Effect of Age, Temperature and Hypoxia on the Response of the Testicular Capsule to Neurohumoral Agents

Arthur M. Horowitz

Loyola University Chicago

1979

Recommended Citation
https://ecommons.luc.edu/luc_diss/1810

This Dissertation is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Dissertations by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

Creative Commons License
This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.

Copyright © 1979 Arthur M. Horowitz
EFFECT OF AGE, TEMPERATURE AND HYPOXIA ON THE RESPONSE OF THE TESTICULAR CAPSULE TO NEUROHUMORAL AGENTS

by

Arthur M. Horowitz

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 1979
EFFECT OF AGE, TEMPERATURE AND HYPOXIA ON THE RESPONSE OF THE TESTICULAR CAPSULE TO NEUROHUMORAL AGENTS

The contractile response of the rat isolated testicular capsule to norepinephrine (NE), acetylcholine (ACh) and prostaglandins (PG) has been found to be dependent upon age of the animal. The testicular capsular contractions attributable to smooth muscle cells located within the tunica albuginea may be involved in the transport of non-motile spermatozoa from the testis and into the epididymis. A relationship appears to exist between the onset of spermiogenesis and the degree of testicular capsular contraction produced by equimolar concentrations of NE and PGA₂. An increase in age of the rat was found to have a concomitant increase in mass of the testis, thickness of the capsule and the amount of smooth muscle fibers present in the tunica albuginea. The response of the rat isolated testicular capsule to neurohumoral agents does not appear to be correlated with the development of an increased mass of contractile elements during prepubertal to early adulthood, or smooth muscle hyperplastic changes associated with advanced age, since the response to ACh remained constant throughout the age range studied and PGF₂α became progressively less stimulatory as the age of the animal increased. The different responses induced by the drugs studied at various ages may be related to changes in the smooth muscle receptors.

Spontaneous contractions of the adult rabbit isolated testicular capsule were found to be influenced by moderate hyperthermic and hypothermic
temperature changes. A progressive increase in temperature from a normal scrotal temperature of 37 to 44°C resulted in an increase in both frequency and tone, accompanied by a significant decrease in amplitude. Further increase in temperature to 48°C resulted in irreversible cessation of spontaneous contractions. On the other hand, decreasing the temperature from 37 to 26°C resulted in a marked decrease in frequency and amplitude progressing to a complete but reversible cessation of spontaneous contractions at 16°C. The addition of NE to the testicular capsule at 32, 40 and 42°C was found to cause the greatest increase and re-initiation of capsular tone.

Spontaneous contractions of the adult rabbit isolated testicular capsule have also been demonstrated to be adversely affected by short exposures to hypoxia. Exposure of the testicular capsule to hypoxia for 10 minutes produced a progressive decrease in frequency and amplitude of spontaneous contractions, along with a corresponding marked increase in tissue tone. Further increases in hypoxic exposure resulted in cessation of capsular contractions. Sodium nitroprusside, papaverine and verapamil each were found to relax the hypoxic-induced contracture of the testicular capsule and to restore capsular tone.

The present data demonstrates that numerous factors such as age, temperature and hypoxia may alter drug-induced and spontaneous contractions of the testicular capsule, which may ultimately interfere with the transport of sperm out of the testis. In addition, neurohumoral agents may play an important role in the maintenance of testicular capsular contractions from prepubertal to advanced age, as well as the maintenance of testicular capsule tone in cases of exposure to extreme temperature and hypoxic conditions.
ACKNOWLEDGEMENTS

It is a pleasure to express my sincere appreciation to Dr. Joseph R. Davis, without whom the opportunity to conduct this research would not have been afforded. I shall always be grateful for his guidance and support throughout my graduate education.

I am sincerely appreciative to Dr. Alexander G. Karczmar, Chairman of the Department of Pharmacology and Experimental Therapeutics for providing a scholarly atmosphere and financial support throughout my graduate education. I wish to acknowledge the staff of the library and the animal research facilities for their technical assistance.

I also wish to express thanks to my parents for their love and support throughout my educational years. My mother has always expressed her compassion and love in some of my difficult hours. My father has through the years been an inspiration and guide. In addition, I wish to thank my grandparents, Irene Fuerst and Joseph and Esther Horowitz for their youthful enthusiasm which has given me great strength and security to achieve this goal.

My utmost appreciation is expressed to my wife, Terrie, for her deep love, encouragement, sacrifices and great patience during my graduate education. Her understanding and strength has made everything now worthwhile and worth looking forward to.

Finally, this dissertation has been written with the same conviction and perseverance as the 19th century Zionist, Theodor Herzl, who wrote in Altneuland, "If you will it, it is no longer a dream."
LIFE

Arthur M. Horowitz was born in Chicago, Illinois on February 1, 1950. He is married to the former Terrie Ship.

His secondary education was obtained at James H. Bowen High School, Chicago, Illinois, where he graduated in 1967. In June, 1972, he received the degree of Bachelor of Science with a major in microbiology from Colorado State University. The author began his graduate education in September, 1972 at Loyola University of Chicago Stritch School of Medicine in the Department of Pharmacology.

During his graduate training at Loyola, the author was a National Institute of Health Pre-doctoral trainee from 1972-1975 and in 1978 was designated as an Arthur J. Schmitt Doctoral Fellow.

The author is currently a member of the following scientific societies: Sigma Xi, American Chemical Society, The New York Academy of Sciences and The Society for the Study of Reproduction.
PUBLICATIONS


# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIFE</td>
<td>v</td>
</tr>
<tr>
<td>PUBLICATIONS</td>
<td>vi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxiii</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxiv</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xxv</td>
</tr>
<tr>
<td><strong>Chapter</strong></td>
<td></td>
</tr>
<tr>
<td>I. STATEMENT OF THE PROBLEM</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF THE RELATED LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>A. MORPHOLOGY OF THE TESTICULAR CAPSULE</td>
<td>5</td>
</tr>
<tr>
<td>1. Tunica vaginalis visceral</td>
<td>5</td>
</tr>
<tr>
<td>2. Tunica albuginea</td>
<td>6</td>
</tr>
<tr>
<td>3. Tunica vasculosa</td>
<td>7</td>
</tr>
<tr>
<td>4. Mediastinum testis</td>
<td>7</td>
</tr>
<tr>
<td>B. VASCULAR SUPPLY OF THE TESTICULAR CAPSULE</td>
<td>9</td>
</tr>
<tr>
<td>1. Arterial supply</td>
<td>9</td>
</tr>
<tr>
<td>2. Venous supply</td>
<td>10</td>
</tr>
<tr>
<td>3. Lymphatic supply</td>
<td>10</td>
</tr>
</tbody>
</table>
### C. INNERVATION OF THE TESTICULAR CAPSULE

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Afferent (sensory) system</td>
<td>10</td>
</tr>
<tr>
<td>2. Efferent (autonomic) system</td>
<td>12</td>
</tr>
<tr>
<td>3. Electrical nerve stimulation</td>
<td>13</td>
</tr>
</tbody>
</table>

### D. MECHANISM OF SPERM TRANSPORT

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cilia</td>
<td>15</td>
</tr>
<tr>
<td>2. Tubular fluid secretions</td>
<td>15</td>
</tr>
<tr>
<td>3. Seminiferous tubules</td>
<td>16</td>
</tr>
<tr>
<td>4. Testicular capsule</td>
<td>19</td>
</tr>
<tr>
<td>a. Rat isolated testicular capsule</td>
<td>19</td>
</tr>
<tr>
<td>b. Rabbit isolated testicular capsule</td>
<td>19</td>
</tr>
<tr>
<td>c. Human isolated testicular capsule</td>
<td>21</td>
</tr>
</tbody>
</table>

### E. ROLE OF PROSTAGLANDINS IN THE TESTIS

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Prostaglandin synthetase system</td>
<td>22</td>
</tr>
<tr>
<td>2. Regulation of rabbit testicular capsular motility</td>
<td>23</td>
</tr>
</tbody>
</table>

### F. CONTRACTILE MECHANISM OF SMOOTH MUSCLE

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Structural basis of contraction and regulation of smooth muscle</td>
<td>25</td>
</tr>
<tr>
<td>2. Spontaneous activity of smooth muscle</td>
<td>27</td>
</tr>
<tr>
<td>3. Mechanisms for activation of smooth muscle</td>
<td>28</td>
</tr>
</tbody>
</table>

### G. PHARMACOLOGY OF SMOOTH MUSCLE

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Catecholamines</td>
<td>30</td>
</tr>
<tr>
<td>2. Acetylcholine</td>
<td>32</td>
</tr>
<tr>
<td>3. Prostaglandins</td>
<td>33</td>
</tr>
</tbody>
</table>
4. Spasmolytic agents
   a. Papaverine
   b. Sodium nitroprusside
   c. Verapamil

H. AGING AND DEVELOPMENTAL CHANGES OF
   THE TESTICULAR CAPSULE
   1. Morphological changes
   2. Vascular changes
   3. Pharmacological responses as it relates
c to developmental and aging changes

I. HYPOXIA
   1. Hypoxia and smooth muscle
      a. Energy production in hypoxia
      b. Calcium utilization in hypoxia
      c. Effects of CO₂ and pH on the
         mechanical properties of smooth
         muscle
   2. Hypoxia and collagen
   3. Effect of hypoxia on the action of
drugs in smooth muscle

J. TEMPERATURE
   1. Vascular heat-exchange
   2. Thermoregulatory apparatus
      a. Dartos muscle
      b. Cremasteric muscle
      c. Scrotal ligament
3. Effect of temperature on spermatogenesis  
   a. Diathermic treatment  51  
   b. Baths  51  
   c. Intrascrotal hyperthermia induced by scrotal insulation  52  
   d. Hyperpyrexia  53  
   e. Control of human spermatogenesis by induced changes of intrascrotal temperature  54

4. Effect of temperature on testicular capsular contractions  55

5. Temperature and smooth muscle  56

6. Temperature and collagen  57

7. Effects of temperature on the action of drugs in smooth muscle  58

8. Congenital disorders of the testicle related to temperature  60  
   a. Cryptorchidism  60  
   b. Varicocele  61

K. PATHOLOGY OF THE TESTICULAR CAPSULE  62

1. Benign lesions  63

2. Malignant neoplasms  63

3. Miscellaneous lesions  63  
   a. Hydrocele  63  
   b. Hematocele  64  
   c. Chylocele  64  
   d. Spermatocele  64

L. MORPHOLOGY OF THE SPLENIC CAPSULE  65
M. VASCULAR SUPPLY OF THE SPLENIC CAPSULE
1. Arterial supply
2. Venous supply

N. INNERVATION OF THE SPLENIC CAPSULE
1. Sympathetic innervation
2. Electrical nerve stimulation

O. PHARMACOLOGY OF THE SPLENIC CAPSULE
1. Catecholamines
2. Cholinergic drugs
3. Prostaglandins
4. Vasoactive amines
5. Barium chloride

P. PATHOLOGY OF THE SPLENIC CAPSULE
1. Perisplenitis
2. Splenic capsule neoplasms

Q. MORPHOLOGY OF THE RENAL CAPSULE
1. Renal fascia
2. Adipose capsule
3. Capsula proper

R. VASCULAR SUPPLY OF THE RENAL CAPSULE
1. Arterial supply
2. Venous supply

S. INNERVATION OF THE RENAL CAPSULE

T. PATHOLOGY OF THE RENAL CAPSULE
III. MATERIALS AND METHODS . . . . 80

A. EXPERIMENTAL CONDITIONS . . . . 81

1. Animals . . . . . . 81
   a. Rat . . . . . . 81
   b. Rabbit . . . . . . 81

2. Isolated organ bath assembly . . . . 82

3. Physiological salt solution . . . . 83

4. Pharmacological agents . . . . 84
   a. Determination of the response of the isolated testicular capsule to autonomic drugs . . . . 84
   b. Determination of the response of the isolated splenic capsule to autonomic drugs . . . . 84

B. PROCEDURE FOR THE ISOLATION OF THE TESTICULAR AND SPLENIC CAPSULES FOR IN VITRO PHARMACOLOGICAL STUDIES . . 85

1. Rat and rabbit isolated testicular capsule preparation . . . . 85

2. Rabbit isolated splenic capsule strip preparation . . . . 86

C. PHYSIOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE . . . . 87

1. Effect of hyperthermic and hypothermic temperature changes on the adult rabbit testicular capsule . . . . 88
   a. Procedure for the determination of optimal temperature . . . . 88
   b. Procedure for rapid reversal of organ bath temperature . . . . 88
c. Procedure to study the effect of temperature on agonist-induced contractions of the isolated testicular capsule 89

2. Effect of hypoxic conditions on the adult rabbit isolated testicular capsule and isolated duodenum 89
   a. Procedure for investigating the effect of hypoxia on spontaneous contractions of the isolated testicular capsule and isolated duodenum 89
   b. Procedure to study the effect of hypoxia on smooth muscle relaxant agents of the adult rabbit isolated testicular capsule 90

D. ANATOMICAL STUDIES ON CAPSULAR TISSUE 90
   1. Tissue preparation 90
   2. Staining procedures 91

E. STATISTICAL ANALYSES 93
   1. Calculation of mean, standard deviation and standard error 93
   2. Calculation of the t distribution and determination of the P value, the degree of significance 93

IV. RESULTS 96
   A. EFFECT OF DEVELOPMENTAL AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS 97
B. EFFECT OF GERIATRIC AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS 100

C. EFFECT OF PROGRESSIVE HYPERTERMIC AND HYPOTHERMIC TEMPERATURE CHANGES ON ADULT RABBIT ISOLATED TESTICULAR CAPSULAR SPONTANEOUS CONTRACTIONS 102

D. EFFECT OF VARIOUS EXPOSURE TIMES TO HYPERTERMIA AND HYPOTHERMIA ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE 103

E. RESPONSE OF THE RABBIT ISOLATED TESTICULAR CAPSULE AT HYPOTHERMIC AND HYPERTERMIC TEMPERATURES TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS F_{2α} 105

F. EFFECT OF HYPOXIA ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE AND DUODENUM 107

G. EFFECT OF HYPOXIA ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL 108

H. COMPARATIVE RESPONSE OF THE ADULT RAT AND RABBIT ISOLATED TESTICULAR CAPSULES TO NEUROHUMORAL AGENTS 110
I. COMPARATIVE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR AND SPLENIC CAPSULES TO NEUROHUMORAL AGENTS 113

J. EFFECT OF HEAVY METALS ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE 115

V. DISCUSSION 118

A. EFFECT OF DEVELOPMENTAL AGE ON THE PHARMACOLOGICAL RESPONSE OF THE RAT TESTICULAR CAPSULE 119

B. EFFECT OF GERIATRIC AGE ON THE PHARMACOLOGICAL RESPONSE OF THE RAT TESTICULAR CAPSULE 124

C. EFFECT OF TEMPERATURE ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE 125

D. RESPONSE OF THE RABBIT ISOLATED TESTICULAR CAPSULE AT HYPERTHERMIC AND HYPOTHERMIC TEMPERATURES TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN $F_{2\alpha}$ 129

E. COMPARATIVE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE AND DUODENUM TO HYPOXIA 131
F. RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAYERINE AND VERAPAMIL EXPOSED TO HYPOXIA . 134

VI. SUMMARY . 137
VII. REFERENCES . 142
VIII. FIGURES . 177
IX. TABLES . 262
X. APPENDICES . 279
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Schematic representation of the procedure for the isolation of the rat and rabbit testicular capsule and its use as an isolated tissue preparation for pharmacological studies.</td>
<td>178</td>
</tr>
<tr>
<td>2.</td>
<td>Schematic representation of the procedure for the isolation of the rabbit splenic capsule and its use as an isolated tissue preparation for pharmacological studies.</td>
<td>180</td>
</tr>
<tr>
<td>3.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to equimolar concentrations (3.0 x 10^{-6} M) of prostaglandins, norepinephrine and acetylcholine.</td>
<td>182</td>
</tr>
<tr>
<td>4.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of norepinephrine.</td>
<td>184</td>
</tr>
<tr>
<td>5.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of acetylcholine.</td>
<td>186</td>
</tr>
<tr>
<td>6.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin E₁.</td>
<td>188</td>
</tr>
<tr>
<td>7.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin A₂.</td>
<td>190</td>
</tr>
<tr>
<td>8.</td>
<td>Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin F₂α.</td>
<td>192</td>
</tr>
</tbody>
</table>
9. Effect of age of the rat on body weight, the weight of the whole testis and the weight of the isolated testicular capsule; Effect of age of the rat on the percent shortening of the isolated testicular capsule to 0.1 $\mu$g/ml acetylcholine and 1.0 $\mu$g/ml norepinephrine.  

10. Effect of age on the tissue contraction of the rat isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandins $A_2$ and $F_2\alpha$.  

11. Effect of age on the response of the rat isolated testicular capsule to equimolar concentrations ($3.0 \times 10^{-6}$ M) of norepinephrine, acetylcholine and prostaglandins $A_2$ and $F_2\alpha$.  

12. Effect of age on the percent shortening of the rat isolated testicular capsule to equimolar concentrations ($3.0 \times 10^{-6}$ M) of norepinephrine, acetylcholine and prostaglandins $A_2$ and $F_2\alpha$.  


14. Typical response of the isolated testicular capsule to hyperthermic and hypothermic temperature change.  

16. Effect of 42° C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. . . . 208

17. Effect of 40° C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. . . . 210

18. Effect of 32° C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. . . . 212

19. Effect of 16° C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. . . . 214

20. Typical response of the isolated testicular capsule exposed for 5 minutes to various hyperthermic and hypothermic temperature changes. . . . . . . 216

21. Typical response of the isolated testicular capsule exposed for 30 minutes to various hyperthermic and hypothermic temperature changes. . . . . . . 218

22. Effect of norepinephrine, acetylcholine and prostaglandin F₂α on the response of the adult rabbit isolated testicular capsule exposed to 37° C. . . . . . . 220

23. Effect of norepinephrine, acetylcholine and prostaglandin F₂α on the response of the adult rabbit isolated testicular capsule exposed to 32° C. . . . . . . 222

24. Effect of norepinephrine, acetylcholine and prostaglandin F₂α on the response of the adult rabbit isolated testicular capsule exposed to 40° C. . . . . . . 224
<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.</td>
<td>Effect of norepinephrine, acetylcholine and prostaglandin ( F_2\alpha ) on the response of the adult rabbit isolated testicular capsule exposed to ( 42^\circ ) C.</td>
<td>226</td>
</tr>
<tr>
<td>26.</td>
<td>Comparative changes of the tone of the adult rabbit isolated testicular capsule by norepinephrine, acetylcholine and prostaglandin ( F_2\alpha ) at ( 32, 40, 42^\circ ) C.</td>
<td>228</td>
</tr>
<tr>
<td>27.</td>
<td>Effect of hypoxia on spontaneous isotonic contractions of the adult rabbit isolated testicular capsule.</td>
<td>230</td>
</tr>
<tr>
<td>28.</td>
<td>Typical response of the isolated testicular capsule exposed for 1, 2, 5, 10 and 15 minutes to hypoxia.</td>
<td>232</td>
</tr>
<tr>
<td>29.</td>
<td>Effect of hypoxia on spontaneous isotonic contractions of the adult rabbit isolated duodenum.</td>
<td>234</td>
</tr>
<tr>
<td>30.</td>
<td>Typical response of the isolated duodenum exposed for 1, 2, 5, 10 and 15 minutes to hypoxia.</td>
<td>236</td>
</tr>
<tr>
<td>31.</td>
<td>Typical effect of sodium nitroprusside, papaverine and verapamil ((10^{-6} \text{ M})) on the response of the adult rabbit isolated testicular capsule exposed to aeration.</td>
<td>238</td>
</tr>
<tr>
<td>32.</td>
<td>Contractile response of the adult rabbit isolated testicular capsule to equimolar additions ((10^{-6} \text{ M})) of sodium nitroprusside, papaverine and verapamil during exposure to aeration.</td>
<td>240</td>
</tr>
<tr>
<td>33.</td>
<td>Typical effect of sodium nitroprusside, papaverine and verapamil ((10^{-6} \text{ M})) on the response of the adult rabbit isolated testicular capsule exposed to hypoxia.</td>
<td>242</td>
</tr>
</tbody>
</table>
34. Contractile response of the adult rabbit isolated testicular capsule to equimolar additions (10^{-6} M) of sodium nitroprusside, papaverine and verapamil during exposure to hypoxia. . . . . . . . . 244

35. Response of the adult rat isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin F_2\alpha. . . . . . . . 246

36. Response of the adult rabbit isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin F_2\alpha. . . . . . . . 248

37. Representative histological cross sections of the rat and rabbit testicular capsules. . . . . . . . 250

38. Response of the adult rabbit isolated splenic capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin F_2\alpha. . . . . . . . 252

39. Comparative response of the adult rabbit isolated testicular and splenic capsules to equimolar concentrations (3.0 \times 10^{-6} M) of norepinephrine, acetylcholine and prostaglandin F_2\alpha. . . . . . . . 254

40. Typical response of the adult rabbit isolated testicular capsule to increasing concentrations of Hg^{++}. . . . . 256

41. Typical response of the adult rabbit isolated testicular capsule to increasing concentrations of Cd^{++}. . . . . 258
42. Prevention of Cd\textsuperscript{++}-induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions by Zn\textsuperscript{++}.

Page 260
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Comparative response of the rat isolated testicular capsule to prostaglandins, norepinephrine and acetylcholine at 30 and 90 days of age.</td>
<td>263</td>
</tr>
<tr>
<td>2.</td>
<td>Development of spermatogenesis of the Sprague-Dawley rat.</td>
<td>265</td>
</tr>
<tr>
<td>3.</td>
<td>Comparative effect of age of the rat on body weight, weight of the intact testis, weight of the isolated testicular capsule, resting length of the testicular capsule and thickness of the testicular capsule of rats aged 30 to 640 days.</td>
<td>267</td>
</tr>
<tr>
<td>4.</td>
<td>Response of the adult rabbit isolated testicular capsule to increasing concentrations of Hg$^{++}$.</td>
<td>269</td>
</tr>
<tr>
<td>5.</td>
<td>Response of the adult rabbit isolated testicular capsule to increasing concentrations of Cd$^{++}$.</td>
<td>271</td>
</tr>
<tr>
<td>6.</td>
<td>Prevention of Cd$^{++}$-induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions by Zn$^{++}$.</td>
<td>273</td>
</tr>
<tr>
<td>7.</td>
<td>Typical temperatures of hot running water in various hotels and hospitals in the United States.</td>
<td>275</td>
</tr>
<tr>
<td>8.</td>
<td>Summer beach water temperatures in selected northern cities of the United States.</td>
<td>277</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Molar equivalent of $\mu$g/ml final bath concentrations of drugs</td>
<td>281</td>
</tr>
<tr>
<td>B. Reproductive data for the rabbit</td>
<td>283</td>
</tr>
<tr>
<td>C. Effect of temperature on spontaneous isometric contractions of the adult rabbit isolated testicular capsule</td>
<td>285</td>
</tr>
<tr>
<td>D. Effect of aeration on the tissue contraction of the rabbit isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin $F_2\alpha$</td>
<td>287</td>
</tr>
<tr>
<td>E. Effect of aeration on spontaneous contractions of the adult rabbit isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin $F_2\alpha$</td>
<td>289</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

acetylcholine ACh
alpha α
beta β
centimeter cm
degree Celsius (Centigrade) °C
degree Fahrenheit °F
drug concentration that produces 50% of the maximal effect ED$_{50}$
gram g
hour h
kilogram kg
liter l
micron μ
microgram μg
microliter μl
milligram mg
milliliter ml
millimeter mm
millimicron μm
millimolar mM
minute min
molar (concentration) M
nanomolar nM
norepinephrine NE
percent %
probability statistical test p
prostaglandin A$_2$ PGA$_2$
prostaglandin E$_1$ PGE$_1$
prostaglandin E$_2$ PGE$_2$
prostaglandin F$_{1α}$ PGF$_{1α}$
prostaglandin F$_{2α}$ PGF$_{2α}$
second sec
times x
CHAPTER 1

STATEMENT OF THE PROBLEM
CHAPTER I

STATEMENT OF THE PROBLEM

Considerable evidence has been reported in support of a role for the testicular capsule in the transport of non-motile sperm out of the testis and into the epididymis. Electrical stimulation of the perivascular nerve and the administration of contractile agents to the isolated testicular capsule and whole testis have been demonstrated to increase the efflux of sperm from the rete testis. The transport of sperm out of the testis has been attributed to the contractile activity of smooth muscle located within the tunica albuginea. The relative contribution of the smooth muscle cells to sperm transport are not equal in all species. Testicular capsules from rabbit and man contract spontaneously, whereas the rat testicular capsule contracts only after electrical or pharmacological stimulation. The absence of spontaneous contractions of the rat testicular capsule has distinct pharmacological advantages in accurately determining the extent of drug-induced contractions. On the other hand, the demonstration of spontaneous contractions of the rabbit testicular capsule represents a unique opportunity to study the physiologic factors involved in the stimulus-contraction coupling process of testicular capsular smooth muscle.

The purpose of this study on the testicular capsule is therefore:

1) To investigate whether a relationship exists between the onset of spermiogenesis and the degree of contraction of the isolated testicular capsule of the developing rat produced by norepinephrine, acetylcholine and prostaglandins.
2) To compare the response of the isolated testicular capsule of older aged rats to norepinephrine, acetylcholine and prostaglandins with respect to an adult rat and to determine whether a relationship exists between the morphological changes observed in the aging testis and the degree of testicular capsular contraction.

3) To determine whether hyperthermic or hypothermic temperature exposure can effect spontaneous contractions of the adult rabbit isolated testicular capsule.

4) To explore the effect of hyperthermic and hypothermic temperature on the action of neurohumoral agents in rabbit testicular capsular smooth muscle.

5) To investigate the comparative effect of various time exposures to hypoxia on spontaneous contractions of the adult rabbit isolated testicular capsule and isolated duodenum.

6) To explore the effect of smooth muscle relaxant agents on the hypoxic-induced contracture of the rabbit isolated testicular capsule.
CHAPTER II

REVIEW OF THE LITERATURE
CHAPTER II

REVIEW OF THE RELATED LITERATURE

A. MORPHOLOGY OF THE TESTICULAR CAPSULE

The testicular capsule surrounding the parenchymal tissue of the testis consists of three layers. These layers include the tunica vaginalis visceral, which is an outer thin serous layer, the tunica albuginea which is the most predominant layer of the capsule and the tunica vasculosa which is a thin layer of loose areolar tissue beneath the tunica albuginea. The scrotal layers are separated from the testicular capsule by the cavity of the tunica vaginalis.

1. *Tunica vaginalis visceral*

Leeson and Adamson (1962) have investigated the ultrastructure of tunica vaginalis visceral of the rat, rabbit and human. The tunica vaginalis visceral is a complete but attenuated mesothelial layer. In the rat and rabbit, thickness of the tunica vaginalis visceral is 200 μm or less, while in the human the thickness of this layer is approximately 500 μm. The nuclei of the mesothelial cells are flattened and protrude slightly into the cavity of the tunica serosa. Mitochondria are small and few in number, with sparse granular endoplasmic reticulum. In addition, microvilli extend into the cavity of the tunica vaginalis visceral. Microvilli are more numerous and often longer in the human, than the rat and rabbit. The mesothelial cells lie on a thin but well-defined extracellular basement membrane, beneath which are many irregularly arranged collagen fibrils.
2. **Tunica albuginea**

The tunica albuginea occupies the middle and most predominant layer of the testicular capsule. It is classified as a dense connective tissue composed of collagen bundles, elastic fibers and numerous fibroblasts. The tunica albuginea is covered by the tunica vaginalis visceral. Contractile cells either of the smooth muscle or myofibroblast type have been found in all mammalian species. Included in this list are capsules of man (Davis and Langford, 1970; Langford and Heller, 1973; Leeson and Cookson, 1974), rat (Holstein and Weiss, 1967; Davis and Langford, 1970; Gorgas and Böck, 1974; Leeson and Cookson, 1974; Leeson, 1974), rabbit (Davis and Langford, 1970), dog and cat (Leeson and Cookson, 1974). These contractile cells lie in one or more layers (Davis et al., 1970) and numerous bundles of cytoplasmic filaments that terminate in plaques of electron-dense material adhering to the inner surface of the plasmalemma. Organelles are located mainly at the pole of the nucleus. Surface vesicles have also been identified that may correspond to the pinocytic vesicles of smooth muscle (Gorgas and Böck, 1974; Leeson and Cookson, 1974).

Smooth muscle is most prominent in the testicular capsule of the rabbit (Davis et al., 1970) and dog (Leeson and Cookson, 1974); and less prominent in the rat (Davis et al., 1970; Leeson and Cookson, 1974) and human (Langford and Heller, 1973). Davis and co-investigators (1970) observed two distinct layers of smooth muscle in the rabbit testicular capsule. The superficial layer runs parallel to the long axis of the testis and the deeper layer is orientated at right angles to the superficial layers. Although the smooth muscle cells of the rabbit testicular capsule achieve uniform distribution, within canine, feline and human capsules these cells are concentrated in the posterior aspect of the testis, where the
testicular capsule becomes continuous with the mediastinum (Davis et al., 1970; Langford and Heller, 1973).

3. **Tunica vasculosa**

The innermost layer of the testicular capsule is the tunica vasculosa. This thin, loose layer has been described to consist of occasional plexiform networks of minute blood vessels held together by a delicate areolar tissue (Davis et al., 1970).

4. **Mediastinum testis**

The mediastinum testis is the intratesticular reflection of the tunica albuginea. Dhingra (1977) has described the mediastinum testis as having tubular and intertubular components. The intertubular mediastinum testis is composed of loose and dense connective tissue. The connective tissue radiates from the mediastinum and divides the testicular parenchyma into lobuli testis (septula testis). The presence of smooth and a few striated muscle fibers have recently been shown in the tunica albuginea adjoining the epididymial margin in the rat (Leeson and Cookson, 1974) and man (Bustos-Obregon and Holstein, 1976). These cells may be embryologically related to the smooth muscle cells of the tunica albuginea (Vossmeyer, 1971).

The tubular component of the mediastinum testis includes the straight tubules (tubuli seminiferi recti) and the rete testis. Kormano (1977) has described the rete testis as a network of irregular labyrinthine spaces and interconnecting tubules located in the mediastinum testis, through which the products of seminiferous tubules transverse before entering the efferent ducts. Roosen-Runge
(1961) has divided the rete testis of the adult rat into three parts: the intratesticular, the intratunical and the extratesticular rete testis.

The intratesticular rete is the largest portion of the rete testis and runs parallel with the longitudinal axis of the testis. A number of seminiferous tubules opens into the intratesticular rete.

The intratunical part of the rete consists of a series of irregular passageways which penetrate the tunica albuginea and connect the intratesticular rete near the superior end of the testis. The cavity of the intratunical rete widens slightly after the channels have penetrated the tunica albuginea and forms the extratesticular rete. The ductuli efferentes join this cavity and lead into the ductus epididymis.

The rete testis epithelium in the adult rat varies from squamous to cuboidal or even low columnar (Leeson, 1962). Bustos-Obregón and Holstein (1976) analyzed the ultrastructure of the human rete testis and two epithelial cell types were distinguished; flat, dark cells exhibiting numerous slender microvilli and numerous apical and basal microvesicles. The second cell type revealed a lighter cell, with abundant cell organelles arranged in a supranuclear position. Both cells were rich in glycogen and fat.

The smooth muscle cells of the tunica albuginea and rete testis may be functionally significant. The later may be involved in changing the volume of the rete testis channels and promoting the flux of the rete testis fluid containing nonmotile spermatozoa from the testis to the epididymis (Holstein and Weiss, 1967; Davis et al., 1970; Rikimaru et al., 1972; Rikimaru and Shirai, 1972; Bustos-Obregón and Holstein, 1976).
B. VASCULAR SUPPLY OF THE TESTICULAR CAPSULE

Despite extensive literature on the vascularization of the testis (Setchell, 1970) testicular angiography has recently provided a detailed assessment of the anatomical arrangement of the vascular supply in the testis (Kormano and Nordmark, 1977).

1. Arterial supply

The paired testicular artery divides into several main branches in the head of the epididymis and surrounding connective tissue. One branch supplies the tunica vaginalis, while the epididymal branch supplies the tunica albuginea and passes toward the mediastinum. The microcirculating bed and distribution of vessels appears to be better developed in the parietal than the visceral layer of the human tunica vaginalis (Maksimova and Maksimov, 1977). Within the testis itself the blood vessels follow the direction of the septa. From the main branches on the testicular surface, the arteries penetrate toward the rete testis as coiled centripetal arteries. Branches of the centripetal arteries which run in an opposite direction are called centrifugal arteries. Both types of arteries divide further and terminate in a system of intertubular arterioles situated among the seminiferous tubules in the interstitial tissue. Their branches produce a network of intertubular capillaries within a single triangular column of interstitial tissue containing Leydig cells.
2. **Venous supply**

Intratesticular venous drainage passes into a series of centripetal veins and is directed towards the rete testis. Peripheral centrifugal veins have an opposite course. These surface veins follow the tunica albuginea toward the mediastinum and anastomose with the centripetal veins to form the pampiniform plexus (Kormano and Suoranta, 1971). Venous drainage of the testis eventually leads into the renal and inferior veins which corresponded to the left and right side, respectively.

3. **Lymphatic system**

The lymphatic drainage occurs by superficial and deep plexuses which is located under the tunica vaginalis. Lymph passes along the spermatic cord in combination with the testicular vessels, which then continues to the lateral aortic and preaortic lymph glands.

C. **INNERVATION OF THE TESTICULAR CAPSULE**

1. **Afferent (sensory) system**

There is abundant clinical evidence that the testis and scrotum are very sensitive to pain and pressure and that the scrotum responds to other modalities of cold, warmth and touch (Hodson, 1970). The sensory receptors of the ram (Waites, 1962) and primate (Iggo, 1966) may play an important role in testicular thermoregulation. This role is reported to be independent of deep body temperature.
The visceral and somatic afferent nerve fibers have their cell bodies in the dorsal (posterior) root ganglia of the spinal nerves. The testis and epididymis do not receive any somatic innervation. Their visceral supply is derived from the tenth thoracic segment, passing via the renal and aortic plexuses.

Two basic types of receptor organs have been distinguished in the testis: free endings and corpuscular endings. Free endings occur in the skin, while encapsulated (corpuscular) endings are located in relatively deep structures. Encapsulated endings have been found in addition to free endings in the tunica vaginalis visceral of the cat, dog (Corona, 1953) and human (Yamashita, 1939). In addition, afferent encapsulated nerve endings have been described in the tunica vasculosa of the bull (Shioda and Nishida, 1966). Kreutz (1964) described a type of encapsulated nerve ending in the human tunica albuginea bearing a resemblance to a Meissner's corpuscle. He found the end organs to be of two distinct types: one surrounded by a capsule composed of interstitial connective tissue and the other type composed of collagen fibers of the tunica albuginea. Afferent nerve endings bearing a resemblance to Pacinian and intercalated corpuscles have been described by Corona (1953) in the tunica vaginalis of the cat. Pacinian corpuscles may be considered as rapidly adapting mechanoreceptors (Iggo, 1966) which can be excited by distension or movement of the surrounding tissue. Intense pain due to pressure applied to the testis may be due to stretching of the testicular capsule, which then would excite the Pacinian corpuscles of the tunica vaginalis visceral.
2. Efferent systems

The autonomic efferent nervous innervation to the testicular capsule receives some fibers from the lumbar sympathetic chain (Kuntz and Morris, 1946). It is thought that the tunica vaginalis visceral and the tunica albuginea receives most of their fibers from the plexus of the intrinsic vessels and from the spermatic plexus (Mitchell, 1935; Risley and Skrepetos, 1964; Norberg et al., 1967).

Recently, it has become possible to use catecholamine-induced fluorescence and acetylcholinesterase as a histochemical index to demonstrate the distribution of adrenergic and cholinergic nerves, respectively, in the testicular capsule.

Adrenergic innervation in the human testis is almost restricted to small blood vessels supplying clusters of Leydig cells (Baumgarten and Holstein, 1967; Baumgarten et al., 1968). Nerve fibers exhibiting a positive acetylcholinesterase reaction are rare in the testis. These fibers appear to be confined to the interstitial tissue and surrounding blood vessels (Shirai et al., 1973). The blood vessels entering the capsule at the rete testis receive a relatively dense adrenergic innervation.

In the mediastinal region of the testicular capsule, specifically in the region surrounding the rete, there are also large numbers of fine, ramifying adrenergic fibers which have the varicose appearance of terminal effector plexuses. In addition, larger non-terminal fluorescent nerves enter the testicular capsule at the rete testis, travel into the non-mediastinal region of the capsule and give rise to scattered, varicose terminal fibers. These fibers branch and terminate into plexuses of geometrical regularity over small areas of the capsular
tissue. In contrast to the distribution of adrenergic fibers, no fibers exhibiting a strong acetylcholinesterase reaction were associated with blood vessels. These cholinergic nerves were sparsely distributed through the non-mediastinal areas of the capsule and appeared to enter the capsule at points some distance from the rete testis. This may suggest the presence of an independent cholinergic supply to the capsular muscle. The smooth muscle of the adult rat testicular capsule appears to be supplied by both adrenergic excitatory and cholinergic excitatory fibers (Bell and McLean, 1973).

3. Electrical stimulation

There is strong evidence for direct innervation of smooth muscle in the testicular capsule. Bell and McLean (1973) using platinum ring electrodes placed around the rostral pole of the rat testis found that electrical field stimulation of the rat isolated testicular capsule in vitro evoked a contractile response. This contractile response produced by electrical field stimulation was reduced in the presence of bretylium which prevents release of nonepinephrine from adrenergic nerves. Atropine and hyoscine caused a slight reduction in responses to electrical field stimulation. Therefore, it can be concluded that the rat testicular capsule receives both adrenergic and cholinergic innervation, with the adrenergic supply predominating.

Responses of the rabbit (Rikimaru and Suzuki, 1972) and human (Rikimaru and Shirai, 1972; Shishito et al., 1975) testicular capsules to electrical stimulation were reduced by bretylium and tetrodotoxin, but not influenced by hexamethonium or atropine. Tetrodotoxin prevents an action potential by blocking the membrane channels to sodium. This eliminates the increase in sodium permeability
associated with the rising phase of the action potential. However, response to acetylcholine was easily abolished by atropine. These data indicate that adrenergic innervation and muscarinic cholinergic receptors are present in the rabbit and human testis.

Rikimaru et al., (1972) stimulated the canine perivascular nerve using a pair of Ag-AgCl ring electrodes which were placed around the spermatic vessels. Stimulation of the perivascular nerve raised the intratesticular pressure and elicited contraction of dog testis. However, in neither the rat nor the rabbit did a rise in intratesticular pressure or a contraction of the testis ensue when the perivascular nerve was stimulated (Hargrove and Ellis, 1976).

Finally, surgical denervation of the hypogastric nerve or pharmacological sympathectomy lead to an increase in the number of spermatozoa in the caput epididymidis of the rat and rabbit (Hodson, 1964, 1965; Swedin, 1971; Bell and McLean, 1973). These data suggest that passage of spermatozoa out of the testis is maintained in the absence of a functional capsular innervation and may be attributable to the existence of other nervous pathways other than the hypogastric nerve supply. Swedin (1971) also proposed the presence of a passive "overflow" of semen into the urethra, which may be due to the continuous production of spermatozoa and secretion fluid even after sympathectomy.

D. MECHANISM OF SPERM TRANSPORT

Cross (1959) first observed contractions of the whole testis in anesthetized rabbits. He enclosed the testis in a fluid-filled chamber that had been implanted in the abdomen and viewed contractile movements with a microscope. There have been suggestions that the rabbit testis as a whole organ might be capable of
undergoing spontaneous movements, as indicated by the report of interstitial pressure changes obtained with a cannula (Holstein and Weiss, 1967) as well as, the observation of irregular movements of the entire testis (Wojcik, 1966).

It has been well established, that the sperm which are formed in the seminiferous tubules of the testis are immotile (Redenz, 1926). The capacity for motility is first attained by sperm during the transit through the epididymis (Yochem, 1930). The mechanisms responsible for the transport of nonmotile sperm out of the seminiferous tubules of the testis and into the epididymis have remained unclear, although a number of explanations for this phenomenon have been offered.

1. **Cilia**

It has been postulated by Zawisch-Ossenwitz (1933) that ciliary action within the efferent ducts could move sperm from the testis. However, Leeson (1962) in examining the fine structure of the rete testis by electron microscopy, suggested that too few cilia were present in the rete epithelium to have any effect on sperm transport. In addition, examination of ram ductuli efferentes flow indicate that cilia cannot generate sufficient propulsive force in the transit of sperm (Winet, 1977).

2. **Tubular fluid secretions**

Secretion of fluid by the seminiferous tubules may play a contributing role in the transport of sperm (Macmillan, 1953; Reid and Cleland, 1959; Barack, 1968; Setchell et al., 1969). Ligation of the ductuli efferentes in adult rats retards sperm flow distal to the region of ligation (Toothill and Young, 1931) and leads to
an increase in testicular fluid (Van Wagenen, 1924). Tubular secretions are thought to create sufficient pressure to transport the spermatozoa through the lumen of the seminiferous tubules and into the rete testis (Smith, 1962; Shishito et al., 1975).

3. **Seminiferous tubules**

Recent observations suggest several additional sources for impelling sperm from the testis. Roosen-Runge (1951) reported slow and undulating movements of the seminiferous tubules which could be seen by direct microscopic visualization. Using a microscope stage chamber, Suvanto and Kormano (1970) observed that the rat seminiferous tubules contract with a mean frequency of 5 per minute and a mean depth of 12 micron. These rhythmic contractions were suggested to be due to contraction and relaxation of the Sertoli cell located within the seminiferous germinal epithelium. It has also been reported that myoid cells which form a continuous layer between the basement membrane of the peritubular tissue may play an important role in seminiferous tubular contractions (Clermont, 1958; Lacy and Rotblatt, 1960; Leeson and Leeson, 1963; Ross, 1967). The cytological features of myoid cells in the human testis resembled those of smooth muscle cells (Ross and Long, 1966; Ross, 1967; Rukosuev, 1976). The myoid cells react with γ-globulin produced against actin from smooth muscle (Straus and Kao, 1968). In addition these myoid cells contain vesicles located beneath the plasma membrane, as well as, desmosome-like junctions seen in smooth muscle cells (Ross and Long, 1966). Ultrastructural studies have revealed tight junctions of myoid cells in the guinea pig and rat (Fawcett et al., 1970). In addition to their
contractile function, these cells comprise a significant component of the blood-testis barrier (Fawcett et al., 1970).

The myoid cell layer of the rat seminiferous tubules differentiate early during postnatal development of the testis. Kormano and Hovatta (1972) observed that seminiferous tubules begin to contract at 15 days, reaching adult values for frequency at 40 days. In early puberty, contractile activity appears to correspond to the development of thin filament bundles appearing in the cytoplasm of the myoid cells. Desmosome-like junctions between the ends of the myoid cells were also well developed at 15 days after birth, corroborating the anatomical data provided by Leeson and Leeson (1963).

Maturation of the contractile myoid cells of the seminiferous tubule during puberty of the rat suggests that such development is endocrine-dependent. Bressler and Ross (1972) tested this hypothesis by removing testes from newborn mice and implanting them in adult hosts. Development proceeded normally, whereas those implanted into hypophysectomized hosts retained an immature appearance. The administration of testosterone to the hypophysectomized mice resulted in partial development of myoid cells.

Under a light microscope, Niemi and Kormano (1965) observed the stimulating effect of oxytocin on seminiferous tubular contractions in vitro. These investigators found that oxytocin in a concentration of $2 \times 10^{-4}$ I.U./ml stimulated contractility, and a concentration of $2 \times 10^{-2}$ I.U./ml induced tonic contractions. Hovatta (1972a, 1972b) maintained rat seminiferous tubules in organ culture and found that testosterone and human chorionic gonadotropin increased the percentage of contractile tubules and density of microfilaments within the myoid cells. It was also observed, that the injection of cyproterone acetate into
rats lead to a disappearance in contractility and a concomitant reduction in the amount of microfilaments. Recently, Urry and co-investigators (1976) implanted 10 and 30 mg implants of estradiol into adult rats or added the steroids \((10^{-8} \text{ to } 10^{-9} \text{M})\) to medium in a chamber containing tubules for microscopic observation. They reported that implants of estradiol or adding the steroid to the medium \textit{in vitro} reduced the percentage of tubules showing contractility.

In addition, the effects of testosterone and dihydrotestosterone on adult rat tubule contractions are biphasic. High doses of testosterone \((10^{-5} \text{ to } 10^{-6} \text{M})\) maintained and actually increased the magnitude of the tubule contractions, whereas, low doses \((10^{-8} \text{ to } 10^{-11} \text{M})\) decreased both the percentage of tubules contracting, as well as the magnitude of the contractions. The authors speculate that steroids may be involved in the control of these contractions and may regulate the transport of sperm from the rat testicle.

In contrast, the isolated testicular parenchyma of both the adult rat and rabbit was found to be devoid of spontaneous contractions and only negligible contractions in response to several autonomic agents were observed. The contractile response of the adult rat and rabbit testicular capsule to \(1 \mu g/ml\) nor-epinephrine appeared to be 280 and 1000 times greater, respectively, as that noted for the isolated testicular parenchyma (Davis and Langford, 1971).
4. Testicular capsule

a. Rat isolated testicular capsule

Davis and Langford (1969a, 1969b, 1969c, 1970) reported that the isolated testicular capsule of the adult rat was capable of responding to several parasympathomimetic agents, acetylcholine, carbachol and pilocarpine. Carbachol and acetylcholine produced a similar contraction at 1 µg/ml final bath concentration, but the response to pilocarpine appeared much less sensitive as compared to acetylcholine. In addition, norepinephrine and epinephrine were observed to cause a contraction of the testicular capsule. Isoproterenol, on the other hand, was found to cause a relaxation of the testicular capsule. Tetramethylammonium, which is a ganglionic-stimulating agent, was observed to cause a contraction of the isolated testicular capsule. The tetramethylammonium-induced contraction suggests the possible presence of parasympathetic ganglia located in the testicular capsule. Maximal contraction of the testicular capsule was reached in 3 to 5 minutes following the addition of the drug, however, the capsule subsequently was found to relax quite slowly after repeated washing in the organ bath (Davis et al., 1970). In contrast to the testicular capsule, the addition of neurohumoral agents to the isolated guinea pig ileum resulted in a very brief contraction.

b. Rabbit isolated testicular capsule

In contrast to the adult rat testicular capsule, the isolated testicular capsule of the adult rabbit was observed to undergo marked spontaneous contractions within one hour of mounting in the organ bath and before the addition of any drug (Langford and Davis, 1970; Davis et al., 1970; Davis and Langford,
Spontaneous contractions of the isolated testicular capsule of the rabbit, but not the rat may be due to its larger size and increased amount of smooth muscle fibers (Davis et al., 1970). The frequency of spontaneous contractions of the isolated testicular capsule of the adult rabbit ranged from 3 to 5 per minute and a force equivalent to 0.2 to 1.0 grams (Hargrove and Ellis, 1976). The amplitude of these spontaneous contractions were found to average 5 percent shortening of the actual entire length of the mounted capsule (Davis and Langford, 1970; Davis et al., 1970). In marked contrast to the periodic spontaneous contractions recorded from both the isolated testicular capsule and intact testis of the rabbit, no spontaneous contractions of the isolated testicular parenchymal tissue were observed (Davis and Langford, 1970, 1971). Therefore, it would appear that the testicular capsule alone may be responsible for the endogenous rhythmic contractions observed with the whole testis and play an important role in the transport of non-motile sperm out of the testis and into the epididymis.

The isolated testicular capsule of the rabbit was also found to resemble that of the rat in that contractions were produced by the addition of acetylcholine, carbachol, norepinephrine, epinephrine, tetramethylammonium and histamine (10^{-6} g/ml). In addition, isoproterenol produced a prolonged relaxation, as well as completely abolishing capsular spontaneous contractions of the rabbit. Contractions produced by the sympathomimetic amines were easily prevented by the addition of the α-adrenoceptor blocking agents, phentolamine (10^{-6} g/ml) or phenoxybenzamine (10^{-6} g/ml). The relaxation observed after the addition of α-blocking agents was prevented by the β-adrenoceptor blocking agent, propranolol (10^{-6} g/ml) (Rikimaru and Suzuki, 1972). In the presence of atropine (10^{-7} g/ml), acetylcholine (10^{-5} g/ml) failed to produce a response. These results demonstrate
the presence of cholinergic muscarinic receptors and both α-adrenergic and β-adrenergic receptors in the rabbit testicular capsule.

c. Human isolated testicular capsule

In addition to rat and rabbit isolated testicular capsule preparations, strips of human testicular capsule contracts to a number of pharmacological agents (Davis and Langford, 1970; Rikimaru and Shirai, 1972; Firlit et al., 1975; Shishito et al., 1975). Shishito and co-investigators (1975) found that the human testicular capsule contracted to norepinephrine (10⁻⁶ g/ml) and the administration of an α-adrenergic blocking agent, phentolamine (10⁻⁶ g/ml) or a β-adrenergic blocking agent, propranolol (10⁻⁶ g/ml) prevented the contraction. Furthermore, the human testicular capsule contracted to acetylcholine (10⁻⁶ g/ml), whereas, atropine (10⁻⁷ g/ml) prevented the response. These results imply the presence of cholinergic muscarinic and adrenergic receptors. The testicular capsule of the human has also been observed to undergo marked spontaneous contractions. The frequency of these spontaneous contractions occurred every 13.6 minutes and the amplitude represented 39 percent shortening of the actual entire length of the mounted strip of the human testicular capsule (Davis and Langford, 1970; Firlit et al., 1975).

E. ROLE OF PROSTAGLANDINS IN THE TESTIS

Prostaglandins are known to be associated with the male reproductive tract. Von Euler (1936) first suggested that prostaglandins might affect the muscular wall of the male reproductive tract and alter the rate of emptying of the luminal contents. An increase in sperm in the deferent duct of rabbits and bulls
with injection of prostaglandin F$_2$$\alpha$ has been recently reported (Hafs et al., 1974a, 1974b). Prostaglandins have been found primarily in the seminal fluid and seminal vesicles (Kurzrok and Lieb, 1930; Von Euler, 1934; Hamberg and Samuelsson, 1965). Prostaglandins E$_1$, E$_2$, F$_1$$\alpha$ and F$_2$$\alpha$ have been isolated from rat testes (Carpenter and Wiseman, 1970; Carpenter, 1974). Furthermore, enzymes for inactivation of prostaglandins (15-hydroxy prostaglandin dehydrogenase and prostaglandin-delta$^{13}$-reductase) have been observed in swine (Anggard, Larsson and Samuelsson, 1971) and rat (Nakano, 1971) testes.

1. **Prostaglandin synthetase system**

The prostaglandin synthetase system has been demonstrated in the interstitial cells and the seminiferous tubules of rat testicular tissue and observed to be gonadotropin- and age-dependent (Ellis and Baptista, 1969; Ellis et al., 1972, 1975). Histochemical localization of prostaglandin synthetase activity was seen in the cauda epididymis with maximal synthesis occurring in the interstitial tissue of the seminiferous tubules and testicular capsule (Johnson and Ellis, 1977). There is no evidence to support the possibility that prostaglandins found in the testis might be synthesized in another tissue and transported to the testis.

Gerozissis and Dray (1977) have measured by radioimmunoassay techniques PGE$_2$ and PGF$_2$$\alpha$ in the isolated testicular capsule of rats aged 22 to 90 days. No significant difference was found with increasing age. However, the isolated rat testicular capsule was found to contain concentrations of PGE$_2$ and PGF$_2$$\alpha$ 100 times higher than those in decapsulated testes.

Prostaglandins have been demonstrated to modulate androgen biosynthesis or release in the mouse and rat testis by decreasing testicular blood flow, adenyl
cyclase activity or cholesteryl ester synthetase; by binding to microsomal enzymes; by interaction at the pituitary region to decrease gonadotropin secretion or through testicular receptors (Ellis and Baptista, 1969; Eik-Nes, 1971; Bartke, 1973; Saksena et al., 1973, 1974; Bartke, 1976). Saksena and co-investigators (1975) demonstrated that the prostaglandin inhibitor, indomethacin, caused a significant decrease in plasma LH and testosterone levels, indicating an action on prostaglandin synthesis at either the testis or pituitary levels.

2. Regulation of rabbit testicular capsular motility

A functional role for prostaglandin-like compounds in regulating rabbit testicular capsular motility in vitro have been demonstrated by several investigators. Spontaneous contractions of the intact testis of the rabbit were inhibited by the addition of PGE$_1$ (Hargrove et al., 1971). In addition, spontaneous contractions ceased when the preparation was rinsed and fresh saline was added. However, contractions were reinitiated when the preparation was placed in the original medium (Hargrove et al., 1973a). Extraction of the original medium for acidic lipids yielded a residue that stimulated contractions of the rabbit testicular capsule. Using thin-layer chromatography, these residues migrated similarly to authentic prostaglandin E$_1$, E$_2$, F$_1\alpha$ and F$_2\alpha$. In addition, PGA$_2$, PGE$_1$, PGE$_2$, PGF$_1\alpha$ and PGF$_2\alpha$ have been demonstrated to initiate increases in tonus and phasic contractions in inactive rabbit testes preparations (Johnson et al., 1971; Seeley et al., 1974). PGF$_1\alpha$ and PGF$_2\alpha$ increased tonus and contractility, whereas, PGE$_1$ and PGE$_2$ produced a bimodal response (Hargrove et al., 1973a; Seeley et al., 1973, 1974; Hargrove et al., 1975). The bimodal effect of E-type prostaglandin which lead to inhibition at higher doses, may be due to the greater
potency of PGE over PGF type prostaglandins to stimulate testicular cyclic AMP production (Butcher and Baird, 1968; Shio et al., 1971; Kuehl et al., 1972). Dibutyril cyclic AMP at 2nM concentrations has been demonstrated to reduce testis tone without significantly reducing the phasic contraction in vivo. In addition, theophylline, a phosphodiesterase inhibitor known to increase cyclic AMP levels, inhibited testicular capsular motility (Seeley et al., 1974). Hargrove and co-investigators (1975) reported the inability of high doses of PGF$_2\alpha$, epinephrine or acetylcholine to overcome PGE$_1$-induced abolition of capsular tonus and motility; which may indicate a non-competitive inhibition or an inability to activate adenyl cyclase.

Low concentrations of PGE$_1$ and PGE$_2$ ($10^{-7}$ to $10^{-8}$M) potentiated rabbit testicular capsular contractions in response to serotonin, acetylcholine, epinephrine, histamine, isoproterenol and CaCl$_2$, but inhibited the response of PGF$_2\alpha$ (Hargrove et al., 1973b, 1975). The potentiating action of PGE may be due to increased cell membrane permeability to Ca$^{++}$, since 5 to 12 mM Ca$^{++}$ produces a contraction when added to an actively contracting rabbit testis in vitro, but not when added to a rinsed, inactive preparation (Hargrove et al., 1973b).

Prostaglandin F$_2\alpha$-induced contractions of the whole rabbit testis were prevented by the prostaglandin synthesis inhibitor, indomethacin. However, the contractions of the testicular capsule in vivo did not decrease after the addition of indomethacin to the bath medium (Hargrove and Ellis, 1976). The authors suggested that during pathological or other abnormal states, the rabbit testes in the intact animal has the capacity to synthesize prostaglandins.
Seeley and co-investigators (1972, 1973) reported that testosterone, androstenedione, progesterone and pregnenolone inhibited PGE\textsubscript{1}-induced and spontaneous contractions of the rabbit testicular capsule \textit{in vitro}. By contrast, testosterone did not inhibit spontaneous contractions \textit{in vivo}, suggesting that testicular capsular contractions may not be regulated by endogenous steroids.

One may conclude that humoral or local hormonal mechanisms may play an important role in the modulation of testicular capsular contractions. In this respect, prostaglandins may participate in the regulation of smooth muscle contractions of the rabbit testicular capsule.

F. CONTRACTILE MECHANISM OF SMOOTH MUSCLE

1. Structural basis of contraction and regulation of smooth muscle

Our knowledge of the structural organization of smooth muscle has advanced greatly since the advent of electron microscopy and x-ray diffraction techniques. Smooth muscle is distinguished from striated muscle by the lack of regular transverse alignment of thick and thin filaments and by the absence of a system of T-tubules. The mechanism involved in the excitation-contraction coupling between the smooth muscle cell membrane and contractile elements is considered more direct than in striated muscle and the diameter of individual muscle fibers are consequently much smaller. Smooth muscle conduction is from fiber to fiber, which may be stimulated by quick stretch. Autonomic nerves have a modulating, but not triggering action.

The surface membrane of most smooth muscle fibers contains rows of pinocytotic vesicles (caveolae). Similar to the T-tubules demonstrated in striated
muscle, these pinocytotic vesicles may be involved with absorption of ions and metabolites or areas of active pumping of calcium out of the cell. Histochemical staining revealed a high concentration of Ca-ATPase in areas close to the caveolae (Lane, 1967). Another important membrane feature of smooth muscle is the nexus, or gap junction, a region of close apposition of adjacent cells which is responsible for electrical coupling between smooth muscle cells. In addition to nexuses, membrane thickenings resembling desmosomes and protrusions of one smooth muscle cell into another cell have been observed (Gabella, 1972). Smooth muscle sarcoplasmic reticulum (SR) are regions of calcium storage and release and participate in the regulation of intracellular free-Ca\(^{++}\) levels during the contraction-relaxation cycle. Additional evidence is provided by the in situ cytochemical demonstration of Ca\(^{++}\)-ATPase (similar to skeletal muscle SR) with the membrane of the smooth muscle SR (Popescu, 1977). In addition, some phasic smooth muscles, such as taenia coli, portal vein and mesenteric artery show few SR vesicles, 1.8 - 2% of cell volume, whereas more tonic muscles such as pulmonary artery and aorta, have 5% SR. It has been speculated that muscles possessing a greater amount of SR maintain a longer contractile response upon stimulation by acetylcholine, whereas those muscles with little SR, the response is lost rapidly (Somlyo et al., 1969).

The contractile apparatus of vertebrate smooth muscle consists of filaments of myosin and actin orientated more or less parallel to the cell axis. A myosin molecule has a long rod-shaped tail region and two globular heads and are arranged in the thick filament with the tails forming the core and the heads at the surface. The myosin molecule arrangement forms a cigar-shaped structure that is studded with projections along its entire length, except for a bare zone in the
middle. Smooth muscle myosin has only two classes of light chains at approximately 20,000 and 16,000 daltons, compared to three for most skeletal muscle myosins (Hartshorne and Aksoy, 1977). Actin is present in the largest amount in smooth muscle. In terms of biological activity it is difficult to distinguish between skeletal and smooth muscle actins. The actin molecules are small, roughly spherical particles that are arranged in the thin filament as if to form a twisted double strand of beads. An important characteristic of the individual actin molecules is that they are not spherically symmetrical. The thick myosin and thin actin filaments interdigitate in an orderly array to form a muscle fiber. In addition to actin, the thin filaments in muscle contain the proteins, tropomyosin and troponin. Tropomyosins are long, thin asymmetric molecules (containing a high proportion of charged amino acids) attached end to end, forming a very thin filament on the surface of each actin filament. Troponin is a complex of globular proteins with a molecular weight of 80,000 daltons. Troponin has frequently been referred to as the "regulatory protein factor" necessary for the interaction of smooth muscle myosin and actin in the presence of ATP. The interaction of myosin and actin is observed only in the presence of this factor and calcium. This is in sharp contrast with vertebrate striated muscle, where troponin in collaboration with tropomyosin depresses the myosin-actin interaction in the absence of calcium (Ebashi et al., 1977).

2. **Spontaneous activity of smooth muscle**

Many smooth muscles, in contrast to striated muscle exhibit spontaneous contractions. These contractions are caused by electrical activity of the muscle fibers. Two components of electrical activity have been classified: slow waves
(slow rhythmic fluctuations in cell membrane potential) and electrical spikes (action potential). Slow waves are generated in the longitudinal muscle of small intestine; they do not trigger contractions in the absence of spikes. The slow potential is generally smaller in amplitude and long in duration, but varies in shape from one tissue to another. The action potential is a simple spike-like potential (in the guinea pig taenia coli), whereas in other tissues it consists of spike-like potential superimposed on a slow plateau potential (in the guinea pig ureter). The spike is evoked by depolarization of the membrane where current passes outward across the surface membrane; it propagates along the tissue and its propagation can be blocked when the membrane is hyperpolarized (Tomita, 1970; Kuriyama et al., 1975).

The origin of the spontaneous activity seems to be myogenic, since various drugs (atropine, hexamethonium, cocaine and tetrodotoxin) which interfere with the nervous activity or transmission do not abolish the activity (Tomita, 1970).

The mechanism of smooth muscle automaticity may be dependent upon the pacemaker potential. Depolarization is known to increase the frequency of the pacemaker potential as well as the spike discharge. The pacemaker potential may be due to (1) an increase in sodium permeability; (2) an increase in calcium permeability; (3) a decrease in potassium permeability or (4) an increase in chloride permeability (Tomita and Watanabe, 1973). The precise ionic basis for the pacemaker potential is currently under investigation.

3. Mechanisms for activation of smooth muscle

When the nerve signal to initiate contraction arrives at the muscle cell, the impulse depolarizes the membrane and causes the release of calcium into the fluid
surrounding the filaments from special storage vesicles in the sarcoplasmic reticulum. The calcium ions bind to the troponin, which then modifies the position of the tropomyosin molecules so that the myosin heads can contact the actin molecules. A myosin head also combines with a molecule of ATP. The myosin-ATP is somehow raised to a "charged" intermediate form that binds to an actin molecule of the thin filament. The combination, the "active complex" undergoes hydrolysis; the ATP splits into adenosine diphosphate and inorganic phosphate and energy is released. It is this energy that forces the cross bridge to swivel to a new angle, pulling the thick filament along with respect to the thin filament and thereby shortening the muscle. The final stage in the hydrolysis reaction, detachment of the cross bridge, is reached only after a new ATP has bound to the actin-myosin complex. The resulting actin-myosin-ATP complex rapidly dissociates to yield a free actin molecule and an uncharged myosin-ATP. The calcium is quickly removed and returned to the storage vesicle by a calcium "pump" located in the membranes of the sarcoplasmic reticulum. The removal of calcium prevents the further cycling of cross-bridges and the muscle relaxes.

During relaxation, the following sequence may operate: calcium removal from the vicinity of the contractile filaments results in gradual cessation of cross-bridge cycling and actinomyosin-ATPase activity, as well as associated energy input. As this occurs, the cross-bridges revert to the disattached state, in which force is transiently maintained. In mammalian smooth muscle, the force maintained during the state of relaxation is determined primarily by the time course of calcium removal and secondarily by stress relaxation of the cross-bridges (Siegman et al., 1977).
G. PHARMACOLOGY OF SMOOTH MUSCLE

1. Catecholamines

The action of adrenergic amines on smooth muscle can be excitatory or inhibitory and are mediated by activation of alpha and beta receptors, respectively (Daniel et al., 1970). In addition, modulation of smooth muscle activity by catecholamines is dependent upon the specific anatomical location of the smooth muscle, species and hormonal status of the individual (Miller, 1967; Marshall, 1970). For example, norepinephrine and epinephrine relax intestinal smooth muscle in most species, but stimulate the uterine muscle of the rabbit, dog and human (Bulbring, 1976). In contrast, epinephrine has been demonstrated to relax the uterus of the rat and of the nonpregnant cat (Marshall, 1959).

The membrane properties of the smooth muscle cell apparently determine whether the muscle will be excited or inhibited by catecholamines. The response to both norepinephrine and epinephrine is characterized by membrane depolarization, a reduction in size of the electrotonic potential and an acceleration of spontaneous spike discharge resulting in an increase in muscle tension in the rat vena portal vein (Shuba et al., 1976) and guinea pig uterus preparations (Szurszewski, 1973). The direction of the potential change suggests an increase in membrane permeability to Na\(^+\) and/or Cl\(^-\) since the equilibrium potentials for both these ions are more positive than the resting potential. Further, a reduction in \((Na)\)_o had an effect on the depolarization, but the removal of Cl\(_o\) drastically reduced or abolished the depolarization (Szurszewski and Bulbring, 1973). Norepinephrine and epinephrine produced a reduced depolarization in a calcium-free solution using the lanthanum technique or in the presence of a calcium-free
solution containing EGTA (Szurszewski and Bulbring, 1973; Marshall and Kroeger, 1973; Shuba et al., 1976). Lanthanum has a much higher affinity for tissue calcium binding sites than does calcium, and therefore it displaces extracellular calcium while preventing calcium flux across the cell membrane (Mayer et al., 1972). These results indicate that calcium is important for the depolarization of the smooth muscle cell membrane. However, calcium influx and its release from intracellular binding sites (which eventually must be replenished by extracellular calcium) are necessary to maintain the increase in tension which normally accompanies the alpha actions of catecholamines (Szurszewski and Bulbring, 1973).

The inhibitory action of epinephrine on the taenia coli of the guinea pig and the rat uterus are associated with hyperpolarization and suppression of spontaneous spike discharge (Bulbring, 1954) followed by subsequent relaxation of the muscle. This hyperpolarization is the result of an increased membrane permeability to potassium (Bulbring and Tomita, 1969; Shuba, 1976). In addition, hyperpolarization is unchanged in chloride-free solution but it is abolished by the addition of Mn$^{++}$ or Ca$^{++}$ replacement with Ba$^{++}$ or lanthanum (Bulbring and Tomita, 1969).

The introduction of specific pharmacological antagonists allowed the separation of $\alpha$- and $\beta$-effects of catecholamines on smooth muscle (Ahlqvist, 1948). Alpha and beta receptors are present in almost every smooth muscle. As a general rule, the response to the $\alpha$ action is contraction and that to the $\beta$ action is relaxation. The exception is that in intestinal smooth muscles, $\alpha$- and $\beta$-adrenergic agents both block the spontaneous electrical activity of the cell membrane and lead to relaxation (Bulbring and Tomita, 1969).
The α response of catecholamines on the taenia coli consists primarily of a change in membrane conductance. The conductance change increases potassium conductance and results in hyperpolarization, leading to cessation in spike activity and subsequent muscle relaxation (Bulbring, 1976). On the other hand, the α-effect of catecholamines on the uterus is excitatory. The cell membrane is depolarized, membrane conductance is increased, spike discharge is accelerated and tension develops (Szurszewski and Bulbring, 1973). Recent evidence suggests that the α action of catecholamines causes calcium release from sarcoplasmic reticulum or calcium activation of a contractile protein in the uterus (Bulbring, 1976).

Beta-adrenergic agents are primarily inhibitory in nature, with the exception of the heart. The β action stabilizes the membrane potential, but produces no change in membrane conductance in the taenia coli. In addition, spontaneous spike discharges are blocked (Bulbring and Kuriyama, 1973). Recent experimental evidence suggest that β-adrenergic effects are associated with an increased intracellular content of cyclic AMP which is believed to activate the process of calcium uptake at intracellular storage sites (Triner, 1971; Bulbring and Hardman, 1976).

2. Acetylcholine

The muscarinic action of acetylcholine and the α action of catecholamines on most smooth muscle preparations are basically similar. The differences between the cholinergic and adrenergic action include differences in potency, latency and duration of action (Szurszewski and Bulbring, 1973).

The stimulant action of acetylcholine on the estrogen-dominated guinea pig uterus and taenia coli caused a rapid discharge of spike potentials and led to a
sustained depolarization in which the electrotonic potential was reduced to zero. Acetylcholine depolarization and subsequent tissue contraction may be due to an increase in sodium, potassium and calcium permeabilities (Bulbring and Kuriyama, 1963; Worcel and Hamon, 1976), but not to chloride ions (Szurszewski and Bulbring, 1973). Acetylcholine in contrast to catecholamines has a longer duration of action on the myometrium resulting in a prolonged contracture (Szurszewski and Bulbring, 1973). Preliminary reports have also indicated that the stimulation of muscarinic cholinergic receptors in perfused hearts, intestinal smooth muscle and the rat ductus deferens preparations may lead to an increased cyclic GMP concentration.

3. **Prostaglandins**

Prostaglandins have been shown to possess distinct actions on a large number of biological systems. Relatively little is known about the mechanism by which prostaglandin (PG) compounds induce contraction and relaxation as well as associated electrophysiological changes of visceral and vascular smooth muscle.

Recent studies have demonstrated that prostaglandins may have a direct action on smooth muscle as well as an indirect effect by depressing or enhancing adrenergic autonomic transmission in smooth muscle (Karim and Hillier, 1972). The first component is a direct effect and may involve the presence of a prostaglandin receptor (Ellatar, 1978). Unfortunately, there is no direct evidence of a prostaglandin receptor site, since its existence is dependent on the effects of structurally related prostaglandin antagonists such as 7-oxa-13-prostynoic acid to inhibit prostaglandin-induced contractile response in smooth muscle (Kuehl, 1974).
Further, α- and β-blocking agents, antihistamines, serotonin antagonists and atropine do not alter the contractile effect of PGE$_1$, PGE$_2$, PGA$_1$, and PGF$_1\alpha$ on the rabbit aorta (Karim and Hillier, 1972). A biphasic effect has also been observed with PGE$_1$, PGE$_2$, PGA$_1$ and PGF$_1\alpha$ on the isolated small resistance arteries of the rabbit, cat and dog. With low dose concentrations, these vessels relax but with higher concentrations, a contraction is observed (Strong and Bohr, 1967). The investigators suggest that low doses of prostaglandins act intracellularly to bind free calcium ions and cause relaxation of the blood vessels, while higher doses act upon the superficial site at the cell membrane to cause depolarization and contraction. Other investigators suggest that PGE$_1$-induced contraction of uterine smooth muscle be related to the release of calcium ions from intracellular stores (Mironneau and Grosset, 1976).

Prostaglandin synthetase inhibitors have been demonstrated to modify the resting tone of many isolated smooth muscle preparations (intestine, uterus and trachea) leading to the speculation that some smooth muscles may continuously release prostaglandins and may be responsible for their resting tone (Vane and Williams, 1973; Bouhuys, 1975). Further, indomethacin, a prostaglandin synthetase inhibitor reduced prostaglandin release and spontaneous tone of human umbilical arteries in vitro (Tuveomo and Wide, 1973).

Cyclic adenosine monophosphate is considered by many investigators to be a key intracellular mediator in tissue response to prostaglandins. E-type prostaglandins have been demonstrated to relax isolated bovine and canine vein strips with a concomitant increase in cyclic AMP formation. In other smooth muscles, the relationship between cyclic nucleotide levels and contraction is not
so clear as angiotensin II contracts the rat myometrium without modifying cyclic AMP levels (d'Auriac and Meyer, 1973). Conversely, PGF failed to increase cyclic AMP levels, but contracted the vein strips and increased tissue cyclic GMP levels (Kuehl, 1974).

Another complex aspect of the action of prostaglandins on the smooth muscle vasculature and other tissues includes: (1) possible release of prostaglandins in the vicinity of autonomic neuroeffector junctions of smooth muscle, both spontaneously and in response to electrical, mechanical and chemical stimulations; (2) potential influence of released prostaglandins on both transmitter release from the nerve terminals and the response of the effector organ to the secreted transmitter and (3) ability of prostaglandins to inhibit or potentiate catecholamines, angiotensin and certain other agonists in smooth muscle preparations of the kidney, intestine, stomach, uterus, vas deferens and adipose tissue (Hedqvist, 1976).

4. Spasmolytic agents

a. Papaverine

Papaverine has been found to act as a non-competitive spasmolytic agent in a variety of smooth muscle preparations. Non-competitive spasmolytics do not interfere with neuromuscular transmission, but rather with the excitation-contraction coupling process (between the intracellular ionization of calcium and its interaction with the contractile system) of the smooth muscle fibers. This direct inhibitory effect on the contractile cells results in an inactivation of both
the phasic and tonic components of smooth muscle (Simonis et al., 1971; Golenhofen, 1976).

The maintenance of intestinal smooth muscle tone is dependent upon aerobic conditions. The tone is abolished by anoxia and cyanide or the addition of 2,4-dinitrophenol which impairs the synthesis of ATP, normally coupled to the electron-transport in the respiratory chain. Papaverine also inhibits oxidative phosphorylation in mitochondria of the guinea pig ileum and rabbit duodenum smooth muscle preparations. These results demonstrate that the spasmolytic effect of papaverine may be due to the inhibition in the electron-transfer reaction chain between nicotinamide adenine dinucleotide and cytochrome b (Santi et al., 1964).

It has also been suggested that the relaxant effects of papaverine on carotid artery and aortic strip preparations might be exerted through cyclic phosphodiesterase inhibition with subsequent accumulation of cyclic AMP (Demesy and Stoclet, 1971; Keatinge and Graham, 1974). Cyclic AMP probably causes relaxation by expelling calcium from the cytoplasm since cyclic AMP promotes calcium uptake by vesicles in rabbit aortic preparations (Baudouin-Legros and Meyer, 1973).

b. Sodium Nitroprusside

Sodium nitroprusside has been demonstrated to have a direct relaxant effect on ventricular and vascular smooth muscle and has become widely used as a vasodilator in the treatment of myocardial disease (Brodie et al., 1976). Studies with aortic smooth muscle have shown that nitroprusside has a relaxant effect independent of changes in calcium ion influx and that there is enhanced calcium
binding to microsomal fractions in the presence of nitroprusside (Kreye et al., 1973). This data suggests that the site of action of nitroprusside may be at the sarcoplasmic reticulum in vascular smooth muscle. Recent studies have confirmed the smooth muscle relaxant characteristics of nitroprusside in the absence of extracellular calcium, as well as nitroprusside-induced elevation of intracellular cyclic GMP levels in the rat ductus deferens (Bohme et al., 1978).

Sodium nitroprusside can selectively suppress tonic activation of guinea pig aortic and stomach smooth muscle. Concentrations of $10^{-8}$ M/l reduced fundus tone and the tonic component in the corpus, without significantly affecting the phasic contractions in the corpus and antrum of the stomach. The addition of higher concentrations ($10^{-6}$ M/l) of nitroprusside, only slightly inhibited the phasic contractions (Boev et al., 1976). The mechanism by which sodium nitroprusside selectively alters the tone is unclear.

c. **Verapamil**

Verapamil, in contrast to sodium nitroprusside selectively blocks phasic contractions from isolated preparations of taenia coli, portal vein, stomach and ureter of the guinea pig (Golenhofen and Lammel, 1972; Boev et al., 1976). Verapamil reduces the frequency of spike discharges mainly by reducing the rate of rise of the pacemaker potential in cardiac and smooth muscle. Inhibition of the spontaneous contractile activity of taenia coli can be antagonized by increasing the extracellular calcium concentration (Golenhofen and Lammel, 1972; Riemer et al., 1974). In addition verapamil inhibits the transmembrane calcium influx into cardiac muscle cells during excitation, without influencing either their resting membrane potential or the action potentials. However verapamil does not
influence the calcium dependent release of norepinephrine from the isolated cat heart produced either by sympathetic nerve stimulation, acetylcholine or by KCl (Haeusler, 1972). Further studies are needed to understand the differential effect of verapamil on excitation-contraction coupling in smooth muscle and on excitation-secretion coupling in adrenergic nerve terminals.

H. AGING AND DEVELOPMENTAL CHANGES OF THE TESTICULAR CAPSULE

Developmental and aging processes of somatic tissues are also characterized by a change in testicular function. However, the testis does not show the abrupt cessation of activity that characterizes the ovary; spermatogenesis may continue to an advanced age. Nevertheless, the tissues do become more refractory to gonadal stimulation. A study of the normal testis as it develops and changes from early fetal life to senility has been extensively reviewed by Sniffen (1950).

1. Morphological changes

Mancini (1955, 1964) demonstrated that the stromal connective tissue of the tunica albuginea of the human testicular capsule shows definite morphological changes, which can be correlated with age. From birth to puberty, the connective tissue undergoes a progressive fibrogenesis characterized by dense collagen bundle formation and a reduction of mucopolysaccharides. During adulthood, the thickness of the tunica albuginea reached maximum size with marked coalescence of the collagen fibers and the beginning of hyalinization of its superficial area. In addition, the fibrous trabeculae had increased in number and thickness. Yoshimura and Fukunishi (1965) reported a progressive increase in the thickness of the
tunica albuginea from 80 μ at 5 years of age to 100 μ at 75 years of age. Capsular smooth muscle hyperplasia has also been reported among healthy men, aged 52-100 years (Honore, 1978).

Denduchis and Mancini (1967) investigated the total and soluble collagen content of the tunica albuginea of prepubertal and adult rat testes. Total collagen content extracted from dry defatted tissue was found to increase significantly with age, in much the same manner as has been observed for the rat, rabbit and guinea pig skin. However, the total collagen content of the testicular glandular tissue did not vary significantly with age, a finding similar to that obtained with rat and human lung and kidney. In contrast, soluble collagen content of the tunica albuginea was found to decrease progressively with age. Since soluble collagen is thought to represent an early stage of collagen synthesis, the authors speculate that there is little turnover of collagen in the tunica albuginea of the adult rat.

2. Vascular changes

With increasing age, other changes have been found to occur in the human testis that may interfere with normal testicular capsule function. Leathem (1977) has indicated that after the fifth decade, thickening of the basement membrane and tunica propria of the seminiferous tubules, can progress to a completely fibrous testis. In addition to the hyalinization of the tunica albuginea and tubular connective tissue, changes in the intratesticular microvasculature have been reported (Sasano and Ichijo, 1969; Suoranta, 1971).

Sasano and Ichijo (1969) suggested that the upper pole and the mediastinum, which are situated at the distal area in the arterial supply, showed advanced senile
changes. Furthermore, vascular alterations may play a role in the pathogenesis of testicular fibrosis, irrespective of a primary etiological factor (Suoranta, 1971).

3. Pharmacological responses as it relates to developmental and aging changes

Measurements of rabbit testicular capsular contractions throughout postnatal development that may provide additional information into the function and regulation of testicular capsular smooth muscle have recently been reported (Mitchell and Seeley, 1977). The rabbit testicular capsule was found to be capable of contracting in vivo and in vitro 2 weeks postpartum, with a frequency and amplitude much reduced in comparison with adult values. Spontaneous contractions reached adult values at 16-18 weeks of age which appeared to coincide with increased development of smooth muscle cells, as well as, connective tissue within the testicular capsule and the onset of spermatids in the maturation phase of spermiogenesis. In addition, the response of the isolated testicular capsule to acetylcholine, epinephrine and prostaglandin F$_2$α remained constant throughout the age group studied. The authors suggest that receptors for each of the agonists may be present in the testicular capsule as early as 2 weeks of age. However, the response of the testicular capsule to high concentrations of prostaglandin E$_1$ became progressively more inhibitory as the rabbits increased in age, which may be indicative of the development of adenylcyclase activity (Seeley et al., 1974).

In contrast to the testicular capsule, Okpako (1976) reported marked age-dependent differences in the smooth muscle of the rat colon and stomach to PGE$_2$ and PGF$_2$α. The colon and stomach strips from immature rats were equally responsive to PGE$_2$ and PGF$_2$α, whereas colons and stomach strips obtained from
mature rats were more sensitive to PGF$_2$\textsubscript{a}, respectively. Okpako suggested that the different responses induced by the prostaglandins studied may indicate a change in the receptors during maturation.

I. HYPOXIA

Several empirical observations have been made concerning the effect of hypoxia on male infertility. These include decreased testicular oxygen tension as a result of exposure to high altitude and in cases of varicocele.

Lowered oxygen tension has been reported to be associated with decreased pressure of atmospheric gases commonly found at high altitude (Lambertson, 1965). Impairment of spermatogenesis, destruction of germinal epithelium and syncytial replacement of the tubular structure with ensuing testicular atrophy in the ram (Monge et al., 1945), as well as, a decrease in sperm transport in rabbits (San Martin, 1950) have been reported. Monge (1942a, 1942b) cited cases of couples who moved to regions of high elevations in Peru and subsequently were unable to have children. Conception was possible when they returned to sea level.

Cohen et al. (1975) reported that subfertile men with varicoceles may have a lowered testicular oxygen tension resulting from an abnormal venous blood circulation of the testis.

The effects of hypoxia on testicular function are poorly understood. Exposure to either high or low levels of oxygen or carbon dioxide has been reported to alter the metabolic activity of the rabbit testis in vitro (Cockett and Johnson, 1970).
1. Hypoxia and smooth muscle

The effects of hypoxia on smooth muscle activity has been studied previously with contradictory results. Investigations at the organ or tissue level have been best studied by respirologists. Widdicombe (1966) has shown in dogs that inhalation of hypoxic gas mixtures results in constriction of airway smooth muscle, initiated by the stimulus of peripheral chemoreceptors. However, in isolated smooth muscle preparations, hypoxia rapidly diminishes the tone and spontaneous activity of isolated uterine (Garry, 1928) and intestinal (Prasad, 1935; Ishida and Urakawa, 1974) preparations, as well as impairs isometric tension development in the trachea (Stephens, 1973; Stephens and Kroeger, 1970). In contrast, the response of isolated helical strips of rabbit aorta during prolonged exposure to anoxia results in a sustained contracture (Detar and Bohr, 1972).

a. Energy production in hypoxia

Hypoxia has been shown to impair smooth muscle contractile function through decreased energy supplies. During hypoxic states, glucose breakdown via glycolysis appears to be the principal source of energy in vascular (Shibata and Briggs, 1967) as well as tracheal (Stephens and Kroeger, 1970) smooth muscle. The finding that the taenia coli rapidly loses its mechanical response to normal electrical activity in oxygenated substrate-free media suggests minimal endogenous energy stores (Axelsson and Bulbring, 1961). Exposure to hypoxia for one hour results in a marked reduction in creatine phosphate and adenosine triphosphate levels in tracheal smooth muscle (Kroeger and Stephens, 1971). During acute ischemia or hypoxia of the smooth muscle cells, the rapid accumulation of lactate and intracellular acidosis result in an early inhibition of
the pH-sensitive mitochondrial creatine phosphokinase leading to loss of essential energy necessary to maintain muscle contraction (Stephens et al., 1977). Hypoxic impairment of contractile function in the trachealis muscle is probably due to reduction in energy utilization as well as intracellular acidosis (Stephens and Chiu, 1970). Potassium-induced tension development in the taenia coli suggests that the phasic response is a passive process, whereas the tonic response and the maintenance of tension during a sustained contracture is an active one dependent upon the aerobic breakdown of carbohydrate (Urakawa and Holland, 1964; Pfaffman et al., 1965). In addition, the tonic response can be abolished in the presence of anoxia and substrate depletion (Urakawa et al., 1969; Urakawa and Holland, 1964). The relaxant response of taenia coli to anoxia was more rapid compared with that due to substrate removal, suggestive that aerobic metabolism is more important for supporting contractile activity (Bose and Bose, 1975).

b. Calcium utilization in hypoxia

The role of calcium in excitation-contraction coupling during hypoxia remains unsubstantiated. Studies on isolated guinea pig taenia coli (Bauer et al. 1965; Goodford, 1965) and isolated helical strips of rabbit aorta (Detar and Bohr, 1972) have shown that when oxygen is abruptly removed, the concentration of intracellular calcium is markedly elevated within a few seconds. The sustained hypoxic-induced contracture in vascular smooth muscle has been explained by a sudden increase in excitation-contraction coupling, due to a net influx of calcium or pre-formed ATP pool available (Mansour and Mansour, 1962). It is thought that calcium diffuses into the fiber and activates the contractile apparatus, the tension being maintained as long as calcium remains at the steady-state level. However,
Urakawa and Holland (1974) have demonstrated that the movement of calcium is dependent on aerobic metabolism which may explain the hypoxic-induced relaxant response of the taenia coli. Further studies of the uptake and binding of calcium by microsomes obtained from normoxic and hypoxic muscles must be undertaken to elucidate the mechanism of excitation-contraction coupling during hypoxia.

c. Effects of $\text{CO}_2$ and pH on the mechanical properties of smooth muscle

Spontaneous activity of the intestine consist of periodic depolarizations of intestinal muscle cells and are associated with an increased spike discharge. The number of spike potentials is related to the magnitude of intestinal contractions (Bortoff, 1976). In taenia coli, one can distinguish two spikes: second rhythm and minute rhythm. The second rhythm of taenia coli is not very sensitive to changes of $\text{pCO}_2$ and pH (Wienbeck et al., 1972), however, the frequency of the minute rhythm of duodenal segments was increased when $\text{CO}_2$ tension was reduced from 43 mm Hg to 0, with corresponding changes in pH (Adelmann, 1956). In contrast, an increase in $\text{CO}_2$ content in the organ bath reduces intracellular pH of the smooth muscle cells in the guinea pig taenia coli leading to a decrease in spontaneous activity (Wienbeck et al., 1972). Therefore changes in the transmembrane pH-gradient appear to alter the phasic component, whereas the intracellular calcium level and available endogenous energy stores may influence the tonic response of vascular and intestinal smooth muscle to hypoxia.
2. **Hypoxia and collagen**

Collagenous tissue is the most prevalent component of the tunica albuginea and accounts for the firm and fibrous appearance of the testicular capsule. In addition to collagen, the tunica albuginea also contains a relatively high content of elastic fibers, fibroblasts and smooth muscle (Davis et al., 1970; Leeson and Cookson, 1974).

It has been demonstrated that fibrogenic cells are more resistant to low oxygen tension and do not develop degenerative changes (Kistler, 1967). Long-term exposure to hypoxia stimulates collagen synthesis in artificially-induced granuloma tissue. An active synthesis of collagenous hydroxyproline and soluble forms of collagen which is thought to represent an early stage of collagen synthesis was observed in hypoxic-treated rats (Chvapil et al., 1970). In addition, the hypoxic effect on rabbit aorta has revealed an increase in the acid mucopolysaccharides and may be due to a stimulated synthesis of sulphated acid mucopolysaccharides as expressed by the increased uptake of $[^{35}S]$ sulphate (Helin et al., 1969). On the other hand, hypoxia inhibits the synthesis of globular proteins (Kim and Han, 1969) and noncollagenous protein formation in rat liver, heart, small intestine and lung (Chvapil et al., 1970). These findings suggest the variable susceptibility of cells to hypoxia.

Long-term exposure to hypoxia is known to produce cardiomegaly associated with an increased collagenous stroma (Bartosova et al., 1969). However, the effect of low oxygen tension on the growth of smooth muscle and collagenous tissue located in the tunica albuginea are unknown. Perhaps chronic exposure to hypoxia may result in an increased collagen content of the tunica albuginea. Hypoxia may thereby interfere with the rhythmic contractions and relaxations of
the testicular capsule and lead of testicular dysfunction or impairment of spermatogenesis.

3. **Effect of hypoxia on the action of drugs in smooth muscle**

Observations in laboratory animals have indicated that acute hypoxia decreases the vasoconstrictor response of rabbit aortic smooth muscle to norepinephrine (Gowdey, 1966), epinephrine (Detar and Bohr, 1972) and sympathetic nerve stimulation (Skinner and Costin, 1969). In the absence of drugs, anoxia usually produces, in addition to the indirect effects of sympathetic discharge, local vasodilation in the canine coronary bed. The local vasodilation effect of anoxia on systemic blood vessels of intact animals and perfused smooth muscle preparations may be mediated by local metabolites (lactic acid and an increase in \( pCO_2 \) resulting in a decrease in pH) rather than by direct action of low \( pO_2 \) on vascular smooth muscle (Somlyo and Somlyo, 1970). The precise mechanism by which hypoxia decreases the contractile response of smooth muscle to drugs remains unclear.

The stimulatory effect of prostaglandins on the spontaneous rhythmic contractions of smooth muscle appears to be dependent on oxidative metabolism. In the rat uterus (Paton and Daniel, 1967) and stomach preparations (Coceani and Wolfe, 1966) bubbling with nitrogen instead of oxygen/carbon dioxide reduced the response to prostaglandins without affecting the contraction induced by acetylcholine or 5-hydroxytryptamine. Similarly in the rat fundus, cyanide, carbon monoxide and nitrogen, all of which block oxidative metabolic processes, also inhibit the response to prostaglandins but not to acetylcholine (Coceani and Wolfe, 1966). The investigators postulate that under anoxic conditions, prostaglandins...
may remain in an inactive form and oxygen may be required for the excitatory contractile response in smooth muscle.

J. TEMPERATURE

It has long been known that high ambient temperature is correlated with impaired fertility in male mammals. The effect of heat on human fertility has been most clearly seen in subtropical and warm temperate latitudes (Pincher, 1945; MacFarlane, 1970). Suppression of spermatogenesis in certain disorders such as cryptorchidism (Nelson, 1951), varicocele (Davidson, 1954; Zorgniotti and MacLeod, 1973) and acute febrile diseases (MacLeod, 1951; Kar, 1953) have been associated with high temperature. The deleterious effect of diathermic treatment (MacLeod and Hotchkiss, 1941) and hot water baths (Fukui, 1923; Procope, 1965) on spermatogenesis in man have been well documented. Direct thermal damage to the germinal epithelium (Moore, 1924b), reduction in testosterone production (Waites, 1976), changes in blood, lymph flow and testicular fluids (Setchell and Waites, 1972) as well as changes in specific metabolic pathways (Davis, 1969) have been reported.

In contrast to heat, short-term exposure to cold temperatures can be tolerated without serious effects on the production of sperm and testicular function. However, reduced androgen production has been reported to be the primary cause of testicular degeneration accompanying prolonged exposure to cold (VanDemark and Free, 1970).

The temperature of the testis has been reported to be 3 to 4°C cooler than the abdominal cavity in a variety of mammalian species (Buyer and Davis, 1966).
For normal spermatogenesis to occur, the scrotal-abdominal temperature gradient must be maintained by vascular heat exchange and thermoregulatory mechanisms.

1. **Vascular heat-exchange**

   Testicular temperature is known to fluctuate rapidly in response to external heating or cooling of the scrotum. The arrangement of the free communicating cord veins within the pampiniform, cremasteric and vasal plexuses has been reported to play an important thermoregulatory function (Shafik, 1974). Blood flowing through the internal spermatic artery of the ram has been demonstrated to cool the testis when intra-testicular temperatures exceeded 33°C (Waites and Moule, 1961). Rapid filling of the superficial testicular veins and increased exposed surface area encouraged heat loss (Waites and Moule, 1961; Kormano, 1967). However, with cold exposure and cremasteric muscle contraction, compression of the cord veins resulted in diminished blood flow, venous surface area and heat radiation (Shafik, 1974).

2. **Thermoregulatory apparatus**

   The structure of the scrotum is characterized by features which enable it actively to control its own temperature. The skin is thin, lacking subcutaneous fat and possessing sheets of smooth muscle (Gutzschebauch, 1935). The thermoregulatory apparatus consisting of the cremasteric and dartos muscles, as well as, the cord and scrotal vessels have been reported to maintain the scrotal-rectal temperature gradient (Shafik, 1974). The cremasteric-dartos muscle component, through its sphincteric mechanism regulates the amount and rate of blood flow within the cord and scrotal vessels.
a. Dartos muscle

The function of the dartos muscle has been extensively studied by Moore and Quick (1924) and Shafik (1973b). Shafik reported that the dartos muscle located in the dermis of the scrotal sac consists of three types of muscle bundles: circular, oblique and longitudinal. Each scrotal compartment has its own dartos muscle and both contribute in the formation of the scrotal septum. Upon exposure to cold, the dartos muscle contracts, elevating the testicle to the warmer abdominal surface and the intervening blood vessels constrict resulting in a diminished scrotal blood flow. Under the influence of heat, the dartos muscle relaxed with vessel dilatation; the testicle was brought away from the body surface through the action of the scrotal ligament.

b. Cremasteric muscle

The cremasteric muscle has been reported to play an important role in the thermoregulatory mechanism of the testicle (Shafik, 1973a). The muscle acts as a suspensory muscle carrying the weight of the testis under normal temperature conditions. In addition, Shafik (1973a) suggested that it may constitute an active component of the fasciomyascular pump of the spermatic cord preventing venous reflux from the abdominal veins and high venous pressure transmitted to the testicle. On contraction, the cremasteric muscle elevated the testicle toward the warmer body surface and compressed the cord veins, diminishing their blood flow and minimizing exposed surface area. Under the influence of heat, cremasteric relaxation resulted in lowered testicular position and increased blood flow within the cord veins, increased surface area which encouraged heat radiation.
c. **Scrotal ligament**

The scrotal ligament may play a significant role in the temperature-regulating mechanism of the testicle. Absence of the ligament has resulted in disordered testicular thermoregulation and may be a factor in the genesis of subfertility in man. The anatomy and physiological role of the ligament has been extensively studied by Shafik (1977).

Histologically, the ligament consisted of collagen impregnated with elastic and smooth muscle fibers; the later abundant in children. However, in adults, the muscle bundles diminished and were absent in a number of surgical specimens analyzed by Shafik (1977). The scrotal ligament connects the dartos muscle with the parietal layer of the tunica vaginalis at the lower testicular pole, as well as, synchronizing the cremasteric-dartos muscular action under normal and varied temperatures. As a result of an absent or poorly developed scrotal ligament, the cremasteric-dartos synchronous action is lost and the two muscles cannot function coordinately. Therefore, at rest, the testicle tends to be elevated toward the abdomen since the cremasteric tone is unopposed by the dartos because the connection between the two muscles along the ligament is lost. Prolonged high testicular position has been reported to create organic changes in the spermatic cord, scrotal wall and disturbances in spermatogenesis (Waites and Setchell, 1969).
3. **Effect of temperature on spermatogenesis**

It has long been known that germinal cells of the testis undergo rapid deterioration when subjected to increased temperature. Specific damage limited to mature spermatocytes have been reported to be the most susceptible to temperature (Steinberger and Dixon, 1959; Chowdhury and Steinberger, 1964).

Crew (1922) first postulated that the damage to the seminiferous epithelium of natural cryptorchid testes was the result of exposure to intra-abdominal temperature higher than the intrascrotal temperature.

Experimental cryptorchidism can similarly result in sterility. Moore (1924b) reported experimental cryptorchidism in guinea pigs resulted in complete dissolution of spermatogenic cells accompanied by degeneration, vacuolation and giant cell formation. A decrease in testicular weight and size, as well as, an increase in the number of interstitial cells were noted. Furthermore, a lack of spermatogenic function was observed while the testis remained in the abdominal cavity. Davis and Firlit (1966) reported similar morphologic changes, including the predominance of a Sertoli-cell retinaculum which contains numerous Type B spermatogonia and occasional pachytene primary spermatocytes. Following 30 days of exposure to an intra-abdominal temperature, Sertoli cells demonstrated cytoplasmic fibrosis and hyalinization, as well as, nuclear pleomorphism.

a. **Diathermic treatment**

Scrotal immersion in warm water has resulted in similar findings. Fukui (1923) and Moore (1924b) demonstrated marked degeneration of germinal epithelium and loss of spermatogenic function when the scrotum of several species
were submerged in water 6 to 7° C above normal body temperature. A direct relationship between an elevation in scrotal temperature and depressed spermatogenesis has recently been reported (Zorgniotti and MacLeod, 1973).

b. Baths

Procope (1965) studied the effect of increased body temperature produced by sauna bathing on the sperm count. The mean total time of bathing was 2 hours and 45 minutes and the mean rise of rectal temperature was 0.93° C. A 50 percent reduction in the mean sperm count occurred about 35 days after the beginning of the bathing, which was followed by rapid recovery.

Rock and Robinson (1965) reported that when male subjects were exposed to bath water temperature of 43° C, the body temperature had risen from a median value of 37.7 to 39.3° C, which corresponded to an increase in scrotal temperature from 36.1 to 40.5° C. An increase in scrotal temperature was accompanied by a decrease in sperm production within 16 weeks. However, in 45 percent of the men, the decrease in sperm count was followed by a rebound to levels higher than had prevailed prior to treatment.

c. Intrascrotal hyperthermia induced by scrotal insulation

There have been reports that tight undergarments such as athletic supporters and Jockey shorts that may hold the scrotum close to the body, interfere with scrotal ventilation and, therefore, could be associated with an artificial elevation of intrascrotal temperature (Davidson, 1954; Robinson and Rock, 1967).
Davidson (1954) has cited the cases of several married men with subnormal sperm counts who had worn scrotal supports for several years. After abandonment of the supports and cold applications to the scrotum, the sperm counts increased from low ranges of 1.25-10 million/ml to ranges of 40-46 million/ml and pregnancies subsequently occurred.

Robinson and Rock (1967) have studied the effects of spermatogenesis, produced by artificial elevation of scrotal temperature which accompanied the use of a scrotal support in 10 healthy, euspermic individuals. The subjects wore athletic supports containing an additional layer of insulating material during waking hours for periods of 6-11 weeks. Use of the scrotal support for 30 minutes increased the scrotal temperature, thereby reducing the scrotal-rectal temperature differential by 0.8°C, as compared to pre-treatment control values. The insulation produced a depression in sperm count in every case, amounting to a mean decline of 10 percent about 6 weeks after the commencement of treatment.

d. Hyperpyrexia

Transitory elevation of testicular temperature due to acute febrile disease has been shown to depress spermiogenesis (Mills, 1919; MacLeod and Hotchkiss, 1941; MacLeod, 1951; Kar, 1953; French et al., 1973).

Mills (1919) was first to describe the pathologic changes in the testes during epidemic pneumonia, which involved degeneration of preformed spermato­cytes, spermatids and spermatozoa. He concluded that the source of the testicular damage was due to circulating toxins, rather than the adverse effects of hyperpyrexia on testicular function.
MacLeod and Hotchkiss (1941) later presented empirical evidence that brief artificial elevations of human body temperature in conjunction with elevated environmental temperature would cause depression in sperm counts. When their subjects remained in a fever cabinet at a temperature of 43°C for less than one hour, pronounced decrease in sperm counts were noted approximately one month later. Typical cytological changes observed were cessation of spermatogenesis and degeneration of pre-formed spermatocytes, spermatids and spermatozoa.

There is also documentation of reduced sperm output in such febrile illnesses as pneumonia and chicken pox (MacLeod, 1951) and in recurrent febrile attacks of familial Mediterranean fever (French et al., 1973). Temperatures of 104°C were reported in all cases with a concomitant decrease in sperm counts approaching subfertile levels within thirty days after the disease was manifested. Sperm counts eventually returned spontaneously to the normal level.

e. Control of human spermatogenesis by induced changes of intrascrotal temperature

Several clinical investigations have been reported to control spermatogenesis by induced changes of intrascrotal temperature (Voegeli, 1962; Robinson et al., 1968).

A regimen of hot sitz baths for inducing temporary male sterility was successfully developed in India by a Swiss physician, Martha Voegeli (1962). The treatment consisted of daily forty-five minute 116°F baths for a period of three weeks. Sterility persisted for 6 months.
In another study, Robinson and co-investigators (1968) found that exposure of the scrotum to the heat from a 150-watt light bulb for 30 minutes resulted in a mean scrotal-rectal temperature differential of 2.9°C. Such treatment on 14 consecutive days caused depression of spermatogenesis, followed by rebounds to temporarily high sperm counts. Conversely, the application of an ice bag to the scrotum for 30 minutes on 14 consecutive days, beginning not less than 12 days following cessation of exposure to heat, elevated the sperm count by three-fold. In addition, the greatest increase in spermatogenesis was noted after the sequential administration of both heat and cold. The authors suggest that stimulating spermatogenesis by intermittent cooling of the scrotum may have clinical importance in the treatment of oligospermic individuals.

4. Effect of temperature on testicular capsular contractions

Davis and co-investigators (1970) reported a progressive increase in temperature from 32 to 38°C, resulted in a 0.2 mm increase in the tone of rat isolated testicular capsule. Further increases in temperature to 44°C were not observed to produce any further change in testicular capsular tone. The authors concluded that an additional explanation for the small size of the cryptorchid testis, which is exposed to the higher abdominal body temperature, may involve the prolonged contraction of the testicular capsule with a resulting pressure atrophy of the seminiferous tubules of the testis.
5. **Temperature and smooth muscle**

Rapid cooling of guinea pig stomach and taenia coli smooth muscle preparations from 32 to $20^\circ C$ produces a tonic contracture and decrease in phasic response with subsequent depolarization of the membrane. In the circular muscle of the stomach, the frequency of slow potential changes (basic minute rhythm) is first reduced followed by a cessation in spontaneous activity below $20^\circ C$. During depolarization of the membrane, an initial decrease in membrane resistance followed by a prolonged increase in membrane resistance has been reported (Prosser, 1977). In contrast, the spike frequency remained markedly sensitive to minimal temperature changes.

The increased membrane resistance changes associated with rapid cooling in the taenia coli and stomach are thought to be due mainly to a nonselective reduction of ionic permeability (Kuriyama et al., 1975). In the cold, the active processes which maintain ionic distribution across the cell membrane are less effective. The taenia coli loses potassium and accumulates sodium when the temperature is lowered and results in a decrease in the membrane potential. By measuring the membrane conductance with the double sucrose-gap technique, Brading and co-investigators (1969) found that between 19 and $37^\circ C$, the membrane conductance increased with rising temperatures and decreased with cooling. In addition the membrane became hyperpolarized with subsequent relaxation of the tissue, as well as an increase in the phasic component. The magnitude of the hyperpolarization is a function of the reduced membrane resistance during the initial phase of rapid cooling. The hyperpolarization is also thought to be due to activation of the electrogenic Na-pump mechanism during recovery from rapid cooling (Magaribuchi et al., 1973).
Cold contracture of the taenia coli and stomach muscle may be produced by the release of calcium ions from sequestered sites (sarcoplasmic reticulum) rather than the influx of calcium ion due to depolarization of the membrane (Kuriyama et al., 1975). It has subsequently been demonstrated in microsomal preparations isolated from longitudinal muscle of the taenia coli that low temperatures markedly decrease microsome capacity to accumulate calcium ions (Hurwitz et al., 1975). Therefore, it may be reasonable to postulate that the tonic contracture and the reduced phasic response at low temperatures is due to release of calcium ions.

The mammalian smooth muscle cells show marked species and tissue variations in response to environmental temperature changes. Unfortunately, the effects of hyperthermic temperature on the electrical and mechanical activity of smooth muscle remains unknown. Further studies are needed to explain the effect of temperature on smooth muscle function observed in many pathophysiological conditions.

6. Temperature and collagen

Little is known about the biological effects of temperature on collagen fiber formation. In addition to smooth muscle located in the tunica albuginea of the testicular capsule, dense connective tissue composed of collagen bundles, elastic fibers and numerous fibroblasts exist (Davis and Langford, 1970).

Contraction and relaxation of collagen fibers have been reported to occur at nonphysiological temperatures (exceeding 60°C) (Banga et al., 1954). In addition, collagen loses its swelling capacity and is solubilized by elastase ten times faster than elastin itself (Banga, 1953). On the other hand, a reduction in
the rate of collagen aging has been demonstrated by artificially cooling isolated collagen fibers or by exposing rats to hypothermia (0-2° C) (Chvapil and Hruza, 1959; Hruza et al., 1966; Hruza and Hlavackova, 1969). These investigators demonstrated that contraction and relaxation of collagen fibers were dependent upon temperature. Cooling decreases the cross-linking of collagen and promotes collagen fiber relaxation. When the cross linking process of collagen molecules is impaired, fiber tensile strength decreases, rendering the fiber more soluble and increasing the fiber's susceptibility to proteolytic turnover.

It is possible to conclude from these studies that temperature may interfere with the elasticity of the testicular capsule and may be responsible for changes in both the testicular size and spontaneous contractions of the testicular capsule.

7. Effects of temperature on the action of drugs in smooth muscle

The ability of temperature to influence the action of drugs in laboratory animals and man has interested pharmacologists and physiologists for a long time. The toxicity of many drugs, particularly those with central effects are influenced by extreme environmental temperatures. An increase in drug toxicity at temperatures below and above the thermoneutral zone (U-shaped response) can best be exemplified by the centrally acting drugs affecting the thermoregulatory center. These drugs include the hypnotics and neuroleptic agents such as phenothiazine, monoamine oxidase inhibitors, rauwolfia alkaloids, morphine and cholinergic blocking agents (Fuhrman and Fuhrman, 1961). The other important type of response is an increase of drug toxicity with a corresponding increase in environmental temperature (linear response). Well known examples include the
sympathomimetic drugs such as amphetamine, epinephrine and norepinephrine as well as cortisone and dinitrophenol. Rewerski and co-investigators (1975) recently reported that the LD$_{50}$ of chlorpromazine studied in mice was markedly effected by ambient temperature. The LD$_{50}$ of chlorpromazine was 5.7 times greater at 32°C than at 20°C. Similarly, chlordiazepoxide was 4.2 times greater than the 20°C control. Significant factors include differences in the rate of metabolism at various environmental temperatures and induced changes in body temperature due to the drug. The mechanism of action of drugs at various body temperatures remains inconclusive.

A reduction in temperature from 30 to 10°C has been demonstrated to inhibit both the uptake of calcium into microsomes and the rate of calcium ion release from the microsomes to the external medium. Further, calcium ions have been associated in a number of drug-induced contractile responses of smooth muscle. An acetylcholine-induced contraction of the longitudinal muscle of the guinea pig taenia coli generated at 30°C has been reported to be markedly different from a response at 10°C. At 30°C, acetylcholine produces an increase in phasic activity followed by a less rapid tonic contraction. However, a prolonged tonic contracture associated with an absence in phasic activity occurred at 10°C. Similarly, temperature markedly affects the response of smooth muscle to prostaglandins. Lowering the muscle bath temperature from 34 to 27°C, markedly reduced the prostaglandin response, while the contractile effect of 5-hydroxytryptamine remained unchanged (Karim, 1972).

The dissimilarity in the mechanical response of smooth muscle to acetylcholine and prostaglandins at the lower temperatures may be associated with the release of calcium (Hurwitz et al., 1975).
8. Congenital disorders of the testicle related to temperature

The sensitivity of spermatogenesis to changes in the environmental temperature of the testes are well known. Disturbances in the quality of semen in the presence of cryptorchidism and varicocele have been related to elevation of testicular temperature.

a. Cryptorchidism

A testis which cannot descend because of some anatomic or physiologic abnormality has been reported to undergo secondary degenerative changes (Brown, 1976). Degenerative changes became apparent in experimentally-induced cryptorchidism in animals. Moore (1924a) demonstrated that the intraperitoneal implantation of the testes of rodents produced total degeneration of the germinal epithelium within 20 days and that re-implantation of the testes to the scrotum was followed by a resumption of spermatogenesis.

The irreversible effect of heat on spermatogenesis occurs in the cryptorchid child with late descent of one or both testes. Hecker and Hienz (1967) demonstrated that if the child's testicle remains outside of the scrotum after the age of 5 years, progressive degeneration of dystopic testicular tissue begins to occur. In addition, if the testicle remains outside of the scrotum until puberty, it is incapable of producing sperm. The intra-abdominal temperature, which is 2 to 3°C higher than the scrotal temperature, is sufficiently elevated to prevent normal spermatogenic development.
b. Varicocele

Varicocele is a well-established cause of male infertility and applies to any abnormal dilatation and tortuosity of the veins of the pampiniform plexus within the scrotum. The age incidence is usually between 15 and 25 years and venographic and anatomical studies clearly indicate that the left gonadal, or internal spermatic vein is the vessel responsible for most instances of varicocele formation (Greenberg, 1977). Improvement in semen quality after surgical ligation of the internal spermatic vein has been repeatedly demonstrated (Dubin and Amelar, 1975).

Several theories have been proposed to explain the effect of varicocele on spermatogenesis, but despite considerable investigation none has been proven. Young (1956) and Hanley and Harrison (1962) believed that the presence of scrotal varicosities caused an elevation in testicular temperatures.

Zorgniotti and MacLeod (1973) and Lazarus and Zorgniotti (1975) demonstrated that intrascrotal temperature was significantly higher in varicocele subjects with poor semen quality than in a control group without varicocele and with normal semen quality.

Thermography has been found valuable in the diagnosis of varicose veins and venous insufficiency. Thermographic recordings of varicocele patients confirmed an elevated scrotal temperature as compared to normal subjects (Kormano et al., 1970, 1973; Amiel et al., 1976).

Insufficient or absent valves in the internal spermatic vein have also been reported to be responsible for the varicocele effect (Agger, 1971). Retrograde flow in the vena testicularis sinistra carrying toxic metabolites from the adrenal or renal vein can produce a bilateral depression of spermatogenesis. El-Sadr and
Mina (1950) have demonstrated the existence of extensive superficial venous collaterals and suprapubic venous anastomoses between the left and right pampiniform plexus in cadavers. Furthermore, Comhaire and Vermuelen (1974) and Cohen et al. (1975) found elevated catecholamine levels in the vena testicularis and peripheral plasma in varicocele patients.

The phlebectasis and the retrograde flow into pampiniform plexus, associated with varicocele, increase the volume of blood in the vicinity of the testis. Due to venous stasis, reduced oxygen tension which may be deleterious to spermatogenesis has been reported (Davidson, 1954; Cohen et al., 1975). However, Donohue and Brown (1969) measured pH, \( pO_2 \) and \( pCO_2 \) in blood from the left testicular veins in the presence of a varicocele and found no abnormality that might account for testicular damage or impairment of spermatogenesis.

K. PATHOLOGY OF THE TESTICULAR CAPSULE

Testicular neoplasms are rare and represent less than one-percent of all malignant disease in men. The peak incidence occurs in the third, fourth and fifth decades, man's most productive years (Gallager, 1972). Approximately 97 per cent of all tumors of the testes are malignant, with the majority of tumor cells originating from germ cells. The non-germinal tumors arising from the interstitial cells of Leydig or the Sertoli cells tend to be small benign lesions (Robbins, 1974).
1. **Benign lesions**

Benign tumors of the tunica albuginea are extremely rare. Fibromas, neurofibromas, leiomyomas and cysts have been reported (Turner et al., 1977). Gowing (1976) has classified these lesions as non-epithelial, paratesticular neoplasms of muscular or connective tissue origin. Hemangioma of the tunica albuginea, a tumor of vascular origin has also been reported (Pfitzenmaier et al., 1975).

2. **Malignant neoplasms**

Hinman and Gibson (1924) have reported that the tunica vaginalis and albuginea are capable of undergoing metaplastic changes. Malignant fibrosarcomas arising from the tunica vaginalis exhibit pleomorphic spindle cells and a considerable number of mitotic figures.

Gowing (1976) has described several cases which have been diagnosed as papillary mesotheliomas of the tunica vaginalis. The tumor cells covering papillary processes are in direct continuity with the mesothelium lining the tunica vaginalis.

3. **Miscellaneous lesions**

   a. **Hydrocele**

The clinical condition known as a hydrocele can be defined as an accumulation of serous fluid either between the layers of the tunica vaginalis or within the processus vaginalis along the spermatic cord. Most hydroceles are of the idiopathic type and are widely believed that asymptomatic inflammation or
trauma may be an underlying cause in many cases. A secondary hydrocele often develops when there is an underlying testicular or epididymal disease, as in cases of orchitis, epididymitis, tuberculosis, filariasis or in neoplastic disease (Cameron and Pugh, 1976). In addition, fluid may accumulate in the tunica vaginalis when there is systemic edema, as in cardiac failure or renal disease (Robbins, 1974).

b. **Hematocele**

Hematocele can be defined as the presence of blood in the tunica vaginalis. This clinical condition usually occurs as a result of local trauma, torsion or an underlying tumor. In chronic cases, the wall of the tunica vaginalis may become increasingly thickened, with possible calcification (Cameron and Pugh, 1976).

c. **Chylocele**

The term chylocele refers to the clinical condition in which lymphatic fluid accumulates in the tunica vaginalis. It is predominantly found in patients with elephantiasis who have widespread, severe, lymphatic obstruction (Robbins, 1974).

d. **Spermatocele**

A spermatocele is a cyst containing spermatozoa occurring as a retention cyst of the spermatic ducts in the rete testis or the head of the epididymis. A characteristic fibrous thickening of the tunica vaginalis has also been observed (Cameron and Pugh, 1976).
L. MORPHOLOGY OF THE SPLENIC CAPSULE

The spleen is the largest lymphatic organ in the body and considered to have numerous immunological (Lennert, 1970), as well as, hemolytic (Wennberg, 1969) functions.

The splenic capsule consists of an outer serosal layer with an inner muscular layer (Tehver and Grahame, 1931). Moore et al. (1964) and Burke and Simon (1970) have investigated the ultrastructure of the splenic capsule of the adult rabbit and have concluded that the outer layer is covered by a single layer of mesothelial cells which have numerous cytoplasmic projections from their free surface. These cells lie on a basement membrane that separates them from the underlying capsule.

The inner and most predominant layer of the splenic capsule has been described as a dense, fibrous membrane composed of bundles of collagen fibers and smooth muscle cells (Chatterjee and Cruickshank, 1929; Tehver and Grahame, 1931; Moore et al., 1964; Burke and Simon, 1970). The important and varying histological feature of this layer is the extent and distribution of the smooth muscle component. In many species such as the dog and cat, the capsular smooth muscle is extensive, while in man and rabbit it is quite sparse (Chatterjee and Cruickshank, 1929; Davies and Withrington, 1973). In contrast to the distribution of smooth muscle in the splenic capsule among different species, smooth muscle is most prominent in the testicular capsule of the rabbit (Davis et al., 1970) and dog (Leeson and Cookson, 1974); and less prominent in the rat (Davis et al., 1970; Leeson and Cookson, 1974) and human (Langford and Heller, 1973). The rabbit splenic capsule, when observed under the electron microscope, was found to consist primarily of cells which have features of both smooth muscle cells and
fibroblasts. These cells have a characteristic stellate appearance with dense fibrillar cytoplasm, small mitochondria and glycogen-like granules. They may also be associated with large bundles of collagen and occasionally with some elastic tissue (Burke and Simon, 1970). Smooth muscle cells are more numerous in deeper layers of the capsule as it invaginates into the pulp of the spleen to form trabeculae (Moore et al., 1964). Davies and Withrington (1973) report that the presence and density of the smooth muscle in the capsule and the number of trabeculae is an indication of the reservoir function of the organ.

McCance and Widdowson (1955) have shown that in contrast to the adult dog, the splenic capsule of the newborn puppy lacks mature muscle fibers. The trabeculae are also very poorly developed and are represented by a delicate and rather inconspicuous network of strands of spindle-shaped cells which lack the distinctive staining reactions of muscle. The capsule and trabeculae were better developed by the seventeenth day, but still resembled those of the newborn rather than the adult. The authors concluded that the spleen of the young puppy does not function as a reservoir of erythrocytes for acute emergencies, but rather as a blood-forming organ. In contrast to the passive role of the postnatal splenic capsule, the rabbit testicular capsule from animals of the same age group, revealed spontaneous contractions which have been attributed to the presence of mature smooth muscle fibers (Mitchell and Seeley, 1977).
M. VASCULAR SUPPLY OF THE SPLENIC CAPSULE

1. Arterial supply

The arterial blood is derived from a major branch of celiac artery, the gastroepiploic arcade system and short gastric branches from the stomach. The splenic arteries enter the spleen at the hilum, divide and terminate in a system of arteries, about 250 μ in diameter, in the trabeculae and capsule. The splenic artery exhibits marked variation in number, mode of origin and distribution of branches in the human (Michels, 1942) and other species (Tischendorf, 1969).

2. Venous supply

The mammalian spleen is divided into a series of compartments by fibrous septa and each segment is drained by its own vein (Braithwaite and Adams, 1957). In the rat, additional drainage occurs by collateral segmental veins rather than diffusion through the pulp.

The venous sinuses empty the pulp veins, lined by endothelium, which unite to form larger veins entering the trabeculae as trabecular veins. Trabecular veins eventually drain into the splenic vein at the hilum. Capsular veins are so inconspicuous, that the nature of their circulation remains unknown.

N. INNERVATION OF THE SPLENIC CAPSULE

The innervation of the mammalian spleen has been investigated by classical histochemical staining techniques and evidence suggests it is almost entirely sympathetic and postganglionic in origin (Tischendorf, 1969; Ballantyne, 1967). Stimulation of the vagus nerve exerts no direct effect on the smooth muscle of
the spleen; a view supported by the lack of histological evidence of any parasympathetic innervation to the organ (Utterback, 1944).

1. **Sympathetic innervation**

Catecholamine-induced fluorescence was confined to the nerve fibers among the smooth muscle of the splenic capsule, trabeculae, arteries and veins in the cat (Gillespie and Kirpekar, 1966). However, fluorescence was not present after treatment with reserpine, a catecholamine depleter, or after degeneration of the postganglionic splenic nerve fibers. The distribution of nonspecific esterases and cholinesterases in the rabbit spleen appears to be confined to the region associated with the vascular system and in extravascular cells. Acetylcholinesterase occurs both in the endothelium of follicular capillaries and in elongated cytoplasmic processes of cells in the follicles, while butyrylcholinesterase is present in nerve fibers arranged as periarterial plexuses (Ballantyne, 1968). However, in the present study, there was no evidence of esterases in the splenic capsule. The autonomic efferent nervous innervation to the splenic capsule may be considered predominantly adrenergic, with the endothelium of follicular capillaries exhibiting a positive acetylcholinesterase reaction. In contrast to the splenic capsule, the smooth muscle of the adult rat testis appears to be supplied by both adrenergic excitatory and cholinergic excitatory fibers (Bell and McLean, 1973).
2. **Electrical nerve stimulation**

Contraction of the spleen in response to electrical stimulation of its pre- or postganglionic nerve supply has been demonstrated in the isolated perfused spleen of the rabbit (Menkin, 1929), cat (Celander, 1954; Salzmann and Pacha, 1976), dog (Davies et al., 1968, 1973) and man (Ayers et al., 1970, 1972). Sympathetic nerve stimulation caused a marked vasoconstriction along with the contraction of the splenic capsule. In addition, the administration of an alpha adrenergic blocking agent abolished the contraction and produced profound vasodilation (Davies et al., 1969; Greenway et al., 1968). The responses of the isolated perfused human spleen to sympathetic nerve stimulation at frequencies of up to 30 Hz produced small contractions of the splenic capsule, despite increases in splenic vascular resistance (Ayers et al., 1972). In contrast to man, similar vascular responses were accompanied by relaxation of the canine splenic capsule as reflected by marked decrease in spleen volume.

O. **PHARMACOLOGY OF THE SPLENIC CAPSULE**

Oliver and Schäfer (1895) were first to describe the contraction of the canine spleen produced by the injection of calf suprarenal gland extracts. These observations were confirmed by the demonstration of noradrenaline in the spleen (Von Euler, 1946) and its identification in the splenic venous effluent after sympathetic nerve stimulation (Peart, 1949).
1. Catecholamines

Epinephrine has been shown to contract isolated whole spleens or splenic strips from many species including: the mouse (Ignarro and Titus, 1968), rat (Magee, 1946), rabbit (Variel, 1933; Saad, 1935; Magee, 1946; Lozowski, 1975), guinea pig (Magee, 1946), cat (Loewe and Faure, 1925; Bickerton, 1963; Innes, 1972; Granata et al., 1974), dog (Vairel, 1933; Saad, 1935), and man (Saad, 1935; Ayers et al., 1972). Norepinephrine has also been demonstrated to contract isolated strips of spleen from the rabbit (Lozowski, 1975), cat (Brandon, 1961; Bickerton, 1963; Granata et al., 1974), and dog (Takano, 1969, 1975). Bickerton (1963) evaluated the responses of isolated strips of cat spleen to sympathomimetic drugs and concluded that epinephrine was more potent than norepinephrine. The epinephrine-induced contraction of splenic strips from several species of animals can be blocked by alpha-adrenergic blocking agents (Saad, 1935; Bickerton, 1963; Kizaki and Abiko, 1966). Low concentrations of isoproterenol ($5 \times 10^{-9}$ to $5 \times 10^{-7}$ M) produced relaxation of the whole mouse spleen (Ignarro and Titus, 1968), whereas, in the cat no contractions of the splenic strip were observed (Bickerton, 1963). However, contractions to isoproterenol have been obtained from the spleen strips of the mouse and cat at higher concentrations ($5 \times 10^{-6}$ to $5 \times 10^{-3}$ M). The isoproterenol-induced contraction of the spleen in cats can be inhibited, by dichloroisoproterenol or dibenamine (Bickerton, 1963).

A species variation has been observed in the effects of beta receptor blocking agents on the responses of splenic strips to catecholamines (Kizaki and Abiko, 1966). Ignarro and Titus (1968) reported that the mouse splenic capsule contains both alpha and beta receptors. Alpha receptor innervation to the capsule of the cat and dog spleen and the presence of innervated alpha and beta receptors
in the splenic vascular bed have been demonstrated (Moerman et al., 1969; Greenway and Stark, 1970). However, Ayers and co-investigators (1972) reported that the human spleen contains predominantly alpha receptors in both the capsule and blood vessels.

2. Cholinergic drugs

It has been reported that the contractile response of isolated cat spleen strips to acetylcholine was smaller than that of epinephrine (Magee, 1946; Bickerton et al., 1962; Kizaki and Abiko, 1966; Takano, 1969). The acetylcholine-induced contraction was blocked by atropine, but not dibenamine (Takano, 1969). In isolated blood perfused spleen preparations of the dog, Daly and Scott (1961) demonstrated that hexamethonium prevented contraction of the spleen produced by acetylcholine. These results suggest that in addition to its muscarinic action, acetylcholine also had a nicotinic action on the spleen. Davies and Withrington (1973) conclude that the effects of acetylcholine appear to be three-fold: a muscarinic effect on the vascular smooth muscle causing vasodilitation, a nicotinic effect on the postganglionic nerve terminals causing capsular contraction, and at high doses a muscarinic effect on the capsular smooth muscle evoking contraction.

3. Prostaglandins

Prostaglandins have been shown to be active and widely distributed in many biological systems; their role as modulators of hormone action, as well as, of neurotransmission have been well documented (Horton, 1969; Karim, 1972).
Two prostaglandins E\(_2\) and F\(_2\alpha\), have been detected in the splenic venous effluent after splenic nerve stimulation in the isolated, blood-perfused spleen of the dog (Davies et al., 1968; Davies and Withrington, 1971), whereas, prostaglandin release from the cat spleen was barely detectable (Bedwani and Millar, 1975). The pressor and the splenic contraction responses to nerve stimulation were found to be reduced by low doses of PGE\(_2\) (Hedqvist, 1970). With higher doses of PGE\(_2\), the splenic contraction response was further depressed, while the pressor response returned to normal. These vascular responses could not be altered by the prior administration of either alpha or beta adrenoceptor blocking drugs to the spleen (Davies and Withrington, 1967).

Prostaglandin release has been demonstrated with infusions of epinephrine or norepinephrine (Gilmore et al., 1968). Contractions induced by norepinephrine or methoxamine of the rabbit splenic capsular strips are accompanied by the release of PGE and PGF (Jobke et al., 1976). Furthermore, the responses of the rabbit splenic capsular strip to norepinephrine and methoxamine are potentiated by indomethacin and 5, 8, 11, 14-eicosatetraynoic acid (ETA), two well-known PG-synthesis blockers. The authors suggest that potentiation of the effects of various agonists by indomethacin and ETA is the result of inhibition of the enzyme fatty acid cyclo-oxygenase in the splenic strips. Angiotensin II-induced contractions of rabbit splenic capsular strips and release of PGE and PGF has also been demonstrated (Diekmann et al., 1977). While the relation between PGE and PGF released is similar to that obtained with norepinephrine and methoxamine as agonists, the absolute amounts of prostaglandin release by angiotensin II were
reportedly higher. Hedqvist (1970) has proposed that the prostaglandins may play a modulatory role on the sympathetic innervation within the spleen by antagonizing the effects of nerve stimulation and circulating catecholamines.

Preliminary observations have revealed all prostaglandins, with the exception of high concentrations of PGE<sub>1</sub>, to be ineffective in eliciting a response of the isolated splenic capsule of the rabbit (Jobke et al., 1976). It has been suggested that the lack of response of the isolated splenic capsule to the exogenous administration of prostaglandins, may be due to their failure to reach the site of action of endogenous prostaglandins.

4. Vasoactive amines

The effects of three polypeptides, angiotensin, vasopressin and oxytocin have been demonstrated to have potent actions on the smooth muscle of the capsule and blood vessels of the dog spleen (Davies and Withrington, 1975). However, there is conflicting evidence whether the action of these polypeptides on the smooth muscle of the perfused spleen, is a direct one on the muscle cells or indirect through the release of norepinephrine stored in sympathetic nerve endings.

5. Barium chloride

Barium chloride, noted for its stimulation of smooth muscle, was observed to produce a slight contraction of the isolated splenic strip of the dog in concentrations ranging from $3 \times 10^{-5}$ to $2 \times 10^{-3}$ M (Takano, 1975). In addition, the barium-induced contraction was greatly increased by changing the bath medium.
from krebs solution to Ca-free-krebs or EDTA-krebs solution. The results suggest that barium may behave like calcium in producing a contraction of the dog spleen strip.

P. PATHOLOGY OF THE SPLENIC CAPSULE

1. Perisplenitis

Hyaline perisplenitis is a lesion characterized by dense, hyaline, collagenous fibrous tissue limited to the splenic capsule. It is postulated that it represents a healed inflammatory perisplenitis attributed to fibrosis of a chronic, capsular edema or chronic history of ascites (Robbins, 1974).

Zipfel and co-investigators (1978) have reported a statistical correlation between fibrosis of the splenic capsule and perisplenitis cartilaginea in the postmortem diagnosis of cor pulmonale. In addition, splenic capsular changes associated with the postmortem diagnosis of myocardial insufficiency and hypertension remain unsubstantiated.

2. Splenic capsule neoplasms

Tumors of the splenic capsule, which may ultimately interfere with its contractile response have not been documented. This is in contrast to the rare occurrence of benign (fibromas, leiomyomas and cysts) and malignant neoplasms (papillary mesothelioma and malignant fibrosarcoma) of the testicular capsule.
Q. MORPHOLOGY OF THE RENAL CAPSULE

The renal capsule surrounding the parenchymal tissue of the kidney consists of three layers. These layers include the outer renal fascia, the adipose capsule and the capsula proper (Itzchak et al., 1974).

1. Renal fascia

The renal fascia is derived from the transversalis fascia and consists of two layers. These layers fuse at the upper pole of the kidney but remain separated at the lower medial border. The anterior layer extends medially in front of the kidney, while the posterior layer is located medially behind the kidney and blends with fascia of the quadratus lumborum and the psoas major muscles.

2. Adipose capsule

The adipose capsule, or commonly known as the perirenal fat, lies in the fascia space of Gerota, between the renal fascia and the fibrous renal capsule. The capsular arteries and veins have been observed in this fat layer.

3. Capsula proper

The fibrous capsule of the kidney is the most predominant layer and covers the kidney completely, in addition to extending over the pelvis of the kidney and ureter. Bulger (1973) investigated the ultrastructure of the capsula proper and concluded that the fibrous capsule of the rat kidney consists of the following layers: mesothelium, dense connective tissue containing collagen and a network of elastic fibers and several sheets of squamous cells separated from the connective tissue by a thick basal lamina. Beneath the capsule, a delicate
vascular connective tissue layer binds the capsule to the renal parenchyma (Mitchell, 1950; Bulger, 1973).

Single squamous mesothelial cells border the peritoneal space in the rat. In addition, slender microvilli extend from the apical border of the mesothelial cells (Cotran and Karnovsky, 1968).

The predominant component of both the testicular and renal capsule is a thick layer of dense, regular connective tissue entwined with occasional elastic fibers. The elastic fibers in the renal capsule are most prevalent in the innermost and outermost collagenous layers, while the elastic fibers within the testicular capsule achieve uniform distribution. The cells in the dense connective tissue of the rat renal capsule are generally fibrocytes (Bulger, 1973), however, in the human renal capsule, smooth muscle cells have been identified (Mollendorff, 1930). In contrast to the sparse distribution of smooth muscle cells in the human renal capsule, a greater distribution of smooth muscle have been observed in the human testicular capsule (Langford and Heller, 1973). In addition, mature smooth muscle cells have also been identified in the testicular capsule of the rabbit (Davis et al., 1970), dog (Leeson and Cookson, 1974) and rat (Davis et al., 1970; Leeson and Cookson, 1974).

Several layers of squamous cells form a continuous layer separating the fibrous aspect of the renal capsule from the loose connective tissue surrounding the parenchymal tissue. The squamous cells comprise thin disk-like plates and differ markedly from the smooth muscle cells. Both sides of the cell layer are uniquely covered by an extremely thick basal lamina. In addition, the cells frequently surround a space or lumen of irregular contour, which may function as a conduction path for lymphatic fluid (Bulger, 1973).
R. VASCULAR SUPPLY OF THE RENAL CAPSULE

Renal angiography has provided a detailed assessment of the anatomical arrangement of the vascular supply in the kidney (Itzchak et al., 1974).

1. Arterial supply

The renal capsular arterial complex has been described to consist of three basic channels: superior, middle and inferior capsular arteries. The arteries are demonstrated on selective angiography, in only one third of normal cases but are frequently seen in pathological conditions. A direct correlation between the visualization and diameter of renal capsular arteries in the presence of renal pathology have been made. Capsular arteries are slightly dilated in space occupying lesions of the renal capsule, inflammatory lesions involving the adipose capsule, cases of subcortical renal cysts and organizing or infected perirenal hematomas. In contrast, a decrease in the diameter of the capsular arteries may be associated with acute renal or subcapsular hemorrhage.

The superior capsular artery usually originates from the inferior adrenal artery or the inferior phrenic artery and follows a characteristic tortuous path, adjacent to the renal border in the adipose capsule.

Two different middle capsular arteries have been identified: the middle recurrent and the middle perforating capsular arteries. The middle recurrent capsular artery has been seen in 40 percent of selective normal renal arteriograms and originates from the dorsal branch of the renal artery. The middle perforating artery arises from one of the interlobular arteries and penetrates the capsula proper. This artery is rarely demonstrated in normal selective angiograms, but is
frequently seen in pathological processes involving the cortical area of the kidney and the renal capsule.

The smaller inferior capsular artery is poorly developed and usually originates from either the gonadal or renal artery. It may anastomose with the superior capsular artery and penetrate the adipose capsule.

2. **Venous supply**

Venous drainage of the renal capsule is dominated by an arcade-shaped vein which communicates with veins of the adrenal, ureter, colon, hemiazygos, or the right subcostal veins and retroperitoneal veins. Angiographic demonstration of renal capsular veins has been strongly associated with renal vein thrombosis and intrarenal venous occlusion.

S. **INNERVATION OF THE RENAL CAPSULE**

Adrenergic and cholinergic nerves have been demonstrated in the renal cortex of the primate by histochemical and electron microscopic techniques (Müller and Barajas, 1972). However, little is known about the innervation of the renal capsule.

Bulger (1973) identified unmyelinated nerves in the dense connective tissue of the renal capsule. While the evidence of adrenergic innervation associated with blood vessels in the renal cortex is well established (Nilsson, 1965; McKenna and Angelakos, 1963), specific histochemical fluorescence of adrenergic innervation of the renal capsule remains undemonstrated.
I. PATHOLOGY OF THE RENAL CAPSULE

Primary tumors of the capsule are rare. The benign tumors are fibromas, leiomyomas, lipomas and angiomas. The malignant tumors comprise the sarcomas derived from the fibrous and fatty elements, mixed tumors, chondromas and chondrosarcomas (Deming and Harvard, 1970). In addition, three types of space occupying lesions may be encountered: cysts, hematomas and inflammatory lesions (Itzchak et al., 1974).

Page (1939) reported the production of experimental hypertension by wrapping the kidneys of animals in cellophane. The resulting thick, dense, perirenal scar did not compromise the main renal vessels; but instead altered the intra-renal hemodynamics to produce ischemia and hypertension. Several cases of hypertension caused by constricting capsular renal lesions, subcapsular hematoma and capsular scar of the kidney, have been documented (Marshall and Castellino, 1971).
CHAPTER III

MATERIALS AND METHODS
CHAPTER III

MATERIALS AND METHODS

A. EXPERIMENTAL CONDITIONS

1. Animals

   a. Rat

      Male Sprague-Dawley rats aged 30, 45, 60, 90, 180, 440 and 640 days and weighing approximately 100, 200, 300, 380, 540, 705 and 655 g, respectively were used in these studies. The animals were purchased from the Holtzman Company of Madison, Wisconsin and fed Purina Rat Chow and supplied with drinking water ad libitum. Rats were housed in 18 x 11 x 8 inch wire cages in groups of 5 per cage, under controlled temperature (22° C) and illumination (12 h light 07:00-19:00 h) / 24 h).

   b. Rabbit

      Adult male New Zealand rabbits aged 18-24 weeks and weighing approximately 2.5-3.2 Kg, respectively, were obtained from Langshaw Farms, Augusta, Michigan and fed Wayne Rabbit Chow and supplied with drinking water ad libitum. Rabbits were housed in 24 x 18 x 14 inch wire cages, 1 per cage under controlled temperature and illumination as described previously.
2. **Isolated organ bath assembly**

Measurements of contractions of the isolated testicular and splenic capsule were performed using a 10 ml isolated-organ bath assembly. An adjustable circulating water bath was employed to provide the desired temperature through the outer jacket of a 125 ml condenser connected to the isolated tissue bath. The condenser provided a constant-temperature reservoir of the physiological salt solution used in the tissue bath. The temperature of the tissue bath was constantly monitored with a thermistor probe connected to a tele-thermometer (Yellow Springs Instrument Company, Inc.). Aeration of the physiological salt solution was carried out through a stainless steel 25 gauge needle. The aeration and thermistor probe needles both entered the side of the tissue bath through a rubber stopper located beneath the isolated tissue support rod.

Tissue contractions by the isolated capsule were detected by a linear motion transducer (Phipps and Bird, Inc., Model ST-2) and a microdisplacement myograph transducer (Narco Bio-Systems, Inc., Model F-20). The 10 cm lever arm of the linear motion transducer was set at a 3:1 ratio with the isolated testicular or splenic capsule attached to the end of the short arm with a 4-0 surgical silk thread leading from the tissue bath. Contractions of the rat and rabbit isolated testicular capsules and rabbit isolated splenic capsule employing a magnification of x 100, x 10 and x 25, respectively, were recorded on a desk model Physiograph (Narco Bio-Systems, Inc.) using a paper speed of 0.25 mm/sec. A tissue load weight of 100 mg added to the end of the long arm of the lever represented a satisfactory resting tension for the subsequent pharmacological studies on the isolated testicular and splenic capsules.
3. Physiological salt solution

The bathing medium used in pharmacological studies on the isolated testicular and splenic capsule was Tyrode's solution (1910). The physiological salt solution freshly prepared for each experiment was made up as follows:

\[
\begin{align*}
\text{NaCl} & \quad 136.9 \text{ mM (8.0 g/l)} \\
\text{D}(+) \text{ Glucose} & \quad 5.6 \text{ mM (1.0 g/l)} \\
\text{KCl} & \quad 2.7 \text{ mM (0.2 g/l; 4.8 ml 25\% solution KCl)} \\
\text{MgCl}_2 \cdot 6\text{H}_2\text{O} & \quad 1.1 \text{ mM (0.1 g/l; 2.4 ml 25\% solution \text{MgCl}_2 \cdot 6\text{H}_2\text{O})} \\
\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O} & \quad 0.4 \text{ mM (0.05 g/l; 3.0 ml 10\% solution \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O})} \\
\text{CaCl}_2 \cdot 2\text{H}_2\text{O} & \quad 1.8 \text{ mM (0.2 g/l; 4.8 ml 25\% solution \text{CaCl}_2 \cdot 2\text{H}_2\text{O})} \\
\text{NaHCO}_3 & \quad 12.0 \text{ mM (1.0 g/l)}
\end{align*}
\]

Aerated with air; pH 7.7

The reagents were prepared and added to approximately 5.0 liter of distilled water in the order indicated, dissolving each reagent thoroughly before adding the next reagent. The solution was then q.s. to 6 liter. Reagents that were not readily soluble in distilled water were made up in concentrated stock solutions and added as indicated in the brackets.
4. Pharmacological agents

   a. Determination of the response of the isolated testicular capsule to autonomic drugs

1. Parasympathomimetic drugs
   a. Acetylcholine chloride (Sigma Chemical Company)

2. Sympathetic stimulating drugs
   a. L-Norepinephrine bitartrate (Sigma Chemical Company)

3. Prostaglandins
   a. Prostaglandin A$_2$ (UpJohn Company)
   b. Prostaglandin E$_1$ (Sigma Chemical Company)
   c. Prostaglandin F$_2$α tromethamine salt (UpJohn Company)

4. Spasmolytic agents
   a. Papaverine hydrochloride (Sigma Chemical Company)
   b. Sodium nitroprusside (Sigma Chemical Company)
   c. Verapamil (Knoll Pharmaceutical Company)

   b. Determination of the response of the isolated splenic capsule to autonomic drugs

1. Parasympathomimetic drugs
   a. Acetylcholine chloride (Sigma Chemical Company)

2. Sympathetic stimulating drugs
   a. L-Norepinephrine bitartrate (Sigma Chemical Company)
Each drug was dissolved in either Tyrode's solution or distilled water. Stock solutions of prostaglandins (PG) $A_2$, $PGE_1$ and $PGF_2\alpha$ were made up in 95% ethanol and dissolved in a 0.2 M-sodium phosphate buffer, pH 6.4. The sodium phosphate buffer was prepared in the following manner: 11.0 g of $NaH_2PO_4\cdot H_2O$ and 8.45 g of $Na_2HPO_4 \cdot 7H_2O$ were added to 800 ml of distilled water and q.s. to exactly 1 liter total volume with distilled water. A dose of 0.1 $\mu g/ml$ ($5.5 \times 10^{-7}$ M) of acetylcholine and 1.0 $\mu g/ml$ ($3.0 \times 10^{-6}$ M) of norepinephrine was added before any dose response studies to establish homogeneity of the population. The addition of aliquots of 200 $\mu l$ or less of each compound was added to the 10 ml organ bath by means of a precision micropipette.

B. PROCEDURE FOR THE ISOLATION OF THE TESTICULAR AND SPLENIC CAPSULE FOR IN VITRO PHARMACOLOGICAL STUDIES

1. Rat and rabbit isolated testicular capsule preparation

Figure 1 presents a schematic diagram of the procedure for the isolation of the testicular capsule of the rat or rabbit. Following sacrifice of the animal by decapitation, a testis is exposed through a mid-line abdominal incision by application of gentle pressure to the scrotum. The testis is then removed, placed in Tyrode's solution and the epididymis and adhering connective tissue removed. A small piece of the inferior end of the testis is cut away as shown in Figure 1a. The seminiferous tubular mass protruding through the resulting opening is then grasped with a forceps (Figure 1b). The lower rim of the testicular capsule is then grasped with a second forceps (Figure 1c) and the seminiferous tubular mass is removed from the inferior of the testicular capsule. In the process of removing
the seminiferous tubular mass from the testicular capsule, the capsule is turned inside-out (Figure 1d). Diagram of the testicular capsule in the inside-out position with only a few seminiferous tubules still attached to its inside surface is presented in Figure 1e. The testicular capsule in its inside-out position is then separated from the seminiferous tubular mass and the testicular artery (Figure 1f). The few remaining seminiferous tubules that are still attached to the inside surface of the testicular capsule are cut away (Figure 1g). The superior end of the testicular capsule is then tied with a long piece of 4-0 surgical silk thread (Figure 1h). The inferior end of the testicular capsule is then tied with a short piece of thread, leaving approximately 25 per cent of the original small hole in the capsule open (Figure 1i). The intact testicular capsule is then mounted in a 10 ml isolated tissue bath with the short inferior thread attached to a support rod and the long superior thread leading to a transducer for detection of tissue contractions, (Figure 1j). The physiological salt solution in the organ bath was Tyrode's solution and gassed with air. Drugs were added to the 10-ml tissue bath in a volume of less than 200 μl by means of a precision micropipette.

2. **Rabbit isolated splenic capsule strip preparation**

Figure 2 presents a schematic diagram of the procedure for the isolation of the splenic capsule of the rabbit. Following sacrifice of the animal by decapitation, the spleen is exposed through a mid-line abdominal incision. The entire spleen is then removed, placed in Tyrode's solution and adhering connective tissue removed. The spleen was fashioned into 5 by 20 mm strips by cutting the ends of the organ with a pair of scissors as shown in Figure 2a. The spleen was placed on a Stadie-Riggs microtome base (Figure 2b) and successive microtome
slices of the splenic pulp were made (Figure 2c). The last remaining slice represented the splenic capsule. Adhering pulp was removed with a curved forceps (Figure 2d). The superior end of the splenic capsule strip is then tied with a long piece of 4-0 surgical silk thread and two short ties at each inferior end of the capsule strip (Figure 2e). The isolated strip of the splenic capsule is then mounted in a 10 ml isolated tissue bath with the short inferior threads attached to a support rod and the long superior thread leading to a transducer for detection of tissue contractions (Figure 2f). The bathing medium was Tyrode's solution aerated with air and maintained at a constant temperature of 39.5°C.

C. PHYSIOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE

The testis occupies a scrotal position in many mammals which is several degrees lower than normal body temperature. A comparison of intraperitoneal and testicular temperatures of the adult rat and rabbit revealed a mean abdominal scrotal temperature difference of 4.3 and 3.1°C, respectively. A temperature of 32°C and 37°C were selected as the control incubation temperatures for the isolated testicular capsule of the adult rat and rabbit because of results indicating the mean intra-testicular temperature for these animals were 33.4°C and 36.9°C, respectively (Buyer and Davis, 1966).
1. Effect of hyperthermic and hypothermic temperature changes on the adult rabbit isolated testicular capsule

a. Procedure for the determination of optimal temperature

Measurements of adult rabbit isolated testicular capsular contractions in response to progressive hyperthermic and hypothermic temperatures were performed using a 10 minute time interval at each 2 degree centigrade change in temperature. The initial temperature of the isolated tissue bath was set at 37°C and an adjustable circulating bath was employed to provide the desired temperature in the outer jacket of the isolated organ bath. Following exposure of the isolated testicular capsule to hyperthermic or hypothermic temperature change, the temperature of the tissue bath was then returned to 37°C for 10 minutes. The temperature of the physiological salt solution within the tissue bath was monitored with a tele-thermometer. Different animals were used for hyperthermic and hypothermic experiments, respectively.

b. Procedure for rapid reversal of organ bath temperature

Measurements of testicular capsular contractions in response to hyperthermic and hypothermic temperature changes were performed using two circulating water baths connected to the outer jacket of the isolated organ bath assembly by a Y-tube. Clamps were arranged to permit only one circulating water bath at a time to fill the outer jacket. In this manner, extreme hyperthermic or hypothermic temperatures in the outer jacket could be replaced with a normal temperature in a period of less than one minute. The isolated testicular capsule was exposed for 5 minutes to 37°C, prior to exposure to 42°C, 40°C, 32°C or 16°C at...
5, 10, 15, 30, 60 and 120 minutes. Following exposure to either a hyperthermic or hypothermic temperature change, the tissue was re-exposed to the 37° C control for 10 minutes.

c. Procedure to study the effect of temperature on agonist-induced contractions of the isolated testicular capsule

The effect of 42°, 40°, 32° and 16° C in response to agonist-induced contractions of the adult rabbit isolated testicular capsule was investigated. An ED$_{75}$ concentration of acetylcholine, norepinephrine and prostaglandin F$_2$ were assayed at the maximum temperature response on spontaneous contractions of the isolated testicular capsule. The percent reversibility of the response of the isolated testicular capsule to the agonist during hyperthermic or hypothermic temperature change was determined.

2. Effect of hypoxic conditions on the adult rabbit isolated testicular capsule and isolated duodenum

a. Procedure for investigating the effect of hypoxia on spontaneous contractions of the isolated testicular capsule and isolated duodenum

The bathing medium is normally aerated by a constant bubbling of filtered air. A simple method of producing hypoxia was achieved by clamping off the air supply. The adult rabbit isolated testicular capsule was exposed to hypoxic conditions at 37° C for an exposure period of 1, 2, 5, 10, 15 and 30 minutes. Following exposure to hypoxia, the tissue was re-exposed to the aerated control
for 10 minutes. An adult rabbit duodenal segment measuring 2.0 cm in length was isolated and mounted in a 10 ml organ bath assembly (Domer, 1971) and the effect of hypoxia on isotonic spontaneous contractions were recorded for suitable comparison. Aliquots of the Tyrode's solution were obtained at each hypoxic exposure period and analyzed for pH, pO₂ and pCO₂ using a Corning Model 165 blood gas analyzer.

b. Procedure to study the effect of hypoxia on smooth muscle relaxant agents of the isolated testicular capsule

The effect of hypoxia in response to smooth muscle relaxant agents on adult rabbit isolated testicular capsular spontaneous contractions was investigated. A concentration of 10^{-6} M of sodium nitroprusside, papaverine and verapamil were assayed at the maximum hypoxic response. Pure nitrogen was bubbled through the bathing medium to ensure proper mixing of the added agonist in the bathing medium. The percent reversibility of the response of the isolated testicular capsule to the agonist during hypoxic states was determined.

D. ANATOMICAL STUDIES ON CAPSULAR TISSUE

1. Tissue preparation

The animals were sacrificed by decapitation. The testes, spleen and kidney were immediately removed and placed in Bouin's solution for overnight fixation.

The testes were bisected longitudinally into two equal halves: one medial and one lateral. The medial and lateral aspects were identified by the rete testis
and the testicular artery, respectively. Superior, middle and inferior segments were subsequently isolated by equidistant transverse cuts. These tissue samples were designated as superior medial (SM), superior lateral (SL), middle medial (MM), middle lateral (ML), inferior medial (IM) and inferior lateral (IL). The tissues were transferred to fresh fixative for an additional 24 hours and processed and embedded using the following procedure:

1. Place specimens in 70% ethanol to remove the excess picric acid (4 one-half hour rinses)
2. 85% ethanol (30 minutes)
3. 95% ethanol (90 minutes)
4. Two solutions of 100% ethanol (30 minutes each)
5. 100% ethanol (60 minutes)
6. 100% ethanol: methyl salicylate (one to two dilution) (overnight)
7. Methyl salicylate (4 one-half hour rinses)
8. Transfer to three baths of paraffin (one hour each at 60°C)
9. Tissues embedded in plastic "boats"

2. Staining procedures

Masson's trichrome stain was used to demonstrate smooth muscle cells and supporting fibrous stroma in the capsules of the testis, spleen and kidney. This procedure results in black nuclei, red cytoplasm and muscle fibers and blue collagen tissue. The tissues were sectioned at 5 microns thickness and stained in the following manner:
1. Deparafinize slides with 2 solutions of xylene (5 minutes each)
2. Wash xylene off in two solutions of 100% ethanol (2 minutes each)
3. Rehydrate with 95% ethanol (2 minutes)
4. 70% ethanol (2 minutes)
5. Distilled water (2 minutes)
6. Weigert’s iron hematoxylin solution (10 minutes)
7. Rinse in running tap water (10 minutes)
8. Rinse in distilled water (1 minute)
9. Biebrich scarlet-acid fuchsin solution (2 minutes)
10. Rinse in two washes of distilled water (30 seconds each)
11. Phosphomolybdic-phosphotungstic acid solution (10 minutes)
12. Aniline blue solution (4 minutes)
13. Rinse in two washes of distilled water (30 seconds each)
14. Acetic acid solution (1%) (3 minutes)
15. Dehydrate with 70% ethanol (dipped once)
16. Two solutions of 95% ethanol (2 minutes each)
17. Two solutions of 100% ethanol (2 minutes each)
18. Clear in two solutions of xylene (5 minutes each)
19. Mount cover slip with permount
E. STATISTICAL ANALYSES

1. Calculation of mean, standard deviation and standard error of the mean

The calculation of the arithmetic mean ($\bar{X}$), the standard deviation (S.D.) and the standard error of the mean (S.E.M.) were computed on a Texas Instruments Model 58 programmable calculator. The statistical program provided by Texas Instruments uses the following formulas:

$$\bar{X} = \frac{\sum x}{n}$$

where $x$ = individual values

$n$ = total number of data points entered

$$S.D. = \frac{\sum x^2 - (\sum x)^2}{n}$$

$$n - 1$$

$$S.E.M. = \frac{S.D.}{\sqrt{n}}$$

2. Calculation of $t$ distribution and determination of the $P$ value, the degree of significance

When data in each group were considered as independent samples from a common population (in accordance with the Null hypothesis), the ratio of the difference between the two sample means to the standard error of the difference was computed as follows:
**Unpaired t-test**

\[
t = \frac{\bar{x} - \bar{y} - \Delta}{\left(\frac{1}{n_x} + \frac{1}{n_y}\right)^{\frac{1}{2}}\left(\frac{\sum x_i^2 - n_x \bar{x}^2 + \sum y_j^2 - n_y \bar{y}^2}{n_x + n_y - 2}\right)^{\frac{1}{2}}}
\]

In instances, where it was necessary to analyze data applicable to the same specimens or subjects the paired t-test was utilized and computed as follows:

**Paired t-test**

\[
t = \frac{\bar{\Delta} \sqrt{n}}{S_\Delta}
\]

where \(\Delta\) = the mean of the difference between the paired values

\(n\) = sample size

\(S_\Delta\) = standard deviation of the differences between the paired values using \(n-1\) degrees of freedom
The P value corresponding to the t-value calculated was found in a table of the significance limits of the Student t-test distribution, using the appropriate degrees of freedom. The P values obtained are expressed as being less than a degree of confidence (percent probability), read on the 2P (2-tailed) scale. A P value less than 0.05 (95% confidence limit) was used as the criterion for significant differences between groups.
CHAPTER IV

RESULTS
CHAPTER IV

RESULTS

A. EFFECT OF DEVELOPMENTAL AGE ON THE RESPONSE OF THE RAT
ISOLATED TESTICULAR CAPSULE TO NOREPINEPHRINE, ACETYLCOLINE AND PROSTAGLANDINS

Figure 3 presents a comparison of the effect of age of the animal on the
typical response of the rat isolated testicular capsule to equimolar concentrations
of norepinephrine, acetylcholine and prostaglandins. The degree of contraction of
the 90 day old rat isolated testicular capsule to NE and PGA₂ was markedly
greater than that of the 30 day old isolated testicular capsule. On the other hand,
the 30 day old isolated testicular capsule displayed a greater contraction of PGF₂α
while the responses of the isolated testicular capsule to ACh and PGE₁ at both 30
and 90 days of age, respectively, were similar in magnitude.

The response of the rat isolated testicular capsule to increasing concentra-
tions of NE is presented in Figure 4. The ED₅₀ values for NE producing a
contraction of the isolated testicular capsule obtained from rats at 30, 45, 60 and
90 days of age were essentially identical, averaging 0.21 µg/ml corresponding to a
final bath concentration of 6.2 x 10⁻⁷ M. However, the addition of an ED₇₅ of 1.0
µg/ml NE to the isolated testicular capsule at 30, 45, 60 and 90 days produced a
total contraction of 0.009, 0.024, 0.084 and 0.152 mm, respectively.
Figure 5 presents the response of the rat isolated testicular capsule to increasing concentrations of ACh. The \( ED_{50} \) values for ACh producing a contraction of the isolated testicular capsule obtained from rats at 30, 45, 60 and 90 days of age were essentially identical, averaging 0.07 \( \mu g/ml \) corresponding to a final bath concentration of \( 4.0 \times 10^{-7} \) M.

Figure 6 presents the response of the rat isolated testicular capsule to increasing concentrations of PGE\(_1\). The \( ED_{50} \) values for PGE\(_1\) producing a contraction of the 30 and 90 day old isolated testicular capsule were essentially identical, averaging 9.65 \( \mu g/ml \) corresponding to a final bath concentration of \( 2.75 \times 10^{-5} \) M.

The response of the rat isolated testicular capsule to increasing concentrations of PGA\(_2\) is indicated in Figure 7. The \( ED_{50} \) values for PGA\(_2\) producing a contraction of the 30 and 90 day old isolated testicular capsule were 1.65 \( \mu g/ml \) or \( 4.9 \times 10^{-6} \) M and 0.27 \( \mu g/ml \) or \( 8.1 \times 10^{-7} \) M, respectively.

Figure 8 presents the response of the rat isolated testicular capsule to increasing concentrations of PGF\(_{2\alpha}\). The \( ED_{50} \) for PGF\(_{2\alpha}\) producing a contraction of the 30 and 90 day old isolated testicular capsule were 0.016 \( \mu g/ml \) or \( 3.4 \times 10^{-8} \) M and 0.320 \( \mu g/ml \) or \( 6.8 \times 10^{-7} \) M, respectively.

Table 1 presents a statistical comparison of the effect of age of the rat on the response of the isolated testicular capsule to prostaglandins, NE and ACh. There was a significant difference in the mm of tissue contraction at 30 and 90 days of age produced by equimolar concentrations of NE, PGA\(_2\) and PGF\(_{2\alpha}\). The equimolar addition of ACh and PGE\(_1\), however, revealed no statistical difference in mm of tissue contraction of the isolated testicular capsule at 30 versus 90 days of age.
As shown in Figure 9a an increase in age of the rat was found to be proportional to a concomitant increase in body weight, the weight of the whole testis and the weight of the isolated testicular capsule as well as the resting length of the isolated testicular capsule in vitro. The weight of the rat isolated testicular capsule at 30, 45, 60 and 90 days of age averaged 18, 30, 38 and 59 mg, respectively, while the in vitro testicular capsule resting length increased 150 percent in the age range studied. The degree of actual tissue contraction of the isolated testicular capsule to the addition of a final bath concentration of 0.1 µg/ml ACh and 1.0 µg/ml NE has been compared in terms of percent shortening of the resting length mounted in vitro (Figure 9b). Percent shortening of the isolated testicular capsule was defined as the mm of actual tissue contraction divided by the resting length of the mounted testicular capsule. At 30 days of age, the response of the isolated testicular capsule to ACh was 10.3 times greater than the corresponding response to NE. However at 90 days of age, the response of the isolated testicular capsule to both ACh and NE was essentially similar. These data indicate that a marked increase in the contraction of the rat isolated testicular capsule to NE occurred from the onset of puberty to adulthood.

Table 2 presents the morphologic changes of the germinal epithelium observed in experimentally induced cryptorchid rats, aged 20 to 90 days. (Davis and Firlit, 1966). Spermatogonial cells first arise in the seminiferous epithelium of the testes about 4 days after birth. Four divisions are necessary for the completion of the spermatogenic cycle, from the production of Type A spermatogonia to Type B spermatogonia, spermatocytes, spermatids and finally mature spermatozoa occurring 50-90 days later. At 20 days of age, the immature rat testis is normally located in an abdominal position and consists of many small
developing seminiferous tubules containing several layers of spermatogonia and transitional to early pachytene primary spermatocytes. However by 30 days, the testis has completely descended into the scrotal sac and approximately half of the tubules contain spermatids of the cap and acrosome phases of spermatogenesis. The remaining tubules contain only spermatogonia and resting or pachytene primary spermatocytes. The onset of spermatid maturation begins at 50 days of age and is considered complete by 90 days. In addition, fertility is at maximum when rats are from 100 to 300 days old.

B. EFFECT OF GERIATRIC AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS

The effect of age on the response of the rat isolated testicular capsule to increasing concentrations of NE, ACh and PGs is indicated in Figure 10. The ED\textsubscript{50} values for NE producing a contraction of the isolated testicular capsule at 30, 90 and 180 days of age were essentially identical, averaging 0.22 \( \mu \text{g/ml} \), corresponding to a final bath concentration of \( 6.5 \times 10^{-7} \text{M} \). However a progressive decrease in the ED\textsubscript{50} values of NE, corresponding to 0.14 \( \mu \text{g/ml} \) and 0.10 \( \mu \text{g/ml} \) at 440 and 640 days, respectively were observed. On the other hand, the ED\textsubscript{50} values for the effect of NE on the isolated testicular capsule of 640 day old (geriatric) rats averaged 0.10 \( \mu \text{g/ml} \), while that of 90 day old (adult) rats averaged 0.21 \( \mu \text{g/ml} \). The ED\textsubscript{50} values for ACh producing a contraction of the isolated testicular capsule obtained from 30, 90, 180 and 440 day old rats were essentially identical, averaging 0.06 \( \mu \text{g/ml} \), corresponding to a final bath concentration of \( 3.4 \times 10^{-7} \text{M} \). The concentration of PGA\textsubscript{2} capable of eliciting a 50
percent maximal response of the testicular capsule was observed to be greater in rats aged 90-640 days than a 30 day old rat. However, PGF$_2\alpha$ was found to be more potent before the onset of puberty (30 day age group) than in older aged rats.

Figure 11 presents the response of the isolated testicular capsule of rats at different ages to equimolar concentrations ($3.0 \times 10^{-6}$ M) of NE, ACh, PGA$_2$ and PGF$_2\alpha$. For rats aged 30 and 640 days, equimolar concentrations of NE or PGA$_2$ induced a significant difference ($p<0.001$) in mm of tissue contraction. The equimolar addition of PGF$_2\alpha$ revealed a statistically significant increase ($p<0.05$) in mm of tissue contraction at 30 days when compared to 640 days of age, while ACh had no significant effect.

Figure 12 presents the response of the isolated testicular capsule of rats at different ages to equimolar concentration ($3.0 \times 10^{-6}$ M) of NE, ACh, PGA$_2$ and PGF$_2\alpha$ in terms of percent shortening. At 640 days, the response of the capsule to NE and PGA$_2$ was 36 and 4.5 times, respectively, as great as that observed at 30 days of age. On the other hand, the response of PGF$_2\alpha$ was 2.8 times greater at 30 days than 640 days of age. In contrast, there was no significant difference in terms of percent shortening of the isolated testicular capsule to equimolar concentrations of ACh at 30 to 640 days of age.

Table 3 demonstrates that an increase in age of the rat from 30 to 640 days was found to be proportional to a concomitant increase in body weight, the weight of the whole testis, the weight of the isolated testicular capsule and the resting length of the testicular capsule in vitro. There was also a uniform increase in thickness of the testicular capsule in rats from the ages of 30 to 440 days. In addition, smooth muscle cells were found to be more prominent along the medial
aspect of the capsule as the capsule becomes continuous with the mediastinum. The accumulation of smooth muscle fibers and vessels of the rete testis may account for the increased thickness of the medial aspect of the capsule throughout the age range studied.

C. EFFECT OF PROGRESSIVE HYPERTHERMIC AND HYPOTHERMIC TEMPERATURE CHANGES ON ADULT RABBIT ISOLATED TESTICULAR CAPSULAR SPONTANEOUS CONTRACTIONS

Figure 13 presents the effect of temperature on spontaneous contractions of the adult rabbit isolated testicular capsule. A progressive increase in temperature from 37 to 44°C resulted in a 73.4 percent increase in frequency, a 9.0 mm increase in tone and a 79.1 percent decrease in amplitude. Further increases in temperature to 48°C resulted in cessation of spontaneous contractions of the testicular capsule. On the other hand, decreasing the temperature from 37 to 34°C produced a 28 percent increase in amplitude with a statistically insignificant change in frequency and tone. A further decrease to 26°C caused a 48.6 and a 35.5 percent decrease from control values at 37°C in both frequency and amplitude, respectively, with a corresponding 3.5 mm decrease in tone. A complete cessation of spontaneous contractions of the testicular capsule occurred at 16°C accompanied by a 12.7 mm decrease in tone.

The typical response of the isolated testicular capsule to hyperthermic and hypothermic temperature changes is shown in Figure 14. Hyperthermia resulted in a progressive increase in frequency and tonus along with a decrease in the amplitude. Hypothermia produced a progressive decrease in the frequency and tonus along with a decrease in amplitude of spontaneous testicular contractions.
The hypothermic inhibition of testicular capsular contractions at $16^\circ C$ could be restored to normal by rapid warming within 20 minutes. However, hyperthermic inhibition at $48^\circ C$ could not be restored to normal by rapid cooling within 2 hours (Figure 15).

D. EFFECT OF VARIOUS EXPOSURE TIMES TO HYPERTHERMIA AND HYPOTHERMIA ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

The effects of $42^\circ C$ on spontaneous isotonic contractions of the adult rabbit testicular capsule are presented in Figure 16. Exposure at $42^\circ C$ revealed a statistically significant decrease in the frequency, amplitude and tissue tone for each of the indicated time periods. However, a recovery from a 30 minute exposure to $42^\circ C$, as reflected in the post-hyperthermic frequency value, was found to be statistically different ($p<0.05$) when compared with $37^\circ C$ control values. In addition, control values at $37^\circ C$ could not be re-established when the tissue was exposed to $42^\circ C$ for periods greater than 30 minutes.

Figure 17 presents the effect of $40^\circ C$ on spontaneous contractions of the adult rabbit testicular capsule. In comparison to the observations made at $42^\circ C$, there was a less marked decrease in frequency. However, a progressive increase in exposure time of the tissue to $40^\circ C$ compared to $37^\circ C$ control values resulted in a $60.4$, $71.9$, $66.5$, $73.0$, $63.7$ and $53.7$ percent decrease in amplitude at a $5$, $10$, $15$, $30$, $60$ and $120$ minute exposure time, respectively. In addition, a $3.47$ mm average decrease in tone was observed at all the exposure times studied.
Following exposure to $40^\circ C$ for 120 minutes and return of the tissue to $37^\circ C$, a complete recovery of the control frequency, amplitude and tone was observed after an exposure to $40^\circ C$ for 120 minutes and following return of the tissue to $37^\circ C$.

The response of the isolated testicular capsule to $32^\circ C$ is presented in Figure 18. Spontaneous contractions ceased for the entire 5 minute exposure period to $32^\circ C$, accompanied by a 8.33 mm decrease in tone. Further increases in exposure times to $32^\circ C$ resulted in a progressive increase in frequency and amplitude. Exposure to $32^\circ C$ for 120 minutes produced a 71.4 percent increase in amplitude from control values at $37^\circ C$. A marked decrease in tone was observed at 5, 10, and 15 minutes, respectively. However exposure to 60 and 120 minutes resulted in a slight increase in tone.

Figure 19 demonstrates that a complete cessation of spontaneous contractions of the testicular capsule occurred at $16^\circ C$, accompanied by a 12.9 mm average decrease in tone for all exposure times studied. The hypothermic inhibition of testicular capsular contractions observed at $16^\circ C$ for each of the exposure times could be restored to control values at $37^\circ C$ within 10 minutes.

The typical response of the isolated testicular capsule to hyperthermic and hypothermic exposures for 5 minutes is shown in Figure 20. An immediate decrease in frequency, amplitude and tone was observed at each of the temperatures studied. The inhibition of testicular capsular contractions at the indicated temperatures could be restored to control values within 10 minutes upon exposure to a normal scrotal temperature of $37^\circ C$. 
Figure 21 presents the typical response of the isolated testicular capsule to hyperthermic and hypothermic exposures for 30 minutes. Hyperthermia resulted in a progressive decrease in frequency, amplitude and tone, along with the inability to restore spontaneous contractions at 42°C by rapid cooling. Exposure to 32°C resulted in large irregular contractions within 10 minutes of the 30 minute exposure time period. In contrast to 32°C, complete cessation of spontaneous contractions occurred at 16°C for the entire exposure time period. The progressive decrease in frequency, amplitude and tone when the tissue was exposed to hypothermic temperatures resulted in complete restoration by rapid warming to 37°C.

E. RESPONSE OF THE RABBIT ISOLATED TESTICULAR CAPSULE AT HYPO- THERMIC AND HYPERTHERMIC TEMPERATURES TO NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F₂α

Figure 22 presents the typical response of the adult rabbit isolated testicular capsule to an ED₇₅ concentration of NE, ACh and PGF₂α at a normal intra-testicular temperature of 37°C. Each of these neurohumoral agents increased the tone of the testicular capsule with NE causing a prolonged contracture throughout the entire drug contact period.

Figure 23 presents the effect of NE, ACh and PGF₂α on the response of the adult rabbit isolated testicular capsule exposed to a hypothermic temperature of 32°C. Spontaneous contractions were initially abolished during the first 5 minutes of exposure to 32°C along with a small decrease in tone. Upon further exposure to 32°C, sporadic spontaneous contractions with a greatly reduced frequency appeared. NE and ACh when added during this exposure of the
testicular capsule to $32^\circ$C were found to increase both the phasic and tonic components of capsular contractions. However, in marked contrast, PGF$_2\alpha$ had no effect in re-initiating normal spontaneous contractions of the isolated testicular capsule exposed to $32^\circ$C.

Figure 24 presents the typical effect of NE, ACh and PGF$_2\alpha$ on the response of the adult rabbit isolated testicular capsule exposed to a hyperthermic temperature of $40^\circ$C. A progressive increase in exposure time to $40^\circ$C compared to $37^\circ$C control values resulted in a 62.5 and 42.5 percent decrease in frequency and amplitude, respectively, of testicular capsular contractions. The addition of NE to the testicular capsule at $40^\circ$C caused a large increase in capsular tone while ACh and PGF$_2\alpha$ produced only a small and temporary increase in capsular tone. In contrast, the phasic component of testicular capsular smooth muscle could not be re-initiated by NE, ACh or PGF$_2\alpha$ at $40^\circ$C. Complete restoration of normal testicular capsular spontaneous contractions occurred upon return of the tissue to a drug-free medium at $37^\circ$C.

Figure 25 indicates the typical effect of NE, ACh and PGF$_2\alpha$ on the response of the adult rabbit isolated testicular capsule exposed to a higher hyperthermic temperature of $42^\circ$C. Cessation of spontaneous contractions of the testicular capsule occurred at $42^\circ$C, accompanied by a small decrease in tone. Only NE was found to re-establish tissue tone at $42^\circ$C as compared to $37^\circ$C pretreatment control values. Following hyperthermic exposure and return of the tissue to $37^\circ$C, a complete recovery of the control frequency, amplitude and tone of testicular capsular contractions was observed.
Figure 26 presents the comparative changes of the tone of the adult rabbit isolated testicular capsule to an ED$_{75}$ concentration of NE, ACh and PGF$_2\alpha$ at 32, 40 and 42° C with regard to drug-induced maximal tissue contractions observed at 37° C. At 32° C, NE and ACh produced an 81.6 and 55.1 percent greater increase in maximal tissue contraction (tone) compared to 37° C control values, while PGF$_2\alpha$ caused a 36 percent smaller contraction. In contrast, at 40° C both ACh and PGF$_2\alpha$ resulted in a significantly greater decrease (p<0.05) in tissue contraction than did NE when compared to 37° C control effects. However, at 42° C, NE, ACh and PGF$_2\alpha$ caused a smaller contraction of the isolated testicular capsule as compared to 37° C drug-induced control values.

**F. EFFECT OF HYPOXIA ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE AND DUODENUM**

The effects of varying periods of hypoxia on spontaneous contractions of the adult rabbit isolated testicular capsule are presented in Figure 27. Exposure to hypoxia for 5 minutes, resulted in a temporary, but significant increase (p<0.01) in frequency, followed by a 60.9, 93.1 and 80.5 percent decrease at a 10, 15 and 30 minute exposure time, respectively, compared to aerated control values. In contrast, a progressive decrease in amplitude and an increase in tissue tone were observed with a corresponding increase in exposure time to hypoxia.

The typical response of the adult rabbit isolated testicular capsule to various exposure periods of hypoxia is shown in Figure 28. Increasing exposure times to hypoxia produced a progressive increase in testicular capsular tone along with a decrease in frequency and amplitude of spontaneous contractions. Prolonged tonic contracture and a maximal decrease in frequency and amplitude
resulting from 15 minutes exposure to hypoxia, corresponded to a maximal
decrease of 14.6 mm Hg oxygen in the organ bath. In contrast to the rapid
restoration of myogenic tone, the phasic component (frequency and amplitude)
returned to control values within 10 minutes upon exposure to aeration.

Figure 29 presents the effects of hypoxia on spontaneous contractions of
the adult rabbit isolated duodenum. In contrast to the isolated testicular capsule,
hypoxia resulted in a much less marked decrease in frequency and amplitude.
Cessation of aeration was accompanied by a relaxation of the duodenum, in
marked contrast to the prolonged contracture observed with the isolated
testicular capsule. A progressive increase in exposure time of the duodenum to
hypoxia compared to aerated control values resulted in a $2.6 \pm 3.2$, $13.9 \pm 3.2$, $21.9$
$\pm 2.76$ and $25.6 \pm 0.96$ mm decrease in tissue tone at a 5, 10, 15 and 30 minute
exposure time, respectively.

The typical response of the adult rabbit isolated duodenum to various
exposure periods of hypoxia is presented in Figure 30. The gradual decrease in
tissue tone, accompanied by large irregular spontaneous contractions and
subsequent atonia, could be restored to control values within 10 minute exposure
to aeration.

G. EFFECT OF HYPOXIA ON THE RESPONSE OF THE ADULT RABBIT
ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL

Figure 31 presents the typical effect of equimolar ($10^{-6}$ M) additions of
sodium nitroprusside, papaverine and verapamil on the response of the adult rabbit
isolated testicular capsule exposed to aeration. An immediate decrease in
frequency and amplitude of capsular spontaneous contractions was observed with the addition of each drug. The sodium nitroprusside-induced inhibition of testicular capsular contractions was rapidly restored to control values by the removal of the drug from the organ bath medium, whereas the recovery of spontaneous capsular contractions after the addition of papaverine was slower. In contrast, recovery of testicular capsular spontaneous contractions after the addition of verapamil did not occur up to a 2 hour drug contact time studied. However, the inhibitory effect of verapamil on testicular capsular contractions could be reversed by the addition of 5 mM CaCl₂.

Figure 32 presents a summary of the contractile response of the adult rabbit isolated testicular caoosule to the equimolar (10⁻⁶ M) addition of sodium nitroprusside, papaverine and verapamil during exposure to aeration. Sodium nitroprusside and papaverine produced a statistically greater decrease (p<0.001) in the phasic activity (frequency and amplitude) of the isolated testicular capsule than did verapamil. In addition, only sodium nitroprusside and papaverine caused a decrease in capsular tone during exposure to aeration.

Figure 33 presents the typical response of equimolar (10⁻⁶ M) additions of sodium nitroprusside, papaverine and verapamil on the response of the adult rabbit isolated testicular capsule exposed to hypoxia. Exposure of the testicular capsule to hypoxia for 10 minutes produced a progressive decrease in frequency and amplitude of spontaneous contractions, along with a corresponding marked increase in tissue tone. Further increases in hypoxic exposure resulted in cessation of spontaneous contractions. The hypoxic-induced inhibition of testicular capsular contractions could be restored to control values within 10 minutes after
exposure to aeration. Sodium nitroprusside, papaverine and verapamil each was found to relax the hypoxic-induced contracture of the testicular capsule and to restore capsular tone toward a control, aerated level.

Figure 34 presents a summary of the contractile response of the adult rabbit isolated testicular capsule to sodium nitroprusside, papaverine and verapamil during exposure to hypoxia. Exposure of the testicular capsule to hypoxia caused a significant decrease \( p<0.001 \) in the frequency and amplitude of capsular spontaneous contractions compared to aerated control values. In addition, a large increase in capsular tone averaging 17.9 ± 2.6 mm was observed upon exposure of the testicular capsule to hypoxia. The addition of sodium nitroprusside, papaverine or verapamil to the testicular capsule at its maximum hypoxic increase in tone did not significantly alter the frequency or amplitude of capsular contractions. However in marked contrast, the hypoxic-induced increase of testicular capsular tone was found to be restored to within aerated control values by an equimolar \( (10^{-6} \text{ M}) \) addition of sodium nitroprusside, papaverine or verapamil.

H. COMPARATIVE RESPONSE OF THE ADULT RAT AND RABBIT ISOLATED TESTICULAR CAPSULES TO NEUROHUMORAL AGENTS

Figure 35 presents the response of the adult rat isolated testicular capsule to increasing concentrations of NE, ACh and PGF\(_2\alpha\) at 32°C. The \( \text{ED}_{50} \) values for NE producing a contraction of the adult rat testicular capsule was 6.2 x 10\(^{-7}\) M (0.21 \( \mu \text{g/ml} \)), whereas the \( \text{ED}_{50} \) values for ACh was 3.3 x 10\(^{-7}\) M (0.06 \( \mu \text{g/ml} \)). In addition, the concentration of PGF\(_2\alpha\) capable of eliciting a 50 percent maximal response of a 90 day old rat testicular capsule averaged
6.8 \times 10^{-7} \text{ M (0.32 } \mu \text{g/ml). Based on the ED}_{50} \text{ values, NE and PGF}_{2\alpha} \text{ were equi-potent. The maximal response of the adult rat isolated testicular capsule to each of the neurohumoral agents studied occurred at a dose of 5.0 } \mu \text{g/ml final concentration; this corresponded to a } 1.5 \times 10^{-5} \text{ M for NE, } 2.7 \times 10^{-5} \text{ M for ACh and } 1.0 \times 10^{-5} \text{ M for PGF}_{2\alpha}. \text{ In every case, maximal contraction of the testicular capsule was reached in approximately 3 minutes following addition of each drug.}

Figure 36 presents the response of the adult rabbit isolated testicular capsule to increasing concentrations of NE, ACh and PGF}_{2\alpha} \text{ at } 37^\circ \text{C. In marked contrast to the rat, the isolated testicular capsule of the adult rabbit was observed to undergo marked spontaneous contractions within 1 hour following mounting in the organ bath and before the addition of any drug. The frequency of these spontaneous contractions of the rabbit isolated testicular capsule ranged from 1.5 to 2.5 per minute. In terms of percentage shortening (mm tissue contraction divided by the resting length of the testicular capsule times 100) of the isolated testicular capsule, the response of the capsule before the addition of any drug was approximately 5.0 percent.}

The ED}_{50} \text{ value for NE producing a contraction of the adult rabbit isolated testicular capsule was } 2.4 \times 10^{-6} \text{ M (0.80 } \mu \text{g/ml), whereas the ED}_{50} \text{ value for ACh was } 4.4 \times 10^{-6} \text{ M (0.80 } \mu \text{g/ml). In addition, the concentration of PGF}_{2\alpha} \text{ capable of eliciting a 50 percent maximal response of the rabbit testicular capsule averaged } 1.8 \times 10^{-6} \text{ M (0.85 } \mu \text{g/ml). Norepinephrine at a final bath concentration of } 3.0 \times 10^{-5} \text{ M (10 } \mu \text{g/ml) was observed to produce a significantly greater maximal response of the isolated testicular capsule than that resulting from a similar dose of ACh or PGF}_{2\alpha}.\)
A comparison of histological sections of the testicular capsule obtained from an adult rat and rabbit provided a reasonable anatomical explanation for both the spontaneous as well as drug-induced contractions of the isolated testicular capsule. Figure 37 presents representative cross sections of the testicular capsule of the adult rat and rabbit. Smooth muscle nuclei and fibers were sparsely located within the dense collagenous tissue of the tunica albuginea of the rat testicular capsule. It appeared that, with respect to the adult rat testis, smooth muscle cells were located predominantly along the posterior border of the testicular capsule. Numerous fibroblasts were also observed along the anterior and posterior borders of the rat testicular capsule.

In contrast to the small amount of smooth muscle present in the testicular capsule of the rat, two distinct layers of smooth muscle fibers were found in the tunica albuginea of the rabbit. A superficial layer of longitudinal smooth muscle which runs parallel to the long axis of the rabbit testis and a second deeper layer of circular smooth muscle which is orientated along the circumference of the rabbit testis at right angles to the superficial layer. In addition, the rabbit testicular capsule was also found to differ from the rat testicular capsule, as numerous thin septa extending from the tunica albuginea into the parenchymal tissue of the rabbit testis. A relationship appears to exist between the extent of the observed pharmacological responses and the amount of smooth muscle present in the testicular capsules of the two species studied.
I. COMPARATIVE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR AND SPLENIC CAPSULES TO NEUROHUMORAL AGENTS

The response of the adult rabbit isolated splenic capsule to increasing concentrations of NE, ACh and PGF$_2$α is indicated in Figure 38. The ED$_{50}$ value for NE producing a contraction of the isolated splenic capsule was $1.7 \times 10^{-6}$ M (0.58 µg/ml), whereas the ED$_{50}$ value for ACh was $1.8 \times 10^{-4}$ M (33.5 µg/ml). In contrast, PGF$_2$α was not found to produce a contraction of the isolated splenic capsule. The maximal response observed for NE and ACh occurred at a dose of $1.5 \times 10^{-5}$ M (5.0 µg/ml) and $1.1 \times 10^{-3}$ M (200.0 µg/ml) final bath concentration, respectively. The maximal response of the splenic capsule of the adult rabbit to NE appeared to be approximately 3 times that observed with ACh.

Figure 39 presents the comparative response of the adult rabbit isolated testicular and splenic capsule to equimolar concentration ($3.0 \times 10^{-6}$ M) of NE, ACh and PGF$_2$α. The isolated testicular capsule was observed to undergo marked spontaneous contractions in the absence of any added drugs, whereas the isolated splenic capsule was found to undergo only a drug-induced contraction. An equimolar addition of NE in the organ bath resulted in a 9 and 7 percent shortening of the actual length of the mounted isolated testicular and splenic capsules, respectively. The rabbit isolated testicular capsule was found to resemble the isolated splenic capsule in that marked contractions were produced by the equimolar addition of NE. In contrast to the isolated testicular capsule, the rabbit isolated splenic capsule was demonstrated to be unresponsive to an equimolar addition of ACh and PGF$_2$α. These data indicate that the response of the isolated splenic capsule is predominantly adrenergic, whereas the response of the adult rabbit testicular capsule is more sensitive to NE, ACh and PGF$_2$α.
In an attempt to provide an anatomical basis for both the spontaneous and drug-induced contractions of the isolated testicular capsule and splenic capsule, histological sections of the adult rabbit testes and spleen were stained with Masson's trichrome stain and examined for smooth muscle. In contrast to the sparse distribution of smooth muscle which was found to be present in the rabbit splenic capsule, two distinct layers of smooth muscle fibers were identified in the tunica albuginea of the rabbit testicular capsule. A quantitative relationship therefore seems to exist between the extent of both spontaneous and drug-induced contractions and the amount of smooth muscle found to be present in the rabbit testicular and splenic capsules.

Finally, the finding that the isolated testicular capsule of the adult rabbit is capable of periodic contractions and relaxations in the absence of any drug may indicate that the testicular capsule is in a constant state of dynamic movement, constantly transporting the non-motile sperm from the seminiferous tubules and into the epididymis where the sperm then attain their motility. Neurohumoral stimulation of testicular capsular smooth muscle cells may regulate the frequency of spontaneous contractions. On the other hand, the splenic capsule may have an active role only in emergencies as in hypovolemic shock. Normally the spleen serves as a reservoir of erythrocytes. However in hypovolemic shock, adrenergic stimulation of the splenic capsular smooth muscle would result in a rapid extrusion of erythrocytes.
J. EFFECT OF HEAVY METALS ON THE RESPONSE OF THE ADULT RABBIT
ISOLATED TESTICULAR CAPSULE

1. Mercury

Figure 40 presents the typical effect of the adult rabbit isolated testicular capsule to increasing concentrations of mercuric acetate. The addition of $10^{-5}$ M Hg$^{++}$ produced no change in the frequency, amplitude or tone of testicular capsular spontaneous contractions. Further increases to $10^{-4}$ M Hg$^{++}$ resulted in a progressive decrease in phasic and tonic activity. However, the addition of $10^{-3}$ M Hg$^{++}$ resulted in cessation of spontaneous contractions of the testicular capsule accompanied by a 10 mm decrease in tone. The Hg$^{++}$-induced inhibition of testicular capsular contractions could not be restored to control values after washing the metal from the organ bath medium.

Table 4 presents a summary of the contractile response of the adult rabbit isolated testicular capsule to increasing concentrations of Hg$^{++}$. The addition of $10^{-5}$ M Hg$^{++}$ produced little change in the frequency and amplitude of capsular spontaneous contractions, as compared to pre-treatment control values. However at $10^{-4}$ M Hg$^{++}$, the amplitude and tone could not be re-established to control values after washing the metal from the organ bath medium. Subsequent exposure to $10^{-3}$ M Hg$^{++}$ resulted in an 18 and 43 percent decrease in frequency and amplitude, respectively. A complete cessation of spontaneous contractions of the testicular capsule, accompanied by a 10 mm decrease in tone occurred after washing the metal from the organ bath medium, which may indicate that the metal was irreversibly bound to the tissue (i.e. sulhydryl groups).
2. Cadmium

The typical response of the adult rabbit isolated testicular capsule to increasing concentrations of cadmium chloride is shown in Figure 41. Exposure of the testicular capsule to increasing concentrations of Cd\(^{++}\) produced a progressive decrease in frequency and amplitude of spontaneous contractions along with a corresponding decrease in tissue tone. Furthermore, the addition of $10^{-3}$ M Cd\(^{++}\) resulted in cessation of spontaneous contractions. The Cd\(^{++}\)-induced inhibition of testicular capsular contractions could not be restored to control values after washing the metal from the organ bath medium.

Table 5 presents a summary of the contractile response of the adult rabbit isolated testicular capsule to increasing concentrations of Cd\(^{++}\). These data indicate that the addition of $10^{-5}$ M Cd\(^{++}\) produced little change in the phasic and tonic activity of capsular spontaneous contractions, as compared to pre-treatment control values. The addition of $10^{-4}$ M Cd\(^{++}\) produced a 93 and 65 percent average decrease in frequency and amplitude, respectively, as compared to pre-treatment control values, along with a corresponding decrease in tissue tone. Rapid recovery of testicular capsular spontaneous contractions after the addition of $10^{-4}$ M Cd\(^{++}\) to control values was observed in a metal-free medium. In contrast, the Cd\(^{++}\)-induced cessation of capsular contractions at $10^{-3}$ M could not be restored to normal after washing the metal from the organ bath medium.

Figure 42 presents the typical response of Zn\(^{++}\) on Cd\(^{++}\)-induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions. The addition of $2 \times 10^{-4}$ M zinc acetate produced no change in the frequency, amplitude or tone of testicular capsular spontaneous contractions. In contrast, an immediate decrease in the phasic and tonic activity of capsular contractions were
demonstrated upon exposure to $10^{-4}$ M Cd$^{++}$. Furthermore, the cadmium-induced cessation of spontaneous contractions of the testicular capsule was prevented by pre-treatment with Zn$^{++}$.

Table 6 presents a summary of the prevention of Cd$^{++}$-induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions by Zn$^{++}$. Testicular capsular contractions were unaffected by the addition of $2 \times 10^{-4}$ M Zn$^{++}$. On the other hand, a maximal decrease in the phasic and tonic activity of capsular contractions were obtained with $10^{-4}$ M Cd$^{++}$. Pre-treatment of the testicular capsule with Zn$^{++}$ for 2 minutes prior to the addition of Cd$^{++}$ prevented the Cd$^{++}$-induced cessation of testicular capsular spontaneous contractions.
CHAPTER V

DISCUSSION
A. EFFECT OF DEVELOPMENTAL AGE ON THE PHARMACOLOGICAL RESPONSE OF THE RAT TESTICULAR CAPSULE

The present data demonstrates that the response of the rat isolated testicular capsule to neurohumoral agents changes with an increase in age of the animal. The rat testis at 20 days of age occupies a position in the abdomen and consists of many small developing seminiferous tubules containing several layers of spermatogonia and transitional to early pachytene primary spermatocytes, being prepubertal in nature. At day 30, the testis has completely descended into the scrotum with approximately half of the tubules containing spermatids of the cap and acrosome phases of spermiogenesis. The onset of spermatid maturation begins at 50 days of age and at 90 days of age spermatogenesis is complete with the testis considered to be adult in nature containing mature sperm (Davis and Firlit, 1966). The sudden increase in the contractile response of the isolated testicular capsule of the rat to NE and PGA₂ observed in the present studies appears to coincide with the onset of spermatids in the maturation phase of spermiogenesis at 50 days of age.

The marked age-dependent differences of drug-induced contractions of the isolated testicular capsule may be attributable to smooth muscle cells located within the tunica albuginea. A recent report describing the action of PGE₂ and PGF₂α on rat colon and stomach smooth muscle preparations in mature (350-
400 g) and immature (35-100 g) rats may indicate a change in the receptors during maturation (Okpako, 1976). The tissues from mature rats were found to exhibit marked differential sensitivities to PGE$_2$ and PGF$_2\alpha$; the colon being more sensitive to PGF$_2\alpha$ than PGE$_2$, while the responses of the stomach to PGE$_2$ were greater than PGF$_2\alpha$. Immature rat colon and stomach preparations revealed a decrease in sensitivity to PGE$_2$ and PGF$_2\alpha$, respectively. Changes in smooth muscle receptor population with respect to the age of the animal appear to be important parameters to consider. Our laboratory is currently investigating histochemical differences that may exist in the testicular capsule of the rat with respect to age.

The ED$_{50}$ values for NE producing a contraction of the rat isolated testicular capsule remained constant in animals 30 to 90 days of age. The increase in testicular capsular contractions following the addition of a standard dose of 1.0 µg/ml NE with age appears to involve an enhancement in the magnitude of the contractile response rather than a greater affinity for the receptor. In addition, the increased contractile response to NE in 90 day old adult rats does not appear to be correlated with the development of the amount of smooth muscle tissue mass since the response of ACh remained constant in animals 30 to 90 days of age. On the other hand, the ED$_{50}$ value for PGA$_2$ producing a contraction of the rat isolated testicular capsule was more potent at 90 days of age. This would indicate that the increased contraction of the testicular capsule with age following the addition of a standard dose of 1.0 µg/ml PGA$_2$ may involve a greater affinity for the receptor as the animal reaches adulthood.
The NE-induced contractions resulted from stimulation of classical adrenergic receptors. The response of the rat isolated testicular capsule to phenoxybenzamine and isoproterenol has demonstrated the presence of adrenergic α and β-receptors. In addition, catecholamine-induced fluorescence associated with nerve fibers have been observed in the rat testicular capsule (Bell and McLean, 1973). The response of NE is characterized by membrane depolarization, a reduction in size of the electronic potential and an acceleration of spontaneous spike discharge resulting in an increase in testicular capsular smooth muscle tension. The depolarization by catecholamines is thought to be due to an increase in chloride permeability and, in addition, sodium is required for the ensuing increase of spike discharge. The response of the rat isolated testicular capsule to ACh can be prevented by atropine. However, hexamethonium has no influence on the response to ACh. These results may imply that cholinergic receptors in the rat testicular capsule are muscarinic in nature. In addition, a sparse distribution of nerves exhibiting an acetylcholinesterase reaction was observed in the rat testicular capsule (Bell and McLean, 1972). The depolarization of the membrane by ACh is due to an increase in sodium and calcium permeability. There is also speculation that ACh acting on muscarinic receptors may cause a rapid and large increase in cGMP in smooth muscle. A protein kinase specifically activated by low concentrations of cGMP may regulate the tone and contractility of testicular capsular smooth muscle (Stephenson, 1976).

It has been observed that an agonist-induced contraction of the isolated testicular capsule of the 30 day old immature rat may 'fade' temporarily from a
rapidly attained peak. This abrupt relaxation at 30 days of age was observed with the addition of every agonist studied and increased with dosage. Receptor occlusion may be insufficient to produce 'fade' (Paton and Waud, 1964). 'Fade' may be an intrinsic property of the tissue profoundly affected or even prevented by altering the calcium concentration (Fastier et al., 1973). It is possible that intracellular calcium levels in the testicular capsule obtained from rats 30 days of age may play an important role in the excitation-contraction coupling mechanism which may limit the smooth muscle response to receptor stimulation and give rise to the 'fade' phenomenon.

The response of the rat isolated testicular capsule to increasing concentrations of PGA₂ and PGF₂α appears to differ with regard to increasing age of the animal. The present data indicate that PGF₂α is most effective at 30 days of age, while PGA₂ is most effective at 90 days of age. In contrast, the ED₅₀ values for PGE₁ producing a contraction of the 30 and 90 day old rat isolated testicular capsule were essentially identical. E-type prostaglandins have been reported to have a bimodal effect on the rabbit testicular capsular tone, with inhibition occurring at doses higher than 10⁻⁵ M (Seeley et al., 1974). Furthermore, exogenous cAMP potentiated the inhibition of rabbit testicular capsular tone to PGE₁. In marked contrast to the rabbit, the response of the rat isolated testicular capsule to PGE₁ were stimulatory throughout the concentration range tested. The present studies may also indicate that the contractile response of the rat isolated testicular capsule to prostaglandins is not mediated by cAMP, as cAMP has been demonstrated to cause relaxation of the rabbit testicular capsule and other
smooth muscle preparations (Triner et al., 1971; Seeley et al., 1974). The age-dependent response of the rat isolated testicular capsule to prostaglandins $A_2$, $E_1$ and $F_2\alpha$ may be related to the development of prostaglandin receptors. Specific compounds capable of blocking the contractile response of prostaglandins are needed to evaluate testicular capsular smooth muscle function. The results of this investigation indicate that in addition to norepinephrine, prostaglandins may be important modulators of testicular capsular contractions with respect to age.

The present data demonstrate that the increase in the contraction of the rat isolated testicular capsule to NE and PGA$_2$, observed from the onset of puberty to adulthood, may play an important role in the transport of non-motile sperm out of the testis into the epididymis, where the sperm may thus attain their motility. The possibility exists that endocrine changes taking place at the onset of puberty may be responsible for the presently observed increase in the contractile response of the rat testicular capsule to NE and prostaglandins.
B. EFFECT OF GERIATRIC AGE ON THE PHARMACOLOGICAL RESPONSE OF THE RAT TESTICULAR CAPSULE

The present investigation demonstrates that the response of the rat isolated testicular capsule to autonomic drugs is dependent on age. The different responses induced by the drugs studied in rats 30 to 640 days of age may be related to changes in smooth muscle receptors occurring during maturation. Further, the responses do not appear to be correlated with the development of an increased mass of contractile elements during prepubertal to early adulthood, or smooth muscle hyperplastic changes associated with advanced age, since the response of the testicular capsule to ACh remained constant throughout the age period studied.

The increased contractile response to NE and PGA₂ in 90 to 640 day old rats may be related to changes in the receptors during the process of ageing. These changes may include conformational changes in the isosteric region of the receptor leading to a greater drug-receptor interaction or an increased NE and PGA₂ receptor population with an increase in age of testicular capsular smooth muscle.

The present data also indicates that the increased contractile response of the isolated testicular capsule to PGF₂α at 30 days of age compared to older aged rats suggests that PGF₂α may be important in the regulation of testicular capsular motility throughout postnatal development and before the onset of spermatid maturation reported to occur at 50 days of age (Davis and Firlit, 1966). The decreased sensitivity of PGF₂α with an increase in age may be the result of increased cyclic adenosine 3',5' monophosphate (cAMP) production, as cAMP has been demonstrated to markedly inhibit rabbit testicular capsular contractions by
suppressing membrane activity of smooth muscle (Seeley et al., 1974). Perhaps the decreased response to PGF$_2\alpha$ in older aged rats may be due to elevated synthesis of cAMP in the testis. This decreased testicular capsular response to PGF$_2\alpha$ in advanced age is consistent with the corresponding increased levels of gonadotropin hormones, serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Stearns et al., 1974) which stimulate cAMP production within seminiferous tubules and Sertoli cells (Heindel et al., 1975). On the other hand, it is possible that oligospermia in certain younger individuals may be due to decreased capsular response to PGF$_2\alpha$ as a result of an anomalous increase in testicular cAMP levels caused by agents such as LH and FSH.

Even though testicular function does not show an abrupt cessation of activity that characterizes the ovary, hypospermatogenesis does occur in the aged. Hypospermia and oligospermia in advanced age have previously been thought to be related to a reduction in male hormone levels and to a thickening of the basement membranes surrounding the sperm-producing seminiferous tubules (Amelar and Dubin, 1977; Bourne, 1960). However, the present experiments suggest that levels of NE and PGA$_2$ may be important modulators of testicular capsular contractions with regard to ageing, thereby playing a role in certain types of oligospermia of unknown etiology responsible for male infertility.

C. EFFECT OF TEMPERATURE ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

The present data demonstrate that exposure to hyperthermic temperatures up to 42°C for more than 15 minutes results in severe injury to spontaneous contractions of the adult rabbit testicular capsule. Temperatures greater than
42° C are beyond normal physiological limits in humans and while providing a useful experimental model in animals may be of doubtful biological significance in man. However, the normal body temperature for the rabbit (40° C) is considerably higher than the normal body temperature of the human (37.5° C). The intra-testicular temperature of the rabbit (36.9° C) (Buyer and Davis, 1966) is higher than that in man (33.5° C) (Amiel et al., 1976). The temperature of 42° C, which we have reported to have deleterious effects on spontaneous contractions of the rabbit testicular capsule may therefore be considered to be within normal pyrexic limits for the rabbit. Given the difference in body and intra-testicular temperatures between rabbit and man, it might therefore be expected that a body temperature of 42° C, which is well within normal pyrexic limits for the rabbit, would be analogous to 40° C in humans and could conceivably have similar deleterious effects on spontaneous testicular contractions in man.

Transitory elevations of intra-testicular temperature have been shown to disrupt testicular thermoregulation. Iggo (1969) demonstrated that a temperature greater than 40° C may represent the maximum temperature at which mammalian cutaneous 'warm' receptors may effectively elicit thermoregulatory compensation. In addition, Lazarus and Zorgniotti (1975) demonstrated that when the oral body temperature of the human reached 37.9° C, testicular thermoregulation seemed to fail and the intrascrotal temperature rose. The finding that increasing the temperature of the in vitro tissue bath from 37 to 40° C resulted in a marked decrease in the amplitude of spontaneous contractions of the adult rabbit testicular capsule suggests that exposure to excessively hot baths or fever may have an inhibitory effect on normal testicular capsular spontaneous contractions which may interfere with sperm transport. In addition, the finding that
hyperthermic temperatures may have an irreversible effect on testicular capsular spontaneous contraction suggests that the temperatures of ordinary household hot water baths and especially sauna baths may represent a serious health hazard. Table 7 presents typical temperatures of hot running water in various hotels and hospitals in the United States.

It is also well established that prolonged heat can produce significant changes in the germinal epithelium of the testis as well as dramatic decrease in sperm count. Tokuyama (1963) reported that single exposure to $43^\circ C$ for 30 minutes produced a significant drop in the sperm count 5 - 7 weeks later, followed by a rapid recovery. These results might be attributable to specific damage limited to young primary spermatocytes (Steinberger and Dixon, 1959).

Therefore it is possible to conclude that hyperthermia may have several deleterious effects in certain cases of oligospermia including cytological changes in the germinal epithelium as well as decreased spontaneous contractions of the testicular capsule.

Prolonged exposure to $32^\circ C$ resulted in a marked increase in the frequency, amplitude and tone of spontaneous contractions of the adult rabbit testicular capsule when compared to the normal scrotal temperature of $37^\circ C$. Moreover, these stimulatory effects were reversible upon warming. These findings suggest that moderate hypothermic temperature change may serve as an important therapeutic tool in certain cases of infertility. Robinson and co-investigators (1968) observed an increase in sperm counts in humans by locally cooling the testis approximately $3^\circ C$ with the application of ice. The elevated sperm counts which were reported might have been due to a stimulation of the amplitude of spontaneous testicular capsular contractions such as was observed in
the present studies with a hypothermic temperature of 3 to $5^\circ$C below the normal $37^\circ$C testicular temperature of the rabbit.

The finding that decreasing the temperature of the in vitro tissue bath from 37 to $16^\circ$C, for a period of 120 minutes, caused a cessation in spontaneous contractions of the adult rabbit testicular capsule suggests that swimming in excessively cold water or exposure to excessive cold weather may also inhibit testicular capsular contractions, thereby interfering with sperm transport. The practical significance of the observed effect of hypothermia on testicular capsular contractions is indicated by the fact that summer beach water temperatures in selected northern cities of the United States were in the temperature range of 10.8 - 26.8$^\circ$C (Table 8).

The effect of temperature on the mechanical activities of testicular capsular smooth muscle may be related to the changes observed in the longitudinal smooth muscle of the rabbit duodenum. Small and Weston (1971) observed that cooling strips of longitudinal smooth muscle of the rabbit duodenum from 37.5 to $25^\circ$C produced a reduction in the resting tension as well as hyperpolarization and multispike complexes. Similar to the present results observed with the testicular capsule, these investigators found the effects of cooling were reversible; with increased frequency and resting tension obtained upon warming to 37.5$^\circ$C. A decrease in temperature has been shown to increase membrane resistance because of the decrease in $K^+$ and $Cl^-$ conductance in the isolated guinea pig taenia coli preparation (Brading et al., 1969). In addition, hypothermia may cause $Ca^{++}$ to be sequestered into the reticulum, thereby decreasing spontaneous activity (Magaribuchi et al., 1973).
The clinical importance of the present experiments is revealed by comparing the human with the rabbit. Given that the intra-testicular temperature of the human is lower than the rabbit, it might therefore be expected that a 5°C increase in intra-testicular temperature in the rabbit, could have a similar deleterious effect on human testicular capsular contractions. Rock and Robinson (1965) reported that when male subjects were exposed to bath water temperature of 43°C, the body temperature had risen from a median value of 37.7 to 39.3°C, which corresponded to an increase in scrotal temperature from 36.1 to 40.5°C. Acute febrile diseases, accompanied by body temperatures between 40-42°C may therefore result in abnormal testicular capsular contractility and ultimately interfere with sperm transport. On the other hand, an optimal temperature for the amplitude of spontaneous contractions of the rabbit testicular capsule occurred at 32-34°C. These findings suggest that a similar 3-5°C decrease in human intra-testicular temperature could lead to a corresponding increase in spontaneous contractions of the testicular capsule and consequently promote sperm transport.

D. RESPONSE OF THE RABBIT ISOLATED TESTICULAR CAPSULE AT HYPER–THERMIC AND HYPOTHERMIC TEMPERATURES TO NOREPINEPHRINE, ACETYLCHEOLINE AND PROSTAGLANDIN F$_2$α

The present data demonstrate that NE, ACh and PGF$_2$α may be involved in the maintenance of testicular capsular homeostasis during exposure to moderate hyperthermic and hypothermic temperature changes. Testicular temperature is known to fluctuate rapidly in response to external heating or cooling of the scrotum. The arrangement of the free communicating cord veins within the
pampiniform, cremasteric and vasal plexuses which aids in vascular heat-exchange, as well as the thermoregulatory apparatus consisting of the cremasteric and dartos muscles have been reported to play a contributing role in the maintenance of the scrotal-abdominal temperature gradient (Shafik, 1974). Impaired fertility in man can be correlated with an inadequate testicular temperature gradient. It has long been known that germinal cells of the testis undergo rapid deterioration when subjected to increased temperature (Nelson, 1951; Steinberger and Dixon, 1959). In contrast to heat, short-term exposure to cold temperatures reduces androgen production, which may ultimately lead to testicular degeneration (VanDemark and Free, 1970). In addition, the marked temperature sensitivity of spontaneous contractions of the adult rabbit isolated testicular capsule may also contribute to male infertility by interfering with sperm transport.

The finding that the adverse effects of the $32^\circ C$ on spontaneous contractions of the adult rabbit isolated testicular capsule can be altered by the addition of NE and ACh suggests new functional roles of neurohormones in various thermoregulatory mechanisms. Sympathomimetic drugs are known to cause an increased mobilization of calorigenic substrates such as glucose and free fatty acids which ultimately lead to an increase in temperature (Maickel, 1970). However, the effect of hypothermic temperatures on drug-induced contraction of smooth muscle remains unclear. Hurwitz and co-investigators (1975) have demonstrated that hypothermic temperature may effect microsomal calcium binding and mechanical activity of intestinal smooth muscle fibers. An ACh-induced contraction of the longitudinal muscle of guinea pig intestine generated at $10^\circ C$, is noticeably larger than $30^\circ C$. The data of the present study indicates in
a similar fashion that both NE and ACh produce a greater increase in tissue tone of the testicular capsule at 32°C as compared to 37°C control values. In contrast, PGF$_2\alpha$ caused a smaller contraction of the testicular capsule at 32°C, indicating the possibility that the response of the testicular capsule to prostaglandins under hypothermic conditions may not be influenced by calcium release.

The finding that NE caused the greatest increase in testicular capsular tone during exposure to hyperthermia indicates that NE may play an important role in the maintenance of testicular capsular tone during exposure to extreme hyperthermic conditions. Therefore the possibility exists that a partial explanation of reduced sperm output in such febrile illnesses as pneumonia and chicken pox (MacLeod, 1951) and in recurrent febrile attacks of familial Mediterranean fever (French et al., 1973) may involve diminished circulating levels of NE reaching the testis. The resulting lack of sufficient testicular capsular tension may therefore influence the rhythmic contraction and relaxation of the testicular capsule which may ultimately interfere with the transport of non-motile sperm out of the testis and into the epididymis.

E. COMPARATIVE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE AND DUODENUM TO HYPOXIA

The present data demonstrates that hypoxia is capable of producing a sudden initial increase in frequency of isolated testicular capsular contractions followed by a progressive decrease in contraction frequency after 5 minutes of hypoxic exposure. However, an immediate decrease in the amplitude of testicular capsular contractions was observed throughout the entire exposure to hypoxia. In
contrast, a progressive but less marked decrease in the frequency and amplitude of isolated duodenal contractions was observed with increasing lengths of hypoxia. At the present time, it is difficult to speculate on the mechanisms responsible for the hypoxic effects of the diminished phasic activity (frequency and amplitude) other than anatomical similarity between the testicular capsule and duodenum with respect to the distribution of longitudinal and circular smooth muscle (Davis and Langford, 1970).

In addition hypoxia resulted in a prolonged contracture of the isolated testicular capsule, whereas in the isolated duodenum a reduction in tissue tone with subsequent atonia was observed. The factors underlying tonic myogenic contraction and phasic activity in smooth muscle are poorly understood. The origin of the spontaneous activity in the intestine and testicular capsule appears to be myogenic in origin, since the addition of atropine, a cholinergic blocking agent; hexamethonium, a ganglionic blocker or tetrodotoxin, an agent which prevents the action potential by blocking the membrane Na$^+$ channels, fails to reduce rhythmic spontaneous contractions (Bortoff and Sacco, 1974; Rikimaru and Suzuki, 1972). These data imply that excitation originates spontaneously in a group of muscle cells acting as pacemakers from which action potentials are propagated to all other smooth muscle cells through tight cell junctions (Ruegg, 1971).

The myogenic mechanical activity initiated by action potentials arising spontaneously in pacemaker cells and maintaining active tension development in a smooth and constant manner over many minutes has been referred to smooth muscle tone (Axelsson, 1970; Golenhofen, 1976). The resting tone of some isolated smooth muscles (frog intestine, bovine iris and rabbit jejunum) have been reported
to be maintained by an intramural synthesis of prostaglandins (Eckenfels and Vane, 1972; Ferreira et al., 1976). Indomethacin, a prostaglandin synthetase inhibitor reduced not only prostaglandin output but also the tone of the rabbit jejunum (Ferreira et al., 1976). In addition, anoxia and dinitrophenol, a powerful uncoupler of oxidative phosphorylation has been found to relax intestinal smooth muscle as oxygen is known to be the rate limiting step in prostaglandin synthesis (Eckenfels and Vane, 1972). It may be speculated that the rapid decrease in intestinal tone to hypoxic exposure may indicate that the intestine is dependent upon a constant intra-mural synthesis of prostaglandins for the maintenance of tone. It is therefore possible that the lack of smooth muscle relaxation in the testicular capsule during hypoxic states may be due to minimal dependence on prostaglandin synthesis for the maintenance of tone or large prostaglandin stores known to exist within the testis. In addition to prostaglandins, the intracellular level of free calcium may be involved in the maintenance of tone as well as hypoxic-induced contraction of the testicular capsule. It is presently thought that calcium diffuses into the fibers and activates the contractile apparatus, the tension being maintained as long as calcium remains at the new steady-state level (Urakawa and Holland, 1974). In contrast, the response of the duodenum to hypoxia resulting in atonia may be the result of calcium remaining bound to the vesicles of the sarcoplasmic reticulum (Ruegg, 1971).

The testicular capsule may represent a unique smooth muscle preparation with respect to the intestine in displaying an increase in tissue tone during states of hypoxia. Our results may have important implications with regard to chronic
spasm of the testicular capsule, since an increase in active tension development within the testis may result in an elevated intra-testicular pressure producing ischemia and ultimately male infertility.

F. RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL EXPOSED TO HYPOXIA

The present data demonstrate that hypoxia is capable of producing a rapid increase in testicular capsular tone accompanied by a progressive decrease in the frequency and amplitude of spontaneous testicular capsular contractions. In addition, sodium nitroprusside, papaverine and verapamil were found to rapidly relax testicular capsular smooth muscle tone during exposure to hypoxia.

Sodium nitroprusside and papaverine have been found to act as non-competitive spasmolytic agents on a variety of aerated smooth muscle preparations. Non-competitive spasmolytics do not interfere with neuromuscular transmission, but rather with the excitation-contraction coupling process between the intracellular ionization of calcium and its interaction with the contractile system of the smooth muscle fibers. The myogenic mechanical activity initiated by action potentials arising spontaneously in a smooth and constant manner over many minutes has been referred to as smooth muscle tone (Axelsson, 1970). Intestinal smooth muscle tone is known to be abolished by anoxia and cyanide or by impairing (by the addition of 2,4-dinitrophenol) the synthesis of ATP normally coupled to the electron transport in the respiratory chain (Santi et al., 1964). Sodium nitroprusside and papaverine has been demonstrated to selectively suppress the tonic component, without significantly affecting the phasic contrac-
tions in guinea pig aortic and stomach smooth muscle (Boev et al., 1976; Golenhofen, 1976). The addition of high concentrations of sodium nitroprusside has been demonstrated to slightly inhibit the phasic contractions in intestinal smooth muscle (Boev et al., 1976). The mechanism by which sodium nitroprusside selectively alters smooth muscle tone is unclear. However, the relaxant effects of papaverine are thought to be due to inhibition of the electron-transfer chain between nicotinamide-adenine dinucleotide and cytochrome b (Santi et al., 1964) or through phosphodiesterase inhibition with subsequent accumulation of cyclic AMP and increased calcium uptake by the vesicles (Baudouin-Legros and Meyer, 1973). The present data demonstrates that sodium nitroprusside and papaverine inhibits both the phasic and tonic component of testicular capsular contraction, rather than a selective inhibition of tone. It is possible that the reduction in both the phasic activity and tone of the isolated testicular capsule during aeration may be due to a high concentration of drug since a lower concentration of sodium nitroprusside and papaverine was found to produce a selective blockade of testicular capsular tone. A drug concentration of $10^{-6}$ M was selected for the present studies since it produced a maximal decrease in capsular tone during exposure to both aeration and hypoxia with recovery to pre-treatment control values following removal of the drug by washing.

On the other hand, verapamil has been demonstrated to selectively block the phasic contractions from isolated preparations of taenia coli, portal vein, stomach and ureter of the guinea pig during exposure to aeration (Golenhofen and Lamel, 1972; Boev et al., 1976). The selective suppression of spontaneous activity in various types of smooth muscle preparations by verapamil has been demonstrated to be due to blockade of transmembrane calcium influx into cardiac and
smooth muscle cells during excitation (Haeusler, 1972; Riemer et al., 1974). The selective blockade of the phasic activity of the adult rabbit isolated testicular capsule to verapamil during exposure to aeration has been confirmed in the present study.

It seems reasonable to postulate from the present data that during prolonged hypoxia, the selective suppression of either the phasic or tonic component of smooth muscle activity by drugs is markedly altered. Sodium nitroprusside, papaverine and verapamil were all found to rapidly restore an elevated testicular capsular tone during hypoxia, whereas during exposure to aeration only sodium nitroprusside and papaverine significantly decreased capsular tension. The observed relaxation of testicular capsular tension during hypoxia by all three drugs may be attributed to a uniform inhibition of calcium activation necessary for electromechanical coupling of testicular capsular smooth muscle.

Our results may have clinical implications with regard to subfertile men with varicoceles, which have been reported to have a lowered oxygen tension (Cohen et al., 1975) or chronic spasm of the testicular capsule resulting in an elevated intra-testicular pressure thereby preventing ischemia and ultimately male infertility. Appropriate in vivo investigations need to be conducted to determine this in the future.
CHAPTER VI

SUMMARY
CHAPTER VI

SUMMARY

The contractile response of the rat isolated testicular capsule to norepinephrine (NE), acetylcholine (ACh) and prostaglandins (PG) has been found to be dependent upon age of the animal. The testicular capsular contractions attributable to smooth muscle cells located within the tunica albuginea may be involved in the transport of non-motile spermatozoa from the testis and into the epididymis. A relationship appears to exist between the onset of spermiogenesis and the degree of testicular capsular contraction produced by equimolar concentrations of NE and PGA$_2$. The response of the aging rat to the addition of equimolar concentrations of NE and PGA$_2$ became progressively greater as the age of the animal increased. An increase in age of the rat was found to have a concomitant increase in mass of the testis, thickness of the capsule and the amount of smooth muscle fibers present in the tunica albuginea. The response of the rat isolated testicular capsule to neurohumoral agents does not appear to be correlated with the development of an increased mass of contractile elements during prepubertal to early adulthood, or smooth muscle hyperplastic changes associated with advanced age, since the response to ACh remained constant throughout the age range studied and the PGF$_{2\alpha}$ became progressively less stimulatory as the age of the animal increased. The different responses induced by the drugs studied at various ages may be related to changes in the smooth muscle receptors.
Spontaneous contractions of the adult rabbit isolated testicular capsule were found to be influenced by moderate hyperthermic and hypothermic temperature changes. An optimal temperature for the amplitude of spontaneous contractions of the rabbit testicular capsule occurred at 32 - 34°C. A progressive increase in the in vitro organ bath temperature from a normal scrotal temperature of 37 to 44°C resulted in an increase in both frequency and tone, accompanied by a significant decrease in amplitude. A complete and irreversible cessation of spontaneous contractions occurred at 48°C. In addition, exposure to 42°C for an exposure period greater than 15 minutes resulted in irreversible changes in spontaneous contractions of the testicular capsule, whereas complete recovery of capsular contractions which had been inhibited by an exposure of 40°C for 120 minutes occurred when the tissue was returned to 37°C. On the other hand, decreasing the temperature from 37 to 26°C resulted in a marked decrease in frequency and amplitude progressing to a complete but reversible cessation of spontaneous contractions at 16°C. These data suggest the possibility that acute febrile diseases or excessive hot baths, as well as swimming in excessively cold water or excessive cold weather exposure may have inhibitory effects of testicular capsular spontaneous contractions which may ultimately interfere with sperm transport.

The marked temperature sensitivity of testicular capsular spontaneous contractions resulting from an exposure to 32, 40 and 42°C have been shown to be influenced by the addition on NE, ACh and PGF$_2\alpha$. Of the three neurohumoral agents studied, only NE was found to cause the greatest increase and re-initiation of testicular capsular tone during exposure to extreme hyperthermic conditions. Therefore the possibility exists that a partial explanation of reduced sperm output
in such febrile illnesses as pneumonia or chicken pox and in recurrent febrile attacks of familial Mediterranean fever may involve diminished circulating levels of NE reaching the testis. The resulting lack of sufficient testicular capsular tension may influence the rhythmic contraction and relaxation of the testicular capsule, thereby interfering with the transport of non-motile sperm out of the testis and into the epididymis where they attain their motility.

Spontaneous contractions of the adult rabbit isolated testicular capsule have also been demonstrated to be adversely affected by short exposures to hypoxia. Exposure of the testicular capsule to hypoxia for 10 minutes produced a progressive decrease in frequency and amplitude of spontaneous contractions, along with a corresponding marked increase in tissue tone. Further increases in hypoxic exposure resulted in cessation of capsular contractions. These results may have important implications with regard to chronic spasm of the testicular capsule, since an increase in active tension development within the testis may result in an elevated intra-testicular pressure producing ischemia and ultimately male infertility. The increase in testicular capsular tension (tone) resulting from hypoxia was demonstrated to be influenced by the addition of smooth muscle relaxant agents. Sodium nitroprusside, papaverine and verapamil each were found to relax the hypoxic-induced contracture of the testicular capsule and to restore capsular tone to a control aerated level. The observed relaxation of testicular capsular tension during hypoxia by all three drugs may be attributed to a uniform inhibition of calcium activation necessary for electromechanical coupling of testicular capsular smooth muscle.
Preliminary experiments have demonstrated that mercury and cadmium are capable of causing a decrease in spontaneous contractions of the rabbit isolated testicular capsule. The present findings suggest that mercury and cadmium released inadvertently into the environment may adversely affect male reproduction by suppressing testicular capsular contractions, and in so doing contribute to male sterility. On the other hand, the administration of zinc may protect the testes from the deleterious effects of cadmium on testicular capsular motility.

In summary, considerable evidence has been presented that numerous factors such as age, temperature and hypoxia may alter drug-induced and spontaneous contractions of the testicular capsule. The possibility exists that certain types of oligospermia of unknown etiology leading to male infertility may be due to one of these factors. The practical significance of the present studies to health problems may be that stimulation of testicular capsular contractions by drugs might enhance sperm transport from the testis to the epididymis, thereby offering a method of treating male infertility.
CHAPTER VII

REFERENCES
CHAPTER VII

REFERENCES


Hodson, N. 1964. Role of the hypogastric nerves in seminal emission in the rabbit. J. Reprod. Fert. 7: 113-122.


CHAPTER VIII

FIGURES
Figure 1. Schematic representation of the procedure for the isolation of the rat and rabbit testicular capsule and its use as an isolated tissue preparation for pharmacological studies. From Davis and Langford (1970).
Figure 2. Schematic representation of the procedure for the isolation of the rabbit splenic capsule and its use as an isolated tissue preparation for pharmacological studies.
METHOD FOR ISOLATION OF A SPLENIC CAPSULE STRIP

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
</tbody>
</table>

Figure 2
Figure 3. Effect of age on the response of the rat isolated testicular capsule to equimolar concentrations (3.0 x 10^{-6} M) of prostaglandins, norepinephrine and acetylcholine. Each large square shown on the chart paper represents 5 mm. The response magnification was x 100. The vertical bars represent the actual mm of tissue contraction for each preparation. In each case, the tissue load was 100 mg. Drug concentrations are expressed as final organ bath concentration. The temperature of the organ bath was maintained at 32^\circ C. The recordings shown are typical of a total of five similar experiments.
EFFECT OF AGE ON THE RESPONSE OF THE
RAT ISOLATED TESTICULAR CAPSULE TO EQUIMOLAR CONCENTRATIONS (3 x 10^-6 M)
OF PROSTAGLANDINS, NOREPINEPHRINE AND ACETYLCHOLINE

30 DAYS

90 DAYS

Figure 3
Figure 4. Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of norepinephrine. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF
THE RAT ISOLATED TESTICULAR CAPSULE
TO INCREASING CONCENTRATIONS OF
NOREPINEPHRINE

(each point represents the average of 5 experiments)

<table>
<thead>
<tr>
<th>AGE OF ANIMALS</th>
<th>E_D50</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 DAYS</td>
<td>0.21 μg/ml (6.2 x 10^{-7}M)</td>
</tr>
<tr>
<td>45 DAYS</td>
<td>0.22 μg/ml (6.5 x 10^{-7}M)</td>
</tr>
<tr>
<td>60 DAYS</td>
<td>0.20 μg/ml (5.9 x 10^{-7}M)</td>
</tr>
<tr>
<td>90 DAYS</td>
<td>0.21 μg/ml (6.2 x 10^{-7}M)</td>
</tr>
</tbody>
</table>

Figure 4
Figure 5. Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of acetylcholine. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF
THE RAT ISOLATED TESTICULAR CAPSULE
TO INCREASING CONCENTRATIONS OF
ACETYLCHOLINE

(each point represents the average of 5 experiments)

AGE OF ANIMALS       ED$_{50}$
30 DAYS               0.08 μg/ml (4.4 x 10$^{-7}$M)
45 DAYS               0.08 μg/ml (4.4 x 10$^{-7}$M)
60 DAYS               0.07 μg/ml (3.9 x 10$^{-7}$M)
90 DAYS               0.06 μg/ml (3.3 x 10$^{-7}$M)

Figure 5
Figure 6. Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin E₁. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF PROSTAGLANDIN E₁

* ± S.E.M. OF 5 EXPS

AGE OF ANIMALS  ED₅₀
30 DAYS  9.5 µg/ml (2.7 x 10⁻⁵ M)
90 DAYS  9.8 µg/ml (2.8 x 10⁻⁵ M)

Tissue Contraction (mm)

Concentration of Prostaglandin E₁ (µg/ml)

Figure 6
Figure 7. Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin $A_2$. Each point represents the mean $\pm S.E.M.$ of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF PROSTAGLANDIN A₂

AGE OF ANIMALS  ED₅₀
30 DAYS  1.65 µg/ml (4.9 x 10⁻⁶ M)
90 DAYS  0.27 µg/ml (8.1 x 10⁻⁷ M)

Figure 7
Figure 8. Effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin F$_2$α. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF PROSTAGLANDIN F\(_{2\alpha}\)

![Graph showing the effect of age on the response of the rat isolated testicular capsule to increasing concentrations of prostaglandin F\(_{2\alpha}\).](image)

- **30 DAYS**: 0.016 μg/ml (3.4 x 10\(^{-8}\) M)
- **90 DAYS**: 0.320 μg/ml (6.8 x 10\(^{-7}\) M)

*Figure 8*
Figure 9. (a) Effect of age of the rat on body weight, the weight of the whole testis and the weight of the isolated testicular capsule. Each point represents the average of five animals.

(b) Effect of age of the rat on the percent shortening of the isolated testicular capsule to 0.1 μg/ml acetylcholine chloride and 1.0 μg/ml L-norepinephrine bitartrate H₂O. Each point represents the average of results obtained from five animals ± S.E.M.
**Figure 9**

(a) **MALE ALBINO RATS**

(Each point represents the average of 3 experiments)

- Testis weight
- Testicular capsule resting length
- Testicular capsule weight
- Body weight

(b) **MALE ALBINO RATS**

Response of isolated testicular capsule to
0.1 µg/ml acetylcholine chloride and
1.0 µg/ml L-norepinephrine bitartrate·H₂O

I = ± S.E.M. of 5 EXPS

- Acetylcholine (ACH)
- Norepinephrine (NE)
Figure 10. Effect of age on the tissue contraction of the rat isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandins A₂ and F₂α. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS A<sub>2</sub> AND P<sub>2α</sub>

Figure 10
Figure 11. Effect of age on the response of the rat isolated testicular capsule to equimolar concentrations (3.0 \times 10^{-6} \text{ M}) of norepinephrine, acetylcholine and prostaglandins A_2 and F_2\alpha. The vertical bar shown on the chart paper represents 10 mm and the response magnification was x 100. The recordings shown are typical of the total of five in each experiment.
Figure 11

EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO EQUIMOLAR CONCENTRATIONS (3.0 x 10^-6 M) OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS A2 AND F2-α.

[Diagram showing the response of the rat isolated testicular capsule to different ages and concentrations of NE, ACh, PGA2, and PGF2α.]
Figure 12. Effect of age on the percent shortening of the rat isolated testicular capsule to equimolar concentrations (3.0 x 10^{-6} M) of norepinephrine, acetylcholine and prostaglandins A₂ and F₂α. Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
EFFECT OF AGE ON THE RESPONSE OF THE RAT ISOLATED TESTICULAR CAPSULE TO EQUIMOLAR CONCENTRATIONS (3.0 x 10^{-6} M) OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDINS A_2 AND F_2α.

Figure 12
Figure 13. Effect of temperature on spontaneous isotonic contractions of the adult rabbit isolated testicular capsule in vitro.
EFFECT OF TEMPERATURE ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

HYPOTHERMIC TEMPERATURE CHANGE 37°C HYPERTHERMIC TEMPERATURE CHANGE

Figure 13
Figure 14. Typical response of the isolated testicular capsule to hyperthermic and hypothermic temperature change. The response magnification was x 10 and recordings shown are typical of the total of five in each experiment.
EFFECT OF TEMPERATURE ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

(10 min time exposure at each temperature)
Figure 15. Reversibility of hypothermic inhibition by rapid warming and irreversibility of hyperthermic inhibition by rapid cooling of adult rabbit testicular capsular spontaneous contractions in vitro. The response magnification was x 10 and recordings shown were typical of the total of five in each experiment.
EFFECT OF TEMPERATURE ON SPONTANEOUS ISO TONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

REVERSIBILITY OF HYPOTHERMIC INHIBITION OF SPONTANEOUS CONTRACTIONS BY RAPID WARMING

IRREVERSIBILITY OF HYPerTHERMIC INHIBITION OF SPONTANEOUS CONTRACTIONS BY RAPID COOLING
Figure 16. Effect of $42^\circ$C on spontaneous isotonic contractions of the adult rabbit testicular capsule *in vitro*. The values are mean $\pm$ S.E.M. for three experiments.
EFFECT OF HYPERTHERMIA (42 °C) ON
SPONTANEOUS ISOTONIC CONTRACTIONS OF THE
ADULT RABBIT TESTICULAR CAPSULE in vitro

Figure 16
Figure 17. Effect of 40°C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. The values are mean ± S.E.M. for three experiments.
EFFECT OF HYPERTHERMIA (40 °C) ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE \textit{in vitro}

![Graph showing the effect of hypothermia on isotonic contractions of the adult rabbit testicular capsule. The graph includes bars indicating control values, 40 °C exposure, and post-hypothermic values at various exposure times.](image)

Figure 17
Figure 18. Effect of $32^\circ$ C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. The values are mean $\pm$ S.E.M. for three experiments.
EFFECT OF HYPOTHERMIA (32 °C) ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

Figure 18
Figure 19. Effect of 16° C on spontaneous isotonic contractions of the adult rabbit testicular capsule in vitro. The values are mean ± S.E.M. for three experiments.
EFFECT OF HYPOTHERMIA (16 °C) ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

Figure 19
Figure 20. Typical response of the isolated testicular capsule exposed for 5 minutes to various hyperthermic and hypothermic temperature changes. The response magnification was x 10 and the recordings shown are typical of three similar experiments.
EFFECT OF HYPERThERMIC AND HYPOTHERMIC EXPOSURE (5 MIN) ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Figure 20
Figure 21. Typical response of the isolated testicular capsule exposed for 30 minutes to various hyperthemic and hypothermic temperature changes. The response magnification was x10 and the recordings shown are typical of three similar experiments.
EFFECT OF HYPER THERMIC AND HYPOTHERMIC EXPOSURE (30 MIN) ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Figure 21
Figure 22. Effect of norepinephrine, acetylcholine and prostaglandin $F_2\alpha$ on the response of the adult rabbit isolated testicular capsule exposed to $37^\circ$ C. The response magnification was x 10 and the recordings shown are typical of four similar experiments.
EFFECT OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F$_2$α (ED$_{75}$ AT 37 °C) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO 37 °C

Figure 22
Figure 23. Effect of norepinephrine, acetylcholine and prostaglandin F$_2\alpha$ on the response of the adult rabbit isolated testicular capsule exposed to 32°C. The response magnification was x 10 and the recordings shown are typical of four similar experiments.
EFFECT OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F$_{2\alpha}$ (ED$_{75}$ AT 37 °C) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO 32 °C

Figure 23
Figure 24. Effect of norepinephrine, acetylcholine and prostaglandin
F$_2$α on the response of the adult rabbit isolated testicular capsule
exposed to 40° C. The response magnification was x 10 and the
recordings shown are typical of four similar experiments.
EFFECT OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F2α (ED75 AT 37 °C) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO 40 °C

Figure 24
Figure 25. Effect of norepinephrine, acetylcholine and prostaglandin 
$F_2\alpha$ on the response of the adult rabbit isolated testicular capsule 
exposed to 42°C. The response magnification was x 10 and the 
recordings shown are typical of four similar experiments.
EFFECT OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F₂α (ED₇₅ AT 37 °C) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO 42 °C.

Figure 25
Figure 26. Comparative changes of the tone of the adult rabbit isolated testicular capsule by norepinephrine, acetylcholine and prostaglandin \( F_2\alpha \) at 32, 40 and \( 42^\circ C \). The tissue was exposed to the indicated temperature for 10 minutes prior to the addition of an \( \text{ED}_{75} \) concentration of the drug for a 5 minute contact time. Control values were re-established at \( 37^\circ C \) between the addition of each drug. The values are mean ± S.E.M. for four experiments.
COMPARATIVE CHANGES OF THE TONE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE
BY NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F₂α
AT 32, 40 AND 42 °C

The tissue was exposed to the indicated temperature for 10 min prior to the addition of an ED₇₅ concentration of the drug (5 min drug contact time). Control values were re-established at 37 °C between the addition of each drug.

- L-NOREPINEPHRINE BITARTRATE (3.0 x 10⁻⁶M)
- ACETYLCHOLINE CHLORIDE (1.1 x 10⁻⁵M)
- PROSTAGLANDIN F₂α (4.2 x 10⁻⁶M)

± ± S.E.M. OF 4 EXPS

Figure 26
Figure 27. Effect of hypoxia on spontaneous isotonic contractions of the adult rabbit isolated testicular capsule. Each point represents the mean ± S.E.M. for five experiments.
EFFECT OF HYPOXIA ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

The tissue was aerated for 15 min and control values re-established between each of the indicated time periods of hypoxia at 37 °C

±: S.E.M. OF 5 EXPS

![Graph showing the effect of hypoxia on the frequency, amplitude, and tone of spontaneous isotonic contractions of the adult rabbit testis capsule.](image-url)

Figure 27
Figure 28. Typical response of the isolated testicular capsule exposed for 1, 2, 5, 10 and 15 minutes to hypoxia. The response magnification was x 10 and the recordings shown are typical of five similar experiments.
EFFECT OF HYPOXIA ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

The tissue was aerated for 15 min and control values re-established between each of the indicated time periods of hypoxia at 37 °C.

Figure 28
Figure 29. Effect of hypoxia on spontaneous isotonic contractions of the adult rabbit isolated duodenum. Each point represents the mean ± S.E.M. for five experiments.
EFFECT OF HYPOXIA ON
SPONTANEOUS ISOTONIC CONTRACTIONS OF THE
ADULT RABBIT ISOLATED DUODENUM

The tissue was aerated for 15 min and
control values re-established between each of
the indicated time periods of hypoxia at 37 °C

S.E.M. OF 5 EXPS

Figure 29
Figure 30. Typical response of the isolated duodenum exposed for 1, 2, 5, 10 and 15 minutes to hypoxia. The response magnification was x 10 and the recordings shown are typical of five similar experiments.
EFFECT OF HYPOXIA ON SPONTANEOUS ISOTONIC CONTRACTIONS OF THE ADULT RABBIT ISOLATED DUODENUM

The tissue was aerated for 15 min and control values re-established between each of the indicated time periods of hypoxia at 37 °C.

Figure 30
Figure 31. Typical effect of sodium nitroprusside, papaverine and verapamil ($10^{-6}$ M) on the response of the adult rabbit isolated testicular capsule exposed to aeration. The vertical bar shown on the chart paper represents a 1.0 mm tissue contraction. The response magnification was x 10. The recordings shown are representative of a total of five experiments.
EFFECT OF SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL (10^{-6} M) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO AERATION

![Figure 31](image-url)
Figure 32. Contractile response of the adult rabbit isolated testicular capsule to equimolar additions ($10^{-6}$ M) of sodium nitroprusside, papaverine and verapamil during exposure to aeration.
CONTRACTILE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL (10^-6 M) DURING EXPOSURE TO AERATION

Figure 32
Figure 33. Typical effect of sodium nitroprusside, papaverine and verapamil (10^{-6} M) on the response of the adult rabbit isolated testicular capsule exposed to hypoxia. The vertical bar shown on the chart paper represents a 1.0 mm tissue contraction. The response magnification was x 10. The recordings shown are representative of a total of five experiments.
EFFECT OF SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL (10^-6 M) ON THE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE EXPOSED TO HYPOXIA

- CONTROL PERIOD (5 min exposure to aeration at 37 °C)
- HYPOXIC EXPOSURE (NITROGEN) FOR EACH OF THE INDICATED TIME PERIODS
- POST-HYPOXIC PERIOD

Figure 33
Figure 34. Contractile response of the adult rabbit isolated testicular capsule to equimolar additions ($10^{-6}$ M) of sodium nitroprusside, papaverine and verapamil during exposure to hypoxia.
CONTRACTILE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO SODIUM NITROPRUSSIDE, PAPAVERINE AND VERAPAMIL (10^{-6} M) DURING EXPOSURE TO HYPOXIA

Figure 34
Figure 35. Response of the adult rat isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin $F_2\alpha$. Each point represents the mean $\pm$ S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
RESPONSE OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF NOREPINEPHRINE, ACETYLCHELINE AND PROSTAGLANDIN $F_2\alpha$

Figure 35
Figure 36. Response of the adult rabbit isolated testicular capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin F\(_2\alpha\). Each point represents the mean ± S.E.M. of results obtained from five testicular capsules, each obtained from different animals.
RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE TO INCREASING CONCENTRATIONS OF NOREpinephrine, Acetylcholine AND Prostaglandin \( F_2 \alpha \)

**Figure 36**
Figure 37. Representative histological cross sections of the rat and rabbit testicular capsules stained with Masson's trichrome stain illustrating the comparative thickness of each capsule as well as the presence of smooth muscle. A-B. Low magnification of the testicular capsule (cap) surrounding the parenchymal tissue of the rat and rabbit testis, respectively (x 22). C. High magnification of the rat testicular capsule demonstrating occasional smooth muscle nuclei (smn) within the collagenous tissue (c) of the tunica albuginea. fib, fibroblast; int, interstitial tissue cell (x 1125). D. High magnification of the rabbit testicular capsule demonstrating the superficial layers of longitudinal smooth muscle (lsm) as well as the deeper layer of circular smooth muscle (csm). st, seminiferous tubule (x 490).
Figure 37
Figure 38. Response of the adult rabbit isolated splenic capsule to increasing concentrations of norepinephrine, acetylcholine and prostaglandin F$_2$$\alpha$. Each point represents the mean $\pm$ S.E.M. of results obtained from five different animals.
RESPONSE OF THE ADULT RABBIT ISOLATED SPLENIC CAPSULE TO INCREASING CONCENTRATIONS OF NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F$_2$α.

Figure 38
Figure 39. Comparative response of the adult rabbit isolated testicular and splenic capsules to equimolar concentrations ($3.0 \times 10^{-6}$ M) of norepinephrine, acetylcholine and prostaglandin $F_{2\alpha}$. The vertical bar shown on the chart paper represents 0.5 mm and the response magnification for the testicular and splenic capsule were $\times 10$ and $\times 25$, respectively. The recordings shown are typical of the total of five in each experiment.
COMPARATIVE RESPONSE OF THE ADULT RABBIT
ISOLATED TESTICULAR AND SPLENIC CAPSULES
TO EQUIMOLAR CONCENTRATIONS (3.0 x 10^{-6} M) OF
NOREPINEPHRINE, ACETYLCHOLINE AND PROSTAGLANDIN F_{2\alpha}

Figure 39
Figure 40. Typical response of the adult rabbit isolated testicular capsule to increasing concentrations of $\text{Hg}^{++}$ ($\text{Hg(CH}_3\text{COO)}_2$). The response magnification was x 10.
EFFECT OF Hg** ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Figure 40
Figure 41. Typical response of the adult rabbit isolated testicular capsule to increasing concentrations of Cd$^{++}$ (CdCl$_2$·$\frac{1}{2}$H$_2$O). The response magnification was x 10.
EFFECT OF Cd++ ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Figure 41
Figure 42. Prevention of Cd\textsuperscript{++} induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions by Zn\textsuperscript{++}. The response magnification was x 10.
PREVENTION OF Cd**-INDUCED INHIBITION OF ADULT RABBIT ISOLATED TESTICULAR CAPSULAR SPONTANEOUS CONTRACTIONS BY Zn**
CHAPTER IX

TABLES
Table 1. Comparative response of the rat isolated testicular capsule to prostaglandins, norepinephrine and acetylcholine at 30 and 90 days of age.
Table 1

**EFFECT OF AGE OF THE RAT ON THE RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO PROSTAGLANDINS, NOREPINEPHRINE AND ACETYLCOLINE**

<table>
<thead>
<tr>
<th></th>
<th>30 days</th>
<th>90 days</th>
<th>30 days</th>
<th>90 days</th>
<th>30 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ED$_{50}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean ± S.E.M.$^+$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine chloride</td>
<td>0.08 ± 0.010 µg/ml (4.4 ± 0.6 x 10$^{-7}$M)</td>
<td>0.06 ± 0.015 µg/ml (3.3 ± 0.8 x 10$^{-7}$M)</td>
<td>0.08 ± 0.015</td>
<td>0.09 ± 0.010</td>
<td>0.17 ± 0.030</td>
<td>0.18 ± 0.020</td>
</tr>
<tr>
<td>L-Norepinephrine bitartrate $\cdot$H$_2$O</td>
<td>0.21 ± 0.047 µg/ml (6.2 ± 1.4 x 10$^{-7}$M)</td>
<td>0.21 ± 0.058 µg/ml (6.2 ± 1.7 x 10$^{-7}$M)</td>
<td>0.004 ± 0.001</td>
<td>0.10 ± 0.020$^{**}$</td>
<td>0.01 ± 0.003</td>
<td>0.15 ± 0.032$^{**}$</td>
</tr>
<tr>
<td>PGF$_1$</td>
<td>9.5 ± 0.916 µg/ml (2.7 ± 0.3 x 10$^{-5}$M)</td>
<td>9.8 ± 1.48 µg/ml (2.8 ± 0.4 x 10$^{-5}$M)</td>
<td>0.11 ± 0.007</td>
<td>0.06 ± 0.007$^{**}$</td>
<td>0.02 ± 0.004</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$ tromethamine salt</td>
<td>0.016 ± 0.002 µg/ml (3.4 ± 0.2 x 10$^{-6}$M)</td>
<td>0.32 ± 0.052 µg/ml$^{***}$ (6.8 ± 1.1 x 10$^{-7}$M)</td>
<td>0.15 ± 0.013</td>
<td>0.11 ± 0.015$^+$</td>
<td>0.30 ± 0.016</td>
<td>0.16 ± 0.008$^{***}$</td>
</tr>
<tr>
<td>PGA$_2$</td>
<td>1.65 ± 0.116 µg/ml (4.9 ± 0.3 x 10$^{-6}$M)</td>
<td>0.27 ± 0.082 µg/ml$^{***}$ (8.1 ± 2.4 x 10$^{-7}$M)</td>
<td>0.11 ± 0.013</td>
<td>0.13 ± 0.008</td>
<td>0.08 ± 0.004</td>
<td>0.26 ± 0.016$^{***}$</td>
</tr>
</tbody>
</table>

*Significantly different compared with 30 days of age: $^+$p < 0.05; $^{**}$p < 0.01; $^{***}$p < 0.001 (student's t test).

$^+$ 5 rats/group
Table 2. Development of spermatogenesis of the Sprague-Dawley rat.

From Davis and Firlit (1966).
Table 2

**MALE SPRAGUE-DAWLEY RATS**

<table>
<thead>
<tr>
<th>AGE OF ANIMAL</th>
<th>POSITION OF TESTIS</th>
<th>DEVELOPMENT OF SPERMATOGENESIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 days</td>
<td>abdominal</td>
<td>The testis consists of many small developing seminiferous tubules containing several layers of spermatogonia and transitional to early pachytene primary spermatocytes. The testis is prepubertal in nature.</td>
</tr>
<tr>
<td>25 days</td>
<td>inguinal</td>
<td>The seminiferous tubules are still loosely lined with spermatogonia, transitional and pachytene primary spermatocytes.</td>
</tr>
<tr>
<td>30 days</td>
<td>scrotal</td>
<td>The testis now begins to demonstrate the onset of spermiogenesis. Approximately half of the tubules contain spermatids of the cap and acrosome phases of spermiogenesis.</td>
</tr>
<tr>
<td>35 days</td>
<td>scrotal</td>
<td>The seminiferous tubules have increased in diameter and contain increasing numbers of spermatids. However, maturing spermatids in the maturation phase of spermiogenesis cannot yet be demonstrated.</td>
</tr>
<tr>
<td>60 days</td>
<td>scrotal</td>
<td>The seminiferous epithelium now contains spermatids in the maturation phase of spermiogenesis.</td>
</tr>
<tr>
<td>90 days</td>
<td>scrotal</td>
<td>Spermatogenesis is complete with the testis considered to be adult in nature containing mature sperm.</td>
</tr>
</tbody>
</table>
Table 3. Comparative effect of age of the rat on body weight, weight of the intact testis, weight of the isolated testicular capsule, resting length of the testicular capsule and thickness of the testicular capsule of rats aged 30 to 640 days.
Table 3

Effect of age of the rat on body weight, the weight of the intact testis, the weight of the isolated testicular capsule, resting length of the testicular capsule and thickness of the testicular capsule.

<table>
<thead>
<tr>
<th>Age of Rat</th>
<th>30 Days</th>
<th>90 Days</th>
<th>180 Days</th>
<th>440 Days</th>
<th>640 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>104.10 ± 3.93</td>
<td>385.10 ± 13.18***</td>
<td>539.75 ± 4.59***</td>
<td>705.00 ± 14.66***</td>
<td>655.00 ± 34.40***</td>
</tr>
<tr>
<td>Weight of Intact Testis (g)</td>
<td>0.42 ± 0.04</td>
<td>1.77 ± 0.04***</td>
<td>2.15 ± 0.04***</td>
<td>2.16 ± 0.16***</td>
<td>2.18 ± 0.08***</td>
</tr>
<tr>
<td>Weight of Isolated Testicular Capsule (mg)</td>
<td>16.26 ± 1.47</td>
<td>60.54 ± 4.58***</td>
<td>74.00 ± 0.004***</td>
<td>99.00 ± 0.008***</td>
<td>97.00 ± 0.004***</td>
</tr>
<tr>
<td>Resting Length of Testicular Capsule (mm)</td>
<td>11.10 ± 0.55</td>
<td>17.60 ± 0.38***</td>
<td>15.60 ± 0.55***</td>
<td>15.40 ± 0.68***</td>
<td>17.70 ± 0.66***</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for single testes obtained from a total of 5-10 animals at each age. Significantly different compared with value at 30 days of age: *p < 0.05; **p < 0.01; ***p < 0.001 (Student's t test). (SH) superior medial; (SL) superior lateral; (MM) middle medial; (ML) middle lateral; (IM) inferior medial and (IL) inferior lateral sections fixed in Bouin's solution.
Table 4. Response of the adult rabbit isolated testicular capsule to increasing concentrations of Hg$^{++}$. 
Table 4

Response of the Adult Rabbit Isolated Testicular Capsule to Increasing Concentrations of Hg^{++}

<table>
<thead>
<tr>
<th></th>
<th>Control 10^{-5} M</th>
<th>Hg^{++}</th>
<th>Post-treatment 10^{-5} M</th>
<th>Control 10^{-4} M</th>
<th>Hg^{++}</th>
<th>Post-treatment 10^{-4} M</th>
<th>Control 10^{-3} M</th>
<th>Hg^{++}</th>
<th>Post-treatment 10^{-3} M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (per min)</td>
<td>2.20</td>
<td>2.40</td>
<td>2.60</td>
<td>1.80</td>
<td>2.00</td>
<td>2.00</td>
<td>2.20</td>
<td>1.80</td>
<td>0</td>
</tr>
<tr>
<td>Amplitude (mm)</td>
<td>0.745</td>
<td>0.517</td>
<td>0.646</td>
<td>1.15</td>
<td>0.790</td>
<td>0.250</td>
<td>0.991</td>
<td>0.567</td>
<td>0</td>
</tr>
<tr>
<td>Tone (mm)</td>
<td>0</td>
<td>0</td>
<td>+1.0</td>
<td>0</td>
<td>-5.0</td>
<td>-8.0</td>
<td>0</td>
<td>-4.0</td>
<td>-10.0</td>
</tr>
</tbody>
</table>

The adult rabbit isolated testicular capsule was exposed to the indicated concentration of Hg^{++} (Hg(CH_3COO)_2) for a 5 min contact time at 37°C. Control values were re-established for 10 min following removal of the metal by washing.
Table 5. Response of the adult rabbit isolated testicular capsule to increasing concentrations of Cd$^{++}$. 


Table 5

Response of the Adult Rabbit Isolated Testicular Capsule to Increasing Concentrations of Cd++

<table>
<thead>
<tr>
<th></th>
<th>Control Cd++ 10^-5 M</th>
<th>Post-treatment</th>
<th>Control Cd++ 10^-4 M</th>
<th>Post-treatment</th>
<th>Control Cd++ 10^-3 M</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (per min)</td>
<td></td>
<td>Frequency (per min)</td>
<td></td>
<td>Frequency (per min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>1.40</td>
<td>1.50</td>
<td>2.20</td>
<td>0.400</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>3.20</td>
<td>0</td>
<td>2.80</td>
<td>1.80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Amplitude (mm)</td>
<td></td>
<td>Amplitude (mm)</td>
<td></td>
<td>Amplitude (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.437</td>
<td>0.586</td>
<td>0.533</td>
<td>0.582</td>
<td>0.450</td>
<td>0.480</td>
</tr>
<tr>
<td></td>
<td>0.725</td>
<td>0</td>
<td>0.628</td>
<td>0.500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Torsion (mm)</td>
<td></td>
<td>Torsion (mm)</td>
<td></td>
<td>Torsion (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-4.0</td>
<td>-7.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-9.0</td>
<td>0</td>
<td>0</td>
<td>-4.0</td>
<td>-4.0</td>
</tr>
</tbody>
</table>

The adult rabbit isolated testicular capsule was exposed to the indicated concentration of Cd++ (CdCl₂·2H₂O) for a 5 min contact time at 37°C. Control values were re-established for 10 min following removal of the metal by washing.
Table 6. Prevention of Cd\(^{++}\)-induced inhibition of adult rabbit isolated testicular capsular spontaneous contractions by Zn\(^{++}\).
Table 6

Prevention of Cd++-induced Inhibition of Adult Rabbit Isolated Testicular Capsular Spontaneous Contractions by Zn++

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Zn++ 2 x 10^-4 M</th>
<th>Post-treatment</th>
<th>Control</th>
<th>Cd++ 10^-4 M</th>
<th>Post-treatment</th>
<th>Control</th>
<th>Zn++ 2 x 10^-4 M</th>
<th>Cd++ 10^-4 M</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (per min)</td>
<td>2.80</td>
<td>2.80</td>
<td>2.60</td>
<td>3.20</td>
<td>0</td>
<td>2.80</td>
<td>3.00</td>
<td>3.00</td>
<td>3.80</td>
<td>2.40</td>
</tr>
<tr>
<td>Amplitude (mm)</td>
<td>0.621</td>
<td>0.664</td>
<td>0.646</td>
<td>0.725</td>
<td>0</td>
<td>0.628</td>
<td>0.567</td>
<td>0.567</td>
<td>0.347</td>
<td>0.442</td>
</tr>
<tr>
<td>Tone (mm)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-9.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

The adult rabbit isolated testicular capsule was exposed to the indicated concentration of Zn++ (Zn(CH3CH2O2)2·2H2O) or Cd++ (CdCl2·2H2O) for a 5 min contact time at 37°C. The tissue was then exposed to Zn++ for 2 min, prior to the addition, without washing, of Cd++. Control periods were re-established for 10 min following removal of the metal by washing.
Table 7. Typical temperatures of hot running water in various hotels and hospitals in the United States.
Table 7

TYPICAL TEMPERATURES OF HOT RUNNING WATER IN VARIOUS HOTELS AND HOSPITALS

<table>
<thead>
<tr>
<th>HOTELS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Location</td>
<td></td>
<td></td>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hotels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOTELS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Location</td>
<td></td>
<td></td>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAN FRANCISCO, CA</td>
<td>St. Francis</td>
<td>138 °F (58.9 °C)</td>
<td></td>
<td>CHICAGO, IL</td>
<td>Sovereign</td>
<td>141 °F (60.6 °C)</td>
</tr>
<tr>
<td>LOS ANGELES, CA</td>
<td>Hyatt Regency</td>
<td>150 °F (66.6 °C)</td>
<td></td>
<td>NEW YORK, NY</td>
<td>Americana</td>
<td>140 °F (60.0 °C)</td>
</tr>
<tr>
<td></td>
<td>Century Plaza</td>
<td>152 °F (66.7 °C)</td>
<td></td>
<td></td>
<td>Hilton</td>
<td>140 °F (60.0 °C)</td>
</tr>
<tr>
<td></td>
<td>Ambassador</td>
<td>139 °F (59.4 °C)</td>
<td></td>
<td></td>
<td>Sheraton</td>
<td>145 °F (62.8 °C)</td>
</tr>
<tr>
<td>DALLAS, TX</td>
<td>Baker</td>
<td>135 °F (57.2 °C)</td>
<td></td>
<td>HOUSTON, TX</td>
<td>Hyatt Regency</td>
<td>135 °F (57.2 °C)</td>
</tr>
<tr>
<td></td>
<td>Holiday Inn Downtown</td>
<td>139 °F (59.4 °C)</td>
<td></td>
<td></td>
<td>Sheraton</td>
<td>138 °F (58.9 °C)</td>
</tr>
<tr>
<td>HOSPITALS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Location</td>
<td></td>
<td></td>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATLANTA, GA</td>
<td>Atlanta Hospital</td>
<td>125 °F (51.7 °C)</td>
<td></td>
<td>CHICAGO, IL</td>
<td>Prentice Women's Hospital</td>
<td>121 °F (49.4 °C)</td>
</tr>
<tr>
<td>NEW YORK, NY</td>
<td>Memorial Hospital</td>
<td>133 °F (56.1 °C)</td>
<td></td>
<td></td>
<td>henrotin Hospital</td>
<td>121 °F (49.4 °C)</td>
</tr>
<tr>
<td></td>
<td>Mount Sinai Hospital</td>
<td>130 °F (54.4 °C)</td>
<td></td>
<td></td>
<td>Illinois College of Podiatric Medicine</td>
<td>132 °F (55.6 °C)</td>
</tr>
<tr>
<td></td>
<td>New York Hospital</td>
<td>130 °F (54.4 °C)</td>
<td></td>
<td></td>
<td>Augustana Hospital</td>
<td>140 °F (60.0 °C)</td>
</tr>
</tbody>
</table>

Data obtained from:
Medical World News, October 17, 1977
Table 8. Summer beach water temperatures in selected northern cities of the United States.
Table 8

FIFTEEN-YEAR SUMMARY (1961-1976) OF MONTHLY BEACH WATER TEMPERATURES
OBSERVED AT SELECTED NORTHERN CITIES OF THE UNITED STATES

<table>
<thead>
<tr>
<th></th>
<th>MAY</th>
<th>JUNE</th>
<th>JULY</th>
<th>AUGUST</th>
<th>SEPTEMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Min</td>
<td>Avg</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>CHICAGO, IL</td>
<td>19.3°F (6.8°C)</td>
<td>46.7°F (8.1°C)</td>
<td>51.7°F (10.9°C)</td>
<td>63.1°F (17.3°C)</td>
<td>52.9°F (11.7°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHILADELPHIA, PA</td>
<td>59.0°F (15.0°C)</td>
<td>58.1°F (14.5°C)</td>
<td>63.7°F (17.6°C)</td>
<td>78.3°F (25.7°C)</td>
<td>65.0°F (18.3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEW YORK, NY</td>
<td>54.7°F (12.6°C)</td>
<td>50.6°F (10.4°C)</td>
<td>57.2°F (13.9°C)</td>
<td>69.1°F (20.6°C)</td>
<td>60.0°F (15.6°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOSTON, MA</td>
<td>40.3°F (4.6°C)</td>
<td>49.3°F (9.6°C)</td>
<td>53.6°F (12.0°C)</td>
<td>69.1°F (20.6°C)</td>
<td>56.2°F (13.4°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PORTLAND, OR</td>
<td>54.0°F (12.2°C)</td>
<td>63.4°F (17.4°C)</td>
<td>48.6°F (9.2°C)</td>
<td>60.2°F (15.7°C)</td>
<td>50.7°F (10.4°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from:
City of Chicago, Water Purification Division
U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration
APPENDIX A
### APPENDIX A

#### Molar Equivalent of μg/ml Final Bath Concentrations of Drugs Used in the Present Study

<table>
<thead>
<tr>
<th>μg/ml</th>
<th>Acetylcholine</th>
<th>Norepinephrine</th>
<th>Prostaglandin A&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Prostaglandin E&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Prostaglandin F&lt;sub&gt;2α&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0010</td>
<td>5.5 x 10&lt;sup&gt;-9&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.10 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.0025</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.25 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.0050</td>
<td>2.75 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.05 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.010</td>
<td>5.5 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>2.96 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>2.10 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.020</td>
<td>-</td>
<td>5.93 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.025</td>
<td>-</td>
<td>-</td>
<td>7.47 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>-</td>
<td>5.25 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.050</td>
<td>2.75 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>1.48 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>1.49 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>-</td>
<td>1.05 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.10</td>
<td>5.5 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>2.96 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>2.33 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>2.82 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>2.10 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.64 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>0.25</td>
<td>-</td>
<td>-</td>
<td>7.47 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>-</td>
<td>5.25 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.50</td>
<td>2.75 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.48 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.49 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
<td>1.05 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0</td>
<td>5.5 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.96 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.33 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.82 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.10 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>-</td>
<td>5.93 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
<td>5.64 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>7.47 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>-</td>
<td>5.25 x 10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.13 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>-</td>
<td>1.48 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>1.49 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>-</td>
<td>1.05 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>10.0</td>
<td>5.5 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>2.82 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>2.10 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>20.0</td>
<td>1.1 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25.0</td>
<td>-</td>
<td>-</td>
<td>7.05 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50.0</td>
<td>2.75 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>1.41 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>100.0</td>
<td>5.5 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>200.0</td>
<td>1.1 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
APPENDIX B
### New Zealand Albino Male Rabbit

*(Oryctolagus cuniculus)*

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight</th>
<th>Reproductive status</th>
</tr>
</thead>
<tbody>
<tr>
<td>weeks</td>
<td>months</td>
<td>kilograms</td>
</tr>
<tr>
<td>36</td>
<td>9</td>
<td>4.5</td>
</tr>
<tr>
<td>32</td>
<td>8</td>
<td>4.1</td>
</tr>
<tr>
<td>28</td>
<td>7</td>
<td>3.6</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>22</td>
<td>5½</td>
<td>3.0</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>2.7</td>
</tr>
<tr>
<td>18</td>
<td>4½</td>
<td>2.5</td>
</tr>
<tr>
<td>14</td>
<td>3½</td>
<td>2.3</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>6</td>
<td>1½</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>
APPENDIX C
APPENDIX C

EFFECT OF TEMPERATURE ON SPONTANEOUS ISOMETRIC CONTRACTIONS OF THE ADULT RABBIT TESTICULAR CAPSULE in vitro

HYPOTHERMIC TEMPERATURE CHANGE 37 °C

HYPERTHERMIC TEMPERATURE CHANGE

- T.S.E.M. OF 3 EXPS PER TEMPERATURE RANGE
- 10 MIN TIME EXPOSURE AT EACH 2 °C TEMPERATURE CHANGE

AMPLITUDE

TONE

FREQUENCY

CONTRACTION FREQUENCY / min

CONTRACTION AMPLITUDE (g/ml)

TISSUE TONE (g/ml)

TEMPERATURE °C

60.8 66.0 75.2 82.4 99.6 96.8 104.8 111.2 118.4

TEMPERATURE °F
APPENDIX D
### APPENDIX D

**COMPARATIVE RESPONSE OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE**

AERATED WITH AIR AND 95% O₂ + 5% CO₂

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>AERATED WITH AIR</th>
<th>95% O₂ + 5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm tissue contraction</td>
<td>mm tissue contraction</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>0.50</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>12.00</td>
<td>12.00</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>22.00</td>
<td>12.55</td>
</tr>
</tbody>
</table>

**NE**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>AERATED WITH AIR</th>
<th>95% O₂ + 5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm tissue contraction</td>
<td>mm tissue contraction</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>0.50</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.70</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.75</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>2.10</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.80</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>2.60</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.55</td>
</tr>
</tbody>
</table>

**ACH**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>AERATED WITH AIR</th>
<th>95% O₂ + 5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm tissue contraction</td>
<td>mm tissue contraction</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.40</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.55</td>
</tr>
<tr>
<td>2.0 µg/ml</td>
<td>1.45</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.90</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>1.60</td>
<td>2.20</td>
</tr>
</tbody>
</table>

**PGF₂α**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>AERATED WITH AIR</th>
<th>95% O₂ + 5% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm tissue contraction</td>
<td>mm tissue contraction</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.10</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>2.0 µg/ml</td>
<td>1.40</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.35</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>1.60</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20</td>
</tr>
</tbody>
</table>

Temperature of tissue bath = 37°C
Aerated with air - pH 7.7
95% O₂ + 5% CO₂ - pH 7.4

Reference which stated Tyrode's buffer should be aerated with air: Pharmacological Experiments on Isolated Preparations, Univ. Edinburgh, 1970.
APPENDIX E
## Appendix E

### Comparative Response of the Adult Rabbit Isolated Testicular Capsule Aerated with Air and 95% O₂ + 5% CO₂

#### Aerated with Air

<table>
<thead>
<tr>
<th>Drug dosage (µg/ml)</th>
<th>Frequency (per min)</th>
<th>Amplitude (mm)</th>
<th>Drug dosage (µg/ml)</th>
<th>Frequency (per min)</th>
<th>Amplitude (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>AFTER DRUG</td>
<td>CONTROL</td>
<td>AFTER DRUG</td>
<td>CONTROL</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>2.00</td>
<td>1.80</td>
<td>0.49</td>
<td>0.54</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>1.20</td>
<td>1.40</td>
<td>0.57</td>
<td>0.61</td>
<td>1.60</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>2.20</td>
<td>2.20</td>
<td>0.44</td>
<td>0.54</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>1.86</td>
<td>1.14</td>
<td>0.32</td>
<td>1.60</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.80</td>
<td>2.00</td>
<td>0.55</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>1.60</td>
<td>0.47</td>
<td>0.57</td>
<td>0.60</td>
</tr>
<tr>
<td>2.0 µg/ml</td>
<td>2.00</td>
<td>2.00</td>
<td>0.30</td>
<td>0.29</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.70</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>2.00</td>
<td>2.00</td>
<td>0.53</td>
<td>0.29</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>1.40</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>1.40</td>
<td>1.40</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
</tbody>
</table>

#### Aerated with 95% O₂ + 5% CO₂

<table>
<thead>
<tr>
<th>Drug dosage (µg/ml)</th>
<th>Frequency (per min)</th>
<th>Amplitude (mm)</th>
<th>Drug dosage (µg/ml)</th>
<th>Frequency (per min)</th>
<th>Amplitude (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>AFTER DRUG</td>
<td>CONTROL</td>
<td>AFTER DRUG</td>
<td>CONTROL</td>
</tr>
<tr>
<td>0.1 µg/ml</td>
<td>2.00</td>
<td>1.80</td>
<td>0.49</td>
<td>0.54</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>1.20</td>
<td>1.40</td>
<td>0.57</td>
<td>0.61</td>
<td>1.60</td>
</tr>
<tr>
<td>0.5 µg/ml</td>
<td>2.20</td>
<td>2.20</td>
<td>0.44</td>
<td>0.54</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>1.86</td>
<td>1.14</td>
<td>0.32</td>
<td>1.60</td>
</tr>
<tr>
<td>1.0 µg/ml</td>
<td>1.80</td>
<td>2.00</td>
<td>0.55</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>1.60</td>
<td>0.47</td>
<td>0.57</td>
<td>0.60</td>
</tr>
<tr>
<td>2.0 µg/ml</td>
<td>2.00</td>
<td>2.00</td>
<td>0.30</td>
<td>0.29</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.70</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>5.0 µg/ml</td>
<td>2.00</td>
<td>2.00</td>
<td>0.53</td>
<td>0.29</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>1.40</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>10.0 µg/ml</td>
<td>1.40</td>
<td>1.40</td>
<td>0.50</td>
<td>0.20</td>
<td>1.00</td>
</tr>
</tbody>
</table>

---

Temperature of tissue bath - 37°C
Aerated with air - pH 7.7
95% O₂ + 5% CO₂ - pH 7.4

Reference which stated Tyrode's buffer should be aerated with air: Pharmacological Experiments on Isolated Preparations, Univ. Edinburgh, 1970.
The dissertation submitted by Arthur M. Horowitz has been read and approved by the following committee:

Dr. Joseph R. Davis, Chairman
Professor, Pharmacology, Loyola

Dr. Michael A. Collins
Associate Professor, Biochemistry, Loyola

Dr. Thomas L. C. Cottrell
Assistant Professor, Urology, Loyola

Dr. Nae J. Dun
Assistant Professor, Pharmacology, Loyola

Dr. Silas N. Glisson
Associate Professor, Pharmacology, Loyola

Dr. Charles C. C. O'Morchoe
Professor, Anatomy, Loyola

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 18, 1979
Date

Joseph R. Davis, M.D., Ph.D.
Director's Signature