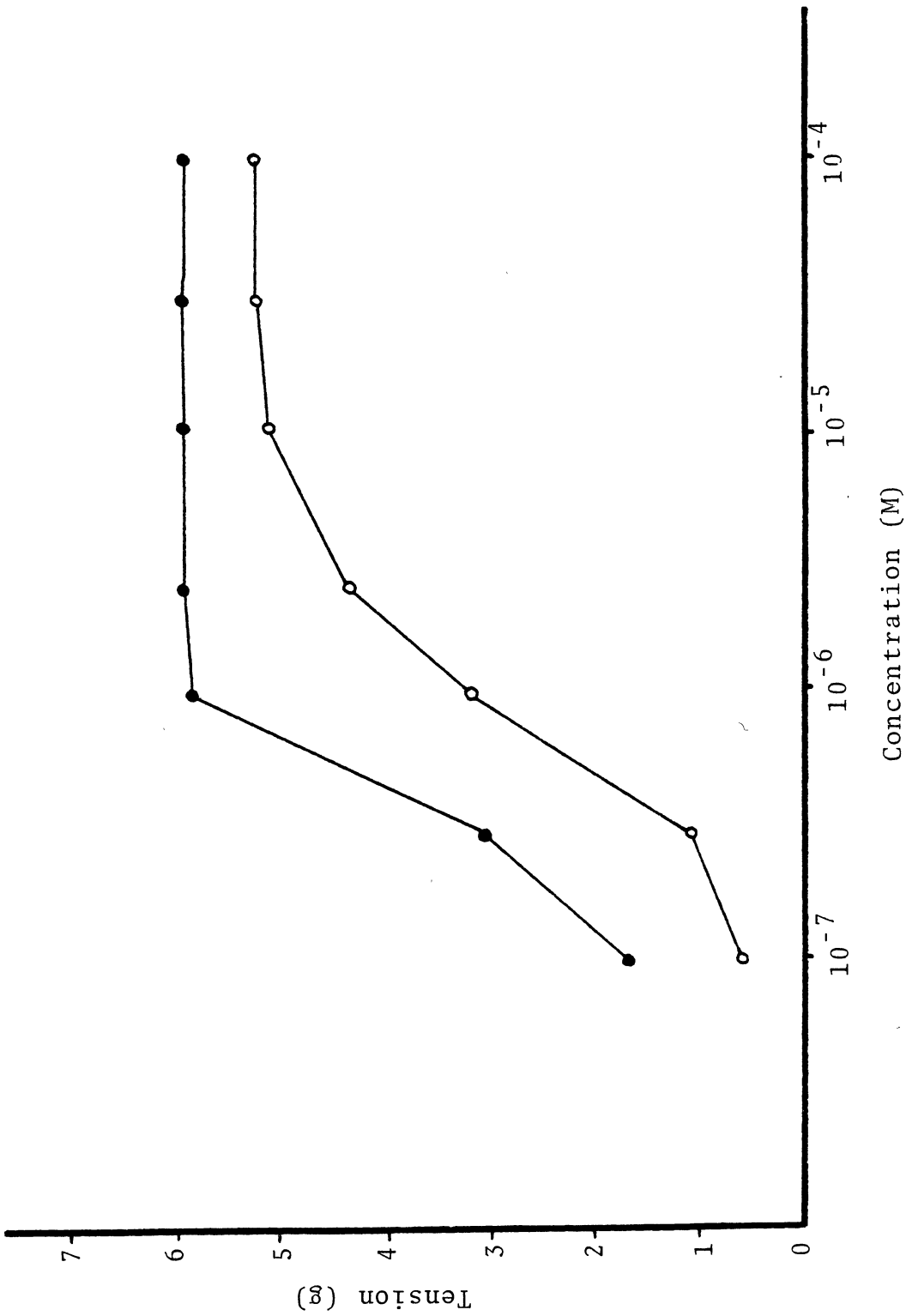
Tension (g)
0
1
2
3
4
5
6
7
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration (M)

TABLE X

EFFECTIVE CONCENTRATION (EC50) VALUES OF CARBACHOL

Group	EC50 ($x10^{-6}M$)	
	Temperature (°C)	
	37	25
Control	0.91±0.05	———
17 β-estradiol	0.66±0.07[a]	———
Progesterone	1.02±0.21	———
Sprague-Dawley 3 month old	0.75±1.47[b]	0.33±0.68[b]
Fischer-344 3 month old	0.81±0.59[b]	0.27±0.39[b]
Fischer-344 12 month old	0.69±0.64[b]	0.20±0.60[b]

[a]Significant difference ($p<0.05$) between control and hormone pretreated groups.

[b]Significant difference ($p<0.05$) between 37°C and 25°C.

cant ($p<0.05$) at $1.0x10^{-7}$ M to $3.0x10^{-5}$ M carbachol in groups IV and VI (Table VII and VIII) and all the concentrations of carbachol in group V (Table IX). Concentration-response curves of all three groups to carbachol shifted to the left as a result of reduction in temperature, indicating a supersensitivity to carbachol at 25°C (Figure 11, 13 and 15). Mean EC50 values at 25°C were significantly ($p<0.05$) lower than the mean EC50 values at 37°C for all three groups (Table X).

CHAPTER V

DISCUSSION

Modulatory effects of reproductive hormones on autonomic responses of various smooth muscles have been the subject of many studies. Successful estrogen therapy in aged menopaused women with urinary incontinence (Salmon et al., 1941) led to subsequent studies on the role of these hormones as modulators of the autonomic function of various organs. Estrogen has been previously shown to increase the contractile responses of rabbit urinary bladder (Schreiter et al., 1976) and guinea-pig gallbladder (Ryan and Pellecchia, 1982) preperations to phenylephrine and acetylcholine respectively. Progesterone, however, has been shown to have the opposite effect in guinea-pig gallbladder. Present study provides further evidence for the modulatory effects of estrogen and progesterone on autonomic responses of isolated rat urinary bladder. The mechanism of the effects of estrogen has not been studied extensively. It appears that an increase in the number of receptors is responsible for increased contractile response (Levin et al., 1980 and Larson et al., 1984). Progesterone may also exert its inhibitory effects at the receptor level by decreasing the number of receptors. Receptor binding studies would provide a clue as to the mechanism of

the effects of progesterone.

Boyd et al. (1982) have shown a significant difference between growth rate, body weight and pulmonary function of Sprague-Dawley and Fischer-344 rats of comparable age. It has also been reported that body weight, kidney weight and kidney function in rat are influenced by strain differences (Hackbarth et al., 1981 and 1983). Effects of strain on pharmacological responses of isolated tissues to various drugs have not been studied. Results of this study indicate that body weight, urinary bladder weight and contractile responses of urinary bladder strips of Sprague-Dawley rats are significantly higher than those of Fischer-344 rats of similar age. An Increase in the weight of the urinary bladders, hence increased number of muscarinic receptors could account for the increased contractlie responses of urinary bladders from Sprague-Dawley rats to carbachol. Thus, strain differences should be considered when pharmacological agents are used experimentally or therapeutically.

Age-related changes in various tissues have been studied extensively. Responses of isolated tissues from the aorta (Tuttle, 1966 and Fleisch et al., 1970), urethra (Smith, 1972), trachea (Hayashi et al., 1980 and Duncan et al., 1985), heart (Narayanan and Derby, 1983 and Baker et al., 1985), urinary bladder (Kolta et al., 1984), vas deferens (Hyland and Docherty, 1985) and small intestine (Kobashi et al., 1985) to pharmaclogical agents are altered by age. Sensitivity of most of these tissues to agonists is

reduced with increasing age. However, some tissues such as the urinary bladder show an increased response while others such as venous vascular smooth muscle (Duckles and Holbert, 1985) show no change. There is a general agreement that age-related changes occur at the level of the receptor. The number of muscarinic cholinergic (Pedigo and Polk, 1985), adrenergic and dopaminergic (Severson et al., 1980 and Misra et al., 1980) receptors in the rat brain decreases with age. Receptors of mononuclear cells in man (Shocken et al., 1977) and submandibular glands in mice (Piantanelli et al., 1980) are also reduced in number with increasing age. Kolta et al. (1984) have reported that the maximum contractile responses of isolated bladder to acetylcholine in 29 month and 17 month old rats were 63% and 15% greater than those of 7 month old rats, respectively. They also showed a 46% and 7% increase in the binding of [^{3}H]-quinuclidinyl benzilate (a muscarinic antagonist) to membrane preparation from bladder of 29 month and 17 month old rats as compared to the binding of membrane preperation from 7 month old animals. Findings of the present study show an increased response in the urinary bladder preparation of 12 month old rats as compared to that of 3 month old rats. Maximum contractile response to carbachol was higher in older rats but the EC50 values did not change significantly, indicating no change in the affinity of the agonist for the receptor. Age-related changes seem to occur in a gradual manner with the more pronounced effects appearing in the latter stages of life. Further studies over a wider

range of age is needed for elucidation of the mechanism of age-related changes in the autonomic function of the urinary bladder.

Recent studies have shown that hypothermia increases the sensitivity of the cardiac tissues to pharmacological agents (Wöppel and Trendelenburg, 1973, Munoz-Ramirez et al. 1973 and Broadely et al., 1977 and 1985). This study provides evidence for the hypothermia-induced supersensitivity in the isolated urinary bladder of the rat. Maximum contractile responses at 37°C and 25°C did not change significantly. A significant reduction in EC50 values of carbachol at 25°c was observed, indicating an increased affinity of the agonist for the receptor and/or an inhibition of enzyme such as acetylcholinestrase at lower temperature. It has been previously shown that hypothermia increases the affinity of the agonist for β-adrenoceptors in guinea-pig cardiac tissues (Broadley and Williams, 1983). Wöppel and Treddelenburg (1973) showed similar effects in corresponding tissues. They also demonstrated that hypothermia-induced supersensitivity is not due to impairment of uptake mechanism (neuronal or extraneuronal) or impairment of enzyme activity. In contrast Runoz-Ramirez et al. (1973) recognize the inhibition of enzymes such as catechol-o-methyl transferase (COMT) or monoamine oxidase (MAO) as the cause of the hypothermia-induced supersensitivity of isolated atria of guinea-pig to adrenergic agonists. Alternative mechanisms for this phenomenon may include factors other than receptors. It is believed that

Ca^{++} influx and intracellular stores of Ca^{++} are increased by hypothermia (Langer, 1973). Changes in ionic movements and/or distribution at lower temperature might also play a role (Nargeot et al., 1982). Further studies at the molecular level are needed to elucidate the mechanism of hypothermia-induced supersensitivity.

In summary, the results of this study, consistent with the previous reports, indicate that: 1) estrogen and progesterone can affect the autonomic responses of rat urinary bladder; 2) strain difference can influence body weight, bladder weight and contractile responses of rat urinary bladder to carbachol - a muscarinic cholinergic agonist; 3) there is an increased contractile response to carbachol in the urinary bladder preparations from older rats which is only significant at two concentrations and 4) hypothermia increases the sensitivity of rat urinary bladder strips to carbachol.

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