INFORMATION TO USERS

While the most advanced technology has been used to photograph and reproduce this manuscript, the quality of the reproduction is heavily dependent upon the quality of the material submitted. For example:

- Manuscript pages may have indistinct print. In such cases, the best available copy has been filmed.

- Manuscripts may not always be complete. In such cases, a note will indicate that it is not possible to obtain missing pages.

- Copyrighted material may have been removed from the manuscript. In such cases, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, and charts) are photographed by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each oversize page is also filmed as one exposure and is available, for an additional charge, as a standard 35mm slide or as a 17”x 23” black and white photographic print.

Most photographs reproduce acceptably on positive microfilm or microfiche but lack the clarity on xerographic copies made from the microfilm. For an additional charge, 35mm slides of 6”x 9” black and white photographic prints are available for any photographs or illustrations that cannot be reproduced satisfactorily by xerography.
The effects of age on urinary bladder function in the male rat: Response to pharmacological agents

Chun, Alexa L., Ph.D.
The Ohio State University, 1987

Copyright ©1987 by Chun, Alexa L. All rights reserved.
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark \checkmark.

1. Glossy photographs or pages 
2. Colored illustrations, paper or print 
3. Photographs with dark background 
4. Illustrations are poor copy 
5. Pages with black marks, not original copy 
6. Print shows through as there is text on both sides of page 
7. Indistinct, broken or small print on several pages \checkmark 
8. Print exceeds margin requirements 
9. Tightly bound copy with print lost in spine 
10. Computer printout pages with indistinct print 
11. Page(s) lacking when material received, and not available from school or author.
12. Page(s) seem to be missing in numbering only as text follows.
13. Two pages numbered . Text follows.
14. Curling and wrinkled pages 
15. Dissertation contains pages with print at a slant, filmed as received 
16. Other 

University Microfilms International
The Effects of Age on Urinary Bladder Function in the Male Rat: Response to Pharmacological Agents

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
Alexa L. Chun, B.S., R.Ph

* * * * *

The Ohio State University
1987

Dissertation Committee: Approved by
Lane J. Wallace
Dennis R. Feller
Dennis B. McKay
Michael C. Gerald

Lane J. Wallace, Adviser
College of Pharmacy
© Copyright by Alexa L. Chun
1987
DEDICATION

To my parents, Marilyn and Alexander Chun
and my sisters, Karen (Chun) Tom and Sandra Chun
and
To my brother Steven Alexander Chun
for giving us so much in such a short time
"If you have built castles in the air, 
your work need not be lost; 
that is where they should be;  
now put foundations under them."

H.D. Thoreau
ACKNOWLEDGEMENTS

I would like to thank:

Richard Couch for his friendship, support, and confidence in my work and for all of his help on the computer

Joni Stevens and Nick Felt for their devoted friendship, support, and empathy

Kathy Brooks and Rose Smith for their kindness and invaluable assistance

National Institute of Aging for funding this research project

Terry Shepard for introducing Columbus to me, for showing me the ropes of graduate school, and for being a close friend

J. Evelyn Lawson and Paul Campbell for their invaluable help on the computer

Cheryl Crooks and Ronald Dent for taking such expert care of my rats

The members of my committee, Lane J. Wallace, Michael C. Gerald, Dennis R. Feller, Dennis B. McKay, and Norman J. Uretsky, for their guidance in my graduate education
Doug Smith, Raye Ann Wallace, Tom Kocarek, Xiao Ying Tien, Jocelyn Burke, Cathy Snider, Florence Kraft, and Gregory Ordway for their friendship

Dr. Robert M. Levin for his interest and input into my research

Dr. Richard Decker for 3 summers of excellent research experience at Abbott, and for encouraging me to go to graduate school

Professor Nicholas Popovich for instilling in me the "pride of pharmacy" and for his encouragement in my graduate studies

Professor George Yim for giving me an opportunity to do pharmacological research as an undergraduate student at Purdue

Phillip Ross for his tailor-made curve fitting and graphic computer programs, and for sharing his knowledge of pharmacology

Frederick S. Ruland for his invaluable assistance with the statistical analysis of my data

Rev. Eugene Winkler for helping me through a very rough time in my life

My relatives in Hawaii, Aunt Charlene, Auntie Lou Lou, and Uncle Alfred and Iris for their letters and goodies

Tara Smith for her friendship, support and thoughtfulness
Grandma Thelma Bonnell for her enthusiasm in my life, and for the memorable times on the farm

Uncle Edward for his interest and enthusiasm in my life and studies

Uncle Henry for his excellent mathematic notes and annual Thanksgiving visits

Linda Airey, Cindy Myers, and Debi Shiozawa, my friends from Purdue who made special trips to Columbus to visit me

Carol (Calhoun) Williams, my best friend from 4th grade who fulfilled her dream of becoming an M.D. and kept me going on mine

Matt and Melanie Huber for making me feel at home in Brazil, In., and for their annual visits to Columbus which I always looked forward to

Bob Myers for my memorable experience as a retail pharmacist in Brazil, Indiana

Dr. Jan Johnson for listening to my research ideas and plans and offering advice, also for the great philosophical conversations on life

Cheryl Abbott for her understanding, and unconditional support, and excellent times we have together
VITA

March 8, 1958..............Born - Lafayette, Indiana


1979-1980 ...................Research Assistant, Dept. of Pharmacology, School of Pharmacy, Purdue University, W. Lafayette, Indiana

1981..............B.S. Pharmacy, Purdue University, W. Lafayette, Indiana

1981-1982 ...................Pharmacist and Assistant Manager, Hook's Drugs, Brazil, Indiana

1982-1985 ...................Graduate Teaching Associate, The Ohio State University, Columbus, Ohio

1985-1987 ...................Graduate Research Associate, The Ohio State University, Columbus, Ohio

Publications


Rat Urinary Bladder Function in vitro: Age Related Effects

Two illustrations in U.S. Pharmacist, August: 37-61, 1981,
Fluoride Dental Therapy, Popovich, N.G. and Popovich, J.G.

Two illustrations in Handbook of Nonprescription Drugs,

**Fields of Study**

Major Field: Urological Pharmacology

Physiology of Aging

Adviser, Lane J. Wallace

Co-Adviser, Michael C. Gerald
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>VITA</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Urinary Incontinence</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Anatomy, Physiology, and Pharmacology of the Lower Urinary Tract</td>
<td>6</td>
</tr>
<tr>
<td>1.2.1 Anatomy and Physiology of the Bladder and Urethra</td>
<td>7</td>
</tr>
<tr>
<td>1.2.2 Neurophysiology of the Bladder and Urethra</td>
<td>12</td>
</tr>
<tr>
<td>1.3 Pharmacotherapy of Urinary Incontinence</td>
<td>18</td>
</tr>
<tr>
<td>1.4 Cystometry</td>
<td>24</td>
</tr>
<tr>
<td>1.5 Micturition</td>
<td>29</td>
</tr>
<tr>
<td>1.6 Urinary Incontinence in the Elderly</td>
<td>32</td>
</tr>
<tr>
<td>1.7 Statement of the Problem</td>
<td>34</td>
</tr>
<tr>
<td>1.8 Animals</td>
<td>38</td>
</tr>
<tr>
<td>1.9 List of Drugs and Chemicals</td>
<td>39</td>
</tr>
</tbody>
</table>
## II. MICTURITION PROFILE: SPECIFIC AIM 1

2.1 Introduction .......................... 40
2.2 Methods ................................ 41
2.3 Results ................................ 45
2.4 Discussion ............................. 55

## III. BLADDER FILLING AND STORAGE: SPECIFIC AIM 2

3.1 Introduction ........................... 62
3.2 Methods ................................ 64
   3.2.1 Whole bladder Preparation ........ 64
      3.2.1.1 Drug Dose-Response Curves
              - Isometric Contraction .... 71
      3.2.1.2 Drug-Dose Response Curves
              - Isotonic Contraction .... 73
      3.2.1.3 Drug Dose Response Curves
              - Expansion .............. 76
   3.2.2 In Vivo Cystometrograms .......... 79
   3.2.3 Natural fill cystometrograms ... 82
3.3 Alpha-Adrenergic Activity: Part A 85
   3.3.1 Introduction ...................... 85
   3.3.2 Results ........................... 87
   3.3.3 Discussion ........................ 93
3.4 Beta-Adrenergic Activity: Part B 95
   3.4.1 Introduction ...................... 95
   3.4.2 Results ........................... 97
   3.4.3 Discussion ........................ 100
3.5 Bladder Function/Structural
    Characteristics: Part C ............ 101
   3.5.1 Introduction ...................... 101
      3.5.1.1 Myogenic Tone .............. 101
      3.5.1.2 Micturition Reflex ....... 106
   3.5.2 Results ........................... 108
   3.5.3 Discussion ........................ 117

## IV. URINARY BLADDER EMPTYING: SPECIFIC AIM 3

4.1 Introduction .......................... 123
4.2 Results ................................ 127
4.3 Discussion ............................. 142

## V. DIURETIC STRESS: SPECIFIC AIM 4

5.1 Introduction .......................... 145
5.2 Methods ................................ 146
5.3 Results ................................ 147
5.4 Discussion .............................. 162

VI. SCREENING SUBSTANCES: SPECIFIC AIM 5 ........ 169

6.1 Introduction ............................. 169
6.2 Methods .................................... 172
6.3 Results .................................... 172
6.4 Discussion ................................. 180

VII. SUMMARY AND CONCLUSIONS ...................... 181

APPENDICES

A. Placement of Tracheal tube ..................... 189

B. Purse String Suture ........................... 194

C. Cannulation of Jugular Vein ...................... 197

D. Expansion and Expulsion Tube Dimensions ........ 201

E. SAS Programs .................................. 202
   E.1 Correlations ................................ 202
   E.2 Analysis of Variance (single classification)/Student Newman Keuls . 203
   E.3 Analysis of Variance (2-way)/Student Newman Keuls ...................... 204
   E.4 Nonlinear Regression Curve Fitting Analysis ......................... 206
   E.5 Nonlinear Regression Curve Fitting Analysis ......................... 209
   E.6 Nonlinear Regression Curve Fitting Analysis ......................... 213
   E.7 Student's T Test ............................ 217

LIST OF REFERENCES .............................. 218
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. List of chemicals and drugs used in the project</td>
<td>39</td>
</tr>
<tr>
<td>2. Log $ED_{50}$ values: phenylephrine pressure D-R curves</td>
<td>88</td>
</tr>
<tr>
<td>3. Maximal pressure response: phenylephrine D-R curves</td>
<td>89</td>
</tr>
<tr>
<td>4. Log $ED_{50}$ values: phenylephrine isotonic D-R curves</td>
<td>90</td>
</tr>
<tr>
<td>5. Time of (phenylephrine) bladder expulsion</td>
<td>92</td>
</tr>
<tr>
<td>6. Isoproterenol expansion: Log $ED_{50}$ values and volume</td>
<td>98</td>
</tr>
<tr>
<td>7. Isoproterenol expansion: percent volume</td>
<td>99</td>
</tr>
<tr>
<td>8. Natural fill cystometrograms</td>
<td>110</td>
</tr>
<tr>
<td>9. Structural bladder capacities</td>
<td>112</td>
</tr>
<tr>
<td>10. Bladder and body weights</td>
<td>116</td>
</tr>
<tr>
<td>11. Log $ED_{50}$ values: ACH and bethanechol pressure D-R curves</td>
<td>128</td>
</tr>
<tr>
<td>12. Maximal pressure response: ACH and Beth D-R curves</td>
<td>129</td>
</tr>
<tr>
<td>13. Log $ED_{50}$ values: ACH and Beth isotonic D-R curves</td>
<td>135</td>
</tr>
<tr>
<td>14. Time of bethanechol bladder expulsion</td>
<td>136</td>
</tr>
<tr>
<td>15. ANOVA table for isoproterenol maximal expansion</td>
<td>152</td>
</tr>
<tr>
<td>16. Log $ED_{50}$ values: isoproterenol D-R curves</td>
<td>154</td>
</tr>
</tbody>
</table>
17. Log ED$_{50}$ values: bethanechol D-R curves  . . . 155
18. Bethanechol pressure D-R curves:
    control/sucrose rats  . . . . . . . . . . . . . . . . 156
19. Cystometrographic plateau pressures:
    sucrose/control rats  . . . . . . . . . . . . . . . . 158
20. Structural bladder capacity: sucrose /
    control rats  . . . . . . . . . . . . . . . . . . . . . 159
22. Urine density: sucrose / control rats  . . . . 161
23. Maximal responses elicited by various drugs . . 179
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Female urinary bladder</td>
<td>5</td>
</tr>
<tr>
<td>2. Urethral anatomy</td>
<td>9</td>
</tr>
<tr>
<td>3. Male urinary bladder</td>
<td>11</td>
</tr>
<tr>
<td>4. Autonomic innervation of the urinary bladder</td>
<td>13</td>
</tr>
<tr>
<td>5. Receptor distribution in urinary bladder</td>
<td>16</td>
</tr>
<tr>
<td>6. Bladder storage mechanisms</td>
<td>17</td>
</tr>
<tr>
<td>7. Normal cystometrogram</td>
<td>26</td>
</tr>
<tr>
<td>8. Abnormal cystometrogram</td>
<td>28</td>
</tr>
<tr>
<td>9. Micturition pressure/flow/EMG</td>
<td>31</td>
</tr>
<tr>
<td>10. Metabolism cage</td>
<td>42</td>
</tr>
<tr>
<td>11. Total 24 h water intake in three age groups of rats</td>
<td>46</td>
</tr>
<tr>
<td>12. Total 24 h urine output in three age groups of rats</td>
<td>47</td>
</tr>
<tr>
<td>13. Average volume of each micturition measured in a 4 h period</td>
<td>49</td>
</tr>
<tr>
<td>14. Total volume of urine voided by rats in</td>
<td>50</td>
</tr>
<tr>
<td>15. Total number of times rats voided</td>
<td>51</td>
</tr>
<tr>
<td>16. Ratio of water intake to urine output in a 24 h period</td>
<td>52</td>
</tr>
<tr>
<td>17. Body weights in three age groups of rats</td>
<td>54</td>
</tr>
<tr>
<td>18. Whole rat bladder preparation</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Description</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>19</td>
<td>Determination of structural capacity</td>
</tr>
<tr>
<td>20</td>
<td>The effect of infusion rate on intravesical pressure</td>
</tr>
<tr>
<td>21</td>
<td>Isotonic expulsion system</td>
</tr>
<tr>
<td>22</td>
<td>Bladder expansion setup</td>
</tr>
<tr>
<td>23</td>
<td>In vivo cystometrogram setup</td>
</tr>
<tr>
<td>24</td>
<td>Natural fill cystometrogram setup</td>
</tr>
<tr>
<td>25</td>
<td>Passive properties of a standard cystometrogram</td>
</tr>
<tr>
<td>26</td>
<td>Natural Fill Cystometrogram</td>
</tr>
<tr>
<td>27</td>
<td>In vitro cystometrograms</td>
</tr>
<tr>
<td>28</td>
<td>Standard cystometrogram</td>
</tr>
<tr>
<td>29</td>
<td>Two part bladder contraction</td>
</tr>
<tr>
<td>30</td>
<td>Bethanechol pressure D-R curves (1/4 full)</td>
</tr>
<tr>
<td>31</td>
<td>Bethanechol pressure D-R curves (3/4 full)</td>
</tr>
<tr>
<td>32</td>
<td>Effect of bladder volume on bethanechol expulsion</td>
</tr>
<tr>
<td>33</td>
<td>Effect of bladder volume on bethanechol contraction</td>
</tr>
<tr>
<td>34</td>
<td>24 h fluid intake for sucrose and control rats</td>
</tr>
<tr>
<td>35</td>
<td>24 h urine output for sucrose and control rats</td>
</tr>
<tr>
<td>36</td>
<td>Geometric representation of isoproterenol expansion</td>
</tr>
<tr>
<td>37</td>
<td>Dose-response curve tracings of various drugs</td>
</tr>
<tr>
<td>38</td>
<td>Maximal pressure response to various drugs</td>
</tr>
<tr>
<td>39</td>
<td>Relationship of bladder usage / sensation / micturition volume</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

1.1 URINARY INCONTINENCE

Urinary incontinence is not a disease or specific condition but merely a symptom of many different types of lower urinary tract dysfunctions. The International Continence Society (ICS) defines urinary incontinence as "a condition where involuntary loss of urine is a social or hygienic problem and is objectively demonstrable" [1].

People who are incontinent often lead isolated lives. Urinary incontinence is responsible not only for physically debilitating problems of inadequate urine control, but also the psychological devastation which centers around the afflicted patient's inability to voluntarily control the micturition reflex in a normal physiological manner. For example, many urine incontinent people go great lengths to keep dry. This might mean going to the toilet every 30 minutes to keep the bladder empty or not drinking fluids for 18 hours [2]. Others may need to wear protective diapers to keep dry. Hopes for a normal social life in these individuals are often shattered.
Concepts in the diagnosis and treatment of urinary incontinence have been around for many years, with one of the earliest references dating back to 1616 [3]. In his dissertation on bladder function (1616), M. V. Goldberg was among the first to classify urinary incontinence into defects of storage and emptying, and into problems of detrusor or outlet function [3]. This classification of urinary incontinence is still valid today and commonly used.

The lower urinary tract performs the two functions of urine storage and urine emptying [4,5]. Normal urine storage requires the three following conditions: 1) the bladder must accommodate increasing volumes of urine at a low intravesical pressure with normal and appropriate sensation; 2) there must be an absence of involuntary bladder contractions until voiding occurs; and 3) the bladder outlet must be closed at rest and during abdominal stress (coughing) [4,6]. Normal urine emptying requires: 1) a coordinated bladder contraction of sufficient magnitude which is associated with; 2) a decrease in outlet resistance at both the proximal (bladder neck and proximal urethra) and distal (intrinsic urethral and striated pelvic floor musculature) levels; and 3) an absence of anatomical obstruction [4,6]. Failure of the bladder to normally fill and store and empty urine results in a variety of
voiding dysfunctions. These voiding functions can be classified into defects of bladder storage and emptying; the same classification system was used by Goldberg in 1616.

Urinary incontinence is a classic symptom of the bladder's inability to fill and store urine adequately [6]. Failure to store may be caused by either increased or involuntary bladder contractility (detrusor hyperreflexia) which may occur with or without sensation of impending micturition [8], a permanent or intermittent decrease in outlet resistance, or both [4,7].

Detrusor hyperreflexia may result from destruction or bypassing of a central nervous system locus or pathway which is normally inhibitory to micturition. Some examples of this type of situation are the urgency incontinence\(^1\) secondary to detrusor hyperreflexia often seen in patients with cerebral vascular disease, a demyelinating disease, and Parkinsonism [8]. Also, stimulation or irritation of a facilitatory pathway to micturition may result in detrusor hyperreflexia [8]. In addition to neurological lesions, detrusor hyperreflexia may be associated with bladder outlet obstruction (secondary to prostate enlargement or external sphincter dyssynergia),\(^2\) or inflammation of the

---

\(^1\) Urgency is the extreme desire to urinate either because of pain or fear of leaking.

\(^2\) The detrusor contracts without associated sphincter relaxation; urine flow is obstructed.
bladder itself. Also, detrusor hyperreflexia may be idiopathic [8].

In addition to detrusor hyperreflexia of various etiologies, failure to store urine can be caused by a decrease in outlet resistance. This decreased outlet resistance may be caused by damage to innervation of the smooth muscle of the bladder neck or proximal urethra, or to intramural striated [6] or pelvic floor muscles (Figure 1). Autonomic or somatic neuropathy, or surgical trauma [4,6], or aging could be responsible for such damage [6]. In elderly hypo-estrogenic women, a deficient mucosal seal mechanism has also been suggested as a contributory factor in decreased outlet resistance [6].

Another etiological category of urinary incontinence may result from urethral instability [8]. In urethral instability the outlet inappropriately relaxes during bladder filling. The cause is unknown.

Failure of the bladder to empty can result from inadequate bladder contractility, increased outlet resistance [7] or both. A paradoxic or overflow incontinence may develop in a patient with long-standing bladder emptying failure. This condition is characterized by a hypotonic, overdistended bladder [8,9] which generally leaks upon increases in intra-abdominal pressures [8].
**Figure 1:** Female urinary bladder. Adapted from [15].
Again, it must be remembered that urinary incontinence, a symptom of bladder storage failure, may be caused by a combination of bladder and outlet abnormalities (increased detrusor contractility, decreased outlet resistance, or both, etc.) [8].

1.2 ANATOMY, PHYSIOLOGY, AND PHARMACOLOGY OF THE LOWER URINARY TRACT

There are large gaps in our knowledge and understanding of the innervation, anatomy, physiology, and pharmacology of the lower urinary tract which includes the urinary bladder and urethra. In addition, there is much controversy over the finer details of the interrelationships of the parasympathetic and sympathetic influences on the bladder and urethra, if any, [6] as well as over the anatomy and physiology of the bladder and urethra.

In this introductory chapter I am going to give a brief review of some of the anatomic, physiologic, and pharmacologic concepts that I feel are necessary to understand the rationales behind the different drug treatments used in urinary incontinence. For a thorough review on the subject Wein [10] should be read.

For the remainder of this chapter and following chapters I will sometimes be referring to the bladder neck and proximal urethra collectively as the bladder outlet (Figure 1).
Also, detrusor will describe the body of the bladder (Figure 1).

1.2.1 Anatomy and Physiology of the Bladder and Urethra

The bladder and urethra comprise the lower urinary tract, and through their unique specialization and coordination provide efficient storage and removal of urine.

The urinary bladder wall consists of an outer adventitial layer of connective tissue, a smooth muscle layer (detrusor muscle), and an inner layer of mucous membrane which totally lines the interior of the bladder [10,11]. The smooth muscle layer of the bladder is further divided into a trigone and detrusor.

The smooth muscle in the triangular trigone region consists of two layers, the superficial and deep trigonal muscles [10,11]. The deep trigonal muscle is continuous with and indistinguishable from the detrusor muscle [11]. The superficial trigonal muscle is continuous with the ureteral musculature [11,12] and becomes continuous with the smooth muscle of the proximal urethra [11].

The ureters penetrate the bladder obliquely through the trigone and course several centimeters under the bladder epithelium [13]. Pressure in the bladder compresses the ureters to prevent backflow of urine during micturition [13].
The detrusor muscle is composed of interlacing bundles of smooth muscle cells arranged as a complex meshwork \[11,12\]. Although discrete layers of smooth muscle are not distinguishable, in the area of the bladder neck longitudinally oriented muscles tend to form the inner and outer layers of the detrusor muscle with a circular middle layer \[10\]. Functionally, the detrusor comprises a single unit of interlacing smooth muscle which, upon contraction, will cause a reduction in all dimensions of the bladder lumen \[11\].

For normal bladder filling and storage the outlet must remain closed to form a watertight seal. Many intrinsic urethral factors have been thought to contribute to urethral closure and this watertight seal. Some of these factors include: mucosal surface tension, submucosal softness, and muscular occlusion \[14,15\].

The mucosa (Figure 2) in both sexes is organized in longitudinal folds apposed to itself \[10,15\]. Mucous secretions in this layer contribute to the development of surface tension \[14\] which may be a factor in urethral closure \[15\].

The submucosal layer (Figure 2) is a vascular plexus \[15\] which acts as a framework of spongy tissue \[14\] to fill between the folds of the mucosal layer as the urethra closes \[15\]. The "fill in" effect of the submucosal layer
Figure 2: Urethral anatomy
 contributes to the watertight seal necessary for urinary continence.

The smooth muscle layer extends throughout the length of the urethra. It exerts a constant tonus under sympathetic control [14] that compresses the mucosal and submucosal layers, thus adding to the watertight seal.

Two groups of striated muscles contribute to urethral closure. These are the intramural and periurethral striated muscles.

The intramural muscle or external sphincter is found close to the urethral lumen, sometimes interdigitating with the smooth muscle of the urethra [15]. The intramural muscle is of the "slow twitch" variety and is adapted to maintain contraction over a long period [15]. It forms a sleeve that is thickest in the middle third of the female urethra (Figure 2) and in the postprostatic region of the male urethra (Figure 3) [11], where closure pressure is the highest [14]. This muscle plays an active role in producing urethral occlusion at rest.

The periurethral muscle is another striated muscle important in urethral function. It is part of the pelvic floor musculature and contains a mixture of fast and slow twitch fibers [15]. The periurethral muscle plays an important role in urinary continence by providing additional occlusive force on the urethral wall during increases in
Figure 3: Male urinary bladder. Adapted from [15].
abdominal pressure (e.g. coughing, sneezing) [14]. This muscle is also important for voluntary interruption of the urinary stream [14].

Consequently, a watertight seal is formed by the coaptation of the mucosal surfaces of the urethral lumen and their compression by smooth and striated muscles [14].

1.2.2 Neurophysiology of the Bladder and Urethra

Under normal conditions the smooth muscles of the urinary bladder and urethra are innervated by both the parasympathetic and sympathetic divisions of the autonomic nervous system [16,17,18] (Figure 4). Sympathetic outflow arises from the thoracolumbar (T10-L2) segments of the spinal cord [18] and courses to the bladder in the hypogastric nerve [16]. Parasympathetic fibers arise from segments S2-S4 of the spinal cord [12,16,18]. The fibers course through the pelvic nerve and synapse with postganglionic fibers in the detrusor muscle [16]. Rich synaptic connections between cell bodies of the sympathetic and parasympathetic divisions permit the complex interaction and coordination of the two divisions needed for normal storage and voiding [18] (Figure 4).

Using functional and biochemical studies we have acquired a pretty good knowledge of the distribution of autonomic receptors in the bladder and urethra. The
Figure 4: Autonomic innervation of the urinary bladder
urinary bladder has an abundance of muscarinic receptors throughout the whole detrusor [10,18]. Parasympathetic stimulation of these receptors results in a coordinated bladder contraction [18] (Figure 5). There are also adrenergic receptors present throughout the bladder muscle. Beta-adrenergic receptors predominate in the body of the bladder [10,18]; sympathetic stimulation of beta-adrenergic receptors causes the smooth muscle in the bladder body to relax. Alpha-adrenergic receptors predominate in the bladder base and proximal urethra; sympathetic stimulation of these receptors causes the smooth muscle in this region to contract [18].

All three types of receptors (alpha and beta-adrenergic and muscarinic) are present in the urethra. Alpha-adrenergic receptors predominate over beta-adrenergic receptors in the urethra (Figure 5) [18]. Therefore, sympathetic stimulation of the predominate alpha-adrenergic receptors causes the urethral smooth muscle to contract. This action compresses the mucosal and submucosal layers of the urethra; the urethral outlet resistance is increased and a watertight seal is formed [14]. It is not presently known what exact purpose the muscarinic receptors serve in the urethra. Hassouna et al. [19] have studied the effects of cholinergic and adrenergic agents on the two muscle layers of the proximal urethra in the cat. They propose that
the cholinergic effect, which is predominate on the longitudinal fibers, is important for voiding; contraction of the longitudinal fibers upon muscarinic stimulation shortens the proximal urethra during voiding, thereby decreasing outlet resistance.

The exact neuropharmacological sequences controlling urinary continence and micturition are not known. It has been suggested that the sympathetic nervous system exerts a tonic influence on the bladder [17] and urethra (Figure 6). This influence acts to facilitate the filling and storage phase of micturition by three mechanisms [10]: 1) sympathetic stimulation of predominantly alpha-adrenergic receptors in the bladder base and proximal urethra would increase muscle tone and closure pressure, thereby, maintaining a watertight seal [10,16,17]; 2) at the same time sympathetic stimulation of predominate beta-adrenergic receptors in the bladder body would cause smooth muscle relaxation and increase accommodation [10,16,17]; and 3) also, activation of alpha-adrenergic receptors in the parasympathetic ganglia of the detrusor may have an inhibitory effect on parasympathetic ganglionic transmission [10,17]. This would increase bladder capacity.

Activation of the parasympathetic motor fibers to the bladder produces intense stimulation of the muscarinic receptors of the detrusor [17]. This produces a strong
Figure 5: Receptor distribution in urinary bladder. Adapted from [18].
Figure 6: Bladder storage mechanisms
coordinated detrusor contraction [10] which lasts throughout the duration of voiding [17]. Release of acetylcholine as the sole neurotransmitter is controversial [10] since muscarinic antagonists only partially block the contraction produced by pelvic nerve stimulation.

During voiding the bladder contracts while the bladder outlet and striated muscles relax. The mediator(s) of reflex relaxation of the bladder outlet are unknown. Tonic influence of the sympathetic nervous system on the lower urinary tract is thought to cease during voiding [17].

1.3 PHARMACOTHERAPY OF URINARY INCONTINENCE

Urinary incontinence is a classic symptom of the bladder's inability to fill and store urine adequately [6]. Failure to store may be caused by an increased bladder contractility or decreased outlet resistance or both. Hence pharmacological management of these defects in the filling and storage phase of micturition is directed toward inhibiting bladder contractility or increasing outlet resistance or both [20]. Current therapy is directed at the level of the end organ structures (bladder and urethra) [20]. Therefore, it is not necessary to totally understand the physiology of normal and abnormal voiding function to effectively treat the clinical signs of voiding dysfunctions [20].
Since acetylcholine-induced stimulation of muscarinic receptors in the bladder smooth muscle is the major stimulus for physiological bladder contractions (voluntary and involuntary), the drugs most commonly used to inhibit involuntary contractions are the atropine-like or anticholinergic drugs. These drugs competitively block muscarinic receptors on bladder smooth muscle and depress true involuntary contractions of any etiology [6].

The total bladder capacity increases with a proportionate reduction in symptomatology in patients with detrusor hyperreflexia who are treated with anticholinergic drugs; the volume to the first contraction is increased and the amplitude of contraction is decreased with anticholinergic drug treatment [6]. However, although there is significant clinical improvement in these patients, only partial inhibition of the contractions generally occurs with anticholinergic drugs such as atropine. This is known as the phenomenon of atropine resistance. Atropine resistance refers to the ability of atropine to only partially antagonize bladder contractions induced by neural or direct electrical stimulation of pelvic nerves as opposed to its ability to totally block acetylcholine-induced bladder contractions [6]. Possibly, noncholinergic neurotransmitters are released in addition to acetylcholine by pelvic nerve stimulation [6]. The clinical significance of this phenomenon
is that it is rare to completely inhibit detrusor hyperreflexia with only an antimuscarinic agent or any single drug treatment [6].

Propantheline bromide (Pro-Banthine®) is the most commonly used anticholinergic agent for uninhibited or involuntary contractions of the bladder. There is little difference between the antimuscarinic effect of propantheline and other clinically used anticholinergic drugs on the bladder smooth muscle [6]. All increase bladder capacity. Anticholinergic drugs clinically used include: glycopyrrolate (Robinul®), isopropamide (Daibid®), hyosyamine (Cystospaz®) and anisotropine methylbromide (Valpin®) [6].

The anticholinergic side effects of these drugs include: inhibition of salivary secretion (dry mouth); blockade of iris sphincter muscle (papillary dilatation); blockade of lens ciliary muscle (blurred vision); inhibition of gut motility; tachycardia; and drowsiness [6,21].

Another group of drugs used to inhibit bladder contractility are the musculotropic relaxants (antispasmodics) or direct acting smooth muscle relaxants. This group of drugs has been found in the laboratory to relax bladder smooth muscle by a papaverine-like mechanism (inhibition of phosphodiesterase activity in the cell) [6]. Also, these drugs have been found to have antimuscarinic and local anesthetic properties [4,5]. It is questionable as to how much of
their clinical efficacy is simply due to their antimuscarinic activity [5]. Drugs in this group are oxybutynin chloride (Ditropan®), dicyclomine hydrochloride (Bentyl®), and flavoxate hydrochloride (Urispas®). The side effects of the drugs in this group are primarily anticholinergic.

The urinary bladder and urethral smooth muscles contain alpha-adrenergic and beta-adrenergic receptors [18]. Therefore, the alpha-adrenergic agonists have the potential to facilitate urine storage at least at the peripheral level [8]. For example, stimulation of contractile alpha-adrenergic receptors in the bladder base and proximal urethra with alpha-adrenergic agonists would facilitate outlet closure (increase outlet resistance). Stimulation of inhibitory beta-adrenergic receptors in the bladder body, if this occurs, would facilitate urine storage by inhibiting muscle contractility [8] and by increasing bladder accommodation by smooth muscle relaxation.

Therapy to facilitate urine storage by increasing bladder outlet resistance has been accomplished with drugs that stimulate contractile alpha-adrenergic receptors in the bladder base and proximal urethra (increase outlet resistance). Ephedrine and phenylpropanolamine are such drugs commonly used to increase outlet resistance [4,5]. Ephedrine is a noncatecholamine sympathomimetic drug which produces peripheral release of norepinephrine and also
directly stimulates alpha and beta adrenergic receptors. Ephedrine's beneficial effect has been attributed to its alpha-adrenergic stimulating effects on smooth muscle of the bladder base and proximal urethra [8]. Theoretically, ephedrine's activation of beta-adrenergic receptors should depress detrusor contractility, further improving accommodation but this effect has not been seen clinically [8]. Pseudoephedrine hydrochloride, a stereoisomer of ephedrine, is used for similar indications. The side effects of ephedrine and pseudoephedrine include hypertension, anxiety, insomnia, headache, palpitations, cardiac arrhythmias, and dizziness [21].

Phenylpropanolamine hydrochloride acts similarly to ephedrine and is approximately equal in potency but has less CNS stimulation [6]. The side effects of phenylpropanolamine are similar to the side effects of ephedrine and include: hypertension, anxiety, insomnia, headache, palpitations, cardiac arrhythmias, and dizziness [21].

Estrogens have been found to improve stress incontinence in elderly postmenopausal women [5]. The effects of estrogens have been attributed to mucosal proliferation and enhancement of the alpha-adrenergic contractile response of urethral smooth muscle to endogenous catecholamines [4,5]. Such hormonal treatment is presently under study.

---

3 Stress incontinence is the involuntary loss of urine from the urethra immediately upon an increase in abdominal pressure [14].
Imipramine hydrochloride, a tricyclic antidepressant, has been found to facilitate urine storage. Clinically, the drug decreases bladder contractility and increases outlet resistance [6].

Imipramine has several mechanisms of action including: peripheral and central anticholinergic actions at some sites; central sedative actions; and blockade of the active transport system that is responsible for the reuptake of released norepinephrine and serotonin [4,6] in the presynaptic nerve endings. Imipramine's anticholinergic effect on bladder smooth muscle is very weak [6] and probably does not contribute significantly to the decreased bladder contractility seen clinically. However, imipramine does have a strong direct inhibitory effect on bladder smooth muscle which is neither anticholinergic nor adrenergic [6]. Imipramine's effect on the bladder may be, at least, partially due to blockade of norepinephrine reuptake by adrenergic nerve terminals [20]. This effect would facilitate urine storage by stimulating alpha-adrenergic contractile receptors of the bladder base and proximal urethra (i.e. produce an increase in outlet resistance). It would also stimulate beta-adrenergic receptors of the bladder body to produce smooth muscle relaxation and increased accommodation, further facilitating urine storage [20].
The most frequent side effects reported with imipramine are anticholinergic. Weakness, fatigue, headache, muscle tremors, and excessive sweating are also seen [6].

1.4 CYSTOMETRY

Cystometry is primarily a test of detrusor function [1] which provides information regarding bladder activity during the filling phase of micturition. The method of cystometry measures the pressure/volume relationship of the urinary bladder. It is one of the principle clinical urodynamic tests used to assess bladder function, and hence, is used in the evaluation of urinary incontinence.

The method of cystometry consists of inflating the bladder, via a catheter inserted into the bladder, with measured volumes of physiological saline or sterile water and simultaneously recording the intravesical or bladder pressure. There are several cystometry techniques and modifications. It is beyond the scope of this document to discuss these. See Khanna [22].

A cystometrogram is obtained from plotting the pressure generated in the bladder from the infusion (Figure 7) against the volume infused into the bladder. The normal cystometric curve shows a low intravesical pressure during filling with no evidence of uninhibited contractions.

---

4 Air or CO₂ are use in gas cystometry.
(Figure 7). The first sensation of filling usually occurs when 100 to 200 mls of fluid have been infused into the bladder [22,23]. As the bladder continues to increase in volume during filling there are minimal increases in intravesical pressure (i.e. the bladder is able to accommodate increasing volumes of fluid with little increase in bladder pressure). At full bladder capacity, which is around 400-500 mls, the patient feels a sensation of fullness and a desire to urinate (V=void) [23]. A reflex micturition contraction occurs at capacity with a strong urge to void. The normal individual can inhibit the contraction voluntarily (Figure 7, H=hold) [22,23]. If voiding is initiated, the normal contraction can deliver a normal urine flow rate of 20-30 mls/sec. and completely empty the bladder if the contraction is sustained [24]. There is a wide individual variation in the intravesical pressure produced by the voiding contractions. Some normal individuals void efficiently at very low intravesical pressures while others void at very high pressures (>100 cm H₂O) [22].

An important observation to be made during cystometry is whether the patient demonstrates a detrusor reflex and whether this reflex can be suppressed on command.

---

5 To obtain true intravesical pressure, rectal pressure must be subtracted from total vesicle pressure. Rectal pressure is a measure of abdominal pressure.
Figure 7: Normal cystometrogram. Adapted from [22]. V = voiding, H = Hold (inhibit contraction voluntarily).
Normally there is an absence of involuntary contractions during the filling phase of micturition until voiding occurs [6]. Figure 8 shows a representative cystometrogram of an individual with uninhibited or involuntary contractions occurring during the filling stage of micturition [23]. (The possible etiologies of these involuntary contractions or detrusor hyperreflexia have been discussed). Sometimes these involuntary contractions are interpreted by the patient as an urge to void. Other times, the contractions are not felt at all [8]. As demonstrated in this cystometrogram, the patient is unable to voluntarily suppress these contractions. Finally, at point A, a strong contraction occurs that forces urine around the catheter and empties the bladder. We can see that this condition of increased bladder contractility greatly reduces the bladder's capacity; the capacity in Figure 8 was only 165 mls compared to 500 mls in the normal cystometrogram (Figure 7).
Figure 8: Abnormal cystometrogram. Adapted from [23].
1.5 MICTURITION

In Figure 9 urethral and bladder pressure along with urine flow and an electromyogram (EMG) were recorded during an actual voiding [25]. The urethral pressure remains greater than detrusor pressure during bladder filling, and thereby, maintains urinary continence (Figure 9). Coaptation of the urethral mucosal lining and the occlusive contraction of smooth and striated intramural and pelvic muscles (contraction of striated muscles manifested by an increase in the electromyographic signal) contribute to both a high urethral pressure and watertight seal during bladder filling. At initiation of voiding there is complete relaxation of the striated muscles of the urethra (intramural muscle) and pelvic floor. This relaxation is seen as cessation of EMG activity (no. 1 in Figure 9) [25]. Urethral pressure falls, partially due to relaxation of these muscles and partially due to an active relaxation of the urethra itself [17]. Concomitant with the fall in urethral pressure is a rise in detrusor pressure (i.e. micturition contraction). Urine flow begins when the detrusor and urethral pressures become equal (no. 2 in Figure 9). The pressures remain equal throughout urinary flow [25]. Voluntary interruption of urinary flow is achieved by contraction of the striated intramural and pelvic floor muscles (EMG signal resumes). This is followed by a transient rise in urethral pressure
above detrusor pressure as the urinary stream is interrupted (no. 3 in Figure 9) [26].
Figure 9: Micturition pressure/flow/EMG. Adapted from [25].
1.6 Urinary incontinence in the elderly

Urinary incontinence is a common distressing symptom of various neurogenic or nonneurogenic bladder disease states which is often disabling, especially in the elderly. Too often, urinary incontinence is accepted by physicians and lay persons as an inevitable consequence of advancing age and is denied the careful investigation it deserves [27]. Certain age-related impairments such as prolapsed uterus, benign prostatic hypertrophy [28], gradual decline in overall muscle tone [29], and periurethral atrophy [29] predispose the elderly to urinary incontinence and can also contribute to urinary incontinence. Central nervous system diseases sometimes prevalent in the elderly, such as dementia, Parkinson's disease, multiple sclerosis, and spinal cord disorders are often accompanied by urinary incontinence because central pathways which control urinary function are located closely to the corticospinal tracts [30]. In addition to age-related impairments and neurological diseases, environmental stresses, adverse stimuli, and iatrogenic illness may complicate the presentation of urinary incontinence in the elderly [33].

Urinary incontinence predisposes persons to skin breakdown, infections, and other health problems and thus, is a major health problem in the elderly. In a Washtenaw county (Michigan) survey [32] the prevalence of urinary
The voiding problems of a vast majority of elderly incontinent probably do not receive careful evaluation. Hence, treatment often times emphasizes the use of diapers [28]. There are advantages to using diapers; however, ultimately, diapers do not cure urinary incontinence. If anything, diapering may encourage incontinence by giving the patient the signal that wetting is medically acceptable [28]. It is, therefore, necessary to study and classify the etiologies of urinary incontinence in the elderly to be able to select the most effective treatment.

The pathophysiology of detrusor instability and other causes of urinary incontinence in the elderly are among the important unresolved issues [36]. Answers to these issues together with a better understanding of factors which predispose the elderly to urinary incontinence will assist in the development of preventative strategies [31] as well
as in improved treatments. The successful treatment of urinary incontinence in the elderly may provide the elderly with an improved quality of life with the opportunity to be independent in the community [28,33].

1.7 STATEMENT OF THE PROBLEM

To date, studies of urinary incontinence have been conducted almost exclusively in the young population [33]. While valuable, this information may not be directly applicable to problems of urinary incontinence in the elderly [33]; the young are not afflicted with the many geriatric illnesses. Also, the underlying urinary pathology may be different between young and old populations [33]. Furthermore, the older population, persons 60 and over, has been growing much faster than the nation's population as a whole during the 20th century; growth of this sector of the population is expected to continue at a faster pace than the rest of the population into the 21st century [37]. The total number of incontinent elderly will increase dramatically because of the overall increasing elderly population. Thus, there is a need for significant experimental and clinical research on problems relating to urinary incontinence in the elderly and more generally, on the effects of aging on urinary bladder function.
Based on the prevalence of urinary incontinence in the elderly, I hypothesize that significant changes are occurring in bladder function with age. The bladder is under autonomic regulation and also has myogenic properties; therefore, changes in bladder function can be evaluated by studying the effects of age on autonomic activity and myogenic properties of the bladder. In the following studies, urinary bladder function was examined in 3 age groups of male Fischer 344 rats (5-7 month, 16-18 month, and 22-24 month) to answer the question: Are there age-related changes in the function of the rat urinary bladder?

Male albino Fischer 344 rats were chosen as the animal subject in this research project for several reasons. The life span of the F344 rat is less than 30 months [40], therefore the cost of rearing the animal is reasonable. Also, F344 rats are currently available from the National Institute on Aging. Further, the isolated rat and human bladders respond very similarly to autonomic agents [38].
The specific aims of this research are as follows:

1) To establish a profile on the micturition behavior of young adult (5-7 months), mature adult (16-18 months), and old (22-24 months) rats. Each of the following measurements were evaluated: a) total 24 h water intake; b) total 24 h urine output; c) average micturition volume; d) micturition frequency; and e) the ratio of water intake to urine output.

2) To evaluate the effects of age on the filling and storage function of the urinary bladder.

Part A: The effects of age on the ability of the bladder to contract in response to stimulation with the alpha-adrenergic receptor agonist phenylephrine were investigated.

Part B: The effects of age on the ability of the bladder to relax (expand) in response to stimulation with the beta-adrenergic receptor agonist isoproterenol were investigated.

Part C: The effects of age on bladder function during natural bladder filling and on structural characteristics revealed by in vitro cystometrograms were evaluated.
3) To evaluate the effects of age on the isotonic and isometric bladder contractions of the emptying phase of bladder function. The effects of age on the ability of the bladder to contract in response to stimulation with muscarinic agonists acetylcholine and bethanechol were investigated.

4) To evaluate the effects of age on bladder function after application of a diuretic stress [sucrose in the drinking water (5%)] to 3 age groups of rats.

i) The effects of age on 24 hour fluid intake and urine output were recorded in control and sucrose treated groups.

ii) The effects of age on bladder relaxation in response to beta-adrenergic stimulation with isoproterenol (filling and storage function) were investigated in control and sucrose treated rats.

iii) The effects of age on bladder filling and capacity (in vitro cystometrograms) were determined in control and sucrose treated rats.

iv) The effects of age on bladder isometric contractions in response to muscarinic stimulation with bethanechol (bladder emptying function) were evaluated in control and sucrose treated rats.
v) The effects of age on bladder weight and urine density were evaluated in control and sucrose treated rats.

5) To screen substances (serotonin, histamine, PGF$_{2a}$ oxytocin, and substance P) that may play a role in urinary bladder function using the in vitro whole rat bladder (isometric) preparation.

1.8 ANIMALS

In the following experiments male Fischer 344 (F344) rats, obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana) through the National Institute on Aging, were studied at 5-7 (young adult), 16-18 (mature adult), and 22-24 (old)$^6$ months of age. The animals were housed in groups of 3 or 4 in stainless steel metal cages (11" by 23" by 7") on ground corn cob bedding (Anderson Bed-o-Cob). Purina Rat Chow # 5012 and tap water were provided to the rats ad libitum. The animal facility was maintained at 21° C with a 12 h on/12 h off lighting schedule.

---

$^6$ Average lifespan of Fischer 344 rats is less than 30 months, thus 24 months is considered to be senescence [40].
1.9 LIST OF DRUGS AND CHEMICALS

Table 1

*List of chemicals and drugs used in the project*

<table>
<thead>
<tr>
<th>Chemical or Drug</th>
<th>Manufacturer</th>
<th>Mol. Wt./ Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-phenylephrine HCl</td>
<td>Sigma</td>
<td>203.7</td>
</tr>
<tr>
<td>DL-isoproterenol HCl</td>
<td>Sigma</td>
<td>247.7</td>
</tr>
<tr>
<td>Bethanechol</td>
<td>Sigma</td>
<td>196.7</td>
</tr>
<tr>
<td>Histamine Di-HCl</td>
<td>Sigma</td>
<td>184.1</td>
</tr>
<tr>
<td>Eserine (Physostigmine)</td>
<td>Sigma</td>
<td>324.4</td>
</tr>
<tr>
<td>Serotonin (5-hydroxytryptamine)</td>
<td>Sigma</td>
<td>212.7</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Sigma</td>
<td>500 IU</td>
</tr>
<tr>
<td>Substance P</td>
<td>Sigma</td>
<td>1348</td>
</tr>
<tr>
<td>Prostaglandin F</td>
<td>Sigma</td>
<td>475.6</td>
</tr>
<tr>
<td>Heparin sodium</td>
<td>Invenex</td>
<td>10,000 IU/ml</td>
</tr>
<tr>
<td>Ketamine HCl</td>
<td>Parke-Davis</td>
<td>100 mg/ml</td>
</tr>
<tr>
<td>Urethane (ethyl carbamate)</td>
<td>Sigma</td>
<td>89.09</td>
</tr>
<tr>
<td>Nembutal (pentobarbital)</td>
<td>Abbott</td>
<td>50 mg/ml</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Sigma</td>
<td>182.2</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Fisher Sci.</td>
<td>58.44</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>Mallinckrodt</td>
<td>74.55</td>
</tr>
<tr>
<td>Sodium Phosphate Monobasic</td>
<td>Fisher Sci.</td>
<td>137.99</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>J. T. Baker</td>
<td>147.02</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>EM Science</td>
<td>203.33</td>
</tr>
<tr>
<td>Dextrose</td>
<td>EM Science</td>
<td>180.16</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>EM Science</td>
<td>84.01</td>
</tr>
</tbody>
</table>
CHAPTER II

MICTURITION PROFILE: SPECIFIC AIM 1

2.1 INTRODUCTION

The purpose of the following experiment was to establish a profile on the micturition behavior of young adult (5-7 month), mature adult (16-18 month), and old (22-24 month) rats to assess any changes in micturition that might be occurring with age. Each of the following measurements was evaluated: a) total 24 h water intake; b) total 24 h urine output; c) average micturition volume; d) micturition frequency; and e) the ratio of 24 h water intake to 24 h urine output. The data and their significance are reviewed in the discussion.
2.2 METHODS

The water intake and urinary output in 5-7, 16-18, and 22-24 month old male Fischer 344 rats were monitored for 24 hours. A four-hour micturition frequency and micturition volume were recorded within the 24-hour period.

Male Fischer 344 rats of three age groups, weighing between 300-450 grams, were weighed and placed into metabolism cages (Figure 10). The cages consisted of a top portion which housed the rat and a bottom portion which funneled urine into the collecting vials. The top portion, which housed the rat, contained a food bin and the water bottle. The floor of the top portion was gridded so as to permit urine and not feces from the rat to fall into the bottom portion of the cage where it was funneled into the collecting vials (Figure 10).

The rats were allowed to acclimate to the cages for 24 hours prior to the experiment (starting at 11 A.M. the day before the experiment.) Food and water were provided ad libitum. At 11 A.M. the day of the experiment, the water bottles and collecting flasks (Erlenmeyer) were weighed and adjusted into place. Parafilm was wrapped around the funnel nipple (bottom portion of cage) and the flask to seal out the air (Figure 10).

Frequency and volume of each micturition were determined between 7 P.M. and 11 P.M. At 7 P.M. the cages were
Figure 10: Metabolism cage
covered with black construction paper to provide a dark cycle similar to the animal room. (The paper was removed the next morning at 8 A.M. providing approximately a 12h/12h on/off cycle). Also, at 7 P.M. the collecting flasks were covered with parafilm and set aside. Preweighed and numbered scintillation vials were used to collect the urine during this 4 h period. Each time a rat urinated, the urine was collected in a preweighed scintillation vial and capped. A new vial was used for each micturition. After the 4 hour micturition frequency and volume determination the Erlenmeyer flasks were replaced under each cage's funnel and again sealed with parafilm to the funnel nipple.

The scintillation vials were weighed, and the results recorded. The volume of micturition was obtained by subtracting the empty scintillation vial weight from that of the vial + urine. Since the density of urine was close to 1 the net weight of each vial was taken as the volume of micturition. The frequency of micturition was equal to the number of scintillation vials filled by each rat in the 4 hour period. The total 24 h urine output volume was obtained by adding the net contents of the flasks to the 4 hour total volumes (net wts. of scintillation vials per rat in 4 hours). The total water intake was calculated by subtracting the water bottle weight after the experiment from the initial bottle weight.
To obtain urine density, 0.100 ml (pipetman) of urine was placed into a preweighed microtube and weighed on a Mettler balance. The net weight of the aliquot of urine was obtained by subtracting the weight of the empty microtube from that of the microtube + urine. The net weight of the aliquot of urine was divided by the volume of the aliquot (0.1 ml). The urine density was recorded in g/ml.

Statistical Analysis

The data were analyzed with analysis of variance (ANOVA) (Appendix E.2). Significant results were further analyzed using Student-Newman-Keuls (SNK) multiple comparisons test (Appendix E.2).
2.3 RESULTS

The 24 h water intake of F344 male rats increased significantly with advancing age (Figure 11). A 39% increase in 24 h water intake was observed in the old rats (22-24 month) as compared to the young adult (5-7 month) rats. The old rats also showed a 20% increase in 24 h water intake as compared to mature adult rats (16-18 month). The mature adult rats showed a 16% increase in 24 h water intake compared to young adult rats.

Similarly, the 24 h urine output of male F344 rats increased significantly with advancing age (Figure 12). A 93% increase in 24 h urine output was observed in old rats as compared to young adult rats. The old rats also showed a 43% increase in 24 h urine output as compared to mature adult rats. Mature adult rats showed a 34% increase in urine output compared to the young adult rats.

The average volume of micturition\(^7\) measured over a 4 h period was significantly lower (50%) in young adult animals as compared to mature adult and old animals (Figure 13). There was no significant difference in the average volume of micturition between mature adult and old animals. The total urine output in this 4 hour period increased with advancing age in the same manner that the 24 h urine output increased with advancing age (5-7 mo. < 16-18 mo. <

\(^7\) Volume of micturition or micturition volume refers to the volume of urine voided in each urination.
Figure 11: Total 24 h water intake in three age groups of rats. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * indicates significant difference from other two age groups (p < 0.05).
Figure 12: Total 24 h urine output in three age groups of rats. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * indicates significant difference from other two age groups (p < 0.05).
22-24 mo.). Since the 4 h urine output pattern was representative of the 24 h urine output pattern, it followed that the 4 h micturition volume and frequency measurements were representative of a 24 h cycle ± the effect of diurnal rhythm (Figure 14).

A significant increase in the frequency of urination was observed in the old animals compared to young adult and mature adult animals (Figure 15). No significant difference in frequency of urination was found between young adult and mature adult animals.

The ratio of water intake to urine output was lowest in the old rats as compared to the ratios of water intake to urine output in the young adult and mature adult rats (Figure 16). Although there was not a significant difference in ratios (I/O) in the young adult and mature adult rats, there was a slight decrease in the ratio (I/O) in the mature adult rats from that of the young adult rats.

Contrary to water intake and urine output values in the male F344 rats, urine density decreased with advancing age. The urine density of the young adult animals (0.9967 ± .0030) was significantly greater (p < 0.01) than the urine density in both the mature adult (0.9858 ± .0030) and old animals (0.9807 ± .0050). There was also a slight decrease in urine density from mature adult to old animals.
Figure 13: Average volume of each micturition measured in a 4 h period. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * = significantly different from 16-18 mo. and 22-24 mo. old rats (p < 0.01).
**Figure 1M:** Total volume of urine voided by rats in a 4 h period. Each horizontal bar represents mean ± S.E.M. with $n = 12$ for 16-18 mo. old rats and $n = 15$ for 5-7 and 22-24 mo. old rats. * indicates significant difference from other two age groups of rats ($p < 0.01$).
Figure 15: Total number of times rats voided in a 4 h period. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * = significantly different from 5-7 mo. and 16-18 mo. old rats (p < 0.01).
Figure 76: Ratio of water intake to urine output in a 24 h period. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * = significantly different from 5-7 mo. and 16-18 mo. old rats (p < 0.05).
The body weights in the young adult, mature adult, and old rats were significantly different from each other (Figure 17). The body weight of the mature adult rats was the highest; the body weight of the young adult rats was the lowest with the body weight of the old rats falling between the young adult and mature adult groups.
Figure 17: Body weights in three age groups of rats. Each horizontal bar represents mean ± S.E.M. with n = 12 for 16-18 mo. old rats and n = 15 for 5-7 and 22-24 mo. old rats. * indicates significant difference from other two age groups of rats (p < 0.01).
Twenty-four hour water intake and urine output was found to increased with advancing age in male F344 rats. The old (22-24 month) rats showed both a greater water intake and urine output in 24 hours than young adult (5-7 month) and mature adult (16-18 month) rats. Further, mature adult rats showed a greater water intake and urine output than young adult rats. Bengele et al. [39] also has shown a greater 24 h water intake and urine output in 23 month old F344 rats than in 4 month old rats. Mature adult rats were not used in their studies. Similarly, Beck et al. [40] found an increase in water intake and urine output in 24 month old male F344 rats compared to 6 mo. old rats. However, they did not find measurable differences in either the 24 h water intake or urine output between 6 mo. and 12 mo. old rats. Measurable differences between 6 mo. and 12 mo. old rats may have been masked because Beck et al. [40] expressed water intake and urine output per 100 grams of body weight. When I express water intake and urine output per 100 grams of body weight in each age group, the significant differences (in both 24 h water intake and 24 h urine output) between young adult and mature adult rats disappear. I chose not to express 24 h water intake or 24 h urine output per 100 grams of body weight because the correlation coefficients (Spearman) (Appendix E.1) between
body weight and water intake ($r=0.13 / p<0.63$, 5-7 mo.; $r=-0.02 / p<0.93$, 16-18 mo.) and body weight and urine output ($r=0.24 / p<0.38$, 5-7 mo.; $r=0.22 / p<0.49$, 16-18 mo.) were not significant in either young adult or mature adult age groups. Although the correlation between body weight and water intake, approached significance\(^8\) in the old rats ($r=0.45 / p<0.09$) and the correlation between body weight and urine output was significant ($r=0.58 / p<0.03$) in the old rats, proper comparison of the old group to the other two age groups would preclude normalization of water intake or urine output to 100 grams of body weight. There is also a possibility that the increases in water intake and urine output are not manifested until 16 months (mature adult) of age in which case the studies of Beck et al. [40] (expressing intake and output as 100 g of body wt.) would probably still not have presented a significant difference in either water intake or urine output between young adult and mature adult animals.

There are at least two components that make up urine output; these are micturition frequency and micturition volume (i.e. the volume in each urination). Since 24 h urine output increased with advancing age in male F344 rats, it would be expected that either the frequency of micturition or the volume of micturition would increase, or both would increase with advancing age. In the old animals

\(^8\) significance is $p < 0.05$
both micturition frequency and volume of micturition increased compared to the young adult rats. Mature adult rats took a split; they shared the same volume of micturition (greater than the young adult) with the old animals but the same frequency (less than the old animals) with the young animals. Thus, the 24 h urine output of the mature adult rats fell between the young adult rats (lowest urine output) and the old (highest urine output) rats.

The 24 h water intake paralleled the 24 h urine output in the male F344 rat (i.e. water intake increased with advancing age). Most (2/3) of the water intake into the human body is in the form of fluid. About (1/3) of the water is also taken into the body in the form of food and a very small amount of water is metabolized by the body through the oxidation of food [13].

Over 50% of the water loss from the body is through urine output. Other avenues of water loss include feces, sweat, and insensible loss (water loss through the lungs and skin) [13,41]. Rats do not have sweat glands, thus, no water loss occurs through this mechanism [41]. However, evaporative loss does occur through the saliva during grooming behavior and through insensible loss (water loss through the the skin and lungs) [42]. Evaporative loss gradually decreases with age. Insensible water loss is almost entirely responsible for this decrease in
evaporative water loss with age and seems to be correlated to the decrease in metabolism with advancing age [42].

There was a decrease in the ratio of water intake to urine output with advancing age. The ratio of water intake to urine output was significantly lower in the old rats than young adult rats. This finding indicated that the old rats were losing more of their water intake through urine than the other two age groups. Theoretically, water intake must equal water loss for an animal to be in a state of equilibrium [43]. If all water loss occurred through the kidneys in the form of urine, the ratio of water intake to urine output would be 1. Since there are several channels of water intake (other than liquids) and water loss (other than urine), the ratio of water intake to urine output was not 1 in these male F344 rats. In fact, the ratio of water intake to urine output ranged from 2.06 in the old animals to 2.94 in the young adult animals reflecting that up to 50% of the water intake through liquids in the old animals was lost through the kidney as urine. Thus, since evaporative water loss appears to decrease with age [42], it seems appropriate that the body would handle the age-related increase in water intake by shunting more water through the other channels of water loss (kidney and feces). As a result the ratio of water intake to urine output would decrease with advancing age, indicating increased
water loss through the kidney as the rat aged. This is just what happened in this set of experiments. The ratio of water intake to urine output decreased significantly from the young adult rats to the old rats.

Similarly, the urine concentrating ability decreases in the male F344 rat with advancing age [39,40]. The urine osmolality before and after 40 h water deprivation is significantly lower in 23-24 month old rats than in 6 month old rats [39,40]. Also, the urine output is higher before and after 40 h water deprivation in 23-24 month old rats compared to 6 month old rats [39,40]. My findings are in agreement with Bengele et al. [39] and Beck et al. [40]. The urine density decreased with advancing age, thereby indicating a decrease in the urine concentrating ability [44]. The urine density of the old rats was significantly lower than that of the young adult rats. Also, as mentioned earlier, the urine output was higher in the old rats than in the young adult and mature adult animals.

Bengele et al. [39] found that the indices of ascending limb solute delivery and transport and solute gradient for water reabsorption are similar between the young adult and old animals. Therefore, they postulated that the urine concentrating ability defect in the aged animals is most likely secondary to a decrease in water permeability along the collecting duct epithelium. Further, from studies
conducted in the presence and absence of vasopressin, Bengele et al. [39] postulated that the decrease in water permeability in the collecting ducts might be due to an impairment of the ability of ADH to increase water permeability of the collecting duct epithelium. Beck et al. [40] further investigated the effect of ADH on the urine concentrating ability in male F344 rats {Vasopressin's hydro-osmotic effect is believed to be mediated through cAMP generation in the collecting duct cells [40]}. The results of their studies suggest that impairment of the urine concentrating ability in 24 month old rats may be, in part, due to a decrease in vasopressin-dependent cAMP generation in old 24 month old rats. This finding supports the postulate of Bengele et al. [40].

In summary, I have established a micturition profile for young adult, mature adult, and old F344 male rats. The following were observed: 1) 24 h urine output as well as 24 h water intake increased significantly with advancing age; 2) the highest urinary output was manifested by both an increase in micturition volume and urination frequency in the old rats compared to the young adult rats; 3) The ratio of water intake to urine output decreased with advancing age (ratio of the old rats was significantly lower than that of the young adult rats); and 4) Urine density decreased with advancing age (urine density in old rats
was significantly lower than in young adult rats). The decline of urine density observed in the aging male F344 rat, suggested a decrease in the urine concentrating ability of the kidney with age, and hence, a diminished water conservation in the kidney. From the data generated in this study it appeared that the diminished water conservation in the aging kidney [45] contributed to the increase in urine output in the aged rat. The polydipsia or increased water intake observed with advancing age may either be an adaptation to the increased urine output observed with advancing age, or it may be a contributory cause of the increased urine output with age. Whether or not the increased water intake seen with aging can be connected to the increased urine output was not determined by this experiment. However, it is important to note in this experiment that increased urine output (micturition frequency and micturition volume) occurred with age in the male F344 rat; therefore, bladder usage increased with age. The implications of this higher urine turnover and bladder usage in the old animals compared to young adult animals is discussed in the next chapter.
CHAPTER III
BLADDER FILLING AND STORAGE: SPECIFIC AIM 2

3.1 INTRODUCTION

The lower urinary tract performs the two functions of urine storage and urine emptying [4,5]. It has been suggested that the sympathetic nervous system exerts a tonic influence on the bladder and urethra [17] and, thereby, facilitates the filling and storage phase of micturition [10]. Facilitation is thought to occur by sympathetic stimulation of predominately alpha-adrenergic receptors in the bladder base and proximal urethra. This action prevents urine loss by increasing muscle tone and closure pressure in the outlet (i.e. maintains a watertight seal) [10,16,17] during bladder filling and storage. At the same time sympathetic stimulation of predominately beta-adrenergic receptors in the bladder body causes smooth muscle relaxation and increases accommodation [10,16,17], further facilitating the filling and storage function.

The following studies were designed to evaluate the effects of age on the filling and storage function of the urinary bladder. For clarity, this chapter has been
divided into three main sections: 1) in part A the effects of age on the ability of the bladder to contract in response to stimulation with the alpha-adrenergic receptor agonist phenylephrine were investigated. 2) in part B the effects of age on the ability of the bladder to relax (expand) in response to stimulation with the beta-adrenergic receptor agonist isoproterenol were investigated; and 3) in part C both the effects of age on bladder function (micturition reflex) during natural filling and the effects of age on the structural characteristics of the bladder were investigated.
3.2 METHODS

3.2.1 Whole bladder Preparation

In the following procedures the \textit{in vitro} whole bladder rabbit preparation of Levin \textit{et al.} [46] was modified to the rat urinary bladder.

Three age groups (5-7 months, 16-18 months, and 22-24 months) of male Fischer 344 albino rats weighing 320-430 grams (Harlan Suppliers) were anesthetized initially with a subcutaneous (s.c.) injection of urethane (1.2 g/kg). After one hour the rats were injected a second time with 1/2 of the initial dose of urethane s.c. The urinary bladder was exposed by making a 3.5 cm midline incision through the lower abdominal wall. The prostate gland and surrounding tissue were gently removed from the bladder exposing the ureters and urethra. The ureters were ligated with 000 suture close to the bladder body and cut distally to the ties. The urethra was isolated and cut as far distally to the bladder body as possible. The bladder was emptied and cannulated via the urethra with PE50 (Intramedic) tubing (16 in.) \{infused with Tyrode's\textsuperscript{9} solution (0.051 ml/min.\} and mounted in a 30 ml tissue bath containing warmed Tyrode's solution (37°) gassed with 95\% O\textsubscript{2} and 5\% CO\textsubscript{2}. The PE50 tubing was attached to a 24 g

\textsuperscript{9} Tyrode's solution contained NaCl 12.5 mM, KCl 2.7 mM, NaH\textsubscript{2}PO\textsubscript{4} 0.4 mM, CaCl\textsubscript{2} 1.8 mM, MgCl\textsubscript{2} 0.5 mM, NaHCO\textsubscript{3} 23.8 mM, Glucose 11 mM.
1/2 in. hypodermic needle which was placed onto a 4-way stopcock connected directly to a Statham pressure transducer (Gould). The third connection to the 4-way stopcock was attached to an infusion/withdrawal pump (Harvard Apparatus Dover, MA) via 2.5 feet of tygon tubing (3 mm i.d.) (Figure 18).

After adjusting the bladder base to the same level as the pressure transducer, the bladder was allowed to equilibrate for 30 minutes. During this time the bladder was washed three times with warmed and gassed Tyrode's solution. The stopcock was open to the pressure transducer, bladder, and infusion pump during this equilibration period and during the infusion. Following equilibration, intravesicular pressure was recorded in response to a continuous infusion of Tyrode's solution (room temperature) at 0.051 ml/min into the bladder (by means of the infusion pump) until a pressure twice the plateau pressure\(^{10}\) was reached (Figure 19). The volume of Tyrode's infused into the bladder that correlated with twice the plateau pressure was defined as the structural capacity of the bladder (Figure 19). In this way a pressure-volume relationship (cystometrogram) was generated for each bladder. The variability in bladder structural capacity between and within age

\(^{10}\) Bladders from all age groups displayed the rapid rise in pressure at the terminal limb of the cystometrogram. The bladder was not infused beyond twice the plateau pressure so as not to overdistend the smooth muscle.
Figure 18: Whole rat bladder preparation. Isometric ("closed system").
groups made plotting intravesical pressure against volume infused infeasible. Therefore, intravesical pressure was plotted against the percent of bladder capacity (ml) instead of volume infused into the bladder.

Figure 20 shows the effect of various infusion rates on intravesical pressures generated in *in vitro* whole bladder cystometrograms. As the infusion rate increased the intravesical filling pressure increased almost proportionately (i.e. starting at 0.104 ml/min doubling the infusion rate approximately doubled the intravesical pressure). The significance of this is discussed in Chapter 7.
Figure 19: Determination of structural capacity
Figure 20: The effect of infusion rate on intravesical pressure. Data shown are means from the in vitro cystometrograms of three young adult rats. Infusion speeds are designated in order of increasing rate as 0.051 ml/min. (Δ); 0.104 ml/min. (○); 0.212 ml/min. (▲); 0.536 ml/min. (●); and 1.072 ml/min. (●). Each point represents the mean ± S.E.M. for 3 bladders (the same 3 bladders were used for each successive rate of infusion). Curves are expressed as percent bladder capacity for each bladder.
Figure 20

[Graph showing pressure (cmHg) against percentage of bladder capacity with different rate (ml/min) values: 1.072, 0.536, 0.212, 0.104, 0.051.]
3.2.1.1 Drug Dose-Response Curves - Isometric Contraction

After generating a cystometrogram and hence, establishing a structural capacity for each bladder, the bladder was emptied by gravity. To empty the bladder by gravity the hypodermic needle hub was removed from the 4-way stopcock at a level below the bladder (i.e. the pressure transducer was lowered below the level of the bladder base before removing the needle hub) and held below the level of the bladder to drain the entire volume of the bladder. The hub was then replaced onto the 4-way stopcock and the transducer returned to bladder base level. The bladder was allowed to equilibrate for 20 minutes after which time it was filled to a specific volume relative to the bladder's structural capacity (i.e. the bladder was filled to 1/4, 1/2, 3/4, of structural capacity or to structural capacity) with the infusion pump open to both the bladder and the pressure transducer. (Filling the bladder to a volume relative to its structural capacity negated any size-related effects on results). When the desired volume had been infused into the bladder, the 4-way stopcock was closed to the infusion pump allowing only the bladder to be connected to the pressure transducer ("closed system") (Figure 18). The bladder was then equilibrated for 40 minutes and washed 3 times within this period. During this time the pressure in the

structural capacity will also be referred to as full bladder throughout this and subsequent chapters.
bladder dropped to a steady baseline slightly above starting pressure. The pressure within the bladder reached the same baseline regardless of the volume of Tyrode's infused.

Bethanechol cumulative dose-response curves were performed on four different bladder volumes (1/4, 1/2, 3/4 of full bladder and full bladder). The dose-response curves for all other drugs (acetylcholine, phenylephrine, oxytocin, substance P, PGF$_2$α, histamine, and serotonin) were performed on the bladder at two different volumes (1/4 and 3/4 capacity). These two volumes were chosen based on their closeness to actual ranges of micturition volume (Section 2.3).

Acetylcholine dose-response curves were generated in the presence of 300 nm physostigmine (Eserine®) for 1/4 full bladder and 100 nm of physostigmine for 3/4 full bladder.

Drugs were added in a cumulative manner starting with a concentration of $3\times10^{-9}$ M and increasing by 1/2 log units to $3\times10^{-3}$ M. Two dose response curves were done on each bladder, one at 1/4 full bladder and the other at 3/4 full bladder. One drug per bladder was assayed in this system. The bladder was washed 3-4 times between dose-response curves and allowed to equilibrate approximately 30-40 minutes after each volume filling to 1/4 full or 3/4 full bladder.
In this "closed system" [46] the increase in intravesical pressure produced by the contracting bladder in response to the addition of smooth muscle stimulating drugs was not associated with a change in bladder volume (isovolumetric). Therefore, there was no change in muscle fiber length as the smooth muscle contracted. Hence, the increases in intravesical pressure upon addition of drugs were due to isometric bladder contractions rather than isotonic contractions, the latter involving a change in muscle length.

3.2.1.2 Drug-Dose Response Curves - Isotonic Contraction

As in the isometric "closed system" a cystometrogram was generated for each bladder to determine the structural capacity of the bladder.

Again, the bladder was emptied after doing the cystometrogram, allowed to equilibrate and filled to a specific volume (1/4, 1/2, 3/4, or full bladder) via the infusion pump. When the bladder was filled to the desired volume the stopcock was closed to the infusion pump. After the intravesical pressure dropped to a steady baseline, a water filled expulsion tube was connected to the system via port A of the 4-way stop-cock attached to the expulsion tube (Figure 21 and Appendix D). The 4-way stop cock on the
transducer was open to the bladder, transducer, and expulsion tube. The expulsion tube was set at a level relative to the bladder which allowed no flow of water. This was also the level of the steady baseline intravesical pressure (polygraph tracing). Drugs were added in a cumulative manner permitting the bladder to finish expelling liquid before adding the next drug concentration. Expelled liquid was collected in preweighed microtubes with caps. Each tube represented a volume expelled by the bladder in response to a concentration of drug.

Bethanechol dose-response curves were performed on the bladders at 4 different volumes (1/4, 1/2, 3/4, and full). Two dose-response curves were done on each bladder, 1 drug per bladder. Acetylcholine curves were done in the presence of physostigmine. The dose response curves for acetylcholine and phenylephrine were performed on the bladder at 2 volumes (1/4 and 3/4 full).

In the expulsion system the volume of the bladder diminished as it expelled a certain quantity of liquid with the addition of each drug concentration (i.e. as the bladder contracted to expel its contents the muscle fibers shortened to conform to the smaller bladder volume). Since a change in muscle length was associated with the contractions in the expulsion system the contractions were isotonic in nature. Therefore, isotonic contractions of the
Figure 21: Isotonic expulsion system
bladder were measured as the amounts of liquid expelled from the bladder.

3.2.1.3 Drug Dose Response Curves - Expansion

As in the isometric "closed system" and isotonic "expulsion" system a cystometrogram was generated for each bladder to determine the structural capacity of the bladder.

Again, the bladder was emptied by gravity after performing the cystometrogram and allowed to equilibrate for 30-40 minutes. The bladder was filled to a 1/4 or 3/4 full via the infusion pump. When the desired volume had been infused into the bladder the stopcock was closed to the infusion pump. The bladder was allowed to equilibrate to a steady baseline pressure. A horizontal expansion tube (Figure 22 and Appendix D) filled with Tyrode's solution was attached to the system at the infusion pump connection in the same manner as the expulsion tube. The expansion tube was set at a level relative to the bladder where the pressure in the tube was equal to the pressure in the bladder. Again, as in the expulsion tube set up, this critical pressure was viewed on the polygraph tracing as the steady baseline pressure.

The beta-adrenergic receptor agonist isoproterenol was added to the tissue bath in a cumulative manner \(3 \times 10^{-9} \text{ M}\).
Figure 22: Bladder expansion setup
to $3 \times 10^{-4}$ M). The bladder expanded upon each addition of isoproterenol. As the bladder expanded Tyrode's solution was withdrawn from the end of the horizontal tube (opposite of expulsion). The distance that the liquid withdrew from the end of the tube after each addition of isoproterenol was measured in mm and later converted to volume (mls).

As in the expulsion system, the bladder volume and hence, muscle length changed with each addition of isoproterenol. Therefore, the bladder expansion produced by isoproterenol was an isotonic phenomenon.

**Statistical Analysis**

The data were analyzed with analysis of variance (ANOVA) (Appendix E.2). Significant results were further analyzed using Student-Newman-Keuls (SNK) multiple comparisons test (Appendix E.2).
3.2.2 In Vivo Cystometrograms

Male F344 rats weighing 300-450 grams were anesthetized with 120 mg/kg s.c. ketamine followed by an injection of 20 mg/kg pentobarbital i.p. ten minutes later. The lower abdomen and neck regions were shaved with electric clippers. The fully anesthetized rat (assessed by lack of corneal reflex and foot pinch withdrawal) was placed onto a wooden dissecting board and secured with tape straps. A tracheal tube was inserted (Appendix A). The rat's body temperature was maintained with a heat lamp connected to a temperature probe inserted into the rat's rectum. One hour after the pentobarbital injection the bladder was exposed through a midline incision into the abdominal wall. The bladder was partially lifted onto the outer abdominal wall which was covered with saline-soaked gauze. A purse-string suture was sewn onto the bladder dome (Appendix B) using 6-0 thread. The PE50 catheter, flushed with saline, was inserted into a hole within the purse string suture and secured. The warmed saline drip was adjusted over the exposed bladder to keep it moist throughout the procedure. The bladder was allowed to equilibrate for approximately 30 minutes or until the rat regained consciousness (assessed by corneal reflex and whisker movement) after which the bladder was emptied by gravity. Following a 5 minute period of equilibration a cystometrogram was run at
0.021 ml/min. until a reflex micturition contraction occurred. One cystometrogram was run on each rat (Figure 23).

The intravesical volume and pressure at which the reflex contraction occurred in each cystometrogram were recorded for each rat. The data were analyzed using Student's t test and ANOVA and SNK (Appendices E.2 and E.7).
Figure 23: In vivo cystometrogram setup. T = trachea; B = bladder.
In the following procedure the urinary bladder of the rat was allowed to fill naturally (i.e. no saline was infused into the bladder). To shorten the duration of the experiment, the rat was given 10% w/v mannitol i.v. to induce a diuresis which sped up the natural filling time. The intravesical pressure was monitored as the bladder filled naturally. Male F344 rats weighing 300-450 grams were anesthetized with 120 mg/kg s.c. ketamine followed by an induction injection of 20 mg/kg pentobarbital i.p. ten minutes later. The lower abdomen and neck regions were shaved with electric clippers. The fully anesthetized rat (assessed by lack of corneal reflex and foot pinch withdrawal) was placed onto a wooden dissecting board and secured with tape straps. A tracheal tube was inserted (Appendix A). The jugular vein was cannulated with PE50 tubing (Appendix C). The rat's body temperature was maintained with a heat lamp connected to a temperature probe inserted into the rat's rectum. One hour after the pentobarbital injection the bladder was exposed through a midline incision into the abdominal wall. The bladder was partially lifted onto the outer abdominal wall which was covered with saline-soaked gauze. A purse-string suture was sewn onto the bladder dome (Appendix B) using 6-0 thread. The PE50 catheter, flushed with saline, was
inserted into a hole within the purse string suture and secured. The catheter was connected to a pressure transducer. The warmed saline drip was adjusted over the exposed bladder to keep it moist throughout the procedure. The bladder was allowed to equilibrate for approximately 30 minutes or until the rat regained consciousness (assessed by corneal reflex and whisker movement)\textsuperscript{12} after which the bladder was emptied by gravity. Following a 5 minute period of equilibration a natural-fill cystometrogram was generated by infusing 10% mannitol into the jugular vein at 0.021 ml/min and allowing the bladder to fill naturally. One cystometrogram was run on each rat (Figure 24).

The intravesical volume and pressure at which the reflex contraction occurred in each cystometrogram were recorded for each rat. The data were analyzed using Student's t test and ANOVA and SNK (Appendices E.2 and E.7).

\textsuperscript{12} Although conscious, the rat was immobilized and anesthetized with ketamine.
Figure 24: Natural fill cystometrogram setup. T = trachea; J = jugular vein; B = bladder.
3.3 ALPHA-ADRENERGIC ACTIVITY: PART A

3.3.1 Introduction

The alpha-adrenergic receptors present in the rat bladder [47] have recently been identified as alpha\textsubscript{1}-adreno-receptors [48]. Although alpha-adrenergic receptors are found throughout the bladder, they have been found to predominate in the bladder base and urethra [18,49].

Activation of alpha-adrenergic receptors in the bladder is thought to mediate smooth muscle contraction [18]. Therefore, it has been suggested that tonic sympathetic stimulation of alpha-adrenergic receptors in the bladder base and urethra produce smooth muscle contraction in this region, and thereby, increase outlet resistance [10]. Hence, leakage of urine during bladder filling and storage is prevented.

Activation of alpha-adrenergic receptors involves a rise in cystolic free Ca\textsuperscript{2+} which exerts its intracellular effects by binding to calmodulin, the Ca\textsuperscript{2+}-dependent regulatory protein. The Ca\textsuperscript{2+}-calmodulin complex activates myosin light-chain kinase which phosphorylates the light chains of myosin. The phosphorylation of myosin is required for formation of cross bridges between actin and myosin that result in contraction [50].

The intent of the following experiments was to determine the effects of age on the ability of the bladder to
contract both isometrically (pressure) and isotonically (expulsion) in response to stimulation with the alpha-adrenergic agonist phenylephrine. Also, the effect of age on the time of expulsion (isotonic) in response to phenylephrine was evaluated.
3.3.2 Results

The alpha-adrenergic receptor agonist phenylephrine was capable of increasing intravesical pressure (isometric) in a dose-related manner. There were no significant age-related differences in the log ED\textsubscript{50} values determined from phenylephrine pressure (isometric) dose response curves for either the 1/4 or the 3/4 filled bladders (Table 2). Similarly, there were no significant age-related differences in the maximal pressure response of bladders filled to 1/4 or 3/4 full in response to phenylephrine stimulation (Table 3).

Phenylephrine was also capable of eliciting bladder expulsion (isotonic) of fluid in a dose-dependent manner. The log ED\textsubscript{50} values determined from phenylephrine isotonic dose-response curves for both the 1/4 and 3/4 filled bladders revealed no significant age-related differences (Table 4). All bladders expelled 100% of their initial volume in response to phenylephrine stimulation. Therefore, there were no age-related differences in the maximal isotonic response of the bladders to phenylephrine stimulation.

Also, the amount of time (min.) it took bladders filled to 1/4 or 3/4 full to expel in response to a 1/2 ED\textsubscript{50} dose of phenylephrine was not significantly different between the age groups (Table 5). Correspondingly, the percent of
Table 2

Log $ED_{50}$ values: phenylephrine pressure D-R curves

Effects of age on the log $ED_{50}$ values obtained from phenylephrine isometric (pressure) dose-response curves performed on in vitro bladders.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Log $ED_{50}$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4 full</td>
</tr>
<tr>
<td>5-7</td>
<td>5</td>
<td>-5.333 ± .089</td>
</tr>
<tr>
<td>16-18</td>
<td>5</td>
<td>-5.328 ± .050</td>
</tr>
<tr>
<td>22-24</td>
<td>5</td>
<td>-5.291 ± .062</td>
</tr>
</tbody>
</table>

p < 0.8966    p < 0.9962

Each value represents the mean ± S.E.M. of 5 bladders in each age group. Phenylephrine pressure dose response curves were performed on bladders filled to either 1/4 or 3/4 capacity (1/4 full or 3/4 full). The log $ED_{50}$ values were obtained from non-linear regression analysis (Appendices E.4,5,6) of phenylephrine dose response curves. No age-related differences were found.
Table 3

Maximal pressure response: phenylephrine D-R curves

Effects of age on the maximal pressure response obtained from phenylephrine isometric (pressure) dose-response curves performed on in vitro bladders.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Maximum Pressure (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4 full</td>
</tr>
<tr>
<td>5-7</td>
<td>5</td>
<td>1.68 ± .20</td>
</tr>
<tr>
<td>16-18</td>
<td>5</td>
<td>1.75 ± .19</td>
</tr>
<tr>
<td>22-24</td>
<td>5</td>
<td>1.92 ± .12</td>
</tr>
</tbody>
</table>

p < 0.6191 p < 0.3186

Each value represents the mean ± S.E.M. of 5 bladders in each age group. Phenylephrine pressure dose-response curves were performed on bladders filled to either 1/4 or 3/4 full. No age-related differences were found.
Table 4

Log ED$_{50}$ values: phenylephrine isotonic D-R curves

Effects of age on the log ED$_{50}$ values obtained from isotonic (expulsion) dose-response curves of phenylephrine performed on in vitro bladders.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Log ED$_{50}$ Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4 full</td>
</tr>
<tr>
<td>5-7</td>
<td>5</td>
<td>-6.696 ± .15</td>
</tr>
<tr>
<td>16-18</td>
<td>5</td>
<td>-6.822 ± .08</td>
</tr>
<tr>
<td>22-24</td>
<td>5</td>
<td>-6.777 ± .16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p &lt; 0.8026</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 bladders in each age group. Phenylephrine isotonic dose-response curves were performed on bladders filled to either 1/4 or 3/4 full. The log ED$_{50}$ values were obtained from non-linear regression analysis of phenylephrine dose-response curves. All bladders expelled 100% of their initial volume. No age-related differences were found.
the initial volume expelled by the bladders filled to 1/4 or 3/4 full in response to the 1/2 ED\textsubscript{50} dose was not significantly different between the age groups.
Table 5

Table 5

Time of (phenylephrine) bladder expulsion

Effects of age on the time of bladder expulsion and the percent initial volume expelled in response to a 1/2 ED$_{50}$ dose of phenylephrine.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Time Expulsion$^a$</th>
<th>% Initial Vol. Expelled$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4 full</td>
<td>3/4 full</td>
</tr>
<tr>
<td>5-7</td>
<td>4</td>
<td>7.5 ± .96</td>
<td>13.5 ± .87</td>
</tr>
<tr>
<td>16-18</td>
<td>4</td>
<td>6.8 ± .48</td>
<td>13.5 ± 1.8</td>
</tr>
<tr>
<td>22-24</td>
<td>4</td>
<td>8.3 ± 1.7</td>
<td>14.8 ± 1.7</td>
</tr>
</tbody>
</table>

$p<0.6599$ $p<0.8017$ $p<0.2364$ $p<0.8411$

Each value represents the mean ± S.E.M. of 4 bladders in each age group. $^a$Time of expulsion is the time (min.) it took the bladders filled to either 1/4 or 3/4 full to finish expelling buffer in response to stimulation with a 1/2 ED$_{50}$ dose of phenylephrine (1/2 ED$_{50}$ dose=8.6x10E-8 M, 1/4 full; 1/2 ED$_{50}$ dose = 3.6x10E-7 M, 3/4 full). $^b$% initial volume expelled is the percent of the initial volume, either 1/4 or 3/4 full, that was expelled by the bladder after stimulation with a 1/2 ED$_{50}$ dose of phenylephrine. No age-related differences were found. Nonparametric statistics (Kruskal-Wallis) were performed on the ratios (%) (Appendix E.3).
3.3.3 Discussion

Stimulation of the *in vitro* rat bladder with the alpha-adrenergic agonist phenylephrine produced a dose-dependent increase in intravesical pressure. Presumably, the increase in intravesical pressure was via smooth muscle contraction caused by activation of alpha-adrenergic receptors in the bladder body and base. Likewise, the dose-dependent expulsion produced by phenylephrine occurred through activation of contractile alpha-adrenergic receptors.

The lack of any significant age-related differences in the log ED$_{50}$ values of either the isotonic (expulsion) or the isometric (pressure) phenylephrine dose response curves suggested that the sensitivity of the bladder smooth muscle to the contractile action of phenylephrine did not change with advancing age. Further, all bladders emptied 100% of their initial volume, thus giving evidence to an absence of age-related change in the maximal isotonic expulsion response of the bladder. Similarly, there were no significant age-related changes in the maximal pressure response of bladders stimulated with phenylephrine, or in the time of phenylephrine-induced expulsion.

Hence, the absence of any age-related differences in the parameters measured in response to phenylephrine stimulation indicated that at least functionally, the
postjunctional alpha-adrenergic activity of the bladder did not change with age.
3.4 BETA-ADRENERGIC ACTIVITY: PART B

3.4.1 Introduction

The rat detrusor muscle contains both excitatory (contractile) alpha-adrenergic receptors and inhibitory (relaxatory) beta-adrenergic receptors [47,51]. Beta-adrenergic receptors have been found to predominate in the bladder body [10]. Attempts to subclassify beta-adrenergic receptors in the bladder body have not been consistent [10]. Some groups have found populations of both beta₁ and beta₂ receptors in the body, and others have found populations of beta receptors not belonging to either class. However, two groups [47,52] have found that the bladder body of the rat contains relaxatory beta₂-adrenergic receptors.

Tonic sympathetic stimulation of beta-adrenergic receptors in the bladder detrusor is thought to facilitate bladder filling and storage by increasing bladder accommodation [10] via smooth muscle relaxation and diminished bladder tone¹³ [18]. Beta-adrenergic stimulation of the in vitro whole rabbit bladder results in increased bladder volume, decreased intravesical pressure, and inhibition of cholinergic stimulated bladder contraction [53]. Thus, these findings support facilitation of bladder filling and storage by adrenergic stimulation of beta-adrenergic receptors located on the bladder body.

¹³ Tone is defined as the resistance of the muscles to passive elongation or stretch [55].
Stimulation of beta-adrenergic receptors by agonists such as norepinephrine or isoproterenol usually results in an increase in cAMP concentration (via activation of adenylate cyclase) and relaxation of smooth muscle [18,54]. A cAMP-dependent phosphorylation event is probably involved, although the precise pathway remains unknown [54].

The intent of the following experiment was to determine the effects of age on the ability of the bladder to relax (expand) in response to stimulation with the beta-adrenergic receptor agonist isoproterenol.
3.4.2 Results

The beta-adrenergic receptor agonist isoproterenol was capable of increasing bladder volume in a dose-dependent manner. The log ED$_{50}$ values determined from isoproterenol dose-response curves for both 1/4 and 3/4 full bladders revealed no significant age-related differences (Table 6). Similarly, the absolute volume accepted by the expanding bladders (filled initially to 1/4 or 3/4 full) in response to isoproterenol stimulation showed no significant age-related differences. In other words, the maximal response of the bladder to isoproterenol did not change with advancing age. However, as might be expected, in all age groups the bladders filled initially to 1/4 full accepted a greater volume of buffer in response to isoproterenol stimulation than did the bladders filled to 3/4 full (Table 6).

For illustrative purposes, absolute volumes accepted by bladders filled initially to 1/4 and 3/4 full in response to isoproterenol stimulation were expressed as percent of total capacity and percent of initial volume (Table 7).
Table 6

Isoproterenol expansion: Log $ED_{50}$ values and volume

Effects of age on isoproterenol-induced bladder expansion: maximal response and log $ED_{50}$ values from isotonic (expulsion) dose-response curves of isoproterenol performed on *in vitro* bladders.

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>Absolute Volume$^a$ (ml)</th>
<th>1/4 full $^b$</th>
<th>3/4 full $^b$</th>
<th>1/4 full</th>
<th>3/4 full</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>0.67±.09</td>
<td>0.37±.04</td>
<td>-5.417±.04</td>
<td>-5.071±.11</td>
<td></td>
</tr>
<tr>
<td>16-18</td>
<td>0.53±.05</td>
<td>0.30±.03</td>
<td>-5.493±.06</td>
<td>-6.103±.13</td>
<td></td>
</tr>
<tr>
<td>22-24</td>
<td>0.62±.06</td>
<td>0.31±.04</td>
<td>-5.511±.06</td>
<td>-5.992±.09</td>
<td></td>
</tr>
<tr>
<td>p&lt;0.3108</td>
<td>p&lt;0.3298</td>
<td>p&lt;0.4217</td>
<td>p&lt;0.7744</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Isoproterenol dose-response curves were performed on bladders that were initially filled to either 1/4 or 3/4 full. Each value represents the mean ± S.E.M. of 5 bladders in each age group for 1/4 full bladders and 7 bladders in 5-7 mo. and 8 bladders in 16-18 mo. and 22-24 mo. for 3/4 full. $^a$Absolute volume was the amount of buffer accepted by the expanding bladder in response to isoproterenol dose-response curves. $^b$Log $ED_{50}$ values were obtained from non-linear regression analysis of isoproterenol dose-response curves. No age-related differences were found.
### Table 7

**Isoproterenol expansion: percent volume**

Effects of age on percent total capacity and percent initial filling volume of isoproterenol-induced bladder expansion.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>% Total Capacity</th>
<th>% Initial Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/4 full</td>
<td>3/4 full</td>
</tr>
<tr>
<td>5-7</td>
<td>61.0 ± 4.3</td>
<td>95.6 ± 1.4</td>
</tr>
<tr>
<td>16-18</td>
<td>66.0 ± 5.6</td>
<td>94.1 ± 1.3</td>
</tr>
<tr>
<td>22-24</td>
<td>67.0 ± 3.4</td>
<td>93.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.5911</td>
<td>p&lt;0.3685</td>
</tr>
</tbody>
</table>

Isoproterenol dose-response curves were performed on bladders that were initially filled to either 1/4 or 3/4 full. Each value represents the mean ± S.E.M. of 5 bladders in each age group for 1/4 full bladders and 7 bladders in 5-7 mo. and 8 bladders in 16-18 mo. and 22-24 mo. for 3/4 full.  

a% Total Capacity was the amount of buffer accepted by the expanding bladder (ml) + initial bladder volume (1/4 or 3/4 full)(ml) divided by the total structural capacity of the bladder (ml).  
b% Initial Volume was the amount the bladder expanded (ml) in response to isoproterenol divided by the initial bladder volume (1/4 or 3/4 full)(ml).  

All values were converted to arcsines for statistical analysis. Structural capacities were not significantly different between the ages. No age-related differences were found.
3.4.3 Discussion

The *in vitro* whole rat bladder expanded in a dose-dependent manner in response to isoproterenol addition to the tissue bath. Presumably, bladder expansion in response to beta-adrenergic receptor stimulation with isoproterenol was via smooth muscle relaxation caused by activation of beta-adrenergic receptors in the detrusor.

Evidence showing a lack of significant age-related differences in the log $ED_{50}$ values determined from isoproterenol dose-response curves suggested that there were no age-related differences in bladder sensitivity to the smooth muscle relaxatory activity of isoproterenol. Similarly, the lack of significant age-related difference in the absolute volume accepted by the bladder in response to isoproterenol addition suggested that the maximal response of the bladder also did not change with advancing age.

Hence, the absence of any age-related effects in the log $ED_{50}$ values or the maximal response of the bladders to isoproterenol stimulation indicated that, at least functionally, the postjunctional beta-adrenergic activity of the bladder did not change with advancing age.
3.5 BLADDER FUNCTION/STRUCTURAL CHARACTERISTICS: PART C

3.5.1 Introduction

3.5.1.1 Myogenic Tone

Smooth muscle tone, that partial steady contraction of muscle which determines firmness [55], is maintained by activity of the contractile elements [10]. Intrinsic characteristics of smooth muscle (myogenic tone), prolonged direct smooth muscle excitation via local tissue factors or circulating hormones, and tonic activity initiated extrinsically in the autonomic nervous system may contribute to the activity of the contractile elements [10] maintaining smooth muscle tone. The intravesical pressure during bladder filling is thought to be a result of this smooth muscle tone, presence or absence of phasic excitatory or inhibitory neuronal impulses at the cell membrane or ganglia, and the elastic properties of smooth muscle [10].

The bladder wall is able to maintain a moderate amount of pressure on fluid in the bladder in the absence of neuronal influences (i.e. myogenic tone [56]). Levin et al. [46] were able to generate a standard 3-part cystometric curve, similar to an in vivo cystometric curve, with their in vitro whole rabbit bladder preparation. The 3-part cystometric curve consisted of: 1) an initial rapid rise in

14 Myogenic tone—tone that is independent of external chemical stimulation and seems to be stretch dependent [57].
pressure concomitant with the start of bladder filling; 2) a large plateau phase with relatively large increases in volume accompanied with only a slight increase in pressure (bladder accommodation); and 3) a rapid rise in pressure with only a slight increase in volume at the terminal limb of the curve. The ability to generate a standard cystometrogram in the *in vitro* whole bladder indicates that bladder tone is an innate property of the smooth muscle and is not dependent on neural innervation [53].

Furthermore, all types of tone are dependent on the presence of extracellular calcium [57]. Levin *et al.* [53] demonstrated that the *in vitro* bladder has significant tone (myogenic) by performing cystometrographic curves in the presence and absence of the calcium chelator EGTA. The volume of the bladder at specific intermediate pressures in the presence of EGTA was approximately double the volume in the absence of EGTA (i.e. in the presence of EGTA the cystometrographic plateau pressure was lower and the curve was shifted to the right compared to control). These results demonstrate an increase in bladder compliancy (decreased bladder tone) in the absence of calcium, and thus, support the $Ca^{2+}$ dependency of bladder tone.

---

15 Bladder tone does not require neuronal innervation, however, the micturition reflex does.
The urinary bladder wall is composed of both passive and active elements. The passive elements (collagen, elastin, and elements of smooth muscle that do not require energy for their function) are important in determining the bladder's response to filling [10]. For example, a gradual increase in bladder volume with a consequent slow stretch on the bladder wall elements results in a very low increase in pressure or wall tension; whereas, a rapid increase in bladder volume with a consequent rapid stretch on the bladder wall elements results in a large increase in pressure or tension [10]. In the latter case, the large increase in pressure gradually returns to almost pre-stretch level even though the muscle is still lengthened by the large increase in bladder volume [10]. The ability of smooth muscle to change length greatly with only transient changes in tension results from a phenomenon called stress-relaxation [56]. This may result from the loose arrangement of actin and myosin filaments in smooth muscle. The filaments of the stretched muscle presumably rearrange their bonds and gradually allow the sliding process to take place, thus allowing the tension to return to its original pre-stretch tension [56]. Reverse stress-relaxation is the converse of stress-relaxation and occurs when smooth muscle is shortened. At first, all tension is lost from the muscle immediately. Gradually, after a few minutes tension
returns [56]. The phenomenon of stress-relaxation is known to be independent of nervous regulation [58] and, therefore, is a characteristic of myogenic tone.

The standard cystometrographic curve (Figure 25) can be divided into two major regions which collectively represent the passive properties of the urinary bladder. The plateau region, 1, represents the function of the elastic properties of smooth muscle [1]. The terminal limb, region 2, represents the function of the elastic properties of collagen. It can be assumed that initial bladder filling stretches primarily smooth muscle fibers (plateau phase, region 1) excluding the majority of loosely aligned [58] or coiled [59] collagen fibers. As the bladder wall is further stretched by filling, more collagen fibers are placed under stress; the collagen fibers uncoil or straighten [59]. Since the collagen fibers have a higher elastic constant\textsuperscript{16} [58] than smooth muscle, they strongly resist further stretching and the terminal limb of the cystometrogram rises rapidly (region 2).

The passive elements of the bladder (collagen, etc.) may also be important in bladder contractility during the emptying phase of micturition [10].

\textsuperscript{16} The elastic constant is the measure of stiffness or rigidity.
Figure 25: Passive properties of a standard cystometrogram. Region 1, the plateau region, represents the function of elastic properties of smooth muscle. Region 2, the terminal limb, represents the function of elastic properties of collagen.
For example collagen fibrils within the smooth muscle bundles have been shown to act as force transmitters between contracting cells of activated muscle [70].

3.5.1.2 Micturition Reflex

Sensations from the bladder and urethra are returned to the CNS via fibers that travel with the sympathetic (T9-L2), parasympathetic and somatic (S2-4) nerves [23]. Sensations from stretch receptors in the bladder are carried by the parasympathetic nerves and sensations of pain, touch, and temperature are carried by sympathetic fibers [23]. A simple reflex arc, formed by afferent and efferent fibers of S2-4 segments of the spinal cord, controls bladder function (micturition reflex). Its activity is under voluntary control of the cerebral cortex [23]. The exact neurophysiological sequences controlling the coordination between motor and sensory input to the bladder are not understood.

Activation of the micturition reflex in the same animal (as a result of sensation of stretch receptors in the bladder) probably occurs at a certain intramural or bladder wall tension [60]. By Laplace's equation\(^{17}\) tension is proportional to pressure, therefore activation of the micturition reflex can also be said to occur at a certain pressure

\[ T = \frac{P \times r}{2}, \text{ where } T = \text{tension}, \ P = \text{pressure}, \text{ and } r = \text{radius} \]
within the bladder. The pressure level at which the micturition reflex is activated is determined by the rate of urinary excretion [60] (i.e. the rate of bladder filling). For example, Klevmark [60] has found that the bladder can achieve large volumes at low intravesical pressures which occur when the rate of excretion is low. Conversely, increases in rate of excretion will increase intravesical pressure, and thus lower the volume threshold for micturition [60].

A common procedure for evaluating bladder function in both animals and humans is to measure the response of the urinary bladder to filling with increasing volumes of fluid (cystometrogram). Structural changes in the bladder can be inferred by the nature of cystometrograms generated in the in vitro whole bladder. However, the micturition reflex which is dependent on neuronal influences must be studied in vivo; the natural filling cystometrogram permits evaluation of the micturition reflex.

The following studies were designed to evaluate the effects of age on bladder function (micturition reflex) during natural bladder filling cystometrograms and on structural characteristics of the bladder during in vitro bladder filling cystometrograms.
3.5.2 Results

In cystometrograms performed on natural filling bladders, the pressure at micturition (IPIMC) (Figure 26) was significantly lower in the 5-7 month rats than in the 16-18 month or 22-24 month old rats (Table 8). The pressure at micturition in the 5-7 month rats was only 50% of the pressure at micturition in 16-18 month and 22-24 month rats. However, there were no significant age-related differences in the bladder volume at micturition in this experiment. Also, the durations of mannitol infusion (duration of the cystometrogram in each rat, minutes) were not significantly different (p < 0.2534) between the age groups (5-7 mo., 54.8 ± 12.7; 16-18 mo., 71.7 ± 6.6; 22-24 mo., 51.2 ± 5.9, means ± S.E.M.).

The in vivo bladders of three age groups of rats (n = 8 for 5-7 mo.; n = 11 for 16-18 mo. and 22-24 mo.) infused with saline at a rate of 0.021 ml/min. showed no age-related differences in either the pressure (cm Hg) at micturition (5-7 mo., 0.63 ± 0.09; 16-18 mo., 0.73 ± 0.08; 22-24 mo., 0.69 /p < 0.7085, means ± S.E.M.) or in the contraction (5-7 mo., 0.54 ± 0.16; 16-18 mo., 0.49 ± 0.05; 22-24 mo., 0.34 ± 0.05 /p < 0.3306, means ± S.E.M.).

Structural bladder capacity determined from cystometrograms performed on in vitro whole bladders (Section 3.2.1)
Figure 26: Natural Fill Cystometrogram. An actual tracing of a cystometrographic curve generated by a natural filling bladder in an *in vivo* 5-7 month old rat. Natural fill cystometrograms in 16-18 month and 22-24 month old rats showed this same pattern with the exception of a higher IPIMC. IPIMC stands for intravesical pressure to initiate the micturition contraction. A 10% IV mannitol solution was infused at the start of the recording.
Table 8

Natural fill cystometrograms

Effects of age on bladder capacity and pressure at micturition in natural fill cystometrograms.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Volume (ml)</th>
<th>IPIMC (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>0.58 ± .160</td>
<td>0.34 ± .047*</td>
</tr>
<tr>
<td>16-18</td>
<td>0.66 ± .070</td>
<td>0.70 ± .069</td>
</tr>
<tr>
<td>22-24</td>
<td>0.45 ± .030</td>
<td>0.68 ± .080</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 6 rats in each group. * indicates significant difference from other 2 age groups (p < 0.01). Bladder capacities were not significantly different between the age groups (p < 0.3368). IPIMC = intravesical-pressure-required-to-initiate-a-micturition-contraction.
did not differ significantly between the three age groups (Table 9). In fact, these structural bladder capacities were remarkably close in value.

The average plateau pressures\textsuperscript{18} generated in the \textit{in vitro} cystometrograms (Section 3.2.1) of isolated whole bladders decreased with advancing age (Figure 27). The average plateau pressure (cm Hg) in the 22-24 month rat bladders (0.88 ± .07, mean ± S.E.M.) was significantly lower (40%) than the average plateau pressures in 5-7 month rat bladders (1.46 ± .095). Bladders from 16-18 month rats demonstrated an average plateau pressure that fell between the two age groups, thus giving further indication of an age-related trend of decreasing plateau pressures in \textit{in vitro} cystometrograms.

\textsuperscript{18} Average plateau pressure is determined by averaging the pressures at 40, 50, and 60% structural bladder capacity for each rat.
Table 9

Structural bladder capacities

Effects of age on structural bladder capacity.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Structural Bladder Capacity (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>75</td>
<td>1.67 ± .063</td>
</tr>
<tr>
<td>16-18</td>
<td>79</td>
<td>1.65 ± .038</td>
</tr>
<tr>
<td>22-24</td>
<td>79</td>
<td>1.69 ± .050</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of rat bladders in 5-7 mo., 16-18 mo., and 22-24 mo. old rats. Structural bladder capacities were not significantly different between the 3 age groups (p < 0.8028).
In vitro cystometrograms. Age groups are designated as 5-7 month (▲); 16-18 month (■); and 22-24 month (●). Each point represents the mean ± S.E.M. for 7 bladders in each age group. The curves are expressed as percent of bladder capacity for each bladder. * indicates the average plateau region (40, 50, and 60%) is significantly different from the 5-7 month group (p < 0.05).
Bladder weights increased significantly with advancing age (Table 10). Bladders from 5-7 month rats were the lowest in weight; the bladders from 22-24 month rats were the highest in weight with the bladders from 16-18 month rats falling between the young adult (5-7 mo.) and old (22-24 mo.) age groups. Bladder weights expressed as percent of body weights were also significantly different between the three age groups. However, the percentages did not increase consistently with advancing age. Bladder weight made up $0.023 \pm 0.0005\%$ (mean $\pm$ S.E.M.) of the body weight in the young adult (5-7 mo.) rats. Bladder weight as a percent of body weight was significantly ($p < 0.05$) lower in the mature adult (16-18 mo.) rats ($0.022 \pm 0.0003\%$) than in either the young adult or old rats. The old rats demonstrated the highest percentage of bladder weight to body weight ($0.024 \pm 0.0004\%$). These results were not surprising since the 16-18 mo. rats had the highest body weight (Table 10).
Table 10

Bladder and body weights

Effects of age on body weights and bladder weights in three age groups of rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Bladder weight (g)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>78</td>
<td>0.0831 ± .0017*</td>
<td>0.356 ± .003**</td>
</tr>
<tr>
<td>16-18</td>
<td>80</td>
<td>0.0931 ± .0014*</td>
<td>0.424 ± .003**</td>
</tr>
<tr>
<td>22-24</td>
<td>80</td>
<td>0.0986 ± .0015*</td>
<td>0.405 ± .003**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 78 rat bladders in 5-7 mo. rats and 80 rat bladders in 16-18 and 22-24 mo. rats. * indicates significant difference from the other 2 age groups (p < 0.05). ** indicates significant difference from other 2 age groups (p < 0.01).
3.5.3 Discussion

The relationship of volume to intravesical pressure is used as an index of the physiological state of the urinary bladder and its reflex pathways [61]. As the normal in vivo bladder is filled, intravesical pressure remains relatively constant until reflex activity of the bladder is stimulated [62].

In the in vivo bladder of young adult (5-7 mo.) rats allowed to fill naturally, the intravesical pressure initiating the micturition reflex was 1/2 of the pressure necessary to initiate a micturition contraction in mature adult and old rats. The higher intravesical pressure required to initiate the micturition contraction (IPIMC) in mature adult and old animals may indicate that the sensitivity to bladder filling decreased in these two age groups. To illustrate, on a standard cystometrographic curve (Figure 28) the pressure to initiate micturition in the older animals (mature adult and old) appeared later on the curve than the young adult animals. The later micturition response of the older animals to bladder natural filling suggested a decrease in their ability to sense bladder wall stretch during filling.

The higher intravesical pressure necessary to initiate the micturition contraction in the older animals translated
into a higher volume of micturition on both the cystome-
trographic curve (Figure 28) and possibly in the actual volume of micturition (Section 2.3) in mature adult and old rats. Thus, the IPIMC paralleled the volume of micturition both in order and magnitude in the 3 age groups of rats. IPIMC in the mature adult and old rats was approximately twice the IPIMC in young mature rats. Similarly, the volume of micturition in the mature adult and old rats was approximately twice that of the young adult rats. Therefore, from the above evidence, it could be hypothesized that initiating a micturition reflex contraction at a higher pressure resulted in a higher micturition volume in this system.

In natural fill cystometrograms, a higher bladder volume (at the time of the micturition reflex contraction) was not associated with the increased IPIMC in the older rats. Although the natural fill cystometrograms were the most physiological of the cystometrograms (in vivo and in vitro), there were some conditions which were not physiological such as the open abdomen and purse string suture in the dome of the bladder that may account for this discrepancy.

The increased micturition volume (Chapter 2, Figure 13) observed in the mature adult and old animals compared to the young animals suggested that the bladder was either
Figure 28: Standard cystometrogram. Positioning of the intravesical pressures to initiate the micturition contraction (IPIMC) on a standard cystometrographic curve for 3 age groups of rats. Y = young mature (5-7 mo.) M = mature adult (26-38 mo.) O = old (22-24 mo.) Volumes of micturition for corresponding IPIMC's are extrapolated from the cystometrographic curve.
becoming structurally larger or more compliant (accommodating)\textsuperscript{19} with advancing age. Since the structural capacities remained the same in all three age groups, the bladder probably became more accommodating or compliant with aging.

Increased accommodating ability of the bladder with advancing age may be manifested as a decreased cystometrographic plateau pressure (in vitro cystometrograms) observed with aging. Changes in plateau pressure indicate a change in the elastic properties of smooth muscle. More specifically, the decrease in cystometrographic plateau pressures in the bladders of old rats suggested a decline in bladder elasticity\textsuperscript{20} or resiliency. Decreased elasticity observed in the bladders of old rats probably occurred through the increased usage of the bladder in these animals. Increased bladder usage was manifested by both an increase in urine frequency and micturition volume (Figure 15 and Figure 13).

Diuretic and diversion data strongly support bladder usage as a major determinant of bladder elasticity. Rats fed 5% sucrose water provide a model for increased urine turnover or diuresis which results in increased bladder usage. In vitro bladders taken from these rats appear to be less elastic and more accommodating than control bladders \textsuperscript{[63]}. The intravesical pressures generated in in vitro

\textsuperscript{19} More accommodating = the bladder is able to accept fluid with less rise in intravesical pressure than before.

\textsuperscript{20} the property of returning to an initial form or state following deformation.
bladders from sucrose-fed rats were much lower than controls [63]. These data along with data from Chapter 5 support the findings of a decrease in bladder elasticity, manifested by decreased cystometrographic plateau pressures, through increased bladder usage. Conversely, the urine-diverted bladders of dogs (a model for bladder disuse) show a very steep volume-pressure curve [61,62]. In other words, the accommodating ability of the bladder is severely decreased. Therefore, in both increased bladder use and bladder disuse, the intravesical pressure developed at any given volume depends upon the previous degree of filling and the amount of fiber stretching to which the bladder has been subjected [61]. Also, muscle strips from disused and nondistended rat bladders showed a higher degree of stiffness or elasticity than controls [64]. Again, bladder tone was altered by bladder disuse. Therefore, the filling-emptying mechanism is the most important factor in establishment of bladder tone [62], and consequently, the accommodating ability of the bladder.

Increases in bladder weight are an indication of bladder distention in diuresed rats [63]. Therefore, the increased bladder weight with advancing age might have possibly been associated with the increased bladder usage [micturition volume (distention) and frequency] with age.
In summary, two main points were presented: 1) The intravesical pressure to initiate the micturition contraction increased with advancing age. Paralleled with this increase in pressure was an increase in micturition volume (mict. profile) with age. Possibly, a decrease in the rat's sensation of bladder pressure with advancing age was responsible for the higher IPIMC and resulting increase in micturition volume. 2) Plateau pressures of in vitro cystometrograms decreased significantly with age. This finding probably indicated a decreased elasticity of the bladder smooth muscle. Evidence from diversion and diuresis studies suggest such changes in bladder elasticity (tone) may be related to bladder usage. The increased urine frequency as well as the increased volume of micturition observed with age suggested that increased bladder usage in the old animals may account for the decreased plateau pressures.
CHAPTER IV

URINARY BLADDER EMPTYING: SPECIFIC AIM 3

4.1 INTRODUCTION

It is generally agreed that the coordinated bladder contraction responsible for bladder emptying is parasympathetically induced [65]. Muscarinic receptors have been demonstrated in the bladder body and base of the rat [10]; concentrations of muscarinic receptors are higher in the bladder body than in the base [66]. Recently, these muscarinic receptors have been identified as the M₂ variety [65,67,68].

Intense stimulation of muscarinic receptors of the detrusor with acetylcholine or other muscarinic agonists results in a strong detrusor contraction which is blocked by atropine [65]. This coordinated contraction which empties the bladder consists of both isometric and isotonic properties [46]. The initial contraction occurs against a closed bladder outlet with a resulting rapid rise in intra-vesical pressure. Since the bladder is contracting against a closed outlet there is no change in bladder volume (i.e. no change in muscle fiber length). thus the contraction is
isometric (isovolumetric). This contraction, which is initially isometric in nature, transforms into an isotonic contraction as the bladder outlet opens to allow outflow of urine (Figure 29). The smooth muscle fibers shorten as the bladder gets smaller with the expulsion of urine. Hence, the in vivo bladder is both an isometric and isotonic organ.
**Figure 29:** Two part bladder contraction. The top portion represents the intravesical pressure in a contracting bladder. The lower portion represents outflow of urine (ml/sec). At the arrows the bladder contraction becomes isotonic (top) as the outlet opens and outflow of urine proceeds (bottom).
Figure 29
The intent of the following experiments was to evaluate the effects of age on both the isometric and isotonic bladder contractions during the emptying phase of bladder function. Isometric contractions were evaluated using the pressure (isometric) in vitro whole bladder preparation (Section 3.2.1.1). Isotonic contractions were evaluated using the expulsion (isotonic) in vitro whole bladder preparation (Section 3.2.1.2).

4.2 RESULTS

The muscarinic receptor agonists acetylcholine and bethanechol were capable of increasing intravesical pressure (isometric) in a dose-related manner. There were no significant age-related differences in the log ED$_{50}$ values determined from either acetylcholine or bethanechol pressure (isometric) dose response curves for either the 1/4 or the 3/4 filled bladders (Table 11). Figure 30 shows bethanechol isometric (pressure) dose-response curves for the 1/4 filled bladders of the 3 age groups. Figure 31 shows bethanechol isometric (pressure) dose-response curves for the 3/4 filled bladders of the 3 age groups. Similarly, there were no significant age-related differences in the maximal pressure response of bladders filled to 1/4 or 3/4 full in response to acetylcholine or bethanechol stimulation (Table 12).
Table 11

*Log ED*$_{50}$ values: ACH and bethanechol pressure D-R curves*

Effects of age on the log ED$_{50}$ values obtained from isometric (pressure) dose-response curves of acetylcholine and bethanechol.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Vol.</th>
<th>5-7 mo.</th>
<th>16-18 mo.</th>
<th>22-24 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>-6.114±.23</td>
<td>-5.920±.16</td>
<td>-6.040±.17</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>-6.345±.087</td>
<td>-6.449±.073</td>
<td>-6.404±.02</td>
<td></td>
</tr>
<tr>
<td>Bethanechol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>-4.407±.080</td>
<td>-4.628±.031</td>
<td>-4.506±.11</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>-4.811±.077</td>
<td>-4.754±.032</td>
<td>-4.756±.04</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 or 6 bladders in each age group for acetylcholine and bethanechol dose response curves. Vol. is the volume of the bladder (1/4, 3/4) at which the dose response curve was performed. Log ED$_{50}$ values were obtained from non-linear regression analysis of dose-response curves. No significant age-related differences were demonstrated for either drug.
Table 12

Maximal pressure response: ACH and Beth D-R curves

Effects of age on maximal pressure responses obtained from isometric (pressure) dose-response curves of acetylcholine and bethanechol.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Vol.</th>
<th>5-7 mo.</th>
<th>16-18 mo.</th>
<th>22-24 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>1/4</td>
<td>2.81±.33</td>
<td>2.89±.15</td>
<td>3.21±.28</td>
</tr>
<tr>
<td></td>
<td>3/4</td>
<td>1.74±.20</td>
<td>1.58±.11</td>
<td>1.78±.18</td>
</tr>
<tr>
<td>Bethanechol</td>
<td>1/4</td>
<td>3.71±.63</td>
<td>3.13±.21</td>
<td>3.06±.23</td>
</tr>
<tr>
<td></td>
<td>3/4</td>
<td>3.02±.51</td>
<td>2.44±.25</td>
<td>2.53±.31</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 bladders in each age group for acetylcholine dose-response curves and 6 bladders in age group for bethanechol dose-response curves. Vol. is the volume of the bladder (1/4 or 3/4) at which the dose response curve was performed. No age-related differences were demonstrated for either drug.
Figure 30: Bethanechol pressure D-R curves (1/4 full). Effects of age on bethanechol isometric (pressure) dose-response curves performed on the 1/4 full bladder are shown. Each point represents the mean ± S.E.M. of 6 bladders in each age group of rats {5-7 mo. (▲), 16-18 mo. (●), and 22-24 mo. (■)}. 
Figure 30
Figure 31: Bethanechol pressure D-R curves (3/4 full). Effects of age on bethanechol isometric (pressure) dose-response curves performed on the 3/4 full bladder are shown. Each point represents the mean ± S.E.M. of 6 bladders in each age group of rats {5-7 mo. (▲), 16-18 mo. (●), and 22-24 mo. (■)}. 
Figure 31
Acetylcholine and bethanechol were also capable of eliciting bladder expulsion (isotonic) of fluid in a dose-dependent manner. The log ED$_{50}$ values determined from acetylcholine and bethanechol isotonic dose-response curves for 1/4 and 3/4 filled bladders revealed no significant age-related differences (Table 13).

Also, the amount of time (min.) it took bladders filled to 1/4 or 3/4 full to expel in response to a 1/2 ED$_{50}$ dose of bethanechol was not significantly different between the age groups (Table 14). Correspondingly, the percent of the initial volume expelled by the bladders filled to 1/4 or 3/4 full in response to the 1/2 ED$_{50}$ dose was not significantly different between the age groups.

Although there were no age-related differences in muscarinic dose-response curves, there were bladder-volume related differences. For example, in the expulsion system the 1/4, 1/2, 3/4, and full bladders were all capable of expelling 100% of their contents (Tyrode’s solution). However, there was a shift in potency; as the initial volume in the bladder (1/4, 1/2, 3/4, or full) increased, a higher concentration of bethanechol was required to empty the bladder. Data graphed as the percentage of total volume expelled reveal an apparent shift to the right (Figure 32). The same pattern was generated by each age group.
Table 13

*Log ED$_{50}$ values: ACH and Beth isotonic D-R curves*

Effects of age on the log ED$_{50}$ values obtained from isotonic expulsion dose-response curves of acetylcholine and bethanechol.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Vol.</th>
<th>5-7 mo.</th>
<th>16-18 mo.</th>
<th>22-24 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>-7.438±.12</td>
<td>-7.254±.16</td>
<td>-7.491±.25</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>-7.058±.055</td>
<td>-6.869±.080</td>
<td>-7.106±.17</td>
<td></td>
</tr>
<tr>
<td>Bethanechol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>-6.259±.21</td>
<td>-6.129±.061</td>
<td>-6.317±.18</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>-5.837±.12</td>
<td>-5.713±.099</td>
<td>-5.822±.11</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 5 or 6 bladders in each age group for acetylcholine and bethanechol dose-response curves. Vol. is the volume of the bladder (1/4, 3/4) at which the dose response curve was performed. Log ED$_{50}$ values were obtained from non-linear regression analysis of dose-response curves. All bladders expelled 100% of their initial volume. No age-related differences were demonstrated for either drug.
Table 14

*Time of bethanechol bladder expulsion*

Effects of age on the time of bladder expulsion and the percent initial volume expelled in response to a 1/2 ED₅₀ dose of bethanechol.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>N</th>
<th>Time Expulsionᵃ</th>
<th>% Initial Vol. Expelledᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>4</td>
<td>25.0±2.1</td>
<td>24.5±5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.8±3.6</td>
<td>22.3±2.4</td>
</tr>
<tr>
<td>16-18</td>
<td>4</td>
<td>18.3±2.7</td>
<td>21.0±5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.0±1.1</td>
<td>21.8±1.7</td>
</tr>
<tr>
<td>22-24</td>
<td>4</td>
<td>21.8±2.9</td>
<td>31.8±7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.0±1.5</td>
<td>18.8±2.0</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.2415</td>
<td>p&lt;0.2289</td>
<td>p&lt;0.7933</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.6727</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 bladders in each age group. ⁴Time of expulsion is the time (min.) it took the bladders filled to either 1/4 or 3/4 full to finish expelling buffer in response to stimulation with a 1/2 ED₅₀ dose of bethanechol (1/2 ED₅₀ dose = 2.9x10⁻⁷ M, 1/4 full; 1/2 ED₅₀ dose = 8.1x10⁻⁷ M, 3/4 full). ⁵% initial volume expelled is the percent of the initial volume, either 1/4 or 3/4 full that was expelled by the bladder after stimulation with a 1/2 ED₅₀ dose of bethanechol. No age-related differences were found. Nonparametric statistics (Kruskal-Wallis) were performed on the ratios (%) (Appendix E.3).
Figure 32: Effect of bladder volume on bethanechol expulsion. Bethanechol dose-response curves were performed on the bladder at 4 different volumes \{1/4 (●), 1/2 (●), 3/4 (▲), and full (■)\}. Each point represents the mean ± S.E.M. of 6 bladders from old rats.
Another bladder-volume related effect occurred in the muscarinic-induced isometric bladder contraction. Bethanechol produced a dose-dependent increase in bladder isometric (pressure) contraction. The largest maximal pressure response was attained by bladders of all age groups filled to 1/4 full (Figure 33). The weakest maximal pressure response was attained by bladders of all age groups filled to full capacity. The effect of bladder volume on bethanechol-induced isometric contraction was clearly demonstrated when the data were graphed as the percent of maximal contraction attained by the 1/4 filled bladders (i.e. as a percent of total maximal contraction) (Figure 33).
Figure 33: Effect of bladder volume on bethanechol contraction. Bethanechol pressure dose-response curves were performed on bladders at 4 different volumes (1/4 (▲), 1/2 (■), 3/4 (●), and full (●)). Each point represents the mean ± S.E.M. of 6 bladders.
Figure 33
4.3 DISCUSSION

Stimulation of the *in vitro* rat bladder with muscarinic receptor agonists acetylcholine or bethanechol produced a dose-dependent increase in intravesical pressure. Presumably, the increase in intravesical pressure was via smooth muscle contraction caused by activation of the abundant muscarinic receptors in the bladder body. Likewise, the dose-dependent bladder expulsion produced by acetylcholine and bethanechol occurred through smooth muscle contraction via activation of muscarinic receptors in the detrusor.

The lack of any age-related differences in the log ED$_{50}$ values of either the isotonic (expulsion) or the isometric (pressure) acetylcholine or bethanechol dose response curves suggested that the sensitivity of the bladder smooth muscle to the contractile action of these muscarinic agonists did not change with advancing age. Further, all bladders emptied 100% of their initial volume, thus giving evidence to an absence of age-related change in the maximal isotonic expulsion response of the bladder. Similarly, there were no age-related changes in the maximal pressure responses of the bladders stimulated with acetylcholine or bethanechol. Again, as with phenylephrine, the time of expulsion in response to a 1/2 ED$_{50}$ dose of bethanechol did not change with advancing age.
Hence, the absence of any age-related differences in the parameters measured in response to acetylcholine or bethanechol stimulation indicated that, at least functionally, the postjunctional muscarinic activity of the bladder did not change with age.

Although there were no significant age-related differences in the parameters of muscarinic activity measured, there were bladder volume effects on both isometric and isotonic contractions. Bladders at all 4 volumes were capable of expelling 100% of their contents in response to bethanechol dose-response curves. The curves shifted to the right in potency; the concentrations of bethanechol necessary to empty the bladders increased as the bladder volumes increased. These results are in agreement with Levin et al. [46].

As the bladder volume increased from 1/4 full to full, the maximal pressure response elicited by bethanechol decreased. These results were in agreement with Levin et al. [46] and Carpenter [63]. Carpenter [63] applies the Laplace relation to this situation. The pressure that develops during a bladder contraction varies inversely with the volume of fluid in the bladder [63]. The Laplace relation is: Tension = (pressure × radius)/2. The equation shows that bladder pressure is proportional to its wall

21 It is assumed that the bladder is a sphere and the bladder radius = 0 when the bladder is empty.
tension. As the bladder muscle develops a fixed amount of tension during a response, it follows that less pressure will be produced if the radius (volume) of the bladder is increased. This phenomenon occurred in this group of experiments.
CHAPTER V
DIURETIC STRESS: SPECIFIC AIM 4

5.1 INTRODUCTION

Autonomic bladder function, specifically postjunctional alpha and beta adrenergic activity of bladder filling and storage and muscarinic activity of bladder emptying, did not change significantly during aging in the rat (Chapters 3 and 4). It could be hypothesized that the aging rat has been able to adapt to various changes that occur with aging, and thus, has been able to maintain autonomic postjunctional bladder function (i.e. the "adaptive capacity" [69] of the aging bladder has been able to maintain postjunctional autonomic bladder function).

It was of interest to test whether or not the "adaptive capacity" of the aging bladder would be reduced under a physiologically stressful situation. In this study diuresis was chosen as the model of physiological stress for the following reasons: 1) it was relatively easy to induce; 2) physiologically, the stress factor was applied in a relative manner to each age group (i.e. each age group controlled their own fluid intake and consequent diuresis);
3) diuresis could and may be a real physiological stress in the elderly human population (e.g. diuretic drugs or adult onset diabetes), and therefore, the diuretic rat could serve as a model for bladder function in this group; and 4) diuresis provided a means to compare extrinsically increased bladder usage in the young adult rats to the natural increase in bladder usage which occurs during aging.

The intent of the following experiments was to evaluate the effects of age on bladder function after application of a diuretic stress (5% sucrose solution for drinking). The parameters of bladder function that were investigated in control and sucrose-fed rats of 3 ages included: 1) a 24-hour fluid intake and 24 hour urine output; 2) bladder relaxation by isoproterenol (filling and storage function); 3) bladder isometric contraction induced by bethanechol (bladder emptying function); 4) cystometrographic plateau pressures and structural capacity; and 5) bladder weight and urine density.

5.2 METHODS

The following group of experiments was performed on the isolated bladders of rats which had received sucrose (5%) in drinking water for 8 weeks prior to sacrifice. The purpose of giving sucrose (5%) in the drinking water was to induce diuresis in each age group of rats (i.e. apply a
diuretic stress to each age group of rats). The filling and storage and emptying functions of the bladder were evaluated in each rat.

An *in vitro* cystometrogram was performed on each isolated bladder by the whole bladder *in vitro* method described in Section 3.2.1. The structural capacity and plateau pressures were obtained from the *in vitro* cystometrograms as previously described. Isometric contractions of the bladder were evaluated by performing pressure dose-response curves with the muscarinic agonist bethanechol (Section 3.2.1.1). Smooth muscle relaxation was evaluated in each bladder by performing expansion dose-response curves with isoproterenol (Section 3.2.1.3).

The urine density and bladder weights were measured for each rat as described in chapter 2.

Results were analyzed by 2-way analysis of variance and Student-Newman Keuls multiple comparisons test (Appendix E.3).

## 5.3 RESULTS

There were no age-related effects on 24 h fluid intake in either the controls (water for drinking) or sucrose-fed (5% sucrose solution for drinking) rats (*p* < 0.1514). However, sucrose-fed rats showed a significant increase in fluid intake (*p* < 0.0006) compared to control rats. The average
fluid intake in sucrose-fed rats was approximately twice the average fluid intake in control animals (Figure 34).

Similarly, there were no age-related effects on 24 h urine output in either the control or sucrose-fed rats \((p < 0.1956)\). However, again, sucrose-fed rats showed a significant increase \((p < 0.0006)\) in urine output compared to control rats. The average urine output in the sucrose-fed rats was approximately 5 times greater than the average output in control rats (Figure 35). (There were no interactions between age and treatment for either 24 h fluid intake or 24 h urine output).

As shown previously, there were no age-related differences in maximal isoproterenol-induced expansion in the control bladders. However, bladders from old sucrose-fed rats showed a significantly greater magnitude of increase in isoproterenol maximal expansion than the mature adult and young adult sucrose-fed groups compared to the controls \((p < 0.05)\) (Figure 36) (interaction between age and treatment was significant, \(p < 0.005\)). Although smaller in magnitude, bladders from mature adult sucrose-fed rats showed a significantly greater increase in isoproterenol maximal expansion than the young adult sucrose-fed rats compared to controls \((p < 0.05)\). Bladders from the young adult sucrose-fed rats did not differ significantly from the young adult or old control bladders but did differ slightly from the mature adult control bladders.
Figure 39: 24 h fluid intake for sucrose and control rats. Effects of age on 24 h water intake in sucrose-fed and control rats. Each bar represents the mean ± S.E.M. of 4 rats in each age group for controls (dark bars) and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats (clear bars).
Figure 35: 24 h urine output for sucrose and control rats. Effects of age on 24 h urine output in sucrose-fed and control rats. Each bar represents the mean ± S.E.M. of 4 rats in each age group for controls (dark bars) and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats (clear bars).
Figure 36: Geometric representation of isoproterenol expansion. Effects of age on isoproterenol maximal expansion in sucrose-fed and control rats. Each symbol represents the mean of 9 rat bladders in control rats and 4 bladders in 5-7 mo. and 22-24 mo. and 3 bladders in 16-18 mo. sucrose-fed rats. Individually circled symbols are significantly different from other groups. Symbols within the same circle or dotted grouping are not significantly different from other symbols in that group. S = sucrose treated group and C = control group.
Table 15

ANOVA table for isoproterenol maximal expansion

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SAPSOS</th>
<th>M Square</th>
<th>F</th>
<th>PROB &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>37</td>
<td>4.40023</td>
<td>XXXXXX</td>
<td>XXXX</td>
<td>XXXXX</td>
</tr>
<tr>
<td>Age</td>
<td>2</td>
<td>0.71258</td>
<td>0.3563</td>
<td>9.64</td>
<td>0.0005</td>
</tr>
<tr>
<td>TRX</td>
<td>1</td>
<td>2.27447</td>
<td>2.2744</td>
<td>61.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>AGE*TRX</td>
<td>2</td>
<td>0.47362</td>
<td>0.2368</td>
<td>6.40</td>
<td>0.0046</td>
</tr>
<tr>
<td>ERROR</td>
<td>32</td>
<td>1.18325</td>
<td>0.0370</td>
<td>XXXX</td>
<td>XXXXX</td>
</tr>
</tbody>
</table>

SASPOS = Sums and partial sums of squares
PROB = Probability
M = mean
TRX = treatment (sucrose-fed and control)
AGE*TRX = interaction between age and treatment
The log ED$_{50}$ values determined from isoproterenol expansion dose-response curves performed on \textit{in vitro} bladders of sucrose-fed rats were not significantly different from control bladders. Also, there were no age-related differences in log ED$_{50}$ values from isoproterenol dose-response curves performed on bladders from either sucrose-fed or control rats (Table 16).

The log ED$_{50}$ values determined from bethanechol pressure dose-response curves performed on \textit{in vitro} bladders of sucrose-fed rats were not significantly different from control bladders. Also, there were no age-related differences in log ED$_{50}$ values from bethanechol dose-response curves performed on bladders from either sucrose-fed or control rats (Table 17).

Although not significant, the maximal pressure attained by the bladders of old sucrose-fed rats was slightly higher than the other 2 sucrose-fed groups as well as the controls (Table 18). Overall, isometric contraction of the bladder in response to bethanechol stimulation was not greatly altered in the sucrose-fed rats.

In the analysis of the plateau pressures taken from \textit{in vitro} cystometrograms, the overall F for age was significant ($p < 0.045$). However, further analysis of the data with conservative Student-Newman-Keuls multiple comparisons test showed no age-related differences. Based on the given
Table 16

Log $ED_{50}$ values: Isoproterenol $D$-$R$ curves

Effects of age on isoproterenol-stimulated bladder expansion in bladders of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Vol.</th>
<th>Control</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>1/4</td>
<td>$-5.588 \pm .14$</td>
<td>$-5.420 \pm .26$</td>
</tr>
<tr>
<td>16-18</td>
<td>1/4</td>
<td>$-5.565 \pm .18$</td>
<td>$-5.406 \pm .27$</td>
</tr>
<tr>
<td>22-24</td>
<td>1/4</td>
<td>$-5.636 \pm .14$</td>
<td>$-5.371 \pm .20$</td>
</tr>
</tbody>
</table>

Each bar represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. Isoproterenol dose-response curves were performed on bladders filled to 1/4 full. Log $ED_{50}$ values were determined from non-linear regression analysis of dose response curves. No significant age-related or treatment-related differences were found ($p < 0.8961$).
Table 17

Log $ED_{50}$ values: *bethanechol* D-R curves

Effects of age on bethanechol-stimulated bladder contraction in bladders of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Treatment</th>
<th>Control</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>1/4</td>
<td>-4.680 ± 0.099</td>
<td>-4.557 ± 0.19</td>
</tr>
<tr>
<td>16-18</td>
<td>1/4</td>
<td>-4.7430 ± 0.044</td>
<td>-4.724 ± 0.055</td>
</tr>
<tr>
<td>22-24</td>
<td>1/4</td>
<td>-4.699 ± 0.033</td>
<td>-4.563 ± 0.025</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. Bethanechol dose-response curves were performed on bladders filled to 1/4 full. Log $ED_{50}$ values were determined from non-linear regression of dose-response curves. No significant age-related or treatment-related differences were found ($p < 0.6342$).
Table 18

*Bethanechol pressure D-R curves: control/sucrose rats*

Effects of age on the maximal contraction elicited by bethanechol in bladders of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Control (cm Hg)</th>
<th>Treatment</th>
<th>Sucrose (cm Hg)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>3.27 ± .44</td>
<td>10</td>
<td>2.88 ± .35</td>
<td>4</td>
</tr>
<tr>
<td>16-18</td>
<td>2.94 ± .16</td>
<td>10</td>
<td>3.56 ± .52</td>
<td>3</td>
</tr>
<tr>
<td>22-24</td>
<td>3.46 ± .28</td>
<td>10</td>
<td>4.19 ± .38</td>
<td>4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of the maximal pressure (cm Hg) attained in bladders filled to 1/4 full in response to bethanechol stimulation. No age-related or treatment-related differences were found.
data and previous data (cystometrographic plateau pressures in Chapter 3), I feel that plateau pressures (Table 19) in bladders of the young adult and mature adult rats group together at a higher mean than the old rat bladders in both control and sucrose-fed rats. Bladders from the sucrose-fed rats demonstrated a decrease in plateau pressure across the age groups (i.e. there was no interaction between age and treatment).

The structural capacity of bladders from sucrose-fed rats increased significantly compared to controls ($p < 0.05$). There were no significant age-related differences in structural bladder capacity in either control or sucrose-fed rats (Table 20).

Age-related increases in bladder weight began to emerge in both sucrose-fed and control rats even with the small $n$ value. Sucrose-fed rats demonstrated an increase in bladder weight compared to control rats (Table 21). There was a slight interaction ($p < 0.085$) between age and treatment which accounted for the bladders of the old sucrose-fed rats showing by far the greatest increase in bladder weight compared to all other groups. This increase was significant ($p < 0.05$).

There appeared to be no age-related or treatment-related differences in urine density (Table 22). However, any differences may have been masked by the small $n$ value.
Table 19

*Cystometrographic plateau pressures: sucrose/control rats*

Effects of age on bladder plateau pressures of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Control (cm Hg)</th>
<th>Sucrose (cm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>1.15 ± 0.20</td>
<td>0.73 ± 0.063</td>
</tr>
<tr>
<td>16-18</td>
<td>1.05 ± 0.17</td>
<td>0.82 ± 0.083</td>
</tr>
<tr>
<td>22-24</td>
<td>0.75 ± 0.096</td>
<td>0.50 ± 0.091</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. Plateau pressures were determined from cystometrographic curves generated on in vitro bladders.
Table 20  

**Structural bladder capacity: sucrose / control rats**

Effects of age on the structural capacity of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>Control (ml)</th>
<th>Sucrose (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>Control</td>
<td>1.50 ± .23</td>
<td>* 3.26 ± .24</td>
</tr>
<tr>
<td>16-18</td>
<td>Sucrose</td>
<td>1.90 ± .38</td>
<td>* 3.10 ± .56</td>
</tr>
<tr>
<td>22-24</td>
<td>Sucrose</td>
<td>1.72 ± .18</td>
<td>* 3.85 ± .46</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. Structural capacities were determined from in vitro cystometrograms as previously described. * indicates significant difference from controls (p < 0.05).
Table 21

*Bladder weights: sucrose / control rats*

Effects of age on the bladder weight of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Control (g)</th>
<th>Sucrose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>0.0714 ± .001</td>
<td>0.1063 ± .004</td>
</tr>
<tr>
<td>16-18</td>
<td>0.0915 ± .01</td>
<td>0.1200 ± .011</td>
</tr>
<tr>
<td>22-24</td>
<td>0.1023 ± .003</td>
<td>*0.1790 ± .021</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. * indicates significantly different from all other groups (p < 0.05).
Table 22

*Urine density: sucrose / control rats*

Effects of age on the urine density of sucrose-fed and control rats.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Treatment</th>
<th>Control</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>Control</td>
<td>0.984 ± 0.009</td>
<td>0.968 ± 0.015</td>
</tr>
<tr>
<td>16-18</td>
<td>Control</td>
<td>0.967 ± 0.009</td>
<td>0.994 ± 0.008</td>
</tr>
<tr>
<td>22-24</td>
<td>Control</td>
<td>0.986 ± 0.014</td>
<td>0.975 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of 4 rats in each age group for controls and 4 rats in 5-7 mo. and 22-24 mo. and 3 rats for 16-18 mo. sucrose-fed rats. No significant age-related or treatment-related effects were found (p < 0.4510).
5.4 DISCUSSION

All age groups of rats fed 5% sucrose in their drinking water showed a significant increase in 24 h fluid intake and 24 h urine output compared to control rats. The age-related increase in 24 h fluid intake and urine output observed previously (Chapter 2) was starting to emerge in control rats, but probably because of the small n, was not significant. A similar pattern was observed in the sucrose-fed rats. The significantly larger urine output observed in sucrose-fed rats indicated that diuresis had been achieved in these rats. Hence, the diuretic stress model was accomplished for the 3 age groups of rats.

As previously shown, the effects of age on isoproterenol-induced *in vitro* bladder expansion were found not to be significant in control rats. This finding suggested that the aging bladder was able to maintain, through its "adaptive capacity" [69], the beta-adrenergic accommodation function important in the filling and storage phase of micturition. This "adaptive capacity" of the bladder was reduced significantly in the old and mature rats subjected to diuretic stress (sucrose-fed rats). Deviation from the isoproterenol-expansion control group (average of 3 ages = 0.61 ml) was by far the greatest in the old sucrose-fed rats (142% increase in maximal expansion) followed by the mature adult sucrose-fed rats (88% increase in
maximal expansion). Interestingly, the maximal isoproterenol expansion in bladders from young adult sucrose fed rats did not differ significantly from that of the controls group. The young adult bladders demonstrated a mere 37% increase in maximal expansion compared to the control group. It appeared that the bladders of young adult rats subjected to the diuretic stress were able to maintain beta-adrenergic function almost to the same level as the control group. These results showed a reduction in "adaptive capacity" of the aging bladder to maintain postjunctional beta-adrenergic function under a physiological stress (diuresis).

In agreement with earlier results, the log ED$_{50}$ values of isoproterenol dose-response curves did not differ significantly among the three age groups. Also, the lack of a significant difference in log ED$_{50}$ values of isoproterenol dose-response curves performed on sucrose-fed rats compared to controls, suggested that the sensitivity of the bladder to isoproterenol did not change with application of the diuretic stress.

Again, in agreement with earlier results, the log ED$_{50}$ values of bethanechol dose-response curves did not differ significantly among the three age groups. Based on the lack of a significant difference in the log ED$_{50}$ values of dose-response curves performed on sucrose-fed and control
rat bladders, it appeared that bladder sensitivity to bethanechol did not change significantly after application of a diuretic stress. However, there was a slight but non significant increase in the maximal pressure response attained by bladders of the old sucrose-fed rats in response to bethanechol. Overall, the isometric bladder contraction in response to bethanechol stimulation was not altered greatly by application of a diuretic stress.

Perhaps bethanechol-induced expulsion dose-response curves would have been a better parameter to measure in this diuretic-stress model. Bethanechol pressure dose-response curves were chosen because in previous experiments they had provided a measurable endpoint (maximal pressure response), whereas, bethanechol expulsion experiments had not; all bladders expelled 100% of their volume, therefore, maximal response or endpoint was the same for all bladders. In this experiment I was concerned that if I had performed bethanechol expulsion instead of pressure dose-response curves, I might have missed a treatment-related change in maximal response of the bladders to bethanechol.

In addition to functioning as a diuretic-stress factor, sucrose induced diuresis provided a means to study extrinsically increased bladder usage in the young adult rats as well as the mature adult and old rats. The increased bladder usage caused by sucrose-induced diuresis resulted in
decreased cystometrographic plateau pressures in all three age groups. As discussed earlier, the decreased plateau pressure represented a decrease in bladder smooth muscle elasticity; the bladders had become more compliant. These data are in agreement with Carpenter [63], who has shown that bladders of sucrose-fed rats are more compliant than controls. Thus, the data generated in these experiments has given support to the hypothesis that increased bladder usage accounts for the decrease in bladder elasticity or a more accommodating bladder.

The structural capacity of sucrose-fed rats increased significantly compared to controls. The structural capacity increased in parallel among the 3 age groups (i.e. there was no age*treatment interaction). It was shown earlier that the structural capacity did not change with advancing age in non-diuresed rats (Table 9). Therefore, the diuretic model of increased bladder usage differed from increased bladder usage observed normally with advancing age in this respect. Perhaps the very gradual increase in urine output (bladder usage) seen with aging as opposed to the rapid diuresis with sucrose accounts for this difference.

Smooth muscle has the ability to adapt to increased functional demands by an increase in mass [70]. For example, the urinary bladders of the rat [70] and rabbit [71]
subjected to short term outlet obstruction show a compensatory increase in bladder mass; the hypertrophied bladder of the rat is capable of developing intravesical pressure as high as the normal detrusor [70]. The increase in bladder weight is caused by hypertrophy of smooth muscle cells [70].

In this study, diuresis accounted for an increase in bladder weight among all three age groups, although not nearly as large as that seen in obstructed bladders. A similar increase in bladder mass occurred with advancing age in the controls of this study and previously, in non-diuresed rats. Based on data from obstructed bladders, the increase in bladder mass seen both with advancing age and in diuresed rats might have been an adaptation of the bladder smooth muscle to increased usage. It was interesting that the bladders of old sucrose-fed rats showed the greatest magnitude of increase in bladder weight; this might have been related to the large deviation of isoproterenol expansion in these bladders from controls.

In summary, a diuretic stress model was accomplished in 3 age groups of rats by adding sucrose (5% w/v) to their drinking water. The results of this study were divided into essentially two parts: 1) the "adaptive capacity" of the bladders to the diuretic stress; and 2) the effects of extrinsically increased bladder usage on the passive properties of the bladders in 3 age groups of rats.
Parameters used to measure the "adaptive capacity" of the bladders subjected to the diuretic-stress included: isoproterenol-induced expansion (filling and storage function); and bethanechol-induced isometric contraction (emptying function). There appeared to be no age-related change in the "adaptive capacity" of the bladder to contract isometrically in response to bethanechol. However, the isotonic expulsion contraction in response to bethanechol might have been altered in the sucrose-fed rats. Levin et al. [71] found that while the obstructed rabbit bladders could attain intravesical pressures as high as control bladders, the obstructed bladders could only expel 28% of their volume. Therefore, it is possible that bladders from sucrose-fed rats could reach equal intravesical pressures with control bladders, yet not empty 100% of their volume. The bladders of old sucrose-fed rats deviated the most from control bladders in isoproterenol maximal expansion. This suggested that the "adaptive capacity" of these bladders to maintain postjunctional beta-adrenergic function was greatly reduced relative to the bladders of young adult rats fed sucrose in this beta-adrenergic system. In other words, the old bladders were less able to adapt to the physiological stress of diuresis; they responded to isoproterenol stimulation with an exaggerated expansion relative to the young adult and mature adult sucrose-fed rats as well as to the controls.
The decreased cystometrographic plateau pressures in the bladders of sucrose-fed rats of all three ages supported the role of increased bladder usage in decreasing bladder elasticity or increasing bladder compliance.
CHAPTER VI
SCREENING SUBSTANCES: SPECIFIC AIM 5

6.1 INTRODUCTION

Endogenous substances, in addition to acetylcholine, have been found to contract bladder smooth muscle in several species of animals and in humans. Some of these substances include: histamine, oxytocin, prostaglandin $F_{2a}$, serotonin, and substance P.

Histamine produces detrusor contractions in vitro in the human [18] and guinea pig [18,72]. In the guinea pig urinary tract histamine receptors have been identified as $H_1$ receptors. Histamine-induced detrusor contractions are abolished by $H_1$ blockers such as promethazine and mepyramine, but not by $H_2$ blockers such as cimetidine [72]. Although histamine is capable of contracting detrusor muscle, it is questionable whether this substance plays a role in bladder function [18].

Significant concentrations of oxytocin have been described in the genitourinary organs such as the testis in the human and rat [73]. Recently, through binding and isolated organ experiments, Romine et al. [73] reported
evidence for oxytocin receptors in the urinary bladder of the rabbit. Oxytocin produces a strong contraction of rabbit strips [73]. However, like histamine, the importance of oxytocin as either a neurotransmitter or neuromodulator in urinary bladder function is unknown.

There is evidence that prostaglandins are produced in the urinary tract [18]. A release of prostaglandins was caused by distension of the urinary bladder of dogs and rabbits [74]. Also, pelvic nerve stimulation in dogs and rabbits caused release of prostaglandins [74]. Of the prostaglandins, \( \text{PGF}_2 \alpha \) is the most potent in producing detrusor contractions [18]. The detrusor contraction induced by prostaglandins develops more slowly and persists longer than the contraction induced by acetylcholine [18,74,75] Choo et al. [75] concluded that \( \text{PGF}_2 \alpha \) does not function as an excitatory neurotransmitter in the bladder because even at high concentrations, the \( \text{PGF}_2 \alpha \) contractions were small and developed more slowly in contrast to the large and rapid contractions evoked by nervous stimulation. Instead, they suggest that \( \text{PGF}_2 \alpha \) may act as a modulator of nervous transmission in the bladder.

Serotonin is present in the lower urinary tract smooth muscle [76]. In vivo, serotonin causes detrusor contractions in dogs and cats. Serotonin also causes contraction of human and pig bladder smooth muscle in vitro [76]. The
serotonin-induced contraction appears to be biphasic; an initial transient contraction (probably due to stimulation of autonomic ganglia) is followed by a more prolonged contraction probably mediated via specific serotonin receptors (different from serotonin $S_2$ receptors) on bladder smooth muscle [76]. Although serotonin is a potential neurotransmitter in bladder smooth muscle, its physiological or pathophysiological importance is unknown.

Nerves containing substance P have been found in the bladder walls of several animal species [74]. Also, small amounts of substance P have been found in the human urinary bladder [10]. Substance P produces contraction of the urinary bladder in several species of animals [74]. For example, substance P produces a contractile effect in in vitro canine bladder [10], and topically applied substance P evokes a dose-dependent contraction in the in vitro rat bladder [77]. Functional studies provide the basis for the hypothesis that substance P plays a role in the afferent, sensory branch of micturition [10,77]. Systemic administration of capsaicin (an agent that depletes substance P and other peptides in the small diameter afferents) depressed the micturition reflex and caused urinary retention [10].

The intent of the following experiment was to evaluate serotonin, histamine, $\text{PGF}_2\alpha$, oxytocin, and substance P for
bladder smooth muscle activity using the in vitro whole rat bladder preparation. Also, the effect of age on the bladder activity of these substances was determined.

6.2 METHODS

Drug dose-response curves were performed on bladders filled to 3/4 full using the in vitro whole bladder isometric "closed" system described in section 3.2.1.1.

Statistical analysis

Data were analyzed using analysis of variance. Significant results were further analyzed using Student-Newman-Keuls multiple comparisons test (Appendix E.2).

6.3 RESULTS

All of the substances (serotonin, histamine, oxytocin, PGF$_{2\alpha}$, and substance P) assayed in the isometric bladder system were able to elicit detrusor contractions (Figure 37). The tracings from actual dose-response curves are shown in Figure 37. Each tracing is representative of dose-response curves performed on all 3 age groups for each particular drug.
Figure 37: Dose-response curve tracings of various drugs. The following tracings are from actual dose-response curves performed on 3/4 full bladders of 5-7 mo., 16-18 mo., and 22-24 mo. rats. The dose-response curve for each drug is representative of dose-response curves for the 3 age groups for that particular drug. Acetylcholine, oxytocin, phenylephrine, and substance P tracings were taken from 22-24 mo. old rats. Histamine tracing was taken from a 16-18 mo. old rat. Bethanechol, PGF$_{2alpha}$, and serotonin were taken from 5-7 mo. old rats. Dose-response curves were recorded at different polygraph sensitivities; the units (cm Hg) are given on the vertical axis by each tracing. The time scale was the same for all tracings. Doses of the various drugs ranged from 3x10E-9 to 3x10E-3.
Figure 37
As expected, the maximal response of the bladders differed among the various drugs (Figure 38). Substance P produced the highest maximal isometric bladder contraction in all three age groups compared to the other non autonomic substances. Oxytocin evoked the next highest detrusor contraction in the 3 age groups. This was followed by serotonin and prostaglandin F$_{2\alpha}$. Histamine produced a very weak bladder contraction in all three age groups.
Figure 38: Maximal pressure response to various drugs. The effects of age on the maximal response of bladders elicited by various smooth muscle contracting drugs were graphed. Each bar (clear, 5-7 mo.; striped, 16-18 mo.; and dark, 22-24 mo.) represents the mean ± S.E.M. of 4 bladders in each age group for substance P (sub P), prostaglandin F$_2$α (PGF$_2$α), oxytocin (oxy), and histamine (hist), 5 bladders in each age group for serotonin (5-HT) and acetylcholine (ACh); and 6 bladders in each age group for bethanechol (Beth). * indicates significant difference from 5-7 mo. old rats (p < 0.05).
Figure 38
There were no age-related effects on the maximal pressure responses elicited by histamine, serotonin (5-HT), or oxytocin (Figure 38) and (Table 23).

Although not significant, there appeared to be the beginnings of an age-related increase in bladder maximal pressure elicited by substance P (Figure 38) and (Table 23).

The maximal pressure response elicited by prostaglandin \( F_{2\alpha} \) was significantly higher in the bladders of 22-24 month old rats than the 5-7 month old rat bladders. The maximal response of the 22-24 month bladders was approximately 75% greater than the maximal response from 5-7 month rat bladders (Figure 38) and (Table 23).
Table 23

*Maximal responses elicited by various drugs*

Effect of age on the maximal responses elicited by various drugs in the in vitro isometric bladder.

<table>
<thead>
<tr>
<th>Drug</th>
<th>5-7 mo.</th>
<th>16-18 mo.</th>
<th>22-24 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>0.15 ± .022</td>
<td>0.12 ± .018</td>
<td>0.12 ± .016</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>0.88 ± .067</td>
<td>0.96 ± .050</td>
<td>0.98 ± .010</td>
</tr>
<tr>
<td>PGF$_2\alpha$</td>
<td>0.33 ± .022</td>
<td>0.46 ± .080</td>
<td>0.58 ± .059 *</td>
</tr>
<tr>
<td>Serotonin</td>
<td>0.53 ± .061</td>
<td>0.60 ± .079</td>
<td>0.62 ± .033</td>
</tr>
<tr>
<td>Substance P</td>
<td>1.63 ± .130</td>
<td>1.74 ± .140</td>
<td>1.89 ± .150</td>
</tr>
</tbody>
</table>

Each value (cm Hg) represents the mean ± S.E.M. of 4 or 5 bladders in age group. All bladders were filled to 3/4 full. The drugs were assayed using the whole bladder isometric system. * indicates significant difference from 5-7 mo. rats (p < 0.05).
All of the substances tested were able to evoke a substantial isometric bladder contraction except for histamine. As far as age-related differences in bladder contractions evoked by these substances are concerned, only substance P and prostaglandin F$_{2\alpha}$ showed any potential of age-related changes. In fact, there was a significant difference in maximal contraction elicited by prostaglandin F$_{2\alpha}$ in the young adult and old rat bladders. It is possible that these changes in bladder isometric contractions with age in response to prostaglandin F$_{2\alpha}$ and substance P stimulation may affect urinary bladder function in the rat. Therefore, further research of these two endogenous substances in the aging bladder is warranted.
CHAPTER VII
SUMMARY AND CONCLUSIONS

As a result of differences in water metabolism, micturition behavior of the male rat changed significantly with advancing age. The increase in urine output that occurred with age might have been caused by a combination of the diminished concentrating ability of the kidneys, and the increased water intake observed with advancing age. Also, the higher intravesical pressure required to initiate a micturition contraction in mature adult and old rats suggested that sensitivity to bladder pressure had decreased in these two age groups relative to the young adult group.

Figure 39 describes a possible relationship of events that leads to Hypothesis 1, which is that the rat's sensation of bladder filling decreases with age. The urine density of the rats decreased with age. This suggested that the urine concentrating ability of the kidney decreased with age. This decline in water conservation (decreased urine density) probably contributed to the increase in urine output in the older\textsuperscript{22} rats. The increase in urine output was manifested by an increase in micturition volume.

\textsuperscript{22} older = mature adult and old rats
and micturition frequency; therefore, bladder usage increased with advancing age. (The increase in water intake that accompanied the increase in urine output in the rat with advancing age could have been either the cause or effect of the increased urine output). The increased bladder usage observed in the aging rat was revealed in the structural characteristics of the in vitro bladder as a decreased cystometrographic plateau pressure. This finding indicated that the bladder had become less elastic or more accommodating with age. In the live aged rat the ability of a more accommodating bladder to hold a larger volume of urine was revealed by the increase in micturition volume.

In addition, it took a higher intravesical pressure to initiate the micturition reflex in the older rats whose bladders were allowed to fill naturally. The gradual increase in urinary excretion rate that must occur with age (increase in urine output/24 h) may have contributed to the higher intravesical pressure during bladder filling (Figure 20) that resulted in a higher IPIMC. The aging rat may have adapted to the increase in intravesical pressure by becoming desensitized or less sensitive to bladder pressure (Hypothesis 1), thereby enabling the rat to accommodate the increased urine output which occurred with age (i.e. the rat was adapting to a larger water load by increasing micturition volume as well as micturition frequency).
Figure 39: Relationship of bladder usage / sensation / micturition volume. Solid boxes surround observations and dotted boxes surround deductions.
Autonomic bladder function, specifically postjuncti­
tional alpha and beta adrenergic activity of bladder fill­
ing and storage, and postjunctional muscarinic activity of
bladder emptying, did not change significantly during aging
in the rat (Chapters 3 and 4). It could be hypothesized
that the aging rat has been able to adapt to various chang­
es that occur with aging, and thus, has been able to main­
tain postjunctional autonomic bladder function (i.e. the
"adaptive capacity" of the aging bladder has been able to
maintain autonomic bladder function) (Hypothesis 2A) (Fig­
ure 40).

Figure 40 describes the effect of stress on autonomic
bladder function. The "adaptive capacity" of the bladder
was reduced significantly in the old and mature rats sub­
jected to diuretic stress (sucrose-fed rats) (Figure 40).

Parameters used to measure the "adaptive capacity" of
the bladders subjected to the diuretic-stress included:
isoproterenol-induced expansion (filling and storage func­
tion); and bethanechol-induced isometric contraction (emp­
tying function). There appeared to be no age-related
change in the "adaptive capacity" of the bladder to con­
tract isometrically in response to bethanechol. However,
the isotonic expulsion contraction in response to bethane­
chol might have been altered in the sucrose-fed rats. In
the isoproterenol maximal expansion, the bladders of the
Hypothesis 2A

As the rat ages the urinary bladder may adapt to various changes (biochemical, myogenic, etc) occurring with age and thereby, maintain autonomic bladder function.

Hypothesis 2B

However, if physically stressed the bladder's ability to compensate (adapt to changes and maintain normal function) decreases with age.

Diuretic Stress

The data suggest that there are no age-related differences in the filling and storage and emptying functions of the rat urinary bladder (postjunctional autonomic activity).

Figure 40: Stress-induced decline of bladder adaptation
old rats deviated the most from controls. Expansion of the old bladders in response to isoproterenol stimulation was greatly exaggerated relative to controls (i.e. the magnitude of isoproterenol expansion in the old bladders was much greater than in the bladders of the young adult sucrose-fed and control rats). This suggested that the "adaptive capacity" of these bladders to maintain postjunctional beta-adrenergic function was greatly reduced relative to the young adult rats in this beta-adrenergic system (Hypothesis 2B) (Figure 40). The decrease in elasticity observed in the bladders of old rats may have contributed to this exaggerated beta-adrenergic response.

Finally, (Figure 41) a decrease in the rat's sensation of bladder pressure (Hypothesis 1) coupled with the decreased adaptive capacity of the stressed bladder with advancing age (Hypothesis 2B) contributes to Hypothesis 3 which is that urinary dysfunction increases with age in the male rat.

There may be implications of this research to urinary incontinence in the elderly. Urinary incontinence in the non-institutionalized elderly [32] is more prevalent among elderly with bladder emptying and irritative symptoms. Bladder emptying symptoms can be related to outlet obstruction or bladder muscle weakness [32]. In women these bladder emptying symptoms are attributed to a reduced tonicity
Hypothesis 1
The rat's sensation of bladder pressure decreases with age.

Hypothesis 2B
If physically stressed, the bladder's ability to adapt and maintain function decreases with age.

Hypothesis 3
Urinary dysfunction increases with age in the rat.

Increased urinary incontinence in the elderly population

Figure 41: Summary
and elasticity of the bladder muscle [32]. Again, the rat bladder became less elastic with age, presumably due to the increased bladder usage that occurred with age. Thus, decreased bladder tonicity or elasticity that occurs with aging might be a common link between the aging rat bladder and the aging human bladder; therefore, perhaps the structural characteristics of the aging bladder, such as bladder elasticity, should receive more attention in future aging bladder research. In this research project the age-related changes in structural characteristics of the bladder seemed to have a strong influence on micturition behavior in the male F344 rat.

Also, the elderly human is subjected to environmental as well as endogenous stress factors. The "adaptive capacity of the human bladder could decline with age, analogous to the aging rat bladder. This, in combination with structural changes in the bladder and the hypothesized loss of bladder sensation, could lead to or complicate bladder emptying and irritative symptoms in the elderly and result in urinary incontinence.
Appendix A

PLACEMENT OF TRACHEAL TUBE

The neck region of the fully anesthetized rat was shaved with electric clippers. The rat was then secured to a wooden dissecting board with tape restraints. Figure 42: (1) A 2 cm cut was made with a scalpel starting approximately 3.5 cm below the jaw. (2) The skin was pulled back with retractors; the glands and longitudinal muscles were exposed. The glands were gently teased away from the longitudinal muscles. (3) Using a needle point probe a 1 cm slit was made into the longitudinal muscles. Figure 43: (4) A closed hemostats was inserted into the slit and then pulled open to expose the trachea. The retractors holding the skin back were removed and placed on opposite sides of the split longitudinal muscles to permit constant exposure of the trachea. Using a blunt-ended probe the trachea was isolated from surrounding muscle tissue. (5) A curved forceps was used to pull two 8 cm suture threads (000) underneath the trachea (A is the top or anterior thread and B is the bottom or posterior thread). (6) While gently
pulling up on thread B a partial cut was made between two rings of the trachea with a fine scissors. Figure 44: (7) A tracheal tube made from 4 cm of PE250 tubing was inserted into the trachea (the end to be inserted into the trachea was slightly beveled to aid in insertion). Also lifting thread B eased insertion of the tube. (8) The tracheal tube was secured into place by tying strings A and B tightly around the tube and the trachea. (9) The incision was closed with two stitches A and B.
Figure 42: Tracheal tube insertion
Figure 43:  Tracheal tube insertion
Figure 44: Tracheal tube insertion
Appendix B

PURSE STRING SUTURE

The lower abdomen of the fully anesthetized rat was shaved with electric clippers. An incision was made just large enough to pop the bladder onto the outer abdominal wall. If the bladder was full, a small amount of urine was squeezed out before the urethra was immediately tied off (a ligature tied around the penis). Figure 45: (1) A continuous suture was sewn onto the outermost layers of the bladder in a circular fashion using 6-0 suture thread. (2) Two 4 cm lengths of thread were left at each end of the completed purse-string suture. (3) The two 4 cm ends of thread were interlooped as in the first stage of tying a knot. (4) Using a fine forceps, the center of the purse string suture was lifted up and a small slit was made into the bladder with a fine scissors. A PE50 catheter infused with saline 0.9% 1 ml/min. was inserted into the slit. The ends of the purse string were pulled, drawing up the bladder tissue within the purse-string suture up around the catheter; the catheter was held in place (5). The infusion

- 194 -
was turned off. The final catheterized bladder using the purse-string suture technique appeared as in part (6).
Figure 45: Purse-string suture
Appendix C
CANNULATION OF JUGULAR VEIN

The neck region of the fully anesthetized rat was shaved with electric clippers. The rat was then secured to a wooden dissecting board with tape restraints. Figure 46: (1) A 3.5 cm cut was made with a scalpel starting approximately 2.5 cm below the jaw. (2) The skin was pulled back with retractors; the glands and longitudinal muscles were exposed. The glands were gently teased away from the longitudinal muscles. (3) An area of tissue located approximately 1 cm laterally to the longitudinal muscles was teased away with a blunt-ended probe to expose the jugular vein. The jugular vein was isolated by stripping away surrounding tissue. Figure 47: (4) A curved forceps was used to pull two 8 cm suture threads (000) underneath the jugular vein. (B is the top or anterior thread and A is the bottom or posterior thread). (5) Thread B, the anterior thread was securely tied to stop blood flow towards the heart. Thread A was slightly elevated. While pulling up on the vein with very fine forceps a small slanted cut
was made between the two threads. A second very fine for-
ceps was gently inserted into the vein to hold it open
while the PE50 cannula was inserted. (6) The cannula was
secured into place by tying threads A and B tightly around
the vein and the cannula. The incision was closed with two
stitches A and B.
Figure 46: Cannulation of jugular vein
Figure 47: Cannulation of jugular vein
Appendix D

EXPANSION AND EXPULSION TUBE DIMENSIONS

Figure 48: Expansion and expulsion tube dimensions
Appendix E

SAS PROGRAMS

E.1 CORRELATIONS

DATA ONE;
TITLE 'BWT, BLWT, CAP CORREL.';
TITLE3 '3 AGEGROUPS';
INPUT AGE BWT BLWT CAP;
CARDS;
1 .371 .0932 2.45
1 .337 .0803 1.84
1 .370 .0889 2.50
2 .388 .0990 2.24
2 .389 .0835 1.84
2 .404 .0905 1.71
3 .390 .0841 2.14
3 .375 .0861 1.07
3 .386 .0752 1.43
PROC SORT;
   BY AGE;
PROC PRINT;
   BY AGE;
PROC PLOT;
   BY AGE;
   PLOT BLWT * BWT;
PROC CORR PEARSON SPEARMAN;
VAR BWT BLWT;
BY AGE;
PROC PLOT;
   BY AGE;
   PLOT CAP * BWT;
PROC CORR PEARSON SPEARMAN;
VAR CAP BWT;
BY AGE;
PROC PLOT;
   BY AGE;
PROC PLOT;
   BY AGE;
   PLOT CAP * BLWT;
PROC CORR PEARSON SPEARMAN;
VAR CAP BLWT;
BY AGE;
DATA ONE;
TITLE 'ANOVA + SNK/ VAR BWT, BLWT, CAP';
TITLE2 '3 AGEGROUPS';
INPUT AGE BWT BLWT CAP;
CARDS;
1  .371  .0932  2.45  
1  .337  .0803  1.84  
1  .370  .0889  2.50  
2  .388  .0990  2.24  
2  .389  .0835  1.84  
2  .404  .0905  1.71  
3  .390  .0841  2.14  
3  .375  .0861  1.07  
3  .386  .0752  1.43  
PROC SORT;
    BY AGE;
PROC PRINT;
    BY AGE;
PROC ANOVA;
    CLASSES AGE;
    MODEL BWT=AGE;
    MEANS AGE/SNK ALPHA = 0.1 LINES;
PROC SORT;BY AGE;
PROC MEANS;BY AGE;VAR BWT;
PROC ANOVA;
    CLASSES AGE;
    MODEL BLWT=AGE;
    MEANS AGE/SNK LINES;
PROC SORT;BY AGE;
PROC MEANS;BY AGE;VAR BLWT;
PROC ANOVA;
    CLASSES AGE;
    MODEL CAP=AGE;
    MEANS AGE/SNK LINES;
PROC SORT;BY AGE;
PROC MEANS;BY AGE;VAR CAP;
E.3 ANALYSIS OF VARIANCE (2-WAY)/STUDENT NEWMAN KEULS

TITLE 'SUCROSE/CONTROL';
TITLE2 '3 AGE GROUPS';
TITLE3 'CAPACITY';
DATA ONE;
INPUT E AGE MLS TRX;
IF AGE=6 AND TRX=20 THEN GROUP=1;
IF AGE=16 AND TRX=20 THEN GROUP=2;
IF AGE=23 AND TRX=20 THEN GROUP=3;
IF AGE=6 AND TRX=22 THEN GROUP=4;
IF AGE=16 AND TRX=22 THEN GROUP=5;
IF AGE=23 AND TRX=22 THEN GROUP=6;
IF AGE=6 AND TRX=20 THEN GROUP=1;
IF AGE=16 AND TRX=20 THEN GROUP=2;
IF AGE=23 AND TRX=20 THEN GROUP=3;
IF AGE=6 AND TRX=22 THEN GROUP=4;
IF AGE=16 AND TRX=22 THEN GROUP=5;
IF AGE=23 AND TRX=22 THEN GROUP=6;
CARDS;
1 6 2.69 22
2 6 3.81 22
3 6 3.48 22
4 6 3.06 22
1 16 2.92 22
2 16 4.12 22
3 16 2.28 22
1 23 2.79 22
2 23 5.0 22
3 23 4.04 22
4 23 3.57 22
1 6 1.22 20
2 6 1.88 20
3 6 1.90 20
4 6 1.0 20
1 16 2.85 20
2 16 1.30 20
3 16 2.16 20
4 16 1.29 20
1 23 1.24 20
2 23 2.0 20
3 23 1.65 20
4 23 2.0 20
PROC PRINT;
PROC GLM;
CLASS GROUP; ..................1-way ANOVA on 6 groups
MODEL MLS=GROUP;
MEANS GROUP/LSD LINES;
MEANS GROUP/LSD CLDIFF;
PROC GLM; ..................2-WAY ANOVA
CLASS AGE TRX;
MODEL MLS=AGE TRX AGE*TRX;
MEANS AGE TRX/SNK LINES;
MEANS AGE*TRX;
PROC SORT;BY AGE;
PROC MEANS;BY AGE;VAR MLS;
PROC SORT;BY TRX;
PROC MEANS;BY TRX;VAR MLS;
PROC SORT;BY AGE TRX;
PROC MEANS;BY AGE TRX;
VAR MLS;
PROC NPARIWAY;........Kruskal-Wallis
E.4 NONLINEAR REGRESSION CURVE FITTING ANALYSIS

written by Phillip Ross,
modified by Frederick S. Ruland and Alexa L. Chun

TITLE 'Bethanechol expulsion D-R curves';
TITLE2 'Individual Log ED50 values';
TITLE3 '3 AGE GROUPS';
OPTIONS LINESIZE=76;
DATA ONE; INPUT TISSUE T CONC AMP A;
LCONC=LOG10(CONC);
CALIBAMP=2;
A1=A*AMP*CALIBAMP;
DROP A AMP CALIBAMP;
CARDS;
1 1 3E-8 0.5 .0168
1 1 1E-7 0.5 .0226
1 1 3E-7 0.5 .0705
2 1 3E-8 0.5 .0004
2 1 1E-7 0.5 .0820
2 1 3E-7 0.5 .1432
3 1 3E-8 0.5 .0274
3 1 1E-7 0.5 .037
3 1 3E-7 0.5 .1379
4 1 3E-8 0.5 .0265
4 1 1E-7 0.5 .0425
4 1 3E-7 0.5 .1025
5 1 3E-8 0.5 .0262
5 1 1E-7 0.5 .0885
5 1 3E-7 0.5 .1388
1 2 3E-8 0.5 .0
1 2 1E-7 0.5 .044
1 2 3E-7 0.5 .0672
2 2 3E-8 0.5 .0086
2 2 1E-7 0.5 .0224
2 2 3E-7 0.5 .0887
3 2 3E-8 0.5 .0317
3 2 1E-7 0.5 .0368
3 2 3E-7 0.5 .1075
4 2 3E-8 0.5 .0218
4 2 1E-7 0.5 .0539
4 2 3E-7 0.5 .0541
5 2 3E-8 0.5 .0192
5 2 1E-7 0.5 .0448
5 2 3E-7 0.5 .0951
1 3 3E-8 0.5 .0
1 3 1E-7 0.5 .0122
1 3 3E-7 0.5 .0122
2 3 3E-8 0.5 .0121
2 3 1E-7 0.5 .0478
2 3 3E-7 0.5 .1308
3 3 3E-8 0.5 .0069
PROC SORT; BY TISSUE T LCONC;
DATA ONE; SET ONE; BY TISSUE T;
  IF FIRST.TISSUE THEN MAXITOT=0;
  IF FIRST.T THEN MAXI=0;
  IF A1>MAXI THEN MAXI=A1;
  IF MAXI>MAXITOT THEN MAXITOT=MAXI;
  RETAIN MAXI MAXITOT;
PROC SORT; BY TISSUE DESCENDING T DESCENDING LCONC;
DATA ONE; SET ONE; BY TISSUE DESCENDING T;
  IF FIRST.TISSUE THEN MAXTOT=MAXITOT;
  IF FIRST.T THEN MAX=MAXI;
  RETAIN MAX MAXTOT;
DROP MAXI MAXITOT;
PROC PRINT; VAR TISSUE T LCONC A1 MAX MAXTOT;
  TITLE A CHECK TO SEE THAT THE MAX
  AND MAXTOT VALUES ARE CORRECT;
PROC SORT; BY TISSUE T LCONC;
DATA ONE; SET ONE;
  A2=(A1/MAX)*100;
  A2TOT=(A1/MAXTOT)*100;
PROC SORT; BY TISSUE T LCONC;
PROC MEANS N MEAN STDERR MAXDEC=2 NOPRINT;
  BY TISSUE T LCONC; VAR A2 A2TOT; OUTPUT
  OUT=MA2 MEAN=MA2 MA2TOT
  STDERR=SMA2 SMA2TOT;
DATA MA2; SET MA2;
  MA2U=MA2+SMA2;
  MA2D=MA2-SMA2;
  MA2TOTU=MA2TOT+SMA2TOT;
  MA2TOTD=MA2TOT-SMA2TOT;
PROC SORT; BY TISSUE T LCONC;
DATA ONE; MERGE ONE MA2; BY TISSUE T LCONC;
  LCONCFOS=12+LCONC;
PROC DATASETS;SAVE ONE;
PROC NLIN DATA=ONE METHOD=MARQUARDT EFORMAT
  CONVERGE=1E-12 MAXITER=100;

BY TISSUE T;
  PARMS M=100 P=1 K=8;
  BOUNDS M>0, 0<P<30, K>0;
X=LCONCPOS; Y=A2;
Q = EXP(P*(X-K));
MODEL Y = ((M*Q)/(1+Q));
DER.M = Q/(1+Q);
DER.K = -M*P*Q/((1+Q)**2);
DER.P = M*Q*(X-K)/((1+Q)**2);
OUTPUT OUT=P1 PREDICTED=PRED RESIDUAL=RESID PARMS=M P K;
TITLE DOSE RESPONSE CURVE AS A PERCENT OF
INDIVIDUAL MAXIMA - CURVE FITTING;

DATA P1;SET P1;
LEC50=K-12;
EC50=10**(LEC50);
P=P/(LOG(10));
PROC SORT;BY TISSUE T LCONC;
PROC MEANS NOPRINT;
   VAR RESID;BY TISSUE T LCONC;OUTPUT
     OUT=MRESID MEAN=MRESID;
PROC SORT;BY TISSUE T LCONC;
DATA P1;MERGE P1 MRESID; BY TISSUE T LCONC;
PROC PRINT;VAR TISSUE T LCONC A2 M P
   LEC50 EC50 PRED MA2 SMA2;
FORMAT EC50 E10. P LEC50 LCONC
   11.3 M PRED MA2 SMA2 A2 11.2;
TITLE DOSE RESPONSE CURVE AS A PERCENT OF
INDIVIDUAL MAXIMA - FITTED PARAMETERS;
PROC PRINT;VAR TISSUE T LCONC A2 RESID MRESID;
TITLE DOSE RESPONSE CURVE AS A PERCENT OF
INDIVIDUAL MAXIMA - FITTED RESIDUALS;
E.5 NONLINEAR REGRESSION CURVE FITTING ANALYSIS
written by Phillip Ross

TITLE 'Bethanechol pressure D-R curves';
TITLE2 'Curves plotted as % total maximum';
TITLE3 'Mature adult rats';
OPTIONS NODATE;
OPTIONS LINESIZE=76;
DATA ONE; INPUT TISSUE T CONC AMP A;
   LCONC=LOG10(CONC);
   CALIBAMP=2;
   A1=A*AMP*CALIBAMP;
   DROP A AMP CALIBAMP;
CARDS;
1 1 3E-7 0.5 0.30
1 1 1E-6 0.5 0.60
1 1 3E-6 0.5 0.75
2 1 3E-7 0.5 0.20
2 1 1E-6 0.5 1.35
2 1 3E-6 0.5 1.45
3 1 3E-7 0.5 0.04
3 1 1E-6 0.5 0.23
3 1 3E-6 0.5 0.43
4 1 3E-7 0.5 0.08
4 1 1E-6 0.5 0.32
4 1 3E-6 0.5 0.74
5 1 3E-7 0.5 0.15
5 1 1E-6 0.5 0.30
5 1 3E-6 0.5 0.90
6 1 3E-7 0.5 0.10
6 1 1E-6 0.5 0.18
6 1 3E-6 0.5 0.65
1 2 3E-7 0.5 0.05
1 2 1E-6 0.5 0.10
1 2 3E-6 0.5 0.20
2 2 3E-7 0.5 0.05
2 2 1E-6 0.5 0.08
2 2 3E-6 0.5 0.30
3 2 3E-7 0.5 0.02
3 2 1E-6 0.5 0.10
3 2 3E-6 0.5 0.22
4 2 3E-7 0.5 0.05
4 2 1E-6 0.5 0.10
4 2 3E-6 0.5 0.28
5 2 3E-7 0.5 0.02
5 2 1E-6 0.5 0.10
5 2 3E-6 0.5 0.25
6 2 3E-7 0.5 0.04
6 2 1E-6 0.5 0.08
6 2 3E-6 0.5 0.28
1 3 3E-7 0.5 0.02
PROC SORT; BY TISSUE T LCONC;
DATA ONE; SET ONE; BY TISSUE T;
   IF FIRST.TISSUE THEN MAXITOT=0;
   IF FIRST.T THEN MAXI=0;
   IF A1>MAXI THEN MAXI=A1;
   IF MAXI>MAXITOT THEN MAXITOT=MAXI;
   RETAIN MAXI MAXITOT;
PROC SORT; BY TISSUE DESCENDING T DESCENDING LCONC;
DATA ONE; SET ONE; BY TISSUE DESCENDING T;
   IF FIRST.TISSUE THEN MAXTOT=MAXITOT;
   IF FIRST.T THEN MAX=MAXI;
   RETAIN MAX MAXTOT;
   DROP MAXI MAXITOT;
PROC PRINT; VAR TISSUE T LCONC A1 MAX MAXTOT;
   TITLE A CHECK TO SEE THAT THE MAX AND
   MAXTOT VALUES ARE CORRECT;
PROC SORT; BY TISSUE T LCONC;
DATA ONE; SET ONE;
   A2=(A1/MAX)*100;
   A2TOT=(A1/MAXTOT)*100;
PROC SORT;BY T LCONC;
PROC MEANS N MEAN STDERR MAXDEC=2 NOPRINT;
   BY T LCONC;VAR A2 A2TOT;OUTPUT OUT=MA2 MEAN=MA2 MEAN=MA2 TOT;
   STDERR=MA2 TOT;
DATA MA2; SET MA2;
   MA2U=MA2+SMA2;
   MA2D=MA2-SMA2;
   MA2TOTU=MA2TOT+SMA2TOT;
   MA2TOTD=MA2TOT-SMA2TOT;
PROC SORT;BY T LCONC;
DATA ONE; MERGE ONE MA2;BY T LCONC;
   LCONCPOS=12+LCONC;
PROC DATASETS; SAVE ONE;
PROC NLIN DATA=ONE METHOD=MARQUARDT EFORMAT
   CONVERGE=1E-12 MAXITER=100;
   BY T;
   PPARMS M=50 K=8;
   BOUNDS M>0, 0<P<30, K>0;
   X=LCONCPOS; Y=A2TOT;
   Q = EXP(P*(X-K));
   MODEL Y = (M*Q)/(1+Q);
   DER.M = Q/(1+Q);
   DER.K = -M*P*Q/((1+Q)**2);
   DER.P = M*Q*(X-K)/((1+Q)**2);
   OUTPUT OUT=P1 PREDICTED=PRED RESIDUEAL=RESID PARM= M P K;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF BLADDER TOTAL - CURVE FITTING;
DATA P1; SET P1;
   LEC50=K-12;
   EC50=10**(LEC50);
   P=P/(LOG(10));
PROC SORT;BY T LCONC;
PROC MEANS NOPRINT;
   VAR RESID; BY T LCONC; OUTPUT OUT=MRESID MEAN=MRESID;
PROC SORT;BY T LCONC;
DATA P1; MERGE P1 MRESID; BY T LCONC;
PROC PRINT;VAR T LCONC A2TOT M P LEC50 EC50
   PRED MA2TOT SMA2TOT;
   FORMAT EC50 E10. P LEC50 LCONC 11.3 M
   PRED MA2TOT SMA2TOT A2TOT 11.2;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF BLADDER TOTAL - FITTED PARAMETERS;
PROC PRINT;VAR T LCONC A2TOT RESID MRESID;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF BLADDER TOTAL - FITTED RESIDUALS;
PROC PLOT;BY T;
   PLOT A2TOT*LCONC PRED*LCONC='X' MA2TOT*LCONC='M'
   MA2TOTU*LCONC='U' MA2TOTD*LCONC='V'
OVERLAY;
TITLE DOSE RESPONSE CURVE AS A PERCENT OF
BLADDER TOTAL - FITTED PARAMETERS;
PLOT RESID*LCONC MRESID*LCONC='M'/OVERLAY VREF=0;
TITLE DOSE RESPONSE CURVE AS A PERCENT
OF BLADDER TOTAL - FITTED RESIDUALS;
**E.6 NONLINEAR REGRESSION CURVE FITTING ANALYSIS**

written by Phillip Ross

TITLE 'Bethanechol pressure D-R curves';
TITLE2 'Curves plotted as % indiv. maximum';
TITLE3 '3 age groups of rats';
TITLE4 '25% full bladders';
GOPTION DEVICE=IBM3287 HSIZE = 6 VSIZE = 8;
OPTIONS NODATE;
OPTIONS LINESIZE=76;
DATA ONE;INPUT TISSUE T CONC AMP A;
   LCONC= LOG10(CONC);
   CALIBAMP=2;
   A1=A*AMP*CALIBAMP;
   DROP A AMP CALIBAMP;
   CARDS;
1 1 3E-7 0.5 0.05
1 1 1E-6 0.5 0.20
1 1 3E-6 0.5 0.30
2 1 3E-7 0.5 0.00
2 1 1E-6 0.5 0.10
2 1 3E-6 0.5 0.17
3 1 3E-7 0.5 0.05
3 1 1E-6 0.5 0.10
3 1 3E-6 0.5 0.20
4 1 3E-7 0.5 0.02
4 1 1E-6 0.5 0.12
4 1 3E-6 0.5 0.20
4 1 1E-3 0.5 4.25
5 1 3E-7 0.5 0.08
5 1 1E-6 0.5 0.15
5 1 3E-6 0.5 0.15
6 1 3E-7 0.5 0.05
6 1 1E-6 0.5 0.15
6 1 3E-6 0.5 0.25
1 2 3E-7 0.5 0.05
1 2 1E-6 0.5 0.10
1 2 3E-6 0.5 0.15
2 2 3E-7 0.5 0.05
2 2 1E-6 0.5 0.08
2 2 3E-6 0.5 0.30
3 2 3E-7 0.5 0.02
3 2 1E-6 0.5 0.10
3 2 3E-6 0.5 0.22
4 2 3E-7 0.5 0.05
4 2 1E-6 0.5 0.10
4 2 3E-6 0.5 0.28
5 2 3E-7 0.5 0.02
5 2 1E-6 0.5 0.10
5 2 3E-6 0.5 0.25
6 2 3E-7 0.5 0.04
214

6 2 1E-6 0.5 0.08
6 2 3E-6 0.5 0.28
1 3 3E-7 0.5 0.08
1 3 1E-6 0.5 0.15
1 3 3E-6 0.5 0.30
2 3 3E-7 0.5 0.05
2 3 1E-6 0.5 0.12
2 3 3E-6 0.5 0.30
3 3 3E-7 0.5 0.00
3 3 1E-6 0.5 0.05
3 3 3E-6 0.5 0.19
4 3 3E-7 0.5 0.00
4 3 1E-6 0.5 0.10
4 3 3E-6 0.5 0.25
5 3 3E-7 0.5 0.05
5 3 1E-6 0.5 0.10
5 3 3E-6 0.5 0.40
5 3 1E-5 0.5 0.70
6 3 3E-7 0.5 0.00
6 3 1E-6 0.5 0.04
6 3 3E-6 0.5 0.06

PROC SORT; BY TISSUE T LCONC;
DATA ONE; SET ONE; BY TISSUE T;
   IF FIRST.TISSUE THEN MAXITOT=0;
   IF FIRST.T THEN MAXI=0;
   IF A1>MAXI THEN MAXI=A1;
   IF MAXI>MAXITOT THEN MAXITOT=MAXI;
   RETAIN MAXI MAXITOT;
PROC SORT; BY TISSUE DESCENDING T DESCENDING LCONC;
DATA ONE; SET ONE; BY TISSUE DESCENDING T;
   IF FIRST.TISSUE THEN MAXTOT=MAXITOT;
   IF FIRST.T THEN MAX=MAXI;
   RETAIN MAX MAXTOT;
   DROP MAXI MAXITOT;
PROC PRINT; VAR TISSUE T LCONC A1 MAX MAXTOT;
   TITLE A CHECK TO SEE THAT THE MAX AND MAXTOT VALUES ARE CORRECT;
PROC SORT;BY TISSUE T LCONC;
DATA ONE; SET ONE;
   A2=(AI/MA1)*100;
   A2TOT=(AI/MAXTOT)*100;
PROC SORT;BY T LCONC;
PROC MEANS N MEAN STDERR MAXDEC=2 NOPRINT;
   BY T LCONC;VAR A2 A2TOT;OUTPUT OUT=MA2 MEAN=MA2 MA2TOT STDERR=SMA2 SMA2TOT;
DATA MA2;SET MA2;
   MA2U=MA2+SMA2;
   MA2D=MA2-SMA2;
   MA2TOTU=MA2TOT+SMA2TOT;
   MA2TOD=MA2TOT-SMA2TOT;
PROC SORT;BY T LCONC;
DATA ONE;MERGE ONE MA2;BY T LCONC;
LCONCPOS=12+LCONC;
PROC DATASETS;SAVE ONE;
PROC NLIN DATA=ONE METHOD=MARQUARDT EFORMAT
   CONVERGE=1E-12 MAXITER=100;
   BY T;
      PARMS M=100 P=1 K=8;
      BOUNDS M>0, 0<P<30, K>0;
      X=LCONCPOS;Y=A2;
      Q = EXP(P*(X-K));
      MODEL Y = ((M*Q)/(1+Q));
      DER.M = Q/(1+Q);
      DER.K = -M*P*Q/((1+Q)**2);
      DER.P = M*Q*(X-K)/((1+Q)**2);
      OUTPUT OUT=P1 PREDICTED=PRED RESIDUAL=RESID PARM=M P K;
      TITLE DOSE RESPONSE CURVE AS A PERCENT OF
         INDIVIDUAL MAXIMA - CURVE FITTING;
DATA P1;SET P1;
   LEC50=K-12;
   EC50=10**(LEC50);
   P=P/(LOG(10));
PROC SORT;BY T LCONC;
PROC MEANS NOPRINT;
   VAR RESID;BY T LCONC;OUTPUT OUT=MRESID MEAN=MRESID;
PROC SORT;BY T LCONC;
DATA P1;MERGE P1 MRESID;BY T LCONC;
PROC PRINT;VAR T LCONC A2 M P LEC50 EC50 PRED MA2 SMA2;
   FORMAT EC50 E10. P LEC50 LCONC 11.3 M
      PRED MA2 SMA2 A2 11.2;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF
      INDIVIDUAL MAXIMA - FITTED PARAMETERS;
PROC PRINT;VAR T LCONC A2 RESID MRESID;
   TITLE DOSE RESPONSE CURVE AS A PERCENT
      OF INDIVIDUAL MAXIMA - FITTED RESIDUALS;
PROC PLOT;BY T;
   PLOT A2*LCONC PRED*LCONC='X' MA2*LCONC='M'
      MA2U*LCONC='U' MA2D*LCONC='V'
      /OVERLAY;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF
      INDIVIDUAL MAXIMA - FITTED PARAMETERS;
PROC PLOT;BY T;
   PLOT RESID*LCONC MRESID*LCONC='M'/OVERLAY VREF=0;
   TITLE DOSE RESPONSE CURVE AS A PERCENT OF
      INDIVIDUAL MAXIMA - FITTED RESIDUALS;
PROC GPLOT; BY T;
written by Richard A. Couch
TITLE1 Dose Response Curve as a Percent of Individual Maxima;
TITLE2 BETHPL2 SAS 25% FULL BLADDER;
LABEL LCONC=Log Concentration;
LABEL PRED=Percent Maximum Pressure;
SYMBOL1 V=NONE I=SM20 C=BLUE;
SYMBOL2 V=DIAMOND C=BLUE;
SYMBOL3 V=PLUS c=blue;
SYMBOL4 V=PLUS c=blue;
PLOT PRED*LCONC MA2*LCONC
MA2U*LCONC MA2D*LCONC
/OVERLAY;
E.7 STUDENT'S T TEST

OPTIONS LINESIZE=76;
DATA ONE;
TITLE 'Isoproterenol D-R curves';
TITLE2 'Maximal expansion response';
TITLE3 '3 age groups of rats';
INPUT AGEGROUP CONC;
CARDS;
1 59.3
1 44.3
1 53.4
1 51.9
1 47.8
2 60
2 47.7
2 66.5
2 48.5
2 52.1
3 57.6
3 61.0
3 46.9
3 56.2
3 54
;
PROC PRINT;
PROC GLM;
CLASS AGEGROUP;
MODEL CONC=AGEGROUP;
MEANS AGEGROUP/DUNCAN LINES;
MEANS AGEGROUP/DUNCAN CLDIFF;
MEANS AGEGROUP/SNK LINES;
MEANS AGEGROUP/SNK CLDIFF;
PROC SORT; BY AGEGROUP;
PROC MEANS; BY AGEGROUP; VAR CONC;
DATA TWO; SET ONE;
IF AGEGROUP=1 OR AGEGROUP=2;
PROC TTEST;
CLASSES AGEGROUP;
VAR CONC;
DATA THREE; SET ONE;
IF AGEGROUP=1 OR AGEGROUP=3;
PROC TTEST;
CLASS AGEGROUP;
VAR CONC;
DATA FOUR; SET ONE;
IF AGEGROUP=2 OR AGEGROUP=3;
PROC TTEST;
CLASS AGEGROUP;
VAR CONC;
LIST OF REFERENCES

1. The Urinary Bladder Neurology and Dynamics, Hald, T. and Bradley W., Eds.; Williams and Wilkins: Baltimore, 1982; Chapter 16.


