GAP JUNCTION FORMATION IN UTERINE SMOOTH MUSCLE

AT PARTURITION AND

ACCOMPANYING CHANGES IN THE ELECTRICAL PROPERTIES

BY

STEPHEN MICHAEL SIMS, BSc

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

May, 1982
GAP JUNCTION FORMATION IN
MYOMETRIUM AT PARTURITION
DOCTOR OF PHILOSOPHY (1982)  McMaster UNIVERSITY
(Medical Sciences)  Hamilton, Ontario

TITLE: Gap Junction Formation in Uterine Smooth Muscle at Parturition 
and Accompanying Changes in the Electrical Properties

AUTHOR: Stephen Michael Sims, BSc. (University of Western Ontario)

SUPERVISOR: Professor Edwin E. Daniel

NUMBER OF PAGES: xiv, 197
The development of synchrony is characteristic of uterine muscle at the end of pregnancy. The coordinated contractile activity of the smooth muscle is effective in delivering the fetuses. A structural change occurs late in gestation, and specialized sites of intercellular communication, gap junctions, appear between the uterine smooth muscle cells. The objectives of this thesis were to characterize the time course of gap junction formation, investigate some factors that govern their appearance, and examine the possible significance of gap junction formation in the myometrium. Electrophysiological methods were used to evaluate the hypothesis that gap junction formation at parturition resulted in improved electrical communication between cells. Improved coupling might allow the synchronization of uterine activity.

Gap junctions were shown by quantitative thin section electron microscopy to be present between uterine smooth muscle cells immediately prior to, during, and for a short time following parturition in the rat. Several experiments involving ovariectomy revealed that the stimulus for gap junction formation was systemic in nature. Bilateral ovariectomy of midterm pregnant rats resulted in premature termination of pregnancy and gap junction formation, both of which were blocked by hormone administration. These and other results suggest that progesterone withdrawal may regulate gap junction formation in rat myometrium, but many factors, such as estrogen and prostaglandins, may also be involved.

Uterine smooth muscle is a functional electrical syncytium, and
properties of electric current flow through the muscle yield indirect estimates of cell-to-cell coupling between cells. Impedance analysis showed that the specific resistance of the cytoplasm of myometrial cells was constant from before term to delivery, but the junctional resistance decreased. Shortly post partum the junctional resistance increased. Cable analysis confirmed that the internal resistance of myometrium was lower at parturition. Thus, improved cell-to-cell communication was associated with a demonstrated increase in gap junction contact between cells. These results are consistent with the hypothesis that gap junction formation at the end of gestation results in improved electrical coupling of uterine smooth muscle cells.
Acknowledgements

Many individuals have assisted me throughout my sojourn at McMaster University. I wish to thank some of these individuals, foremost, my supervisor, Dr. Ed Daniel, who offered guidance, yet freedom. From him I have learned much and I owe him my gratitude. Dr. Bob Garfield provided a considerable amount of help and constant encouragement, and for that I am grateful. Dr. Ethel Cosmos offered guidance in an impartial and forthright manner.

Many other members of the Department of Neurosciences helped to ease my burden. From my very start at McMaster, Dr. Dr. Mathur Kannan has offered advice and companionship. Colin Ikeson unsparingly gave time, advice and expert technical support. Debbie Merrett provided help with some of the drudgery of some of this work. To these folks and others I haven't mentioned (such as the fine occupants of the lab, 4N75), I express thanks.

It was an honour to receive the comments and criticisms of Dr. T. Tomita. I appreciate the effort he spent thoroughly reviewing my thesis.

The Medical Research Council of Canada, on behalf of the taxpayers of Canada, generously supported me during my studies.

Finally, my wife Terri put up with considerable bother through the course of my studies, albeit, not without comment. As well, my mother put up with me. The hospitality of Kirkton was a welcome reward for completing various stages of my mission. For all these, I offer thanks. But wait, I have just one more experiment to complete......
# TABLE OF CONTENTS

## Chapter 1

**General Introduction and Review of the Literature**

- General Introduction and Objectives ........................................ 2
- Review of the Literature
  - Anatomy and Ultrastructure of the Uterus ............................... 4
  - Electrical Properties of Smooth Muscle ................................. 8
  - Mechanisms of Parturition ................................................ 11
  - Junctional Cell-to-Cell Communication ................................. 18

## Chapter 2

**Formation of Gap Junctions in Rat Myometrium**

- Introduction .................................................................................. 32
- Methods and Materials
  - Electron Microscopy ................................................................. 33
  - Quantitation of Gap Junctions in Myometrium .......................... 34
  - Ovariectomy ............................................................................ 36
  - Statistical Comparisons ........................................................... 36
- Results
  - General Ultrastructural Features of Myometrium ..................... 37
  - The Presence of Gap Junctions in Myometrium from
    - Pregnant and Post Partum Animals ........................................ 37
    - Ovariectomy .......................................................................... 46
Discussion

Presence of Gap Junctions in the Myometrium
Mechanisms for Control of Gap Junction Formation in Myometrium
Overview

Chapter 3
Changes in the Longitudinal Impedance Characteristics of Rat Uterine Smooth Muscle at Parturition

Introduction

Methods

Experimental Preparation
Measurement of the Longitudinal Impedance

Results

Electrode Impedance
Frequency Response of Nonpregnant Myometrium
Equivalent Circuit
Estimating Resistance Values
Changes in the Impedance due to Isosmotic Sucrose
Comparison of Late Term, Delivering and Post Partum Myometrium

Junctional Time Constant and Capacitance
Method of Standardizing the Results

Discussion

Occurrence of Gap Junctions in the Muscle
Sources of Error
Changes in the Impedance properties of other Smooth Muscles ................................. 104

Conclusions ......................................................... 106

Chapter 4
Changes in the Cable Properties of Myometrium Associated with
the Formation of Gap Junctions at Parturition

Introduction .......................................................... 108

Methods
Tissue Preparation .................................................. 109
Electrical Recording ................................................. 110
Analysis of the Spatial Decay of Electrotonic Potentials 112
Electron Microscopy ................................................ 113
Statistical Analysis ................................................. 113

Results
Electron Microscopy ................................................ 114
Electrical Properties .............................................. 119
Spatial Decay of Electrotonic Potentials ....................... 122
Membrane Time Constant ........................................ 131

Discussion
Gap Junctions in the Myometrium ............................... 135
Changes in the Electrical Characteristics ...................... 137
Length Constant .................................................... 137
Membrane Resistance ............................................. 140
Internal Resistance ............................................... 141
Chapter 5

General Discussion
Distinctive Nature of This Research ......................... 146
Control of Gap Junction Formation .............................. 151
Associating Increased Area of Gap Junction Contact with
  Improved Coupling in Myometrium .............................. 153
A Model for Predicting the Change Brought About by
  Gap Junction Formation ........................................ 156
Conclusions .......................................................... 160
Further Studies ...................................................... 161

Appendix 1
Frequency Dependent Characteristics of a Model Circuit ...... 165

Appendix 2
Equations and Assumptions of One-dimensional, Linear
  Cable Theory ...................................................... 170

Appendix 3
Effect of Stretch on Cable Parameters ............................ 177

References .......................................................... 186
List of Tables

I  Gap Junctions in Pregnant and Post Partum Myometrium ... 43
II Effect of Ovariection on Formation of Gap Junctions .... 47.
III Impedance Components of Smooth Muscle ................. 81
IV Specific Resistance of Myometrium During Pregnancy
   and Post Partum ............................................. 93
V  Junctional Time Constant and Capacitance ................. 95
VI Gap Junctions in Myometrium Following Electrical
   Recordings .................................................. 116
VII Electrical Parameters of Myometrium ...................... 132
VIII Electrical Parameters of Delivering and
    Post Partum Myometrium .................................. 179
IX  Effect of Stretch on Cable Parameters .................... 183
**List of Figures**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematic view of current flow in a cable-like syncytium</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Low power electron micrograph of uterine wall</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>Electron micrograph of longitudinal myometrium</td>
<td>41</td>
</tr>
<tr>
<td>4</td>
<td>Gap junctions in parturient myometrium</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>Gap junctions in ovariectomized rats</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A) Nongravid horn at parturition</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>B) Bilateral ovariectomy</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>Diagram of impedance measuring chamber</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>Tests of electrode impedance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A) Schematic diagram of end-to-end measuring system</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>B) Variations in interelectrode distance</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>Impedance characteristics of nonpregnant myometrium</td>
<td>73</td>
</tr>
<tr>
<td>9</td>
<td>Model equivalent circuit of smooth muscle</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>Impedance of nonpregnant myometrium increases in sucrose</td>
<td>78</td>
</tr>
<tr>
<td>11</td>
<td>Conductivity of nonpregnant myometrium</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>Longitudinal impedance of taenia coli smooth muscle</td>
<td>82</td>
</tr>
<tr>
<td>13</td>
<td>Impedance responses of pregnant myometrium</td>
<td>85</td>
</tr>
<tr>
<td>14</td>
<td>Impedance increases with time in sucrose</td>
<td>88</td>
</tr>
<tr>
<td>15</td>
<td>Conductivity of pregnant myometrium</td>
<td>89</td>
</tr>
<tr>
<td>16</td>
<td>Electron micrograph of gap junctions in myometrium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A) 30 min in sucrose</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>B) 90 min in sucrose</td>
<td>91</td>
</tr>
<tr>
<td>17</td>
<td>Linear relationship between sample weight and admittance</td>
<td>97</td>
</tr>
</tbody>
</table>
Diagram of partition bath ........................................111
Timing of delivery ..............................................115
Electron micrographs of gap junctions in myometrium
   A) Parturient myometrium .......................................118
   B) Before term ................................................118
Size distribution of gap junctions ..........................120
Spontaneous membrane electrical activity ..................121
Electrotonic potentials during action potentials .......123
Current-voltage relationship before term
   A) Membrane responses .........................................125
   B) I-V plots ..................................................125
   C) Length constant plots ......................................125
Current-voltage relationship at delivery
   A) Membrane responses .........................................127
   B) I-V plots ..................................................127
Spatial decay of electrotonic potentials ...................129
Time dependence of electrotonic potentials ..............133
Frequency response of test circuit .........................168
Membrane responses of post partum myometrium
   A) Membrane responses .........................................185
   B) I-V plots ..................................................185
### LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cross sectional area</td>
</tr>
<tr>
<td>a</td>
<td>Cable radius</td>
</tr>
<tr>
<td>C</td>
<td>Centigrade</td>
</tr>
<tr>
<td>C_m</td>
<td>Membrane capacitance</td>
</tr>
<tr>
<td>C_j</td>
<td>Junctional capacitance</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeters</td>
</tr>
<tr>
<td>EM</td>
<td>Electron microscopy</td>
</tr>
<tr>
<td>f</td>
<td>Frequency</td>
</tr>
<tr>
<td>f_o</td>
<td>Characteristic frequency</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GJ</td>
<td>Gap junction</td>
</tr>
<tr>
<td>j</td>
<td>Imaginary operator</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>i_m</td>
<td>Membrane current</td>
</tr>
<tr>
<td>i_long</td>
<td>Longitudinal current along the core</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz (cycles per second)</td>
</tr>
<tr>
<td>λ</td>
<td>Length constant</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>mM</td>
<td>MilliMolar</td>
</tr>
<tr>
<td>mOsm</td>
<td>MilliOsmolar</td>
</tr>
<tr>
<td>mV</td>
<td>Millivolt</td>
</tr>
<tr>
<td>μF</td>
<td>Microfarad</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>Ω</td>
<td>Ohm</td>
</tr>
<tr>
<td>ϕ</td>
<td>Phase angle</td>
</tr>
<tr>
<td>ω</td>
<td>Radial frequency</td>
</tr>
<tr>
<td>OVX</td>
<td>Ovariectomy</td>
</tr>
<tr>
<td>R_i</td>
<td>Specific internal resistance (resistivity) of cable</td>
</tr>
<tr>
<td>R_j</td>
<td>Specific internal resistance due to junctions</td>
</tr>
<tr>
<td>R_m</td>
<td>Specific membrane resistance</td>
</tr>
<tr>
<td>R_myo</td>
<td>Specific internal resistance due to myoplasm</td>
</tr>
<tr>
<td>R_o</td>
<td>Input resistance</td>
</tr>
<tr>
<td>R_0 Hz</td>
<td>Specific internal resistance at zero Hz</td>
</tr>
<tr>
<td>R_∞ Hz</td>
<td>Specific internal resistance at ∞ Hz</td>
</tr>
<tr>
<td>r_e</td>
<td>Resistance of extracellular space</td>
</tr>
<tr>
<td>r_i</td>
<td>Internal resistance per unit length of fiber</td>
</tr>
<tr>
<td>r_m</td>
<td>Membrane resistance per unit length of fiber</td>
</tr>
<tr>
<td>θ</td>
<td>Conduction velocity</td>
</tr>
<tr>
<td>S</td>
<td>Siemens</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>τ_m</td>
<td>Membrane time constant</td>
</tr>
<tr>
<td>τ_j</td>
<td>Junctional time constant</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>Y</td>
<td>Admittance</td>
</tr>
<tr>
<td>Z</td>
<td>Impedance</td>
</tr>
<tr>
<td>Z_t</td>
<td>Total tissue impedance</td>
</tr>
</tbody>
</table>
NEW DISCOVERIES

A discovery is rarely, if ever, a sudden achievement, nor is it the work of one man; a long series of observations, each in turn received in doubt and discussed in hostility, are familiarized by time, and lead at last to the gradual disclosure of truth.

-- Sir Berkely Moynihan (1865-1936) --
CHAPTER 1

General Introduction and Review of the Literature
GENERAL INTRODUCTION

"Broadly speaking, the functions of the uterus are twofold, first, to provide a safe and adequate environment for the developing ovum, and second, to deliver its contents at term by the processes of parturition" (Reynolds, 1949). The transformation of the uterus from a relatively quiescent organ to one that contracts forcefully to expel the fetal contents represents a remarkable transition in functional behaviour at the end of gestation. Factors governing the conversion are of primary importance in regulating the onset and progression of labour. Reynolds recognized the complexity of parturition and stated a principle which still warrants emphasis, i.e. "in late pregnancy a train of events is initiated that ultimately results in the delivery of the fetus. ...we still do not know how and where the train starts, or exactly how it exerts its ultimate action on the myometrial cell."

Since 1949 significant advances have been made in the field of reproductive biology. Many steps in the cascade of events which culminates in normal labour have been elucidated, but, the complete sequence of events and, specifically, many factors which directly regulate myometrial activity are still incompletely understood.

The transition in uterine character at the end of gestation becomes apparent as the development of synchronous electrical activity of cells in different regions of the uterus. The synchronized electrical events may in turn result in the coordinated contractile activity of a large population of smooth muscle cells, leading
eventually to the orderly evacuation of uterine contents.

The basis for the conversion of uterine muscle from a quiescent to an active state is uncertain. Many factors appear to be involved in regulating the conversion, but few can account directly for the rapid change in contractile function of the uterus during the onset of parturition.

A recent discovery revealed a change in the structure of uterine smooth muscle at the end of gestation, a change that may have a bearing on the altered function. Garfield and Daniel found that specialized sites of communication between cells, gap junctions or nexuses, which were normally not visible between uterine smooth muscle cells, could be identified in the myometrium of rats close to term or in labour. Gap junctions are generally thought to represent sites of low resistance electrical coupling between many cell types. It was considered possible that the development of gap junctions might be the basis for improved electrical communication between the individual muscle fibers and thereby promote synchronization of electrical and mechanical activity within the uterus.

With these preliminary observations in mind, the following objectives were detailed for this thesis:

i) Characterize the time course of gap junction formation between uterine smooth muscle cells at the end of gestation in the rat.

ii) Determine some of the mechanisms that operate in vivo to regulate gap junction formation.

iii) Investigate one possible functional significance of gap
junction formation on the physiological properties of the myometrium. Specifically, examine the hypothesis that gap junction formation at parturition is associated with improved electrical coupling of smooth muscle cells.

REVIEW OF THE LITERATURE

A) Anatomy and Ultrastructure of the Uterus

General Anatomy. The uterus duplex of the rat consists of two separate tubes or horns, fused externally at the cervical end. They open separately into two cervical canals, which in turn open separately into the vagina (Mossman, 1977). Each uterine horn is a hollow organ whose wall is composed of three distinct layers (Finn and Porter, 1975; also see Results of Chapter 2). The inner endometrium is a columnar epithelial cell layer which lines the entire horn. The middle circular smooth muscle cell layer consists of cells arranged concentrically around the longitudinal axis of each horn. Contraction of the circular muscle layer would tend to constrict a region of the uterine lumen. The outer longitudinal muscle layer consists of bundles of fibers that are oriented parallel to the longitudinal axis of the horn, and contraction of this muscle would tend to shorten the horn. There is some morphological evidence that the two muscle layers are continuous in places (Csapo, 1962; Finn and Porter, 1975). Electrophysiological studies confirm that electrical events are transmitted in both directions between the two layers (Osa and Katase, 1975).

Muscle Cell Structure. In common with most smooth muscle types, myometrial cells are markedly anisotropic, being long and tapered. The
dimensions of uterine smooth muscle cells are largest late in gestation, and range from 5-10 μm wide in the middle, to 300-600 μm long (Csapo, 1962; Finn and Porter, 1975). The cells appear round or oval when viewed in transverse orientation, if the cells are in a relaxed or stretched state. Kao (1977) estimates that a single myometrial cell from a rat late in gestation might occupy about 21,000 μm$^3$. Taking an average volume to surface area ratio of 0.9 μm, the surface area would be approximately 23,000 μm$^2$. This estimate is several fold greater than Gabella's figure, for the surface area of a taenia coli smooth muscle cell (1976). Flask shaped infoldings, termed caveolae, occur in the surface membrane of smooth muscle cells. Some caveolae open to the extracellular compartment and could effectively increase the surface area of the smooth muscle cell by 25-75% (Gabella, 1973). It is difficult to evaluate the contribution of the caveolae to the overall electrical and metabolic properties of the cells, and an understanding of their precise function is lacking.

The ultrastructural details of myometrial cells appear to be similar to many other smooth muscle types (Burnstock, 1970; Gabella, 1973). The cells have single nuclei usually located centrally and the cytoplasm is occupied largely by contractile filaments, although other structures such as microtubules, 10 nm filaments and numerous cytoplasmic organelles are also present. These include mitochondria, sarcoplasmic reticulum, golgi apparatus, ribosomes and rough endoplasmic reticulum.

In addition to the endometrial and smooth muscle cells, other cell types are present in the uterine wall (see Results, Chapter 2).
Fibroblasts are often seen surrounding bundles of muscle cells, separating the various layers and bordering the serosal surface of the uterus. Sympathetic nerve branches from the inferior mesenteric ganglia do penetrate into the uterine wall, but nerve terminals are present only infrequently in the rat uterus (Mossman, 1977). In the rat, the nerves are usually seen only in close association with blood vessels which are present in the uterus, often in the connective tissue regions separating various layers. This suggests that direct innervation of the rat myometrium is infrequent, at best. Functional nerve-muscle relationships may exist in the myometrium of other species (Mossman, 1977; Marshall, 1981).

Striking changes in the myometrium occur during pregnancy. A 10-fold increase in the weight of the myometrium is due to both hypertrophy and hyperplasia (Reynolds, 1949; Afting and Elce, 1978). Estrogens and distention of the uterine wall both play a role in stimulating growth of the uterus (Finn and Porter, 1975). Following delivery of the young, the uterus undergoes post partum involution. Collagen is degraded, the amount of DNA in the myometrium decreases and the amount of protein returns to prepregnancy levels within about 10 days (Afting and Elce, 1978).

**Cell-to-Cell Contacts in Smooth Muscle.** At certain regions smooth muscle cells come into close contact with their neighbours and form several types of junctions. Four major types of cell-to-cell contacts occur in smooth muscle, and include the gap junction or nexus, the intermediate contact, interdigitations and simple appositions (Gabella, 1973; Garfield and Daniel, 1974; Henderson, 1975).
Intermediate contacts are regions where the plasma membranes run parallel, with a separation of 30-50 nm. Submembranous densities exist below the junction and often a dense line and granular material is seen in the extracellular space of the junction. Interdigitations occur where a portion of one cell protrudes into a neighbouring cell. The shape and frequency of interdigitations are determined in part by the plane of section and the contractile state of the cells (Gabella, 1976). Simple appositions are regions where the membranes of neighbouring cells run parallel, less than approximately 30 nm apart. The precise functions of these three types of cell-to-cell contacts are not known. They may provide sites of physical attachment or contribute to intercellular communication in some manner.

The gap junction, or nexus, has been described in smooth muscle (Dewey and Barr, 1962; Burnstock, 1970; Gabella, 1973; Henderson, 1975) and a variety of other cell types (Bennett, 1977; Perrachio, 1980). Its appearance varies somewhat depending upon the method of fixation and staining of the tissue. Properly oriented gap junctions in glutaraldehyde fixed, en bloc stained, mammalian tissues are visualized in thin section electron microscopy (EM) as seven-lined structures, 15-20 nm wide. They are composed of two apposing membranes separated by a 2-3 nm gap between the outer membrane leaflets. Colloidal lanthanum and other tracers have been used to delineate the extracellular spaces. These electron dense markers penetrate the gap between the cells and outline an array of structures spanning the gap. The plaque-like nature of gap junctions on the cell surface is evident when tissues are examined with freeze-fracture EM. With this method gap junctions are
identified as aggregations of membrane particles (each 6-7 nm in
diameter) on P-(protoplasmic) fracture faces or corresponding pits on
E-(ectoplasmic) faces. The particle spacing ranges from 7-14 nm
center-to-center, and the packing pattern varies, depending on
experimental handling and source of the tissue (Parrachia, 1980; Raviola
et al., 1981). The proposed role of gap junctions in cell-to-cell
communication is described below.

B) Electrical Properties of Smooth Muscle

The principal factor responsible for control of contraction in
smooth muscle is the Ca** ion. Increased levels of Ca** in the
cytoplasm, due either to influx across the membrane or liberation from
sites within the cell, initiates a train of events which leads
eventually to the generation of force (Hartshorne and Seimenkowsk, 1981). Internal Ca** appears to be elevated by Ca** entry during the
action potential (Vassort, 1981), and tension in uterine smooth muscle
is determined by the duration and frequency of action potentials
(Marshall, 1959; Kuriyama and Csapo, 1961; Marshall and Csapo, 1961;
Casteels and Kuriyama, 1965; Kao, 1977; Anderson et al., 1981). Bursts
of action potentials, of the type illustrated in Chapter 4, are
associated with phasic contractions. It is apparent therefore, that
consideration of the electrical properties of smooth muscle is
fundamental to the understanding of its contractile characteristics.

Functional Electrical Synctium. The multicellular nature of
uterine smooth muscle was described in an earlier section. However,
just as in many other smooth muscle types, excitation does spread
through the intact tissue, and often the muscle behaves as a single unit. Four possible mechanisms may underlie the spread of excitation in smooth muscle. These include nervous, mechanical, humoral and electrical mechanisms. Because of the speed of propagation and failure of various nerve and pharmacological blocking agents to alter transmission of impulses, direct electrical interaction between cells is thought to be the principal means of impulse spread through the tissue. Impulses generated in one region of a smooth muscle spread from cell-to-cell through the muscle, just as in a single cell, by local circuit currents (Barr et al., 1968; Tomita, 1970; 1975; Bennett, 1972). Because of the continuous nature of impulse movement and its reliance upon local circuit currents, the term propagation is considered more appropriate than transmission, which emphasizes the discontinuous nature of the process. Subthreshold electrotonic potentials can be measured many cells distant from the site of stimulation, which is interpreted to mean that smooth muscle cells are joined by low resistance pathways. In view of the spread of active and passive potentials, uterine smooth muscle, like most smooth muscle types, is considered to be a functional electrical syncytium.

Cable Properties. Studies of the passive electrical properties of tissues are relevant to the problem of impulse spread through a coupled network because the passive spread of current generated by the action potential is responsible for propagation of the impulse (Katz, 1964; Tomita, 1970; 1975; Fozzard, 1979). An effective strategy for quantitatively characterizing the passive electrical properties of many tissues has been to model tissues as cables. The application of cable
theory to biological tissues was pioneered by Hodgkin and Rushton (1946) and later applied to many other excitable tissues (see Katz, 1964 and Cole, 1968). The principle of cable theory is that the tissue behaves as a core conductor enveloped by a high resistance membrane.

The one-dimensional cable model has been profitably applied to smooth muscle (Abe and Tomita, 1968). Intact smooth muscle can be represented as a series of independent cables, functionally connected by end-to-end and side-to-side connections. Under the appropriate experimental conditions of extracellular polarization the transverse flow of current is negligible and ideally can be ignored, since the tissue is equipotential in the transverse direction. Abe and Tomita (1968) first showed that electrotonic potentials in smooth muscle decayed exponentially with distance, the time course of the electrotonic potentials was described by the cable equation, and the response of the muscle to alternating current was as predicted for a uniform cable. The derivation of the cable equations is described in Appendix 2, and some assumptions of the model as it applies to smooth muscle are considered.

Properties of passive current flow in a cable can be characterized according to the time required for potentials to develop, described by the time constant, and the rate of decay of the size of electrotonic potentials with distance. The length constant of the cable describes the distance over which the amplitude of the steady-state electrotonic potential decays to 1/e (approximately 37%) of its size. The length constant is determined by two factors, the resistance to flow of current in the axial and in the radial direction. Axial current flow in the core of an homogenous cable is impeded solely by the resistance
of the cytoplasm in the core. An additional impediment to the axial flow of current in an electrical syncytium is the resistance between cells. A schematic representation of this concept is shown in Figure 1. The internal resistance of a multicellular cable is composed of the resistance of the cytoplasm plus the resistance of the junctions between cells.

In view of the foregoing discussion, it is apparent that the characteristics of current flow in a syncytium such as smooth muscle are determined not only by the properties of the individual cells, but also by the communication between cells. If other factors, such as the resistance of the cytoplasm remain constant, changes in the internal resistance can be attributed to changes in the coupling between cells. It is for this reason that characterization of the cable properties is of value in assessing the extent of coupling in smooth muscle.

C) Mechanisms of Parturition

Phenomenon of Labour. In vivo studies have documented the transition in uterine activity at the end of gestation. Kao (1959) and Daniel (1960) first used chronically implanted wire electrodes to show that large amplitude electrical signals, indicating synchronous excitation of cells, were prevalent at parturition. More recent studies have used, in addition to multiple extracellular electrodes, intrauterine pressure transducers to measure luminal pressure. Such recording methods monitor the activity of a large population of smooth muscle cells, and can give indirect indications of the degree of synchrony.
FIGURE 1. A schematic diagram shows that axial flow of current in a homogenous cable, such as a nerve axon, is impeded solely by the resistance of the cytoplasm of the cable. In a syncytium such as smooth muscle, axial current flow in the core is impeded not only by the resistance of the cytoplasm of individual cells, but also by the resistance of the current pathways (i.e. junctions) between cells. Therefore, the core resistance of a syncytial tissue can give an indication of electrical coupling between the cells.
In all species studied, uterine electrical and mechanical activity before term is localized and segmental. Different regions behave as if they were functionally independent of each other. As term approaches trains of impulses begin to propagate readily throughout the uterus, leading eventually to synchronous electrical and mechanical activity at various parts of the uterus (Csapo, 1962; Csapo and Takeda, 1965; Fuchs, 1969; 1973; Wolfs and van Leeuwan, 1979). Synchronous and well coordinated electrical and mechanical activity appear to be prerequisites for successful labour.

What is the basis of the transition in uterine activity? No simple answer yet exists. The sequence of events leading to labour contractions can be summarized as follows.

1) Excitation of the individual smooth muscle cells
2) Spread of excitation between cells of the uterus
3) Excitation-contraction coupling in the cells
4) Synchronized contractile activity causes increased intrauterine pressure.

Changes occurring at one or more of these stages may contribute to the effective activation of the uterus as a whole. Whether the transition from relative quiescence to the activity of parturition is caused by release from an inhibition or by stimulation is still a matter of controversy. It is known, however, that samples of myometrium from both before term and delivering animals are capable of generating similar tension when optimally stimulated (Csapo, 1977). Therefore, a change in the working capacity of the muscle cannot explain the onset of labour.
According to one theory the myometrium is intrinsically active but suppressed throughout gestation. Relief of the suppression results in the initiation of labour. Csapo has been the primary advocate of this scheme and advanced much evidence, from studies of various animals, to support the progesterone block hypothesis. Inhibitory actions of progesterone on uterine smooth muscle are proposed to be due to interference with excitation and impairment of electrical coupling of myometrial cells (Csapo, 1962; 1977). The effect of high tissue progesterone levels throughout gestation is proposed to reduce the synchrony of contractions, so that activity is localized. At the end of term, when plasma and tissue progesterone levels decline, the suppression is ended and synchronized contractions are effective in delivering the young. Many attempts have been made to define the actions of progesterone on myometrium, but the precise manner in which progesterone might "block" activity has not been defined (Csapo, 1962; 1977 and see the critical review of Kao, 1977). The results presented in this thesis provide a plausible mechanism to explain some effects of high progesterone levels during gestation.

An alternate view for regulation of uterine activity is that the uterus is inert throughout gestation, but is reactive to various agents. Sufficient concentrations of agonists are not present until the end of pregnancy, at which time they initiate labour. Prostaglandins are synthesized by the uterus and may be the stimulating agents present late in gestation (Thorburn and Challis, 1979; Phillips and Poyser, 1981).

A compromise that accommodates both alternate hypotheses may provide the most suitable explanation for the onset of labour. One
expression of this idea is the "see-saw" theory by Csapo (1977). Uterine activity represents the balance of two opposing forces, that of inhibition by progesterone and that of uterine activation by prostaglandins. Physiological mechanisms of parturition differ considerably among species, making it difficult to present a single sequence of general significance. It seems probable that events common to many (or all) species would be of fundamental importance to the process of parturition.

Endocrine Changes at Parturition. A cascade of endocrine events occurs late in pregnancy. Concurrent changes in many hormonal profiles, and the integration of hormone actions, makes it difficult to isolate each individual event. However, in certain experimental animals, in particular the sheep, cow and goat, the cascade of endocrine events has been elucidated. The fetal pituitary-adrenal axis has been shown to play a central role in the mechanisms that initiate parturition (Nathanielsz, 1978; Thorburn and Challis, 1979). In sheep, fetal glucocorticoid secretion stimulates placental production of estrogen from progesterone, thereby leading to a fall in maternal plasma and tissue progesterone concentrations and a concomitant rise in estrogen levels. Estrogens are potent stimulators of prostaglandin synthesis and the decline in progesterone levels further allows prostaglandin release to occur.

The role of the individual fetus in regulating the onset of labour in polytocous species, such as the rat, is not clearly defined. The trigger for labour could arise from one, some, or all the fetuses in a litter. While many hormones may play important roles in preparation
for labour, the final site of action must occur at the level of the myometrium.

Endocrine changes in the pregnant rat have been well characterized (Thorburn and Challis, 1979). Progesterone production is predominantly ovarian, but a placental contribution late in gestation is supplementary. Plasma levels of progesterone remain high throughout pregnancy and begin to decline several days before term. Progesterone is essential for maintenance of pregnancy, and ovariectomy before day 18 causes abortion (Csapo and Weist, 1969; Pepe and Rothchild, 1973). Exogenous progesterone will maintain pregnancy in ovariectomized animals and, when administered to intact animals late in term, will prolong pregnancy (Fuchs, 1973).

Estrogen production during pregnancy is largely ovarian. Plasma levels are characterized by an increase beginning 1 to 2 days before labour. Estrogens in vivo have a stimulatory effect on the uterus, which may largely be due to the stimulation of prostaglandin production (A.B.M. Anderson et al., 1981). The increase in estrogen levels late in term is thought to be an important step in the cascade of events leading to parturition in the rat, as in other species (Thorburn and Challis, 1979). For reasons that are unclear, administration of low doses of exogenous estrogen can inhibit uterine activity and delay parturition (Downing et al., 1981).

A growing body of evidence has implicated prostaglandins in the final chain of events resulting in parturition in many species. If prostaglandin synthesis inhibitors are administered to the pregnant rat and other animals during late gestation, parturition is delayed and
prolonged (Thorburn and Challis, 1979). While this observation does not enable us to define their precise role, we can conclude that endogenous prostaglandins are involved in parturition. The rat uterus synthesizes a variety of prostaglandins as well as thromboxanes (Phillips and Poyser, 1981). The endometrium is the major source of uterine prostaglandins, and compounds released may serve two purposes. The primary action would be to enter the circulation and have a luteolytic effect, causing regression of the corpus luteum. The fall in plasma progesterone levels that results from luteolysis further enhances prostaglandin release. This in turn may result in a sizable secondary action of prostaglandins, which is to diffuse within the uterine wall and directly stimulate the myometrial layers. Some of the prostaglandins are potent stimulators of rat myometrium, especially late at term, causing depolarization of the membrane and increased frequency of action potential bursts (Kuriyama and Suzuki, 1976b).

A variety of other agents influence myometrial activity in the rat. Oxytocin, like prostaglandins, stimulates electrical activity of the muscle (Kuriyama and Suzuki, 1976b). Administration of oxytocin more than 6-8 hours before labour is ineffective in inducing labour (Fuchs, 1973) and its role in triggering the onset of, or supporting labour, is uncertain. Alexandrova and Soloff (1980) demonstrated that there was a marked increase in oxytocin binding by particulate fractions of rat myometrium 1-2 days before delivery. It is possible that oxytocin could influence uterine activity without any change in plasma levels. Both vasoactive intestinal polypeptide (VIP) (Bolton et al., 1981) and relaxin (Cheah and Sherwood, 1981) inhibit activity of the
myometrium, but the role, if any, of these agents in regulating the onset of parturition is not yet clear.

D) Junctional Cell-to-Cell Communication

Evidence for direct electrical communication between cells was first obtained several decades ago in studies of cardiac tissue and neurons (Weidmann, 1952; Furshpan and Potter, 1959). Flow of current could be expected to play a role in signalling in both cases. Further instances of cell-to-cell coupling were uncovered in electrically excitable tissues, but an important observation revealed that not only small ions, but a large hydrophilic molecule, fluorescein, could traverse a junctional pathway between non-excitable cells (Bennett, 1977; Loewenstein, 1981). The observation that direct communication between cells was not limited to the flow of current between excitable cells prompted more thorough studies of cell-to-cell communication in a wide range of tissues. Numerous investigations have revealed that almost all cell types exhibit the capacity for junctional communication at some stage of their life cycle.

The cumulative evidence indicates that cells in organized tissues commonly form interconnected systems by way of hydrophilic channels. As a result, cytoplasmic constituents can flow directly from one cell interior to the next. Circumstantial evidence has accumulated that the gap junction is the structural basis for junctional cell-to-cell communication.

Coupling. The term coupling in its broadest sense refers to communication between cells. Electrical coupling exists when two or
more cells behave as if connected by a low resistance. In the experimental sense, this implies that current applied to one cell produces a potential in a second cell larger than it does in the bulk medium. Strictly speaking, this observation does not require that there be a specialized pathway for flow of current between cells, but such pathways can be demonstrated with tracers. A second means of demonstrating coupling does not require (but does not exclude) movement of charge between cells, and instead involves the passage of dye tracer molecules between cells. This occurs via a pathway that is insulated from the extracellular space. A third way of demonstrating coupling, a subgroup of the dye tracer category, is metabolic coupling, which involves the passage of (radiolabelled) nucleotides between cells (Hooper and Subak-Sharpe, 1981).

A firm theoretical basis exists for the analysis of electrical coupling between cells (Bennett, 1977). Quantitative analysis of junctional ionic conductance can be achieved with several electrical methods. In the simplest situation of two cells, current applied to one cell (called cell 1) will cause a voltage change in that cell ($V_1$) and in the coupled, adjoining, cell ($V_2$). The extent of coupling can be described with a commonly used measure, the coupling coefficient (CC), which refers to the ratio of $V_2/V_1$. The coupling coefficient is determined not only by the junctional resistance ($r_j$), but also by the nonjunctional membrane resistance ($r_m$) of cell 2, and is given by

$$CC = \frac{V_2}{V_1} = \frac{r_m}{r_m + r_j}$$

(Bennett, 1977).

As the ratio $r_j/r_m \to 0$, CC $\to 1$. In an extreme case, the two cells act as a single, isopotential cell. As the ratio $r_j/r_m \to \infty$, CC
\[ \rightarrow 0, \text{i.e. as the junctional resistance between cells increases,} \]
coupling decreases.

Changes in the coupling coefficient may reflect changes in the junctional resistance, but care must be taken to eliminate possible errors arising from changes in the nonjunctional membrane resistance. The coupling coefficient is not a direct or linear measure of junctional conductance (Socolar, 1977). Coupling coefficients have been applied principally to simple cell systems, and are not applicable to more complex systems such as smooth muscle.

Recently, direct measures of junctional conductance were made in several cell types, utilizing dual voltage-clamp instrumentation (Spray et al., 1979; 1981a and b; Harris et al., 1981). This is a powerful method of analysing coupling, for the results are direct and unambiguous. One can only hope that such technology will be applicable to a wide variety of tissue types in the future.

Measurements of coupling by passage of dye molecules or radioisotopes rely upon the same principles as flow of ions between cells, but are not as amenable to quantitative analysis. With proper controls, they provide valuable qualitative information regarding the nature and extent of cell-to-cell coupling. For example, the use of a series of fluorescent probes has given some insight into the size of the channels. Molecules of less than 1000-2000 MW, having dimensions less than approximately 1.5-2.0 nm, can permeate the channels (Loewenstein, 1981). Most complete evidence for junctional cell-to-cell communication comes from studies that examine all three types of coupling: electrical, metabolic and dye transfer (e.g. Gilula et al., 1972). When combined
with electron microscopy, such studies can help to resolve the question of what is the morphological basis of junctional cell-to-cell communication. The picture that emerges from electrical and dye tracer experiments is that cells are linked by membrane channels that provide structural continuity between cytoplasmic compartments.

**The Morphological Basis of Junctional Cell-to-Cell Communication.**

It is desirable to combine morphological, biochemical and physiological techniques in order to identify the membrane differentiation responsible for the communication between cells. While it is true that no single experimental finding is in itself conclusive, the cumulative evidence strongly suggests such a role for the gap junction, or nexus. A comprehensive picture of the communicating unit is emerging. Each membrane particle, as seen in freeze fracture electron microscopy, is thought to constitute one half of a membrane channel. Each particle has been termed a connexon, and bonding of the connexons in symmetric pairs forms the channel unit (Makowski et al., 1977; Bennett and Goodenough, 1978).

Various chemical and physical methods have been used to study isolated gap junctions. X-ray diffraction, electron-optical and chemical analyses have revealed that the connexon units of liver gap junctions are composed of six protein subunits. Each pair of connexons constitutes a membrane channel and appears to have a hydrophilic core (Makowski et al., 1977). Three dimensional Fourier synthesis of electron microscopic views of isolated gap junctions confirmed the hexameric structure of the gap junction particles (Unwin and Zampighi, 1980). The results also suggested that the subunits are roughly rod
shaped and have a slight tilt with respect to the axis of the channel. This last study fell short of demonstrating continuity of the water core through the connexon pair. However, an impressive amount of evidence is accumulating to support the notion that the gap junction unit is a paired structure capable of linking cell interiors by way of a central hydrophilic core which spans the distance of two cell membranes. As such, the gap junction is a credible cell-to-cell channel.

A number of observations relating to the distribution of the gap junction preceded the physico-chemical analysis of the junction. The major evidence implicating gap junctions in coupling is inductive, and is outlined below.

i) Gap junctions are the only discernable junctional specialization present in some communicating cell types (Gilula et al., 1972; Johnson et al., 1974; Bennett, 1977; Sheridan et al., 1978).

ii) The size of gap junctions increases progressively as electrical coupling improves progressively (Johnson et al., 1974).

iii) Gap junctions are absent in some non-communicating cell lines. Fusion of such communication incompetent cells with communicating cells yields communication competent hybrids. The acquisition, and eventual loss, of coupling, goes hand in hand with the presence then loss of gap junctions (Loewenstein, 1979; 1981).

iv) Experimental treatments that modulate gap junction frequency result in corresponding changes in the degree of coupling. Thus, increased gap junction contact between cells is associated with improved coupling (e.g. Flagg-Newton et al., 1981) and decreased gap junction contact is associated with increased internal resistance (i.e.
decreased coupling, e.g. Meyer et al., 1981).

Thus, although circumstantial, the cumulative evidence for the gap junction as the morphological correlate of the junctional cell-to-cell channel is good. No comparable case has been made for any other junctional structure. This does not mean, however, that the gap junction is the sole means of communication between cells (see below). Because of the uncertainty of this matter, it is wise to keep the morphological and physiological terminology separate.

If one accepts the premise that gap junctions are sites of low resistance coupling between cells, the physiological and morphological results can be combined to derive some junctional characteristics. Estimates of specific junctional resistance are typically several orders of magnitude lower than the specific resistance of the nonjunctional membrane. Sheridan et al. (1978) performed both the physiological and morphological studies on the same cell culture system and calculated a value of 0.01 Ω cm² for gap junctional membrane resistance. This is lower than previous estimates, such as those by Spira (1974), but conforms with the idea that the gap junction is composed of channels, approximately 2 nm in diameter and 15-20 nm long, having core resistivity comparable with that of cytoplasm.

Are Gap Junctions Necessary for Cell-to-Cell Coupling? Results of some electron microscope studies of several smooth muscle types, including myometrium, indicated that ultrastructurally detectable gap junctions could not be demonstrated between muscle cells (Garfield and Daniel, 1974; Daniel et al., 1976; Gabella, 1976; Paton et al., 1976). However, physiological evidence supported the notion that electrical
coupling between cells did exist (e.g. Abe and Tomita, 1968; Daniel and Lodge, 1975; Kuriyama and Suzuki, 1976). The failure to find gap junctions in coupled systems lead to the proposal that gap junctions may not be necessary for coupling in smooth muscle (Daniel et al., 1976). A similar suggestion regarding coupling between cardiac cells has also been made (Sperelakis, 1979; Williams and DeHaan, 1981) and the possibility that some other structure may subserve electrical coupling has been acknowledged by Loewenstein (1981).

One tractable approach to the question of whether gap junctions are necessary for cell-to-cell coupling is to determine whether gap junctions exist in certain coupled systems. Careful morphological studies, ideally using freeze fracture EM, are required to resolve the problem. Negative results are inconclusive, but do shift some of the burden of proof to others who might claim that gap junctions exist.

Part of the problem of resolving this issue, I believe, is due to lack of precision of the terminology. The term gap junction refers to a certain morphological structure, an aggregate of membrane particles when viewed with freeze fracture EM. But the individual components of the structure, connexons, are recognized as being the functional units of the junction. A single particle may represent a functional communicating link between cells. But does a single connexon warrant the term gap junction when it is not, by definition, an aggregate? Also, what is the minimum number of particles that constitute an aggregate, and hence a gap junction? Clearly, these are concepts that must be considered when setting criteria for the acceptance of certain morphological structures. If the term gap junction is to encompass
small aggregates, or even single particles, it is important to include
the requirement that the putative junctions exist in regions where
adjacent cells come into close proximity. It must be stressed that not
all membrane proteins seen by freeze fracture EM are necessarily
connexons. Also, an aggregate of membrane particles may not necessarily
have corresponding partners on an adjacent cell. Therefore,
demonstration of membrane particles is equivocal evidence for the
presence of connexons.

Only when explicit nomenclature is formulated, used, and widely
accepted can the underlying problem, that of identifying the structural
basis of cell-to-cell coupling, be resolved. Several proposals have
been made to explain coupling in the absence of ultrastructurally
detectable gap junctions. Alternative structures, such as cellular
interdigitations, may serve as low resistance pathways between cells
(Daniel et al., 1976). In addition, isolated, but functional gap
junction channels may exist scattered throughout the area of closely
apposed cell membrane (Williams and DeHaan, 1981).

Control of Coupling. The extent of junctional cell-to-cell
communication can be regulated in several general ways. Modulation of
size, number or distribution of junctions is one control step. Control
of junctional permeability of preexisting junctions is the other.
Examples of both methods of regulating coupling exist (Hertzberg et al.,

Changes in the size and number of gap junctions have been
semiquantitatively related to coupling properties. Improved coupling
was associated with increased area of gap junction contact between cells
(Johnson et al., 1974; Albertini and Anderson, 1975; Flagg-Newton et al., 1981). In the most recent case, cyclic AMP was implicated in stimulating improved coupling. With the knowledge that gap junctions represent aggregates of channels connecting two cells, the increase in gap junction size or number can be viewed as the placement of additional low resistance pathways in parallel with the preexisting channels. This could occur either by insertion of new channel units in the membrane or aggregation of preexisting membrane proteins. Such an increase in the number of junctional pathways would cause a decrease in junctional resistance, which would facilitate the flow of current between cells. Evidence to support the notion of a step-by-step increase in junctional pathways has come from high resolution studies by Loewenstein and coworkers of the quantal steps of junctional conductance (Loewenstein, 1981). The step-like nature of junctional conductance was interpreted as representing the insertion of functional channels between cells.

The loss of communication between cells is also subject to control. Excision of portions of liver tissue causes the frequency of gap junctions in the remaining tissue to decrease. This structural change is accompanied by increased junctional resistance (Meyer et al., 1981). The modulation of gap junctions in this and the previous examples may be related to growth regulation or functional activity of the tissue.

The second major method of regulating coupling is the modulation of existing channels. Most evidence that exists concerns mechanisms of channel closure, resulting in uncoupling of cells. However, one can imagine a situation where a tonic uncoupling influence could be removed,
resulting in improved communication.

Several mechanisms for control of junctional permeability have been described. Increased intracellular levels of free Ca\(^{++}\) cause a rapid and reversible decrease of junctional conductance (Rose and Loewenstein, 1976; see also Loewenstein, 1981). The uncoupling effects of Ca\(^{++}\) in Chironomus cells do not appear to involve changes in intracellular pH (Rose and Rick, 1978). But an abundance of evidence in several other systems indicates that decreased intracellular pH decreases junctional conductance, independent of any changes in Ca\(^{++}\) levels (Iwatsuki and Peterson, 1979; Spray et al., 1981b). Species differences may account for the discrepancy, but methodological deficiencies may disguise any common regulatory role of Ca\(^{++}\) and H\(^{+}\).

Another mechanism for control of coupling is found in amphibian embryos. Junctional conductance is highly sensitive to transjunctional voltage (Spray et al., 1979; 1981a; Harris et al., 1981). This form of junctional regulation is a novel application of voltage dependent mechanisms, a principle so fundamental to the operation of excitable membranes. The general significance of this junctional control mechanism is not yet known. Several other treatments cause junctional conductance to decrease. Secretagogues such as acetylcholine cause uncoupling of pancreatic cells, but Ca\(^{++}\) may act as a mediator in this case (Iwatsuki and Peterson, 1979). Treatment of tissues with aldehydes, of the sort used for electron microscopy, closes gap junctions as well (Bennett, 1977; Johnson and Ramon, 1981).

The question arises of how Ca\(^{++}\) or H\(^{+}\) might regulate junctional permeability. Loewenstein (1981) speculates that Ca\(^{++}\) could either
directly occlude the channel or cause a change in channel conformation and indirectly result in blockage. Ca\textsuperscript{++} or H\textsuperscript{+} could cause realignment of channel protein subunits, and thereby regulate permeability (Unwin and Zampighi, 1980). Harris et al. (1981) present some kinetic analysis of channel closure which they interpret as being consistent with a gating mechanism in the junctional channel. Electrical coupling of internally perfused neurones is extremely insensitive to direct elevation of Ca\textsuperscript{++} or H\textsuperscript{+} in the perfusion fluids (Johnson and Ramon, 1981). One interpretation of these observations is that the uncoupling agents act through a mobile intermediate compound, one that is absent in the perfusate. Calmodulin is a possible candidate for such a role.

Some attention has focused on the question of whether a detectable change in gap junction structure accompanies the transition from the conducting to the nonconducting state. Perrachia (1980) has provided evidence that the gap junction particle packing, as seen in freeze fracture EM, is regulated by the Ca\textsuperscript{++} and H\textsuperscript{+} levels. Shifts from a disordered, to an ordered hexagonal array, occur in response to uncoupling stimuli, and have been suggested to reflect the process of channel closure. The validity of this hypothesis has been questioned, in the light of morphological evidence obtained by rapid freezing of tissues with gap junctions. Such procedures are thought to preserve the native state, thus avoiding artefactual effects of chemical fixation. Uncoupling of cells precedes any detectable change in gap junction structure (Hanna et al., 1981) and considerable heterogeneity in the packing array of connexons exists in healthy tissues (Raviola et al., 1980).
Physiological Roles of Cell-to-Cell Communication. Most cell types in organized tissues are capable of communication by way of gap junctions. Cells that do not exhibit gap junction interaction are more the exception in both developing and mature organisms (Perrachia, 1980; Herzberg et al., 1981; Loewenstein, 1981). In view of the wide distribution of gap junctional communication between cells, it is not surprising that many roles for such communication have been suggested.

The most basic physiological role of the cell-to-cell channel is probably homeostatic. Channel permeant molecules could diffuse within a tissue, buffering variations between cells. In this way channels may be involved in the distribution of nutrients or coordination of cellular functions. Metabolic cooperation is a well documented phenomenon in which compounds diffuse between cells. Mutant cells, which are deficient of an enzyme required for the synthesis of a nucleotide, can survive in certain culture media only when they are cocultured and contact normal cells (Hooper and Subak-Sharpe, 1981). The missing nucleotide is distributed amongst the population of cells by way of cell-to-cell junctions.

Much evidence has implicated gap junctions in the transmission of regulatory signals. The transmission of electrical signals between excitable cells, such as neurons, cardiac and smooth muscle cells, is an obvious example of the synchronizing function of junctional cell-to-cell communication (Furshpan and Potter, 1959; Tomita, 1975; Bennett, 1977; Fozzard, 1979). Other forms of signalling could include passage of regulatory molecules between cells. Lawrence et al. (1978) demonstrated that a communicated mediator, probably cyclic AMP, diffused between
cultured cells and regulated the contractile and metabolic activity of the recipient cells. The role of junctional communication in the dissemination of developmental or growth regulating signals is appealing, but, as yet, largely speculative (Loewenstein, 1979; 1981).
CHAPTER 2

Formation of Gap Junctions in Rat Myometrium
INTRODUCTION

Uterine smooth muscle, like most smooth muscle types, is a functional, though not anatomical syncytium (Abe, 1970; Tomita, 1970). It consists of bundles of small smooth muscle cells in series and in parallel, each cell from 300-600 μm long and 5-10 μm wide at term (Csapo, 1962; Finn and Porter, 1975). Uterine electrical activity is initiated at mobile, but discrete pacemaker regions (Marshall, 1959; Lodge and Sproat, 1981) and spreads from one part of the tissue to the next in much the same way as within a single cell. It is apparent, therefore, that electrical interaction between cells in vivo is necessary for coordinated activity of the myometrium. The electrical interaction between cells in smooth muscle tissues is usually taken to imply coupling of cells by low resistance pathways of some sort and spread of local circuit currents (Barr et al., 1968).

Studies of the electrical properties of myometrium in vitro indicate that impulses propagate through the muscle and that the tissue exhibits cable-like properties (Abe, 1970; Daniel and Lodge, 1973; Kuriyama and Suzuki, 1976; Chapter 4). The structural basis of such cell-to-cell communication in myometrium, and indeed, in several other smooth muscle types, is a matter of some dispute (Daniel et al., 1976). Gap junctions between smooth muscle cells have generally been considered to provide the basis for low resistance electrical coupling within the tissue (Dewey and Barr, 1962; Barr et al., 1968; but see Chapter 1). However, no gap junctions were identified between rat uterine smooth
muscle cells (Garfield and Daniel, 1974; Daniel et al., 1976), leading to the question, what is the structural basis of cell-to-cell communication in myometrium?

Following extensive investigations into the morphological basis of coupling in smooth muscle by Daniel and Garfield, observations revealed that gap junctions were present in rat myometrium only late in term or during the process of delivery.

There were two objectives of this ultrastructural study of rat myometrium. The first was to characterize the time course of appearance and disappearance of gap junctions at the end of pregnancy. The second objective was to investigate some of the factors that govern the appearance of gap junctions in vivo. This study was done in collaboration with R.E. Garfield, and the results have been published elsewhere (Garfield et al., 1977 and 1978).

METHODS AND MATERIALS

Electron Microscopy

Uterine tissues were obtained from Wistar rats at various stages of the reproductive cycle. These included pregnant rats (day 14 and days 20–22 of gestation), delivering animals (days 22–23) and post partum (1–27 h and 16 days after completion of delivery). Day 1 was the day sperm was identified in a vaginal smear. Some animals at term received oxytocin (0.1 to 3 units of Syntocinon in a subcutaneous or intraperitoneal injection) in an effort to initiate labour. To prepare uterine tissues for electron microscopy (EM) animals were anesthetized with ether vapour and the abdominal cavity was filled with fixative
solution, consisting of 2% glutaraldehyde in 0.075 M cacodylate buffer (pH 7.4) containing 4.5% sucrose and 1 mM CaCl₂. After 20-30 min fixation, tissues were removed from the mid portions of the uterine horns and placed in fresh fixative for an additional time period of 2 h to overnight at 4°C. Following fixation tissues were washed in 0.1 M cacodylate buffer containing 4.5% sucrose and 1 mM CaCl₂ for 60 min before being postfixed for 90 min in 2% osmium tetroxide in 0.05 M cacodylate buffer. Tissues were stained en bloc for 30 min with saturated, aqueous, uranyl acetate, then dehydrated in graded alcohols and embedded in Spurr resin.

Thin sections of the longitudinal and circular muscle layers were cut with glass knives on a Porter Blum MT-2B microtome and mounted on 200 mesh grids. In all cases myometrium was examined in transverse orientation. Sections were stained for 30 sec with lead citrate and examined in a Philips 300 or 301 electron microscope.

Quantification of Gap Junctions in Myometrium

Eight to twelve nonoverlapping photographs of smooth muscle cells from one grid square of tissue were taken at 11,000 X magnification. Prints were made at an enlargement of 3 X (final magnification of 33,000 X) and structures thought to be gap junctions were further enlarged to 110,000 X for identification and length determination. The criteria used to identify gap junctions were as follows: A) Each junction must be composed of 5 or 7 lines at some point along its length. If seven lines were evident, the gap between the two unit membranes must be about 2 nm wide. B). The total thickness of the junction must be about 15 to 20 nm. [If the thickness
of the unit membrane (7-8 nm) was used as an index, these criteria could realistically be applied. The amount of nonjunctional membrane in the photographs was quantitated by measuring the linear distance around the perimeter of cells with a map tracer. The final values that were obtained were the number and size of gap junctions and the length of nonjunctional membrane. From these values the number of gap junctions per 1000 um of membrane was obtained, a value which was used for comparison between various groups of tissues.

An improved method of quantitating frequency of gap junctions was used in some later EM studies, described in Chapter 4, 20-24 nonoverlapping photographs of smooth muscle were taken at 4,200 X magnification and enlarged 8 times to a final print magnification of 33,600 X. A transparent test grid was laid over each photograph and the number of intersections of the grid with the plasma membrane was used to calculate the length of nonjunctional membrane (Garfield et al., 1980a), according to the following formula.

\[ L_n = \frac{\pi}{2} \times \left( \frac{I}{L_p} \right) \times A \times CF \]

where \( L_n \) is the length of non gap junctional membrane; \( I \) is the average number of intersections determined by two estimates taken at right angles to each other; \( L_p \) is the total length of probe lines on the grid; \( A \) is area of the photograph and \( CF \) is a correction factor for magnification. Gap junctions were identified using the same criteria described above. This method represented an improvement over the earlier technique because the measurements were less tedious and a larger amount of tissue was examined in each series, improving the sensitivity of the measurement.
Ovariectomy

In order to ensure that animals became pregnant in one horn only, some nonpregnant animals were unilaterally ovariectomized, either the left or right ovary being removed. The procedure involved anesthetizing the rats with ether, making a midline incision through the dorsal skin, pulling the skin over to one side, and performing blunt dissection through the abdominal wall over the ovary. One ovary was externalized, a single suture was placed around the tubal end of the uterus, the ovary excised, and the skin sewn closed. Animals were allowed to recover for several weeks before being bred to adult males.

Other rats at 16–17 days of gestation were bilaterally ovariectomized, using the same procedure as described above, only removing both left and right ovaries. Some animals were sham operated, in which case the procedure progressed to the stage where the ovaries were externalized, then the animals were sewn up intact. Bilaterally ovariectomized rats were randomly divided into two groups. One group received subcutaneous injections daily of 4 mg progesterone and 100 µg estrone in 0.1 ml sesame oil. The second group received the vehicle only. 72–80 h after surgery the untreated, ovariectomized (OVX) animals showed signs of vaginal bleeding or abortion. At this time uterine tissues from all 3 categories of animals (sham, OVX and OVX plus progesterone and estrogen) were fixed in situ for EM. In addition, animals were examined to determine whether complete excision of the ovaries had been accomplished.

Statistical Comparisons

All results are expressed as the mean ± SD. Independent
Student's t tests were used for most comparisons between groups of data. The Mann-Whitney U test was used for comparison of groups exhibiting markedly non-normal distribution (e.g. results of bilateral ovariectomy, below). A significance level of 5% was used in the interpretation of two-tailed tests.

RESULTS

General Ultrastructural Features of Myometrium

The uterine wall is comprised of an outer longitudinal muscle layer and an inner circular muscle layer, both enclosing the endometrium (Finn and Porter, 1975; Mossman, 1977). A low magnification micrograph showing the whole uterine wall fixed in situ, in a 20 day pregnant rat, illustrates the relationship of the different layers (Figure 2). A medium power micrograph illustrates some of the ultrastructural details of myometrium (Figure 3). The smooth muscle cells (in this case from a midterm pregnant animal) are arranged into bundles, surrounded by fibroblasts and collagen fibers. A portion of a blood vessel is seen in the upper part of the micrograph, with a nerve varicosity enclosed in a Schwann cell alongside. As shown in Figure 3, the infrequent nerves that I detected were closely associated with blood vessels.

The Presence of Gap Junctions in Myometrium from Pregnant and Post Partum Animals

No gap junctions were observed between smooth muscle cells in either the longitudinal or circular muscle layers of rat myometria fixed on day 14 of gestation (Figure 3; Table I). Gap junctions were
FIGURE 2. Low power electron micrograph of uterine wall from a 20 day pregnant rat, fixed in situ. The uterine lumen is to the top of the field, and is bordered by a thin layer of endometrial cells (ENDO) and some connective tissue. The inner circular muscle layer (CIRC) is shown in longitudinal section and the outer longitudinal muscle layer (LONG) is shown in transverse orientation. The total wall thickness in this late term uterus is approximately 65 μm. Bar, 10 μm. X 6,600.
FIGURE 3. An electron micrograph of myometrium from a rat fixed in situ at day 14 of gestation. A bundle of smooth muscle cells is bordered by fibroblasts (F) and collagen (COLLAGEN). Several nuclei of smooth muscle cells (N) are apparent and mitochondria (M) are visible in the muscle cells. A portion of a nonmuscular blood vessel (BV) (possibly a capillary) is present at the top of the field and a small nerve (NERVE) enclosed in a Schwann cell is situated to its left. Bar, 5 μm. X 18,000.
identified in some of the tissues from animals before term, but not delivering (20-22 days gestation), at a frequency of 4 gap junctions /1000 μm of membrane (Table I). In other tissues from animals at the same late stage of pregnancy, no gap junctions were identified between smooth muscle cells (Table I). Treatment of rats late in gestation with oxytocin (0.1-3 Units, 1-2 h before fixation) did not induce all animals to deliver or produce formation of gap junctions in all tissues.

Gap junctions were observed in both the circular and longitudinal muscle layers of all animals fixed during parturition, when 1-12 fetuses had been delivered (Table I, data from the two muscle layers are lumped together in the results). Gap junctions from delivering myometrium are illustrated in Figure 4. The frequency of gap junctions in delivering myometrium was 6.7 gap junctions /1000 μm membrane (Table I).

The frequency of gap junctions was the same in samples of muscle from both distended and evacuated portions of the uterus from the same animal. Five quantitative series were obtained each from stretched portions and contracted portions of the myometrium of two delivering rats. The frequency of gap junctions in the stretched muscle was $7.7 \pm 2.3$ gap junctions/1000 μm of membrane, compared to $5.7 \pm 3.6$ gap junctions/ 1000 μm of membrane in the contracted muscle. These values were not significantly different ($p > .05$).

Tissues from animals fixed 1-8 h following the completion of delivery had 5.3 gap junctions/ 1000 μm, a frequency comparable to that observed at delivery. The number of contacts was significantly smaller by 19-24 h post partum (0.6 gap junctions / 1000 μm of membrane) and by
TABLE I

GAP JUNCTIONS IN PREGNANT AND POST PARTUM MYOMETRIUM

<table>
<thead>
<tr>
<th></th>
<th>Length of Membrane</th>
<th>Mean No. GJ's/1000</th>
<th>Fractional Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. GJ's</td>
<td>Surveyed (µm)</td>
<td>µm membrane ± SD</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 day</td>
<td>7</td>
<td>0</td>
<td>4,770</td>
</tr>
<tr>
<td>20-22 days</td>
<td>23</td>
<td>62</td>
<td>14,019</td>
</tr>
<tr>
<td>with GJ's</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No GJ's</td>
<td>11</td>
<td>0</td>
<td>6,897</td>
</tr>
<tr>
<td>22-23 days</td>
<td>14</td>
<td>50</td>
<td>7,955</td>
</tr>
<tr>
<td>Delivering</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Partum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-8 h</td>
<td>6</td>
<td>14</td>
<td>3,334</td>
</tr>
<tr>
<td>19-27 h</td>
<td>11</td>
<td>4</td>
<td>6,541</td>
</tr>
<tr>
<td>16 days</td>
<td>6</td>
<td>0</td>
<td>4,739</td>
</tr>
</tbody>
</table>

TABLE I. Number of gap junctions (GJ's) in myometrium at different stages of gestation. n is the number of uterine horns examined, which is approximately twice the number of animals in each group. Data from longitudinal and circular muscle layers are combined. The number of GJ's indicates the total number of 5 or 7 lined junctions found in 8-12 electron micrographs of each tissue. Length of membrane surveyed refers to the total length of nonjunctural membrane measured in the series of micrographs. P indicates the level of confidence between the GJ frequencies of different groups. Fractional area is the amount of cell membrane occupied by GJ's.
FIGURE 4. Electron micrographs of myometrium from delivering rats shows the presence of gap junctions between the smooth muscle cells. A). Several features of myometrium are illustrated, including part of a smooth muscle cell nucleus (N), a mitochondrion (M) and membrane caveolae (CAV). Interdigitation of cell processes is apparent in the left portion of the micrograph and a gap junction (ARROW) joins two cells. The washed out area to the upper right of the gap junction is probably an artefact of tissue processing, where glycogen was leached out of the cell during staining with uranyl acetate. Bar, 0.5 μm. X 55,000. B). A high power micrograph illustrates the seven layered appearance of a gap junction between two muscle cells. The gap region is shown especially well between the two arrows, where the total junctional width is approximately 20 nm. Bar, 100 nm. X 117,000.
16 days post partum no gap junctions were observed (Table I).

The average measured length of gap junctions from animals before term was 0.15 ± 0.11 (SD) μm. The mean junctional length at delivery was 0.20 ± 0.10 μm and 0.25 ± 0.13 μm in tissues from animals 1-8 h post partum. The fractional area of cell surface membrane occupied by gap junctions was determined by doubling the total length of all gap junctions (since each junction joins two cells) and dividing by the total length of nonjunctional membrane. The fractional area (Table I) was largest in muscle from delivering animals, where gap junctions occupied 0.25 % of the cell surface.

Ovariectomy

Gap junctions were present at parturition in both the gravid and nongravid horns of animals that were unilaterally ovariectomized prior to conception (Figure 5a). There were, however, significantly fewer gap junctions observed in the nongravid horn (3.7 gap junctions /1000 μm membrane) compared to the gravid horn (8.2 gap junctions /1000 μm membrane, Table II). As observed in intact pregnant animals, no gap junctions were evident before term.

72-80 h following bilateral ovariectomy of 16-17 day pregnant rats, premature delivery or vaginal bleeding had commenced. Gap junctions were present in the myometrium at this time (3.5 gap junctions /1000 μm membrane, Table II, Figure 5b). Treatment of bilaterally ovariectomized animals with exogenous hormones (4 mg progesterone, 100 μg estrone per day) prevented premature delivery and reduced significantly the appearance of gap junctions (only one was found in all the tissues, frequency of 0.12 gap junctions /1000 μm. Table II). The
TABLE II

EFFECTS OF OVARIECTOMY ON FORMATION
OF GAP JUNCTIONS IN MYOMETRIUM

<table>
<thead>
<tr>
<th></th>
<th>Length of Membrane</th>
<th>No. GJ's</th>
<th>No. GJ's /1000 μm</th>
<th>Membrane</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>GJ's</td>
<td>(μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNILATERAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravid</td>
<td>4</td>
<td>0</td>
<td>3,458</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Nongravid</td>
<td>2</td>
<td>0</td>
<td>1,549</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Delivering</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravid</td>
<td>17</td>
<td>79</td>
<td>10,503</td>
<td>7.6 ± 3.3</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>Nongravid</td>
<td>13</td>
<td>32</td>
<td>9,024</td>
<td>3.4 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>BILATERAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>6</td>
<td>0</td>
<td>5,265</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>16-17 days gestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operated</td>
<td>2</td>
<td>0</td>
<td>1,303</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>No Treatment</td>
<td>12</td>
<td>24</td>
<td>6,943</td>
<td>3.5 ± 3.3</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>Progesterone (4 mg) and Estrone (100 μg) daily</td>
<td>12</td>
<td>1</td>
<td>9,029</td>
<td>0.12 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE II.** Data as described in Table I. Some animals were unilaterally ovariectomized prior to mating. Tissues were obtained from 2 such animals before term and from 4 animals during spontaneous delivery. Bilateral ovariectomy was performed on 3 nonpregnant rats and others at 16-17 days of gestation, including 2 sham operated control animals, 4 rats which received injections of progesterone and estrone and 5 rats which received vehicle only. Tissues for gap junction analysis were obtained 72-80 h following surgery, at which time vaginal bleeding or premature delivery of fetuses had commenced in the untreated experimental group of animals. P values indicate confidence levels.
FIGURE 5. Electron micrographs of gap junctions in the myometrium of animals subjected to ovariectomy. A]. Gap junctions (ARROWS) between several myometrial cells in the nongravid horn of a spontaneously delivering animal. A small annular gap junction is indicated by the asterisk. Bar, 0.5 μm. X 53,000. B]. Gap junctions are present between muscle cells in the myometrium of rats bilaterally ovariectomized at day 16 of gestation. Tissue was fixed 72 h after the operation; the animal received only oil vehicle as treatment. Bar, 0.5 μm. X 53,000. In the inset the 7 lined structure of a gap junction is apparent. Bar, 100 nm. X 270,000. As before, N labels the nuclei and M the mitochondria.
distribution of gap junction frequencies was markedly non-normal (since many "zeros" occurred), so the Mann-Whitney U test was used to evaluate effectiveness of the hormone treatment on control of gap junction formation. Nonparametric tests, such as the Mann-Whitney U test, are less affected than parametric tests by failure of the data to fit a normal distribution. Two sham operated control animals showed no signs of premature delivery when tissues were fixed 72-80 h after surgery and no gap junctions were observed in the myometrium of these animals. No gap junctions were present in the myometrium of nonpregnant animals ovariectomized 24-72 h prior to fixation (Table II).

DISCUSSION

Presence of Gap Junctions in the Myometrium

Gap junctions were shown to be present between uterine smooth muscle cells in some tissues from animals at late term, not delivering, but not in other animals. In all tissues taken from animals in which delivery had commenced, gap junctions were observed. Uterine tissues fixed 1-8 h post partum had about the same number of gap junctions as observed in parturient tissues, but the number of gap junctions was greatly reduced by 27-36 h post partum and none were apparent 16 days post partum. This temporal distribution of visible gap junctions suggests a sequential formation of gap junctions prior to parturition, then disappearance after the completion of delivery. This proposed sequence could explain the inconsistent appearance of gap junctions before term and their inevitable presence during labour. Uncertainties in the timing of conception or variability in the duration of pregnancy
would result in variations in the timing of parturition. Only those animals close to the onset of delivery would have gap junctions.

The presence of gap junctions between smooth muscle cells of the uterus has important implications for the maintenance and termination of pregnancy. Throughout gestation the myometrium exhibits only localized, asynchronous electrical and mechanical activity. The absence of gap junctions during the course of pregnancy may be a major factor in restricting the spread of electrical impulses through the tissue. At the end of term, the uterus undergoes a transition and electrical and mechanical activity throughout becomes synchronized (Kao, 1959; Csapo and Takeda, 1965; Fuchs, 1969; 1973; Marshall, 1973; Wolfs and van Leeuwen, 1979; Anderson et al., 1981). Large amplitude electrical spike activity measured in parturient muscle indicated the synchronous activation of muscle fibers (Kao, 1959; Marshall and Csapo, 1961). The appearance of gap junctions between neighbouring smooth muscle cells immediately prior to parturition, and their presence during labour, may promote impulse propagation within the muscle. The improved coupling between different regions may favour the evolution of synchronous activity throughout the uterine musculature, resulting in the coordinated mechanical activity and expulsion of the uterine contents.

In the following chapters I examine some passive electrical properties of myometrium at the time of parturition in order to evaluate the functional significance of gap junction formation in the tissue. Two different methods of measuring cell-to-cell coupling in smooth muscle are used to examine the proposal described above, i.e., that the presence of large numbers of gap junctions in parturient muscle is
associated with improved conduction of electrical signals.

The relative amount of membrane occupied by gap junctions at parturition (0.25%, Table I) is comparable to the value described for some intestinal smooth muscles (Gabella and Blundell, 1979; 1981) and for cultured cardiac cells (Williams and DeHaan, 1981), but is more than twice that found for tracheal smooth muscle (Kannan and Daniel, 1978). The fractional area of gap junctions in these muscle types is considerably smaller than that observed in some other tissues, such as liver (1.5%) intact cardiac cells, (1.5%) and lens fibers of the eye (80%) (Peracchia, 1980).

Mechanism for Control of Gap Junction Formation in Myometrium

Several studies were performed to investigate the nature of control over gap junction formation in vivo. The presence of gap junctions during delivery in both the gravid and nongravid horns of unilaterally pregnant animals provides a clue to the control of junction formation. The stimulus apparently is systemic in nature, being common to both horns. Gap junction formation does not depend exclusively upon the presence of the fetoplacental unit or stretch of the uterine wall. However, these factors could play some role in regulating gap junction formation, because significantly fewer gap junctions were observed in the nongravid horns of delivering animals (Table II).

These results are consistent with the physiological data describing parturition in unilaterally pregnant rats. Nongravid, as well as gravid horns show a change from asynchronous activity during gestation to intense activity at term (Kao, 1959; Fuchs, 1973).
However, the sterile horn does not attain the same, highly coordinated state. Both uterine volume and circulating estrogens are thought to be more important than the fetoplacental unit in the evolution of circular muscle activity (Kawarabayashi and Marshall, 1981). In view of these results, the smaller number of gap junctions in the nongravid horn might be due to the lack of distention, and may explain the physiological differences observed by Kao (1959) and Fuchs (1973). Thus, while stretch and/or the fetuses may be contributing factors to activation of the myometrium and regulation of gap junction formation, the primary stimulus is systemic in nature.

Some of the evidence implicating hormonal changes in control of parturition were described in Chapter 1. The objective of surgical manipulation of the pregnant animals was to duplicate some of the hormonal changes that are normally seen at term. Bilateral ovariectomy (OVX) causes rats to be deprived of their primary source of the sex hormones (the corpora lutea of the ovaries) and, as such, to be subjected to the "withdrawal" of circulating progesterone, as well as estrogen (Csapo and Weist, 1969; Pepe and Rothchild, 1973). The physiological repercussions of the regulatory imbalance induced by OVX is the premature termination of pregnancy. Ovariectomy early in gestation results in the fetuses being reabsorbed in utero, while after approximately day 16 abortion occurs. OVX-induced termination of pregnancy is accompanied by the evolution of synchronous electrical and mechanical activity in the uterus (Fuchs, 1973; Talo and Karki, 1977). Estrogen is required for the transition in uterine activity to fully resemble that which occurs at normal parturition (Kawarabayashi and
Marshall, 1981). Treatment of bilaterally OVX rats with exogenous progesterone and estrogen is sufficient to maintain circulating hormone levels and prolong pregnancy (Csapo and Weist, 1969; Pepe and Rothchild, 1973; Mantalenakis, 1976; Garfield et al., 1982).

In the experiments reported here bilateral OVX induced premature delivery (abortion), vaginal bleeding and reabsorption of the fetuses by 72–80 h following surgery. Such manipulation was also an appropriate stimulus for the formation of gap junctions in myometrium (Table II). The appearance of gap junctions in midterm pregnant myometrium was associated with changes similar to those observed in normal labour. The surgical procedure alone was not the cause of junction formation, as indicated by the absence of gap junctions in the sham operated controls.

Administration of exogenous hormones, in this case both progesterone and estrogen, resulted in the maintenance of pregnancy and significantly blocked the development of gap junctions in the myometrium. These findings suggest that progesterone withdrawal may be involved in regulation of gap junction formation in vivo.

These results are consistent with the results of the unilateral ovariectomy, which showed that the control of gap junction formation was systemic in nature. The hormones progesterone and/or estrogen may represent the circulating factors common to both the gravid and nongravid uterine horns that regulate, directly or indirectly, the appearance of gap junctions. The absence of gap junctions prior to parturition and their presence during a time when progesterone levels decline may be the structural and functional basis for the progesterone block hypothesis (Csapo, 1962; 1977; Csapo and Weist, 1969).
Recently, a more thorough investigation into the regulatory imbalance accompanying bilateral OVX of pregnant rats was completed (Garfield et al., 1982b). The results confirm and extend the findings reported here. Ovariectomy of pregnant rats at 16 days of gestation was shown to result in a precipitous decline of plasma and uterine tissue levels of estrogen and progesterone but increased levels of prostaglandin $F_{2\alpha}$. 48 h following OVX, during premature delivery, large numbers of gap junctions were shown to be present in the myometrium of animals treated with estradiol 17B, while administration of progesterone and estradiol together inhibited premature labour and gap junction formation. The frequency of gap junctions was greater in tissues from estrogen treated rats fixed 48 h following surgery than in the samples I studied (72-80 h post surgery, no estrogen). Thus, while estrogen increases the likelihood of abortion (Csapo and Weist, 1969; Pepe and Rothchild, 1973), it may also stimulate gap junction formation. Administration of progesterone decidedly blocks the formation of gap junctions in midterm myometrium.

Several general themes emerge from experiments involving changes of hormone levels in vivo in the rat. Whenever progesterone levels decline, at normal labour and in ovariectomy induced premature termination of pregnancy, the uterus is activated and gap junctions appear in the myometrium. Administration of progesterone in both cases inhibits the contractile activity (Fuchs, 1973; Kishikawa, 1981) and significantly lowers the number of gap junctions in the myometrium (Table II, and Garfield et al., 1978). Therefore, progesterone appears to inhibit gap junction formation in rats.
Progesterone withdrawal may not be involved in regulation of gap junction formation in all species. In the guinea-pig, gap junctions are present in low frequency throughout the last third of gestation, when circulating progesterone levels are high. The number of gap junctions increases at the time of parturition or during prostaglandin induced abortion, but it is uncertain in these situations whether progesterone withdrawal occurs in the myometrium or plays a causal role in gap junction formation (Garfield et al., 1982a).

Estrogen may act to promote gap junction formation. Circulating estrogens increase prior to parturition in many species including the rat (Thorburn and Challis, 1979). Estrogen plays a central role in activation of the uterus at the end of term (A.B.M. Anderson et al., 1981). Administration of estrogen to ovariectomized animals promotes abortion (Csapo and Weist, 1969) and stimulates gap junction formation (Garfield et al., 1982).

The interaction between estrogen and progesterone was emphasized in studies which showed a positive correlation between gap junction frequency in sheep myometrium and increased estrogen/progesterone ratios in maternal and fetal blood (Garfield et al., 1979b). Further evidence for a stimulatory effect of estrogen is described below.

Treatment of nonpregnant animals with progesterone results in irregularity in uterine spike activity, while estrogen promotes regularity and improves coupling of uterine muscle (Marshall, 1959; Kao, 1977; Bortoff and Gilloteaux, 1980). Large doses of estrogen are known to cause gap junction appearance in nonpregnant myometrium (Dahl and Berger, 1978; Merk et al., 1980; McKenzie and Garfield, unpublished.)
observations). In the absence of high doses of estrogen, administration of progesterone is not known to modulate gap junctions in nonpregnant myometrium.

An in vitro culture system has been developed and utilized by Garfield and colleagues to investigate gap junction formation in rat myometrium. Large numbers of gap junctions form in tissues incubated in vitro in the absence of any hormonal influences (Garfield et al., 1978). This finding is consistent with the hypothesis that withdrawal of an inhibitory factor, such as progesterone, can result in gap junction formation.

The principle value and strength of the in vitro culture system (over studies in vivo) is that it allows a variety of agents that may influence gap junction formation to be tested in relative isolation from other complicating factors. Gap junction formation in myometrium does not require either estrogen or progesterone, but estrogen stimulates and progesterone, when coadministered with estrogen, inhibits their formation.

Several other features of gap junction formation in myometrium have been examined with the culture system. Protein synthesis is an essential step in the process. Low concentrations of protein synthesis inhibitors significantly reduce the frequency of gap junctions (Garfield et al., 1980 b). Prostaglandins appear to regulate gap junction formation, for prostaglandin synthesis inhibitors also reduce the frequency of gap junctions (Garfield et al., 1980 a,b). Prostaglandins are synthesized by the uterus (Phillips and Poyser, 1981) and are thought to play a role in regulating uterine contractions (Thorburn and
Evidence has accumulated that many factors, which are known to be involved in the regulation of parturition, influence gap junction formation in the myometrium. It would be premature (and perhaps overly optimistic) to suggest that many of the agents described above act solely through regulation of gap junction formation. But it certainly is apparent that modulation of gap junction formation is a common mode of action. This fact suggests that gap junction formation may be a critical step, one that is subject to many restraints and stimuli, in the sequence of events leading to activation of the myometrium at parturition.
CHAPTER 3

Changes in the Longitudinal Impedance Characteristics of Rat Uterine Smooth Muscle at Parturition
INTRODUCTION

The appearance of gap junctions between uterine smooth muscle cells at the end of gestation has been described in the preceding chapter. In this, and in the following chapter, I examine some passive electrical properties of the myometrium to determine the functional significance of gap junction formation.

Gap junctions are generally thought to be the structures that mediate ionic and metabolic coupling between many cell types (for review, Bennett and Goodenough, 1978; Loewenstein, 1981; but see Chapter 1). The proposal was made that gap junction formation might be instrumental in improving the electrical coupling between smooth muscle cells at the time of delivery (Garfield et al., 1977 and 1978). The improved electrical communication amongst cells could facilitate synchronous excitation of a large number of fibers and permit the evolution of effective, coordinated uterine activity, resulting in termination of pregnancy.

In this chapter I examine the question of whether improved coupling between uterine smooth muscle cells is evident at a time when gap junctions are present in large numbers. The small size of smooth muscle cells and the complex 3-dimensional geometry of the intact, syncytial tissues preclude the direct measurement of coupling between adjacent cells. But properties of electric current flow through the muscle can yield indirect estimates of ionic coupling between smooth muscle cells. Measurements of the specific resistance of syncytial
tissues can give an indication of the ionic coupling between cells (Tomita, 1969; Mathias et al., 1981a). I chose to use an alternating current technique to determine the specific resistance of myometrium in order to distinguish between the resistance of the cytoplasm and the junctions between cells.

Alternating current (AC) impedance analysis of biological samples is a valuable method for study of details of electric current flow in tissues. The major advantage of AC analysis over steady-state square pulse analysis of electrical parameters can be attributed to the fact that patterns of current flow in many circuits depend upon the frequency of excitation. Thus, pathways for current flow change as the frequency changes, depending on the circuit details.

AC analysis has been profitably applied to numerous cells and tissues. Early studies of the impedance of cell suspensions provided evidence that cells were composed of a conducting medium, of slightly greater resistivity than sea water, enclosed in a high resistance membrane. Remarkably accurate estimates of specific membrane capacitance (\( \sim 1 \, \mu F/cm^2 \)) were obtained from the impedance analyses of cell suspensions (for the history, see Cole, 1968; Schanne and Ceretti, 1978). Extracellular electrodes have been used to measure the transverse impedance of whole tissues (e.g., Fatt, 1964). Although this method is unsuitable for obtaining certain parameters of the equivalent circuit, it does provide a valuable complement to measurements with intracellular electrodes, where they exist.

A wide variety of excitable, as well as nonexcitable cell and tissue types have been subjected to the scrutiny of AC impedance studies.
with intracellular microelectrodes and sensitive measurement techniques (for review, Jack et al., 1975; Schanne and Ceretti, 1978; for recent methodology and applications see Mathias et al., 1981 a and b). Multiple parameters, such as specific membrane resistance and capacitance and internal resistance can readily be determined in those studies. Quantitative ultrastructural data is an essential corequisite of electrical impedance data (Eisenberg and Mathias, 1980). Such information is required to obtain realistic estimates of the above-mentioned specific parameters and, in addition, to develop an electrical model of a cell or tissue, test the model and interpret the electrical properties of the tissue.

Relatively few AC measurements have been made on smooth muscle systems. Those that have been reported lack the sophistication and power of many of the AC analyses performed on other cell systems, described above. Impedance studies of smooth muscle have been limited almost exclusively to measuring the longitudinal impedance with extracellular electrodes and assessing a single parameter, the specific internal resistance of the tissue (see references in Discussion).

Several factors may account for the limited application and scope of AC impedance analysis of smooth muscle. These include not only the small size of the individual cells, the complex syncytial geometry and the large and tortuous extracellular space (all factors that introduce technical difficulties into making and interpreting electrical measurements) but also the considerable morphological and electrical heterogeneity amongst smooth muscle types. While some of these problems are common to other cell systems, such as cardiac tissues, they are not
trivial obstacles to overcome, and they have plagued smooth muscle
physiologists for decades.

Nevertheless, as stated above, the objective of this study was
to use an AC method to evaluate the coupling in uterine smooth muscle.
Some limitations of the technique (which will become apparent below) are
of fundamental nature and the errors introduced by them are serious.
Therefore, the results are not as quantitatively precise and
interpretation is not as straightforward as might be hoped for.
However, the information that is obtained is useful in a comparative
sense, because errors inherent in the technique should be common to all
categories of tissues studied.

In the following chapter the results presented here are
validated with an independent experimental technique. Some of these
results have been reported (Sims and Daniel, 1979) and have been
submitted for publication (Sims et al.).

METHODS

Experimental Preparation

Wistar rats at various stages of the reproductive cycle were
used in this study. This included animals that were nonpregnant, at
17-22 days gestation, delivering, and 1 1/2 - 2 days after completion
of delivery. Animals were stunned then killed by cervical dislocation.
Uterine horns were excised and placed in Krebs solution (20-22°C) in a
petri dish lined with Sylgard (Dow Corning). The composition of the
Krebs solution was, in mM: NaCl 115.5; KCl 4.6; CaCl₂ 2.5; MgSO₄ 1.2;
NaH₂PO₄ 1.2; NaHCO₃ 22.1; glucose 11.1. The Krebs solution had been equilibrated with 95% O₂-5% CO₂ and had a pH of 7.4. Each horn was pinned at its in vivo length and a longitudinal cut was made along the mesometrial attachment. In the case of pregnant animals fetal contents were removed before uterine tissues were laid flat, serosal side up, pinned at the original length. Under a binocular microscope longitudinal muscle pieces (2.7 cm long and approx. 1 mm wide) were dissected from the antimesometrial area alongside the linea uteri. Successful dissections yielded uninterrupted bundles of longitudinal muscle.

**Measurement of the Impedance**

I measured the longitudinal impedance of uterine smooth muscle with a technique essentially the same as that described by Ohba et al. (1976). Strips of muscle were quickly blotted in a standard manner to remove excess fluid and weighed before being mounted in the experimental chamber. Tissues were held at their in vivo length in the horizontal part of a T-tube cut in a Plexiglas block, as shown in Figure 6. Platinum-platinum black electrodes (see below) were placed at either end of the horizontal tube and made contact with the tissue. The impedance of the muscle between the electrodes was determined by measuring the current produced by a sinusoidal voltage applied across the chamber (Hewlett Packard Vector Impedance Meter, 4800A). Tissue responses were independent of variations in current magnitude ranging from $3 \times 10^{-7}$ A to $3 \times 10^{-6}$ A. The phase shift between the current and voltage was also recorded.
FIGURE 6. Diagram of the impedance measuring chamber. The impedance magnitude (|Z|) and phase angle was measured across a horizontally mounted strip of muscle, between Pt-Pt Bk electrodes. Solutions were superfused from the vertical tube at 1 ml/min. The interelectrode distance was 2 cm, the muscle strip was 2.7 cm long.
Platinum-platinum black electrodes were prepared by the method of Ferris (1974). Pure platinum electrodes were polished to a bright surface with fine grit (microtome blade sharpening powder) then thoroughly cleaned with organic solvents to remove any residue of grease. The electrodes were then immersed in platinizing solution, 3% chloroplatinic acid in 0.025 N HCl, containing 0.025 % lead acetate (Hellige Ltd., N.Y.). To ensure even coating, all four electrodes were simultaneously connected to a power supply, with the electrodes being the cathode and a large plate of platinum as the anode. With the current limiting, the potential difference between the recipient and indifferent electrodes was gradually increased until chlorine bubbles just began to emanate from the anode and platinum was deposited on the cathode. Plating continued for approximately 20 min, by which time the electrodes had taken on a soft black appearance. Freshly plated electrodes were externally short circuited and allowed to stand for at least 6 h in distilled water to allow any charge produced during the plating process to decay. This procedure served to deposit colloidal platinum on the electrode surfaces, effectively increasing the surface area of the electrodes by as much as 10,000 times (Ferris, 1974) and thereby reducing the electrode polarization impedance (Schwan, 1963).

After being mounted in the experimental chamber the tissues were superfused with Krebs solution through the vertical tube and allowed to recover for 45 minutes at 20–22°C. At the beginning of each experiment Krebs was replaced with isosmotic sucrose (92 g/l, 274 mOs) (Aristar grade, BDH Chemicals) flowing at the rate of 1 ml/min. Contamination of the water and sucrose would result in Ca^{++} concentrations of less than 1
µM. Impedance measurements were made at 18 frequencies between 5 Hz and 10 KHz at regular intervals during superfusion with sucrose. Each frequency sequence took approximately 1.5 min.

Results are expressed as specific tissue impedance, based on the observed impedance, tissue weight and the interelectrode distance (2 cm). In order to correct for differences in the size of tissue samples, and also to express the results as values of specific resistance, I applied a correction factor to the measured impedance magnitude values. The rationale for the procedure was as follows. The resistance \((r, \Omega)\) of a conducting cylinder is determined by the specific resistance \((R, \Omega/cm)\) of the material, the length of the object between the measuring sites \((L, \text{cm})\) and the cross sectional area \((A, \text{cm}^2)\).

\[
r \ \Omega = R \ \Omega/cm \cdot L \ \text{cm} / A \ \text{cm}^2
\]

Knowing the weight and length of each tissue sample, and assuming uniform dimensions along the length and a specific gravity of 1.06 g/cm³, the effective cross sectional area can be calculated. By rearranging Eq'n 1, and assuming a value of 35% extracellular space (hence, 65% cellular space) (Kao, 1977), an equation for determining the specific impedance of a sample of tissue is given by:

\[
Z(t) \ \Omega/cm = Z \ \Omega \cdot A \ \text{cm}^2 \cdot 0.65 / 2 \ \text{cm}
\]

where \(Z(t)\) is the specific impedance of the sample

\(Z\) is the measured impedance magnitude

\(A\) is the effective cross sectional area of muscle

\(2 \ \text{cm}\) is the interelectrode distance

This is the correction factor applied to the results. No correction was made for the impedance of the measuring system because the electrodes
were shown to make a negligible contribution.

While some shunting of current through the experimental chamber and extracellular space was inevitable, the effect on my measurements was small since the resistivity of the sucrose solution was about 0.5 MΩcm. This was more than two orders of magnitude greater than the calculated resistivity of the tissue at short times in sucrose.

In order that I might directly compare the results of my experimental methods to those of Ohba et al. (1976), several experiments were performed on the taenia coli muscle of male guinea-pigs. The interelectrode distance in these experiments was 2.5 or 2.7 cm. All other methods were the same as described above for myometrium.

Results are expressed as mean values ± SD. The Student's t-test was used to test for differences between groups using a 5% significance level and two-tailed tests. The assumption of homogeneity of variances was tested by the F-test (Snedecor and Cochran, 1974). When evidence of heterogeneity of variances was found, the t-values were corrected according the method of Snedecor and Cochran, resulting in a more conservative testing.

RESULTS

The results of studies on the impedance of rat myometrium are organized in the following manner. I first present evidence to show that the impedance of the electrodes is negligible. I then describe the longitudinal impedance characteristics of myometrium from nonpregnant animals as well as my methods of quantitating the response. An equivalent circuit model of smooth muscle is described in order that the impedance components can be interpreted. Finally, I present results of
the comparative study of myometrium from animals close to term, at delivery and post partum. Hereafter, I refer to the various categories of tissue as, respectively; nonpregnant, before term, delivering and post partum muscle.

Electrode Impedance

An end-to-end measuring system of the type described here measures the total impedance between the electrodes. This is composed of the impedance of the tissue, in series with the impedance of the electrodes, and the interface between the electrodes and tissue (Figure 7a). The electrode impedance was shown to be small by varying the interelectrode distance while measuring the impedance of an electrolyte solution (Figure 7b). The results show that no sizeable series impedance, which might be attributed to the electrodes, was detected. In addition, theoretical considerations show that the electrode polarization impedance is likely to be small when the current density across the metal surface is sufficiently small (Schwan, 1963). The current density of the electrodes used here ranged from 0.3 to 0.03 mA/cm², well below the level considered to be acceptable (Schwan, 1963; Schanne and Ceretti, 1978). Measurements of the electrode impedance by substitution of electrolyte solutions for the tissue sample showed that the electrodes exhibited little frequency dependence. The impedance magnitude of sucrose with Krebs added, or of Krebs solution alone, decreased only 1.6 ± 0.36 % (SD, n=15) and the phase angle remained approximately zero over the frequency range from 5 Hz to 10 KHz. A further piece of evidence to show that the measuring system itself did not exhibit any frequency dependence was obtained by measuring the
FIGURE 7. Characteristics of the measuring system used for the impedance analysis. A). Measurements of the end-to-end impedance are composed of the impedance of the tissue (Z_t) plus the contribution of the impedance of the electrodes and the electrode-tissue interface (Z_e), also termed the polarization impedance. B). Impedance of 0.9% saline was measured at 10 Hz at several interelectrode distances. Each circle represents a single measurement. The solid line was drawn through the origin. The good fit to the data points indicates that the series resistance of the electrodes was negligibly small.
impedance of pieces of myometrium which had been briefly placed in boiling Krebs solution. The impedance of such samples was constant over the frequency range from 5 Hz to 10 KHz and the phase angle was around zero. Therefore, negligible resistance and capacitance were associated with the measuring system, suggesting that the measured impedance can be attributed to the tissue.

**Frequency Response of Nonpregnant Myometrium**

The longitudinal impedance characteristics of nonpregnant myometrium are illustrated in Figure 8. These measurements were made 5 minutes after the flow of sucrose began. The impedance magnitude (|Z|) exhibited a marked dependence on the frequency of the alternating current, decreasing with increasing frequency (Figure 8A). At the low and high frequency extremes the magnitude was relatively independent of frequency. The phase angle of the impedance (ϕ) was always negative that is, capacitive, and showed a single peak around 1000 Hz (Figure 8B). The frequency response of nonpregnant myometrium was displayed on the Real(R)-Imaginary(X) plane according to the relationships |Z| = (R^2 + X^2)^1/2 and tan ϕ = X/R (see Appendix 1). The results described the arc of a circle (Figure 8C). The center of the circle was always depressed slightly below the real axis. At frequency extremes the impedance was essentially resistive, there being little capacitive reactance.

These results show that there exists in myometrium a frequency dependent component, a capacitance, in the longitudinal direction. Studies of several smooth muscle types (Tomita, 1969; Goto et al., 1976; Ohba et al., 1976; Bortoff, 1978; Bortoff and Gilloteaux, 1980) and of
FIGURE 8. Impedance characteristics of nonpregnant myometrium after five
min. superfusion with sucrose. A). Impedance magnitude decreased with
increasing frequency of stimulation, but was independent of frequency
at low and high extremes. B). Phase angle between voltage and current
showed a single peak. C). Impedance locus, where resistance is the
real part and reactance is the imaginary part of the impedance.
Numbers near the circles indicate the measuring frequency. The center
of the locus is depressed below the real axis with a phase angle of
84°. The characteristic frequency (f_o) occurs at 390 Hz.
cardiac tissues (Sperelakis and Hoshiko, 1961; Freygang and Trautwein, 1970; Stibitz andMcCann, 1974; Chapman and Fry, 1978) have provided evidence for a capacitance oriented in the longitudinal direction. Since the cytoplasm of cells is purely resistive (nerve, Cole and Hodgkin, 1936; skeletal muscle fiber, Mobley et al., 1975; invertebrate muscle cell, Caillé, 1975) it is reasonable to assume that the capacitance is associated with the current pathways, i.e., junctions, between cells.

**Equivalent Circuit**

An equivalent circuit has been proposed by Tomita (1969) to account for the impedance properties of smooth muscle when current is constrained to flow in the longitudinal axis. As shown in Figure 9, the model describes the junctional resistance between cells as being in parallel with the capacity component of the junction and the surrounding cell membrane, all in series with the resistance of the myoplasm. According to the model the junctional impedance would decrease with increasing frequency due to a bypass of current through the capacitance. Therefore the impedance measured at high frequencies would probably be due solely to the resistance of the myoplasm, while the impedance which disappears with increasing frequency could be attributed to the junctional membrane.

The frequency dependent characteristics of nonpregnant myometrium (Figure 8) are generally consistent with the frequency response of the circuit in Figure 9 (Appendix 1). However, the model is insufficient in several respects. It does not, for instance, take into
FIGURE 9. Model equivalent circuit of smooth muscle under various conditions [after Tomita (1969) and Ohba et al., (1976)].

A). Total impedance of tissue with shunt resistance of extracellular medium. $R_j$ and $C_j$ are junctional resistance and capacitance. 

B). Tissue impedance after washout of extracellular medium with sucrose. 

C). Tissue resistance at zero Hz, $R_0$, is the sum of the myoplasmic and junctional components. 

D). $R_{\infty}$ represents myoplasmic impedance. 

E). Model of the current pathways between smooth muscle cells.
account the impedance of the plasma membrane of the muscle cells. From the cable-like properties of smooth muscle it would be expected that current must first cross the cell membrane near the current supplying electrodes, flow longitudinally from cell to cell through junctions, then across the cell membrane at the other electrode. However, the contribution of the plasma membrane to the longitudinal impedance has been shown to be negligible when large interelectrode distances are used and at frequencies greater than 5 Hz (Ohba et al., 1976). The equivalent circuit of smooth muscle in Figure 9 also cannot explain the depression of the impedance locus (Fig 8c) below the Real axis. The deviation from the predicted locus (center on the Real axis) is even more pronounced in the pregnant myometrium, as described below.

Despite these deficiencies, it was not my objective to formulate a model equivalent circuit for smooth muscle. Rather, I accepted the model in Figure 9 in order to interpret the impedance response of myometrium and to compare the quantitative aspects of several types of myometrium.

**Estimating Resistance Values**

I have used two methods for estimating the myoplasmic and junctional resistance from the impedance measurements. Extrapolation of the low frequency impedance magnitude values, plotted on a linear frequency scale, gave a value of the impedance at zero Hz, $R_{0\text{Hz}}$. This parameter corresponds to the internal (core) resistance in the steady state, $R_i$. It is composed of the resistance of the myoplasm and the junctions, and has been termed the effective resistivity by Mathias et al. (1981). Since the impedance magnitude was nearly independent of
frequency at high frequencies, the 10 KHz value of the impedance was used as an estimate of $R_{\infty \text{Hz}}$, the myoplasmic resistance. The difference between 0 Hz and 10 KHz was assumed to represent the junctional resistance, $R_j$.

A second method of deriving resistance values utilized the impedance locus form of presentation (Ohba et al., 1976). The resistance at zero and infinite frequencies was estimated from the points where the locus intersected the real axis in Figure 8c. The myoplasmic resistance was the impedance at $\infty \text{Hz}$ and the junctional resistance was taken to be the difference between 0 Hz and $\infty \text{Hz}$. Both procedures gave similar values of myoplasmic and junctional resistance (see Table III, below). However, the use of impedance magnitude values had the advantage of simplicity, because it did not require the plotting of an impedance locus and the fitting of an arc to the data.

Changes in the impedance due to isosmotic sucrose

The myometrium was superfused with isosmotic sucrose in order to reduce the shunting of current through the tissue chamber and extracellular space (Figure 9). As a result, the tissue impedance increased with time, although the form of the response was unchanged (Figure 10 and Tomita, 1969; Ohba et al., 1976). Five minutes is likely to be sufficient time for washout of ions from the extracellular space of rat myometrium (Hamon et al., 1976) so the increase in impedance that is seen after five minutes may be due in part to the leaching of ions from the intracellular compartments. In order to correct for this change I analysed the results according to the methods used by Ohba et
FIGURE 10. Changes in impedance of nonpregnant myometrium with time in sucrose. The impedance increased with time, but the form of the response was constant. A). Impedance magnitude measured over a range of frequencies at various times in sucrose as indicated. B). Numbers by the loci represent the time after starting superfusion.
al., (1976). Conductivity values (resistivity $^{-1}$) were plotted as a function of time in sucrose (Figure 11) and estimates of the conductivity before exposure to sucrose (i.e. time zero) were obtained by extrapolating the curves to the ordinate. This form of presentation was used (Ohba et al., 1976) because of the similarities between the decay of conductivity (presumably due to washout of ions) and the fluxes of radioisotopes from smooth muscle (for e.g. Hamon et al., 1976). In both instances the changes have been shown to consist of several exponential processes. The need to extrapolate my results over time is a serious drawback of this experimental technique. Because of this step my results can only be viewed as rough estimates of the true resistivity. But the extrapolation procedure does not appear to contribute to the differences between groups, so the comparative aspects of this study are not compromised.

Resistivity values at zero time in sucrose obtained in the manner described are summarized in Table III for nonpregnant myometrium. There were no significant differences between values of myoplasmic or junctional resistance calculated from the impedance magnitude values or the impedance loci (paired t tests). Included in Table III are the quantitative results of several experiments performed on the the taenia coli muscle of the guinea-pig. Myoplasmic and junctional resistance values were estimated from the impedance magnitude at 5 Hz and 20 KHz, as described above. I obtained results which were similar to those reported by Ohba et al. (1976), which are also included in Table III. The form of the impedance response of taenia coli (Figure 12) was essentially the same as that of nonpregnant myometrium (Figure 8).
FIGURE 11. Changes in conductivity (resistivity$^{-1}$) with time in sucrose. Specific resistance values were determined by extrapolating lines to the ordinate.
TABLE III

IMPEDANCE COMPONENTS OF SMOOTH MUSCLE

<table>
<thead>
<tr>
<th></th>
<th>Myoplasmic Resistance</th>
<th>Junctional Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonpregnant Myometrium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A) Impedance Magnitude</td>
<td>595 ± 80</td>
<td>1333 ± 325</td>
</tr>
<tr>
<td>Values (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B) Impedance Locus Values</td>
<td>593 ± 83</td>
<td>1141 ± 254</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taenia coli (n=11)</td>
<td>233 ± 69</td>
<td>419 ± 119</td>
</tr>
<tr>
<td>Published values of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taenia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ohba et al., 1976)</td>
<td>214 ± 42</td>
<td>372 ± 92</td>
</tr>
</tbody>
</table>

Table III. Values of specific resistance (ohm·cm) are means ± SD. 

n = number of animals studied, except for taenia coli, where 11 
tissues from 7 animals were studied. Junctional resistance was 
determined as \( R_0 \text{ Hz} - R_\infty \text{ Hz} \). Values of resistance determined by 
impedance locus did not differ from values determined by impedance 
magnitude, p > .05, paired Student's t test.
FIGURE 12. Impedance characteristics of guinea-pig taenia coli smooth muscle at various times in sucrose, as indicated on the plots. A). Impedance magnitude versus frequency and B). Impedance loci. The impedance increased with time but the form of the response stayed the same.
Confirmation of the previous reports on taenia coli reassured me that my techniques were adequate and yielded comparable results.

**Comparison of Late Term, Delivering and Post Partum Myometrium**

My primary object was to determine whether a change in the impedance properties was evident at parturition. I examined tissues from 7 delivering rats, 22-23 days of gestation, and compared their characteristics to those of 8 rats before term, 17-22 days gestation. A comparison of these two categories is the most appropriate, because differences in the geometry of the two tissue types, owing to hypertrophy and hyperplasia of the uterus during gestation, are kept to a minimum. In addition, I have examined 4 animals at 1 1/2-2 days post partum, a time at which few gap junctions, if any, remain between myometrial cells (Garfield et al., 1978).

Examples of the impedance characteristics at 5 minutes after superfusion with sucrose are given in Figure 13. In all cases the impedance magnitude showed the same response, decreasing with increasing frequency. The post partum response (Figure 13C) is plotted with a different scale than before term (Figure 13A) and delivering (Figure 13B). The difference between low and high frequencies is smallest in the delivering tissue and the quantitative differences are described in detail below. The phase responses of myometrium are also displayed in Figure 13. Before term (Figure 13A) and post partum (Figure 13C) tissues showed the same phase response as nonpregnant myometrium, a single peak. The maximum phase angle was always less in before term tissues. Delivering tissues displayed no comparable peak in the phase response, only a gradual increase in the phase with increasing frequency.
FIGURE 13. Impedance responses of A) before term, B) delivering and C) post partum myometrium at 5 min in sucrose. Responses of before term and post partum myometrium were similar to nonpregnant (Figure 2). But delivering myometrium (B) showed no peak in phase angle and no locus was evident.
Locii of before term and post partum tissues could be described as arcs of circles (Figures 13A and 13C). The centers of before term loci were depressed below the real axis to a greater extent than either post partum (Figure 13C) or nonpregnant myometrium (Fig. 8). Impedance loci could not be fitted to the frequency response of delivering tissues (Figure 13B). Therefore I was unable to quantitate the impedance response by the locus method, and instead relied entirely upon the impedance magnitude values.

Illustrated in Figure 14, for before term, delivering and post partum tissues, are the changes in impedance due to superfusion with sucrose. The impedance responses are shown at nine times after the flow of sucrose began, as indicated on some of the plots. In all cases the impedance increased with time but the impedance at the lower frequencies increased slightly more rapidly than at the higher frequencies.

Throughout the experiments the form of the frequency response remained the same. Conductivity curves for estimating the impedance at time zero are shown in Figure 15 for before term and delivering samples.

Examination of tissue samples after superfusion with sucrose showed that shrinkage of the cells had occurred. Cells of delivering myometrium, fixed after 30 min in sucrose, appeared shrunken with many processes, but gap junctions were structurally intact (Figure 16). After prolonged exposure to sucrose (90 min) muscle cells were shrunken to an even greater extent, although some intercellular junctions appeared intact (Figure 16B).

Estimates of the electrical parameters are summarized in Table
FIGURE 14. Impedance of before term, delivering and post partum myometrium increased with exposure to sucrose. A). Centers of the impedance loci before term were depressed below the real axis. B). Delivering myometrium did not describe loci when the impedance was plotted on the real-imaginary plane. The plotting program fitted some very depressed arcs to the data points for some of the frequency dispersions at long times in sucrose. This occurred infrequently. C). The impedance response of post partum myometrium increased with exposure to sucrose but the form of the response was constant.
FIGURE 15. Conductivity of the myometrium at zero and infinity Hz decreased with exposure to sucrose. Plots such as these for before term (A) and delivering (B) muscles were used to determine the tissue resistivity at zero time in sucrose.
FIGURE 16. Electron micrographs of gap junctions in myometrium from delivering animals after impedance measurements had been made. A). Delivering myometrium after 30 min exposure to isosmotic sucrose. The cells were slightly shrunken, indicated by the scalloped appearance of the cell profile. The nucleus (N) and the mitochondria (M) appear normal and a structurally intact gap junction is shown between a cell process and the body of a cell (ARROW). Bar, 1.0 μm. X 32,000. B). Longer exposure to sucrose (90 min) resulted in further shrinkage of the cells and distortion of the normal geometry. Some gap junctions were structurally intact at this time, as indicated by the arrow. Bar, 0.5 μm. X 78,000.
IV. The total tissue resistance ($R_{0Hz}$) decreased from before term to delivery. This change occurred in the presence of constant myoplasmic resistivity, $319 \pm 113 \, \Omega \text{cm}$ before term and $340 \pm 93 \, \Omega \text{cm}$ at delivery. The decrease in total tissue resistance was attributable to a decrease in the junctional component, from $323 \pm 161 \, \Omega \text{cm}$ before term to $134 \pm 64 \, \Omega \text{cm}$ in delivering tissues. Both myoplasmic and junctional resistance values were larger in post partum tissues.

Independent t tests were used for the comparison of specific resistance values of before term to delivering and delivering to post partum myometrium. Correction for non-homogeneity of variance was applied for the comparison of junctional resistance before term and at delivery, and for all the comparisons of delivering to post partum muscle (Table IV).

Extrapolation of the conductivity values to zero time (Fig 15) gave estimates of tissue resistivity that were about 25% lower than the values recorded at 5 minutes in sucrose (time of the first measurements). However, the extrapolation procedure itself did not contribute to the differences observed between tissues from before term, delivering and post partum animals. This is evident from two observations. First, at five minutes the myoplasmic resistivity values were the same before term ($400 \pm 170 \, \Omega \text{cm}$), and at delivery ($405 \pm 95 \, \Omega \text{cm}$). But the junctional resistivity values were $519 \pm 217 \, \Omega \text{cm}$ before term and significantly lower at delivery ($187 \pm 91 \, \Omega \text{cm}$, p<.001).

Junctional and myoplasmic resistivity was higher post partum, at $918 \pm 221 \, \Omega \text{cm}$ and $2694 \pm 920 \, \Omega \text{cm}$ respectively. This is the same pattern that was shown in Table IV. Second, analysis of the conductivity versus time
<table>
<thead>
<tr>
<th></th>
<th>Total Tissue Resistance (R₀ Hz)</th>
<th>Myoplasmic Resistance (Rₘ Hz)</th>
<th>Junctional Resistance (Rₖ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-22 Days Gestation</td>
<td>642 ± 220</td>
<td>319 ± 113</td>
<td>323 ± 161</td>
</tr>
<tr>
<td>(n=18)</td>
<td>(p&lt;.05)</td>
<td>(p&gt;.05)</td>
<td>(p&lt;.01)</td>
</tr>
<tr>
<td>Delivering</td>
<td>474 ± 126</td>
<td>340 ± 93</td>
<td>134 ± 64</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Partum 1 1/2 - 2 days</td>
<td>2121 ± 924</td>
<td>756 ± 196</td>
<td>1358 ± 940</td>
</tr>
<tr>
<td>(n=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table IV values of specific resistance (ohm cm) are means ± SD. 

n = number of tissues examined, from 8 animals before term, 7 animals delivering and 4 animals post partum. All values were determined from the impedance magnitude, and junctional resistance was determined by $R_0 \text{ Hz} - R_∞ \text{ Hz}$. Resistance of post partum myometrium was significantly greater than delivering myometrium in all categories (p < .01). P values indicate levels of confidence between before term and delivering samples.
curves by curve peeling techniques showed that the rate of change of the conductivity was about the same for both before term and delivering tissues.

**Junctional Time Constant and Capacitance**

Estimates of the junctional time constant ($\tau_j$) and capacitance ($C_j$) were determined according to the relationship

$$\tau_j = \frac{1}{2\pi f_0} = R_j C_j.$$  

The characteristic frequency ($f_0$) was the frequency at which the reactance of the tissue was greatest when the results were plotted as impedance loci. Because of a gradual decrease in $f_0$ and increase in $R_j$ with increasing time in sucrose, the calculated values of $\tau_j$ and $C_j$ changed with time. For comparison I have presented the measured values at 5 minutes in sucrose in Table V. The mean $f_0$ for before term was used to calculate $\tau_j$ and $C_j$ for delivery, since no impedance loci were obtained from these tissues. The $\tau_j$ remained relatively constant in all types of tissues at approximately 0.2 - 0.3 msec, while $C_j$ varied, depending on $R_j$. The values of $\tau_j$ are less that 1% of the membrane time constant determined from studies of the cable properties of myometrium (Chapter 4).

**Method of Standardizing the Results**

I have standardized all of my results to units of specific resistance using the individual sample weights as a measure of the effective cross sectional area of conducting tissue (see Methods). If the weight of the samples is a good measure of the cross sectional area and provides a valid means of correcting for variations in sample size, I would predict that the following relation would hold true,
### TABLE V

JUNCTIONAL TIME CONSTANTS AND CAPACITANCE

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Time Constant $\tau_j$ (msec)</th>
<th>Junctional Capacitance $C_j$ ($\mu$F/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (n=7)</td>
<td>$534 \pm 156$</td>
<td>$0.33 \pm 0.12$</td>
</tr>
<tr>
<td>Before Term (n=18)</td>
<td>$673 \pm 383$</td>
<td>$0.28 \pm 0.20$</td>
</tr>
<tr>
<td>Delivering (n=14)</td>
<td>$---$</td>
<td>$0.24$</td>
</tr>
<tr>
<td>Post Partum (n=7)</td>
<td>$753 \pm 306$</td>
<td>$0.26 \pm 0.12$</td>
</tr>
</tbody>
</table>

All values are mean ± SD, determined as described in the text.
(rearranging the correction equation given above).

\[ Y = \text{Weight (g)} \times \frac{K}{Z(t)} \]

\( Y \) is the measured admittance, impedance\(^{-1}\), units Siemens, weight is the weight of the muscle sample in grams, and \( K \) is a constant comprised of the cellular space, tissue length, interelectrode distance and density. The admittance should be directly related to the tissue weight and inversely proportional to the true specific impedance of the tissue, \( Z(t) \).

To test whether this relation held true I plotted the measured admittance as a function of tissue weight. Before term and delivering tissues are illustrated in Figure 17. The distribution of data points is roughly linear, in agreement with my prediction and could be fitted by lines using least squares regression analysis. At 10 KHz (Figure 17a), before term and delivering tissues overlap and the slopes of the regression lines are about the same. Thus, the specific impedance of the myoplasm is the same before term and at delivery, as described in Table IV. The admittance at 5 Hz (Figure 17b) illustrates the difference between the total tissue impedance of before term and delivering tissues. Other factors being equal, the steeper slope for delivering tissues is evidence for a smaller specific impedance. Thus, without any correction factors, the "raw data" illustrate the different properties of before term and delivering tissues. The difference between the specific impedances predicted from the slopes is slightly greater than the difference at time zero presented in Table IV.
FIGURE 17. Linear relationship between sample weight and admittance (impedance$^{-1}$) at 5 min in sucrose. Open circles and broken lines represent before term tissues. Closed circles and solid lines represents delivering tissues. Lines were fitted by least squares method. A) Admittance at 10 KHz, the slopes of the two tissue types were similar. B) At 5 Hz the admittance versus weight line was steeper for delivering samples, suggesting that the specific resistance of the tissue was smaller (see text).
DISCUSSION

I have presented data which show that the longitudinal impedance characteristics of rat myometrium change during pregnancy and at parturition. The total specific resistance of longitudinal myometrium was lower at delivery than before term. A decrease in the contribution of the junctional resistance was the basis of the change, there being no difference in the myoplasmic resistance of before term and delivering tissues. One and a half to two days following completion of delivery the junctional resistance was again increased and the myoplasmic resistance was greater. This sequential change in the junctional resistance correlates temporally with, and may be related to, the appearance and subsequent disappearance of large numbers of gap junctions between uterine smooth muscle cells. Several other less likely explanations, which may contribute to the observed changes, are considered below.

Occurrence of Gap Junctions in the Muscle

Because I did not measure gap junction frequency in this study my interpretation of the change of the impedance relies upon the premise that gap junctions are present in large numbers at delivery and absent, or present in low frequency, at other times. It is likely, for two reasons, that some gap junctions were present in the tissues obtained from animals that were sacrificed before term. First, the possibility exists that variations in the timing of delivery lead me to examine tissue from animals not yet in labour, but in whom gap junction formation had commenced (see results of Garfield et al., 1977 and 1978). Second, it is known that gap junctions begin to form between uterine
smooth muscle cells after removal of the pregnant uterus from the donor and incubation at 37°C under organ culture conditions. (Garfield et al., 1978 and 1980a). It is not known if in vitro incubation of delivering or post partum tissues results in formation of gap junctions. If they do form the rate of formation may be reduced at 22°C (Johnson et al., 1974; Kam et al., 1978).

The possibility of in vitro formation of gap junctions in before term tissues is an important consideration because all samples of tissue were studied in vitro, some as much as ten hours after excision from the donor. Quantitative electron microscope (EM) studies of the experimental tissues were not made because the exposure to sucrose for 90 minutes resulted in cell shrinkage and distortion of the tissue geometry. Examination of some samples after shorter times in sucrose did reveal the presence of a small number of gap junctions in before term tissues and many gap junctions in delivering tissues.

In the following investigation (Chapter 4) I include the results of a quantitative EM study of gap junctions in muscle strips fixed following electrical recordings. In that study I found that gap junctions were present in all delivering tissues and in some of the before term tissues examined. However, the amount of junctional membrane was much smaller before term than at delivery. In light of these findings, the changes in electrical properties that I observed at parturition should be viewed as being consistent with an increased number of gap junctions between uterine smooth muscle cells.

To be sure, the in vitro formation of gap junctions during the dissection and recording procedure is a serious artefact inherent in the
experimental design. The object of my in vitro study, the differential
distribution of gap junctions, begins to change at some time after
removal of the uterus from the donor. But I must emphasize that any
change in the properties of before term tissues brought about by gap
junction formation should tend only to obscure, and not cause,
differences between that category and delivering tissues. This same
argument applies to the presence of gap junctions which may have formed
in vivo, before delivery. Thus, the fact that I do observe a
significant reduction in the specific resistance at delivery suggests
the in vivo formation of large numbers of gap junctions at delivery, is
responsible for a considerable functional change in the property of
myometrium. The magnitude of the differences in vivo, with and without
gap junctions, might be greater than that reported here.

Sources of Error

Measurements of the longitudinal impedance components of smooth
muscle, as reported here, are subject to several errors. Some of the
uncertainties encountered in these experiments are common to all the
categories of tissue, and should not, therefore, interfere with the
interpretation of my results on a comparative basis. Some of these
errors will be described then other factors, which could contribute to
the differences observed, will be considered.

Effects of isosmotic sucrose. The strips of muscle used in these
experiments were superfused with ion-free, isosmotic sucrose. This step
was necessary to reduce the shunting of current in the extracellular
space and constrain the current flow through the muscle primarily to the
longitudinal direction. This procedure has the drawback of causing the measured impedance to increase with time, as shown in Figures 10 and 14. As described above, it is likely that one reason for the increase is due to the washout of ions from the extracellular and cellular spaces (Ohba et al., 1976). Diffusion of ions from the cells into the ion-free sucrose would result in loss of cellular volume, as was indicated by EM observation (Figure 16). Two steps were taken to deal with the effects of sucrose and derive meaningful estimates of the specific resistance in physiological saline. I began my measurements after five minutes then extrapolated a time series of measurements back to time zero (Figures 11 and 15). As described in the Results, the extrapolation procedure did yield values which were lower than those measured at 5 minutes and later, but it did not cause the differences observed between groups.

Irreversible uncoupling of cardiac muscle cells, indicated by an increased internal resistance, is thought to occur after 15–60 minutes in Ca\textsuperscript{++}-free solutions (New and Trautwein, 1972; Kléber, 1973). However, the longitudinal impedance of intestinal smooth muscle was insensitive to the external Ca\textsuperscript{++} concentration (Tomita, 1969). Functional uncoupling of uterine smooth muscle cells may have occurred in my experiments and contributed to the increase in specific resistance which was observed, but the form of the frequency responses did not change with time. Structural uncoupling may have occurred to some extent, but some gap junctions remained structurally intact after moderate or prolonged superfusion with sucrose (Figure 16). The extrapolation to time zero should reduce the likelihood of these factors influencing my estimates of tissue resistance to any great extent. If
Ca++-free solutions cause closure of gap junctions in myometrium, parturient muscle, with the greatest number of gap junctions, should be most susceptible to this effect. Therefore it seems unlikely that uncoupling can account for the observed decrease in junctional resistance.

**Effects of damaged tissue.** While the weight of each piece of tissue was a good, relative measure of the cross sectional area (Figure 17), it does not account for the effect of tissue damage during dissection or discontinuous bundles of cells. I would expect that cells uncoupled due to damage and improperly aligned bundles would be of higher resistance than undamaged, well aligned bundles of cells. Irreparably damaged, leaky, cells can probably be considered as extracellular space, decreasing the effective cross sectional area of conducting tissue. All three artefacts result in an overestimation of the true specific resistance. I have not taken these factors into consideration in my calculations, but did attempt to minimize the impact with careful dissection procedures.

**Changes in the extracellular space.** In calculating the specific resistance values reported here I have assumed in all cases that 65% of the tissue cross sectional area was cellular space (Kao, 1977) and that the extracellular space was very high resistance to be neglected. If the actual size of the cellular space in myometrium were to change at the end of pregnancy, some error would be introduced into the calculation of the specific resistance, both the myoplasmic and junctional components being affected equally. For example, if the cellular space were to increase to a size greater than 65% of the
tissue, then multiplying the measured impedance by the (now incorrect) factor of 0.65 would cause the calculated specific resistance to be an underestimate of the "true" specific resistance. It is improbable that such an occurrence was the sole basis of the decrease in internal resistance seen at delivery, for the ratio of junctional:myoplasmic resistance decreased. The ratio was 1.1 before term, 0.4 at delivery and 1.8 post partum, suggesting that a real decrease in junctional resistance did occur at delivery. In addition, no remarkable change in the extracellular space of rat myometrium was observed in late pregnancy (Casteels and Kuriyama, 1965). A slight increase in the extracellular space was observed in post partum tissues, which could contribute to the increase in both myoplasmic and junctional resistance that I observed.

Assumed equivalent circuit

I have assumed from the outset that the equivalent circuit described in Figure 9 (after Tomita, 1969) was adequate for representing myometrium. Some differences from the predicted frequency responses were evident, especially in myometrium from before term and delivering animals. The phase responses were relatively flat and the centers of the impedance loci were depressed far below the real axes (before term) or loci were nonexistent (delivering). For quantitative comparisons of delivering myometrium to other stages I relied upon the impedance magnitude values, so these deviations from the expected responses should not affect my interpretation of the results. The basis of the depressed center of the impedance locus is not known, although the presence of a constant phase shift element or inhomogeneity of the sample, resulting in multiple time constants, have been suggested as causes (Cole, 1968;
Schanne and Ceretti, 1978; Poon and Choy, 1981). The equivalent circuit may not be appropriate for delivering myometrium, which exhibited none of the predicted characteristics other than decreased impedance magnitude with increased frequency. Such a nonspecific change would be expected of any linear network composed of resistors and capacitors and offers no information about the arrangement of the circuit elements involved. It is unclear how a quantitative change in the number of gap junctions could lead to qualitative alteration in the equivalent circuit.

Regardless of the uncertainties associated with the equivalent circuit, my values of the impedance at zero Hz are good estimates of the total specific resistance or effective resistivity of myometrium. The decrease in longitudinal impedance that I observed at parturition supports the hypothesis that the internal resistance of myometrium is lower at delivery. But of course, without the additional information obtained from the measurement of the impedance at high frequencies I would not have been able to distinguish between a decrease in the resistance of junctions or myoplasm.

**Changes in impedance properties of other smooth muscles**

There have been other reports correlating differences in the impedance characteristics of smooth muscle with changes in the conduction properties or the ultrastructural details of the tissue. The circular and longitudinal muscle layers of the small intestine are structurally different, there being identifiable gap junctions between smooth muscle cells in the circular layer only (Daniel et al., 1976). Bortoff (1978) found that while the myoplasmic impedance of the two
layers was the same, the junctional impedance of the circular layer was less than that of the longitudinal layer. The improved coupling in the circular layer was attributed to the abundant gap junction contacts between the smooth muscle cells.

Denervation of the rat vas deferens results in synchronization of contractile activity (analogous to the change in activity of the myometrium at term) and has been associated with a decrease in junctional impedance (Goto et al., 1976). It is doubtful that gap junctions play any role in this case of improved coupling, because some investigators were unable to identify any gap junctions between the smooth muscle cells of either intact or chemically denervated vas deferens (Paton et al., 1976).

Finally, Bortoff and Gilloteaux (1980) showed that the junctional impedance of nonpregnant cat myometrium decreased when the animal was under the hormonal influence of estrogen. Such treatment stimulates growth of the myometrium and is said to result in improved spike propagation within the tissue. Estrogens are thought stimulate gap junction formation in the myometrium (Garfield et al., 1980). Increased estrogen and decreased progesterone levels at the end of pregnancy in the rat (Thorburn and Challis, 1979) may contribute to the improved coupling that I observed at parturition. It should also be noted that large doses of synthetic estrogen can induce gap junction formation in nonpregnant myometrium (Dahl and Berger, 1978; Merk et al., 1980; MacKenzie and Garfield, unpublished observations). As yet there is no direct evidence to suggest that the hormonal treatments used by Bortoff and Gilloteaux to modify the junctional impedance influenced the
occurrence of gap junctions in cat myometrium.

Conclusions

These experiments demonstrate that the longitudinal impedance characteristics of rat myometrium change during pregnancy. A transient improvement of the electrical coupling was measured at parturition. I interpret this change to be the result of increased numbers of gap junctions in the tissue, but I cannot entirely eliminate other factors as contributing to the observed change. My results are indirect evidence that the gap junctions observed in thin section electron microscopy represent functional sites of communication between smooth muscle cells. Therefore, the results are consistent with, but do not prove, the hypothesis that formation of gap junctions at parturition results in improved coupling.

In the following chapter I validate these results by showing a similar decrease in internal resistance of myometrium at parturition, using an independent technique (Chapter 4). In addition, I present ultrastructural evidence to support the contention that increased numbers of gap junctions are present in the experimental samples of delivering tissue.
CHAPTER 4

Changes in the Cable Properties of Myometrium

Associated With the Formation of Gap Junctions at Parturition
INTRODUCTION

In this chapter I use a second experimental technique to evaluate the internal resistance of uterine smooth muscle. As in the preceding chapter, my aim was to examine the functional significance of gap junction formation at parturition.

Under the appropriate experimental conditions of extracellular polarization, multicellular smooth muscle preparations can be modelled as one dimensional cables (Abe and Tomita, 1968). (The theory and assumptions of cable analysis are described in Appendix 2). Measurements of the cable parameters such as the length constant can yield estimates of, amongst other things, the longitudinal internal resistance of the smooth muscle. Axial current must flow from cell to cell along the length of tissue, so the internal resistance of the cable is composed of the resistance of myoplasm of individual cells in series with the resistance of the junctions between cells. As such, the internal (core) resistance can be considered an indirect measure of cell-to-cell coupling in smooth muscle tissues. If other factors remain constant, relative changes in the internal resistance can be attributed to modulation of ionic coupling within the tissue.

The object of this study was to characterize the cable properties of rat longitudinal myometrium before term and at delivery. Several reports on the electrical properties exist (Abe, 1971; Daniel and Lodge, 1973; Kuriyama and Suzuki, 1976a; Kanda and Kuriyama, 1980), but the selection and grouping of tissues were not adequate to determine
if a change in electrical properties accompanied gap junction formation. In this study I utilize intracellular microelectrodes to measure the internal resistance of the muscle. This experimental technique is somewhat more commonly used and is independent of that technique employed for the longitudinal impedance studies (Chapter 3), yet yields results that are comparable. In addition, I have measured the gap junction frequency in some of the same samples after the experimental procedures, thereby strengthening the association of increased frequency of gap junctions with improved electrical coupling in the myometrium. Preliminary reports of some of these results have been published (Sims et al., 1981; 1982) and a full report has been submitted for publication (Sims et al.).

METHODS

Tissue Preparation

Wistar rats were used for all experiments. The timing of pregnancy was determined by examination of vaginal smears. The presence of sperm the morning after a male rat was placed in the cage with a female indicated day one of pregnancy.

Uterine tissues were obtained in the manner described in Chapter 3. Each horn was pinned at its in vivo, distended, length. With the aid of a binocular microscope, longitudinal muscle strips (2 cm long and 1-2 mm wide) were dissected from nonplacental areas alongside the linea uteri. Samples were taken from either the ovarian or mid horn regions.

Tissues were transferred to a partitioned organ bath of a design
described by Abe and Tomita (1968) (see Figure 18). I arranged the specimens so that 1 cm of the ovarian end of the myometrium was located between the stimulating electrodes and the other 1 cm protruded into the recording chamber. Tissues were allowed to recover for 30-45 minutes while being superfused with Krebs solution at a rate of 1-2 ml/min. The solution was prewarmed to 30°C and equilibrated with 95% O₂-5% CO₂.

Electrical Recording

The membrane responses of superficial smooth muscle cells were recorded with intracellular microelectrodes. Electrodes were prepared from 1.2 mm O.D. capillary tubing (Frederick Haer, Brunswick, Maine), pulled on an Industrial Scientific Associates (Woodside, NY) M7 micropipette puller. The length of the electrodes from shoulder to tip ranged from 5 to 10 mm. Electrodes were back-filled with filtered 3 M KCl and had resistances of 30-50 MΩ. Tip potentials, determined by measuring the shift in potential upon breaking of the tip, were always less than 5 mV. Microelectrodes were connected to the probe of a W-P Instruments M4A electrometer (New Haven, Connecticut) and the voltage signal was displayed on one channel of a Tektronics D-13 dual beam storage oscilloscope (Beaverton, Oregon). Measurements of electrical parameters were made by applying rectangular current pulses of one second duration with large external electrodes (two silver-silver chloride plates 8 x 8 mm). The stimulator used was a Grass S-88. The voltage gradient applied to the tissues was measured with two platinum wires, 1.5 mm apart, in the stimulating chamber. Film records were obtained by directly photographing the oscilloscope screen with a
Diagram of the type of experimental chamber used to pass current into a piece of smooth muscle tissue
(Based on the method described by Abe & Tomita, 1968)

FIGURE 18. Partition style bath used for measuring the cable parameters of smooth muscle (after the design of Abe and Tomita, 1968). A sample of muscle is pinned between two stimulating electrodes and protrudes into the recording chamber, where intracellular microelectrodes are used to measure membrane responses. (Figure taken from Holman and Nield, 1979).
Nihon-Kohden PC2A camera.

Analysis of the Spatial Decay of Electrotonic Potentials

In order to characterize the linear electrical properties of strips of uterine smooth muscle the amplitude and time course of steady-state electrotonic potentials were measured. A minimum of 22 cells were sampled in each experiment, measurements being made at three to five distances from the nearest stimulating plate. The mean number of impalements per tissue in the experiments reported here was $31 \pm 6.6$ (SD, $n=33$). The length constant ($\lambda$) was determined as the distance for decay of the electrotonic potential to $e^{-1}$ when plotted as $\log_{10}$ Voltage versus distance. To obtain the membrane time constant ($\tau_m$), the time to reach 50% of the steady-state potential of some of the electrotonic potentials was plotted against distance from the nearest electrode (Gage and Eisenberg, 1969; Jack et al., 1975). A single value of $\lambda$ and $\tau_m$ was determined for each experiment and mean values were determined for each category of tissue.

Microelectrode impalements were considered satisfactory if there was a sudden shift in the baseline potential and if the membrane potential was steady for at least 5 sec. In some cases cells were spontaneously active, exhibiting bursts of action potentials. In these cases the most negative potential was considered the membrane potential.

Assessing the position of the microelectrode tip relative to the stimulating electrode presented some difficulties. The position in the horizontal plane could be seen through the microscope, but a direct measure of distance from the electrode could not be obtained by the
eyepiece micrometer because no reliable reference was available. Thick insulation on the plate obscured the muscle at short distances. In addition, variations in the angle of the electrode and optical distortion due to the air-Krebs interface made it difficult to rely upon reference points out of the plane of the muscle surface. However, estimates of the distance of the microelectrode tip from the stimulating electrode were made by the use of landmarks inscribed in the Sylgard base. Scratches every 1 mm provided consistent markers of the distance from the plate. Some errors in measurement were inevitable, but they were of a random nature and would not have biased my results.

**Electron Microscopy**

Some of the samples of longitudinal uterine muscle were fixed for electron microscopy following completion of the experiments. Tissues were processed for thin section electron microscopy, sectioned in transverse orientation and quantitatively analysed to determine gap junction frequency. The methods were described in detail in Chapter 2. Fractional area of junctions was calculated by doubling the total length of gap junction membrane and dividing by the length of nonjunctional membrane.

**Statistical Analysis**

All results are expressed as mean values ± SD. In most instances the Student's t-test was used to test for differences between groups, but the Mann-Whitney U test was used to compare the morphological data. A significance level of 5% was used for the tests.
The F-test was used to test for homogeneity of variances, but no corrections were required for the comparisons reported below.

RESULTS

The timing of parturition in 28 rats that actually went into labour in this, and the preceding study, is shown in Figure 19. Delivery occurred over a range of times, but the majority of animals delivered in the afternoon of day 22 of gestation or later. In only 2 instances did animals begin to deliver pups before noon on day 22, and they both occurred between 11 am and noon. The mean litter size of all the animals used in these experiments was 13.0 ± 2.8 (SD, n=51).

Electron Microscopy

Quantitative electron microscopic studies were performed on some of the myometrial samples that were used for physiology experiments (3-5 hours after excision from the donor). Results in Table VI show that tissues from delivering animals (hereafter referred to as delivering myometrium) had a large number of gap junctions (Figure 20a). Both the frequency of gap junctions (6.75 gap junctions/1000 μm) and the fractional area of cell membrane occupied by gap junctions (0.24%) was comparable to the values from tissues fixed in situ or immediately after removal from naturally delivering animals (Chapter 2). A small number of gap junctions (0.41/1000 μm of cell membrane), occupying a small percentage of the total cell membrane (0.005%), were identified in 3 of the 8 tissues from animals before term (i.e., 20-22 days of gestation,
FIGURE 19. Timing of parturition in the rats used in these studies. The onset of labour in the majority of animals occurred in the afternoon of the 22nd day of gestation, or later. In only two cases did animals deliver before noon on day 22, and they were both between 1100 and 1200 hours.
### TABLE VI

GAP JUNCTIONS IN MYOMETRIUM

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Length of Membrane (µm)</th>
<th>No. of GJ's</th>
<th>GJ/1000 µm</th>
<th>Fractional Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE TERM</td>
<td>8 (3)</td>
<td>11373</td>
<td>5</td>
<td>0.41±0.61</td>
<td>0.005±0.008</td>
</tr>
<tr>
<td>(20–22 days of gestation, not delivering)</td>
<td></td>
<td></td>
<td></td>
<td>(p&lt;.001)</td>
<td>(p&lt;.001)</td>
</tr>
<tr>
<td>DELIVERING</td>
<td>6 (6)</td>
<td>8366</td>
<td>56</td>
<td>6.75±3.83</td>
<td>0.24±0.14</td>
</tr>
</tbody>
</table>

Table VI. Frequency of gap junctions (GJ's) in tissues fixed following completion of electrical recordings. n is the number of tissues examined; following in brackets is the number of tissues in which GJ's were identified. Length of membrane refers to the total length of membrane surveyed in 20–24 micrographs of each tissue. Number of GJ's is the total of all 5 or 7 lined junctions identified and is used to calculate the frequency of GJ's, expressed as the mean number of GJ's /1000 µm of nonjunctional membrane. The fractional area of GJ's was calculated by doubling the total length of GJ membrane and dividing by the length of nonjunctional membrane. Values are means ± SD. P values determined by the Mann-Whitney U test.
FIGURE 20. Electron micrographs of gap junctions present in samples of myometrium fixed following physiology experiments. A). Several gap junctions in parturient myometrium. Low magnification Bar, 1 µm. X 50,000. Inset, Bar, 100 nm. X 150,000. B). A small gap junction between smooth muscle cells of myometrium taken from an animal before term. Bar, 0.5 µm. X 53,000.
but not delivering, Figure 20b) (hereafter referred to as before term myometrium). When individual samples were considered, the greatest incidence of gap junctions before term (1.4/1000 µm) was less than the smallest frequency seen at delivery (2.2/1000 µm). These results show that gap junctions could be identified in some tissues from before term animals but they were 16 times less frequent than in delivering samples. The fractional area of membrane occupied by junctions was about 48 times less before term than in delivering tissues, owing to the smaller size as well as the lower frequency of the junctions. The size distribution of the gap junctions observed is shown in Figure 21. A clear distinction between groups is evident.

Both the frequency and fractional area of gap junctions were significantly greater in delivering muscle than in samples from before term ($p<.001$). A nonparametric test, the Mann-Whitney U test, was used for these comparisons because the distribution of data points was not normal. An indication of the non-normal distribution came from the mean values presented in Table VI, where some of the means minus one standard deviation were less than zero. The failure to observe any gap junctions in some samples before term resulted in several values of zero being included, causing the non-normal distribution.

**Electrical Properties**

Membrane electrical activity before term and at delivery consisted of bursts of action potentials, alternating with silent periods (Kuriyama and Csapo, 1961; Casteels and Kuriyama, 1965; Kuriyama and Suzuki, 1976a; Kanda and Kuriyama, 1980; Anderson et al., 1981).
**FIGURE 21.** Size distribution histogram of the length of gap junctions observed in (A) before term and (B) delivering myometrium. The bin sizes include junctions greater than the lower limit and less than or equal to the upper limit. Approximately 35% more nonjunctional membrane was surveyed in before term than in delivering myometrium, as shown in Table VI.
FIGURE 22. Membrane electrical activity of myometrial cells.

Spontaneous bursts of action potentials occurred in both before term (A) and delivering (B) myometrium. These records are montages of continuous records, showing impalements of approximately 4 min in (A) and 10 min in (B).
Records of the bursting activity of the membrane are shown in Figure 22 for before term and delivering tissues. The amplitude of electrotonic potentials evoked during quiescent phases was slightly larger than that obtained during active periods (Figure 23). The decreased amplitude of the electrotonic potentials during bursts may have resulted from increased membrane conductance, possibly due to increased Ca\(^{++}\) conductance or Ca\(^{++}\)-activated K\(^+\) conductance (Walsh and Singer, 1980). Membrane responses to electrical stimulation were linear in the hyperpolarizing direction (Abe, 1971; Kuriyama and Suzuki, 1976a), a critical requirement of the cable analysis. The responses of myometrium to four different stimulus intensities at four distances from the nearest stimulating electrode are shown in Figure 24 for before term tissue and Figure 25 for delivering muscle. Depolarizing stimuli usually initiated active membrane responses, as is also shown in Figures 24 and 25.

The resting membrane potentials of longitudinal myometrial cells were determined upon withdrawal of the microelectrode from the cells, and are summarized in Table VII. The grand mean of the membrane potentials from before term tissues was \(-52 \pm 4.7\) mV, significantly greater than the value of \(-46 \pm 2.6\) mV observed during delivery.

**Spatial Decay of Electrotonic Potentials**

Examples of responses to hyperpolarizing current pulses are shown at various distances from the nearest stimulating electrode in Figure 26A for before term and delivering tissues. The amplitude of the steady state hyperpolarizing potential decayed exponentially with
FIGURE 23. Some hyperpolarizing current pulses were applied during spontaneous bursts of action potentials as well as during quiescent periods in both before term (A) and delivering myometrium (B). The amplitude of the electrotonic potentials was slightly smaller during the active phases, probably due to decreased membrane resistance.
FIGURE 24. Membrane responses of myometrial cells from before term.

Several hyperpolarizing current pulses of one second duration and of
different amplitudes were applied and responses measured at 4
distances from the stimulating plate. A). The upper trace in all
records is the current monitor, recording the voltage gradient in the
stimulating chamber. The middle line is at zero potential and the
bottom trace is the voltage responses of the cells. Depolarizing
pulses initiated active responses. B). Membrane responses at 4
distances from the stimulating electrode were linear in the
hyperpolarizing direction. C). Length constant measurements were
made at 4 stimulus intensities and were independant of the applied
potential. Each dot in this and the preceding current-voltage plot
is the mean of measurements from two cells.
BEFORE TERM

A

1 mm

2 mm

3 mm

4 mm

1 V/cm

-50 mV

5 sec

B

\( \frac{V}{cm} \)

C

AMPLITUDE OF ELECTROTONE POTENTIALS (mV)

DISTANCE (mm)

1 mm

2 mm

3 mm

4 mm

1.29 mm

2.43 mm

2.30 mm

2.33 mm
FIGURE 25. Membrane responses of myometrial cells at delivery. Records are as described in figure legend 24. A). Hyperpolarizing responses were measured at 4 distances from the stimulating electrode. B). Responses of the membrane were linear in the hyperpolarizing direction.
FIGURE 26. Spatial decay of hyperpolarizing potentials is shown for before term and delivering myometrium. A). Responses are shown at four distances, 1, 2, 3, and 4 mm from the nearest stimulating electrode. B). Geometric means ± SD of the steady state amplitudes are plotted as log\(_10\) voltage versus distance from the electrode. The lines were determined by least squares regression analysis of all the data points. For these graphs there were 40 impalements before term and 33 impalements in the delivering tissue. The length constant (\(\lambda\)) was calculated as the distance for decay of the electrotonic potentials to \(e^{-1}\). The voltage at the anode (\(V_o\)) was determined from the intercept of the regression line with the ordinate.
distance from the anode. Semilog plots of the geometric mean amplitudes ± SD at each distance are shown in Figure 26B. The lines represent the least squares fit to data plotted as logarithm of voltage versus distance for all data points. This form of data presentation and analysis was used to convey and account for the variation in the size of electrotonic potentials encountered at any given distance in individual experiments. Acknowledgement of this variability, which probably results from true nonuniformities of the muscle and variations in the quality of impalements, is important when characterizing the electrical properties of smooth muscle. This type of analysis is essential to eliminate bias in a comparative study, such as this one, and desirable in others.

Before term the length constants ranged from 1.7 to 4.1 mm, with a mean value of 2.6 ± 0.8 mm (Table VII). Delivering tissues had length constants ranging from 2.4 to 6.1 and a mean value of 3.7 ± 1.0 mm (Table VII). The mean value of λ was significantly larger (a 42% increase) in delivering animals (p<.05, Student's t test).

Inasmuch as rat longitudinal myometrium exhibits cable-like properties when polarized with extracellular electrodes (Abe, 1971; Kuriyama and Suzuki, 1976a; Kanda and Kuriyama, 1980; the present findings), the equations of one dimensional cable theory (Hodgkin and Rushton, 1946) can be applied to the tissue. Measurements of λ and the input resistance (R\textsubscript{in}) can be used to determine values of membrane resistance (r\textsubscript{m}) and internal resistance (r\textsubscript{i}) (Gage and Eisenberg, 1969; Jack et al., 1975; for smooth muscle, Haeusler and Thorens, 1980).

Relative values of R\textsubscript{in} were obtained from the ordinate intercept
(voltage at the anode, $V_o$) of the voltage versus distance lines (Figure 27). While no direct measure of the amount of current passed across the cell membrane in the stimulating chamber was available, I monitored the magnitude of the voltage gradient in each case. The assumption was made that the amount of current which flowed across the membrane was proportional to the applied potential. Therefore $R_o$ was calculated in arbitrary units ($R_o = V_o / I$, mV/V cm$^{-1}$). Relative values of $r_m$ and $r_i$ were obtained from the following relations and are given in Table VII.

\[ \lambda = (r_m / r_i)^{1/2} \quad \text{and} \quad R_o = 1/2(r_m \cdot r_i)^{1/2} \]

Therefore, \[ r_m = 2R_o \lambda \quad \text{and} \quad r_i = 2R_o / \lambda \]

During delivery the membrane resistance was 47% larger and the internal resistance was 33% lower than before term (Table VII). Both of these factors contributed to the increased $\lambda$ observed in delivering tissues.

Membrane Time Constant

The membrane time constant ($\tau_m$) was estimated from plots of the time to reach 50% of the steady state electrotonic potential versus distance from the nearest stimulating electrode (Figure 27). The slope of the line approaches $\tau_m / 2\lambda$ when the length of the tissue between the stimulating electrodes is greater than about 3$\lambda$ (Bywater and Taylor, 1980). The values of $\tau_m$ were 158 $\pm$ 61 msec before term ($n=10$) and 225 $\pm$ 72 msec during delivery ($n=14$) (Table VII). (The $n$ values for the $\tau_m$ measurements were different from those of the rest of the cable parameters because records of electrotonic potentials were not obtained
TABLE VII

ELECTRICAL PARAMETERS OF MYOMETRIUM
FROM BEFORE TERM AND DELIVERING MYOMETRIUM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Term (n=17)</th>
<th>Delivering (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Membrane Potential (mV)</td>
<td>51.7 ± 4.7</td>
<td>45.5 ± 2.6</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>Length Constant ( \lambda ) (mm)</td>
<td>2.59 ± 0.84</td>
<td>3.68 ± 1.0</td>
<td>p&lt;.05</td>
</tr>
<tr>
<td>Membrane Resistance ( r_m ) (arbitrary units)</td>
<td>413 ± 177</td>
<td>607 ± 240</td>
<td>p&lt;.05</td>
</tr>
<tr>
<td>Internal Resistance ( r_i ) (arbitrary units)</td>
<td>64 ± 21</td>
<td>43 ± 14</td>
<td>p&lt;.01</td>
</tr>
<tr>
<td>Time Constant ( \tau_m ) (msec)</td>
<td>158 ± 61</td>
<td>225 ± 72</td>
<td>p&lt;.05</td>
</tr>
</tbody>
</table>

Table VII. Summary of electrical parameters of the longitudinal myometrium from 12 animals before term and from 11 animals delivering. All values are mean ± SD. The membrane resistance and internal resistance are expressed in arbitrary units, as explained in the text. P values were determined by the Student's t test.
FIGURE 27. Graphs of time to reach half maximum amplitude versus distance from the nearest stimulating electrode were used to estimate the membrane time constant ($\tau_m$) and conduction velocity ($\theta$). Circles are mean values ± SD of several measurements at each distance, in this case for delivering myometrium. The line was fitted by least squares regression analysis, the slope was used to calculate $\tau_m$ and $\theta$ (see text). $\tau = 2\lambda$/slope = 248 msec. $\theta = $ slope$^{-1}$ = 2.3 cm/sec.
on a fast time base in all experiments). These values of $\tau_m$ could represent underestimates, especially in the case of delivering tissues, since less than 3A of tissue were present in the stimulating compartment in some instances. An alternate method of determining $\tau_m$ was to consider the ordinate intercept in Figure 27 (Time-intercept $= 0.23 \tau_m$). Using the time-intercept method, the values of $\tau_m$ before term were 207 ± 52 msec. However, in the case of delivering myometrium $\tau_m$ was 171 ± 51 msec. The reasons for the differences in the values of $\tau_m$ determined in the two ways are not known.

The conduction velocity ($\theta$) of the half maximal amplitude of the electrotonic potential is given by the inverse of the slope of the line in Figure 27. Since the conduction velocity along a cable is inversely proportional to the square root of the internal resistance (Hodgkin, 1954; Jack et al., 1975) I would predict that $\theta$ would be greater at delivery, when the internal resistance is decreased (see also Chapter 3 and Appendix 2). However, in these experiments, I found that $\theta$ was the same for both categories of muscle, 3.4 ± 0.5 cm·sec$^{-1}$ (n=10 before term, n=14 at delivery). Simultaneous changes in one or more of the other parameters that determine $\theta$ (e.g., an increase in membrane resistance) may have concealed any change due to a decrease in internal resistance. Changes in the propagation velocity of action potentials, rather than electrotonic potentials, might better reflect the decreased internal resistance observed at parturition (see Weingart, 1977; Sharp and Joyner, 1980). Characterization of active membrane responses would also give a more direct indication of electrical synchrony throughout the tissue (e.g., Clapham et al., 1980).
DISCUSSION

My aim has been to investigate the significance of gap junction formation in the myometrium at parturition. In this report I have established a temporal relationship between a change in structure and a change in cable properties of the myometrium. The results are consistent with the view that increased numbers of gap junctions between smooth muscle cells facilitate the flow of current in the parturient uterus.

Gap Junctions in the Myometrium

Ultrastructural studies of some of the same samples used for in vitro physiology experiments have confirmed that gap junctions were present in all delivering tissues (Table VI). A small number of gap junctions could be identified in some of the tissues from animals 20-22 days pregnant but not in labour. The mean values of gap junction frequency and percentage of total cell surface were markedly and significantly different in the two groups. When individual samples were considered, the groups were nonoverlapping. Thus, evidence shows that I have studied two distinct groups of myometrium, different with regards to the incidence of gap junctions. Moreover, if I assume that the gap junctions I observe are functional and are the basis of the physiological change at term, then the presence of gap junctions before term should serve only to conceal, and not cause, the differences between the two categories of muscle. The fact that I was able to discern differences at delivery suggests that the two groups were
functionally distinct.

When individual samples were considered, a meaningful (inverse) relationship between gap junction frequency or area and the internal resistance was not evident. This is probably a result of my deliberate attempts to select animals from distinct categories (before term and delivering) in order to study a dichotomous variable (i.e., gap junctions were either present or not). My attempts were only partially successful, insofar as gap junction frequency was shown to be a discontinuous variable. Gap junctions were present in some, but not all tissues from animals before term, while all delivering samples had gap junctions. In contrast to the ultrastructural data, the physiological studies showed that the internal resistance was a continuous variable, with measurable values of resistance in all cases.

There are two possible explanations for the presence of gap junctions in my tissues before term. I may have selected animals in which in vivo formation of gap junctions preceded identifiable labour. Alternatively, some gap junctions may have formed during the course of the in vitro experiments (Garfield et al., 1978). The frequency of gap junctions in my tissues was less than that observed after 2 or 6 hours in vitro in culture (Garfield et al., 1980a) suggesting that some factor(s) influencing the rate of gap junction formation is different in the two cases. The difference is perhaps related to the fact that in these experiments only longitudinal muscle was present, and it was kept at lower temperatures (see Johnson et al., 1974; Kam et al., 1978) in Krebs medium.

I cannot exclude entirely the possibility that changes in the
myometrium at the end of gestation other than gap junction formation (e.g., changes in cell geometry) contribute to the observed differences. However, I have reduced the likelihood of this happening, and have made the best comparison possible, by studying 20-22 day pregnant myometrium and delivering myometrium. Growth of the myometrium (Afting and Elce, 1978) and the fetuses (Knox and Lister-Rosener, 1978) are both reduced at the end of term. Therefore it is unlikely that any significant change in myometrial structure took place at the end of term associated with uterine or fetal growth.

**Changes in the electrical characteristics**

Several differences in the electrical characteristics of before term and delivering myometrium were apparent. The resting membrane potential decreased from before term to delivery, consistent with previous reports on rat myometrium (Casteels and Kuriyama, 1965; Kuriyama and Suzuki, 1976a; Kanda and Kuriyama, 1980; Anderson et al., 1981). Several factors may account for the values of membrane potential (46-52 mV) being slightly lower than those reported by Kuriyama and Suzuki (1976a), who obtained values of 54-64 mV at 35°C. My measurements were obtained from samples of muscle dissected from nonplacental regions (see Kanda and Kuriyama, 1980) which were maintained at 30°C to reduce the spontaneous activity (Kuriyama and Suzuki, 1976a).

**Length Constants**

The mean values of λ that I obtained (Table VII) were larger than that reported by Abe (1971) for mid and late term myometrium (1.8
Kuriyama and Suzuki grouped before term with delivering tissues and reported a mean $\lambda$ of 2.9 mm, close to the value I found for before term animals alone. They also reported that a small sample (n=3) of delivering tissues had a $\lambda$ of 3.7 mm (see Figure 9, Kuriyama and Suzuki, 1976a). Bearing in mind that tissues at the end of gestation must be properly categorized to take into account changes due to gap junction formation, these values are very similar to mine.

My results differ from those of Zelcer and Daniel (1979) who did not identify any change in $\lambda$ at parturition. I believe that the values reported in this study accurately reflect the changes that occur at parturition. Several factors may account for the contradictory results. I used longer and wider muscle samples aligned so that the decay of potentials was measured in the ovarian-cervical direction, and the experiments were conducted at 30° C. In addition, I consistently maintained the samples at their in vivo, distended length (see Methods). I have not systematically analysed the effect of the first three factors but the amount of stretch imposed on samples of myometrium did influence the measured cable parameters (see Appendix 3). Because of the experimental protocol used to ensure consistent alignment and stretch, these variables did not enter into my experiments, allowing me, I believe, to resolve the increase in $\lambda$ at parturition.

The degree of stretch placed on smooth muscle tissues in vitro is an important variable that has not always been adequately acknowledged and controlled for in studies of cable properties. The possibility has been raised that variations in stretch may have contributed to the large scatter in values of $\lambda$ reported for various
smooth muscle (Tomita, 1975). My procedure for maintaining the muscle at its in vivo length during experiments may not be universally applicable (for e.g. if a particular smooth muscle exhibits marked variation in its length in vivo) but it is an attempt to deal with this problem systematically.

The λ in pregnant myometrium is many times the length of each cell (300-600 μm), as is the case for many smooth muscle tissues (Tomita, 1975) and single smooth muscle cells (Singer and Walsh, 1980). The relatively good coupling before term existed when gap junctions were either present in low frequency or were absent. In view of the widely held belief that the gap junction is the morphological correlate of low resistance coupling (Bennett and Goodenough, 1978), the basis of the coupling before term is uncertain. Perhaps only a very small area of gap junction contact between smooth muscle cells is required for coupling. The increased frequency at parturition would serve, as I have suggested, to further reduce the junctional resistance between cells. Alternatively, coupling could occur in the absence of visible gap junctions. Small gap junctions, undetectable by thin section electron microscopy, may exist (see Williams and DeHaan, 1981) or other morphological structures may provide low resistance pathways for current flow between cells (Daniel et al., 1976). Whether gap junctions are necessary for cell coupling is a subject of debate, but available evidence, including results described in this investigation, suggests that the junctions are sufficient for coupling.

The length constant has been defined as \( \lambda = \left( \frac{r_m}{r_i} \right)^{1/2} \), or if we consider the specific cable parameters, \( \lambda = \left( \frac{R_m a}{R_i} 2 \right)^{1/2} \), where \( r_m = \).
\( R_m/2\pi a, r_1 = R_1/\pi a^2 \). The cable radius (a) is the radius of the individual smooth muscle fibers. An increase in the specific membrane resistance, a decrease in the specific internal resistance or increased cable radius could be the cause of the change in \( \lambda \) observed at term. I have assumed that the fiber radius is constant before term and at delivery, having no reason to believe that cells of the myometrium undergo any significant growth at the end of pregnancy (Afting and Elce, 1978). (If any change at all is occurring, the cells are being stretched further by the growth of the fetuses, so the the cable radius should decrease. This would cause a change opposite to that I have observed.)

Membrane Resistance

The increase in \( \lambda \) at delivery can be attributed in part to an increase in membrane resistance (Table VII). Consistent with my observation at delivery, several workers have reported that membrane depolarization in myometrium is accompanied by an increase in membrane resistance (Bülbring and Kuriyama, 1973; Kuriyama and Suzuki, 1976a). Tomita and Watanabe (1973) have suggested that the increased membrane resistance which precedes active membrane responses may be the result of a decreased potassium conductance. This relationship of decreased membrane potential and increased membrane resistance is also suggested by the results of Kanda and Kuriyama (1980) who showed regional differences in myometrium. Smooth muscle overlying placental regions exhibited more negative resting potentials and smaller \( \lambda \), when compared to muscle from nonplacental sites. The larger \( \lambda \) in nonplacental sites may have resulted from increased membrane resistance.
I observed membrane time constants that were similar to those reported by Kuriyama and Suzuki (1976a). The larger $\tau_m$ at delivery (Table VII) was probably the result of the increased membrane resistance. Both parameters increased by the same amount (approximately 45%) from before term to delivery.

If a fiber radius of 5 $\mu$m is assumed, the values of specific internal resistance (from chapter 3) and $\lambda$ can be used to derive estimates of specific membrane resistance ($R_m$). The membrane time constant is given by the relation $\tau_m = R_m C_m$, so estimates of membrane capacitance were also made. Before term $R_m$ was 87 k$\Omega$cm$^2$ and $C_m$ was 1.8 $\mu$F/cm$^2$. At delivery $R_m$ was 130 k$\Omega$cm$^2$ and $C_m$ was 1.7 $\mu$F/cm$^2$. These values are subject to considerable error, but are within the range found for a variety of smooth muscle tissues (Tomita, 1975), including, myometrium (Vassort, 1981) and single gut smooth muscle cells (Singer and Walsh, 1980).

Internal Resistance

The second factor which contributed to the change in $\lambda$ at delivery was the 33% decrease in internal resistance (per unit length) from before term to delivery (Table VII). This finding agrees reasonably well with the 26% decrease in internal resistivity ($R_i$, also termed $R_0$ Hz) determined in the impedance studies (Chapter 3). (Relative changes of the two units of internal resistance are directly comparable, having assumed earlier that the cable radius is constant). Thus, two independent experimental methods for assessing the internal resistance of myometrium have yielded similar results. The internal resistance of myometrium is composed of the resistance of the myoplasm
of individual cells plus the resistance of junctions between cells. From the data presented here, I am unable to determine which component caused the decrease in $r_1$ at delivery. However, in the impedance study (Chapter 3) I showed that the myoplasmic resistance was constant and that the junctional resistance decreased by approximately 60% from before term to delivery. With this evidence to support the (usual) assumption of constant cytoplasmic resistivity, I interpret the decrease in internal resistance measured in these experiments to indicate decreased junctional resistance between muscle cells.

Therefore my results provide electrophysiological evidence for closer coupling of myometrial cells during delivery. Taken together with ultrastructural evidence also reported here, these data support the hypothesis that gap junction formation causes an improvement in electrical coupling in parturient myometrium.

Gap junction formation might also serve to improve metabolic coupling (Hooper and Subak-Sharpe, 1981) between cells of the uterus. Passage of compounds other than inorganic ions between cells may certainly play a role in the development and maintenance of labour contractions.

I suggest that improved coupling of myometrial cells is one factor involved in the transition of the uterus from a relatively quiescent organ to one that expels the fetal contents in an orderly manner. In principle, a transition of this sort is not unique, and several reports have correlated changes in the mechanical activity of muscle with alterations in the electrical properties. Some normally quiescent smooth muscles respond to treatment with tetraethylammonium
ion by developing phasic contractions, representing the synchronous mechanical activity of many muscle cells. This transition has been associated with increased $\lambda$ (Kroeger and Stephens, 1975), increased membrane resistance and decreased internal resistance (Haeusler and Thorens, 1980). The latter change could be related to an increased incidence of gap junctions between smooth muscle cells (Kannan and Daniel, 1978). Denervation induced phasic activity of vas deferens is accompanied by an increased $\lambda$ in some tissues, although the basis of the change is not clear (Goto et al., 1978). The onset of synchronous beating of heart cell aggregates has been correlated with a decrease in coupling resistance, which is thought to be associated with increased area of gap junction contact between the cells (Clapham et al., 1980).

It is apparent, therefore, that regulation of mechanical activity of several muscle syncytia, including myometrium, can be achieved by modulation of junctional communication. This could involve modulation of the number, size or functional state of existing gap junctions (for review, Hertzberg et al., 1981).

Not only the longitudinal, but the circular muscle layers of the rat uterus exhibit the characteristic transition of electrical and mechanical activity that culminates in normal parturition (Anderson et al., 1981). Gap junctions appear at parturition in both muscle layers in approximately equal numbers (Chapter 2). Furthermore, in all other species studied to date, increasing numbers of gap junctions between myometrial cells accompany the evolution of coordinated activity at labour. This includes mice (Dahl and Berger, 1978), guinea-pigs, sheep and humans (Garfield et al., 1979a; b; 1982a). Electrophysiological
changes of the sort described here may accompany the structural changes in these other instances. The widespread occurrence of gap junction formation at parturition attests to the fundamental nature of the phenomenon. On a speculative note, this mechanism of regulating uterine activity may be of general significance, universal amongst eutherian mammals (those having placentas).

Finally, it is worth emphasizing that many factors are involved in the initiation and progression of labour (Liggins, 1979; Thorburn and Challis, 1979; see Chapter 1). Hormones and prostaglandins act directly on smooth muscle cells to regulate excitability and contractility. Improved ionic coupling resulting from gap junction formation represents just one mechanism that may operate to ensure synchronous activation of the myometrium and safe, effective delivery of the young.
CHAPTER 5

General Discussion
Distinctive Nature of this Research

In this study I have documented the formation of gap junctions between uterine smooth muscle cells. Quantitative thin section electron microscopy of samples of rat myometrium revealed that no gap junctions were visible between uterine smooth muscle cells at day 14 of gestation. Gap junctions were present in the myometrium of some animals fixed at 20–22 days of gestation and gap junctions were present in all tissues from parturient animals and from animals shortly post partum. I interpret this distribution of gap junctions to be evidence that gap junctions are present only in the final stages of gestation. Since the timing of delivery varies, the occurrence of gap junctions before term would also vary.

The structural change in the myometrium coincides with, and may be associated with, the conversion of uterine behaviour at term i.e., from a relatively quiescent organ to one that contracts forcefully to expel its contents. The evidence that gap junctions are related to the activation of the uterus is circumstantial. Gap junctions were present in increased numbers when uterine motility was increased. Furthermore, treatments that block activation of the myometrium inhibited gap junction formation, as is summarized below.

1) Gap junctions were present in both the gravid and nongravid horn at the time of parturition (Chapter 2).

ii) Gap junctions were present in the myometrium of aborting animals (Chapter 2; Garfield et al., 1982b).
iii) Ovariectomy induced premature labour in rats was blocked with progesterone, and gap junction formation was inhibited (Chapter 2; Garfield et al., 1982b).

iv) Administration of estrogen to nonpregnant animals causes synchrony of uterine activity and can promote gap junction formation. (Marshall, 1959; Fuchs, 1973; Kao, 1977; Dahl and Berger, 1978; Merk et al., 1980; MacKenzie and Garfield, personal communication). (These two findings have not been examined in the same experiments, so this association is tenuous).

v) Administration of progesterone at term blocks labour and inhibits gap junction formation (Garfield et al., 1978).

vi) An increased incidence of gap junctions has been observed at parturition in every animal species studied to date (Garfield et al., 1979a and b; 1982a).

Thus, in many of the situations examined, increased frequency of gap junctions between uterine smooth muscle cells is associated with synchronization of uterine activity. A similar association has been made in other smooth muscle types, as described in chapter 4 (Kannan and Daniel, 1978; Haeusler and Thorens, 1980). Burden et al. (1979) examined the effects of severing the nerve trunk innervating the uterus of pregnant rats. Gap junctions formed in denervated uteri, but delivery of young was delayed and prolonged, leading to the suggestion that gap junction formation was not sufficient for safe delivery of young. Failed labour may have been due to other effects of nerve section, such as delay in the process of cervical maturation. This suggestion was made because the fetuses became entrapped in the cervix
during delivery.

In view of the role that gap junctions are thought to play as mediators of cell-to-cell communication, the proposal was made that gap junction formation might regulate activation of the myometrium. Improved communication between individual cells may permit propagation of action potentials through the myometrium and result in synchronous excitation. Thus, gap junction formation may represent an important step in the sequence of events leading to the onset of parturition.

This hypothesis is a novel explanation of the transition of the uterus at term, but does have some precedent. The progesterone block hypothesis advanced by Csapo (1962; 1977) proposed that high levels of progesterone impaired the excitation and electrocoupling within the myometrium throughout gestation. The mechanisms of the block were uncertain (Kao, 1977). A decline of plasma or tissue progesterone levels would somehow release the inhibition and facilitate the initiation and spread of excitation. The absence of gap junctions throughout gestation and their formation at the end of term could be the basis of the progesterone block theory (Garfield et al., 1978; 1982b).

A principal objective of this thesis has been to examine the electrical significance of gap junctions in the myometrium at parturition. I have established a temporal correlation between increased area of gap junction contact and changes in the passive electrical properties of the tissue. Measurements of the longitudinal impedance of uterine smooth muscle were interpreted as indices of the internal resistance of the myometrium (Chapter 3). Comparison of the longitudinal impedance before term and at delivery revealed that the
effective resistivity, or internal resistance at zero Hz, was 26% lower in parturient muscle. The high frequency impedance, thought to represent the myoplasmic component, was approximately the same before term and at delivery. The difference between the low and high frequency impedance was assumed to represent the junctional resistance between cells, which decreased 60% from before term to delivery. 1 1/2 to 2 days post partum, a time at which few, if any, gap junctions remain in the myometrium (Garfield et al., 1978; Berezin et al.) the junctional resistance, as well as the myoplasmic resistance had increased significantly. Therefore, the results of the longitudinal impedance study demonstrated a sequential decrease then increase in the junctional resistance, accompanying the presumed appearance and disappearance of gap junctions.

The decrease in the internal resistance of myometrium from before term to delivery was verified with an independent method (Chapter 4). The cable parameters were measured with an intracellular microelectrode technique and estimates of the internal resistance per unit length decreased by 33% from before term to delivery. Assuming that the cable (fiber) radius remained constant from before term to delivery [and evidence indicates that growth of the myometrium has levelled off at the end of gestation (Afting and Elce, 1978)] the 33% change in internal resistance per unit length ($r_i$) is directly comparable to the 26% decrease found in the impedance studies (see below). The close agreement between the findings of two independent methods is reassuring.

Quantitative thin section electron microscopy confirmed that a
significantly greater area of gap junction contact existed between muscle cells at parturition than before term. Therefore, the results show that increased area of gap junction contact is associated with decreased internal resistance of the tissue. These results support, but do not prove, the hypothesis that gap junction formation at the end of gestation causes improved coupling between smooth muscle cells.

It is worth considering how improved coupling could promote synchronization of excitation in the uterus. Studies of the passive electrical properties of excitable tissues are relevant to studies of impulse conduction through a coupled network, since the passive spread of current generated by the action potential is responsible for propagation of the impulse (Katz, 1965; Cole, 1968; Tomita, 1975; Fozzard, 1979).

Action potentials in the myometrium are initiated at discrete pacemaker areas (Marshall, 1959; Lodge and Sproat, 1981). Successful propagation of an action potential into surrounding, non-pacemaker, regions is determined by the excitability characteristics of the surrounding cells and the passive cable properties of the tissue, including the coupling resistance between cells. Other factors remaining constant, the amount of local circuit current which flows into neighbouring cells is inversely proportional to the junctional resistance. Therefore, improved coupling (decreased junctional resistance) between cells would facilitate the spread of local circuit currents, promoting action potential propagation and excitation throughout the tissue. Such a relationship has been demonstrated for cultured heart cell aggregates (Clapham et al., 1980). Synchronization
of action potentials was directly related to the decrease of junctional resistance between clumps of cells.

The preceding discussion has served two purposes. First, I have reviewed the salient findings from the experimental chapters of this thesis. The formation of gap junctions in the myometrium at parturition was associated with activation of the uterus. Furthermore, the presence of gap junctions was associated with improved coupling in the myometrium. Second, I have described a plausible connection between improved coupling and enhanced propagation of action potentials throughout the uterus. Therefore, my experimental results provide a credible explanation for the evolution of synchronous electrical activity in the parturient uterus.

**Control of Gap Junction Formation**

Regulation of gap junction formation is intrinsically interesting, but tangible rewards might be achieved if information regarding control of gap junction formation were to have clinical relevance. The capacity to regulate gap junctions could be of value in the management of pregnancy, for it may lend itself to a rational means of intervention in cases of abnormal labour.

Several experiments reported here have provided the groundwork for more detailed investigations into the mechanisms existing in vivo to control gap junction formation. Unilateral ovariectomy of rats prior to conception resulted in fetal development in the intact, but not the sterile horn. Gap junctions were present in both gravid and nongravid horns during parturition. This observation indicates that the stimulus
for gap junctions to form was systemic in nature, being common to both horns. The fetoplacental unit or stretch of the uterine wall was not necessary for gap junction formation, but may have influenced the process since fewer gap junctions were present in the nongravid horn.

Some further clues to the factors responsible for control of gap junction formation were obtained from experiments which involved bilateral ovariectomy of midterm pregnant rats. Removal of the endogenous source of estrogen and progesterone resulted in the termination of pregnancy, which was accompanied by the formation of gap junctions. Replacement therapy of estrone and progesterone significantly reduced the number of gap junctions in the muscle. In a similar experiment, ovariectomy of rats was shown to cause withdrawal of progesterone and estrogen from the plasma and uterus, while administration of these steroids maintained the hormonal levels. Only when progesterone levels were abnormally low did gap junctions form in the myometrium (Garfield et al., 1982).

In other studies, both in vivo and in vitro, and in other species besides the rat, gap junction formation in myometrium has been shown to be subject to a wide range of controlling factors. Collectively, the results suggest that estrogen and some prostaglandins or endoperoxides may promote gap junction synthesis. At least in some species, progesterone appears to inhibit gap junction formation.

There is convincing evidence that gap junction formation is influenced, if not regulated, by the various hormones and prostaglandins. Endocrine control of parturition is known to involve a multitude of stimulatory and inhibitory mechanisms in both the fetal and
maternal compartments (Nathanielsz, 1978; Thorburn and Challis, 1979). A good correspondence exists between the timing of gap junction formation and the timing of hormonal changes in vivo (e.g. Garfield et al., 1979b; 1982b). Thus, control of gap junction formation is integrated into the cascade of endocrine events that occurs at parturition.

Mechanisms for regulating gap junction disappearance in myometrium are inadequately understood and warrant further study. Two possible mechanisms for degradation of gap junctions are thought to exist. One involves internalization of whole junctions (see Albertini and Anderson, 1975) and the other involves the dispersal of channel units throughout the surrounding membrane (e.g. Lane and Swales, 1980). The disappearance of gap junctions post partum can occur in the absence of ovaries, so an ovarian factor does not appear to regulate disappearance (Berezin et al.).

**Associating Increased Area of Gap Junction Contact With Improved Coupling in Myometrium**

Changes in the longitudinal impedance and the cable properties of myometrium at parturition have been documented in Chapters 3 and 4. The results of the studies indicate that the internal resistance of parturient myometrium was less than that before term or post partum. Having established an (inverse) association between a pair of variables (frequency of gap junctions and internal resistance) I have suggested that a causal relation may exist between the two. Clearly, this inference must be accepted with caution, because no direct evidence for
a cause-effect relationship was presented.

More than one variable may have changed from before term to delivery, leading to an illusory correlation between gap junction formation and decreased internal resistance. Other factors certainly did change with the passage of time from before term to the onset of delivery. For example, the membrane potential was shown to decrease and the membrane resistance increase. However, there are no compelling reasons to believe that either of these two factors in particular could cause a change in the internal resistance. And, at the risk of making a circular argument, much evidence from other studies suggests that gap junction formation would cause improved coupling, which would result in a decrease in internal resistance.

It is impossible to rule out all other factors as possible sources of interference when dealing with an in vivo phenomenon such as gap junction formation. The best possible comparison was made, comparing tissues from animals just before term and shortly post partum to those actually delivering. Long term changes due to fetal and myometrial growth were eliminated, as much as possible. Other physiological changes of parturition could not be eliminated. With this concession in mind, I should emphasize the importance of validating any finding with independent methods. The hope, of course, is that different methods might be subject to different sources of bias. For this reason it is reassuring that the impedance and cable results were in close agreement.

It is appropriate here to stress the potential value of studying gap junction formation in vitro. Physiological changes that may
accompany the structural change might be examined in relative isolation from the multitude of changes that occur in vivo. One might better be able to attribute any change in electrical or metabolic properties to the presence of gap junctions in the muscle. However, it is possible that the functioning of gap junctions may be altered as a result of the in vitro culture system.

Another source of uncertainty concerns the functional state of the gap junctions observed in the myometrium. The functional coupling of cells is known to vary, depending on cytoplasmic Ca$^{++}$ or H$^+$, and also transjunctional potential (Loewenstein, 1981; Spray et al., 1979 and 1981). It is impossible to distinguish between the conducting and nonconducting states of gap junctions with thin section electron microscopy (or, likely, even with freeze fracture microscopy, see Chapter 1). Therefore the question arises, were the gap junctions that I observed in myometrium functional? No direct answer is available. (I do not intend to introduce the argument that any "fixed" sample is non-functional). Uncoupling of cells occurs in response to tissue injury, but proper handling and care of tissues (maintaining proper pH, oxygen levels, lack of trauma etc.) are factors conducive to a healthy state. The health and vigour of my samples was evident from their appearance in the electron microscope and the large membrane potentials. Therefore, the implicit assumption throughout has been that the conditions were at least suitable for functional coupling by gap junctions. Furthermore, my results demonstrated without question that some coupling existed in the tissue. Whether good electrical communication between cells exists in vivo is uncertain, and warrants
further investigation.

A Model for Predicting the Change Brought About by Gap Junction Formation

It is worth considering a model to predict the change in internal resistance that would result from the increase in gap junction area found in Chapter 4. The geometry is simplified if I assume that I am dealing with a cable composed of individual cells arranged end-to-end. Also, assume that all longitudinal current along the core passes between cells through gap junctions and that the relative increase in gap junction area observed in the ultrastructural studies is representative of increased junctional contact at the end of cells. Using a formula similar to one described by Spira (1971) for cardiac tissue, the internal resistance \( r_i \) of the cable can be written as

\[
 r_i = r_{myo} + r_{junction}
\]

When \( r_i = \frac{R_i}{\pi a^2} \), \( r_{myo} = \frac{R_{myo}}{\pi a^2} \) and \( r_j = nR_{gj}/\pi a^2 \).

\( R_{myo} \) is the specific resistance of the myoplasm (\( \Omega \text{cm} \)), \( R_{gj} \) is the specific resistance of gap junctional membrane (\( \Omega \text{cm}^2 \)), \( n \) is the number of cells per unit length (cm\(^{-1} \)), \( a \) is the cable radius (cm) and \( f \) is the fractional area of the apposed cell membranes occupied by gap junctions.

If the cable radius is constant, the ratio of \( r_i \) (before term) to \( r_i^* \) (delivering) (or \( R_i/R_i^* \)) simplifies to

\[
 r_i/r_i^* = (R_{myo} + nR_{gj}/f)/(R_{myo}^* + n^*R_{gj}^*/f^*)
\]

Assuming that \( R_{gj} = R_{gj}^* \), \( n = n^* \), \( R_{myo} = R_{myo}^* \) and, from the ultrastructural data, \( f^* = 48f \), then

\[
 r_i/r_i^* = (R_{myo} + nR_{gj}/f)/(R_{myo} + nR_{gj}/48f)
\]
The fraction $nR_{ij}/f$ corresponds to the junctional resistivity $(R_j, \Omega \text{cm})$, examined in Chapter 3.

$$r_1/r_1^* = (R_{myo} + R_j)/(R_{myo} + 0.02R_j)$$

The value of the ratio can be determined if relative values of $R_j$ and $R_{myo}$ are assigned. If we set $R_j = R_{myo}$, (as shown for before term tissues in the impedance study), then for any value of myoplasmic resistivity, the ratio $r_1/r_1^* = 1.96$. The predicted ratio is 24% larger than the actual ratio of $r_1/r_1^* = 1.49$ determined by the length constant study, and 31% larger than the ratio of $R_1/R_1^*$ determined by the impedance study. Therefore, I did not observe as large a decrease in internal resistance as predicted from the ultrastructural studies and this model. If, in the above analysis, the $R_j$ is less than $R_{myo}$, then the predicted ratio approaches the observed. Alternatively, if $R_j$ is greater than $R_{myo}$, then the predicted values would deviate by an even greater amount from the ratios that I obtained.

My initial choice of $R_{myo} = R_j$ was, in fact, what I found to hold true for before term muscle in the impedance study. Observations in coupled neurons reveal a similar situation. The resistance of the cytoplasm is about the same as the resistance of the electrotonic junction between two neurons (Johnson and Ramon, 1981).

If the junctional resistance alone is considered, I measured a decrease of 62% from before term to delivery. The ultrastructural data would have lead to predicted reduction of 98%.

Several factors could contribute to some deviation between the predicted ratio of internal resistance and the actual ratios. The value describing the increase in gap junction area ($48 X$ increase at delivery)
is subject to considerable uncertainty because such a small and variable number of gap junctions were observed in the myometrium from animals before term. The addition or removal of just a few junctions would result in a substantial change in the ratio.

In addition, the possibility exists that the increase in gap junction area observed does not accurately reflect the increase in junctional area contributing to longitudinal current flow. Recognizing that all the gap junctions observed in this study were oriented "side-to-side," the possibility must be considered that a portion of the junctions may facilitate radial current flow only, and not contribute to the longitudinal spread of potential. A schematic model of longitudinal current flow in a smooth muscle syncytium through lateral junctions was shown in Figure 1 of Chapter 1.

It is worth noting that gap junctions between cardiac muscle cells are located over the entire cell surface, but the density is greatest in the intercalated disc regions (Spira, 1971). Gap junctions in the discs are usually situated on folds of the cell membrane. Most gap junctions in the discs, and all those on the sides of cells, are oriented parallel to the longitudinal axis of the fibers. Therefore, on a microscopic level, apposing cardiac fibers are joined "side-to-side" by gap junctions. On a macroscopic level however, such junctional organization contributes to effective coupling of cells in the longitudinal axis (Fozard, 1979). In two studies in which this variable was examined, gap junctions were found to be distributed more-or-less evenly over smooth muscle cells (Gabella and Blundell, 1979; 1981).
Koide (1967) has developed a network model of smooth muscle in which cells are arranged with half the length of each cell overlapping the next cell. In such a model the junctions are lateral, rather than end-to-end, as I have assumed. Altering my model to incorporate this refinement would more realistically describe the physical situation, since I do observe lateral junctions exclusively and the cells do overlap in length. A different factor, to replace the number of cells per unit length, would be required. But in a comparative analysis, as presented here, no numerical improvement would derive from such a modification, because this factor is common to both parts of the ratio and cancels out.

Another assumption of this comparative analysis was that longitudinal current flowing between cells did so exclusively by way of gap junctions. It has been argued that this may not be so (Daniel et al., 1976; Loewenstein, 1981; Williams and DeHaan, 1981). If a parallel low resistance pathway (unspecified in nature) were to exist between cells, a large increase in gap junction area could result in only a moderate change in total junctional communication, as I have observed. (This follows from the calculation of total junctional resistance being the combined resistance of two parallel pathways).

A final possible cause of the deviation is that the techniques used to evaluate the internal resistance may not have revealed the true change in cell-to-cell coupling that occurred at parturition. This possibility seems unlikely in view of the close agreement between the impedance and cable studies, but warrants further study. Some different experimental approaches to examine the problem of changes in coupling at
parturition are described below.

Conclusions

I will recapitulate the objectives of this thesis that were described in Chapter 1 and summarize my conclusions.

i) What is the time course of gap junction formation?

Gap junctions were shown to be present between uterine smooth muscle cells immediately prior to, during, and for a short time following parturition in the rat. During parturition, gap junctions were present at a frequency of approximately 6 per 1000 μm of nonjunctional membrane, comprising 0.25% of the total cell surface area.

ii) What controls gap junction formation?

The appearance of gap junctions in myometrium was governed by systemic factors. Distention of the uterine wall or presence of the fetuses were not necessary, but they may have influenced gap junction formation. Bilateral ovarioectomy of midterm pregnant rats resulted in premature termination of pregnancy and gap junction formation. Both effects were blocked by administration of progesterone and estrogen. These results suggest that progesterone withdrawal at the end of gestation in the rat may be one factor regulating gap junction formation at parturition.

iii) What is the functional significance of gap junction formation?

Two independent methods were used to test the hypothesis that gap junction formation at parturition results in improved electrical communication between uterine smooth muscle cells. Measurements of the longitudinal impedance were used to estimate the internal resistance of
myometrium. Impedance analysis showed that the specific resistance of the cytoplasm of myometrial cells was constant from before term to delivery, but the junctional resistance decreased. Shortly post partum the junctional resistance increased. Cable analysis confirmed that the internal resistance of myometrium was lower at parturition than before term. Thus, improved cell-to-cell communication was associated with a demonstrated increase in gap junction contact between smooth muscle cells. These results are consistent with the hypothesis that gap junction formation at the end of gestation results in improved electrical coupling of uterine smooth muscle cells.

Further Studies

I have described studies that establish a temporal correlation between increased number of gap junctions and changes in the passive electrical properties of smooth muscle. Furthermore, I have suggested that changes in the passive properties could influence the spread of action potentials, which in turn would cause synchronous activation of the muscle. Both of these topics require further study in order to elucidate the functional significance of gap junction formation at parturition.

It is desirable to establish a causal relationship between gap junction formation and coupling in uterine smooth muscle. Short term modulation of gap junctional communication has been shown in other systems to be under the control of various agents. If smooth muscle cells could be reversibly uncoupled with some treatment (e.g. by increasing intracellular Ca²⁺ or H⁺), then a causal relationship between
gap junctions and coupling in smooth muscle could be deduced. This particular approach has been used in cardiac muscle (Weingart, 1977; Fozzard, 1979) but has not yet been attempted with smooth muscles. Knowledge of the factors that regulate coupling in smooth muscle will undoubtedly be forthcoming, and will lead to more definitive studies. Ideally, junctional permeability could be regulated in all the following experiments in which the role of gap junctions in myometrium is investigated.

Several other experimental techniques could be used to corroborate the changes in cell-to-cell coupling reported here. The passage of labelled compounds between cells, both fluorescent and radiolabelled molecules, is routinely used to monitor coupling in other systems (Bennett, 1977; Hooper and Subake-Sharpe, 1981; Loewenstein, 1981). Application of tracer methodology to studies of coupling in smooth muscles would require that some technical difficulties be resolved. But such an approach would be of value because it would be independent of the electrical methods that predominate in this field.

The propagation velocity of action potentials in cardiac tissues depends upon the local excitability characteristics and the cable characteristics, which includes the cell-to-cell coupling resistance of the core (Fozzard, 1979). Changes in the degree of cell-to-cell coupling have been shown to alter the propagation velocity of action potentials (Weingart, 1977; Sharp and Jöyner, 1980). A decrease in the internal resistance of myometrium, attributable to gap junction formation, may be evident as an increased propagation velocity of evoked action potentials.
Finally, one last technique will be considered that should be applicable to the problem of coupling in uterine smooth muscle. Measurements of the input resistance of biological samples can be obtained with a single microelectrode, by injecting current into a cell and simultaneously measuring the voltage response (utilizing a bridge circuit). The input resistance $R_o$ is the resistance "seen" by the tip of the electrode and is defined by the ratio of voltage/current. In a single, isopotential cell, $R_o$ is determined solely by the membrane resistance ($R_o = \text{membrane resistance } R_m / \text{area of membrane}$, neglecting any effects due to leakage around the electrode tip). However, in a 2 or 3 dimensional synoytium measurements of $R_o$ by this method are remarkably insensitive to the membrane resistance ($R_o \propto R_m^{1/4}$, Tomita, 1970). Instead, $R_o$ is determined largely by the coupling resistance with the surrounding tissue (Jack et al., 1975). Therefore, the input resistance is quite sensitive to changes in coupling between cells, and can be used to determine the extent of coupling in smooth muscle (Koide, 1966; Holman and Nield, 1979).

Measurements of $R_o$ in myometrium could be made quickly, with relatively uncomplicated electronic instrumentation, and the results could be readily appraised. In a comparative study such as the one considered here, the other factors that could contribute to a change of input resistance must still be recognized. These might include a number of geometrical considerations in addition to the aforementioned membrane resistance.

Additional studies are required to relate the formation of gap junctions to changes in the electrical and mechanical characteristics of
myometrium at parturition. Some questions that could be examined are:

- Do action potentials propagate more readily (or rapidly) through myometrium when gap junctions are present?

- Do various areas of the uterus exhibit truly "synchronous" electrical and mechanical activity during parturition?

- Does the circular muscle layer exhibit changes in the passive or active electrical properties at parturition, which might be associated with gap junction formation?

- Do gap junctions play a vital role in permitting passage of permeant signal molecules between cells at parturition?

- Do physiological characteristics (action potential propagation, spread of electrotonic potentials, contractions etc.) change gradually with gap junction formation before term, or is there a threshold number of gap junctions, at which point there is a sudden change in electrical characteristics?

- Is it possible to monitor cell-to-cell coupling over an extended period of time while gap junctions form in vitro?
APPENDIX 1

Frequency Dependant Characteristics of a Model Circuit

In this section I will consider the theory behind the impedance responses of the circuit model described in Chapter 3. A similar test circuit is shown in the inset of Fig. 28, composed of a parallel resistor ($R_2$) and capacitor ($C$) in series with a resistor ($R_1$). The response of the circuit to sinusoidal currents varies as the frequency of excitation changes. Both the impedance magnitude and impedance locus forms of presentation are used to illustrate how the values of the circuit elements can be determined experimentally by impedance analysis (Jack et al., 1975; Schanne and Ceretti, 1978).

Alternating current (AC) impedance analysis of any circuit has the major advantage that a steady sinusoidal excitation produces a steady sinusoidal response. The magnitude of the impedance ($|Z|$) is defined as the ratio of the maximum voltage ($V$) to the maximum current ($i$), and has the dimensions of resistance (ohms). While the impedance of a pure resistance is independent of the applied current, the impedance of a capacitance ($Z_C$) is highly frequency dependent. The ability of a capacitance $C$ to pass current is determined by the rate of change of voltage ($dV/dt$). Such that capacity current $i_C = C \frac{dV}{dt}$ and $Z_C = 1/j\omega C$, where $j$ is the complex operator $-1^{1/2}$ and $\omega = 2\pi$ frequency, the frequency in radians per second.

The $|Z|$ of the circuit is shown as a function of frequency in Fig. 28A. These results were obtained for the test circuit described in the
inset of Fig. 28A, with the impedance meter used for the measurements in Chapter 3. The frequency dependant behaviour of the circuit, that is, the decrease in impedance is attributable to the capacitor C.

The total impedance of the circuit is given by

\[ Z = R_1 + \frac{R_2}{(1 + j\omega C R_2)} \]  

**equation 1**

There are two limiting resistances, at low and high frequencies. By inspection of Fig 28A and by substitution into equation 1, \( R_{OHZ} = R_1 + R_2 \) and \( R_{HZ} = R_1 \). Therefore, measurement of the impedance of the circuit at extremely low and high frequencies can yield estimates of the resistive elements.

The voltage across the circuit being considered will lag behind the current flowing by a certain phase angle \( \phi \), due to the time required for charging and discharging of the capacitor. The phase angle also is a function of frequency, as shown in Fig 28B. The single peak in the \( \phi \) versus frequency curve indicates the presence of a single time constant in the circuit.

The presence of the phase lag means that the impedance cannot be directly compared to the resistance, which has no phase lag. The difficulty comparing the impedance with the resistance is overcome by presenting the impedance as being composed of two separate components. These are the **resistive** and **reactive** components (also termed **Real** and **Imaginary** parts, respectively). The equation describing the impedance of the circuit (eq'n 1) can be separated into its real and imaginary parts. The equations are simplified if we use the relation \( \tau = \frac{R_2}{2C} \) to describe the time constant of the circuit.

\[ Z = R_1 + \frac{R_2}{1 + j\omega \tau^2} - j \frac{\omega \tau R_2}{1 + \omega \tau^2} \]  

**equation 2**
FIGURE 28. The model circuit used to display frequency dependant characteristics is displayed in the inset. The values of the circuit elements are as labelled. A). The impedance magnitude decreased with increasing frequency of stimulation, but was independent of applied frequency at extremes of low and high frequencies. B). The phase angle (\( \phi \)) of the test circuit is shown as a function of frequency. The single peak indicates the presence of a single time constant. C). The impedance locus form of presentation combines both the impedance magnitude and phase responses. One point is shown as the vector position of a point in a plane, composed of the real and imaginary parts of the impedance (see the text). The distribution of different frequencies describes a semicircle with the center on the real axis.
This is the sum of the real (R) and imaginary (X) parts of the impedance in the form of \( Z = R + jX \). The imaginary part is distinguished by the \( j \) factor and is negative when dealing with a capacitive reactance.

The real and imaginary components defined in equation 2 can be presented graphically as the impedance locus (Fig. 28C), which, in principle, describes the \( Z \) at each frequency as the vector position of a point on a plane. The resistive component \( R \) is plotted on the abscissa and the reactive component \( X \) is plotted on the ordinate (by convention, \(-X\) is above the real axis). Any pair of values \( R \) and \( X \) will specify a point in the plane which completes a right angle triangle. The distance from the origin to the point is the magnitude of the impedance (\(|Z|\)). The angle formed with the \( R \) axis is the phase angle \( \phi \) between voltage and current. \( R \) and \( X \) are related such that

\[
|Z| = (R^2 + X^2)^{1/2} \quad \text{and} \quad \tan \phi = X/R
\]

Alternatively, \( R = |Z|\cos \phi \) and \( X = |Z|\sin \phi \)

Equation 2 describes a semicircle (Fig. 28C) when the frequency is changed between zero and infinity. This curve is called the impedance locus. When \( \omega = \infty \), reactance is zero and \( Z = R \). When \( \omega = 0 \), reactance again is zero and \( Z = R_1 + R_2 \). The impedance locus can be determined analytically from equation 2 or experimentally. The maximum reactance of the curve will occur at the point \( \omega = 1/\tau \), which falls at 1/2 \([R_1 + (R_1 + R_2)]\). This relation allows the time constant \((\tau)\) be determined from the locus.

In practice, the impedance loci obtained for many biological tissues, including myometrium are semicircular, but differ from that
shown in Fig 28C, in that centers of the semicircles are depressed below the real axis. The explanation for this deviation is not certain (Schanne and Ceretti, 1978), although the presence of a constant phase shift element (Cole, 1968) or inhomogeneity of the sample (e.g. Poon and Choy, 1981) has been suggested to be the cause. In Chapter 3 the values of circuit elements $R_1$ and $R_2$ were determined by the points of intersection of the locus with the real axis. By definition, the points of intersection were purely resistive, there being no capacitative reactance.

APPENDIX 2

Equations and Assumptions of One-Dimensional, Linear Cable Theory

Several authors have provided the solutions for the transmembrane voltage responses of infinite, one-dimensional linear cables to external polarization (Hodgkin and Rushton, 1946; Katz, 1966; Jack et al., 1975). If a sufficiently long sample of smooth muscle is polarized with external electrodes, using the partition stimulation method of Abe and Tomita (1968), the muscle can be modelled as an infinite cable with uniform core conductor properties (Abe and Tomita, 1968; Tomita, 1970; Bennett, 1972; Bywater and Taylor, 1980). Some of the equations describing one-dimensional cable theory will be developed and then some assumptions of the cable model, as they apply to smooth muscle, will be described.

Models to describe current flow in a cable and the equivalent circuit are shown below.
The Steady State

If a signal of steady state amplitude \( V \) is applied at one point along the cable, its forward spread depends on the amount of longitudinal current \( i_{\text{long}} \) which flows along the core. [Note: using the partition stimulating method of Abe and Tomita (1968) the source of current appears to be a point source located at the nearest stimulating electrode]. The spread of potential \( dV/dx \) is described by

\[
i_{\text{long}} = -\frac{1}{r_i} \cdot \frac{dV}{dx}
\]

where \( x \) is the distance along the cable. The negative sign indicates that the direction of electrical current is positive along a falling (negative) gradient of potential.

The longitudinal current diminishes with distance, since some current leaks out across the membrane (\( i_m \)). This loss is described as

\[
- \frac{di_{\text{long}}}{dx} = i_m = \frac{V}{r_m}
\]

Combining equations 1 and 2

\[
\frac{r_m}{r_i} \cdot \frac{d^2V}{dx^2} = V
\]

The solution of this differential equation is given by Katz (1966) as
\[ V = A \exp\left(\frac{-x}{(r_m/r_i)^{1/2}}\right) + B \exp\left(\frac{x}{(r_m/r_i)^{1/2}}\right) \]

equation 4

where A and B are constants.

As \( x \to +\infty \), \( V \to 0 \) (i.e. the signal decays to zero infinitely far away) and the factor \( B=0 \). According to common nomenclature \( V=V_o \) at \( x=0 \), and the solution of equation 4 is

\[ V = V_o \exp\left(-\frac{x}{(r_m/r_i)^{1/2}}\right) \]

equation 5

The ratio \( (r_m/r_i)^{1/2} \) is termed the length constant \( (\lambda) \). Strictly speaking, \( \lambda = (r_m/r_i + r_e)^{1/2} \) but the resistance of the extracellular fluid can usually be ignored, as described in the assumptions, below.

For the special case when \( x=\lambda \), equation 5 simplifies to

\[ V_{\lambda} = V_o e^{-1} \]

Therefore the voltage signal that is imposed decays exponentially with distance, falling to \( 1/e \) (approximately 37\%) of its initial value \( (V_o) \) at one length constant.

**Time Dependent Changes in Potential**

The equations thus far have considered the steady state voltage across the cable membrane. If we consider the time-dependent properties of the cable, due to the presence of a membrane capacitance \( (C_m) \) in parallel with the membrane resistance, the cable equations are similar to those above. The extra complexity is explained by the fact that total membrane current \( (I_m) \) is time dependent, and is the sum of ionic and capacity currents \( (i_o) \). The amount of current which flows "across" a capacitance is determined by the value of the capacitance and the rate of change of voltage, such that

\[ i_c = C_m \cdot \frac{dV}{dt} \]
Total membrane current is the sum of ionic and capacity currents given by

\[ I_m = V/r_m + C_m \cdot \frac{dV}{dt} \]

The equivalent form of equation 3, when the capacity current is considered and the equation is multiplied by \( r_m \), is

\[ \frac{r_m}{r_i} \frac{d^2V}{dx^2} = V + C_m r_m \frac{dV}{dt} \]

\[ \frac{r_m}{r_i} \frac{d^2V}{dx^2} = V + C_m r_m \frac{dV}{dt} \]

\[ r_m/r_i \text{ is } \lambda^2 \text{ and } c_m r_m \text{ is the membrane time constant } \tau_m. \]

This can be rearranged to give

\[ -\lambda^2 \frac{d^2V}{dx^2} + \tau_m \frac{dV}{dt} + V = 0 \]

\[ -\lambda^2 \frac{d^2V}{dx^2} + \tau_m \frac{dV}{dt} + V = 0 \]

This is the cable equation, a partial differential equation where the voltage across the membrane (V) is variable and depends upon two independent variables, distance x and time t. If we standardize x and t to dimensionless variables where \( X = x/\lambda \) and \( T = t/\tau_m \), equation 7 becomes

\[ \frac{d^2V}{dx^2} + \frac{dV}{dT} + V = 0 \]

\[ \frac{d^2V}{dx^2} + \frac{dV}{dT} + V = 0 \]

The response of a non-inductive cable to a sudden, steady application of current (I_0) at time T=0, applied at X=0 is described by equation 9 (from Tomita, 1970; Bennett, 1972).

\[ V(x) = \sum_{n=0}^{\infty} \frac{1}{2} \left( \frac{e^{-X}}{X^{1/2}} \left[ 1 - \text{erf} \left( \frac{X}{(2t)^{1/2}} \right) \right] - e^{X} \left[ 1 - \text{erf} \left( \frac{X + T^{1/2}}{(2t)^{1/2}} \right) \right] \right) \]

The general properties of this solution can be made clear by considering some limiting cases.

As \( T \to \infty \), the signal will reach a steady state, \( \text{erf} \ T \to 1. \)

As \( X \to +\infty \) (i.e. the site of measurement is far away), the signal
approaches zero, and equation 9 simplifies to

\[ V_x = V_0 e^{-x/\lambda} \]

Equation 10, which describes the steady state condition, is identical to equation 5, and shows that voltage decays exponentially with distance. On a semi-logarithmic plot this relation is a straight line with slope of $-1/\lambda$. $\lambda$ can be determined experimentally from the measurement of the slope.

At the point of stimulation $V_{x=0} = 1/2 I_0 r_i (r_m/r_i)^{1/2}$

The factor $1/2$ is necessary because one half of the current goes to $+\infty$ and the other to $-\infty$. From this equation we arrive at the definition of input resistance $R_o$, the resistance seen at the site of current injection.

\[ R_o = V_0/I_0 = 1/2 (r_m/r_i)^{1/2} \]

If we consider the time dependent change at $x=0$, then

\[ V(t, x=0) = V(t=\infty, x=0) \text{erf}\left(t/\tau_m\right)^{1/2} \]

The voltage at the point of stimulation rises to 84% of its steady state value in one time constant. The time to reach half-maximum amplitude $T_{1/2}$ (max at $x=0$) = 0.23 $\tau_m$. The value of $\tau_m$ can be determined by measuring the time to reach half-maximum amplitude at the point of stimulation.

Since $\lambda = (R_m a/R_i)^{1/2}$ and $\tau_m = R_m C_m$, then

\[ 2\lambda/\tau_m = (2a/R_m R_i C_m^2)^{1/2} \]

The value of $2\lambda/\tau_m$ is the propagation velocity of the electrotonic potential and can be determined experimentally from a plot of the time to reach half-maximum amplitude versus distance from the point of stimulation. Velocity is given by the inverse of the slope of the line.
Assumptions of the Cable Analysis

The basic simplifying assumptions of cable theory are designed to reduce the spatial dependence of a signal V to one dimension, namely, distance along the length of cable. Some of the assumptions of cable analysis, as they apply to smooth muscle are considered below.

1) The membrane acts passively. That is, the transmembrane impedance and the electromotive force remain constant (as opposed to active membrane, which exhibits changes in the impedance with time, as ion permeabilities change). This assumption can be restated as requiring that the membrane resistance is ohmic (i.e. exhibits linear response characteristics). The membrane response of myometrium was shown to be linear in the hyperpolarizing direction in Chapter 4.

2) The core, or internal medium of the cable is assumed to provide a simple, homogenous, ohmic resistance to electrical current flow. The validity of this assumption clearly is compromised by the multicellular composition of smooth muscle. In addition, the impedance studies of Chapter 3 show that current flow in the longitudinal axis is frequency dependent, therefore the core is not ohmic. The effect of the capacitance oriented in the longitudinal axis is minimal in this analysis for the following reasons. The time constant of the junctions between cells, determined in Chapter 3, was less than 1% of the membrane time constant. Its contribution to the total charging time of the membrane would therefore be negligible. More importantly, in this, as
in many other studies, the steady state response is considered, so the frequency dependance will not cause error in length constant measurements.

3) The extracellular medium is assumed to be of negligible resistance. This is usually true when the tissues are bathed in a large volume of an ionic solution. This assumption can be restated as requiring that the extracellular space is isopotential, for \( R_{\text{out}} \) must be zero if \( V_{\text{out}} \) is zero. If leakage of current from the stimulating compartment causes a voltage change in the recording compartment, it is possible to eliminate this artefact by measuring the difference between the inside and outside potential. For example, Bywater and Taylor (1980) applied this correction to measure the true transmembrane potential.

4) The radial dimensions of the cable must be small with respect to the length constant of the system. This condition must be met to satisfy the requirements of a negligible voltage drop in the radial direction, that is, current flow in the tissue occurs essentially in one dimension only. The cable diameter of uterine smooth muscle cells is approximately 5-10 \( \mu \text{m} \), the cell diameter (Tomita, 1970; 1975). This dimension is considerably smaller than the length constant of 2-4 mm. Individual cells, rather than the strip of muscle, constitute the cable-like structure, owing to the method of extracellular polarization. Assuming uniform polarization of the muscle (see Assumption 5) doubling the size of the sample would cause twice as many parallel cables to be
present, but should have no effect on the measured cable parameters. As such, the tissue size is an arbitrary, experimental variable.

5) Longitudinal current flow must be uniform across the cross sectional area of the cable. This requirement of uniform stimulation is a more specific component of assumption 4, above. Under conditions of uniform, extracellular polarization, there is no radial voltage drop. It is especially applicable to these studies, owing to the method of stimulation and the large samples of muscle employed. Bennett (1972) showed evidence that the amplitude of the electrotonic potentials varied by less than 20% across a strip of muscle, supporting this requirement. It is not known whether the variation that is seen is due to true non-uniformities within the muscle or variations of the quality of electrode penetrations.

6) Membrane capacity is assumed to act as a perfect dielectric.

**APPENDIX 3**

**Effect of Stretch on Cable Parameters**

A) Physiological Contraction of the Uterus is Associated with Changes in Cable Parameters

The degree of stretch imposed on uterine smooth muscle appears to be a variable affecting the electrophysiological parameters measured in vitro with the partition bath technique. In Chapter 4 I described
how all the samples compared had been maintained at their in vivo, distended length. Any effects of stretch were therefore eliminated from that comparison, because such select groups of tissues were examined. In this section I present some data collected from samples of delivering myometrium that were naturally contracted, the fetuses having already been expelled from these regions. In addition, I include results of experiments on samples of myometrium obtained from animals 1/2 to 2 1/2 h post partum (Figure 29). These tissues have undergone considerable shortening, the post partum uterine length being as little as 1/3 to 1/4 of the distended length. The objective of making this comparison was to determine whether in vivo changes in the contractile state of the uterus were accompanied by changes in the cable parameters.

A summary of the physiological parameters of contracted myometrium from delivering and post partum animals is given in Table VIII. For comparison, the corresponding values for distended delivering myometrium (from Table VII, Chapter 4) are included. The resting membrane potentials were the same in all categories, but some differences in the cable parameters were apparent between the contracted and distended states. Differences between the three categories of muscle were examined by one way analysis of variance, followed by Student's t tests where appropriate. The length constant of delivering (contracted) muscle was less than that of delivering (distended) muscle. In addition, the internal resistance of both delivering (contracted) and post partum muscle was greater than that of delivering (distended). Other parameters were not significantly different between the categories.
## TABLE VIII

### ELECTRICAL PARAMETERS OF DELIVERING AND POST PARTUM MYOMETRIUM

<table>
<thead>
<tr>
<th></th>
<th>Delivering (Contracted)</th>
<th>Post Partum (n=8)</th>
<th>Delivering (Distended) (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Membrane Potential (mV)</td>
<td>48.4 ± 4.3</td>
<td>45.6 ± 1.7</td>
<td>45.5 ± 2.61</td>
</tr>
<tr>
<td>Length Constant λ (mm) (p&lt;.05)</td>
<td>2.37 ± 0.79</td>
<td>2.61 ± 0.78</td>
<td>3.68 ± 1.0</td>
</tr>
<tr>
<td>Membrane Resistance r_m (arbitrary units)</td>
<td>377 ± 172</td>
<td>422 ± 200</td>
<td>607 ± 240</td>
</tr>
<tr>
<td>Internal Resistance r_i (arbitrary units) (p&lt;.02)</td>
<td>71 ± 28</td>
<td>61 ± 33</td>
<td>43 ± 14</td>
</tr>
<tr>
<td>Time Constant τ_m (msec)</td>
<td>153 ± 37</td>
<td>141 ± 41</td>
<td>225 ± 72</td>
</tr>
<tr>
<td>Propagation velocity θ (cm/sec)</td>
<td>3.3 ± 0.4</td>
<td>3.7 ± 0.4</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>

TABLE VIII. Electrical parameters obtained from 4 delivering (contracted) and 5 animals 1/2 to 2 1/2 h post partum. The delivering (distended) data is taken from Table VII. The τ_m and θ values were taken from 3 experiments only in the delivering (contracted) category and 7 for post partum. P values indicate a comparison with the delivering (distended) samples, using the Student's t test.
These results show that the internal resistance (and, as a result, the $\lambda$) was different at various physiological states of contraction. The number of gap junctions should be the same in delivering (contracted) and (distended) muscle (see Chapter 2) and electron microscopic observation confirmed that gap junctions were present in post partum myometrium. A preliminary study of two samples of muscle 1/2 to 2 1/2 h post partum revealed that 24 gap junctions were observed in 2502 $\mu$m of membrane. There were $9.5 \pm 6.5$ gap junctions/1000 $\mu$m of membrane, with a fractional area of $0.34 \pm 0.09 \%$, values that are slightly larger than those of delivering myometrium (see Table VI).

Therefore, the internal resistance was greater in samples of muscle that were contracted, compared to distended, in vivo length muscle. The frequency of gap junctions was comparable. These observations do not provide evidence for a causal relationship between stretch and cable parameters, but they are consistent with the hypothesis that variations in stretch affect the measured electrical properties.

B) Varying the Length of Samples of Smooth Muscle

Some further experiments were conducted to determine whether a change of the stretch imposed on a sample caused the electrophysiological parameters to vary. The protocol for examining this variable was as follows. Samples of myometrium 2 cm long were mounted in the organ bath at their in vivo length and the cable parameters were measured. Samples were then stretched to 3 cm and reexamined.

Therefore, the comparison I present here is between myometrium at 100%
of its in vivo (i.e. "natural") length and at 150% of its original length. Hereafter I will refer to these categories as in vivo and stretched. In one instance I performed the experiment in the reverse order. After being examined at the in vivo length, the muscle was allowed to contract to 66% of its in vivo length, then reexamined. This particular sample is marked in Table IX. The shorter muscle (which in this case was not "in vivo" length), is kept on the left side of the results, and its relatively stretched counterpart is kept on the right.

Summary of Effects of Stretch on Samples of Myometrium

The results of individual paired experiments (i.e. before and after stretching) are presented in Table IX. Paired Student's t tests were used to analyse the data. Because the sample sizes were small, various groups were combined together for the analyses. If a change was not significant (i.e. p<.05) I have not made note of it here.

1) Before term + post partum (n=7). $\lambda$ increased and $r_i$ decreased upon stretching.

2) Before term + post partum + delivering (contracted) (n=9). Membrane potential decreased, $\lambda$ increased, and $r_i$ decreased upon stretching.

3) Just delivering (distended, n=4). No change.

4) Combine ALL (n=13). Membrane potential decreased and $r_i$ decreased upon stretching.

Conclusions

These results are incomplete, but do suggest that the amount of stretch imposed on a sample of smooth muscle can affect the physiological parameters. This conclusion is based on two findings.
First, paired experiments showed that the internal resistance decreased upon stretching (Table IX). Second, physiological shortening of myometrium in vivo was accompanied by increased internal resistance (Table VIII). Therefore, increased stretch decreases \( r_i \) which probably causes the increase in \( \lambda \), since \( \lambda \) is inversely related to the internal resistance. The manner in which stretch causes this change is not apparent. While maintaining constant volume, the geometrical effect of increasing the length of a deformable cylinder is to cause a decrease in the radius of the structure. Since \( r_i = R_i / 
abla a^2 \), decreasing the radius \( a \) would cause \( r_i \) to increase, opposite to that I observed. The length of cells may be a critical factor in determining the internal resistance that is measured by this technique (\( r_i \) is the internal resistance per unit length of cable). A more thorough analysis, possibly involving several types of smooth muscle, is required to understand the mechanism whereby stretching brings about the change.

The important point I wish to make here, however, is that the stretch is a variable that should be accounted for in experiments on smooth muscle. Efforts should be made to ensure that consistent methods are utilized for mounting tissues for in vitro experiments. Awareness of this artefact of in vitro electrical recording is essential for comparative analyses of smooth muscle characteristics.
|                | In Vivo |                | Stretched |                |            |            |
|----------------|---------|----------------|------------|----------------|------------|
|                | RMP     | $\lambda$  | $r_m$ | $r_i$ | RMP      | $\lambda$ | $r_m$ | $r_i$ |
| **Before Term**| 54.3    | 2.18  | 401  | 84  | 49.5     | 3.93  | 562  | 52   |
| **            | 47.4    | 3.28  | 530  | 59  | 45.5     | 3.99  | 412  | 36   |
| **            | 46.0    | 1.72  | 328  | 111 | 36.8     | 2.14  | 261  | 57   |
| **            | 56.4    | 1.82  | 261  | 79  | 45.3     | 2.32  | 248  | 46   |
| **Delivering (distended)** | 47.2    | 2.73  | 673  | 90  | 45.9     | 2.47  | 497  | 82   |
| **            | 51.1    | 4.02  | 896  | 55  | 49.2     | 3.38  | 701  | 61   |
| **            | 48.2    | 5.64  | 924  | 29  | 44.9     | 3.19  | 361  | 51   |
| **            | 45.9    | 3.51  | 367  | 30  | 41.3     | 2.32  | 242  | 45   |
| **Delivering (contracted)** | 48.6    | 2.03  | 452  | 110 | 40.4     | 2.86  | 295  | 36   |
| **            | 51.2    | 2.53  | 525  | 83  | 43.7     | 3.18  | 419  | 41   |
| **Post Partum** | 46.0    | 2.48  | 586  | 97  | 47.0     | 2.50  | 625  | 105  |
| **            | 46.4    | 2.42  | 631  | 108 | 46.0     | 3.52  | 682  | 52   |
| **            | 44.1    | 2.39  | 287  | 52  | 41.4     | 3.74  | 647  | 46   |

TABLE IX. Individual paired experiments show the effects of stretch on myometrium. RMP is resting membrane potential, other symbols as described earlier. Samples were pinned at 100% of their in vivo length, then stretched to 150% of that length. The data marked with asterisks were collected in reverse order, going from 100% (on the right) to 66% of the in vivo length (on the left). Paired Student's t tests were used to test for differences between the two groups, as described in the text.
FIGURE 29. Membrane responses of myometrium taken from an animal 1/2 to 1 1/2 h post partum. A). Hyperpolarizing and depolarizing current pulses of various magnitudes were applied and the responses measured at several distances from the stimulating electrode. B). Membrane responses of a different sample of post partum myometrium were linear in the hyperpolarizing direction.
$1\frac{1}{2}$ h POST PARTUM

A

1 mm

1.5 mm

2 mm

1 V/cm

0

-50 mV

2.5 mm

3 mm

500 msec

B

$V/cm$

0.8

0.6

0.4

0.2

mV

-5

-10

-15

-20

-25

-30

3 mm

2 mm

1 mm
REFERENCES


