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Pharmacological and Anatomical Studies of the Testicular Capsule

George A. Langford
Loyola University Chicago

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PHARMACOLOGICAL AND ANATOMICAL STUDIES
OF THE TESTICULAR CAPSULE

by
George A. Langford B.Sc., M.Sc.

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

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George A. Langford was born in Vancouver, British Columbia, Canada on April 2, 1941. He is married and has one son.

Mr. Langford began his studies at The University of British Columbia where he received both the B.Sc. degree (zoology and biochemistry, 1964) and the M.Sc. degree (zoology, 1968). In November, 1967, Mr. Langford continued his graduate studies at Loyola University Stritch School of Medicine in the Department of Pharmacology. In January, 1971, he became Senior Investigator in the Division of Reproductive Physiology at the Pacific Northwest Research Foundation in Seattle, Washington.

During his graduate training at The University of British Columbia, Mr. Langford studied the nature of the morphological changes in the germinal epithelium of the testis during various experimental conditions. At Loyola, he has been concerned with biochemical and pharmacological parameters of the testis.

The academic career of Mr. Langford has coincided with eight years of active service in The Royal Canadian Naval Reserve. He received the Queen's Commission in 1964 and reached the rank of Lieutenant by 1965. During his service with the R.C.N.R., Lt. Langford spent time on both the east and west coasts of Canada, and became specialized in marine navigation.

Mr. Langford is a full member (1968) of The Society for the Study of Reproduction. In June, 1971, he was awarded a Postdoctoral Fellowship by the Medical Research Council of Canada.
BIBLIOGRAPHY


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It is also with pride but with deep regret that I acknowledge the excellent research and accomplishments of Patrick J. Kirby on the biochemistry of the testicular capsule - which was tragically halted by his recent death from cancer. Pat was a rare and courageous person whom I shall never forget.

There are many other fine people whom I would like to acknowledge: my father, Mr. Albert E. Langford, for steering me toward this goal; Professor Alexander G. Karczmar, Chairman of The Department of Pharmacology; Dr. Alexander H. Friedman, for his encouragement and help; Professor Joseph T. Velardo, Chairman of The Department of Anatomy; Miss Helen Huelsman, for her help as a Librarian and as a friend; and to the other members of my dissertation committee.

My final acknowledgment belongs to my wonderful wife, Sonia and my son, Michael who have made everything now worth while, and worth looking ahead to.
ABSTRACT

The testicular capsule of the adult rat, rabbit and human has been prepared for the first time as an intact isolated tissue which is suitable for the investigation of effects of pharmacological drugs. Various autonomic drugs were found to produce a contraction or relaxation of the isolated testicular capsule, the predominant response being a contraction. In addition, periodic spontaneous contractions of the isolated testicular capsule of the adult rabbit and human were observed in the absence of any added pharmacological agent. A testicular capsule contraction was stimulated by parasympathomimetic drugs, sympathomimetic drugs stimulating alpha receptor sites, ganglionic stimulating drugs, histamine, direct-acting smooth muscle stimulants, oxytocin and vasopressin. A testicular capsule relaxation was produced by sympathomimetic drugs stimulating beta receptor sites. Experiments utilizing pharmacological blocking drugs suggest that each stimulatory drug may occupy its own specific receptor site on the effector tissue in the capsule. Histological examination of the three types of testicular capsules revealed the presence of smooth muscle, offering a reasonable anatomical explanation for both spontaneous and drug-induced contractions of the testicular capsule. The present pharmacological data suggest the possibility that both sympathetic and parasympathetic nerve fibers may innervate these smooth muscle fibers of the testicular capsule.
The isolated intact whole testis was also shown to undergo rhythmic spontaneous contractions. In contrast, the isolated testicular parenchymal tissue does not spontaneously contract. It therefore appears that the testicular capsule alone is responsible for the endogenous spontaneous contractions of the whole testis. It seems very likely that these rhythmic contractions and relaxations of the testicular capsule serve to massage the seminiferous tubules, providing a pumping action which may assist lymphatic and venous drainage of the testis, may influence the secretion of testosterone from the Leydig cells or may transport the non-motile sperm from the seminiferous tubules toward the efferent ducts out of the testis and into the epididymis where the sperm then attain their motility. The possible therapeutic usefulness of testicular capsule contractions is discussed involving the administration of drugs shown to cause contraction of the testicular capsule to stimulate the transport of sperm out of the testis in some cases of male infertility. Conversely, it is suggested that a possible approach to male contraception may involve the administration of drugs which may cause prolonged relaxation of the testicular capsule thereby preventing the sperm-propelling action of the testicular capsule. Moreover, as a separate intact tissue, the isolated testicular capsule appears to offer a unique opportunity in screening drugs for possible pharmacological activity relating to testicular physiology.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>STATEMENT OF THE PROBLEM</td>
</tr>
<tr>
<td>II</td>
<td>REVIEW OF THE LITERATURE</td>
</tr>
<tr>
<td>A.</td>
<td>MORPHOLOGY OF THE TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>1.</td>
<td>GENERAL CONSIDERATIONS</td>
</tr>
<tr>
<td>2.</td>
<td>TUNICA VAGINALIS VISCERAL</td>
</tr>
<tr>
<td>3.</td>
<td>TUNICA ALBUGINEA</td>
</tr>
<tr>
<td>4.</td>
<td>TUNICA VASCULOSA</td>
</tr>
<tr>
<td>5.</td>
<td>MEDIASTINUM TESTIS</td>
</tr>
<tr>
<td>B.</td>
<td>COLLAGENOUS TISSUE OF THE TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>C.</td>
<td>INNERVATION OF THE TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>1.</td>
<td>GENERAL CONSIDERATIONS</td>
</tr>
<tr>
<td>2.</td>
<td>TUNICA VAGINALIS VISCERAL</td>
</tr>
<tr>
<td>3.</td>
<td>TUNICA ALBUGINEA</td>
</tr>
<tr>
<td>4.</td>
<td>TUNICA VASCULOSA</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxiv</td>
</tr>
<tr>
<td>Chapter</td>
<td>PATHOLOGY OF THE TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>D.</td>
<td>1. HYDROCELE</td>
</tr>
<tr>
<td></td>
<td>2. TUMORS OF THE TESTICULAR CAPSULE</td>
</tr>
<tr>
<td></td>
<td>3. MISCELLANEOUS LESIONS OF THE</td>
</tr>
<tr>
<td></td>
<td>TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>E.</td>
<td>MORPHOLOGY OF SMOOTH MUSCLE</td>
</tr>
<tr>
<td></td>
<td>1. INTRODUCTION.</td>
</tr>
<tr>
<td></td>
<td>2. GENERAL MORPHOLOGY.</td>
</tr>
<tr>
<td></td>
<td>3. SPECIALIZED REGIONS OF CONTACT.</td>
</tr>
<tr>
<td></td>
<td>4. FINE STRUCTURE OF THE SMOOTH</td>
</tr>
<tr>
<td></td>
<td>MUSCLE CELL</td>
</tr>
<tr>
<td></td>
<td>5. FINE STRUCTURE OF THE CONTRACTILE</td>
</tr>
<tr>
<td></td>
<td>APPARATUS</td>
</tr>
<tr>
<td>F.</td>
<td>NEUROMUSCULAR TRANSMISSION OF SMOOTH</td>
</tr>
<tr>
<td></td>
<td>MUSCLE.</td>
</tr>
<tr>
<td></td>
<td>1. INTRODUCTION.</td>
</tr>
<tr>
<td></td>
<td>2. AUTONOMIC NEUROMUSCULAR</td>
</tr>
<tr>
<td></td>
<td>TRANSMITTERS.</td>
</tr>
<tr>
<td></td>
<td>a. Acetylcholine.</td>
</tr>
<tr>
<td></td>
<td>b. Norepinephrine.</td>
</tr>
<tr>
<td>G.</td>
<td>INNERRVATION OF SMOOTH MUSCLE</td>
</tr>
<tr>
<td></td>
<td>1. INTRODUCTION.</td>
</tr>
<tr>
<td></td>
<td>2. MORPHOLOGY OF AUTONOMIC INNERRVATION</td>
</tr>
<tr>
<td></td>
<td>3. VARIATIONS OF AUTONOMIC INNERRVATION</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>4. SYMPTOMATIC VESICLES OF AUTONOMIC NERVES</td>
<td>47</td>
</tr>
<tr>
<td>a. Small Granular Vesicles</td>
<td>47</td>
</tr>
<tr>
<td>b. Small Agranular Vesicles</td>
<td>48</td>
</tr>
<tr>
<td>c. Large Granular Vesicles</td>
<td>50</td>
</tr>
<tr>
<td>H. ELECTRICAL PROPERTIES OF SMOOTH MUSCLE</td>
<td>51</td>
</tr>
<tr>
<td>III MATERIALS AND METHODS</td>
<td>56</td>
</tr>
<tr>
<td>A. EXPERIMENTAL CONDITIONS</td>
<td>57</td>
</tr>
<tr>
<td>1. TISSUES</td>
<td>57</td>
</tr>
<tr>
<td>a. Animal</td>
<td>57</td>
</tr>
<tr>
<td>b. Human</td>
<td>57</td>
</tr>
<tr>
<td>2. ISOLATED TISSUE BATH ASSEMBLY</td>
<td>58</td>
</tr>
<tr>
<td>3. PHYSIOLOGICAL SALT SOLUTIONS</td>
<td>59</td>
</tr>
<tr>
<td>a. Tyrode's Solution</td>
<td>59</td>
</tr>
<tr>
<td>b. De Jalon's Solution</td>
<td>60</td>
</tr>
<tr>
<td>c. Krebs-Henseleit Solution</td>
<td>60</td>
</tr>
<tr>
<td>B. PROCEDURES FOR THE ISOLATION OF THE TESTICULAR CAPSULE AND RELATED TISSUES FOR PHARMACOLOGICAL STUDIES</td>
<td>62</td>
</tr>
<tr>
<td>1. ADULT RAT ISOLATED TESTICULAR CAPSULE PREPARATION</td>
<td>62</td>
</tr>
<tr>
<td>2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE PREPARATION</td>
<td>64</td>
</tr>
<tr>
<td>3. HUMAN ISOLATED TESTICULAR CAPSULE PREPARATION</td>
<td>64</td>
</tr>
<tr>
<td>Chapter</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4.</td>
<td>ADULT RAT AND RABBIT ISOLATED TESTICULAR PARENCHYMAL TISSUE PREPARATION</td>
</tr>
<tr>
<td>5.</td>
<td>ADULT RAT AND RABBIT ISOLATED WHOLE TESTIS PREPARATION</td>
</tr>
<tr>
<td>6.</td>
<td>ADULT RAT ISOLATED EPIDIDYMIS PREPARATION</td>
</tr>
<tr>
<td>7.</td>
<td>ADULT RAT ISOLATED VAS DEFERENS PREPARATION</td>
</tr>
<tr>
<td>8.</td>
<td>ADULT GUINEA PIG ISOLATED ILEUM PREPARATION</td>
</tr>
<tr>
<td>9.</td>
<td>ADULT RABBIT ISOLATED DUODENUM PREPARATION</td>
</tr>
<tr>
<td>C.</td>
<td>PHARMACOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE</td>
</tr>
<tr>
<td>1.</td>
<td>DETERMINATION OF THE RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO</td>
</tr>
<tr>
<td></td>
<td>AUTONOMIC DRUGS</td>
</tr>
<tr>
<td>a.</td>
<td>Parasympathetic Stimulating Drugs</td>
</tr>
<tr>
<td>b.</td>
<td>Sympathetic Stimulating Drugs</td>
</tr>
<tr>
<td>c.</td>
<td>Ganglionic Stimulating Drugs</td>
</tr>
<tr>
<td>d.</td>
<td>Histamine</td>
</tr>
<tr>
<td>e.</td>
<td>Direct-acting Smooth Muscle Stimulants</td>
</tr>
<tr>
<td>f.</td>
<td>Naturally occurring Hormones Known to Effect, Smooth Muscle</td>
</tr>
<tr>
<td>2.</td>
<td>DETERMINATION OF THE EFFECT OF PHARMACOLOGICAL BLOCKING DRUGS ON THE</td>
</tr>
<tr>
<td></td>
<td>RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>a. Parasympathetic Blocking Drug</td>
<td>71</td>
</tr>
<tr>
<td>b. Sympathetic Blocking Drug.</td>
<td>72</td>
</tr>
<tr>
<td>c. Ganglionic Blocking Drug.</td>
<td>72</td>
</tr>
<tr>
<td>d. Antihistamine</td>
<td>72</td>
</tr>
<tr>
<td>e. Skeletal Neuromuscular Blocking Drug</td>
<td>72</td>
</tr>
<tr>
<td>f. Direct-acting Smooth Muscle Blocking Drug</td>
<td>72</td>
</tr>
<tr>
<td>3. DETERMINATION OF THE EFFECT OF PHARMACOLOGICAL BLOCKING DRUGS ON</td>
<td>73</td>
</tr>
<tr>
<td>SPONTANEOUS CONTRACTIONS OF THE ISOLATED TESTICULAR CAPSULE.</td>
<td></td>
</tr>
<tr>
<td>a. Parasympathetic Blocking Drug</td>
<td>73</td>
</tr>
<tr>
<td>b. Sympathetic Blocking Drug.</td>
<td>73</td>
</tr>
<tr>
<td>c. Direct-acting Smooth Muscle Blocking Drug</td>
<td>73</td>
</tr>
<tr>
<td>d. Skeletal Neuromuscular Blocking Drug</td>
<td>74</td>
</tr>
<tr>
<td>D. ANATOMICAL STUDIES ON THE TESTICULAR CAPSULE.</td>
<td>75</td>
</tr>
<tr>
<td>1. LIGHT MICROSCOPY</td>
<td>75</td>
</tr>
<tr>
<td>a. Tissue Preparation</td>
<td>75</td>
</tr>
<tr>
<td>b. Staining Procedures</td>
<td>77</td>
</tr>
<tr>
<td>2. ELECTRON MICROSCOPY</td>
<td>80</td>
</tr>
<tr>
<td>E. PHYSIOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE</td>
<td>82</td>
</tr>
</tbody>
</table>
# Chapter

## 1. EFFECT OF TEMPERATURE ON THE ADULT RAT ISOLATED TESTICULAR CAPSULE

Page 82

## 2. EFFECT OF TEMPERATURE ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Page 83

## 3. EFFECT OF CRYPTORCHIDISM ON THE RESPONSE OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS

Page 83

## F. QUANTITATIVE BIOASSAY OF ATROPINE USING THE ISOLATED TESTICULAR CAPSULE

Page 85

### IV RESULTS

Page 88

#### A. ISOLATION OF THE TESTICULAR CAPSULE

Page 89

1. ADULT RAT ISOLATED TESTICULAR CAPSULE

Page 89

2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE

Page 90

3. HUMAN ISOLATED TESTICULAR CAPSULE

Page 91

#### B. RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS

Page 91

1. ADULT RAT ISOLATED TESTICULAR CAPSULE

Page 91

   a. Response of the Testicular Capsule to Acetylcholine and Norepinephrine

Page 91

   b. Recovery Time of the Testicular Capsule Following the Response of the Capsule to Acetylcholine

Page 92

   c. Influence of Physiological Salt Solutions on the Response of the Testicular Capsule to Acetylcholine

Page 93
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>d. Influence of Resting Tensions on the Response of the Testicular</td>
<td>94</td>
</tr>
<tr>
<td>Capsule to Acetylcholine</td>
<td></td>
</tr>
<tr>
<td>e. Response of the Testicular</td>
<td>95</td>
</tr>
<tr>
<td>Capsule to Autonomic Drugs</td>
<td></td>
</tr>
<tr>
<td>2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE</td>
<td>98</td>
</tr>
<tr>
<td>a. Spontaneous Contractions</td>
<td>98</td>
</tr>
<tr>
<td>b. Influence of Resting Tensions on Spontaneous Contractions of the</td>
<td>100</td>
</tr>
<tr>
<td>Testicular Capsule</td>
<td></td>
</tr>
<tr>
<td>c. Response of the Testicular</td>
<td>100</td>
</tr>
<tr>
<td>Capsule versus the Testicular</td>
<td></td>
</tr>
<tr>
<td>Parenchymal Tissue to Autonomic</td>
<td></td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
</tr>
<tr>
<td>d. Response of the Isolated Whole</td>
<td>102</td>
</tr>
<tr>
<td>Testis to Acetylcholine and</td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
</tr>
<tr>
<td>e. Response of the Testicular</td>
<td>103</td>
</tr>
<tr>
<td>Capsule versus the Testicular</td>
<td></td>
</tr>
<tr>
<td>Parenchymal Tissue of the Adult Rat to Autonomic Drugs</td>
<td></td>
</tr>
<tr>
<td>3. HUMAN ISOLATED TESTICULAR CAPSULE</td>
<td>105</td>
</tr>
<tr>
<td>a. Spontaneous Contractions of the Testicular Capsule</td>
<td>105</td>
</tr>
<tr>
<td>b. Response of the Testicular</td>
<td>105</td>
</tr>
<tr>
<td>Capsule to Autonomic Drugs</td>
<td></td>
</tr>
<tr>
<td>C. ANATOMY OF THE TESTICULAR CAPSULE</td>
<td>106</td>
</tr>
<tr>
<td>1. ADULT RAT TESTICULAR CAPSULE</td>
<td>106</td>
</tr>
<tr>
<td>a. Identification and Distribution of Smooth Muscle in the Tunica</td>
<td>106</td>
</tr>
<tr>
<td>Albuginea</td>
<td></td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>b. Fine Structure of the Collagenous Tissue of the Tunica Albuginea</td>
<td>108</td>
</tr>
<tr>
<td>2. ADULT RABBIT TESTICULAR CAPSULE</td>
<td>109</td>
</tr>
<tr>
<td>a. Identification and Distribution of Smooth Muscle in the Tunica Albuginea</td>
<td>109</td>
</tr>
<tr>
<td>3. HUMAN TESTICULAR CAPSULE</td>
<td>110</td>
</tr>
<tr>
<td>a. Identification and Distribution of Smooth Muscle in the Tunica Albuginea</td>
<td>110</td>
</tr>
<tr>
<td>D. EFFECT OF AUTONOMIC BLOCKING DRUGS ON THE RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS</td>
<td>112</td>
</tr>
<tr>
<td>1. ADULT RAT ISOLATED TESTICULAR CAPSULE</td>
<td>112</td>
</tr>
<tr>
<td>a. Parasympathetic Postganglionic Blocking Drug</td>
<td>112</td>
</tr>
<tr>
<td>b. Ganglionic Blocking Drug</td>
<td>113</td>
</tr>
<tr>
<td>c. Sympathetic Blocking Drugs</td>
<td>115</td>
</tr>
<tr>
<td>d. Antihistamine</td>
<td>117</td>
</tr>
<tr>
<td>e. Skeletal Neuromuscular Blocking Drug</td>
<td>117</td>
</tr>
<tr>
<td>f. Direct-acting Smooth Muscle Blocking Drug</td>
<td>117</td>
</tr>
<tr>
<td>2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE</td>
<td>118</td>
</tr>
<tr>
<td>a. Effect of Pharmacological Blocking Drugs on Spontaneous Contractions of the Isolated Testicular Capsule</td>
<td>118</td>
</tr>
<tr>
<td>E. FACTORS INFLUENCING THE TESTICULAR CAPSULE</td>
<td>119</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1. TEMPERATURE</td>
<td>119</td>
</tr>
<tr>
<td>a. Effect of Temperature on Contraction of the Adult Rat Isolated Testicular Capsule</td>
<td>119</td>
</tr>
<tr>
<td>b. Effect of Temperature on Spontaneous Contractions of the Adult Rabbit Isolated Testicular Capsule</td>
<td>120</td>
</tr>
<tr>
<td>2. CRYPTORCHIDISM</td>
<td>120</td>
</tr>
<tr>
<td>a. Effect of Cryptorchidism on the Response of the Adult Rat Isolated Testicular Capsule to Autonomic Drugs</td>
<td>120</td>
</tr>
<tr>
<td>3. ANOXIA</td>
<td>121</td>
</tr>
<tr>
<td>a. Effect of Anoxia on Spontaneous Contractions of the Adult Rabbit Isolated Testicular Capsule, Isolated Whole Testis and Isolated Duodenum</td>
<td>121</td>
</tr>
<tr>
<td>4. IRREGULAR SPONTANEOUS CONTRACTIONS OF THE ISOLATED TESTICULAR CAPSULE</td>
<td>123</td>
</tr>
<tr>
<td>5. STRETCHING</td>
<td>123</td>
</tr>
<tr>
<td>a. Comparison of the Effect of Stretching of the Isolated Rat Testicular Capsule versus the Isolated Guinea Pig Ileum on the Response to Acetylcholine</td>
<td>124</td>
</tr>
<tr>
<td>F. QUANTITATIVE BIOASSAY OF ATROPINE USING THE ISOLATED TESTICULAR CAPSULE</td>
<td>125</td>
</tr>
<tr>
<td>1. LEFT ISOLATED TESTICULAR CAPSULE OF THE ADULT RAT</td>
<td>125</td>
</tr>
<tr>
<td>a. Data Terminology</td>
<td>125</td>
</tr>
<tr>
<td>b. Data</td>
<td>125</td>
</tr>
<tr>
<td>c. Graphic Solution</td>
<td>126</td>
</tr>
</tbody>
</table>
# 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 testicular capsule of the adult rat and its use as an isolated tissue preparation for pharmacological studies.</td>
<td>180</td>
</tr>
<tr>
<td>2.</td>
<td>Photograph of the isolated testicular capsule of the adult rat.</td>
<td>182</td>
</tr>
<tr>
<td>3.</td>
<td>Photograph of the isolated tissue bath assembly employed for measurement of contractions of the isolated testicular capsule.</td>
<td>184</td>
</tr>
<tr>
<td>4.</td>
<td>Comparison of the size of the isolated testicular capsule of the adult rat with the removed seminiferous tubular mass; and photograph of the intact isolated testicular capsule in the inside-out position as it would appear following its removal from an isolated tissue bath.</td>
<td>186</td>
</tr>
<tr>
<td>5.</td>
<td>Contraction of the isolated testicular capsule of the adult rat with various doses of acetylcholine and norepinephrine.</td>
<td>188</td>
</tr>
<tr>
<td>6.</td>
<td>Dose-response curve for the effect of acetylcholine and norepinephrine on the isolated testicular capsule of the adult rat.</td>
<td>190</td>
</tr>
<tr>
<td>7.</td>
<td>Time taken for the adult rat isolated testicular capsule to return to the initial resting tension following washing out of various concentrations of acetylcholine.</td>
<td>192</td>
</tr>
<tr>
<td>8.</td>
<td>Time taken for the adult guinea pig isolated ileum to return to the initial resting tension following washing out of 0.01 µg/ml acetylcholine.</td>
<td>194</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>9. Influence of various resting tensions (load) on the response of the isolated testicular capsule to acetylcholine</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>10. Response of the isolated testicular capsule of the adult rat to various autonomic drugs</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>11. Comparison of the effect of isoproterenol on the adult rat isolated testicular capsule during contraction of the capsule in response to acetylcholine and norepinephrine</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>12. Representative preparations of the adult rabbit testis used for pharmacological studies</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>13. Spontaneous contractions of the adult rabbit testis in the absence of any added drugs</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>14. Effect of load on spontaneous contractions of the isolated whole testis and the isolated testicular capsule of the adult rabbit</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>15. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rabbit testis to various autonomic drugs</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>16. Photographs of the isolated capsule and parenchymal tissue of the adult rat testis and the adult rabbit testis used for pharmacological studies</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>17. Response of the isolated whole testes of the adult rat and adult rabbit to acetylcholine and norepinephrine</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>18. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rat testis to various autonomic drugs</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>19. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rat testis to oxytocin and vasopressin.</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>20. Schematic representation of the procedure used in the isolation of the human testicular capsule and its use as an isolated tissue preparation for pharmacological studies.</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>21. Spontaneous contractions of the isolated testicular capsule of the human in the absence of any added drugs.</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>22. Effect of autonomic drugs on the isolated testicular capsule of the human.</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>23. Schematic diagram of the various layers of the testicular capsule and the scrotum.</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>24. Representative cross sections of the testicular capsule of the adult rat employing Masson's trichrome stain.</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>25. Smooth muscle cells of the tunica albuginea of the adult rat testis.</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>26. Relationship of the mediastinal region of the tunica albuginea to the rete testis and vasa efferentia of the adult rat testis.</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>27. Electron microscopy of collagen fibrils in the tunica albuginea of the adult rat testis.</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>28. Cross sections of the testicular capsule of the adult rabbit illustrating the distribution of smooth muscle in the tunica albuginea.</td>
<td>234</td>
<td></td>
</tr>
<tr>
<td>29. Cross sections of the human testicular capsule illustrating the presence of smooth muscle in the tunica albuginea.</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>30. Representative sections of the testicular capsules of the rat, rabbit and human stained with Masson's trichrome stain and illustrating the comparative thickness of each capsule as well as the presence of smooth muscle.</td>
<td>238</td>
<td></td>
</tr>
<tr>
<td>31. Color photomicrographs of the testicular capsules of the rat, rabbit and human stained with Masson's trichrome stain and illustrating the presence of smooth muscle</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>32. Effect of atropine sulfate on the response of the adult rat isolated testicular capsule to acetylcholine</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>33. Effect of tetraethylammonium on the response of the adult rat isolated testicular capsule to tetramethylammonium</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>34. Effect of various pharmacological antagonists on the percent response of the adult rat isolated testicular capsule to pharmacological agonists</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>35. Initial stimulatory response of the adult rat isolated testicular capsule to two adrenergic blockers, propranolol and phenoxybenzamine</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>36. Comparison of actual millimeters of tissue contraction produced by various temperatures acting on the isolated testicular capsule of the adult rat</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>37. Comparison of the response of the isolated capsules from the right cryptorchid testis and the contralateral left scrotal testis of a 650 day old rat to various autonomic drugs</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>38. Effect of various temperatures on the spontaneous contractions of the adult rabbit isolated testicular capsule</td>
<td>254</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>39. The nature of the inhibitory effect of low and high temperatures on spontaneous contractions of the adult rabbit isolated testicular capsule</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>40. Effect of anoxia on the tone and spontaneous contractions of the adult rabbit isolated testicular capsule</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>41. Effect of anoxia on tone and spontaneous contractions of the adult rabbit isolated whole testis</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>42. Effect of anoxia on tone and spontaneous contractions of the adult rabbit isolated duodenum</td>
<td>262</td>
<td></td>
</tr>
<tr>
<td>43. Irregular spontaneous contractions of the adult rabbit isolated testicular capsule in the absence of any drugs</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>44. Effect of preliminary stretching of the isolated testicular capsule of the adult rat on the response of the isolated capsule to acetylcholine</td>
<td>266</td>
<td></td>
</tr>
<tr>
<td>45. Effect of preliminary stretching of the adult guinea pig isolated ileum on the response of the ileum to acetylcholine</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>46. Use of the adult rat isolated testicular capsule for bioassay of an unknown concentration of atropine sulfate</td>
<td>270</td>
<td></td>
</tr>
<tr>
<td>47. Graphical analysis of the data obtained from the bioassay of atropine sulfate shown in figure 46</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>48. Use of the adult rat isolated testicular capsule for bioassay of an unknown concentration of atropine sulfate</td>
<td>274</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>49.</td>
<td>Graphical analysis of the data obtained from the bioassay of atropine shown in figure 48.</td>
<td>276</td>
</tr>
<tr>
<td>50.</td>
<td>Response of the isolated vas deferens (pars inguinalis) of the adult rat to various autonomic drugs.</td>
<td>278</td>
</tr>
<tr>
<td>51.</td>
<td>Comparison of the response of the adult rat isolated vas deferens and isolated epididymis to carbachol and norepinephrine</td>
<td>280</td>
</tr>
</tbody>
</table>
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Comparison of the contraction of the adult rat isolated testicular capsule in various physiological salt solutions</td>
<td>281</td>
</tr>
<tr>
<td>2.</td>
<td>ID₅₀ of pharmacological antagonists in inhibiting the response of the adult rat isolated testicular capsule to various agonists</td>
<td>282</td>
</tr>
<tr>
<td>3.</td>
<td>Effect of blocking agents on the response of the adult rat isolated testicular capsule to various autonomic drugs</td>
<td>283</td>
</tr>
</tbody>
</table>
CHAPTER I

STATEMENT OF THE PROBLEM
The testicular capsule in virtually every anatomical representation of the testis has only been indicated by a simple line drawn around this organ. In many references, the testicular capsule is commonly referred to as the "tunica albuginea", even though the tunica albuginea is but one of three layers comprising the complete capsular membrane. Moreover, the very brief histological descriptions of the testicular capsule which are available in the standard textbooks of anatomy imply only that it is a membrane composed of dense white fibrous tissue.

It has long been assumed that the testicular capsule merely forms an outer covering of the testis with no other function than to contain the underlying seminiferous tubular mass. However, recent data from this laboratory has indicated that not only is it possible to obtain the testicular capsule in an intact isolated form, but that the testicular capsule is capable of contractions when exposed to acetylcholine and norepinephrine (Davis and Langford, 1969). These observations made it apparent that the testicular capsule may indeed be a most important component of the testis and could no longer be considered a mere covering to be ignored during the study of the seminiferous epithelium and interstitial tissue.
Precisely what the physiological function of the testicular capsule is, remains unknown. However, by isolating the testicular capsule as a separate intact tissue, a unique opportunity is provided to study the physiology and pharmacology of a component of the testis which has previously been neglected.

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

1) To establish for the first time that the testicular capsule is not an inert tissue but rather is a highly dynamic tissue capable of responding to pharmacological agents.

2) To elucidate physiological and pharmacological properties of the testicular capsule in its isolated intact state, using in vitro tissue techniques.

3) To provide a reasonable anatomical explanation for the observed contractions of the testicular capsule when exposed to exogenous pharmacological agents.

4) To investigate the possible role of the testicular capsule in the regulation of various aspects of testicular physiology.
CHAPTER II

REVIEW OF THE LITERATURE
A. MORPHOLOGY OF THE TESTICULAR CAPSULE

1. GENERAL CONSIDERATIONS

The testicular capsule surrounding the parenchymal tissue of the testis consists of three tissue layers. All too often, though, the layers of the scrotum have been erroneously included as part of the outer capsular covering of the testis. Figure 23 presents a typical schematic diagram of the various layers of the testicular capsule and scrotum.

The seven layers of the scrotum include the skin, dartos muscle, superficial perineal fascia, external spermatic fascia, cremasteric fascia, internal spermatic fascia and the tunica vaginalis parietal. The three layers of the testicular capsule include (1) the tunica vaginalis visceral which is an outer thin serous layer, (2) the tunica albuginea which forms the substance of virtually the entire capsule and (3) the tunica vasculosa which is a thin delicate layer of loose areolar tissue directly beneath the tunica albuginea. The scrotal layers are separated from the testicular capsule by the cavity of the tunica vaginalis.

The tunica albuginea in most mammalian species gives off numerous very thin septa which radiate down into the testicular
parenchymal tissue. These septa which are also known as trabeculae divide the testis into many lobules and form a connective tissue support for the passage of blood vessels to the substance of the gland. It is interesting to note that the testis of the rat, however, has no septa (Albert, 1961). The tunica albuginea becomes widened along the posterior border of the testis where it is known as the mediastinum testis. The mediastinal area of the tunica albuginea serves to contain the channels of the rete testis as well as the initial portions of the vasa efferentia.

2. **TUNICA VAGINALIS VISCERAL**

The extremely thin outer layer of the testicular capsule is known as the tunica vaginalis visceral as diagrammed in figure 23. The tunica vaginalis is formed by a downward growth of the vaginalis process of the peritoneum as described by Gier and Marion (1969). At the stage of development when the testis still lies behind the peritoneum in the abdomen, a diverticulum called the vaginalis process, passes over the pubic bone into both halves of the scrotum. The testes later pass down into the scrotum to occupy a position behind and outside the cavity of the tunica vaginalis in approximately the same relationship with the cavity as they had with the peritoneal cavity before their descent into the scrotum. As indicated by Gier and Marion, the
entire testis is completely invested by the cavity except on its posterior border. In the adult, it is the smallest serous cavity derived from coelomic tissue, and in most animals, communicates directly with the peritoneal cavity. In the human and in arthropod apes, however, the cavity becomes discontinuous with the peritoneal cavity and covers only the testis, epididymis and lower part of the spermatic cord due to an obliteration of the stalk of the vaginalis process. A serous fluid forms in the cavity in both man and animals. In animals, the fluid can drain through the patent vaginalis process (Allen, 1946) but in man, a lymphatic drainage system in the parietal lamina of the tunica vaginalis develops due to the obliteration of the proximal region of the vaginalis process (Allen, 1943, 1946).

The ultrastructure of the tunica vaginalis visceral has been investigated by Leeson and Adamson (1962). These authors have concluded that in the rat and rabbit, as well as in the human, the tunica vaginalis visceral is a complete layer composed of mesothelial cells that are quite attenuated. In the rat and rabbit, the thickness of the tunica vaginalis visceral is 200 μ or less, while in the human, the thickness of this layer approximates 500 μ. The tunica vaginalis visceral can be so thin that according to Leeson and Adamson, on occasion it may be beyond the resolution of the light microscope which may explain why some authors have considered it to be an incomplete layer. The nuclei
of the mesothelial cells comprising the tunica vaginalis visceral are flattened and protrude to only a slight extent into the cavity of the tunica vaginalis. The mesothelial cells of the tunica vaginalis visceral contain small mitochondria which are few in number along with a very sparse endoplasmic reticulum. Mitotic figures are virtually non-existent in these cells. These authors have described numerous microvesicles occurring in the attenuated cytoplasm of the tunica vaginalis visceral. In addition, microvilli extend into the cavity of the tunica vaginalis. These microvilli are much shorter and less numerous in the rat and rabbit as compared to the human. The cell interfaces between adjacent cells of the tunica vaginalis visceral appear relatively free of interdigitations and desmosomes. The mesothelial cells of this layer of the capsule lie on a thin but well-defined extracellular basement membrane which serves to separate them from the tunica albuginea layer of the capsule (Khaysman, 1957).

3. TUNICA ALBUGINEA

The middle and most predominant layer of the testicular capsule is known as the tunica albuginea as shown in figure 23. The tunica albuginea occupies essentially the entire substance of the testicular capsule and has been classically described by
current textbooks of anatomy as a tough external covering of the testicular parenchymal tissue consisting of dense, bluish-white, fibrous connective tissue with inelastic properties and composed of collagen fibers and bundles interlacing in every direction enclosing numerous fibroblasts.

In the human, the tunica albuginea develops from a narrow peripheral zone of the indifferent gonad (Gillman, 1948; Van Wagenen and Simpson, 1965). The zone is three to five cells wide at six weeks of embryonic age, and is composed of small closely packed cells which separate the sex cords from the surface epithelium. The cells of this peripheral zone are unevenly distributed as a result of the sex cords in the area. During the next several weeks, the spherical nuclei of the cells of the tunica albuginea begin to elongate and become less closely packed due to a cytoplasmic proliferation. At eight weeks of embryonic age, the tunica albuginea begins to acquire characteristics of loose connective tissue. The cells are more flattened and dispersed than at earlier stages of development, and the cytoplasm is vacuolated. The fibrous tissue in the tunica albuginea increased particularly in the outer region until at four months of embryonic age, the tunica albuginea differentiates into an outer fibrous zone and an inner loose vascular layer which is the tunica vasculosa. According to Van Wagenen and Simpson, the
connective tissue forming the septa increases initially in the region of the rete testis and extends irregularly toward the periphery of the testis until at approximately five months of embryonic age, some of the collagenous fibrils reach the tunica albuginea to form incomplete septa between the testicular lobules.

The tunica albuginea is covered by the tunica vaginalis visceral except where the tunica vaginalis visceral is reflected over the epididymis and along the posterior border of the testis including where the testicular vessels enter the testis. According to Mancini et al. (1964), the tunica albuginea itself contains relatively few blood vessels. The testicular artery, upon entering the testis, immediately penetrates the tunica albuginea and runs along the deep surface of the tunica albuginea around the inferior pole and up the anterior border of the testis, at which point it breaks into small terminal branches which enter the testicular parenchymal tissue.

4. TUNICA VASCULOSA

The very thin, loose and innermost layer of the testicular capsule adjacent to the testicular parenchymal tissue is known as the tunica vasculosa and is diagrammed in figure 23. This layer consists of plexiform networks of minute blood vessels held
together by delicate areolar tissue. The tunica vasculosa also forms an internal investment to all spaces of the testicular parenchymal tissue.

5. MEDIASTINUM TESTIS

The region of the tunica albuginea of the testicular capsule which is widened along the posterior of the testis in some species or reflected into the interior of the testis where it forms an incomplete vertical septum is known as the mediastinum testis.

Roosen-Runge (1961) has divided the rete testis of the adult rat into three major parts. The first and largest portion of the rete testis, known as the intratesticular rete, runs parallel to the longitudinal axis of the testis. The seminiferous tubules open into the intratesticular rete as it extends from the lower to the upper end of the testis. The second portion of the rete testis, known as the intratunical rete, consists of irregular passageways which penetrate through the tunica albuginea near the upper end of the testis. The intratunical rete then widens to form the third portion of the rete testis, known as the extratunical rete, from which the ductuli efferentia leave to join with the ductus epididymis. The only large blood
vessels surrounded by the tunica albuginea are found in this region of the tunica albuginea near the superior extremity of the testis, where the rete testis penetrates through this structure and where the vascular supply to the testis enters. Various types of epithelium have been found by Leeson (1962) to line different regions of the rete testis. A squamous type of epithelium lines the main cavity of the intratesticular rete which is embedded in the collagenous tissue of the mediastinum. A cuboidal type of epithelium lines the cavity of the intratesticular rete near the openings of the tubuli recti whereas a low columnar epithelium lines the cavity of the intratunical rete. An extracellular basement membrane separates the cells of the epithelial lining of the cavity of the rete from the collagenous tissue of the mediastinum.
B. COLLAGENOUS TISSUE OF THE TESTICULAR CAPSULE

The testicular capsule appears to be composed of the three main morphological components typical of connective tissue in general, namely cells, ground substance and fibers. These cellular components include fibroblasts which have been demonstrated to occur in the testicular capsule (Mancini et al, 1955). The ground substance of connective tissue, which seems to be secreted from the interior of the fibroblast, consists of protein-polysaccharide complexes such as the chondroitin sulfates and hyaluronic acid as well as neutral carbohydrates such as glucose, galactose, mannose and fucose (Neustadt, 1963). In addition, the ground substance of connective tissue also contains non-collagen protein, which is tightly combined with the protein-polysaccharide complexes. The fibers of the testicular capsule consist predominantly of collagen protein which is also apparently synthesized within the fibroblast and extruded into the ground substance (Neustadt, 1963).

Mancini et al. (1955; 1964) has studied the changes in the histochemical appearance of the tunica albuginea of the human testicular capsule which occur with age. From birth to puberty,
the connective tissue of the tunica albuginea progressively undergoes a change from an embryonic type rich in juvenile fibroblasts to a more mature type characterized by adult fibroblasts and dense collagen bundles. During adult life, the tunica albuginea undergoes a further thickening with coalescence of the collagen fibers and the beginning of hyalinization of its superficial area. In addition, the fibrous trabeculae increase both in terms of number and thickness. Finally, during the period of senescence, the fibroblasts appear less numerous along with a progressive hyalinization that now includes the middle layer of the tunica albuginea as well. The thickness of the tunica albuginea of the human testis was also found to increase with age by Yoshimura and Fukunishi (1965). These authors were able to demonstrate a progressive increase in the thickness of the tunica albuginea from 80 µ at 5 years of age to approximately 1,000 µ at 75 years of age. Moreover, it was possible to accurately estimate the age of an individual at autopsy for medicolegal purposes by the measurement of the tunica albuginea within two days after death.
C. INNERVATION OF THE TESTICULAR CAPSULE

1. GENERAL CONSIDERATIONS

Four general types of fibers have been described to exist in the peripheral nervous system (Goodman and Gillman, 1970). These include (1) somatic efferent motor fibers and (2) somatic afferent sensory fibers which provide innervation to striated muscle and skin; (3) visceral efferent fibers or autonomic nerves and (4) visceral afferent fibers which provide innervation to smooth muscles and glands.

The nerves which innervate the testis include the peripheral autonomic nerves and the visceral afferent nerves (Mitchell, 1935).

2. TUNICA VAGINALIS VISCERAL

The autonomic efferent nervous innervation to the testicular capsule is not entirely clear, although it is currently believed that the testis receives fibers from the lumbar sympathetic chain (Albert, 1961). It is thought that both the tunica vaginalis visceral and the tunica albuginea receive autonomic
efferent nerve fibers from the internal spermatic plexus or nerves which follow the testicular artery and innervate the blood vessels of the testis (Mitchell, 1935; Kuntz and Morris, 1946). The fibers of the internal spermatic plexus may also be afferent in nature since testicular pain has long been considered a classical example of visceral pain.

Afferent nerve endings bearing a resemblance to both Pacinian and intercalated corpuscles have been demonstrated by Corona (1953) in the tunica vaginalis of the cat. Encapsulated nerve endings have also been identified in the tunica vaginalis visceral of the dog (Corona, 1953) and the human (Yamashita, 1939). It is well known that the testis is very sensitive to pain or pressure and the possibility therefore exists that many of these encapsulated nerve endings may be sensory since Pacinian corpuscles according to Iggo (1966) are rapid-adapting mechanoreceptors which can be stimulated by movement or distension of the surrounding tissue. During testicular damage or when pressure is applied to the testis, the intense pain experienced may therefore be due to a stretching of the testicular capsule which then would excite the Pacinian-like corpuscles of the tunica vaginalis visceral.
3. TUNICA ALBUGINEA

According to Risley and Skrepetos (1964) and Norberg et al. (1967), the majority of the autonomic efferent nerve fibers from the internal spermatic plexus which pass through the tunica albuginea appear to accompany and supply blood vessels. However, another type of encapsulated nerve ending has been described in the human tunica albuginea by Kreutz (1964), namely a nerve ending bearing a resemblance to a Meissner's corpuscle. It is probable that the referred pain perceived in the caudal areas of the tenth thoracic segment caused by deep visceral pain in the testis (Woollard and Carmichael, 1933) is the result of stimulation of these deep encapsulated afferent nerve endings present in the tunica albuginea.

4. TUNICA VASCULOSA

In addition to the nerve endings observed in the tunica vaginalis visceral and the tunica albuginea, occasional afferent encapsulated nerve endings have also been described in the tunica vasculosa of the bull by Shioda and Nishida (1966).
D. PATHOLOGY OF THE TESTICULAR CAPSULE

1. HYDROCELE

The clinical condition known as a hydrocele can be defined as an increased amount of fluid present in the cavity of the tunica vaginalis of the testis. The cavity of the tunica vaginalis as discussed in section A, 'The Morphology of the Testicular Capsule', is the smallest of the various serous cavities derived from the coelom, which also include the pleural, pericardial and peritoneal cavities. Not only is the cavity of the tunica vaginalis unique in that it is normally located at the lower environmental temperature of the scrotum, but it is the only coelomic cavity in which a large increase of fluid can occur in healthy individuals that are otherwise normal.

One little known feature of the cavity of the tunica vaginalis is that only the human and arthropoid apes possess a tunica vaginalis which does not communicate with the peritoneal cavity (Allen, 1943, 1946). Most of the common laboratory animals, on the other hand, have a patent vaginal process which communicates directly with the peritoneal cavity. It is for this
reason that experimental procedures designed to study hydrocele in laboratory animals has been virtually impossible. Huggins and Entz (1931) have, however, studied the pathogenesis of hydrocele in the human. Following the injection of phenolsulphonphthalein into the cavity of the tunica vaginalis, it was noted that the dye appeared in the urine within 40 to 60 minutes with the total excretion of the dye amounting to 50 percent in 24 hours. When a similar injection was performed in patients with a hydrocele, it was observed that the absorption of the dye from the cavity of the tunica vaginalis was so slow as to indicate that a delayed absorption of fluid might be the prime factor responsible for the condition of hydrocele. It was therefore suggested that a hydrocele was composed of a stagnant pool of fluid, rather than being due to an overproduction of fluid.

Allen (1943) has studied the distribution of the lymphatic supply of the tunica vaginalis in the human. No lymphatic plexuses were found to occur in the visceral layer of the tunica vaginalis. However, the parietal layer of the tunica vaginalis was found to possess both superficial and deep lymphatic plexuses composed essentially of patches of channels. The appearance of these lymphatic plexuses of the parietal layer of the tunica vaginalis of the testis was found to be quite comparable to the subserous lymphatic plexuses of the pleural/
pericardial and peritoneal cavities (Allen, 1946). It was postulated that the obliteration of the proximal processus vaginalis occurring when man adapted to the erect posture was accompanied by the development of a lymphatic absorptive apparatus; and that if obliteration of the proximal processus vaginalis would occur during gestation before the establishment of an effective lymphatic absorptive system, this then could result in a congenital hydrocele. In addition, Rinker and Allen (1951) were then able to demonstrate a scarcity or absence of lymphatics in the parietal layer of the tunica vaginalis of hydroceles, the tunica vaginalis comprising the sac of the hydrocele. This was in marked contrast to the abundance of lymphatics observed in a normal tunica vaginalis sac. It would therefore appear that a hydrocele results from an impairment of the normal resorption of the fluid of the cavity of the tunica vaginalis by the insufficiency of the lymphatics located in the parietal layer of the tunica vaginalis (Ozdilek, 1957). In this regard, Shah (1963) has reported a very successful operation for hydrocele, which removes the barrier of the alymphatic layer of the sac of the tunica vaginalis, the removal of which then exposes the testis to the scrotal lymphatics which can then absorb the fluid of the hydrocele.
2. TUMORS OF THE TESTICULAR CAPSULE

Both benign and malignant tumors are capable of arising from the capsule of the testis. Benign fibromas of the tunica vaginalis have been reported not only of the single type (Kawaichi, 1949) but of the multiple variety as well (Goodwin and Vermooten, 1946). These fibromas have the gross appearance of nodules loosely attached to the testicular capsule by means of short stalks. The nodules are freely movable within the cavity of the tunica vaginalis. The microscopic appearance of these fibromas is that of dense fibrous connective tissue which is arranged in whorled masses. The connective tissue contains dense bundles of collagen running in various directions with scattered zones of calcification. The principle cell type found in these fibromas is the mature fibroblast. As many as 15 to 20 individual nodules can be attached to the testicular capsule (Lewis and Pierce, 1962). Malignant fibrosarcomas of the tunica vaginalis of the testicular capsule have also been reported to occur (Trinidad and Sutton, 1953; McDonald, 1955). These fibrosarcomas exhibit marked cellularity with bizarre shapes and hyperchromatic nuclei. Fibromas can also arise from the tunica albuginea of the testicular capsule (Hinman and Gibson, 1924; Silverton and Welsh, 1937; Bodner, 1946; Levant and Chetlin, 1948). Such lesions are often well-
circumscribed and are composed essentially of collagenous tissue in the shape of concentric layers arranged in whorles, with only a few young fibroblasts present.

3. MISCELLANEOUS LESIONS OF THE TESTICULAR CAPSULE

King (1954) has reported that the visceral layer of the tunica vaginalis of the testicular capsule is capable of undergoing a metaplastic change. Under normal conditions, the visceral lining of the tunica vaginalis is that of a single layer of mesothelial cells. However, on occasion, there can be proliferation and metaplasia of this lining, resulting in the appearance of a stratified squamous type of epithelium instead of the normal single layer of mesothelial cells. It is not certain at the present time, whether this metaplasia might possibly represent a preneoplastic change or not.

Meyer (1928) has demonstrated the presence of calcified bodies laying free in the cavity of the tunica vaginalis. These plaques of calcification presumably arise in the tunica albuginea of the testicular capsule and then become free bodies within the cavity of the tunica vaginalis. These calcified bodies can on occasion be associated with either a hydrocele or a chronic inflammation of the testis.
Cystic lesions within the tunica albuginea of the testicular capsule can also be associated with the presence of an inflammatory reaction in the testis (Arcadi, 1952). In addition, an abscess of the tunica vaginalis of the testicular capsule may occur as a complication of a perforated duodenal ulcer (Spencer, 1963). Such an abscess may be due to the passage of duodenal contents through a processus vaginalis opened by an inguino-scrotal hernia.
E. MORPHOLOGY OF SMOOTH MUSCLE

1. INTRODUCTION

Among the first studies on the structure of smooth muscle were those of Schwann (1847), and since then a voluminous amount of literature has been published on the light microscopy of smooth muscle. Studies on the fine structure of smooth muscle have only appeared in the literature during the last fifteen years.

Among the first electron microscope studies were those which considered the visceral smooth muscle, especially that of the uterus (Mark, 1956; Bergman, 1958). The first description of the fine structural relationships of autonomic nerves to smooth muscle cells was that reported by Caesar et al. (1957). Early studies of the fine structure of smooth muscle in blood vessels included those by Parker (1958), Fawcett (1959) and Karrer (1959).

Until the discovery by Dewey and Barr (1962; 1964) of the existence of structural connections between smooth muscle cells, much of the early studies on the fine structure of smooth muscle considered possible relationships between the different muscle
fibers (Mark, 1956; Bergman, 1958; Thaemert, 1959; Prosser et al., 1960). More recent studies of smooth muscle have been concerned with the nature of the contractile apparatus (Needham and Shoenberg, 1964; Lane, 1965; Panner and Honig, 1967; Kelly and Rice, 1968) and the nature of the autonomic neuro-effector junction and intra-axonal vesicles (Richardson, 1962, 1963, 1964, 1966; Merrillees et al., 1963; Burnstock and Merrillees, 1964; Evans and Evans, 1964; Van Orden et al., 1966; Bennett and Merrillees, 1966; Burnstock and Robinson, 1967).

2. GENERAL MORPHOLOGY

In general, the largest smooth muscle cells are found in the pregnant uterus, measuring up to 10 \( \mu \) in width and 600 \( \mu \) in length (Csapo, 1962). The smallest smooth muscle cells as indicated by Rhodin (1967) are found in arterioles and measure approximately 2 \( \mu \) in width and 20 \( \mu \) in length.

Smooth muscle cells are arranged in branching bundles or fasciae which are surrounded by connective tissue sheaths (Prosser et al., 1960; Csapo, 1962; Merrillees et al., 1963). The diameter of effector muscle bundles has been estimated by Burnstock and Prosser (1960) and confirmed by Bennett and Burnstock (1968) to be approximately 100 \( \mu \). Muscle bundles of
approximately 100 µ have been identified in most tissues studied (Prosser et al., 1960; Csapo, 1962).

The location of smooth muscle bundles has been demonstrated in most hollow organs to be in the form of an outer longitudinal muscle coat and an inner circular muscle coat. In the rat vas deferens, there is an additional inner longitudinal smooth muscle coat. The location of smooth muscle in blood vessels is restricted to the tunica media (Strong, 1938) where the muscle bundles are arranged in a spiral manner, although the dominant orientation is circular. According to Rhodin (1967), in small arterioles, the smooth muscle bundles are arranged in a circular orientation.

From the fine structural studies using serial section sampling methods on the guinea pig vas deferens (Merrillees, 1968), and those on the intestine of the mouse (Thaemert, 1966), a more accurate concept of the shape of smooth muscle cells has been made possible. For example, the nucleus of smooth muscle cells is not always found centrally located but varies up to 50 µ from the center of the muscle cell. The shape of smooth muscle cells is not exactly fusiform because of extremely uneven contours along their length. In the guinea pig vas deferens, the shape of smooth muscle cells in cross section vary from polyhedral, triangular, flattened ellipses, ribbon and rod-like.

The size of smooth muscle cells appears to have a range but
this may be due to the different degrees of contraction of the muscle fibers during fixation of the tissue for electron microscopy. The studies of Lane (1965) on the changes in the form of smooth muscle cells located in the outer longitudinal muscle layer of the mouse jejunum during various stages of muscle contraction point to the above conclusion. During relaxation, the smooth muscle cell is long, narrow and has smooth contours. During contraction, the cell becomes ellipsoid, and numerous invaginations occur at the points where the myofilaments are closely associated with the plasma membrane. The nuclear membrane also becomes convoluted, producing a shorter, wider nucleus.

The precise arrangement of smooth muscle cells within muscle bundles has been partially elucidated by the studies of Bennett and Merrillees (1966) on the guinea pig vas deferens using serial sectioning sampling. The thick central portion of the cell containing the nucleus lies approximately adjacent to the long tapering ends of adjacent smooth muscle cells. In contrast to the guinea pig vas deferens, Rhodin (1962) has indicated that the smooth muscle cells in many blood vessels branch and appear to meet end-to-end. In addition, interdigitations between smooth muscle cells are prominent in the pregnant uterine smooth muscle (Kameya, 1964). The studies on the guinea pig vas deferens by Merrillees (1968) reveal that in any one
cross sectional plane, a smooth muscle cell is surrounded by 6 other cells and since the tapering ends of adjacent cells end approximately midway along any one smooth muscle cell, Merrillees concluded that each smooth muscle cell is surrounded by 12 other smooth muscle cells.

According to Prosser et al. (1960) and Merrillees (1968), adjacent smooth muscle cells in most organs are separated from each other within effector bundles by a basement membrane-filled gap of 500 to 800 Å. In large arteries, intercellular separations of 2000 to 3000 Å are characteristic. In addition to the basemembrane material located in the extracellular space between adjacent smooth muscle cells, Caesar et al. (1957) have described a number of other structures including scattered collagen filaments, fibroblasts, blood vessels, nerves and Schwann cells, elastic tissue, mucopolysaccharides and macrophages.

3. SPECIALIZED REGIONS OF CONTACT.

Early electron microscope studies by Mark (1956) and Thaemert (1959) described protoplasmic continuity between smooth muscle cells, but later studies using better fixation techniques have not confirmed these observations. However, the presence of intercellular bridges or areas of close contact, where the separation of smooth muscle cells is only 100 - 200 Å have been
described by numerous workers (Richardson, 1958; Prosser et al., 1960; Merrillees et al., 1963; Bennett and Rogers, 1967). The number of points of contact between an intestinal smooth muscle cell and the adjacent cells have been estimated by Rhodin (1962) to be approximately 150.

Another specialized region of contact between smooth muscle cells includes "peg and socket" structures, described by Lane and Rhodin (1964) and Verity and Bevan (1966). These structures involve evaginations of the plasma membrane fitting into invaginations of adjacent smooth muscle cells. In smooth muscle layers of the mouse vas deferens, the plasma membranes of such areas are about 100 Å apart and occupy about 3 - 5% of the total cell area.

Probably the most interesting specialized regions of contact between different smooth muscle cells are those areas where the plasma membrane of two different cells have fused to produce a 5-layered structure consisting of protein-lipid-protein-lipid-protein layers instead of the usual 3 layers per plasma membrane separated by the intercellular space. Such areas of contact have been reported in the smooth musculature of the intestine (Dewey and Barr, 1962, 1964; Lane and Rhodin, 1964; Bennett and Rogers, 1967), in the smooth musculature of the uterus (Bergman, 1968), in the vas deferens (Yamauchi and Burnstock, 1969) and in the vascular smooth muscle (Verity and Bevan, 1966; Cliff, 1967;
Rhodin, 1967). This specialized area of contact was first described as the "nexus" by Dewey and Barr (1962, 1964) and occurs either as a simple abutment of adjacent cells as is frequently seen in the intestinal smooth muscle of the dog, or as projections of one cell into another, such as that seen in guinea pig intestine. The 5 layers of the nexus are approximately 140 Å in total thickness, and it has been suggested by Dewey and Barr (1964) that the simple type of nexus may act to increase the mechanical tie between contracted cells while the projected nexus may be involved in electrical coupling. This latter possibility is apparent from the studies of Furshpan (1964) who demonstrated that electrical coupling can occur across tight junctions between cells.

Still another type of area of close contact between smooth muscle cells are desmosomes or desmosome-like structures consisting of electron-dense areas on adjacent cell membranes. The common occurrence of desmosomes has been described in the smooth musculature of the chick amnion (Evans and Evans, 1964) and they have been functionally implicated in playing an important role in maintaining the integrity of the tissue during contraction or extension.
4. FINE STRUCTURE OF THE SMOOTH MUSCLE CELL

The plasmalemma or plasma membrane of smooth muscle cells resembles that of most other tissues, being 70 - 110 Å thick. Several features, however, are not characteristic of other muscle cells such as the presence of numerous micropinocytotic vesicles termed caveolae intracellulaires (Caesar et al., 1957; Prosser et al., 1960; Simpson and Devine, 1966) which have also been called plasmalemmal vesicles (Bruns and Palade, 1968). According to Verity and Bevan (1966), approximately 45 percent of the plasmalemma surface of the rabbit pulmonary artery contains vesiculation. While Yamauchi and Burnstock (1964) have indicated that caveolae are rare in the mouse vas deferens up to 9 days after birth, Kameya (1964) has shown that the number of caveolae increases in the smooth muscle cells of the uterus during pregnancy.

The exact function of these micropinocytotic vesicles is not clear but many authors have suggested that they may be involved in active transport processes. Caesar et al. (1957) have suggested that these micropinocytotic vesicles may be a compensating mechanism for the sparse supply of blood capillaries in smooth muscle. In the rat vas deferens, Burnstock and Merrillees (1964) observed continuity between the endoplasmic reticulum and the micropinocytotic vesicles. Moreover, Rostgaard and Barrnett (1964) and Lane (1967) have localized high levels of ATPase.
activity in these vesicles.

The mitochondria of smooth muscle cells are found mainly near the regions of micropinocytotic vesicles or near the end regions of the cell where they extend out from the nucleus in chain-like fashion.

When compared to striated muscle cells, the endoplasmic reticulum of smooth muscle cells is not well developed. The exception is in fast contracting smooth muscles such as the vas deferens of the rat (Burnstock and Merrillees, 1964). As shown by Caesar et al. (1957) the granular endoplasmic reticulum which is presumably involved in protein synthesis is located either near the cell surface, and usually near regions of micropinocytotic vesicles, or in the perinuclear region. In developing smooth muscle cells, as in most other developing cells, rough endoplasmic reticulum is abundant (Leeson and Leeson, 1965a,b; Yamauchi and Burnstock, 1969).

The sarcoplasmic reticulum or tubular system is also poorly developed in smooth muscle as compared to skeletal and cardiac muscle (Yamauchi, 1964; Lane, 1965, 1967). The sarcotubules lie in the longitudinal axis of the cell and according to Lane (1967) contain high ATPase activity which he interprets to imply involvement in the process of excitation-contraction coupling.

The most prominent features of the nucleus of smooth muscle cells are the spiral indentations. These indentations become
more conspicuous during contraction (Lane, 1965) and disappear following stretching. The flexibility of the nucleus is seen to an even greater extent when comparing its diameter, which changes from 3 µ in the contracted state to 1 µ in the stretched state.

5. FINE STRUCTURE OF THE CONTRACTILE APPARATUS

The presence of tightly packed myofilaments 30 - 80 Å in diameter or approximately the same size as actin filaments in striated muscles have been reported in smooth muscle cells (Mark, 1956; Caesar et al., 1957; Shoenberg, 1958; Pease and Molinari, 1960; Karrer, 1961; Lane, 1965; Danner and Konig, 1967). These myofilaments run parallel to the long axis of the cell and occupy the entire cell except for the terminal perinuclear zones. The greatest density of myofilaments, according to Merrillees et al. (1963) occurs at the ends of the muscle cell. When isolated and purified from uterine smooth muscle, the properties of actin are almost identical to those of actin isolated from cardiac and skeletal muscles (Carsten, 1965). In addition, Needham and Williams (1963) have shown that purified preparations of uterine actin react normally with skeletal muscle myosin to produce a viscous actomyosin preparation.

The presence of distinct myosin filaments in smooth muscle
cells has not been definitely elucidated. Thicker filaments of uneven length and irregular distribution have been described only in fine structural studies where a rapidly penetrating fixative such as glutaraldehyde has been used (Needham and Shoenberg, 1964; Yamauchi and Burnstock, 1969). Most workers feel that myosin is probably present in smooth muscle as myosin molecules (Needham and Shoenberg, 1964; Elliot, 1964; Shoenberg et al., 1966). X-ray diffraction studies of smooth muscle from guinea pig taenia coli by Elliot (1964, 1967) have indicated the presence of actin, but no myosin patterns were observed.

The proportion of actin to myosin in smooth muscle actomyosin has been shown by Needham and Williams (1963b) to be 1:4 which is comparable to that of skeletal actomyosin although smooth muscle contains only 6 - 10 mg per gram wet weight which is much less than the actomyosin content of skeletal muscle which is approximately 70 mg per gram wet weight (Needham and Williams, 1963a).

Numerous dense areas formed of protein have been observed in the cytoplasm of most smooth muscle cells, either randomly dispersed or evenly dispersed (Mark, 1956; Prosser et al., 1960; Rhodin, 1962; Lane, 1965; Rogers and Burnstock, 1966). These dense areas have been observed to alternate with the regions containing the micropinocytototic vesicles, and it has been suggested by Pease and Molinari (1960) that the myofilaments attach to the plasma membrane of the smooth muscle cell at these dense areas. This concept
has received general support. The later work of Panner and Honig (1967) has indicated a resemblance of the dense bodies to Z-discs of skeletal muscle since the free ends of myofilament bundles appear to interact with other bundles at these dense areas. During contraction, forces are thought to be applied to the entire periphery of the cell, with the main force extended from end to end since most fibers lie along the longitudinal axis. The greatest force according to Panner and Honig would therefore be applied to the plasma membrane at each end of the cell, since the ends of the cell contain the majority of filaments per unit area of the membrane.
F. NEUROMUSCULAR TRANSMISSION OF SMOOTH MUSCLE

1. INTRODUCTION

The pharmacological basis for the action of drugs on smooth muscle has been only partially elucidated in a voluminous number of reports describing the effects of drugs and chemical agents on smooth muscle. Interpretation and classification of experimental results has been made difficult due to the variability of the observed pharmacological effects from one smooth muscle system to another. In some smooth muscles such as the rat and mouse vas deferens (Merrillees et al., 1963; Lane and Rhodin, 1964; Yamauchi and Burnstock, 1969b), the innervation is dense. Yet in other smooth muscles such as the longitudinal layer of the intestinal smooth muscle, the uterus or vascular smooth muscle, the innervation is sparse (Caesar et al., 1957; Clementi, 1962; Thaemert, 1963, 1966; Lane and Rhodin, 1964; Devine and Simpson, 1967; Rhodin, 1967).

Interpretation of various drug effects on smooth muscle takes into consideration the possibility of not only a direct action of the drug on the smooth muscle cells as is the case in neuromuscular transmission, but also an indirect action mediated by the drug effects on the nerves innervating the tissue. Yet, compared with the working knowledge of the skeletal muscle system, the
morphology, innervation and physiology of smooth muscle is not clear. Neither is the mechanism of drug action on the smooth muscle cell membrane, nor the release of transmitters, their uptake or storage completely clear.

2. AUTONOMIC NEUROMUSCULAR TRANSMITTERS

The concept that a neurohumoural agent is involved in transmission of nerve impulses between autonomic nerves and smooth muscle effector cells has developed from the idea that the nervous system does not exist as a syncitium. The existence of at least a physiological discontinuity between nerve endings and muscle effector cells was first demonstrated in skeletal muscle by Claude Bernard (1856). Bernard showed that it was possible to inhibit nervous transmission to skeletal muscle in the presence of the drug, curare. The action of curare appeared to be at the region of the junction between nervous tissue and muscle, since either nerve or muscle alone could still function.

An anatomical separation, however, between individual neurons or between neurons and skeletal or smooth muscle cells was not demonstrated until 100 years later when a definite inter-neural and neuromuscular gap was demonstrated using the electron microscope (Palade and Palay, 1954; Palay, 1956). This gap was
shown to be approximately 75 Å wide for the skeletal neuromuscular junction (Robertson, 1956), but at the smooth muscle neuromuscular junction, the gap was 100 - 200 Å.

The initial evidence that the neurotransmission was chemically mediated was shown by Lewandowsky (1899) who according to Dale (1937) described the effects of the administration of extracts of the adrenal glands. Elliott (1904, 1905) in similar but more extensive studies suggested that the liberation of adrenaline may be the mechanism by which sympathetic nerves function.

a. Acetylcholine

Acetylcholine was considered to be the probable parasympathetic transmitter following the studies of Dale (1914). However, acetylcholine was not demonstrated to be released following nerve stimulation until Otto Loewi (1921a,b; 1924) showed that the perfusion fluid collected from the isolated frog heart during stimulation of the vagus nerve could inhibit the heart beat. Loewi called the inhibitory substance "Vagusstoff" which was subsequently shown to generally mimic parasympathetic effects. This type of identification of acetylcholine, however, relied on bioassay techniques.

Confirmation by chemical methods that acetylcholine is indeed released following nerve stimulation has recently been
demonstrated using choline-methyl-\(^{14}\)C and choline-methyl-\(^{3}\)H.

Wallach et al. (1967) reported that by labeling acetylcholine in this manner, the isolated cat heart can be shown to release labeled acetylcholine following stimulation of the vagus nerves. This was also shown at the skeletal neuromuscular junction by Saelens and Stoll (1965) following stimulation of the phrenic nerve to the rat diaphragm.

Although the specific site of the released acetylcholine has not been conclusively established to be the postganglionic axonal endings, there is overwhelming evidence to support this hypothesis. For example, the stored acetylcholine in the bladder smooth muscle innervation has been shown by Carpenter and Rand (1965) and Carpenter and Rubin (1967) to be entirely due to the presence of cholinergic nerves. These authors found that degeneration of the bladder post-ganglionic nerves, produced depleted acetylcholine levels in the bladder.

The biosynthesis of acetylcholine has been shown by Korkes et al. (1952) to require the presence of only acetylcoenzyme A, choline and choline acetylase. As discussed by Hebb (1957), choline acetylase is distributed very selectively in the autonomic nervous system, and its presence is generally indicative of the cholinergic nature of the particular neuron. Most of the choline acetylase has been located in the terminals of cholinergic neurons and as shown by Hebb and Waites (1956), following severing of
a cholinergic axon, the choline acetylase becomes concentrated several days later at the nerve ending, proximal to the cut. On the basis of these results, these authors have suggested that the choline acetylase is probably synthesized in the region of the neuronal perikarya and transported along the axon to the neuronal terminals. Such a continuous flow of axonal material has been demonstrated by Weiss et al. (1962). The storage site of acetylcholine in small vesicles located in the varicosities of cholinergic neurons will be discussed below in section G "Innervation of Smooth Muscle".

b. Norepinephrine

As discussed above, the early studies of Elliott (1904, 1905) suggested that sympathetic nerves may function by releasing epinephrine. Later studies by Loewi (1921a) who found a substance resembling epinephrine released following stimulation of cardioaccelerator nerves confirmed this hypothesis. Still later studies by von Euler (1946), Gaddum and Goodwin (1947) and others indicated that the transmitter released by sympathetic nerves was norepinephrine.

The presence of norepinephrine in adrenergically innervated organs was shown chemically using column and paper chromatography (Goodall, 1951) and confirmed by Rexed and von Euler (1951) and
von Euler (1951) who indicated that the presence of norepinephrine was located only in post-ganglionic unmyelinated nerves since the content of norepinephrine in organs was found to be proportional to the amount of unmyelinated nerves present.

The storage site of norepinephrine in small vesicles located in the varicosities of adrenergic neurons will be discussed below in section G "Innervation of Smooth Muscle".

The biosynthesis of norepinephrine in adrenergic neurons was first suggested by Blaschko (1939) and later demonstrated by Goodall and Kirshner (1958) to involve the rate limiting step of converting tyrosine to dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase. DOPA then undergoes decarboxylation to 3,4-dihydroxyphenylethylamine (dopamine) by aromatic amino acid decarboxylase. Dopamine is then converted to norepinephrine by dopamine β-hydroxylase. The studies of Goodall and Kirshner (1958) and Austin et al. (1967) demonstrated the formation of labelled norepinephrine from the labelled precursor, tyrosine.
G. INNERVATION OF SMOOTH MUSCLE

1. INTRODUCTION

The morphology of the autonomic innervation of smooth muscle has been described in numerous reports using silver and methylene blue staining techniques for light microscopy. Using these methods, some of the details of the organization of intramural plexuses and their relationships with extrinsic nerves have been eloquently worked out (Hillarp, 1959; Richardson, 1960). The more intimate relationships between the single autonomic nerve fibers and individual smooth muscle cells have been elaborated only with the fine structural studies made possible with the electron microscope.

2. MORPHOLOGY OF AUTONOMIC INNERVATION

Among the first description of the fine structure of the innervation of smooth muscle was the report by Caesar et al. (1957) on the urinary bladder of the mouse. These authors described membranes of nerve and muscle cells that were separated by
only 70 - 200 Å and therefore indicated the existence of neuromuscular synapses in smooth muscle. Later studies confirmed the presence of neuromuscular synapses (Thaemert, 1963; Lane and Rhodin, 1964; Richardson, 1964; Evans and Evans, 1964).

The concept that the transmitter could also be released at various sites along the nerve within the effector organ as well as at terminal synapses was proposed by Richardson (1962) and Merrillees et al. (1963). This hypothesis has received support from fluorescent histochemical studies of sympathetic nerves (Norberg and Hamberger, 1964; Malmfors, 1965; McLean and Burnstock, 1966, 1967).

The fluorescent technique is very sensitive and relies on the localization of monoamines in sympathetic nerves (Falck, 1962; Falck et al., 1962; Eranko, 1967). These studies have established that the fibers of sympathetic nerves terminate only after running long distances through the smooth musculature of organs. The length of a sympathetic neuron in the smooth muscle effector system has been calculated to be up to 10 cm (Dahlstrom and Haggendal, 1966b).

The structure of the nerve fiber within smooth muscle has been shown by serial sampling electron microscopy (Thaemert, 1966; Bennett and Rogers, 1967; Merrillees, 1968) to consist of up to 30 irregular enlargements per 100 μ along its length. The diameter of each enlargement or varicosity is approximately 2 μ
while the intervaricose regions are only 0.1 - 0.2 µ. The localization of high concentrations of monoamines at the varicosities has suggested that amines can be released at these varicosities within the smooth muscle effector system and can therefore influence numerous smooth muscle cells (Bennett and Burnstock, 1968).

Three functional varicose regions have been elucidated by Merrillees (1968). The first functional region consists of that portion of the nerve before it penetrates the smooth muscle when Varicosities first appear. The release of transmitter at these varicosities is doubtful since Schwann cell processes completely surround the neuron. The second functional region contains the majority of varicosities and is only partially surrounded by Schwann cell processes, making the release of transmitter possible at the varicosities. The third functional region consists of the few remaining preterminal varcosities before the terminal varicosity and contains no Schwann cell processes. These represent functional synapses.

Quantitative histochemical studies by Dahlstrom and Haggenerdal (1966a) and Ritzen (1966) have indicated that most of the norepinephrine content of sympathetic nerves is located in the varicosities and terminal portions of the neuron. The ratio of norepinephrine content between the varicosities and the nerve cell body has been calculated to be approximately 300:1.

Despite the fact that there are as yet no reported methods
either histochemical or radioautographic available for localising of acetylcholine in nervous tissue, indirect methods have been described to demonstrate cholinergic neurons (Koelle and Friedenwald, 1949; Davis and Koelle, 1967) for both light and electron microscopy. The methods are based on the correlation of acetylcholinesterase activity being present in known cholinergic neurons, and its apparent absence from other neurons. By using this method, Bell and McLean (1967) were able to identify by electron microscopy, acetylcholinesterase activity and norepinephrine in separate neurons of the guinea pig vas deferens. Esterhuizen et al. (1968) were also able to localize acetylcholinesterase activity and norepinephrine in separate neurons of the cat nictitating membrane using a combined electron microscopic radioautographic localization of norepinephrine and separate localization of acetylcholinesterase activity.

3. VARIATIONS OF AUTONOMIC INNERVATION

There is a wide range in the degree of innervation in various smooth muscle systems, which probably reflects form underlying physiological function. The greatest degree of innervation in which every smooth muscle fiber synapses with at least one neuron through a distance of 200 Å occurs where fast
co-ordinated responses are necessary such as the vas deferens of the rat and mouse (Merrillees et al., 1963; Lane and Rhodin, 1964; Yamauchi and Burnstock, 1969b) and the ciliary muscles of the cat (Ishikawa, 1962).

A second type of dense innervation of smooth muscle has been observed in a number of smooth muscle systems, but differs from that seen in the rat and mouse vas deferens in that 200 Å neuromuscular junctions have not been observed for every smooth muscle fiber. According to Merrillees (1968), however, transmitter which is released from nerves that are within 1000 Å from smooth muscle cells could also be effective. Included in this type of smooth muscle innervation is the guinea pig vas deferens (Merrillees et al., 1963; Merrillees, 1968), the urinary bladder (Caesar et al. 1957; Thaemert, 1963; Burnstock and Merrillees, 1964), circular intestinal muscle (Thaemert, 1963; Rogers and Burnstock, 1966) and the nictitating membrane of the cat (Esterhuizen et al., 1967).

A third type of smooth muscle innervation is characterized by the presence of only a relatively few 200 Å neuromuscular junctions. A prominent feature, though, is the presence of numerous tight junctions between adjacent smooth muscle cells. This type of innervation has been described in the longitudinal intestinal muscle (Thaemert, 1963, 1966; Lane and Rhodin, 1964), the uterus (Caesar et al., 1957; Clementi, 1962), and vascular

4. SYNAPTIC VESICLES OF AUTONOMIC NERVES

Three main types of vesicles have been observed in the varicosities located in the terminal regions of autonomic nerves. These consist of small granular vesicles 250 - 600 Å in diameter, large granular vesicles 700 - 1600 Å in diameter, and small agranular vesicles 250 - 600 Å in diameter.

a. Small Granular Vesicles

These vesicles have been observed mainly in mammalian sympathetic nerves (Burnstock and Merillees, 1964; Simpson and Devine, 1966) and are characterized by having a dense 150 Å core which has been postulated to consist of stored norepinephrine (Burnstock and Robinson, 1967; van Orden et al., 1967). This concept is in agreement with the fluorescent histochemical studies of Norberg and Hamberger (1964) and many others as discussed above which have shown the varicose regions of sympathetic nerves to contain high levels of norepinephrine. Moreover, drugs such as reserpine and L-methyl-D-tyrosine and guanethidine which deplete the norepi-
nephrine stores in sympathetic nerve endings have been shown to also deplete the granules from the granular vesicles (Pellegrino de Iraldi and De Robertis, 1961, 1963; Richardson, 1963; Devine et al., 1967). For example, van Orden et al. (1966) have shown a reduction from 53 - 64 percent to 6 - 11 percent in the number of granular vesicles in sympathetic nerves of the rat vas deferens following administration of \( \alpha \)-methyl-m-tyrosine. Conversely, in norepinephrine-depleted animals, Pellegrino de Iraldi, Zieher and De Robertis (1965) and Bondareff and Gordon (1966) have shown that the depleted vesicles of sympathetic nerve terminal varicosities can be replenished with granules following the administration of norepinephrine. Further evidence which strongly indicates that granular vesicles functionally store norepinephrine has been reported by Bloom and Barrnett (1966). Using electron microscope histochemical techniques, these authors have localized norepinephrine in 80 percent of the granular vesicles of the sympathetic nerve terminal varicosities in the rat vas deferens.

b. Small Agranular Vesicles

The occurrence of small agranular vesicles has been observed in both sympathetic and parasympathetic nerves. In smooth muscles predominantly innervated by parasympathetic nerves such as the lung, urinary bladder or ciliary muscle, the main type of vesicle
found is agranular (Burnstock and Merrillees, 1964; Richardson, 1964). These small agranular vesicles have been described as identical to those vesicles found at the neuromuscular junction, and central nervous system ganglionic synapses which have been shown to contain acetylcholine (De Robertis and Bennett, 1955; Robertson, 1956; Palay, 1956). In the sympathetic nerves of the mouse vas deferens, the proportion of small vesicles that were agranular was only 12 - 20 percent compared to 81 - 85 percent for the granular vesicles (Yamauchi and Burnstock, 1969b). Earlier studies on the rat vas deferens by van Orden et al. (1966, 1967) reported similar findings. These results suggest that either the agranular vesicles may contain acetylcholine and that a cholinergic link may be involved in the transmission of nerve impulses in sympathetic nerves as proposed by Burn and Rand (1959, 1965), or that the agranular vesicles reflect empty granular vesicles. This problem has been partially resolved by the data of Pellegrino de Iraldi and De Robertis (1961, 1963) and van Orden et al. (1966, 1967). These authors have demonstrated that the small proportion of agranular vesicles in the varicosities of sympathetic nerves are capable of storing norepinephrine since the number of agranular vesicles was decreased with a corresponding increase of stored norepinephrine.
c. Large Granular Vesicles

The presence of large granular vesicles ranging in size from 700 Å to 1600 Å have been reported in the terminal regions of autonomic nerves (Bennett and Rogers, 1967; Burnstock and Merrillees, 1964; Thaemert, 1963). According to Yamauchi (1964), the origin of these large granular vesicles may be the smooth endoplasmic reticulum in the terminal axoplasm, but the precise nature of these vesicles has not yet been resolved. However, since large granular vesicles are prominent in nerve terminals containing the small granular vesicles, Dahlstrom and Haggendal (1966a,b) have suggested that the large granular vesicles represent sites of synthesis of catecholamines while the small granular vesicles act as sites of storage of the transmitter.
H. ELECTRICAL PROPERTIES OF SMOOTH MUSCLE

A survey on the literature of smooth muscle - which is enormous, reveals that the physiological and pharmacological properties of smooth muscle are not nearly as well elucidated as those properties of skeletal muscle. As stated by Burton (1962) "we can safely generalize about all skeletal muscles, but we cannot do so about smooth muscles as a group". Burton also states that most muscle physiologists have a strong "faith" that the basic mechanisms of transducing chemical into mechanical energy must be the same in skeletal and in all kinds of smooth muscle, but that it would be dangerous if it were not recognized as pure "faith" and not "science".

Much of the pioneer research on the electrical activity of smooth muscle was carried out by Bozler (1938 a,b; 1939) using extracellular recording techniques. His later experiments on the mechanisms which appeared to govern smooth muscle activity (Bozler, 1948) suggested that mechanical changes of smooth muscle were associated with the initiation of impulses which were probably discharged by a depolarization of the cell membrane caused by a change in the metabolism of the smooth muscle cell. According to Bozler, the membrane potential and the resting meta-
bolism of the smooth muscle cell fluctuate spontaneously. Later studies using intracellular electrical recording techniques have both confirmed Bozler's studies and have advanced his interpretations.

The more precise measurements of smooth muscle electrical activity, made possible by micro-electrodes, were first reported by Bülbring and Hooton (1954) and Woodbury and McIntyre (1954). Despite the modern techniques in electrophysiology, however, most studies have been restricted to reporting the observable electrical phenomena. The basic mechanisms of drug actions and the physiological function of smooth muscle as indicated above by Burton (1962) still remain to be elucidated.

A large proportion of the electrophysiological studies of smooth muscle has originated in the laboratory of Bülbring (1954, 1955, 1962) using the longitudinal layer of the guinea pig intestine, the taenia coli, which undergoes spontaneous changes in length and tension. Attempts to measure the membrane potential of smooth muscle were hindered because of spontaneous fluctuations of the potential (Bülbring, 1954) and the presence of a continuous rhythmic discharge of spontaneous spikes averaging one per second, reported first by Bozler (1939). As a consequence, only average values of the membrane potential of smooth muscles have been described. For the taenia coli, the average value was found to be 50 mv with a range of 25 mv to 75 mv (Bülbring, 1954). However,
the potential was found to depend on the degree of stretch of the smooth muscle. The more the muscle was stretched, the lower the membrane potential (Bülbíng, 1954, 1955). In addition, the frequency of spontaneous spike discharge was found to be proportional to the length of the muscle, increasing as the muscle is stretched (Bülbíng, 1954, 1955). Non-spontaneously active smooth muscle such as the vas deferens have been reported to have a higher membrane potential of approximately 65 mv (Burnstock and Holman, 1961; Kuriyama, 1963 a) than that of spontaneously active smooth muscle such as the intestine.

Bülbíng (1955) demonstrated a close correlation between the membrane potential and the rate of spontaneous spike discharge and the tension. When the taenia coli was stretched in steps of 2 mm, both the tension and the number of spikes increased, while the membrane potential fell. When the muscle preparation was relaxed in steps of 2 mm, the membrane potential increased again, while both the tension and spike frequency decreased. Bülbíng concluded that the changes in membrane potential and spike frequency were a function of the tension produced in response to the increased length of the muscle. A similar correlation between depolarization, increased spike frequency and increased tension was obtained when the taenia coli was stimulated by acetylcholine or histamine. The action of acetylcholine appeared to be indistinguishable from that of histamine. Bülbíng (1955, 1957 a,b)
also demonstrated that the effect of acetylcholine on the spontaneously active taenia coli, when fixed isometrically at a moderate tension, was very similar to the effect of electrotonic depolarization since the result was an increased rate of spike discharge and an increased tension. According to Bülbring, the increase in tension is caused by a prolonged spike duration as a result of a slower rate of repolarization. The next spontaneous spike may therefore occur before complete repolarization of the previous spike. The net result would be an increase in tension.

On the other hand, the relaxation of smooth muscle produced by either epinephrine or electrotonic hyperpolarization has been shown by Bülbring (1957a,b) to be the result of a cessation or decrease in the rate of spontaneous spike discharge. The increased membrane potential results in a shorter spike duration and an increased prepotential duration before the firing level is reached. Consequently, the intervals between spontaneous spike discharges are increased.

The level of tension of the smooth muscle is therefore determined by the spacing of the spikes which in turn is governed by the state of polarization of the cell membrane. Although the tone of smooth muscle was originally defined by Evans (1926) as the muscle's resistance to extension, smooth muscle tone according to Breeding and Bülbring (1964) can now be considered as a fused or partially fused tetanus determined by the rate of discharge of
the spontaneous spikes.

The ionic basis of the resting potential, as in most cells, is mainly determined in smooth muscle cells by the potassium ion. This conclusion is based on observations following changes in the external ionic concentrations (Kuriyama, 1963b, 1964; Bulbring et al., 1968; Casteels and Kuriyama, 1965, 1966). However, there is evidence which indicates that the regulation of ions across the smooth muscle cell membrane may differ from that of other excitable tissues which obey the Donnan distribution of ions. According to Casteels and Kuriyama (1966), the concentration of intracellular potassium of the taenia coli remains relatively constant following the addition of KCl to Krebs' solution or when the NaCl of Krebs' solution is replaced with KCl. The studies of Casteels and Kuriyama (1965, 1966) also indicate that the resting membrane potential of smooth muscle cells is determined not only by potassium ions but most probably by the diffusion potentials of other ions such as sodium and chloride. The effect of chloride ions on the membrane potential of some mammalian smooth muscles may be very significant. In some invertebrate muscles such as the elasmobranch (Hagiwara and Takahashi, 1967) and Ascaris (Del Castillo et al., 1964), the resting potential is dependent more on chloride ions than potassium ions.
CHAPTER III

MATERIALS AND METHODS
A. EXPERIMENTAL CONDITIONS

1. TISSUES

   a. Animal

   90 day old male Sprague-Dawley rats weighing approximately 325 g, and adult male New Zealand White rabbits weighing approximately 3.0 Kg were used in these studies on the testicular capsule. The animals were obtained from the Abrams Small Stock Breeders, Inc. of Chicago, Illinois and were fed Rockland Laboratory Rat Chow or Rockland Rabbit Diet and supplied with drinking water ad libitum. Rats were housed in 18 x 11 x 8 inch wire cages in groups of 4 - 6 animals per cage. Rabbits were housed in 24 x 18 x 14 inch cages, 1 animal per cage. All animals were maintained in a controlled environmental room at approximately 25 °C.

   b. Human

   Orchiectomy, surgical segments of normal testes were obtained from males aged 60 - 70 years whose testes were removed as a therapeutic measure for prostatic carcinoma at Hines Veterans Administration Hospital. The testicular segments were then transpor-
ted from Hines Hospital in oxygenated Krebs-Ringer bicarbonate buffer, packed in an insulated ice container within 15 minutes of removal of the testes at surgery.

2. ISOLATED TISSUE BATH ASSEMBLY

Figure 3 shows a photograph of the isolated tissue bath assembly employed for the measurement of contractions of the isolated testicular capsule. A constant-temperature bath with a pump serves to provide a circulating stream of water at the desired temperature through the outer jacket of the isolated tissue bath and through the outer jacket of a 2 foot condenser connected to the isolated tissue bath. The condenser serves to provide a constant-temperature reservoir of the physiological salt solution used in the tissue bath. The temperature of the tissue bath was monitored directly below the mounted testicular capsule by a tele-thermometer (Yellow Springs Instruments Company, Inc.). Aeration of the physiological salt solution was carried out through a stainless steel 30 gauge needle. The aeration and telethermometer needles both entered the side of the tissue bath through a rubber stopper located just below 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). The 10 cm lever arm of the transducer
was set at a 3:1 ratio with the isolated testicular capsule attached to the end of the short arm with a long piece of 4-0 surgical silk thread leading from the tissue bath below. Recordings of each tissue response were made by an oscillo-riter (Texas Instruments, Inc.) which was connected to the transducer. The speed of the recorder paper was set at 0.25 mm per second. Any contraction or relaxation of the isolated testicular capsule is therefore magnified and appears as a deflection of the pen on the recorder chart paper.

3. PHYSIOLOGICAL SALT SOLUTIONS

The following three physiological salt solutions were used in pharmacological studies on the isolated testicular capsule:

a. Tyrode's Solution* aeration: air

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>8.0 g/l</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0 g/l</td>
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</table>
| KCl       | 0.2 g/l  
|           | (4.8 ml 25% solution KCl) |
| MgCl₂     | 0.1 g/l  
|           | (2.4 ml 25% solution MgCl₂) |
| NaH₂PO₄   | 0.05 g/l  
|           | (3.0 ml 10% solution NaH₂PO₄) |
| CaCl₂     | 0.2 g/l  
|           | (4.8 ml 25% solution CaCl₂) |
| NaHCO₃    | 1.0 g/l |

* used for all pharmacological studies except buffer experiments.
b. De Jalon's Solution  

\[ \text{aeration: 95\% O}_2 \text{ & 5\% CO}_2 \]

- NaCl 9.0 g/l
- Glucose 0.5 g/l
- KCl 0.42 g/l (3.4 ml 25\% solution KCl)
- CaCl₂ 0.03 g/l (0.24 ml 25\% solution CaCl₂)
- NaHCO₃ 0.5 g/l


c. Krebs-Henseleit Solution  

\[ \text{aeration: 95\% O}_2 \text{ & 5\% CO}_2 \]

- NaCl 6.87 g/l
- Glucose 2.0 g/l
- KCl 0.4 g/l
- MgSO₄ 0.14 g/l
- NaH₂PO₄ 0.14 g/l (2.8 ml 10\% solution NaH₂PO₄)
- CaCl₂ 0.28 g/l (2.2 ml 25\% solution CaCl₂)
- NaHCO₃ 2.1 g/l

All reagent concentrations shown are for 1 liter of physiological salt solution. Each solution was prepared by adding the reagents to approximately 0.7 l of distilled water in the order
indicated, dissolving each reagent thoroughly before adding the next reagent. The solution was then q.s. to 1 liter. Reagents that were not readily soluble in distilled water were made up in concentrated stock solutions and the necessary volume added as indicated in the brackets. In every case, the physiological salt solution was prepared fresh for each daily experiment.
B. PROCEDURES FOR THE ISOLATION OF THE TESTICULAR CAPSULE AND RELATED TISSUES FOR PHARMACOLOGICAL STUDIES

1. ADULT RAT ISOLATED TESTICULAR CAPSULE PREPARATION

The first testicular capsule that was isolated in this laboratory and investigated for pharmacological properties was that of the adult rat. Figure 1 presents a schematic diagram of the procedure for the rapid isolation of the testicular capsule of the adult rat and its use as an isolated tissue preparation for pharmacological studies. The animals used were 90 day old male Sprague-Dawley rats weighing approximately 325 g. 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, leaving a hole in the testis which measures approximately 5 mm in diameter. The seminiferous tubular mass protruding through the hole in the testis 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 one begins to gently remove the seminiferous tubules from the interior of the testis. In the process of removing the seminiferous tubular mass from the testicular capsule, the capsule is turned inside out (figure 1d), leaving only a few seminiferous tubules still attached to its inside surface (figure 1e). The testicular capsule in its inside-out position is then separated from the mass of seminiferous tubules and the large testicular artery (figure 1f). The few remaining seminiferous tubules that are still attached to the inside surface of the testicular capsule are then cut away (figure 1g). The intact isolated testicular capsule is then tied at its superior end with a long piece of 4-0 surgical silk thread (figure 1h). A second smaller tie is placed at the inferior end of the testicular capsule with care taken to leave approximately one-fourth 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 assembly by attaching the lower thread to the tip of the support rod within the bath assembly and the upper long thread to the lever arm of a linear motion transducer connected to a recorder for detection of tissue contractions (figure 1j). The physiological salt solution in the isolated tissue bath was Tyrode's solution (Tyrode, 1910) at 32 °C and gassed with air as indicated by the
bubbles leading from the aeration tube. Each drug was dissolved in Tyrode's solution and added to the 10 ml tissue bath in a volume of 0.1 ml by means of a suitable pipette.

2. **ADULT RABBIT ISOLATED TESTICULAR CAPSULE PREPARATION**

The animals used in these experiments were New Zealand White male rabbits weighing approximately 2.5 Kg. Following sacrifice of each animal by a sharp blow to the head and dislocation of the cervical vertebrae, both testes and adjoining epididymis were exposed by a mid-line inguinal incision, removed and placed in Tyrode's solution. The epididymis was then carefully removed as well as the adhering connective tissue. The isolation of the testicular capsule of the adult rabbit is similar to that shown for the adult rat in figure 1 with the exception that it is somewhat more laborious to remove the internal parenchymal tissue of the adult rabbit testis because of the numerous thin septa extending from the capsule into the testicular parenchyma.

3. **HUMAN ISOLATED TESTICULAR CAPSULE PREPARATION**

Figure 20 presents a schematic diagram of the procedure used in the isolation of the human testicular capsule and its use as an isolated tissue preparation for pharmacological studies.
Human testes were obtained from males aged 60-70 years whose testes had been removed as a therapeutic measure for prostatic carcinoma. Following surgical orchietomy, the medial half of the human testis is cut away and transported to the Pathology Department for routine histological examination. The lateral segment is transported in oxygenated Krebs-Ringer bicarbonate buffer, packed in ice within an insulated contained to our laboratory within 15 minutes of removal of the testis at surgery. Inasmuch as the entire isolated testicular capsule of the human is too large to be mounted in a tissue bath, the following strips of the testicular capsule, as indicated in figure 20, were cut to approximate 20 mm in length and 6 mm in width: (1) lateral horizontal strip; (2) superior, posterior, vertical strip; (3) inferior, posterior, vertical strip; (4) inferior, anterior, vertical strip; (5) superior, anterior, vertical strip. Each capsular strip was tied with silk thread at both ends and mounted in a 10 ml isolated tissue bath with the upper long thread leading to a linear motion transducer connected to a recorder. The physiological salt solution in the isolated tissue bath was Tyrode's solution gassed with air at 32 °C.
4. ADULT RAT AND RABBIT ISOLATED TESTICULAR PARENCHYMAL TISSUE PREPARATION

In the process of obtaining the testicular capsule as an isolated tissue preparation, one can also easily obtain the testicular parenchymal tissue (seminiferous tubular mass) as an intact isolated tissue preparation as indicated in figure 1. A long thread is attached to the upper end of the isolated seminiferous tubular mass and leads to a linear motion transducer connected to a recorder. The lower end of the isolated seminiferous tubular mass is mounted to a support rod within a 50 ml isolated tissue bath. It was therefore of interest to investigate whether drugs found to cause a contraction of the testicular capsule may also cause a contraction of the seminiferous tubules. This was especially pertinent in view of postulated "contractile cells" that have been described to lie within the basement membrane of the seminiferous tubules.

5. ADULT RAT AND RABBIT ISOLATED WHOLE TESTIS PREPARATION

Following the removal of a testis and adjoining epididymis from an adult rat or rabbit, the preparation is placed in Tyrode's solution. The epididymis is carefully removed and the intact testis is then tied at its superior end with a long piece of sur-
gical silk thread. A second smaller tie is placed at the inferior end and the testis is then mounted in a 50 ml isolated tissue bath assembly, attaching the inferior thread to the support rod within the bath and the superior thread to a linear motion transducer. The physiological salt solution is Tyrode's solution gassed with air at 32 °C for the rat testis and at 37 °C for the rabbit testis.

6. ADULT RAT ISOLATED EPIDIDYMIS PREPARATION

The epididymis of the adult rat can be isolated in its entire intact form and used as a single isolated tissue preparation, or it can be bisected into capit and caudal portions and each portion used as a separate isolated tissue preparation. Using the single isolated tissue preparation, the caudal epididymis is mounted to a support rod within a 10 ml isolated tissue bath while a long thread is attached to the capit epididymis and leads to a linear motion transducer connected to a recorder.

7. ADULT RAT ISOLATED VAS DEFERENS PREPARATION

The isolation of the vas deferens for isolated tissue studies is carried out by removing the pars inguinalis portion of the vas deferens of the adult rat and mounting a 2 cm length in a 10 ml isolated tissue bath using Tyrode's solution at 32 °C, gassed with air.
8. ADULT GUINEA PIG ISOLATED ILEUM PREPARATION

The animals used for these experiments were English strain guinea pigs weighing approximately 300 - 400 g, obtained from Abrams Small Stock Breeders Inc., Chicago, Illinois. The animal is sacrificed by a blow on the head and the abdominal cavity exposed. Approximately 12 inches of ileum is removed and placed in a beaker of aerated Tyrode's solution from which a 1/2 inch segment of ileum is then prepared by carefully cutting away attached mesentery. In addition, a water tube is inserted at one end of the lumen to flush any contained material out. The preparation is then mounted in a 10 ml isolated tissue bath with an upper long thread attached to a transducer. Approximately 500 mg load on the lever arm is necessary to produce a satisfactory tension on the isolated ileum.

9. ADULT RABBIT ISOLATED DUODENUM PREPARATION

The isolated duodenum tissue preparation is obtained in a similar manner as that described above for the guinea pig ileum with the exception that a slightly heavier load is required on the lever arm to produce a satisfactory tension of the rabbit duodenum.
C. PHARMACOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE

The isolation of the testicular capsule as a separate intact tissue appeared to offer a unique opportunity to study the pharmacological properties of a component of the testis which has been previously completely neglected.

1. DETERMINATION OF THE RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS

Members of the following classes of pharmacological drugs were studied to determine whether the drug produced a contraction or relaxation of the isolated testicular capsule. Each drug was dissolved in the physiological salt solution used in the isolated tissue bath and added to the 10 ml bath by means of a 100 lambda pipette.

a. Parasympathetic Stimulating Drugs

1) acetylcholine chloride (City Chemical Corporation).
2) pilocarpine hydrochloride (Sigma Chemical Company).
3) carbachol chloride (Carcholin; Merck, Sharp and Dohme).

b. Sympathetic Stimulating Drugs

1) norepinephrine (Levophed Bitartrate; Winthrop Laboratories).
2) epinephrine chloride (Adrenalin Chloride; Parke, Davis and Company).
3) isoproterenol hydrochloride (Isuprel Hydrochloride; Winthrop Laboratories).

c. Ganglionic Stimulating Drug

1) tetramethylammonium chloride (Aldrich Chemical Company, Inc.).

d. Histamine

1) histamine phosphate (Ergamine Phosphate; Burroughs Wellcome and Company).
2) histamine free base (Sigma Chemical Company).
e. Direct-acting Smooth Muscle Stimulant

1) barium chloride (Baker Analyzed Reagent; J.T. Baker Chemical Company).

f. Naturally Occurring Hormones Known to Effect Smooth Muscle

1) oxytocin (Pitocin; Parke, Davis and Company).
2) vasopressin (Pitressin; Parke, Davis and Company).

2. DETERMINATION OF THE EFFECT OF PHARMACOLOGICAL BLOCKING DRUGS ON THE RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS

Members of the following classes of pharmacological blocking agents were studied to determine whether any contraction or relaxation of the isolated testicular capsule produced by specific autonomic stimulating agents could be antagonized. In every case, the blocking agent was allowed to act in the isolated tissue bath for two minutes before the addition of an autonomic stimulating agent.

a. Parasympathetic Blocking Drug

1) atropine sulfate (Mallinckrodt Chemical Works).
b. **Sympathetic Blocking Drugs**

1) phenoxybenzamine hydrochloride (Dibenzyline; Smith, Kline and French).

2) propranolol hydrochloride (Inderal Hydrochloride; Ayerst Laboratories Inc.).

c. **Ganglionic Blocking Drug**

1) tetraethylammonium chloride (Eastman Organic Chemicals).

d. **Antihistamine**

1) tripelennamine hydrochloride (Pyribenzamine Hydrochloride; Ciba Pharmaceutical Company).

e. **Skeletal Neuromuscular Blocking Drug**

1) D-tubocurarine chloride (K. and K. Laboratories, Inc).

f. **Direct-acting Smooth Muscle Blocking Drug**

1) papaverine hydrochloride (Lilly and Company).
3. DETERMINATION OF THE EFFECT OF PHARMACOLOGICAL BLOCKING DRUGS ON SPONTANEOUS CONTRACTIONS OF THE ISOLATED TESTICULAR CAPSULE

Members of the following classes of pharmacological blocking drugs were studied to determine their effect on spontaneous contractions of the adult rabbit isolated testicular capsule in the absence of any autonomic stimulating agents. In every case, the blocking drug was allowed to act in the isolated tissue bath for 20 minutes before washing out the drug.

a. Parasympathetic Blocking Drug

1) atropine sulfate (Mallinckrodt Chemical Works).

b. Sympathetic Blocking Drugs

1) phenoxybenzamine hydrochloride (Dibenzyline; Smith, Kline and French).
2) propranolol hydrochloride (Inderal Hydrochloride; Ayerst Laboratories Inc.).

c. Direct-acting Smooth Muscle Blocking Drug

1) papaverine hydrochloride (Lilly and Company).
d. Skeletal Neuromuscular Blocking Drug

1) D-tubocurarine chloride (K. and K. Laboratories, Inc.).
D. ANATOMICAL STUDIES ON THE TESTICULAR CAPSULE

1. **LIGHT MICROSCOPY**

   a. Tissue Preparation

   The testicular capsules of the adult rat and adult rabbit were prepared for histological studies by first removing the whole testes from the animals and placing them in Bouin’s fluid for 1/2 hour. The testes were then dissected in equal halves and transferred to fresh Bouin's fluid for at least 24 hours.

   Human testicular capsules were prepared for histological studies by placing approximately 5 mm portions of human testicular capsules and adjoining parenchymal tissue (obtained from males aged 60 - 70 years whose testes had been removed as a therapeutic measure for prostatic carcinoma) in Bouin's fluid for at least 24 hours. Larger portions of post mortum human testicular capsules and adjoining parenchymal tissue were also fixed in Bouin's fluid for several days.

   Bouin's fluid was chosen as the fixative for preserving the architecture of the testicular capsules of the adult rat, adult rabbit and human because it penetrates the tissue well while caus-
ing very little shrinkage to the testicular capsule. Moreover, tissues fixed in Bouin's fluid afford brilliant staining by the trichrome methods.

**Bouin's fluid**

750 ml saturated aqueous picric acid
250 ml formalin (40% formaldehyde)
50 ml glacial acetic acid

The excess picric acid which produces the yellow color of the testicular tissues was removed by at least 3 one-hour rinses in 70% ethanol. Following the 3 ethanol rinses, the testicular tissues were processed and embedded using the following procedure:

85% ethanol (1 hour)
95% ethanol (1 hour)
100% ethanol (1 hour - first bath)
100% ethanol (1 hour - second bath)
100% ethanol (1 hour - third bath)
benzene (1/2 hour - first bath)
benzene (1 hour - second bath)
benzene (1/2 hour - third bath)

SLUSH (overnight - liquid mixture of paraffin* saturated with benzene at 40 °C)

*paraffin used in all embedding procedures was Tissuemat with a melting point of 57-58 °C (Fisher Scientific Co.).
paraffin (1/2 hour - first bath at 59 °C)
paraffin (1 hour - second bath at 59 °C)
paraffin (1 hour - third bath at 59 °C)
↓
Tissues embedded in plastic "boats"

b. Staining Procedures.

The general connective tissue stain employed was Masson's trichrome stain. Tissue sections, 4 microns in thickness, were stained in Weigert's iron hematoxylin, followed by Biebrich scarlet-acid fuchsin and then aniline blue. Differentiation was carried out in 1% acetic acid. This procedure results in blue-black nuclei, red cytoplasm and muscle fibers and brilliant-blue collagen tissue.

The following outlines are the recipes for the various staining solutions used in the Masson's trichrome staining procedure:

1) Weigert's Iron Hematoxylin Working Solution

25 ml of Weigert's iron hematoxylin solution A were mixed with 25 ml of Weigert's iron hematoxylin solution B. This working solution was stable for 1 week.
Weigert's Iron Hematoxylin Solution A

1 g hematoxylin
100 ml absolute ethanol

Weigert's Iron Hematoxylin Solution B

4 ml 30% aqueous ferric chloride
100 ml distilled water
1 ml concentrated HCl

2) Biebrich Scarlet-acid Fuchsirn

1 g Biebrich scarlet
0.3 g phosphotungstic acid
5 ml glacial acetic acid
50 % ethanol q.s. to 100 ml

3) Phosphomolybdic-Phosphotungstic Acid Solution

This solution was prepared fresh for each staining cycle. 25 ml distilled water were mixed with 12.5 ml of 10% phosphotungstic acid solution and 12.5 ml of 10% phosphomolybdic acid solution.
4) **Aniline Blue**

A 2.5% aqueous solution of aniline blue was found to be stable for several months.

5) **1% Acetic Acid Solution**

This solution was prepared fresh for each staining cycle.

**Staining Procedure for the Masson's Trichrome Method**

1. Deparaffinize slides with 2 solutions of xylene (2 minutes each).
2. Wash xylene off in two solutions of 100% ethanol (1 minute each).
3. Hydrate to distilled water through steps of 95% ethanol, 70% ethanol, distilled water (1 minute each).
4. Transfer to Weigert's iron hematoxylin working solution (5 - 10 minutes).
5. Wash in running tap water (10 minutes).
6. Rinse in distilled water.
7. Transfer to Biebrich scarlet-acid fuchsin (2 minutes).
8. Rinse thoroughly in distilled water.
9. Transfer to phosphomolybdic-phosphotungstic acid solution (10 minutes).
10. Transfer to aniline blue (1 minute).
11. Rinse in 2 washes of distilled water.
12. Transfer to 1% acetic acid solution (3 minutes).
13. Dehydrate through steps of 70% ethanol, 95% ethanol, 100% ethanol, 100% ethanol (1 minute each).
14. Clear in 2 solutions of xylene (2 minutes each).
15. Mount in Permount.

2. ELECTRON MICROSCOPY

The adult rat testicular capsule was isolated in fresh phosphate buffered 6% glutaraldehyde (pH 7.3) at 4°C. The intact isolated capsule was then dissected by razor blades into the following specific regions: the posterior border and adjacent areas; the anterior border and adjacent areas; the lateral and medial sides; and the superior and inferior ends. Each dissected area of the capsule was cut into small squares of approximately 1 square mm and transferred to fresh phosphate buffered glutaraldehyde at 4°C for 1 hour. Following 3 washings in separate cold buffer solutions, the capsular squares were then post-fixed in 1% osmium tetroxide (pH 7.3) at 4°C for 1 hour, washed twice in cold buffer solutions and dehydrated in an ethanol-propylene oxide series, bring the temperature to room temperature following
the 95% ethanol step. The capsular tissues of the adult rat were then embedded in Epon and polymerized at 37 °C for 48 hours and then at 60 °C for 12 hours. Thin sections were cut on a Porter-Blum MT 1 microtome, transferred to 300 mesh carbon-coated copper grids, and stained in a saturated solution of uranyl acetate in 70% ethanol. Additional staining was carried out with lead citrate. The sections were then examined with a RCA EMU 3F-2 electron microscope and photographed on Kodak projection plates.
E. PHYSIOLOGICAL STUDIES ON THE ISOLATED TESTICULAR CAPSULE

The testis occupies a scrotal position in many animals which is several degrees lower than normal body temperature. Because of this condition, it was of interest to investigate the effect of various temperatures on tissue contraction of the isolated testicular capsules of both the adult rat and the adult rabbit.

1. EFFECT OF TEMPERATURE ON THE ADULT RAT ISOLATED TESTICULAR CAPSULE

The effect of increasing temperatures on the tissue contraction of the isolated testicular capsule of the adult rat was investigated in the absence of any drug. This was carried out by increasing the temperature of the isolated tissue bath 2°C every 15 minutes from 32°C (scrotal temperature) to 44°C. The temperature of the tissue bath was monitored by a tele-thermometer located directly below the isolated testicular capsule in the tissue bath.
2. **EFFECT OF TEMPERATURE ON SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE**

The effect of various temperatures on the spontaneous contractions of the adult rabbit isolated testicular capsule was investigated in the absence of any drugs. The initial temperature of the isolated tissue bath was set at 32 °C and monitored with a tele-thermometer. At 20 minute intervals, the temperature of the tissue bath was either lowered 6 °C until 14 °C was attained, or raised 6 °C until 50 °C was attained. Following exposure of the different capsules at either the minimum or the maximum temperature, the temperature of the tissue bath was then returned to the original starting temperature.

3. **EFFECT OF CRYPTORCHIDISM ON THE RESPONSE ON THE RESPONSE OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS**

The effect of cryptorchidism on the response of the isolated testicular capsule to various autonomic drugs was studied by comparing the response of the isolated testicular capsule from a cryptorchid testis with that of the isolated testicular capsule from the contralateral scrotal testis.

Cryptorchidism was experimentally induced in male Sprague-Dawley rats 50 to 60 days old at the beginning of the experiment.
A mid-line abdominal incision was made under ether anesthesia, and gentle pressure was applied to the bottom of the scrotal sac forcing the right testis through the inguinal canal into the abdomen. A fine 6-0 surgical silk suture was then passed under the tunica albuginea and attached to the dorsolateral body musculature. Care was taken to avoid damage to the testicular artery by placing the suture beyond the point where the testicular artery enters the testicular parenchyma. Closure of the inguinal canal was not performed because it was found that suturing alone was capable of preventing testicular descent. The contralateral left scrotal testis of the same animal was left in the scrotum to serve as the source for a control isolated testicular capsule.
F. QUANTITATIVE BIOASSAY OF ATROPINE USING THE ISOLATED TESTICULAR CAPSULE

1. PROCEDURE

The isolated testicular capsule of the adult rat was selected to perform a bioassay of an unknown concentration of a pharmacological drug to determine if the testicular capsule could be used as a practical biological tool for quantitative bioassay procedures. The bioassay chosen to test the capabilities of the isolated testicular capsule was the determination of an unknown concentration of atropine sulfate, using capsules from the left and right testes of the same animal.

The percent inhibition of the response of the isolated testicular capsule to 1.0 μg/ml acetylcholine chloride by atropine was determined using a 4 point assay consisting of two doses of the test solution (T) of atropine, and two doses of a standard solution (S) of atropine by the following manner:

A = low concentration of test solution (T₁)
B = high concentration of test solution (T₂)
C = low concentration of standard solution (S₁)
D = high concentration of standard solution (S₂)
In each application of atropine to the isolated testicular capsule in the tissue bath, the atropine was allowed to act for two minutes before the addition of acetylcholine to the tissue bath.

2. METHOD FOR CALCULATING POTENCY

1) The mean percent response was calculated for each solution of atropine as follows:

\[
\text{mm Response (atropine + 1.0 µg/ml acetylcholine)} \times 100
\]
\[
\text{mm Response (1.0 µg/ml acetylcholine)}
\]

2) The mean values of \( S_1, S_2 \) and \( T_1, T_2 \) (percent response) were graphically plotted against the corresponding log dose of atropine (ml).

3) Doses (ml) were obtained from the graph for the standard (X ml) and test (Y ml) solutions of atropine which permitted the same percent response of the testicular capsule to 1.0 µg/ml acetylcholine and used in the following formula:

**Graphic Solution Formula**

\[
(X \text{ ml}) \times (\text{concentration of standard solution}) = (Y \text{ ml}) \times (\text{concentration of test solution})
\]
Concentration of test solution = \( \frac{(X \text{ ml}) \times \text{ (concentration of standard solution)}}{Y \text{ ml}} \)

Total concentration of test solution = \( \text{ (concentration of test solution) \times (dilution factor)} \)
CHAPTER IV

RESULTS
A. ISOLATION OF THE TESTICULAR CAPSULE

1. ADULT RAT ISOLATED TESTICULAR CAPSULE

The procedure for the isolation of the testicular capsule of the adult rat and its subsequent use as an isolated tissue preparation for pharmacological studies is shown in figure 1. The resulting intact isolated testicular capsule of the adult rat obtained by this method can be seen to resemble a thin hollow sac (figure 2) with a wet weight of approximately 75 mg. Figure 3a indicates a comparison of the size of the intact isolated testicular capsule of the adult rat with the mass of removed seminiferous tubules. The fact that the removed seminiferous tubular mass was observed to expand to a size approximately twice that of the testicular capsule would seem to suggest that the pressure the capsule would appear to apply to the seminiferous tubules must be quite appreciable. The isolated parenchymal tissue mass had a wet weight of approximately 1,500 mg. Figure 3b presents a photograph of the intact isolated testicular capsule of the adult rat in the inside-out position with the two attached pieces of thread, as it would appear following its removal from an isolated tissue bath.
2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE

The procedure for the isolation of the testicular capsule of the adult rabbit and its subsequent use as an isolated tissue preparation for pharmacological studies was found to be essentially the same as that described for the adult rat (figure 1) with the exception that it was somewhat more laborious to remove the internal parenchymal tissue because of the numerous thin septa extending from the capsule into the testicular parenchyma.

The resulting intact isolated testicular capsule of the adult rabbit resembles a thin hollow sac (figure 12c-d) approximately the same size as the isolated capsule of the adult rat testis, but considerably thicker. The testicular capsule of the adult rabbit had a wet weight of about 125 mg or approximately twice that of the adult rat testicular capsule. Figure 12a-b indicates the comparative sizes of the isolated whole testis and the isolated testicular parenchymal tissue of the adult rabbit with the isolated testicular capsule seen in figure 12c. The removed testicular parenchymal tissue had a wet weight of approximately 1500 mg and was not observed to expand as much as the isolated testicular parenchymal tissue of the adult rat (figure 4a).
3. **HUMAN ISOLATED TESTICULAR CAPSULE**

The procedure for the isolation of the human testicular capsule and its subsequent use as an isolated tissue preparation for pharmacological studies is shown in figure 20. Inasmuch as the entire isolated testicular capsule of the human was too large to be mounted in an isolated tissue bath, strips of the testicular capsule were cut to an approximate size of 20 mm in length and 6 mm in width. Each human capsular strip had an average wet weight of approximately 400 mg.

**B. RESPONSE OF THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS**

1. **ADULT RAT ISOLATED TESTICULAR CAPSULE**

   a. Response of the Testicular Capsule to Acetylcholine and Norepinephrine

   Figure 5 presents the response of the isolated testicular capsule of the adult rat to acetylcholine and norepinephrine. Both acetylcholine and norepinephrine were observed to produce a marked contraction of the testicular capsule. In each case, the contractions induced by each neurohumoral agent appeared to
be dose dependent and maximal contraction of the testicular capsule was reached in approximately 3 minutes following addition of the drug.

Figure 6 presents a dose-response curve for the effect of acetylcholine and norepinephrine on the isolated testicular capsule of the adult rat. Tissue contractions were detected at concentrations of 0.001 µg/ml for acetylcholine and 0.01 µg/ml for norepinephrine. The maximal response observed for both acetylcholine and norepinephrine occurred at a dose of 1 µg/ml final concentration. The maximal response of the capsule to acetylcholine appeared to be approximately twice that observed with norepinephrine.

These data, then, represent the first reported use of the testicular capsule as an isolated tissue preparation as well as the first reported response of the testicular capsule to a pharmacological agent, namely acetylcholine and norepinephrine.

b. Recovery Time of the Testicular Capsule Following Response of the Capsule to Acetylcholine

The testicular capsule was found to relax slowly after it had been caused to contract with acetylcholine or norepinephrine. As shown in figure 7, the time taken for the testicular capsule to return to the initial resting tension following washing out of 0.01 µg/ml acetylcholine was in excess of 12 minutes. With
increasing doses of 0.1 µg/ml and 1.0 µg/ml acetylcholine, the recovery time increased.

In contrast to the testicular capsule, the time taken for a standard smooth muscle preparation such as the guinea pig isolated ileum (figure 8) to return to the initial resting tension following washing out of 0.01 µg/ml acetylcholine is approximately 40 seconds.

Since the testicular capsule was observed to relax slowly following contraction, it was found that by adding a slight extra weight to the initial resting tension load of 100 mg, its recovery time was greatly shortened. This procedure was found to conveniently allow doses to be added about once every 15 minutes.

c. Influence of Various Physiological Salt Solutions on the Response of the Testicular Capsule to Acetylcholine.

Since the testicular capsule was a new isolated tissue preparation, it was necessary to determine a suitable physiological salt solution in which the isolated capsule could be maintained while studying its physiological and pharmacological properties. Table 1 presents the relative responses of the isolated testicular capsule of the adult rat to acetylcholine in Tyrode's solution, Krebs solution (1932) and DeJalon solution.
The greatest contraction of the testicular capsule in response to 0.01 µg/ml acetylcholine was obtained in Tyrode's solution. Smaller contractions of the capsule were obtained using Krebs and DeJalon solutions, and these contractions were expressed as a per cent contraction of that obtained in Tyrode's solution. Contractions of the testicular capsule in Krebs and DeJalon solutions were only 56% and 36% respectively of that obtained in Tyrode's solution. On the basis of these results, Tyrode's solution was used in all subsequent isolated tissue bath studies.

d. Influence of Various Resting Tensions on the Response of the Testicular Capsule to Acetylcholine

Figure 9 presents the influence of various resting tensions (load) on the testicular capsular contractions produced by 0.1 µg/ml acetylcholine. The abscissa is the load on the lever arm in mg while the left ordinate is mm of tissue contraction. The degree of contraction of the testicular capsule in response to acetylcholine was found to increase from 0.19 mm at an initial load of 75 mg to a maximal response of 0.34 mm observed at a load of 400 mg. The contraction strength of the testicular capsule in response to acetylcholine appears to be relatively great since the isolated rat testicular capsule weighing only 50-75 mg is capable of still lifting load weights of over 1 gm.
The work done by the testicular capsule as each load is lifted is shown on the right ordinate of figure 9. Load weights were added to the end of the long arm of a 3:1 lever so that each load was lifted a distance equal to 3 times the actual contraction of the capsule. The work done by the capsule to lift each load was therefore obtained by the equation:

\[
\text{WORK} = \text{LOAD (mg)} \times \text{CONTRACTION (mm)} \times 3
\]

The work performed by the testicular capsule was found to increase from 43 mg-mm at the initial load of 75 mg to a maximum of 462 mg-mm at a load of 700 mg. While performing the greatest amount of work, however, the capsule undergoes 0.22 mm contraction which was approximately the same as that contraction (0.23 mm) observed with a load of only 100 mg. Moreover, the work done by the capsule with a load of 100 mg was much less at only 69 mg-mm. It was therefore decided to adopt the load of 100 mg as the resting tension for the subsequent pharmacological studies on the isolated testicular of the adult rat.

e. Response of the Testicular Capsule to Various Autonomic Drugs.

Figure 10 presents the results of further pharmacological studies of the testicular capsule of the adult rat involving the
effects of various autonomic agents. Two additional parasympathomimetic agents, carbachol and pilocarpine, were compared with acetylcholine as to their effect on the isolated testicular capsule. Carbachol, a synthetic cholinomimetic agent, seemed to resemble acetylcholine in producing a marked contraction of the capsule. The maximal response of the capsule to carbachol was also found to occur at a dose of 1 µg/ml final concentration. Pilocarpine, a naturally-occurring cholinomimetic alkaloid was also observed to cause a contraction of the isolated testicular capsule. However, the response of the capsule to pilocarpine appeared markedly less sensitive as compared to acetylcholine as indicated by a dose of 50 µg/ml final concentration of pilocarpine being required to cause only a slight contraction of the capsule.

The effects of two additional sympathomimetic agents, namely epinephrine and isoproterenol, were compared with norepinephrine as to their effect on the isolated testicular capsule. Epinephrine, at a final concentration of 1 µg/ml, was observed to cause a contraction of the isolated capsule which was somewhat greater than that resulting from a similar dose of norepinephrine. Isoproterenol, on the other hand, was found to cause a relaxation of the isolated testicular capsule.

Tetramethylammonium (TMA), which is a ganglionic stimulating agent, produced a contraction of the isolated capsule of the rat.
Histamine, at a concentration of 50 µg/ml, also resulted in a contraction of the isolated testicular capsule of the adult rat. The histamine-induced contraction of the capsule seemed somewhat unusual with respect to the other agents studied, in that the capsule began to relax immediately after reaching maximal contraction. In the case of the other drugs causing contraction of the rat testicular capsule, the capsule was found to relax quite slowly and only after washing out the added drug.

Barium chloride, noted for its intense stimulation of smooth muscle fibers, was observed to produce only a very slight contraction of the capsule and only at the extremely high final concentration of 1 mg/ml of tissue bath volume.

Since isoproterenol was the only autonomic drug found to produce a relaxation of the testicular capsule, it was of interest to determine the effect of isoproterenol on the contracted testicular capsule. Figure 11 (top recording) indicates the response of the testicular capsule to 5 µg/ml isoproterenol during the maximal contraction of the capsule to norepinephrine. Only a very slight relaxation of the capsule was observed approximately 1 minute following the addition of isoproterenol. The middle recording of figure 11 indicates the response of the testicular capsule to isoproterenol during maximal contraction of the capsule to acetylcholine. A marked relaxation of the capsule was observed immediately following the addition of iso-
proterenol which was almost sufficient to restore the initial tone of the capsule. These data indicate that the relaxation effect of isoproterenol is enhanced during an acetylcholine-induced contraction of the testicular capsule, but during a norepinephrine-induced contraction of the capsule, the relaxation effect of isoproterenol is reduced. It was therefore of interest to determine the effect of isoproterenol during a contraction of the testicular capsule produced by the combined effects of both norepinephrine and acetylcholine. The bottom recording of figure 11 indicates that the total contraction of successive doses of norepinephrine followed 5 minutes later by acetylcholine consists of 2 separate components, an initial norepinephrine-induced contraction followed by a marked acetylcholine contraction. This second contraction of the capsule had an additive effect to the initial sustained contraction produced by norepinephrine. The addition of isoproterenol to the tissue bath during this contracted state of the capsule produced an immediate relaxation which was sufficient to almost eliminate the acetylcholine component of the testicular capsular contraction.

2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE

a. Spontaneous Contractions

Figure 13 presents the results of the initial pharmacologi-
cal investigations of the isolated testicular capsule of the adult rabbit. In contrast to the adult rat testicular capsule, the isolated testicular capsule of the adult rabbit as shown in the middle recording was observed to undergo marked spontaneous contractions within 1 hour following mounting of the capsule in the tissue bath and before the addition of any drug. The amplitude of the spontaneous contractions was found to average a 5 per cent shortening of the actual entire length of the mounted capsule, and indeed, it was even possible to observe these rhythmic movements of the capsule with the unaided eye. The frequency of the spontaneous contractions ranged from 3 to 5 per minute. As shown in the top recording, spontaneous contractions were also observed in the isolated whole testis of the adult rabbit without removal of the capsule. In marked contrast to the periodic spontaneous contractions recorded from both the isolated testicular capsule and the isolated whole testis, no spontaneous contractions of the isolated testicular parenchymal tissue were observed, even at a response magnification of $x 100$.

It would therefore appear that the testicular capsule alone is responsible for the endogenous rhythmic spontaneous contractions observed with the whole rabbit testis. In addition, the fact that the whole testis with its contained seminiferous tubules and interstitial tissue offering a mass of tissue resistance was observed to undergo spontaneous contractions would seem to
lend great physiological importance to the contractions described for the isolated capsule alone.

b. Influence of Various Resting Tensions on Spontaneous Contractions of the Testicular Capsule.

Figure 14 presents the influence of various resting tensions (load) on spontaneous contractions of the isolated testicular capsule and the isolated whole testis of the adult rabbit. The initial load was 100 mg for both the testicular capsule and the whole testis, and increasing loads of 200 mg, 300 mg or 500 mg were added at intervals of approximately 20 minutes. Spontaneous contractions were observed to be nearly completely inhibited at a load of 1000 mg for the whole testis and at a load of 1500 mg for the isolated testicular capsule. The initial load of 100 mg appeared to be a satisfactory resting tension and it was therefore decided to adopt this load for subsequent pharmacological studies on the isolated testicular capsule and whole testis of the adult rabbit.

c. Response of the Testicular Capsule versus the Testicular Parenchyma to Autonomic Drugs.

Figure 15 presents a comparison of the response of the isolated capsule versus the isolated parenchymal tissue to the
same autonomic drugs employed for the adult rat isolated testicular capsule. The response of the isolated testicular capsule to each drug is shown at the top of each series of recordings. Like the capsule of the rat testis, the rabbit isolated capsule was found to undergo a contraction following the addition of acetylcholine and norepinephrine. A final concentration of 1 µg/ml of acetylcholine in the tissue bath resulted in a contraction of the rabbit isolated testicular capsule which amounted to approximately a 10 per cent shortening of the actual length of the mounted capsule. A final concentration of 1 µg/ml of norepinephrine in the tissue bath produced a 20 percent shortening of the actual entire length of the mounted capsule.

The rabbit isolated testicular capsule was also found to resemble the isolated testicular capsule of the rat in that contractions were produced by the addition of carbachol, epinephrine, tetramethylammonium (TMA) and histamine. The rabbit isolated testicular capsule was found to be unusually sensitive to histamine in that a final concentration of only 5 µg/ml of histamine resulted in a marked contraction. In addition, isoproterenol also produced a prolonged relaxation of the testicular capsule, as well as completely abolishing capsular spontaneous contractions. However, two differences were noted between the isolated testicular capsule of the rat and rabbit in that pilocarpine, in a comparable dose, did not elicit any response while barium chloride, in a comparable dose, produced a very large contraction.
of the rabbit testicular capsule.

The isolated testicular parenchymal tissue displayed only extremely small and quite negligible contractions with several of the autonomic agents studied, even at a response magnification of x 100. For example, the addition of 1 µg/ml final concentration of both acetylcholine and norepinephrine to the isolated testicular capsule of the rabbit resulted in tissue contractions of 0.975 and 1.185 mm/100 mg respectively. However, similar additions of both acetylcholine and norepinephrine to the isolated testicular parenchymal tissue of the rabbit resulted in tissue contractions which averaged not more than 0.001 mm/100 mg.

Figure 16b presents a photograph of the isolated capsule and parenchymal tissue of the adult rabbit testis used for the above pharmacological studies.

d. Response of the Isolated Whole Testis to Acetylcholine and Norepinephrine.

Now that the effect of various autonomic drugs had been determined on the isolated capsule and isolated parenchymal tissue of the adult rabbit, it seemed of interest to investigate the effect of pharmacological agents on the isolated whole testis. Figure 17 presents the response of the isolated whole testes of
the adult rabbit as well as the adult rat to 1 µg/ml acetylcholine and 1 µg/ml norepinephrine.

The isolated rat testis underwent a contraction in response to both neurohumoral agents but the response was approximately half that usually observed for the isolated capsule of the adult rat testis.

The isolated rabbit testis was observed to undergo marked spontaneous contractions. Following the addition of acetylcholine or norepinephrine, a marked contraction was observed which appeared to be biphasic. As observed for the isolated testicular capsule, the response of the whole testis to norepinephrine was larger than that observed with acetylcholine.

e. Response of the Testicular Capsule versus the Testicular Parenchyma of the Adult Rat to Autonomic Drugs.

Figure 18 presents a comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rat testis to the same autonomic drugs employed for the comparison of the isolated capsule and parenchymal tissue of the rabbit testis. The response of the testicular capsule to each drug is shown at the top of each series of recordings and are similar to those responses shown previously in figure 10.

The lower recording of each series presents the response of
the isolated testicular parenchymal tissue of the adult rat to these same nine autonomic drugs. The isolated parenchymal tissue had a wet weight of 1,500 mg as compared to only 75 mg for the isolated capsule. None of the autonomic agents studied were observed to have any effect on the isolated parenchymal tissue except for norepinephrine and epinephrine, and these two agents caused only a negligible degree of contraction. For example, the addition of 1 \( \mu g/ml \) final concentration of acetylcholine or norepinephrine to the testicular capsule produced a tissue contraction of 0.47 and 0.28 mm/100 mg respectively. However, the corresponding addition of both drugs to the isolated parenchymal tissue caused a negligible tissue contraction which averaged not more than 0.001 mm/100 mg.

Figure 19 presents a comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the rat testis to oxytocin and vasopressin. 0.5 U/ml vasopressin was observed to produce a slight contraction of both the testicular capsule and testicular parenchymal tissue. On the other hand, 0.05 U/ml oxytocin was observed to produce a slight contraction of only the isolated testicular parenchymal tissue.
3. **HUMAN ISOLATED TESTICULAR CAPSULE**

   a. Spontaneous Contractions of the Testicular Capsule.

   The question arose as to whether the pharmacological responses of the isolated testicular capsule of the rat and rabbit would also occur in the human testicular capsule. Figure 21 presents the results of the initial pharmacological studies on the isolated testicular capsule of the human. The human testicular capsule was observed to undergo very marked spontaneous contractions which reached an actual tissue contraction of 1.381 mm/100 mg. These extremely strong spontaneous contractions represented a remarkable 39 percent shortening of the actual entire length of the mounted strip of the human testicular capsule. The average frequency of these spontaneous contractions of the human testicular capsule was such that one extremely powerful contraction occurred every 13.6 minutes. It is interesting to note that in most instances a large spontaneous contraction was followed by a slightly smaller spontaneous contraction and that the larger contraction was usually biphasic in nature.

   b. Response of the Testicular Capsule to Various Autonomic Drugs.

   Figure 22 presents the effects of various autonomic drugs
on the isolated testicular capsule of the human. Each drug was added to the tissue bath shortly after the recording of a spontaneous contraction had returned to baseline so as to be sure that the response was due to the added drug alone. Acetylcholine, carbachol, norepinephrine, tetramethylammonium (TMA) and barium chloride were all observed to induce a contraction of the isolated capsule of the human. In addition, the human testicular capsule seemed extremely sensitive to norepinephrine and indeed, norepinephrine was found to induce spontaneous contractions of the testicular capsule at the peak of its effect, which was within 2 minutes after addition to the tissue bath. Whereas norepinephrine was found to produce a shortening of the actual entire length of the mounted capsule of the rat and rabbit testis which amounted to approximately 2 and 10 percent respectively, norepinephrine produced a 20 percent shortening of the actual entire length of the mounted testicular capsule of the human.

C. ANATOMY OF THE TESTICULAR CAPSULE

1. ADULT RAT TESTICULAR CAPSULE

   a. Identification and Distribution of Smooth Muscle in the Tunica Albuginea.

   Figure 24 presents representative photomicrographs of the
testicular capsule of the adult rat as it continues around the circumference of the testis. The widened mediastinal region of the testicular capsule encloses the cavities of the rete testis which are lined with a cuboidal type of epithelium (figure 26). Because of the pharmacological effects on the rat isolated testicular capsule which have been observed with various autonomic drugs, it became of special interest to attempt to ascertain the presence of smooth muscle fibers in the testicular capsule of the adult rat. Although Rolshoven (1936) had suggested that smooth muscle was "apparently lacking" in the tunica albuginea of the rat, it was possible by employing Masson's trichrome stain to identify occasional smooth muscle fibers as well as smooth muscle nuclei which were located within the collagenous tissue of the mediastinal region of the rat testicular capsule (figures 24, 25, 31). In addition, these smooth muscle fibers and nuclei were also seen in sparse but definite amounts in areas of the tunica albuginea of the capsule which were adjacent to both sides of the mediastinum as indicated in figure 24. It appeared that with respect to the testis of the adult rat, smooth muscle cells of the tunica albuginea were located predominantly along the posterior border of the testicular capsule and are extremely difficult to find along the anterior border of the testicular capsule. Fibroblasts, on the other hand, are readily apparent along the anterior and posterior borders of the capsule,
being present in all portions of the capsule as it envelops the testis.

As shown in figure 31, the use of Masson's trichrome stain results in blue-black nuclei, red cytoplasm and muscle fibers and brilliant-blue collagen tissue. Smooth muscle nuclei can easily be distinguished from fibroblast nuclei since the former have a characteristic lightly-stippled appearance with an oval shape whereas the latter appear much darker and thinner.

b. Fine Structure of the Collagenous Tissue of the Tunica Albuginea.

In addition to the presence of smooth muscle fibers as described above, the tunica albuginea also contains fibroblasts and collagen fibrils. Collagenous tissue is by far the most prevalent component of the tunica albuginea, accounting for the firm, fibrous nature of the testicular capsule. Figure 27 presents the ultrastructure of collagen fibrils of the tunica albuginea of the rat testicular capsule. Each collagen fibril was found to exhibit regularly occurring light and dark bands with the usual average axial periodicity of 640 Å, characteristic of normal collagen. The numerous fibroblasts present in the tunica albuginea when observed with the electron microscope, were found to have an extremely thin cytoplasm with irregular extensions projecting into the adjacent collagen. These irregu-
lar fibroblastic extensions appeared to separate the collagenous tissue into collagen fibers.

2. **ADULT RABBIT TESTICULAR CAPSULE**

   a. Identification and Distribution of Smooth Muscle in the Tunica Albuginea.

   The presence of smooth muscle fibers were also found in the tunica albuginea of the adult rabbit testicular capsule (figures 28, 31). Figure 28 presents representative cross-sections of the testicular capsule of the adult rabbit, illustrating the distribution of smooth muscle in the tunica albuginea of this animal. In contrast to the rat, two distinct layers of smooth muscle fibers were found in the tunica albuginea of the rabbit testicular capsule. A superficial layer of longitudinal smooth muscle runs parallel to the long axis of the rabbit testis. A second, deeper layer of circular smooth muscle is oriented along the circumference of the rabbit testis at right angles to the superficial layer. Collagen occurring on either side of the deeper circular layer of smooth muscle was found to separate this layer from the longitudinal layer of smooth muscle. In addition, numerous fibroblasts were observed within the dense collagenous tissue of the tunica albuginea. These results
indicate that whereas the rat testicular capsule has only a sparse amount of smooth muscle present, the rabbit testicular capsule has an abundance of smooth muscle which is oriented in two distinct layers. In addition, the rabbit testicular capsule was also found to differ from the rat testicular capsule in that it displayed numerous thin septa extending from the tunica albuginea into the parenchymal tissue of the rabbit testis.

3. HUMAN TESTICULAR CAPSULE

a. Identification and Distribution of Smooth Muscle in the Tunica Albuginea.

Smooth muscle fibers were also found in the tunica albuginea of the human testis as shown in figures 29, 30, 31. The human testicular capsule appears to resemble the testicular capsule of the adult rat in that smooth muscle fibers do not enclose the entire organ but are found predominantly on the posterior side of the testis which is adjacent to the epididymis.

In contrast to the sparse 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 human testicular capsule. A superficial layer of longitudinal smooth muscle which contains the majority of smooth muscle fibers
found in the human testicular capsule, appears to run parallel to the long axis of the testis while a second, deeper layer of circular smooth muscle appears to be oriented along the circumference of the testis at right angles to the superficial layer. In addition, numerous fibroblasts were observed within the dense collagenous tissue of the tunica albuginea. A definite 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 adult rat, rabbit and human.
D. EFFECT OF AUTONOMIC BLOCKING AGENTS ON THE RESPONSE OF
THE ISOLATED TESTICULAR CAPSULE TO AUTONOMIC DRUGS

1. ADULT RAT ISOLATED TESTICULAR CAPSULE

a. Parasympathetic Postganglionic Blocking Drug

Figure 32 presents the effects of increasing concentrations of atropine sulfate on the response of the adult rat isolated testicular capsule to acetylcholine. A through J represent the order of recordings obtained in a typical dose-response procedure for the blocking effects of atropine on acetylcholine-induced contractions of the testicular capsule. The control responses of the capsule to 1.0 µg/ml acetylcholine are presented on the left, and were obtained immediately preceding the response of the capsule to acetylcholine in the presence of increasing doses of atropine as shown on the right. Between F and G and between H and I, fifteen-minute acetylcholine dose cycles were required to return the acetylcholine-induced response of the capsule to the control responses shown at G and I respectively.

Figure 34 presents the effective blocking ability of various
doses of atropine in terms of percent response of the adult rat testicular capsule to 1 µg/ml acetylcholine. At a minimum dose of 0.01 µg/ml, atropine had no observable effect on the acetylcholine-induced contraction of the capsule, whereas, at a dose of 1.0 µg/ml, atropine was observed to completely inhibit the acetylcholine-induced contraction. The inhibitory dose of atropine found to produce a 50 percent block ($I_{D_{50}}$) of the response of the testicular capsule to acetylcholine was calculated to be 0.1 µg/ml atropine (table 2).

Table 3 presents the effect of atropine on the response of the testicular capsule to other autonomic agents. At a dose of 1 µg/ml, atropine was observed to completely block the contraction of the capsule produced by 100 µg/ml tetramethylammonium. However, atropine had no observable effect on either the contraction or relaxation of the capsule produced by 1 µg/ml norepinephrine or isoproterenol respectively.

b. Ganglionic Blocking Drug

Figure 33 presents the effects of increasing concentrations of tetraethylammonium (TEA) on the response of the adult rat isolated testicular capsule to 50 µg/ml tetramethylammonium (TMA). A through I represent the order of recordings obtained in a typical dose-response procedure for the blocking effects of TEA
on TMA-induced contractions of the capsule. The control responses of the capsule to 50 µg/ml TMA are presented on the left and were obtained immediately preceding the response of the capsule to TMA in the presence of increasing doses of TEA as shown on the right. Following a partial block of the response of the capsule to TMA, several fifteen minute TMA dose cycles were required to return the TMA-induced response of the capsule to the control responses shown at E and G. At a dose of 500 µg/ml, TEA was observed to completely inhibit the TMA response as shown in the bottom recording. However, by increasing the dose of TMA to 200 µg/ml, the block was partially reversed.

Figure 34 presents the effective blocking ability of various doses of TEA in terms of percent response of the testicular capsule of the adult rat to 50 µg/ml TMA. At a minimum dose of 50 µg/ml, TEA had no observable effect on the TMA-induced contraction of the capsule, whereas, a dose of 250 µg/ml TEA produced almost a complete block. The ID$_{50}$ of TEA in inhibiting TMA was calculated to be 115 µg/ml (table 2).

Table 3 presents the effect of TEA on the response of the testicular capsule to other autonomic agents. In every case, 200 µg/ml TEA had no effect on the response of the capsule to 1 µg/ml acetylcholine, 1 µg/ml norepinephrine or 5 µg/ml isoproterenol.
c. Sympathetic Blocking Drugs

1) Phenoxybenzamine

Figure 34 presents the percent response of the adult rat testicular capsule to 1 µg/ml norepinephrine in the presence of various doses of phenoxybenzamine. A dose of 1 µg/ml phenoxybenzamine was observed to completely block the norepinephrine-induced contraction, while a dose of 0.1 µg/ml phenoxybenzamine produced only a 20 percent block. The ID₅₀ of phenoxybenzamine in inhibiting norepinephrine was calculated to be 0.3 µg/ml (table 2). It was of interest to note that 10 µg/ml phenoxybenzamine had no observable effect on the testicular capsular relaxation produced by 5 µg/ml isoproterenol. (table 3).

Phenoxybenzamine was also observed to have an antagonistic effect on the acetylcholine-induced contraction of the testicular capsule (figure 34). A dose of 1 µg/ml phenoxybenzamine (which completely blocks a norepinephrine-induced contraction of the capsule) had no effect on 1 µg/ml acetylcholine. However, a higher dose of 10 µg/ml phenoxybenzamine was found to produce a 30 percent inhibition of acetylcholine-induced contractions. Also at this higher dose of 10 µg/ml, phenoxybenzamine was found to completely inhibit the contraction of the testicular capsule to 100 µg/ml tetramethylammonium (table 3).

Occasionally, as shown in figure 35, phenoxybenzamine was
observed to cause an initial small contraction of the adult rat isolated testicular capsule. This stimulatory response was never observed at doses lower than 10 µg/ml.

2) Propranolol

Propranolol was found to have no observable effect on the response of the testicular capsule to acetylcholine, tetramethylammonium (TMA) or norepinephrine (table 3), but was found to be completely effective in blocking the testicular capsular relaxation produced by isoproterenol. Figure 34 presents the effective blocking ability of various doses of propranolol in terms of percent response of the capsule to 5 µg/ml isoproterenol. A dose of 100 µg/ml propranolol was observed to completely block the capsular relaxation produced by isoproterenol, while 10 µg/ml propranolol had no observable effect. The ID$_{50}$ of propranolol in blocking isoproterenol was calculated at 27 µg/ml (table 2).

Occasionally, Propranolol was observed to cause a contraction of the testicular capsule of the adult rat as shown in figure 35. This response occurred only after a latent period of approximately 1 minute following the addition of the drug and characteristically was a slow contraction most often occurring following doses of at least 100 µg/ml propranolol. This was in contrast to the immediate contraction often observed following
high doses of phenoxybenzamine.

d. Antihistamine

The contraction of the adult rat testicular capsule observed with 50 µg/ml histamine was observed to be completely inhibited by a dose of 1 µg/ml tripelennamine. However, tripelennamine had no observable effect on the response of the capsule to acetylcholine, norepinephrine or isoproterenol.

e. Skeletal Neuromuscular Blocking Drug

Table 3 presents the effect of d-tubocurarine on the response of the adult rat testicular capsule to several autonomic agents. In every case, 10 µg/ml tubocurarine was observed to have no effect on the response of the capsule to acetylcholine, tetramethylammonium, norepinephrine or isoproterenol. At a higher dose of 1 mg/ml, tubocurarine still had no observable effect on the acetylcholine-induced contraction of the capsule.

f. Directing-Acting Smooth Muscle Blocking Agent

The testicular capsular contraction produced by 1 mg/ml BaCl₂ was found to be inhibited by 1 mg/ml papaverine.
2. ADULT RABBIT ISOLATED TESTICULAR CAPSULE

a. Effect of Pharmacological Blocking Drugs on Spontaneous Contractions of the Isolated Testicular Capsule

Since drug-induced contractions or relaxation of the adult rat isolated testicular capsule were capable of being antagonized by specific pharmacological blocking agents, it was of interest to study the effects of several of these blocking agents on the spontaneous contractions of the adult rabbit isolated testicular capsule. The following blocking agents were found to have no observable effect on spontaneous contractions during a 20 minute contact period in the isolated tissue bath: 1 mg/ml atropine, 1 mg/ml phenoxybenzamine, 1 mg/ml propranolol, 1 mg/ml D-tubocurarine and 1 mg/ml papaverine.
E. FACTORS INFLUENCING THE ISOLATED TESTICULAR CAPSULE

1. TEMPERATURE

a. Effect of Temperature on Contraction of the Adult Rat Isolated Testicular Capsule

Figure 36 presents the effect of increasing temperatures on the tissue contraction of the isolated testicular capsule of the adult rat. The abscissa indicates the temperature of the tissue bath which was increased 2 degrees every 15 minutes from an initial temperature of 32 °C to 44 °C. The ordinate indicates the actual tissue contraction in mm. An increase in temperature above 32 °C in the absence of any drug was observed to produce a marked contraction of the capsule at each two degree increase up to 38 °C. However, increases in temperature above 38 °C were not observed to produce any further testicular capsular contraction. The total contraction of the isolated testicular capsule between 32 °C and 38 °C was found to be 0.2 mm of tissue contraction.
b. Effect of Temperature on Spontaneous Contractions of the Adult Rabbit Isolated Testicular Capsule

Figure 37 presents the effect of various temperatures on the spontaneous contractions of the adult rabbit isolated testicular capsule. Spontaneous contractions were observed to be largest at a temperature of 32 °C, the initial temperature of the tissue bath. As the temperature of the tissue bath was increased or decreased 6 °C at 20 minute intervals, the amplitude of the spontaneous contractions was observed to decrease. At a minimal temperature of 14 °C or at a maximal temperature of 50 °C, the spontaneous contractions were observed to be completely inhibited.

The inhibition of the spontaneous contractions resulting from a low temperature (14 °C) was found to be completely reversible when the temperature of the tissue bath was increased to 26 °C (figure 38). However, the inhibition of the spontaneous contractions resulting from high temperatures (50 °C) was found to be irreversible since no spontaneous activity was observed when the temperature was returned to 32 °C (figure 38).

2. CRYPTORCHIDISM

a. Effect of Cryptorchidism on the Response of the Adult Rat Isolated Testicular Capsule to Autonomic Drugs
The general appearance of the adult rat right testis rendered cryptorchid for approximately 600 days was that of a small sac weighing about 250 mg. The contralateral left scrotal testis resembled that of a normal testis and weighed about 2,100 mg. The isolated capsule of the cryptorchid testis was smaller but appeared much thicker than that of the isolated capsule of the contralateral scrotal testis.

Figure 39 presents the response of the isolated capsule from a 600 day cryptorchid testis to the same autonomic agents previously studied on the adult rat isolated testicular capsule. The response of the contralateral scrotal testis to these same autonomic agents is shown in the top series of recordings in figure 39. The body weight of the 650 day old rat was 535 g. In every case, the response of the isolated cryptorchid capsule to each drug was smaller although similar to that of the contralateral scrotal testicular capsule, and the 90 day old adult rat isolated testicular capsule.

3. ANOXIA

a. Effect of Anoxia on Spontaneous Contractions of the Adult Rabbit Isolated Testicular Capsule, Isolated Whole Testis and Isolated Duodenum

A commonly observed response of the adult rabbit isolated
testicular capsule before the addition of any drug occurs when previously unaerated buffer is washed into the tissue bath.

Immediately following the buffer wash, the isolated testicular capsule undergoes a marked contraction for several minutes before returning to the initial resting tension (figure 40A). Following this phenomenon, the spontaneous contractions of the capsule usually increase in amplitude although the frequency of spontaneous contractions returns to the initial rate. It was found that this response of the testicular capsule could be approximately duplicated by turning the aeration in the tissue bath off for several minutes. Figure 40B-C illustrate a marked contraction of the testicular capsule and an accompanied inhibition of spontaneous contractions following cessation of aeration in the tissue bath. Following re-aeration of the tissue bath, the testicular capsule quickly recovers its original tone and spontaneous activity. An increase in the amplitude of the spontaneous contractions, however, is usually observed following the response.

Similar responses during anoxic conditions in the tissue bath were observed with the intact isolated whole testis of the adult rabbit (figure 41A-C).

In marked contrast to the responses of the isolated testicular capsule and isolated whole testis of the adult rabbit during anoxic conditions in the tissue bath, the isolated duodenum of the adult rabbit was observed to undergo a decrease in tone during
anoxic conditions in the tissue bath (figure 42A–C). A decrease in spontaneous contractions accompanies the relaxation. Following re-aeration of the tissue bath, the tone and frequency of spontaneous contractions return to the initial levels.

4. **IRREGULAR SPONTANEOUS CONTRACTIONS OF THE ADULT RABBIT ISOLATED TESTICULAR CAPSULE**

Very occasionally, the adult rabbit isolated testicular capsule was observed to undergo irregular spontaneous contractions before the addition of any drug to the tissue bath. Figure 43 presents a continuous recording of consecutive irregular spontaneous contractions from an apparently normal isolated testicular capsule observed throughout a period of 95 minutes. Despite the irregular nature of the spontaneous contractions, a definite pattern was observed which consisted of large sustained contractions followed by short, relatively inactive periods. Each sustained contraction of the capsule precipitated rapid spontaneous activity, the frequency of which was not unlike that usually observed with the isolated rabbit testicular capsule.

5. **STRETCHING**
a. Comparison of the Effect of Stretching of the Isolated Rat Testicular Capsule Versus the Isolated Guinea Pig Ileum on the Response to Acetylcholine.

As described earlier in figure 9, an increase in the weight of the resting tension (load on the lever arm) up to 400 mg resulted in an increase in testicular capsular contraction in response to 1 µg/ml acetylcholine. It seemed of interest to speculate that the increase in capsular contraction with an increasing load weight may be due to the heavier loads stretching the capsule. The effect of preliminary stretching of the testicular capsule was therefore studied with respect to the response of the isolated capsule to acetylcholine. Figure 44 indicates that following preliminary stretching of the testicular capsule, an increase in the response of the testicular capsule occurs despite a constant resting tension of 100 mg.

This increase in response of the testicular capsule was found to be in contrast to that observed with a standard smooth muscle preparation such as the guinea pig ileum. Figure 45 illustrates that following preliminary stretching of the isolated guinea pig ileum, no observable change in the contraction of the ileum occurs in response to 0.01 µg/ml acetylcholine.
F. BIOASSAY OF ATROPINE USING THE ISOLATED TESTICULAR CAPSULE

1. LEFT ISOLATED TESTICULAR CAPSULE OF THE ADULT RAT

a. Data Terminology

Standard solution = S
Test solution = T
Low concentration of standard solution = S₁ (C)
High concentration of standard solution = S₂ (D)
Low concentration of test solution = T₁ (A)
High concentration of test solution = T₂ (B)

b. Data

Dose sequence: C D A B D C B A A B C D
Standard solution of atropine = 10 µg/ml
Inhibition of acetylcholine response was obtained with the following doses:
S₁ = 0.02 ml
S₂ = 0.05 ml
T₁ = 0.02 ml
T₂ = 0.05 ml
The following responses in mm were obtained from figure 46, 40 seconds following the addition of 1.0 µg/ml acetylcholine:

Top figure = response of the testicular capsule to 1 µg/ml acetylcholine in the presence of atropine.

Bottom figure = control response of the testicular capsule to 1 µg/ml acetylcholine.

<table>
<thead>
<tr>
<th>Sum Response</th>
<th>Mean Response</th>
<th>Mean % Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 = 17/23.5</td>
<td>12/20</td>
<td>11.5/18</td>
</tr>
<tr>
<td>S2 = 9/21</td>
<td>6/24</td>
<td>5/20</td>
</tr>
<tr>
<td>T1 = 11.5/22</td>
<td>5/22.5</td>
<td>7/17.5</td>
</tr>
<tr>
<td>T2 = 5/22.5</td>
<td>3.5/22</td>
<td>1.5/20</td>
</tr>
</tbody>
</table>

The mean % response is plotted against the log dose of atropine as illustrated in figure 47.

c. Graphic Solution

From figure 47, the following values were obtained for the standard (X ml) and test (Y ml) solutions of atropine which permitted the same % response of the testicular capsule to
1.0 µg/ml acetylcholine: \[ X = 0.0460 \text{ ml} \]
\[ Y = 0.0233 \text{ ml} \]

\[(X \text{ ml}) \times (\text{conc. Standard}) = (Y \text{ ml}) \times (\text{conc. Test})\]

\[(0.0460 \text{ ml}) \times (10 \text{ µg/ml}) = (0.0233 \text{ ml}) \times (\text{Test})\]

Test Solution = \[
\frac{(0.0460 \text{ ml}) \times (10 \text{ µg/ml})}{0.0233 \text{ ml}}
\]

Test Solution = 19.74 µg/ml

The actual concentration of the test solution of atropine was 19.8 µg/ml and was supplied by Dr. A.F. Friedman, Nov., 1969. The percent error was 0.03%.

2. **RIGHT ISOLATED TESTICULAR CAPSULE OF THE ADULT RAT**

   a. **Data Terminology**

   Standard solution = \( S \)
   Test solution = \( T \)
   Low concentration of standard solution = \( S_1 \) (C)
   High concentration of standard solution = \( S_2 \) (D)
   Low concentration of test solution = \( T_1 \) (A)
   High concentration of test solution = \( T_2 \) (B)
b. Data

Dose sequence: A B C D B A D C

Standard solution of atropine = 20 µg/ml

Inhibition of the acetylcholine response was obtained with the following doses of atropine:

- \( S_1 = 0.010 \text{ ml} \)
- \( S_2 = 0.025 \text{ ml} \)
- \( T_1 = 0.010 \text{ ml} \)
- \( T_2 = 0.025 \text{ ml} \)

The following responses in mm were obtained from figure 48, 40 seconds following the addition of 1.0 µg/ml acetylcholine:

Top figure = response of the testicular capsule to 1 µg/ml acetylcholine in the presence of atropine.

Bottom figure = control response of the testicular capsule to 1 µg/ml acetylcholine.

<table>
<thead>
<tr>
<th></th>
<th>Sum Response</th>
<th>Mean Response</th>
<th>Mean % Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( S_1 )</td>
<td>13.5 ( \frac{17.5}{29} )</td>
<td>31 ( \frac{15.5}{61.5} )</td>
<td>50.4%</td>
</tr>
<tr>
<td>( S_2 )</td>
<td>6 ( \frac{6}{27} )</td>
<td>12 ( \frac{6}{53} )</td>
<td>22.6%</td>
</tr>
<tr>
<td>( T_1 )</td>
<td>16.5 ( \frac{14}{29} )</td>
<td>30.5 ( \frac{15.3}{56.5} )</td>
<td>54.0%</td>
</tr>
<tr>
<td>( T_2 )</td>
<td>9.5 ( \frac{6}{31} )</td>
<td>15.5 ( \frac{7.8}{63} )</td>
<td>24.6%</td>
</tr>
</tbody>
</table>
The mean percent response is plotted against the log dose of atropine as illustrated in figure 49.

c. Graphic Solution

From figure 49, the following values were obtained for the standard (X ml) and test (Y ml) solutions of atropine which permitted the same percent response of the testicular capsule to 1 µg/ml acetylcholine: 

\[
X = 0.0150 \text{ ml}  \\
Y = 0.0165 \text{ ml}  
\]

\[
(X \text{ ml}) \times (\text{conc. Standard}) = (Y \text{ ml}) \times (\text{conc. Test})  
\]

\[
(0.0150 \text{ ml}) \times (20 \mu g/ml) = (0.0165 \text{ ml}) \times (\text{Test})  
\]

Test Solution = \[
\frac{(0.0150 \text{ ml}) \times (20 \mu g/ml)}{0.0165 \text{ ml}} 
\]

Test Solution = 18.18 µg/ml

The actual concentration of the test solution of atropine was 19.8 µg/ml and was supplied by Dr. A.F. Friedman, Nov., 1969. The percent error was 8.2%. 
G. RESPONSE OF ISOLATED EXCURRENT SPERM DUCTS TO AUTONOMIC DRUGS

1. ADULT RAT ISOLATED VAS DEFERENS

Figure 50 presents the response of the isolated vas deferens (pars inguinalis) of the adult rat to the same autonomic drugs employed in the study of the adult rat isolated testicular capsule. A final concentration of 1 µg/ml acetylcholine in the tissue bath was found to have no observable effect on the isolated vas deferens. This was in marked contrast to the contraction observed with the testicular capsule. Similarly, 50 µg/ml pilocarpine was not observed to have any effect on the vas deferens. However, a final concentration of 1 µg/ml carbachol produced an increase in tone or overall shortening of the vas deferens and stimulated very rapid spontaneous contractions approximately 1 minute following the addition of the drug. When compared to the spontaneous contractions of the adult rabbit testicular capsule in the absence of any drug, the carbachol-induced spontaneous contractions of the vas deferens were much more rapid and
more numerous. Figure 51 indicates that prolonged exposure of the isolated vas deferens to carbachol results in a gradual return of the tone of the vas deferens to the initial resting level. Spontaneous contractions remain just as rapid but decrease in number.

The isolated vas deferens appeared to be very sensitive to sympathetic agents as shown in figure 50, since 0.1 µg/ml norepinephrine and only 0.02 µg/ml epinephrine resulted in an immediate contraction and the onset of spontaneous activity. It is of interest to note that the vas deferens is markedly more sensitive to epinephrine than to norepinephrine. Prolonged exposure of the vas deferens to norepinephrine as shown in figure 51 results in a relatively fast return of the tone to the initial resting tone. Spontaneous contractions, however, continued for over 10 minutes.

The response of the vas deferens to 5 µg/ml isoproterenol was of interest since although no change in tone was observed, several very rapid spontaneous contractions were observed. This was in marked contrast to the response of the isolated testicular capsule of the adult rat and adult rabbit. 5 µg/ml isoproterenol was observed to completely inhibit spontaneous contractions of the adult rabbit isolated testicular capsule (figure 15), and produce a relaxation of the isolated capsules of both the adult rat and rabbit testes (figures 10 and 15 respectively).
Small contractions of the vas deferens were also produced by 50 µg/ml tetramethylammonium and 50 µg/ml histamine (figure 50). However, 50 µg/ml barium chloride was observed to stimulate rapid spontaneous activity only after a latent period of approximately 3 minutes.

2. **ADULT RAT ISOLATED EPIDIDYMIS**

Figure 51 presents the response of the isolated caput epididymis and the isolated cauda epididymis to 1 µg/ml carbachol and 1 µg/ml norepinephrine respectively. Both drugs were observed to cause a contraction of the epididymis and in addition, norepinephrine produced spontaneous contractions which persisted for as long as the drug was in contact with the tissue.

A comparison of the effect of carbachol and norepinephrine on the epididymis and vas deferens (figure 51) indicates that while norepinephrine appears to have a greater stimulatory effect on the epididymis, carbachol appears to have a greater stimulatory effect on the vas deferens.
CHAPTER V

DISCUSSION
A. BIOLOGICAL SIGNIFICANCE OF THE PRESENT STUDIES

The present studies show for the first time that both the isolated capsule of the testis and the isolated whole testis undergo rhythmic spontaneous contractions and can also be stimulated to undergo a marked contraction or relaxation in response to various pharmacological drugs. The isolated parenchymal tissue does not spontaneously contract or significantly respond to pharmacological drugs. It therefore appears that the testicular capsule alone is responsible for the endogenous rhythmic spontaneous contractions observed with the whole testis. Moreover, the fact that the whole testis with its contained seminiferous tubules and interstitial tissue, offering a mass of tissue resistance, was observed to undergo spontaneous and drug-induced contractions, seems to lend great physiological importance to the contractions described for the isolated capsule alone.

The present studies also demonstrate the presence of smooth muscle fibers located in the tunica albuginea of the testicular capsule, offering a reasonable anatomical explanation for both spontaneous and drug-induced contractions of the testicular capsule.
It seems of interest to speculate that a fundamental and far-reaching significance of these initial studies on the testicular capsule as an isolated tissue preparation could well be the testicular capsule serving uniquely as a model system for the pharmacological investigation not only of capsular tissue but of fibrous membranes in general.
B. PHARMACOLOGY OF THE TESTICULAR CAPSULE

The simplest method for studying the action of drugs on various muscles has been to remove the muscle from the animal and suspend it in a physiological salt solution. One end of the muscle is fixed and the other end is attached to a lever designed to write on a drum or transmit the response to a transducer-recorder system. In this manner, drugs can be added to an isolated muscle preparation, and contractions or relaxations observed and measured in biological assays. The present studies demonstrate that the capsule of the testis can be prepared as an intact isolated tissue suitable for such pharmacological investigations.

The possibility of the presence of smooth muscle fibers located in the testicular capsule became apparent when the isolated capsule was observed to contract in response to acetylcholine and norepinephrine (Davis and Langford, 1969). The subsequent histochemical demonstration of smooth muscle fibers in the tunica albuginea of the testicular capsule indeed provided a reasonable anatomical explanation for the observed testicular capsular contractions. Smooth muscle fibers have also been identified in the tunica albuginea of the rabbit (Holstein and Weiss, 1967) and human (Holstein, 1967) to support the observations of the present studies. There appears to be a definite relationship, however, between the number of smooth muscle fibers in the tunica albuginea
of the testicular capsules of the adult rat, rabbit and human, and the extent of the observed pharmacological responses. For example, the sparse amount of smooth muscle present in the testicular capsule of the rat produced no observed spontaneous contractions of the capsule, and only produced drug-induced contractions at high magnifications of approximately x 100. In the adult rabbit and human testicular capsules, however, there is a marked increase in the amount of smooth muscle present in the form of two distinct layers of smooth muscle fibers producing marked spontaneous contractions as well as drug-induced contractions at much lower magnifications.

The testicular capsular responses to barium chloride, a direct-acting smooth muscle stimulant, provides additional evidence to suggest that quantitative differences in the pharmacological responses of the adult rat, rabbit and human testicular capsules are based on differences in the quantity of smooth muscle present in each capsule. While noted for its intense stimulation of smooth muscle fibers, barium chloride produces only a slight contraction of the isolated capsule of the rat, and only at an extremely high dose. However, at identical doses, barium chloride produces very large contractions of the rabbit and human testicular capsules. This would seem to suggest that the rat testicular capsule has either a very low sensitivity to barium chloride or that the low response to barium ions is indicative of
the small quantity of smooth muscle fibers present in the capsule of the rat.

A pronounced feature of the contraction of the testicular capsule is the markedly slow rate of contraction and relaxation as compared with most other isolated smooth muscle preparations. This slow rate of contraction can be attributed to at least two factors: an imposing amount of collagenous tissue and a relatively small quantity of smooth muscle fibers. The collagenous tissue which comprises most of the testicular capsule would appear to offer a substantial resistance to the contracting or relaxing smooth muscle fibers.

The responses of the isolated testicular capsule to pharmacological drugs can be summarized as either excitatory or inhibitory, producing a contraction or relaxation respectively. The various pharmacological agents producing an excitatory response include (1) parasympathomimetic drugs, (2) sympathomimetic drugs stimulating alpha receptor sites, (3) ganglionic stimulating drugs, (4) histamine, (5) direct-acting smooth muscle stimulants, (6) oxytocin and (7) vasopressin. The pharmacological agent producing an inhibitory response of the isolated testicular capsule includes only sympathomimetic drugs stimulating beta receptor sites.

The predominant pharmacological response of the testicular capsule is excitatory. This suggested the possibility of a
common excitatory receptor site through which most or all of the excitatory drugs were acting. However, the experiments carried out using autonomic blocking drugs suggest not only the presence of more than one excitatory receptor site but that each excitatory drug may occupy its own specific receptor site. The various blocking agents used were highly specific in their blocking ability and effective at approximately similar doses used for other smooth muscle preparations. It is interesting to note, however, that both sympatholytic drugs, phenoxybenzamine and propranolol, at relatively high doses, produced initial small contractions of the isolated testicular capsule. Phenoxybenzamine specifically blocks a norepinephrine-produced capsular contraction and hence presumably acts to stimulate the alpha excitatory receptor sites. However, the nature of the contraction produced by propranolol remains unclear. Propranolol specifically blocks the isoproterenol-produced testicular capsular relaxation. If propranolol acts by competing with isoproterenol for the beta inhibitory receptor sites, then stimulation of these receptor sites by propranolol would be expected to produce a capsular relaxation.

In addition to the blocking experiments, there is evidence to suggest that there may be a quantitative difference between the various types of receptor sites, either in the degree of excitability or in the number of receptor sites, as indicated by consecutive applications of norepinephrine and acetylcholine to the
isolated testicular capsule. In its contracted state following a maximal dose of norepinephrine, the capsule further contracts by approximately the same amount following the addition of a maximal dose of acetylcholine.

As indicated above, the innervation of the testicular capsule remains unclear. According to Risley and Skrepetos (1964) and Norberg et al. (1967), the majority of the autonomic efferent nerve fibers from the internal spermatic plexus pass through the tunica albuginea and appear to accompany and supply blood vessels. However, the possibility certainly exists that smooth muscle fibers present in the tunica albuginea can also be innervated by these sympathetic nerve fibers. The fact that the isolated testicular capsule can be stimulated to contract by sympathomimetic drugs strongly suggests sympathetic innervation of the smooth muscle fibers of the capsule. In addition, nerve fibers of the internal spermatic plexus may have afferent components located in the tunica albuginea.

While the testis has been known for many years to receive sympathetic innervation (Kuntz and Morris, 1946), it is generally believed that the testis does not receive any parasympathetic innervation (Monnier, 1968). It is of great interest, then, to note that tetramethylammonium (TMA), which is a ganglionic stimulating drug, produces a contraction of the isolated testicular capsule. The available reports in the literature indicate that
the testis is apparently negative for cholinesterase activity, employing both acetylthiocholine and butyrylthiocholine as substrates (Risley and Skrepetos, 1964; Burnstock and Merrillees, 1964). However, the fact that tetramethylammonium was observed to cause contraction of the isolated testicular capsule, but can be specifically blocked from causing a contraction by tetraethylammonium, a specific ganglionic blocking drug, would seem to suggest the presence of parasympathetic ganglia and postganglionic parasympathetic nerve fibers located in the testicular capsule. Additional support for the presence of a parasympathetic innervation of the testicular capsule is the muscarinic action of acetylcholine on the isolated testicular capsule. Atropine, a belladonna alkaloid which specifically blocks the postsynaptic membrane sites of postganglionic parasympathetic nerves, was observed to produce a complete block of the excitatory effects of acetylcholine as well as tetramethylammonium on the isolated testicular capsule.

An extraordinary feature of the pharmacological responses of the isolated testicular capsule is the apparent lack of a parasympathetic-sympathetic antagonism. If the testicular capsule is indeed innervated by both parasympathetic and sympathetic nerve fibers, then the testicular capsule can not be catagorized with the general hypothesis that the activity of autonomic organs is controlled by a balance between parasympathetic and sympathetic
influences. The isolated epididymis and vas deferens of the adult rat also undergo a contraction in response to both acetylcholine and norepinephrine. However, they differ from the isolated testicular capsule since the application of isoproterenol produces a tissue contraction rather than a relaxation.

Since the predominant pharmacological response of the isolated testicular capsule is excitatory involving a capsular contraction, it would be of interest to investigate whether these contractions have a physiological role both in the regulation of testicular size and in testicular function such as sperm transport.
C. RELATIONSHIP OF THE TESTICULAR CAPSULE TO SPERM TRANSPORT

It has long been known that the sperm which are formed in the seminiferous tubules of the testis are not capable of motility (Rendenz, 1926). The capacity for motility is first attained by sperm during their transit through the epididymis (Yochem, 1930). However, the mechanisms responsible for the initial transportation of the non-motile sperm out of the seminiferous tubules of the testis to the epididymis remains unclear, although a number of possible explanations for this phenomenon have been offered. It has been postulated by Macmillan (1953) as well as Reid and Cleland (1957) that the non-motile sperm are passed from the seminiferous tubules to the proximal region of the epididymal duct through the continuous flow of fluid from the testis to the epididymis. It has been suggested that this flow of fluid from the testis to the epididymis results from movements of cilia of the epithelium of the rete testis and vasa efferentia. However, Leeson (1962) in investigating the fine structure of the rete testis by electron microscopy came to the conclusion that the number of cilia present in the rete epithelium was virtually negligible.
and that ciliary movement would be insufficient to have any effect on sperm transport.

Another mechanism that has been postulated to explain the transport of non-motile sperm from the testis to the epididymis involves possible undulating motions of the seminiferous tubules. Roosen-Runge (1951) reported that minute undulating motions of individual seminiferous tubules could be seen by direct microscopic visualization. This undulating motion was suggested to be due to a contraction and relaxation of the Sertoli cells located within the seminiferous germinal epithelium. However, a number of other investigators have suggested that if there are contractile elements located in the seminiferous tubules, these contractile elements might involve cell types other than the Sertoli cell. Clermont (1958) has suggested the presence of fibrous elements lying in the wall of the seminiferous tubule of the rat which seemed to bear some resemblance to smooth muscle cells. Lacy and Rotblat (1960) suggested that the main elements of the boundary tissue connecting the seminiferous tubules of the rat testis to the testicular interstitial tissue were elongated narrow cells which were interspaced between two layers of the basement membrane of the seminiferous tubule. These elongated narrow cells were said to exhibit microscopic features that were similar to those of smooth muscle. Ross and Long (1966),
however, stated that while the cytological features of the peritubular connective tissue in the human testis resembled those of smooth muscle cells, there were several microscopic features that the peritubular cells shared with fibrocytes. Niemi and Kormano (1965) also have suggested that the basement membrane of the seminiferous tubules of the rat testis is composed of double lamellae with spindle-shaped cells between the two layers of the basement membrane. In addition, these authors have suspended 2-5 cm long pieces of seminiferous tubules obtained from the rat testis in a few drops of Tyrode's solution on cover slips and examined them under a microscope. It was stated that these adult rat seminiferous tubules contracted irregularly with a period of about 10 seconds and a depth of about 15 µ. However, it was also stated that not all the seminiferous tubules were observed to contract in an equal manner and that there were always some tubules that did not contract. It seems apparent that it is most difficult at the present time to adequately ascertain the importance of any of the suggested contractile cells located either within the seminiferous tubule itself or in the peritubular connective tissue with regard to sperm transport. The possibility exists that any in vitro undulating motions of the seminiferous tubules observed with a high-power microscope may be due to a type of Brownian movement of the suspending solution. In this regard,
it should be noted that when large coiled masses of seminiferous tubules were viewed directly with a microscope through an incision of the abdominal wall of an anesthetized rabbit, the seminiferous tubules did not exhibit any contractions of their own (Gross, 1959). There has, however, been some recent suggestions that the testis as a whole appears capable of spontaneous movements, as indicated by the report of rhythmic interstitial pressure changes obtained with a canula puncturing the testis through the scrotum of an anesthetized rabbit (Holstein and Weiss, 1967) as well as a kymographic recording of irregular movements of the entire testis (Wojcik, 1966). It would therefore seem reasonable to expect something other than possible minute undulating motions of the seminiferous tubules to be responsible for movements of the entire testis and it would appear, on the basis of the data of the present studies, that the responsible factor could well be the testicular capsule contracting and relaxing.

The finding that the isolated testicular capsule exhibits periodic and powerful spontaneous contractions in the absence of any drug would seem to indicate that under normal conditions, the testicular capsule is in a constant state of dynamic movement, capable of exerting great force against the contained seminiferous tubular mass. It seems likely that these rhythmic contractions of the testicular capsule then
serve to massage the seminiferous tubules and in so doing provides a pumping action which transports the non-motile sperm from the seminiferous tubules towards the efferent ducts leading to the epididymis as shown in figure 26.
D. REGULATION OF MALE FERTILITY BY THE TESTICULAR CAPSULE

In addition to the demonstration of spontaneous contraction of the isolated testicular capsule, the present investigations also indicate that both of the naturally-occurring neurohumoral agents of the autonomic nervous system, namely acetylcholine and norepinephrine, as well as numerous other autonomic agents, are capable of causing a contraction of the isolated testicular capsule. The sympathetic nervous system has been shown by a number of investigators to be essential for the general function of the testis in experimental animals. King and Langworthy (1940) as well as Coujard (1952; 1954) have demonstrated that any injury to the sympathetic ganglionic chain along the vas deferens results in hypoplasia and aspermatogenesis of the testis. Moreover, atrophy of the testis accompanied by loss of potency has been reported to occur in the human following lumbar sympathectomy (Bandmann, 1950). It has also been shown that only about one-twentieth of male paraplegic patients are fertile and that human male paraplegics commonly have testicular atrophy (Bars et al., 1950; Cooper et al., 1950; Stemmermann et al., 1950; Talbot, 1955). The possibility therefore exists that one partial
explanation for the loss of fertility in males with sympathetic nerve damage may involve a lack of norepinephrine, thereby resulting in an impairment of testicular capsular contractions necessary to propel sperm into the epididymis. It is interesting to speculate that if those impaired contractions of the testicular capsule could be artificially stimulated by a sympathomimetic agent, then such cases of male infertility might be corrected. Indeed, it may even be more feasible to administer a parasympathomimetic agent such as carbachol which is also capable of stimulating contractions of the testicular capsule.

The speculation can also be made that one possible approach to the investigation of contraception in the male may involve the retardation of contractions of the testicular capsule. This may be accomplished by the administration of a drug capable of causing prolonged relaxation of the testicular capsule as has been demonstrated with isoproterenol. Such a drug could conceivably cause relaxation of the testicular capsule by having a direct action on the smooth muscle of the capsule. In addition, relaxation of the testicular capsule may also be accomplished by a drug with the property of interfering with the endogenous effect of either acetylcholine or norepinephrine, such as would be the case of an autonomic nervous system blocking agent.
E. REGULATION OF TESTICULAR SIZE BY THE TESTICULAR CAPSULE

The testicular capsule may also have the function of serving to regulate the size of the testis. Masters and Johnson (1966) have indicated that the human testes achieve an appreciable increase in size during the period of elevated sexual tensions that occurs before the orgasmic phase. The possibility may therefore exist that an inhibition of testicular capsular contractions with a resulting relaxation of the capsule may be partially responsible for this phenomenon. It would certainly seem of interest to investigate the relationship of the testicular capsule to the normal physiology of the testis occurring during the sexual response of the male. In addition, it would also seem of interest to investigate the relationship of the testicular capsule to the size of the small testes found in hypogonadism of an endocrine-linked origin as well as in cases of cryptorchidism.

In the instance of an undescended testis, a prolonged contraction without relaxation of the testicular capsule resulting from the effects of an elevated abdominal temperature as demonstrated in figures 36 and 37 may produce excessive
pressure on the seminiferous tubules leading to atrophy of the germinal epithelium with a decrease in testicular size. There may be two possible mechanisms to explain how a prolonged testicular capsular contraction could lead to excessive pressure on the seminiferous tubules. The first mechanism simply involves external pressure being applied by the contracted capsule to the seminiferous tubular mass within the testis.

The second mechanism involves the possible clamping off of the efferent ducts of the testis by a prolonged contraction of the testicular capsule inasmuch as both the rete testis and vasa efferentia pass directly through and are completely surrounded by the mediastinal region of the testicular capsule as indicated in figure 26. This clamping off of the efferent ducts of the testis by the prolonged contraction of the testicular capsule could then exert internal pressure on the seminiferous epithelium in much the same way as the experimental ligation of the vasa efferentia.

Early studies of ligation of the vasa efferentia in rats reported a rapid and complete degeneration of the germinal epithelium (Van Wagenen, 1924, 1925, 1926; Osling, 1926). Ligation of the vasa efferentia appears to affect the germinal epithelium in a nonspecific manner since degenerative changes to spermatids and spermatocyes occur in variable numbers at all stages of the cycle of the germinal epithelium (Langford, 1968). In addition to atrophy of the germinal epithelium, Mason and Shaver (1952) indic-
ated the accumulation of sperm on the testicular side of the ligation. The cause of the atrophy of the seminiferous epithelium has been suggested by Smith (1962) and by Langford (1968) to be the result of an increase in the intratesticular pressure due to the accumulation of intratubular fluid and germinal products. This would account for the increase in the size of the seminiferous tubules observed shortly after the ligation of the vasa efferentia is made (Langford, 1968).

It seems likely, therefore that the atrophic changes in the seminiferous epithelium found in the small cryptorchid testis may be due not only to the damaging effects of an elevated temperature on the metabolism of the seminiferous epithelium (Davis, 1969), but may also involve the application of an excessive amount of both external and internal pressure on the seminiferous tubules exerted by a prolonged and increased contraction of the testicular capsule.
F. USE OF THE ISOLATED TESTICULAR CAPSULE AS A TOOL
IN BIOLOGICAL ASSAY PROCEDURES

While the demonstration of spontaneous contractions employing the adult rabbit isolated testicular capsule has important physiological implications, the isolated testicular capsule of the rat would seem to have distinct pharmacological advantages involving the qualitative assay of drug effects. Indeed, bioassay procedures in pharmacology are often designed to eliminate spontaneous contractions of an isolated tissue in order to more accurately determine the extent of drug-induced contractions.

The results of the bioassay for an unknown concentration of atropine indicate that the isolated testicular capsule can also be utilized as a biological tool for quantitative measurement with a high degree of accuracy.

In addition to the isolated testicular capsule now being included with other biological tissues useful for qualitative or quantitative assay procedures, the isolation of the testicular capsule as a separate intact tissue now appears to offer a unique opportunity in the screening of drugs for possible pharmacological activity relating to the testis.
CHAPTER VI

SUMMARY
The testicular capsule of the adult rat, rabbit and human has been prepared for the first time as an intact isolated tissue which is suitable for the investigation of effects of pharmacological drugs.

Various autonomic drugs were found to produce a contraction or relaxation of the isolated testicular capsule. The predominant pharmacological response was a capsular contraction. In addition, periodic spontaneous contractions of the isolated testicular capsule of the adult rabbit and human were observed in the absence of any added pharmacological agent.

Pharmacological agents producing a testicular capsular contraction include (1) parasympathomimetic stimulating drugs, (2) sympathomimetic drugs stimulating alpha receptor sites, (3) ganglionic stimulating drugs, (4) histamine, (5) direct-acting smooth muscle stimulants, (6) oxytocin and (7) vasopressin. Only one pharmacological agent was found to produce a testicular capsular relaxation and this was sympathomimetic drugs stimulating beta receptor sites. Experiments utilizing pharmacological blocking drugs suggest that each stimulatory drug may occupy its own specific receptor site on the effector tissue.

The present studies also demonstrate that the testicular capsule contains smooth muscle, offering a reasonable anatomical
explanation for both spontaneous and drug-induced contractions of the testicular capsule.

The autonomic innervation of the testis has previously been considered to be restricted to sympathetic nerve fibers passing through the tunica albuginea to accompany and supply blood vessels. The present studies suggest sympathetic innervation of the smooth muscle fibers of the testicular capsule in addition to smooth muscle fibers of testicular blood vessels. Although it is generally believed that the testis does not receive any parasympathetic innervation, the present studies also suggest the presence of parasympathetic ganglia and parasympathetic postganglionic nerve fibers located in the testicular capsule. If the testicular capsule is therefore innervated by both parasympathetic and sympathetic nerve fibers, these two autonomic nerve systems appear to act in a synergistic manner in the testicular capsule.

In addition to the isolated testicular capsule, the isolated intact whole testis also undergoes rhythmic spontaneous contractions. In contrast, the isolated testicular parenchymal tissue does not spontaneously contract. It therefore appears that the testicular capsule alone is responsible for the endogenous spontaneous contractions of the whole testis. The finding that the isolated testicular capsule is capable of periodic contractions and relaxations in the absence of any drug indicates that under normal circumstances, the testicular capsule is in a const-
ant state of dynamic movement, exerting force against the contained seminiferous tubular mass. It seems very likely that these rhythmic contractions and relaxations of the testicular capsule serve to massage the seminiferous tubules, providing a pumping action which may assist lymphatic and venous drainage of testis, may influence the secretion of testosterone from the Leydig cells or may transport the non-motile sperm from the seminiferous tubules toward the efferent ducts out of the testis and into the epididymis where the sperm then attain their motility.

The possibility also exists that the administration of drugs shown to cause contraction of the testicular capsule may be of aid in stimulating the transport of sperm out of the testis in some cases of male infertility. On the other hand, a possible approach to male contraception has been suggested involving administration of drugs which may cause prolonged relaxation of the testicular capsule, thereby preventing the sperm-propelling action of the testicular capsule. It would also seem of interest to explore the possibility that some drugs which are administered to males may have as yet undetected side-effects involving either the stimulation or inhibition of testicular capsular contractions, thereby affecting sperm transport from the testis.

Testicular capsular contractions may also serve to regulate the size of the testis, both under normal and abnormal conditions. An inhibition of the contractions of the testicular capsule to naturally occurring neurohumoral agents may be responsible for
the increase in testicular size which can occur prior to the orgasmic phase of the sexual response in the male. In contrast, a prolonged contraction without relaxation of the testicular capsule as may result from the effects of an elevated abdominal environmental temperature may be partially responsible for the decrease in testicular size which occurs in cases of cryptorchidism.

It is hoped that the present studies will draw attention to a previously neglected structure of the testis, namely the testicular capsule. It would seem that the testicular capsule can no longer be considered an inert tissue serving only as a supporting membrane for the testicular parenchymal tissue. Rather, the testicular capsule appears to be a highly dynamic tissue in view of its response to pharmacological drugs. It seems likely that the testicular capsule not only plays a vital role in transporting sperm out of the testis but may be involved in several aspects of testicular physiology under both normal and pathological conditions. Moreover, as a separate intact tissue, the isolated testicular capsule appears to offer a unique opportunity in screening drugs for possible pharmacological activity relating to the testis.
CHAPTER VII

REFERENCES


CHAPTER VIII

FIGURES
Figure 1. Schematic representation of the procedure for the isolation of the testicular capsule of the adult rat and its use as an isolated tissue preparation for pharmacological studies.

a. Cut a small piece off the inferior end of the testis.  
b. Grasp the seminiferous tubular mass protruding through the resulting opening with a forceps.  
c. Grasp the lower rim of the testicular capsule with a second forceps and gently remove the seminiferous tubules from the interior of the testicular capsule.  
d. After removing most of the seminiferous tubules, turn the testicular capsule inside-out.  
e. Diagram of the testicular capsule in the inside-out position with only a few seminiferous tubules still attached to its inside surface.  
f. Separate the testicular capsule from the mass of seminiferous tubules, along with the large testicular artery.  
g. Cut away the few remaining seminiferous tubules still attached to the inside surface of the testicular capsule.  
h. Tie the superior end of the testicular capsule with a long piece of silk thread.  
i. Tie the inferior end of the testicular capsule with a short piece of silk thread, leaving approximately 24 percent of the original small hole in the capsule open.  
j. Mount the testicular capsule 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 2. Photograph of the isolated testicular capsule of the adult rat. The capsule has been turned inside-out in order to completely remove all of the interior tissue of the testis (x 7.5).
Figure 3. Photograph of the isolated tissue assembly employed for measurement of contractions of the isolated testicular capsule. The capsule is mounted in a 10 ml isolated tissue bath as indicated by the arrow. A linear motion transducer above the tissue bath is connected to a recorder shown at the left. A constant temperature water bath shown at the right serves to maintain the desired temperature of the tissue bath.
Figure 4. (a) Comparison of the size of the isolated testicular capsule (left) of the adult rat with the removed seminiferous tubular mass (right). (b) Photograph of the intact isolated testicular capsule in the inside-out position as it would appear following removal from an isolated tissue bath. The superior thread led to a transducer while the inferior tie was attached to the tip of the support rod within the tissue bath (x 4).
Figure 5. Contraction of isolated testicular capsule of the adult rat with various doses of acetylcholine and norepinephrine. Each large square shown on the chart paper represents 5 mm. Drug concentrations are expressed as ug/ml of organ bath volume for acetylcholine chloride and norepinephrine base. Response magnification was x 25.
Figure 6. Dose-response curve for the effect of acetylcholine chloride and norepinephrine base on the isolated testicular capsule of the adult rat. Each point on the curves represents an average of at least 8 experiments using different isolated capsules.
Figure 7. Time taken for the adult rat isolated testicular capsule to return to the initial resting tension following washing out of various concentrations of acetylcholine chloride. In each case, washing out of the drug occurred 4 minutes after the addition of acetylcholine to the tissue bath. No extra weight was added to the initial resting tension load of 100 mg in order to assist relaxation. Response magnification was x 100.
Figure 8. Time taken for the adult guinea pig isolated ileum to return to the initial resting tension following washing out of 0.01 µg/ml acetylcholine chloride. Washing occurred 4 minutes following the addition of acetylcholine to the tissue bath. No extra weight was added to the initial resting tension load in order to assist relaxation. Response magnification was x 10.
Figure 9. Influence of various resting tensions (load) on the response of the isolated testicular capsule to acetylcholine chloride. The actual millimeters of tissue contraction produced by 0.1 µg/ml acetylcholine at different resting tensions are shown on the left ordinate. The work done by the capsule as each load is lifted is shown on the right ordinate. Loads were added to the end of the long arm of a 3:1 lever so that each load was lifted a distance equal to 3 times the actual contraction of the capsule. The work done by the capsule to lift each load was obtained from the following equation:

\[
\text{WORK} = \text{LOAD} \times \text{CONTRACTION} \times 3
\]
Figure 10. Response of isolated testicular capsule of the adult rat to various autonomic drugs. Each large square shown on the chart paper represents 5 mm. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, pilocarpine hydrochloride, norepinephrine base, epinephrine base, isoproterenol hydrochloride, histamine base, tetramethyl ammonium chloride (TMA) and barium chloride. Response magnification was x 50.
Figure II. Comparison of the effect of isoproterenol hydrochloride on the adult rat isolated testicular capsule during contraction of the capsule to norepinephrine base (top), to acetylcholine chloride (middle), or to both norepinephrine and acetylcholine (bottom). Response magnification was x 100.
Figure 12. Representative preparations of the adult rabbit testis used for pharmacological studies. A-C. Photographs of the isolated whole testis, the isolated testicular parenchymal tissue and the isolated testicular capsule. Each isolated preparation has a single long thread tied to the superior end which extends to the lever of a transducer lever arm. The two shorter threads tied to the inferior end anchor the preparation in the tissue bath to a support rod. (x 0.75). D. Photograph of the isolated testicular capsule inside out. The testicular parenchymal tissue has been completely removed. The arrow indicates the small hole at the inferior end of the capsule which is always left open. (x 2).
Figure 13. Spontaneous contractions of the adult rabbit testis in the absence of any added drugs. The top recording was obtained from the whole testis weighing approximately 1,700 mg at a response magnification of x 10. The middle recording was obtained from the isolated testicular capsule weighing 135 mg at a response magnification of x 25. The bottom recording was obtained from the isolated testicular parenchymal tissue weighing approximately 1,550 mg at a response magnification of x 100. In every case, the load on the lever arm was 100 mg and the temperature of the tissue bath was maintained at 37 °C.
Figure 14. Effect of load on spontaneous contractions of the isolated whole testis (left) and the isolated testicular capsule (right) of the adult rabbit. The initial load was 100 mg for both the testicular capsule and the whole testis, and increasing loads of 200 mg, 300 mg or 500 mg were added at intervals of approximately 20 minutes. Response magnification was x 10 for both tissues.
Figure 15. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rabbit testis to various autonomic drugs. In each case, the temperature of the tissue bath was maintained at 37°C and the load on the lever arm was 100 mg. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, pilocarpine hydrochloride, norepinephrine base, epinephrine base, isoproterenol hydrochloride, histamine base, tetramethylammonium chloride (TMA) and barium chloride. Response magnification was x 10 and x 100 for the capsule and the parenchymal tissue respectively.
Figure 16. Photographs of the isolated capsule and parenchymal tissue of the adult rat testis (top) and the adult rabbit testis (bottom) used for pharmacological studies. Each isolated preparation has a single long thread tied to the superior end which extends to the lever of a transducer. The two shorter threads tied to the inferior end, anchor the preparation in the tissue bath to a support rod. (x 0.75).
Figure 17. Response of the isolated whole testes of the adult rat (left) and the adult rabbit (right) to acetylcholine chloride and norepinephrine base. In each case, the load on the lever arm was 100 mg. The temperature of the tissue bath was maintained at 32 °C for the rat testis and 37 °C for the rabbit testis. Response magnification was x 100 and x 10 for the rat and rabbit testis respectively.
Figure 18. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rat testis to various autonomic drugs. The isolated parenchymal mass had a wet weight which averaged 1,450 mg as compared to only 75 mg for the isolated testicular capsule. The load on the lever arm was 100 mg for both the isolated capsule and parenchyma tissue. The temperature in each tissue bath was maintained at 32 °C. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, pilocarpine hydrochloride, norepinephrine base, epinephrine base, isoproterenol hydrochloride, histamine base, tetramethylammonium chloride (TMA) and barium chloride. Response magnification was x 100 for both tissues.
Figure 19. Comparison of the response of the isolated capsule versus the isolated parenchymal tissue of the adult rat testis to oxytocin (top) and vasopressin (bottom). In each case, the load on the lever arm was 100 mg. Response magnification was x 100.
Figure 20. Schematic representation of the procedure used in the isolation of the human testicular capsule and its use as an isolated tissue preparation for pharmacological studies. Human testes were obtained from males aged 60-70 years whose testes had been removed as a therapeutic measure for prostatic carcinoma. Following orchietomy, the testis is bisected into medial (M) and lateral (L) segments, and the medial segment is transported to the Pathology Department (Path.) for routine histological examination. The lateral segment is transported to our laboratory within 15 minutes of removal of the testis at surgery. A, B, C. Anterior, posterior and lateral views respectively, of the lateral half of the human testis. Inasmuch as the entire isolated testicular capsule of the human is too large to be mounted in an organ bath, the following strips of the testicular capsule are cut to approximate 20 mm in length and 6 mm in width: 1 = lateral horizontal strip; 2 = posterior, superior, vertical strip; 3 = posterior, inferior, vertical strip; 4 = anterior, inferior, vertical strip; 5 = anterior, superior, vertical strip. D. Each capsular strip is tied with silk thread at both ends and mounted in a 10 ml isolated tissue bath with the upper long thread leading to a transducer for detection of tissue contractions.
Figure 21. Spontaneous contractions of the isolated testicular capsule of the human in the absence of any added drugs. A through J represent consecutive spontaneous contractions observed throughout a time period of 134 minutes. The time beneath each spontaneous contraction represents minutes after the preceding contraction. The temperature of the tissue bath was maintained at 32 °C. The preparation employed was a longitudinal strip of the isolated testicular capsule 18 mm in length and 7 mm in width which was obtained from the superior half of the posterior border of the human testis. The load on the lever arm was 250 mg. Response magnification was x 5.
Figure 22. Effect of autonomic drugs on the isolated testicular capsule of the human. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, tetramethylammonium chloride (TMA), norepinephrine base, and barium chloride. The temperature of the tissue bath was maintained at 32°C and the load on the lever arm was 250 mg. The preparation employed was a circular strip of the isolated testicular capsule measuring 20 mm in length and 6 mm in width which was obtained from the middle third of the medial surface of the human testis. Response magnification was x 10.
Figure 23. Schematic diagram of the various layers of the testicular capsule and the scrotum.
Figure 24. Representative cross sections of the testicular capsule of the adult rat employing Masson's trichrome stain. A. Mediastinal region of the testicular capsule enclosing a cavity of the rete testis (rt) which is lined with a cuboidal type of epithelium (ep). Occasional smooth muscle fibers (smf) can be seen within the collagenous tissue (c) of the tunica albuginea (x 350). B-H. Continuation of the testicular capsule around the circumference of the testis. smn, smooth muscle nucleus; fib, fibroblast; ext, cytoplasmic extension of a fibroblast; int, interstitial tissue cells; tvv, tunica vaginalis visceral; ta, tunica albuginea; tv, tunica vasculosa. The smooth muscle cells of the tunica albuginea are predominantly located along the posterior border of the testis. (x 700).
Figure 25. Smooth muscle cells of the tunica albuginea of the adult rat testis. A. Mediastinal region of the tunica albuginea surrounding a cavity of the rete testis (rt) lined by a cuboidal epithelium (ep). B. A portion of the tunica albuginea adjacent to the mediastinum illustrating the tunica vasculosa (tv) and an occasional adherent interstitial tissue cell (int). smn, smooth muscle nucleus; smf, smooth muscle fiber; fib, fibroblast; c, collagenous tissue of the tunica albuginea. Masson's trichrome stain. (x 1,150).
Figure 26. Relationship of the mediastinal region of the tunica albuginea to the rete testis and vasa efferentia of the adult rat testis. A. Illustration of the pathway of sperm transport from the seminiferous tubules (st) to the rete testis (rt) and vasa efferentia (va). The vasa efferentia then lead directly to the epididymis by passing through the tunica albuginea (ta) of the mediastinum (x 75). B. Demonstration of the complete enclosure of the cavities of the rete testis and vasa efferentia by the tunica albuginea of the mediastinum (x 175). Masson's trichrome stain.
Figure 27. Electron microscopy of collagen fibrils in the tunica albuginea of the adult rat testis. Glutaraldehyde and osmium tetroxide fixation. Uranyl acetate and lead citrate stain. A. Portion of a fibroblast (fib) illustrating thin cytoplasm (cy) with irregular extensions (ext) projecting into adjacent collagen (c) (x 33,600). B. Transverse section of collagen fibers (cf) separated by irregular cytoplasmic extensions of the fibroblasts (x 23,600). C. Longitudinal section of individual collagen fibrils (f) (x 73,600). D. Higher magnification of the fine structure of a collagen fibril illustrating the regular occurrence of light and dark bands with an average periodicity of 640 Å (x 204,000).
Figure 28. Cross sections of the testicular capsule of the adult rabbit illustrating the distribution of smooth muscle in the tunica albuginea. A. Low magnification of the testicular capsule (cap) completely surrounding the parenchymal tissue of the testis. A cavity of the rete testis (rt) can be seen toward the posterior border of the testis (x 8.5).
B. Higher magnification of the testicular capsule indicating the continuity of the septum (sep) with the tunica albuginea (x 70).
C. Demonstration of two distinct layers of smooth muscle fibers in the tunica albuginea. A superficial layer of longitudinal smooth muscle (lsm) runs parallel to the long axis of the testis. A second, deeper layer of circular smooth muscle (csm) is oriented along the circumference of the testis at right angles to the superficial layer. Collagen (c) occurs on either side of the circular layer of smooth muscle separating this layer from the longitudinal layer of smooth muscle (x 175).
D. Higher magnification of the circular smooth muscle layer of the capsule illustrating several smooth muscle nuclei (smn). Numerous fibroblasts (fib) can be seen within the dense collagenous tissue (c) of the tunica albuginea (x 750).
Masson's trichrome stain.
Figure 29. Cross section of the human testicular capsule illustrating the presence of smooth muscle in the tunica albuginea. A. Low magnification of the testicular capsule (cap) surrounding the seminiferous tubules (st) of the parenchymal tissue. (x 45). B. Higher magnification of the dense collagenous tissue (c) of the tunica albuginea where there is an abundance of smooth muscle nuclei (smn), smooth muscle fibers (smf) and fibroblasts (fib). (x 490). Masson's trichrome stain.
Figure 30. Representative sections of the testicular capsules of the rat, rabbit and human stained with Masson's trichrome stain and illustrating the comparative thickness of each capsule as well as the presence of smooth muscle. A-C. Low magnification of the testicular capsule (cap) surrounding the parenchymal tissue of the rat, rabbit and human testis, respectively (x 22). D. 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 cell (x 1125). E. High magnification of the rabbit testicular capsule demonstrating the superficial layer of longitudinal smooth muscle (lsm) as well as the deeper layer of circular smooth muscle (csm). st, seminiferous tubule (x 490). F. High magnification of the human testicular capsule demonstrating the abundance of smooth muscle fibers (smf) located within the dense collagenous tissue of the tunica albuginea (x 490).
Figure 31. Color photomicrographs of representative sections of the testicular capsules of the adult rat (top, x 300), adult rabbit (middle, x 200) and human (bottom, x 40), stained with Masson's trichrome stain and illustrating the presence of smooth muscle fibers. The use of the trichrome stain results in blue-black nuclei, red cytoplasm and muscle fibers and brilliant-blue collagen tissue. Smooth muscle nuclei can be readily distinguished from fibroblast nuclei by possessing a characteristic lightly-stippled appearance with an oval shape. Fibroblast nuclei appear much darker and thinner.
Figure 32. Effect of atropine sulfate on the response of the adult rat isolated testicular capsule to acetylcholine chloride.

A through J represent the order of recordings obtained in a dose-response procedure for the blocking effects of atropine on acetylcholine induced contractions of the capsule. The recordings on the left are control responses of the capsule to 1.0 ug/ml acetylcholine and were obtained directly preceding the response of the capsule to acetylcholine in the presence of increasing doses of atropine as seen on the right. Between F and G and between H and I, three 15 minute acetylcholine dose cycles were required to return to the control responses seen at G and I respectively. Response magnification was x 100.
Figure 33. Effect of tetraethylammonium chloride (TEA) on the response of the adult rat isolated testicular capsule to tetramethylammonium (TMA). A through I represent the order of recordings obtained in a dose-response procedure for the blocking effects of TEA on TMA induced contractions of the capsule. The recordings on the left are control responses of the capsule to 50 μg/ml TMA, and were obtained directly preceding the response of the capsule to TMA in the presence of increasing doses of TEA as seen on the right. Between D and E, one 15 minute TMA dose cycle was required to return the response of the capsule to a control response seen at E. Similarly, between F and G, two 15 minute TMA dose cycles were required to return to the control response seen at G. Response magnification was x 100.
Figure 34. Effect of various pharmacological antagonists on the per cent response of the adult rat isolated testicular capsule to pharmacological agonists. The log dose of each antagonist is shown on the abscissa. Each point on the graph represents the average of at least 3 experiments.
Figure 35. Initial stimulatory response of the adult rat isolated testicular capsule to the 2 adrenergic blocking agents, propranolol hydrochloride and phenoxybenzamine hydrochloride. Response magnification was x 100.
Figure 36. Comparison of actual millimeters of tissue contraction produced by various temperatures acting on the isolated testicular capsule of the adult rat.
Figure 37. Effect of various temperatures on the spontaneous contractions of the adult rabbit isolated testicular capsule. The initial temperature of the isolated tissue bath was set at 32 °C and monitored with a tele-thermometer. At 20 minute intervals, the temperature was either lowered 6 °C until 14 °C was attained, or raised 6 °C until 50 °C was attained. In each case, the load on the lever arm was 100 mg. Response magnification was x 10.
Figure 38. The nature of the inhibitory effect of low and high temperatures on spontaneous contractions of the adult rabbit isolated testicular capsule. The top set of recordings indicate that the inhibition of spontaneous contractions caused by low temperatures (14°C) is reversible. The bottom set of recordings indicate that the inhibition of spontaneous contractions caused by high temperatures (50°C) is irreversible. The initial temperature of the isolated tissue bath was set at 32°C and successive temperature changes of 6°C as indicated by the arrows were maintained for 20 minutes and monitored with a tele-thermometer. In each case, the load on the lever arm was 100 mg. Response magnification was x 10.
Figure 39. Comparison of the response of the isolated capsules from the right cryptorchid testis and the contralateral left scrotal testis of a 650 day old rat to various autonomic drugs. Unilateral cryptorchidism was experimentally induced in rats which were 50 to 60 days old at the beginning of the experiment. The temperature of the tissue bath was maintained at 32 °C and the load on the lever arm was 100 mg in each case. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, pilocarpine hydrochloride, norepinephrine base, epinephrine base, isoproterenol hydrochloride, histamine base, tetramethylammonium chloride (TMA) and barium chloride. Response magnification was x 100.

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<th>EFFECT OF AUTONOMIC DRUGS ON THE ISOLATED CAPSULES OF THE SCROTAL AND CRYPTORCHID RAT TESTIS</th>
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<td>SCROTAL CAPSULE</td>
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Figure 40. Effect of anoxia on the tone and spontaneous contractions of the adult rabbit isolated testicular capsule.
A. Typical response of the capsule when fresh buffer is washed into the tissue bath. Response magnification was x 25.
B-C. Response of the capsule when the aeration in the tissue bath is stopped for a period of 3 minutes and then turned on again. Response magnification was x 10.
Figure 41. Effect of anoxia on tone and spontaneous contractions of the adult rabbit isolated whole testis. A. Typical response of the whole testis when fresh buffer is washed into the tissue bath. B-C. Response of the whole testis when aeration in the tissue bath is stopped for a period of 10 and 9 minutes respectively, and then turned on again. Response magnification was x 10.
Figure 42. Effect of anoxia on the tone and spontaneous contractions of the adult rabbit isolated duodenum. A. Typical response of the duodenum when fresh buffer is washed into the tissue bath. B-C. The response of the duodenum when aeration in the tissue bath is stopped for a period of 9 and 5 minutes respectively and then turned on again. Response magnification was x 10.
Figure 43. Irregular spontaneous contractions of the adult rabbit isolated testicular capsule in the absence of any added drugs. The consecutive spontaneous contractions were observed throughout a time period of 95 minutes. Response magnification was x 10.
Figure 44. Effect of preliminary stretching of the isolated testicular capsule of the adult rat on the response of the isolated capsule to 1 µg/ml acetylcholine chloride. In every case, maximal contraction of the capsule was reached in approximately 3 minutes following the addition of the drug. The load on the lever arm was 100 mg. A. Initial response of the capsule to acetylcholine. B - D. Consecutive responses of the capsule to acetylcholine following 15 minute stretching periods with 500 mg additional load on the lever arm. Response magnification was x 100.
Figure 45. Effect of preliminary stretching of the adult guinea pig isolated ileum on the response of the ileum to 0.01 μg/ml acetylcholine chloride. In every case, maximal contraction was reached in approximately 20 - 40 seconds following the addition of the drug. A. Initial response of the ileum to acetylcholine. B - C. Consecutive responses of the ileum to acetylcholine following 15 minute stretching periods with 1,000 mg additional load. Response magnification was x 10.
Figure 46. Use of the adult rat isolated testicular capsule for bioassay of an unknown concentration of atropine sulfate. The per cent inhibition of the response of the capsule of the left testis to 1.0 μg/ml acetylcholine chloride was determined with a 4 point assay using two doses of the test solution of atropine and two doses of a standard solution of atropine (10 μg/ml). A = 0.02 ml test solution; B = 0.05 ml test solution; C = 0.02 ml standard solution; D = 0.05 ml standard solution. In every case, the dose of atropine was allowed to act for 2 minutes before the addition of acetylcholine to the tissue bath. The dose sequence of atropine was C D A B D C B A A B C D. Between each dose of atropine, two to four 15 minute acetylcholine dose cycles were required to return the response of the testicular capsule to a control response. Response magnification was x 100.
Figure 47. Graphical analysis of the data obtained from the bioassay of atropine shown in figure . Each value plotted for $T_1$, $T_2$, $S_1$, $S_2$ represents the average of the three values obtained where $T_1 = A$, $T_2 = B$, $S_1 = C$, $S_2 = D$. The dotted lines indicate the doses of the standard atropine solution and the test atropine solution (0.0460 ml and 0.0233 ml respectively) which permitted only a 34% response of the adult rat isolated testicular capsule to 1.0 μg/ml acetylcholine chloride.
Figure 48. Use of the adult rat isolated testicular capsule for bioassay of an unknown concentration of atropine sulfate. The per cent inhibition of the response of the capsule of the right testis to 1.0 µg/ml acetylcholine chloride was determined with a 4 point assay using two doses of the test solution of atropine and two doses of a standard solution of atropine (20 µg/ml). A = 0.01 ml test solution; B = 0.025 ml test solution; C = 0.01 ml standard solution; D = 0.025 ml standard solution. In every case, the dose of atropine was allowed to act for 2 minutes before the addition of acetylcholine to the tissue bath. The dose sequence of atropine was A B C D B A D C. Between each dose of atropine, two to four 15 minute acetylcholine dose cycles were required to return the response of the testicular capsule to a control response. Response magnification was x 100.
Figure 49. Graphical analysis of the data obtained from the bioassay of atropine shown in figure. Each value plotted for $T_1$, $T_2$, $S_1$, $S_2$ represents the average of the two values obtained where $T_1 = A$, $T_2 = B$, $S_1 = C$, $S_2 = D$. The dotted lines indicate the doses of the standard atropine solution and the test atropine solution (0.0150 ml and 0.0165 ml respectively) which permitted only a 38% response of the adult rat isolated testicular capsule to 1.0 μg/ml acetylcholine chloride.
Figure 50. Response of the isolated vas deferens (pars inguinalis) of the adult rat to various autonomic drugs. The temperature of the tissue bath was maintained at 32 °C. The load on the lever arm was 100 mg. Drug concentrations are expressed per ml of organ bath volume for acetylcholine chloride, carbachol chloride, pilocarpine hydrochloride, norepinephrine base, epinephrine base, isoproterenol hydrochloride, histamine base, tetramethylammonium chloride (TMA) and barium chloride. Response magnification was x 100.
Figure 51. Comparison of the response of the adult rat isolated vas deferens (top) and isolated epididymis (bottom) to 1 µg/ml carbachol chloride and 1 µg/ml norepinephrine base. Response magnification was x 100.
**TABLE I**

COMPARISON OF THE CONTRACTION OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE IN VARIOUS PHYSIOLOGICAL SALT SOLUTIONS

<table>
<thead>
<tr>
<th>SOLUTION</th>
<th>AERATING GAS</th>
<th>% MAXIMAL CONTRACTION WITH 0.01 µg/ml ACh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrode</td>
<td>Air</td>
<td>100</td>
</tr>
<tr>
<td>Krebs</td>
<td>95% O₂ + 5% CO₂</td>
<td>56</td>
</tr>
<tr>
<td>De Jalon</td>
<td>95% O₂ + 5% CO₂</td>
<td>36</td>
</tr>
</tbody>
</table>
**TABLE II**

**ID$_{50}$ OF PHARMACOLOGICAL ANTAGONISTS IN INHIBITING THE RESPONSE OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE TO VARIOUS AGONISTS**

<table>
<thead>
<tr>
<th>AGONIST</th>
<th>ANTAGONIST</th>
<th>ID$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µg/ml Acetylcholine</td>
<td>Atropine</td>
<td>0.1</td>
</tr>
<tr>
<td>50 µg/ml Tetramethylammonium</td>
<td>Tetraethylammonium</td>
<td>115</td>
</tr>
<tr>
<td>1 µg/ml Norepinephrine</td>
<td>Phenoxybenzamine</td>
<td>0.3</td>
</tr>
<tr>
<td>5 µg/ml Isoproterenol</td>
<td>Propranolol</td>
<td>27</td>
</tr>
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</table>
EFFECT OF BLOCKING AGENTS ON THE RESPONSE OF THE ADULT RAT ISOLATED TESTICULAR CAPSULE TO VARIOUS AUTONOMIC DRUGS

<table>
<thead>
<tr>
<th></th>
<th>Acetylcholine (1 µg/ml)</th>
<th>Tetramethylammonium (100 µg/ml)</th>
<th>Norepinephrine (1 µg/ml)</th>
<th>Isoproterenol (5 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATROPINE (1 µg/ml)</td>
<td>CB</td>
<td>CB</td>
<td>nb</td>
<td>nb</td>
</tr>
<tr>
<td>TETRAETHYLAMMONIUM (200 µg/ml)</td>
<td>nb</td>
<td>CB</td>
<td>nb</td>
<td>nb</td>
</tr>
<tr>
<td>D-TUBOCURARINE (10 µg/ml)</td>
<td>nb</td>
<td>nb</td>
<td>nb</td>
<td>nb</td>
</tr>
<tr>
<td>PHENOXYBENZAMINE (10 µg/ml)</td>
<td>PB</td>
<td>CB</td>
<td>CB</td>
<td>nb</td>
</tr>
<tr>
<td>PROPRANOLOL (50 µg/ml)</td>
<td>nb</td>
<td>nb</td>
<td>nb</td>
<td>CB</td>
</tr>
</tbody>
</table>

CB = complete block  
PB = partial block  
nb = no block
APPROVAL SHEET

The dissertation submitted by George A. Langford, B.Sc., M.Sc. has been read and approved by five members of the faculty of Loyola University.

The final copies have been examined by the Dissertation Director 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 with reference to content, form and mechanical accuracy.

This dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

DATE

SIGNATURE OF ADVISOR

Dec. 3, 1971

Joseph R. Davis, M.D., Ph.D.