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UNIVERSITY OF ALBERTA

CONTROL AND COORDINATION OF ELECTRICAL OSCILLATIONS AND CONTRACTIONS IN THE CANINE COLON

BY

PAUL JOHN SABOURIN (C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ELECTRICAL ENGINEERING

EDMONTON, ALBERTA

FALL 1990

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ISBN 0-315-65026-5

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Paul Sabourin

Paul J. Sabourin

14016 89 A Avenue

Edmonton, Alberta

Canada

T5R 4S5

Date: Oct. 9/90

The words of the wise are like goads,

And the words of scholars are like well driven nails...,

And further, my son, be admonished by these.

Of making many books there is no end,

And much study is wearisome to the flesh.

Solomon, circa 975 B.C.

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SUBMITTED BY PAUL JOHN SABOURIN,

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ELECTRICAL ENGINEERING.

Y. Jack Kingma, supervisor

Kenneth L. Bowes

Nelson G. Durdle

Jan D. Huizinga

Peter A. Smith

Richard S. Smith

Date: Aug 24 - 1990

ABSTRACT

In vitro investigations of the electrophysiology in the canine colon's smooth muscle layers are presented. The main goal of this thesis was to understand how electrophysiological events in the colon cause contractions coordinated between the circular and longitudinal muscle layers. Previous in vitro results reported dissimilar activities in the two muscle layers whereas in vivo results suggested that the two layers could coordinate their activities. As a possible basis for coordination, the hypothesis that the frequency of canine colonic electrical oscillations are dependent on applied force was tested. An active feedback system that applies a constant force to a muscle specimen was designed. Slow wave electrical oscillations from circular muscle are positively correlated to applied force. This relationship was significantly reduced in the presence of tetrodotoxin; in spite of this reduction a significant relationship still persisted after tetrodotoxin was administered. A 20 cycle per minute electrical oscillation associated with the longitudinal muscle is also positively correlated to applied force. It is concluded that contractions in one muscle layer may cause force mediated changes in the electrical activity of the other muscle layer thus providing a basis for coordinated contractions. This relationship appears to be mediated in part by tetrodotoxin sensitive nerves. In other studies a unique L-shaped muscle preparation that has isolated circular and longitudinal muscle legs and a full thickness hub was designed. A

dual sucrose gap apparatus was designed that records electrical and contractile activities simultaneously from both muscle layers. Patterns of interaction between the muscle layers were thereby observed. Interactions occurred rarely in the basal state. Cholinergic stimulation with carbachol and neostigmine significantly increased the incidence of interactions. Two main patterns of interaction were observed: contractions in both muscle layers coordinated with a) the circular muscle slow wave (Type C), and b), the longitudinal muscle electrical spike burst (Type L). Both types of interaction persisted in the presence of tetrodotoxin suggesting that they were not mediated by tetrodotoxin sensitive nerves. In further studies selective stimulation of the muscle layers by carbachol showed that Type L interaction results when *either* muscle layer is stimulated, and, Type C interaction results when *both* muscle layers are stimulated. Interactions were evoked more consistently by barium chloride stimulation than by cholinergic stimulation. A lower concentration of barium chloride elicited Type L interaction, whereas a higher concentration elicited Type C interaction. In summary, this thesis reports an in vitro basis for coordinated contractions that are observed in vivo. Two patterns of interaction which give rise to both short and prolonged contractions were found. The two patterns of coordinated contractions may have different functions in vivo since these patterns can be distinguished by different levels of stimulation or by selective stimulation of the muscle layers.

ACKNOWLEDGEMENT

I wish to express my appreciation to Professor Y. J. Kingma and Dr. K. L. Bowes whose generous support for those many 'extras' made my Ph. D. studies a more enriching experience.

I would also like to thank the Alberta Heritage Foundation For Medical Research for their support in the form of a full time studentship.

Various portions of the work presented in this thesis were supported by grant A 2169 from the Natural Sciences and Engineering Research Council of Canada, grant MA 5946 from the Medical Research Council of Canada, and grant RA 8448 from the Alberta Heritage Foundation for Medical Research.

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CHAPTER I. INTRODUCTION

A. THE GASTROINTESTINAL TRACT AND MOTILITY

Studies of the gastrointestinal tract have involved many and varied approaches due to the many functions of the gut. One can concentrate on a particular region of the gut including the esophagus, the stomach, the small intestine, the colon or the anal canal, considering the wall movements required at each point. Many have considered in detail what nutrients and substances are extracted at each stage along the gut. Studies of secretions, hormones and blood flow occupy volumes. Studies can be performed in health or disease, clinical or basic science, in vivo or in vitro.

The present study is focussed on colonic function from the point of view of motility. Colonic motility is itself a broad area (Phillips) covering:

1) the cellular properties of smooth muscle
2) neurogenic coordination of smooth muscle, both intrinsic and extrinsic
3) movements of the colonic wall resulting from contractions of the smooth muscle layers
4) propulsion of the colonic contents along the lumen.

The colon acts to store for periods of hours to days material emptied out of the small intestine (Metcalf), and occasionally to evacuate the accumulated material into the anal canal. Underlying

this contractile activity is the electrical activity of the constituent smooth muscle. The smooth muscle cells of the mammalian colon, and many other smooth muscle organs, demonstrate periodic and transient electrical oscillations that determine the muscle's contractile behavior (Szurszewski). This myogenic electrical activity is influenced by intrinsic and extrinsic neural elements (Roman et al., Wood 1987) but many details have yet to be determined.

B. SCOPE OF THE PRESENT STUDY

The present study concentrates on the electrical activity of the colonic muscle layers, its effect upon contractile activity, and mechanical influences upon electrical activity. This is an attempt to understand electrical events in the *normal* colon and to explain physiological colonic function. This is in contrast to questions of electrical abnormalities and pathological conditions.

In vivo, it appears that the electrical and contractile activities of the colonic muscle layers can become coordinated. The present study specifically involved determining the electrophysiological basis for coordinated activity. As a possible factor contributing to coordinated activity this study investigated how contractile activity in a muscle layer could affect its own electrical activity and the electrical activity of other layers. This study also identified factors that enhanced or blocked coordinated activity.

The colon consists of two primary muscle layers, the circular and the longitudinal, which are perpendicularly oriented. The longitudinal layer is outermost and separated from the inner circular layer by a conglomeration of neural tissue, the myenteric plexus (Wood 1987). Between the colonic lumen and the circular layer are the mucosa, the muscularis mucosae, the submucosa and the submucosal plexus (Warwick et al.) as shown in Figure 1.

The electrical and contractile activities in the circular and longitudinal muscle layers are different when they are studied in isolated in vitro strips (Barajas-López et al. 1988, Chow et al., Debski et al., Durdle et al. 1983a, El-Sharkawy, Sabourin et al. 1990b, Smith et al. 1987b). Contractile activity observed in vivo (Kingma et al. 1980, Sarna 1986) and ex vivo (Kocylowski et al.) suggest, however, that the activity in the two layers can become coordinated. The mechanisms by which this occurs are not understood and the main goal of the present study was to investigate this matter further.

In vivo motility experiments often yield complex results that are difficult to understand. Electrical and contractile recordings are usually a composite of activity resulting from a mixture of the activities of the various muscle layers. In such a situation it is difficult to associate a particular event with a particular region or muscle layer within the organ being recorded from.

In vitro experiments can yield less ambiguous results since

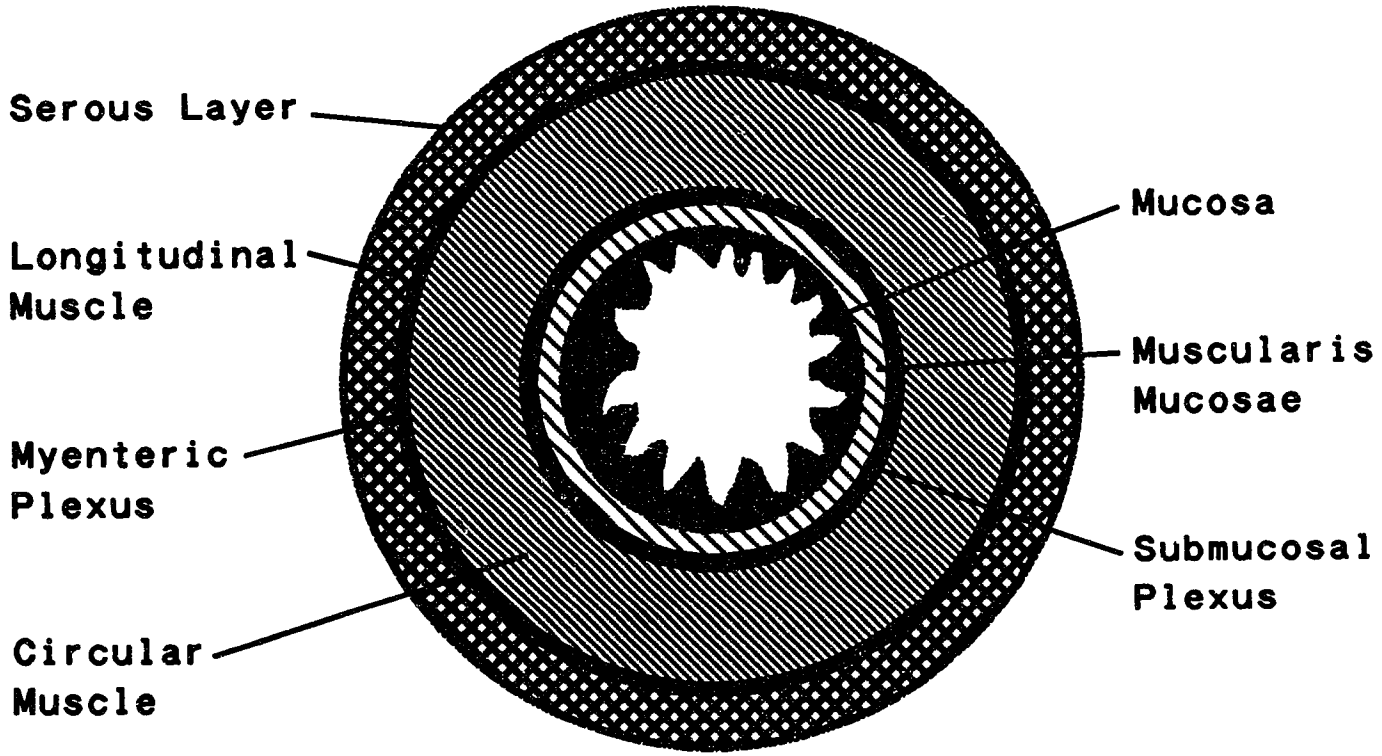

Figure 1. Cross section of the canine colon

preparations are dissected and manipulated at will. In the present case, however, in vitro findings do not explain the in vivo findings. The present study was all performed in vitro. Special apparatus and tissue preparations were developed in an attempt to bridge the current gap between the in vivo and the in vitro findings.

Ultimately, the human colon is of course the organ of interest. Obtaining an adequate number of healthy and suitable tissue specimens is a major difficulty, so a canine animal model was chosen. An animal model has the advantages of: easy (relatively) availability, the tissue is usually fully healthy, the samples can be chosen to be as large as desired, and a more consistent and regular schedule can be followed than is possible when working with human tissue. Since new apparatus were developed, an animal model was appropriate in the present case to prove the techniques first on the model, perhaps to apply them more efficiently in the future on human tissue.

The question of suitability of the animal model arises. The dog was chosen primarily because our group has extensive experience with this model. Also, it was essential for this study to use large tissue preparations. Such preparations can be readily obtained from canine colon. In both dogs and human the electrical and contractile activity of the two muscle layers are dissimilar when the two muscle layers are studied separately in vitro so they are comparable in this respect. Moreover, the electrical and contractile activity in

the longitudinal muscle layers of both human and dog colon are quite similar. However, activities in the two circular muscle layers are less similar so certain results found in the dog are not directly comparable to the human case. The human colon has haustra and the longitudinal muscle layer has three thickened portions, the taenia coli, while the dog colon has neither. In this respect, pig colon may be a better model since it has both (Huizinga et al. 1987a). Despite the species differences, the techniques developed and the general findings would prove useful in any further study of the human colon.

To summarize, the present study is an in vitro investigation into the electrophysiology and contractile activity of healthy canine colon. Of particular interest are the phenomena by which the dissimilar electrical and contractile activities of the circular and longitudinal muscle layers interact, and the electrophysiological basis for coordinated contractions.

The research was conducted in several stages. In the first stage, a system was designed and applied to perform isotonic studies of smooth muscle specimens from the individual muscle layers. Of specific interest was the manner in which applied force altered the electrical activity of the specimens. The intent was to understand how distension of the colonic wall by the luminal contents might initiate or control coordination within the colon. The work of stage 1 appears in chapters 3 and 4.

In the second stage, a dual sucrose gap apparatus that permitted in vitro simultaneous study of electrical and contractile activity from the circular and longitudinal muscle layers was designed and validated. The purpose was to allow direct observation of interaction between the two muscle layers. With this capability, the intent was to subject the tissue specimens to various pharmacological protocols to identify how coordinated activity could be facilitated or blocked, and thereby to possibly reveal the underlying mechanisms of coordination. Within the colonic wall different regions demonstrate different electrical and contractile activity. The involvement of the various regions in coordinated electrical and contractile activity was delineated by: 1) making microelectrode impalements in various locations, 2) using various tissue preparations, and 3) selectively transecting the tissue preparations during periods of interaction. The work of stage 2 appears in chapters 5, 6 and 7.

Finally, in chapter 8 a model is proposed that integrates the findings obtained in this study.

CHAPTER II. BACKGROUND

A. CELLULAR MEMBRANE BIOPHYSICS

Colonic smooth muscle cells possess an electrical potential difference across their cellular membrane that has a steady state and a periodically changing component (Szurszewski). The steady state, or resting, membrane potential arises from an unequal distribution of ions on the intracellular and extracellular side of the membrane (Hille). The periodically changing component of electrical potential is a manifestation of ion movement through the membrane (Hille).

Ions can cross the cellular membrane in a number of ways. Active processes have been identified where ions are 'pumped' into or out of the cell or into intracellular sequestering sites. Pumping may be the result of ion transport or a type of ion exchange across the membrane (Hille). These pumping actions are generally concerned with maintaining a certain distribution of ions. This distribution is compromised by continual diffusion down electrochemical gradients or as a result of sudden ion movements associated with, for example, an action potential.

The conductivity of the membrane is the result of ionic channels which are embedded in the membrane (see Figure 2). The membrane of the cell is basically a phospholipid bilayer which is less than 10 nanometers (10^{-9} m) thick. Pure lipid bilayer has a very high resistivity (as high as 10^{15} ohm centimeters) and a

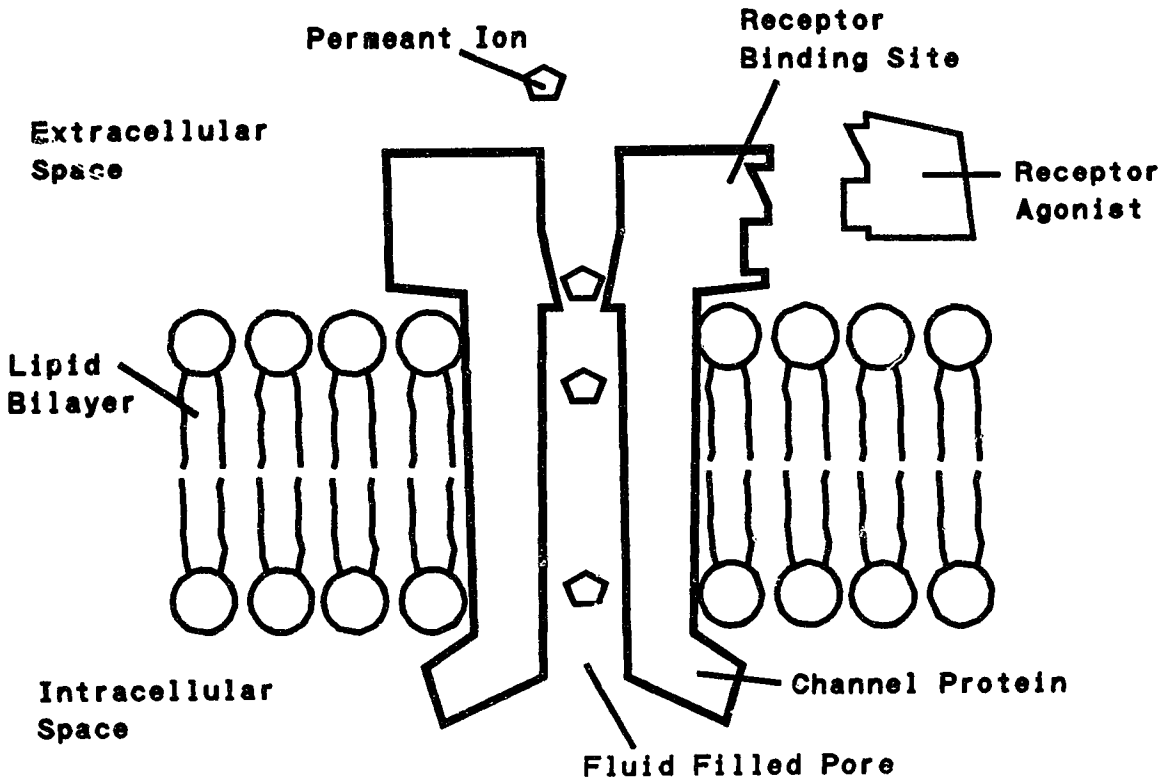

Figure 2. Channel protein in the cellular membrane

significant capacitance on the order of one microFarad per centimeter squared (Hille). The properties of a particular region of cellular membrane are determined by the type and quantity of the ionic channels present within it. These ionic channels which have variable conductances are thought to be mainly responsible for the periodic and transient electrical oscillations observed in the colon.

Ionic channels have nonlinear conductances which change with membrane voltage, are selective to particular ions, and can be activated or inactivated by specific agents. Ionic channels are macromolecular proteins which are incorporated in the membrane and connect the intracellular and the extracellular space by a fluid filled pore. The physical conformation of the channel, and hence, its conductance, varies with the membrane voltage so that a particular channel may open, for example, as the membrane voltage is decreased. The shape of the pore causes it to be selective to a greater or lesser degree to particular ions. Hence, we speak of calcium channels, potassium channels, etc. Certain channels are receptor operated so that a particular agonist, if present, will bind in some way to a receptor associated with the channel thereby causing it to open. Other agents act to inactivate specific channels, acting at a receptor, or perhaps even 'plugging' the pore.

The distribution of ions and the cellular membrane potential are determined by a combination of chemical and electrical gradients. Given an unequal distribution of ions of a given species

across a permeable membrane the ions will diffuse down the concentration gradient. Permeability is a measure of the ease with which ions can diffuse across a membrane and has units of m/s. However, an electrical potential difference will develop across the membrane when there is an unequal distribution of charge. An equilibrium is reached when electrical forces equal chemical diffusional forces. The equilibrium potential for a given ion species is given by the well known Nernst equation:

$$E_{ION} = \frac{RT}{F} \ln \frac{[ION]_o}{[ION]_i}$$

where R is the Gas constant equal to 8.314 Volt Coulomb per Kelvin mole, F is Faraday's constant equal to 9.648 X 10^4 Coulomb per mole, T is absolute temperature, $[ION]_i$ is the concentration of the ion inside the cell, and $[ION]_o$ is the concentration of the ion outside the cell. The membrane potential at which no net current flows when multiple ions are present is given by the Goldman-Hodgkin-Katz equation (Hille):

$$E_{reversal} = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}$$

if Na^+, K^+ and Cl^- are the permeant ions and P_K is the membrane permeability to K^+. Therefore, changes in the permeability of the various channels will cause changes in the potential measured across the membrane.

Action potentials and electrical oscillations in cells are the result of voltage and time dependent opening and closing of ionic channels. A typical sequence in an electrical oscillation is as

follows:

1) a membrane depolarizing stimulus arises from a pacemaker, or from the release of neurotransmitter,
2) the decrease in membrane potential causes one or more voltage sensitive channels to open causing a regenerative depolarization of the cell as ions flow passively down a concentration gradient,
3) the depolarization will cause one or more other channels to open which will act to repolarize the membrane as other ions flow down a concentration gradient,
4) the channels activated by the depolarization-repolarization sequence will close again, and are ready to generate another oscillation.

The permutations of the above sequence are many as electrical events can be depolarizing or hyperpolarizing, fast or slow, composite, etc..

B. EXCITATION-CONTRACTION COUPLING

Electrical oscillations in canine smooth muscle cells control contractile events. However, not all electrical events have a contractile response. Certain electrical events act as pacemakers, or as 'electrical control activity' that determine when other electrical events can occur which cause contractions.

In muscle a mechanical threshold is defined (Hodgkin et al.)

which is a value of membrane potential that, when exceeded, will give rise to a contraction. This mechanical threshold varies depending upon the tissue. Values reported in different regions of the stomach, for example, vary from -45 milliVolts to -55 milliVolts (Szurszewski). In canine colon circular muscle this value has been reported at -44 milliVolts (Barajas-López et al. 1989). In canine colon, the normal resting membrane potential is below the mechanical threshold (Barajas-López et al. 1988, Chow et al.).

The basis of the mechanical threshold is the voltage dependent entry of calcium into the cell. For example, if the mechanical threshold of a cell is determined to be -44 milliVolts then this suggests that calcium channels open at around -44 milliVolts. Since the intracellular calcium concentration is maintained at a low level by the cell, extracellular calcium enters readily through the open channels driven by the strong concentration gradient. The elevated level of intracellular calcium then triggers the contractile apparatus causing the smooth muscle cell to contract.

The circular muscle layer demonstrates periodic slow waves (Figure 3) that occur in a frequency range of 4-7 cycles per minute. The slow wave can cause phasic contractions (Figure 3) since the plateau exceeds the mechanical threshold. The slow wave also acts as a pacemaker for spike potentials (Huizinga et al. 1987b) and superimposed higher frequency electrical oscillations which cause more powerful contractions (Huizinga et al. 1984). The mechanical threshold in the circular muscle appears to be constant throughout

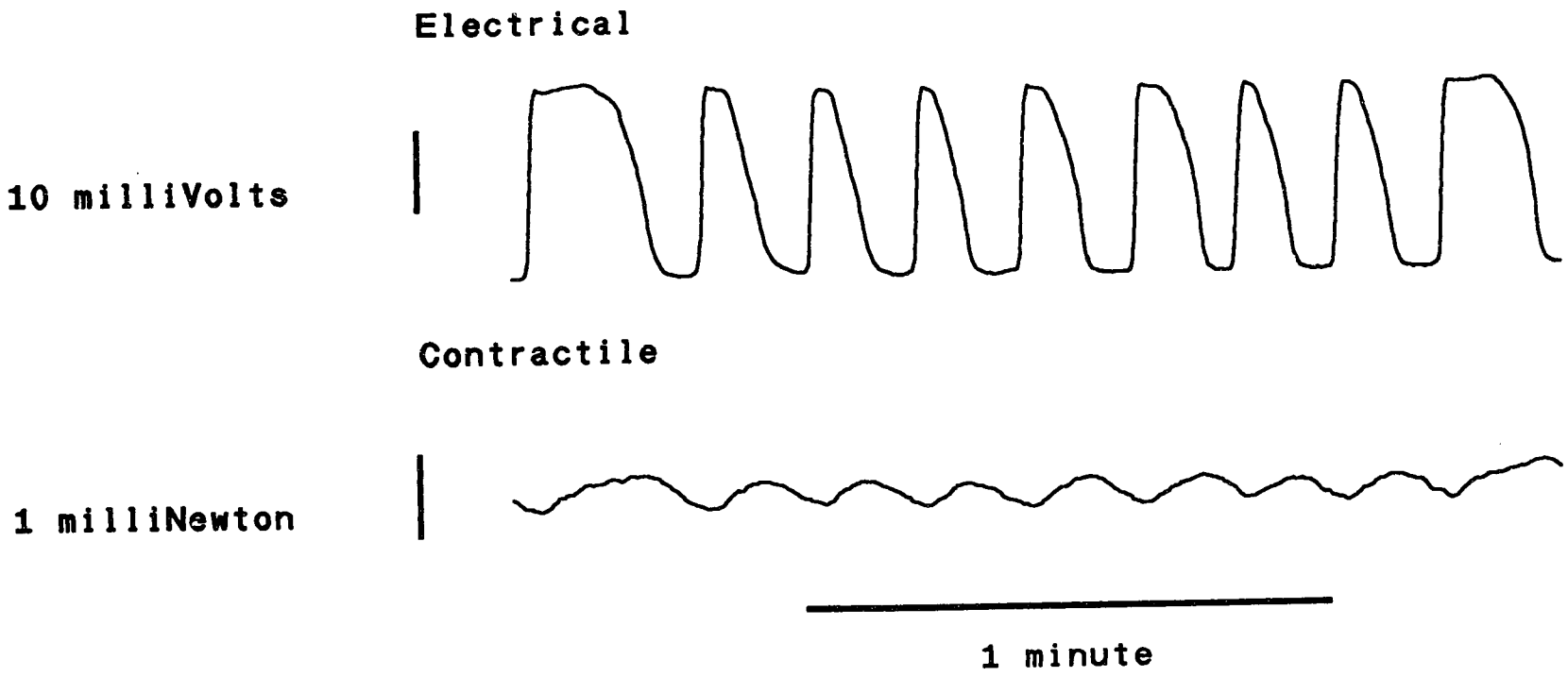

Figure 3. Activity of canine colon circular muscle

(Barajas-López et al. 1989). In contrast, heterogeneity in the amplitude of the circular muscle slow wave and the amount of spiking and higher frequency electrical oscillations at the submucosal and myenteric plexus border have been noted (Barajas-López et al. 1988,1989, Smith et al. 1987b).

The electrical activity of the longitudinal muscle layer consists of higher frequency, 20 to 30 cycles per minute, electrical oscillations which occur continuously and in bursts (Figure 4). Spike potentials occur superimposed on these oscillations and cause sustained tone or prolonged contractions, respectively. The contractions are prolonged because the muscle does not relax completely between spikes and the individual smaller contractions 'accumulate' to generate the prolonged contraction.

The circular muscle slow wave is generated at the submucosal border and propagates towards the myenteric plexus (Durdle et al., Smith et al. 1987a). Circular muscle slow wave generation has been associated with interstitial cells of Cajal which form gap junction contacts with smooth muscle at the submucosal border of circular muscle (Berezin et al.). The location and structures responsible for the longitudinal muscle's electrical activity are not known.

Considering the whole organ, slow waves appear to spread quickly around the circumference and more slowly along the long axis of the colon. Adjacent regions of the colon have independent slow wave pacemakers (Christensen 1987a). Considering migrating spike

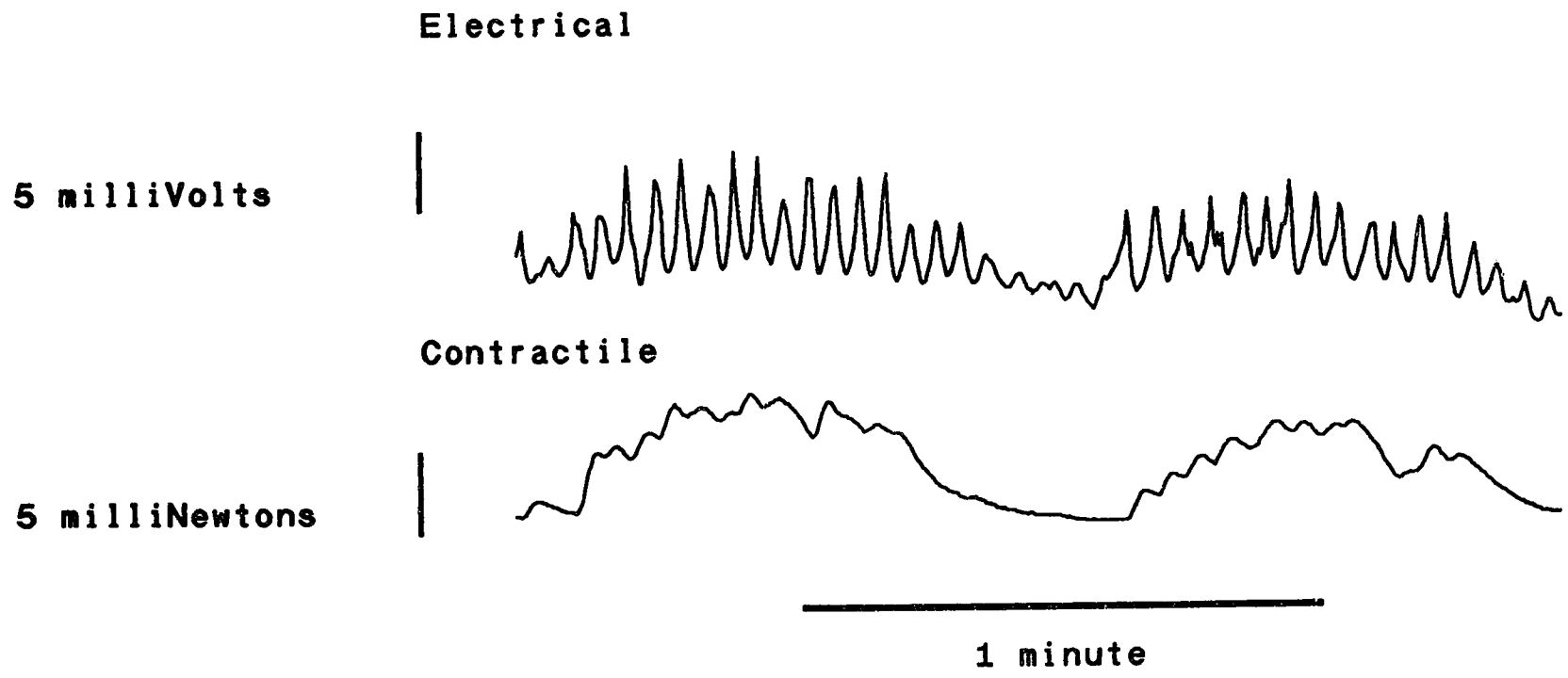

Figure 4. Activity of canine colon longitudinal muscle

bursts, these are apparently associated with the longitudinal muscle, and were noted to propagate over large sections of the colon, both orad and caudad (Christensen 1987a).

PHARMACOLOGY

Pharmacological agents cause changes in the electrical and contractile activity of smooth muscle by acting upon ionic channels in the cellular membrane. Regarding the resting membrane potential, these agents will change the conductances of these channels causing the cells to hyperpolarize or depolarize. Membrane hyperpolarization causes inhibition since a greater stimulus is then required to decrease the membrane potential to the threshold required to generate spontaneous electrical and contractile events. Conversely, depolarization of the membrane causes excitation since the membrane is then closer to or actually above threshold.

Some agents act at receptor operated channels in the membrane. This is typical of neurotransmitters which are released from bulbous enlargements of the nerve fibers in the vicinity of smooth muscle fibers (Kelly et al.). The neurotransmitter first diffuses to the cell membrane then binds to receptors there causing either depolarization or hyperpolarization (Figure 5). Exogenously applied agents may act at the same receptors.

Other exogenous agents will block specific channels. If the channel is open at the resting membrane potential then a change in membrane potential will result. Blocking a channel can also block

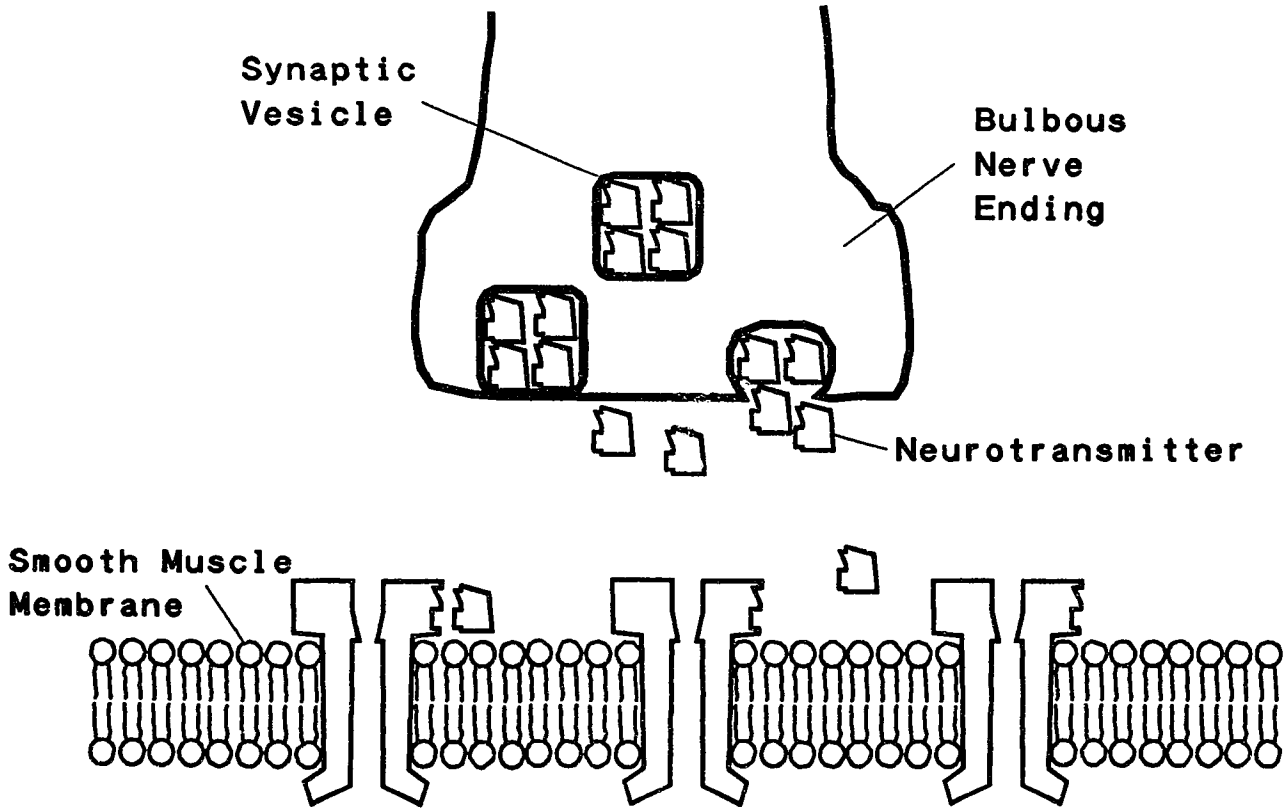

Figure 5. Neurotransmitter release from nerve ending

certain electrical events that rely upon that channel.

Four main pharmacological agents were used in the present study:

1) carbamylcholine chloride (carbachol) is an analog of acetylcholine which will act at muscarinic and nicotinic receptors. It mimics neural input from the parasympathetic nervous system (a parasympathomimetic) and so has excitatory effects in the colon. Carbachol, in contrast to acetylcholine, is not rapidly inactivated by cholinesterase (Krogh). Carbachol activates muscarinic receptors in the smooth muscle cell membrane which depolarize the cell leading to excitation. Carbachol causes an increase in circular muscle slow wave duration and amplitude, apparently by suppressing calcium dependent potassium current (Cole et al.).
2) neostigmine (Prostigmin™) is a parasympathomimetic which inhibits the destruction of acetylcholine by cholinesterase (Krogh). Neostigmine has excitatory effects on colonic smooth muscle, enhancing intrinsic cholinergic activity. It has a depolarizing effect on the smooth muscle cells.
3) barium (barium chloride, $BaCl_2$) has excitatory effects when applied to colonic smooth muscle preparations. Barium is a potassium channel blocker (Hille). In colonic smooth muscle, potassium channels are open at the resting

membrane potential. When barium is applied the potassium channels are blocked causing a depolarization which in turn leads to excitation (actually there are many types of potassium channels and barium does not block them all with the same efficacy (Hille)). Barium also permeates open calcium channels and enhances the amplitude of calcium action potentials (Hille).

4) tetrodotoxin (TTX) is a very specific sodium channel blocker (Hille). TTX blocks the propagation of action potentials that have an upstroke that is caused by an influx of sodium into the cell. In this capacity TTX is often used to block the neural component of nerve-muscle tissue preparations while leaving myogenic activity intact. In preparations from the colonic wall the constituent structures are not all fully characterized. The idea that only myogenic activity remains after TTX administration is convenient but simplistic. Although TTX also blocks sodium channels in the smooth muscle cells, this does not block the smooth muscle's associated electrical activity (El-Sharkawy, Huizinga et al. 1984, Sabourin et al. 1990b)

D. RECORDING AND MEASURING TECHNIQUES

Sucrose Gap Technique

A number of techniques for measuring electrical activity from

smooth muscle have appeared in the literature and each has had its proponents. In the present study, the sucrose gap technique and microelectrodes were used.

With the sucrose gap technique a smooth muscle sample is exposed to three solutions in three compartments (see Figure 23 in chapter 5). A central compartment is filled with sucrose solution which has a high impedance. Adjacent to the sucrose is a compartment containing potassium chloride which depolarizes and inactivates the cells it contacts. On the opposite side of the sucrose is a compartment containing a physiological Krebs-Ringer solution which keeps the tissue alive and active. The electrical potential that appears across the sucrose compartment is an attenuated version of the intracellular electrical activity of the cells in the Krebs compartment (Coburn et al.)[1]. Recording a signal with the same shape as the intracellular waveform is an advantage over extracellular recording techniques since they provide time derivatives of the intracellular waveform (Bortoff 1975). Also, the sucrose gap amplifier is usually dc coupled. Uninterrupted long duration recordings, which were essential in the present study, can be obtained with the sucrose gap technique. This technique tolerates vigorous contractile activity in the tissue preparation.

When recording bioelectrical events it is necessary to couple electrical potentials, that occur in an electrolyte solution as a result of ionic currents, to electronic amplifiers that are based

---

1 The theory of the sucrose gap technique is discussed in more detail in Chapter 5.

upon the flow of electrons through metals and semiconductors. An electrode that serves well to measure bioelectric events is the silver/silver chloride electrode (Durdle 1983b, Familoni, Kingma et al. 1983). These and other electrodes have an electrical potential associated with the electrode-electrolyte interface (Geddes). This potential will appear as a dc offset in the recorded potential. The silver/silver chloride electrode has a relatively small low frequency impedance (Lenhard) which is important when measuring the low frequency signals from the gut. This electrode is quite stable with time and has low thermal noise (Durdle 1983b, Familoni).

Microelectrodes

The microelectrodes used in the present study are the glass micropipette type which are filled with a 3 molar potassium chloride solution (Kuriyama and Ito 1975). A silver/silver chloride pellet couples the signal from the pipette solution to the amplifier (Figure 6). A reference electrode is placed in the bath solution. Microelectrodes are positioned with very stable precision manipulators with micron resolution. The electrode is advanced to contact the cell membrane and punctures it to provide a contact to the cytoplasm. Unlike the sucrose gap, microelectrodes can be used to measure the resting membrane potential. In contrast to most other recording techniques, the location from which the electrical signal is actually measured can be determined with great precision.

Although microelectrodes give definitive results there are

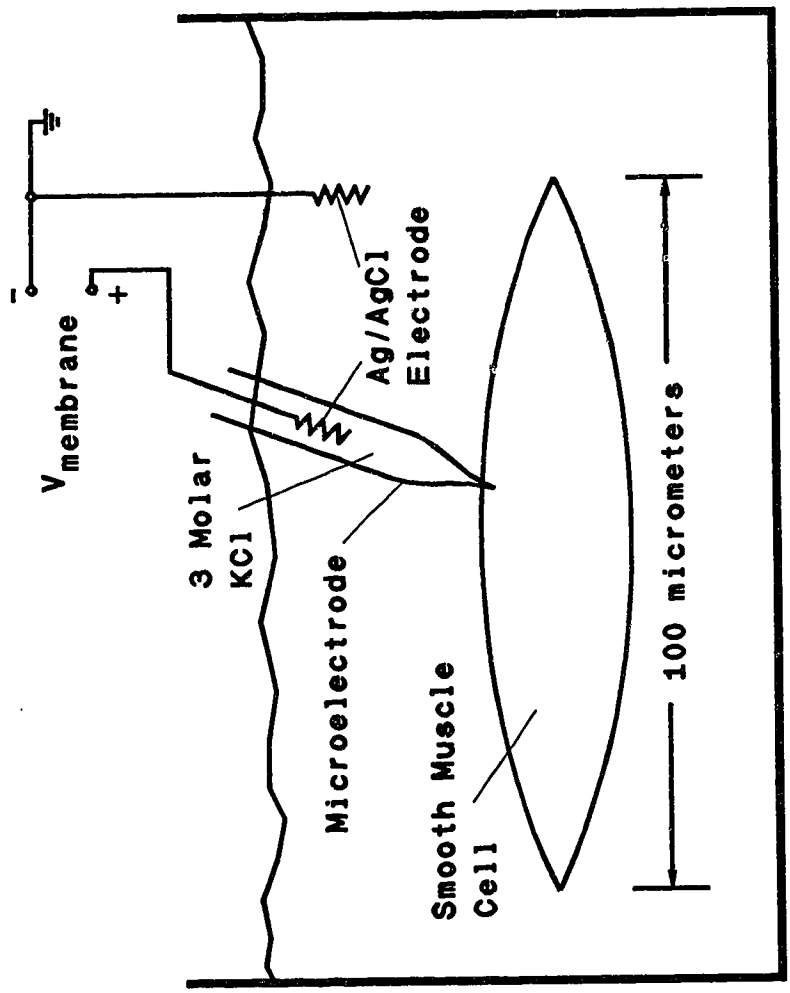

Figure 6. Microelectrode penetration of smooth muscle cell

drawbacks to their use. It is relatively difficult to impale smooth muscle cells, especially since with many experimental setups the cells cannot be properly visualized. Once a cell is impaled a muscle contraction will often break the electrode or pull it out. This makes long duration (hours) or uninterrupted recording impractical. Experimenters who record from smooth muscle often immobilize the tissue with elaborate pinning and occasionally block contractions with the muscarinic antagonist atropine, as well. For these reasons the present study depended primarily on the sucrose gap apparatus.

Data Acquisition

A common problem in a study of this type is management and analysis of acquired data (which fills a number of cabinets). To partially address this problem a computer based data acquisition system was developed. This integrated system had the following goals:

1) digitize all signals and store them on computer disk, acquisition rates should be easily adjustable, files backed up on floppy disk provide archival storage in place of or in addition to the traditional paper chart records,
2) store information regarding the experiment and the acquired signals together with the data, all points within the record are addressable by a unique time stamp referenced to the start of the recording,

3) provide facilities that permit detailed examination of one or more channels (zooming, amplification, offset, etc.),

4) provide signal processing capabilities including frequency spectrum determination (fast Fourier transform), auto- and cross-correlation, lowpass, highpass, bandpass and bandreject filtering which are performed after the data has been acquired, filtered signals are to be stored in files separate from the original which is preserved,

5) provide high resolution output to a color computer monitor and/or a computer printer to aid in signal analysis and to facilitate documentation of results.

All the above requirements were achieved using an IBM PC AT™ computer with a Scientific Solutions Labmaster™ analog to digital converter unit. The acquisition, storage, analysis and display software were all programmed using the ASYST Scientific System™ environment. This integrated system has proven to be very useful in all respects.

CHAPTER III. AN ACTIVE FEEDBACK SYSTEM FOR ISOTONIC STUDIES OF SMOOTH MUSCLE

A. ABSTRACT

A feedback system used to perform isotonic studies of smooth muscle is presented. This system is capable of applying a constant force to muscle samples regardless of their contractile activities. The force applied to the tissue is controlled using a proportional integral control system which drives a linear motor. The device is integrated into a sucrose gap tissue bath apparatus where measurements of displacement and electrical activity are also possible.

B. INTRODUCTION

Studies of smooth muscle from human colon have shown that cyclic electrical depolarization waves occur in both the circularly and longitudinally oriented muscle layers. This phenomenon is also observed in canine colon. When these muscle samples are mounted in a tissue bath they often exhibit powerful contractions. In the usually semi-isometric measuring arrangement the force applied to the specimen will thus vary considerably, and reliable force versus frequency measurements cannot be made.

In order to perform a study of the effects of force applied to smooth muscle tissue, a feedback system has been developed that applies a constant force to the muscle samples. This force clamping system enables the imposition of truly isotonic conditions on the

tissue regardless of any contractile activity. As a whole, the system provides simultaneous measurement of force, displacement and electrical activity from the smooth muscle samples.

The idea of isotonic studies is not new. Devices are available which use a lever held in tension by a spring which develops an approximately constant force over a limited distance (e. g. Harvard Apparatus #52-9511). Such a device would not interface well to the small 1 by 10 mm tissue samples and the confined space of the tissue bath used in our and similar studies. A different modality for isotonic studies using active components and feedback was designed. Friction and inertia that the tissue sample has to overcome in a mechanical spring loaded lever system are not a concern with our apparatus.

C. FEEDBACK SYSTEM

The quantity of interest, force, is obtained by attaching a force transducer to one end of the tissue. This transducer consists of strain gauges placed in a bridge network (Peura et al.). The strain gauges are mounted on a cantilever, one on each side. The tissue specimen is perpendicularly attached to the free end of the cantilever such that contractions deflect the cantilever. A differential amplifier placed across the bridge senses the unbalance in the bridge due to an applied force and develops a voltage that is approximately linearly related to this force. This voltage is used as the feedback signal which is compared to a reference voltage to

develop an error signal. The reference voltage corresponds to the desired force which is to be applied to the tissue (Figure 7).

The error signal, developed by subtracting the actual force transducer voltage from the desired force reference voltage, is applied to a proportional integral controller realized with operational amplifier circuits. The control voltage developed by this stage is applied to a voltage to current power stage which drives a linear motor which in turn displaces the tissue to adjust the force. Since the controller integrates the error signal the system has a theoretical steady state error of zero for step inputs.

The linear motor chosen to vary the force on the tissue is a long travel low frequency loudspeaker (Radio Shack-Realistic 4 inch woofer, 8 Ω,5 W,#40-1022). A small arm is attached to the center of the woofer cone and allows attachment to the remaining free end of the tissue. The direction of travel of the woofer cone is along the long axis of the tissue which is usually prepared as a long thin strip. As the tissue is stretched or released the force applied to the muscle changes. The woofer has a travel of about 7 mm which limits the size of the tissue specimens that can be used.

Testing showed that the closed loop system was slightly underdamped (ζ=0.5) and a second order approximation to the system was of the form (Ogata):

$$T(s) = \frac{K}{(s^2+66s+4356)} \qquad \omega_n=66 \text{ rad/sec}$$

This damping ratio was dependent on the loop gain chosen. A ζ

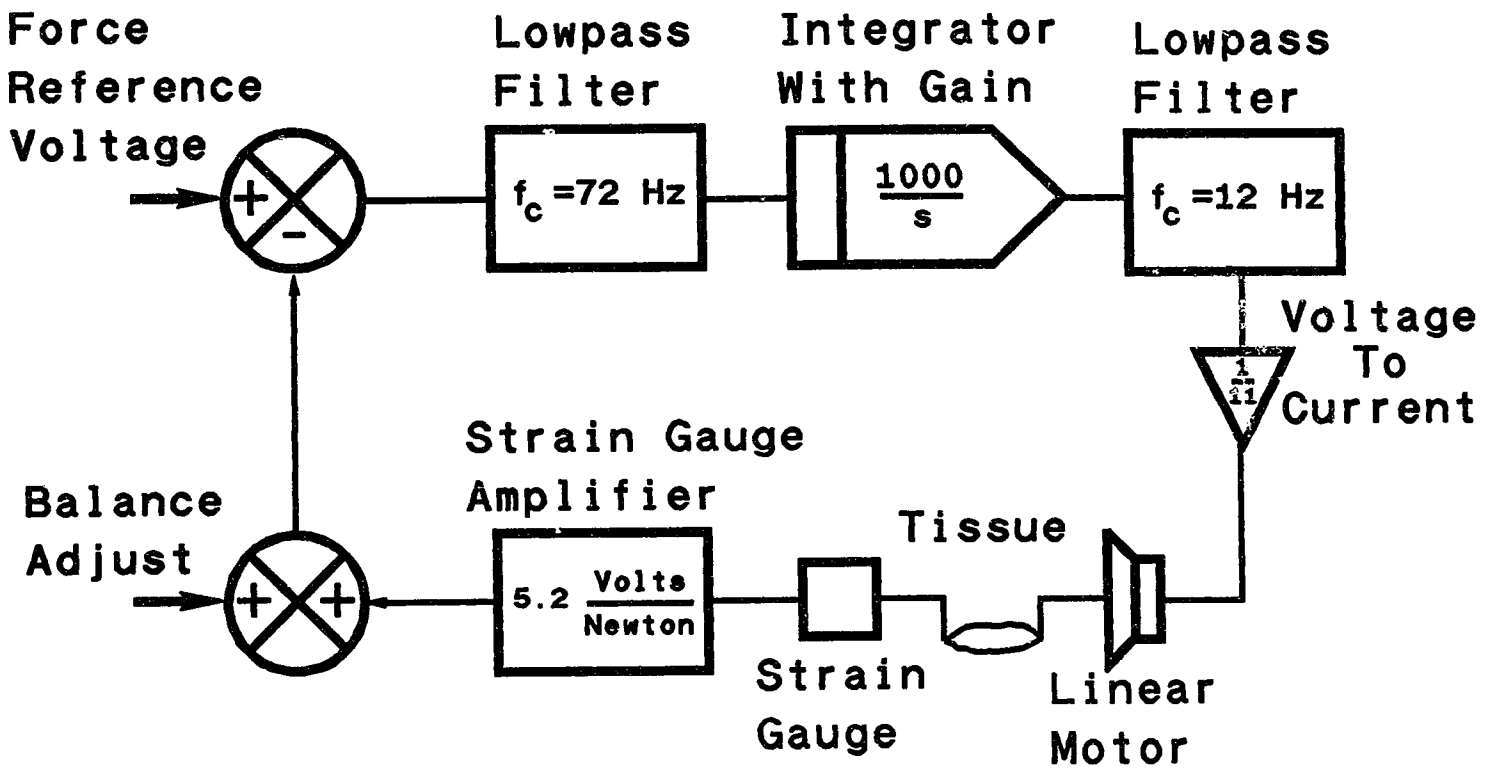

Figure 7. Block diagram of feedback system

of 0.5 was achieved with the settings shown in Figure 7. Frequency compensation was not required. With this bandwidth the system was able to track any physiologically occurring contractions in the tissue samples (see Figure 8). An advantage of active feedback was that the system's effective inertia was reduced. Therefore, the tissue did not experience an increased load (non isotonic conditions) even during rapid contractions. In figure 8 the top trace was tissue length and the bottom trace was tissue force. In isometric mode the apparatus records periodic changes in force. After enabling the feedback system the force was held constant and the system measured periodic changes in length.

D. LENGTH MEASURING SYSTEM

Since isotonic recordings necessarily mask any information about contractile force, a length measuring circuit was included in the system. This consisted of a light source and solar cell (Radio Shack-Archer 25X5 cm silicon solar cell, #276-124) mounted around the woofer cone and inside a light proof enclosure (Figure 9). The light source and solar cell were mounted in such a way that the movement of the woofer cone occluded the light path between solar cell and light source. Since the tissue was attached to the cone this movement represented the shortening or lengthening of the tissue sample. The solar cell converted the incident light energy to electric current flowing into the resistive load. The potential generated across the resistor was approximately linearly related to the displacement of the tissue (Figure 10). Although the curve has

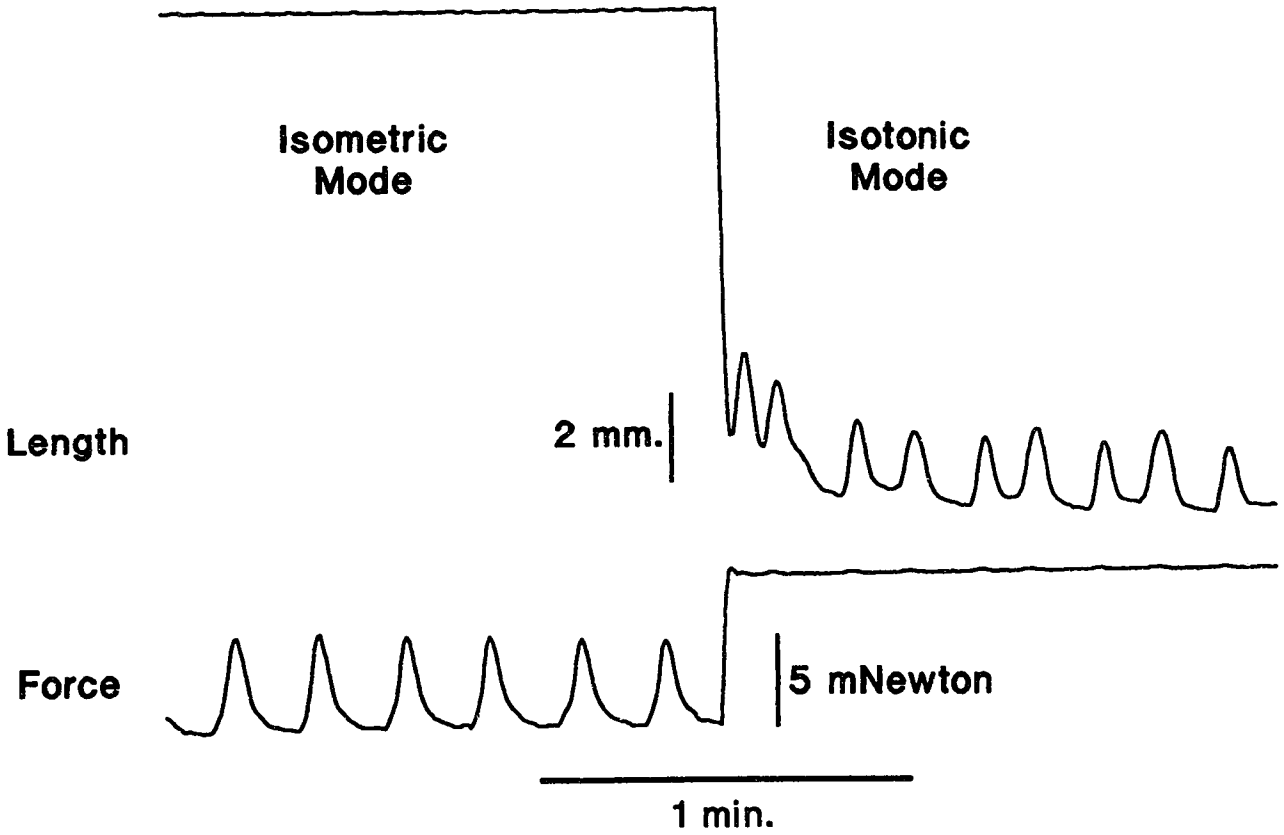

Figure 8. Transition from isometric to isotonic recording

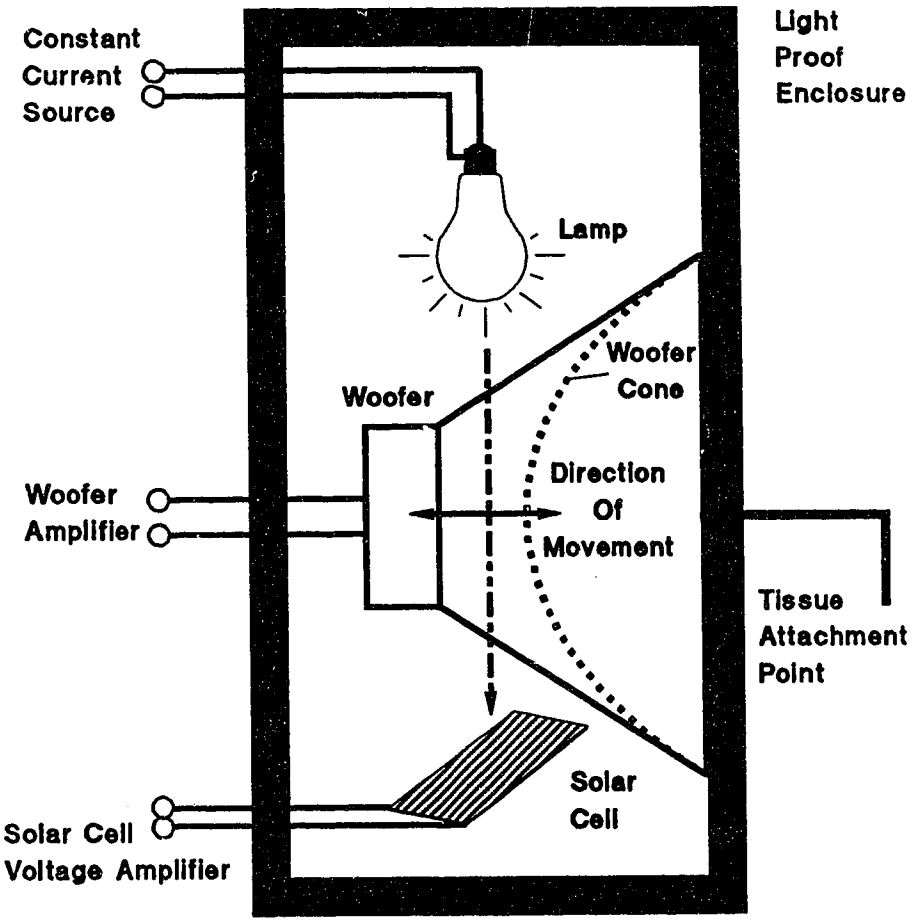

Figure 9. Length measuring system

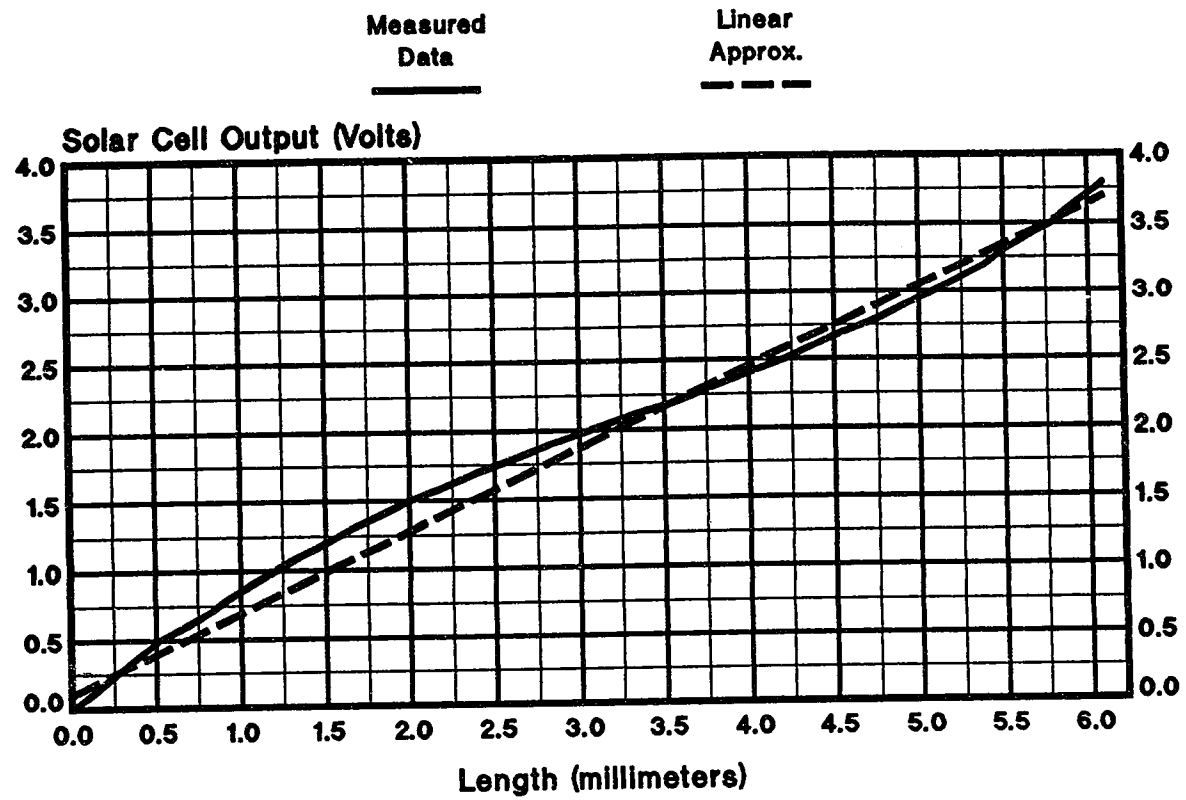

Figure 10. Plot of solar cell voltage versus tissue length

a complex shape a linear fit was a good approximation. This signal served as a good indicator of contractile activity and permitted a continuous assessment of muscle length.

By mounting the strips simultaneously in a sucrose gap apparatus (the apparatus is described in Chapters 2 and 5) a continuous assessment of the muscle sample's electrical activity was possible. The results which were obtained with this device are discussed in Chapter 4.

E. CONCLUSION

An active feedback system coupled with a small linear motor achieved size and performance improvements over the traditional passive spring loaded lever type of isotonic device.

CHAPTER IV. A RELATIONSHIP BETWEEN APPLIED FORCE AND ELECTRICAL PACEMAKER ACTIVITY IN CANINE COLON

A. ABSTRACT

The frequencies of electrical and contractile oscillations in both circular and longitudinal muscle preparations were noted to have a direct correlation to applied force. Neural conduction blockage by tetrodotoxin significantly reduced this correlation.

B. INTRODUCTION

The application of quick stretch to smooth muscle can elicit an excitatory response (Bulbring 1955, 1963, Burnstock et al. 1960, Gillespie, Kosterlitz et al.) and some smooth muscles apparently require stretch before they will generate spontaneous electrical and contractile activity (El-Sharkawy et al. 1982, 1983, Huizinga et al. 1987a). Stretch has also been shown to overcome the inhibitory effect of atropine (Burnstock et al. 1960, El-Sharkawy 1983).

In vivo, colonic function is modified by the amount and type of luminal content (Phillips). The colon has the capability to modify its activity depending on whether it is full and distended or if it is empty and not distended. This ability must be related in part to changes brought about in the electrical activity that controls the contractile events in the colon.

In canine colonic circular smooth muscle a repetitive slow wave is observed at a frequency around 6 cycles per minute and has

associated phasic contractions. The purpose of the present study was to examine in a quantitative and controlled manner the relationship between oscillator frequency and applied force in the canine colon's circular smooth muscle pacemaker. The hypothesis that tetrodotoxin (TTX) sensitive nerves mediate this relationship was investigated. The relationship between applied force and oscillator frequency in the longitudinal muscle's 20 cycle per minute oscillator was also studied.

C. METHODS

Tissue Preparation

Tissue specimens were obtained from healthy mongrel dogs of either sex. The dogs were anesthetized with pentobarbital sodium 30 mg/kg and the colon was accessed via a midline abdominal incision. A 10 to 12 cm segment of proximal colon was excised. The excised segment was cut along the mesenteric border and colonic content was removed. The resulting muscle sheet was maintained at room temperature in oxygenated Krebs solution. The mucosa was removed by sharp dissection.

The muscle sheet was pinned to the Sylgard (Dow Corning Corp.) bottom of a dissection dish and strips were cut along the long axis of the circular or longitudinal muscle cells. The strips were 15 mm. long and 2 mm. wide. The longitudinal muscle layer was removed from the circular strips and the circular layer was removed from the longitudinal, both by sharp dissection. All dissection was

performed with the aid of a dissection microscope. The strips were mounted in a sucrose gap apparatus (Coburn et al.).

Solutions and Drugs

The Krebs solution contained the following (mM): NaCl, 116; Glucose, 10.1; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2 and $NaHCO_3$, 22. The other solutions were 8% sucrose and 117 mM KCl.

Tetrodotoxin 10^{-6} M (Sigma) was administered in Krebs solution to the compartment of the sucrose gap apparatus where electrical activity was recorded. All solutions were equilibrated with 95% O_2-5% CO_2.

Data Analysis

P values comparing the frequency of membrane potential oscillations were calculated using the Student's T test. Individual values were expressed as mean ± standard deviation.

Experimental Protocol

Muscle strips were mounted in the sucrose gap apparatus and attached to a force transducer on one end and a woofer loudspeaker on the other end with silk suture thread. The sucrose compartment was bounded by dental rubber dam material (Dental Dam, HCC Corp.). The muscle strips were pulled through small holes in the rubber dam. Since the rubber dam is pliable it offers little resistance to contractions while maintaining proper isolation of the solutions.

Temperature was maintained at 37°C and recording was begun immediately.

The apparatus can record from the muscle sample in isometric or isotonic mode (Sabourin et al. 1987). In isometric mode the woofer is disabled and the force transducer measures changes in force as a result of the contractile activity of the muscle strip. In isotonic mode a feedback system is enabled which measures the force from the muscle sample using the same force transducer. This force is compared to a desired force value and the woofer is driven by means of an electronic circuit to stretch or release the muscle strip to bring the force to the desired value (see Figure 11). In essence the force applied to the tissue is 'clamped' regardless of any contractile activity occurring in the strip (see Figure 12). During isotonic recording conditions the position of the woofer is sensed and this provides a continuous assessment of the tissue's length, and therefore also its contractile activity.

Muscle strips were mounted under stretch and allowed to develop spontaneous activity during isometric recording conditions. A protocol of stepwise increases and decreases in applied force would then be followed. This consisted of several increases in force followed by several decreases, or, fewer increases with larger steps in force followed by larger decreases. The frequency of electrical oscillations before and after force changes was determined both visually, and by means of a Fast Fourier Transform program running on a personal computer (Stanley et al.). In other

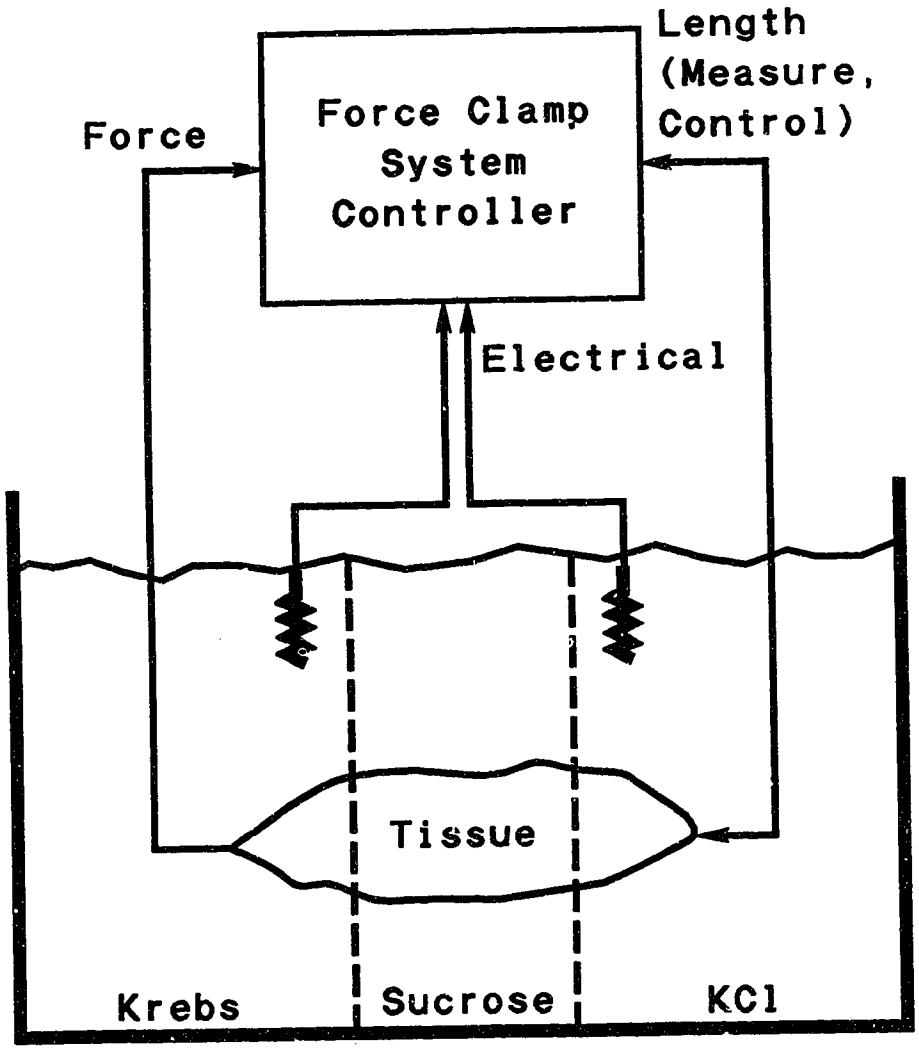

Figure 11. Schematic of force clamping system

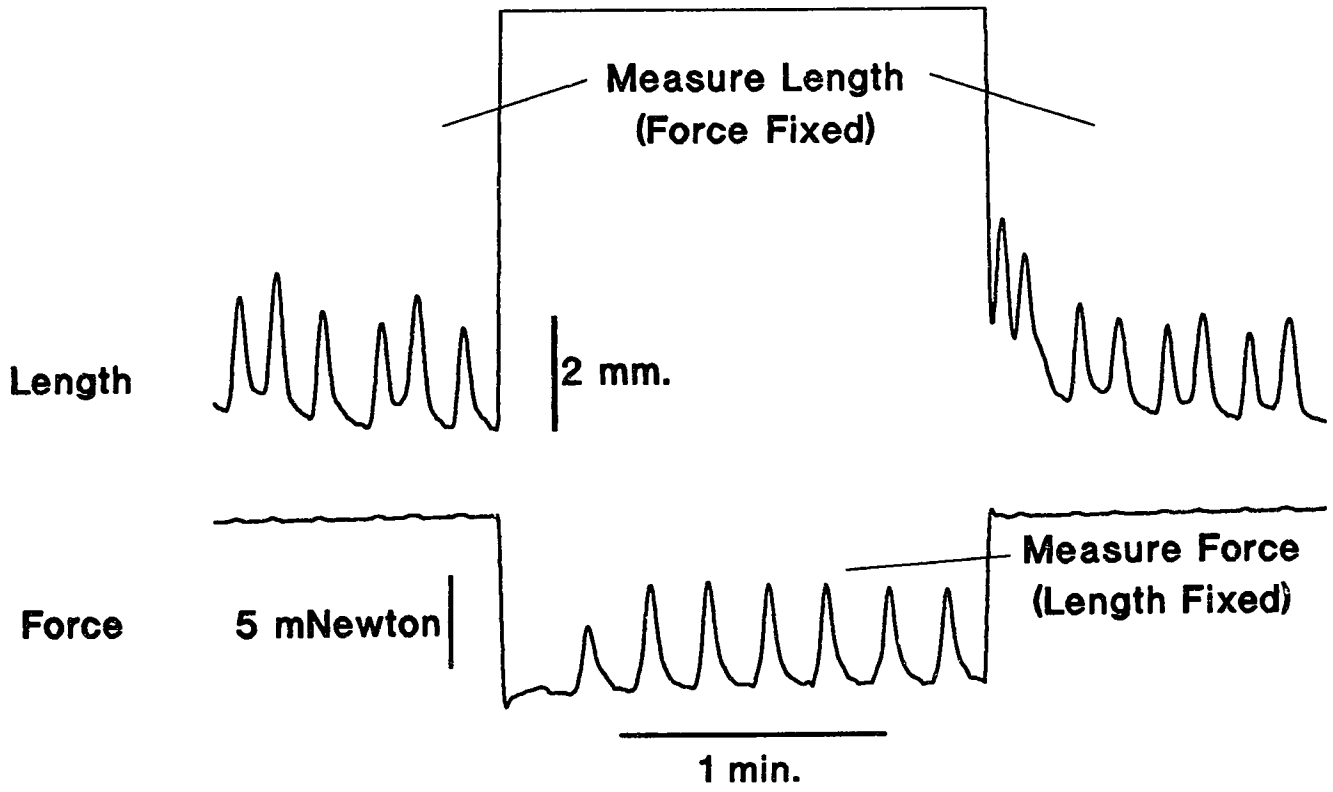

Figure 12. A demonstration of length and force measuring

experiments the same stretch-release protocols were followed before and after TTX administration.

Recording Conditions

Electrical activity in the sucrose gap was measured with Ag/AgCl electrodes. Contractile activity was measured using isometric force transducers (Grass FT.03C). Tissue length was measured using the intramurally developed system.

All signals were recorded using a six-channel Beckman Type R Dynograph (Beckman Instrument Inc.). The signals were also digitized and stored on a PC-AT (IBM Corp.) using a Lab Master input/output board (Scientific Solutions).

D. RESULTS

Circular Muscle

Twenty specimens from eight dogs were subjected to a stretch release protocol. Thirteen of the twenty specimens were subjected to a stretch release protocol before and after treatment with tetrodotoxin. The intention was to identify a relationship between applied force and electrical oscillation frequency if it existed and to identify the tetrodotoxin sensitive neural component of this relationship.

Before quantitative results are presented it is necessary to explain some of the calculations. In an attempt to normalize the

relationship of force to frequency and to allow comparison between samples, a figure of merit was devised. Force was adjusted in steps and for each step the percentage of the change was calculated. For example, an increase in force from 10 Newtons to 11 Newtons represents a positive 10% change. Similarly, the resulting percentage of change in the electrical oscillation frequency was calculated. For example, after an increase in force of 10% the oscillation frequency may have changed from 6 cycles per minute to 6.6 cycles per minute reflecting a positive 10% change. The ratio obtained by dividing percent change in frequency by percent change in force yields a dimensionless figure of merit. In the above example the ratio would be 10%/10% = 1. A positive ratio indicates a positive correlation between applied force and electrical oscillation frequency. Note that this is true for decreases in force also. For example, decreasing the force from 10 Newtons to 9 Newtons is a negative 10% change. A positive correlation suggests an associated decrease in oscillation frequency, for example from 6 cycles per minute to 5.4 cycles per minute, also a negative 10% change. The ratio is calculated as -10%/-10% = 1, still a positive ratio.

Some complications arise when calculating this ratio. Often, a muscle sample demonstrates a spontaneous decrease in frequency with time which is more rapid initially and decreases or stabilizes as the experiment progresses. The effect of this spontaneous decrease upon the frequency-force ratio is different for increases

in applied force than it is for decreases. If a spontaneous background decrease in oscillation frequency is occurring, the ratio for an increase in force will be more negative than it 'actually' is and the ratio for a decrease in force will be more positive that it 'actually' is. To estimate the actual frequency-force ratio an average of ratios resulting from both increases and decreases in applied force must be used. This has the effect of cancelling out the positive and negative artifacts. An unfortunate result of this situation is that frequency-force ratios for increases in applied force can not be directly compared to the ratios for decreases. Instead, the average must be compared to zero (no effect) or compared, for example, before and after tetrodotoxin administration. All ratios from one specimen were averaged, or an average was taken from the entire period before and the entire period after tetrodotoxin administration.

As an example of the changes caused by a force adjustment Figure 13 shows a series of slow waves before and after a force increase. The slow waves in the 'after' tracing appear to be occurring at a higher frequency. Figure 14 show the frequency spectrum calculated by fast Fourier Transform before the force increase. The frequency is approximately 4.5 cycles per minute. In Figure 15 the frequency spectrum of the slow waves after force adjustment is shown. The frequency peak has increased to approximately 5.5 cycles per minute, a +22% change.

Table 1 shows the data that were obtained from a typical

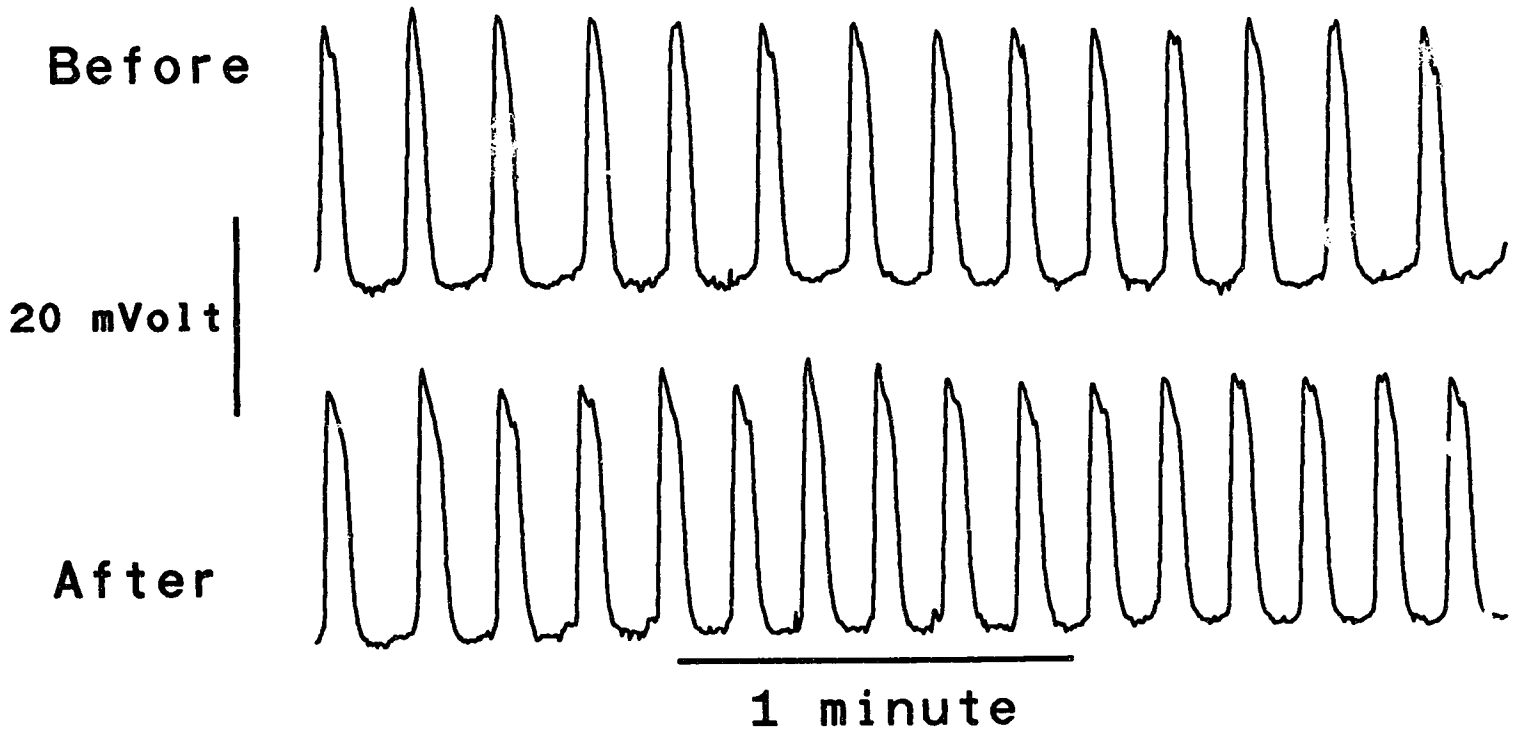

Figure 13. Slow waves before and after a force increase

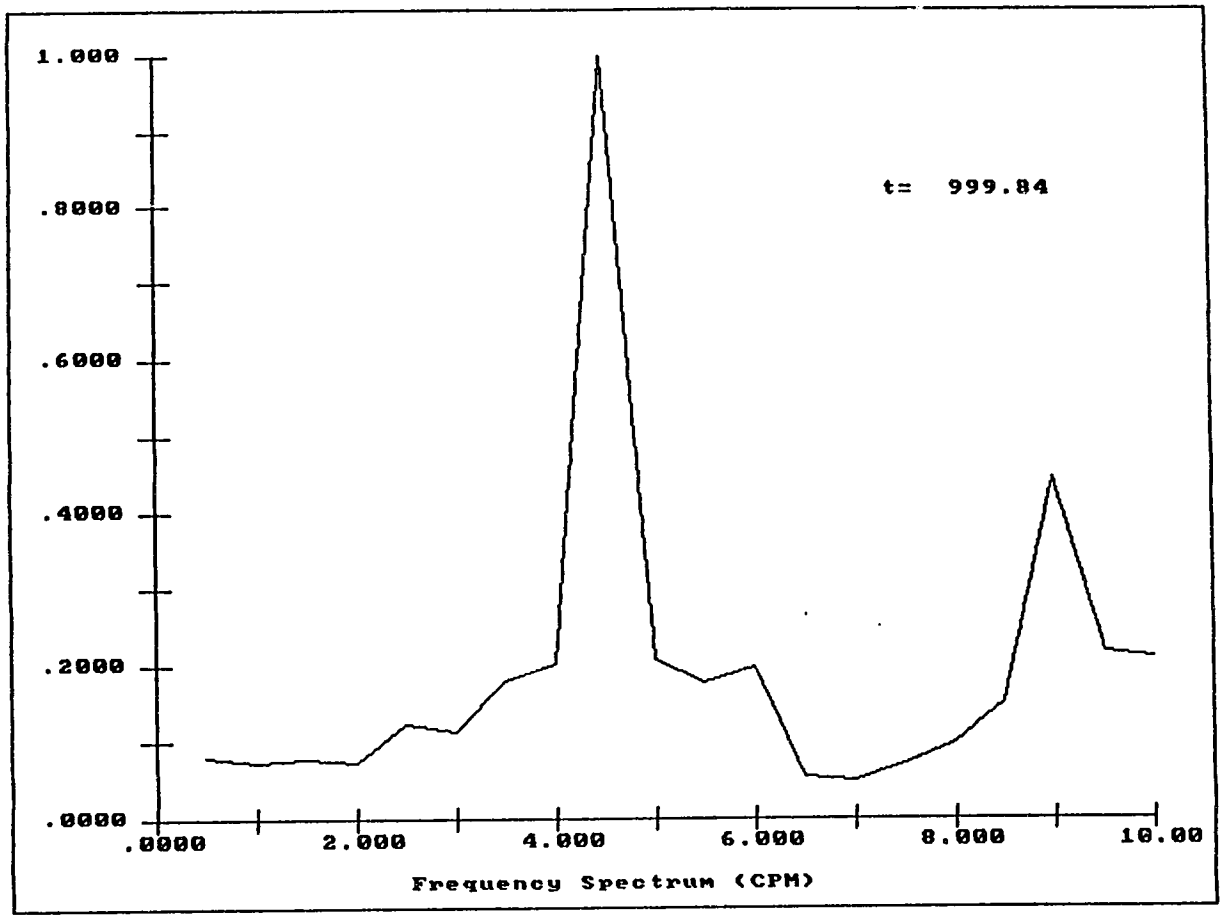

Figure 14. Frequency spectrum of slow waves before force increase

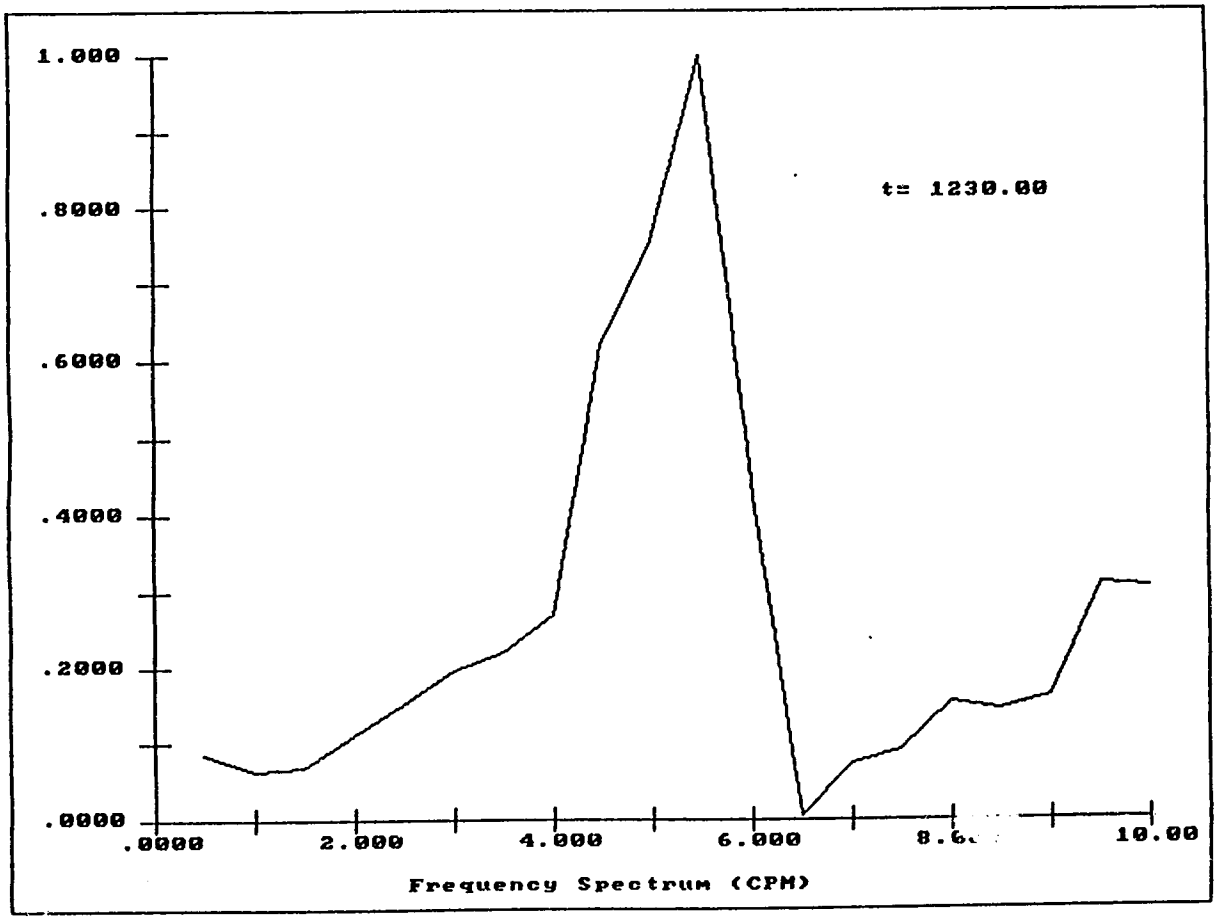

Figure 15. Frequency spectrum of slow waves after force increase

Force (mNewtons)	Frequency (cycles per minute)	% Change In Force	% Change In Frequency	Freq.-Force Ratio
10	7.08			
11	6.5	10%	-8%	-0.82
12	6.5	9%	0%	0.00
13	6.74	8%	4%	0.44
14	6.6	8%	-2%	-0.27
13	5.4	-7%	-18%	2.55
12	4.6	-8%	-15%	1.93
11	4.24	-8%	-8%	0.94
10	3.94	-9%	-7%	0.78
9	3.84	-10%	-3%	0.25
8	3.6	-11%	-6%	0.56
10	3.8	25%	6%	0.22
12	3.74	20%	-2%	-0.08
14	3.34	17%	-11%	-0.64
8	3	-43%	-10%	0.24
14	3.3	75%	10%	0.13
			Average Ratio = 0.41	

Table 1. Frequency and force data, Specimen #1, June 17, 1987

specimen. Considering the columns of data from left to right, force and frequency are measured at 5 minute intervals. From these data per cent changes in force and frequency were derived. Finally, from the per cent changes the frequency-force ratio is calculated for each change then averaged over the entire sample, yielding a value of +0.41 in this case.

The data are best understood graphically so three graph types were developed. In Figure 16 the frequency-force ratio is plotted versus the % change in force. This shows at a glance whether the ratio is tending positive or negative. It is also easy to see if the ratio is different for increases in force when compared to decreases in force. In this specimen the ratio is clearly more positive for decreases than it is for increases. This is a result of the background decrease in electrical oscillation frequency mentioned earlier. This is best seen in Figure 17. In this graph oscillation frequency is plotted against time. Superimposed on this are bars representing the force applied to the specimen during each time interval. From the force bars we get a clear picture of the magnitude and direction of force changes. It is also easy to see the effect of force changes on the oscillation frequency. In this specimen oscillation frequency is clearly decreasing with time. However, it is also clear that oscillation frequency is responding to the changes in applied force. The peaks and valleys in the force bars are matched with peaks and valleys on the oscillation frequency curve. The last graph type that was used is shown in Figure 18.

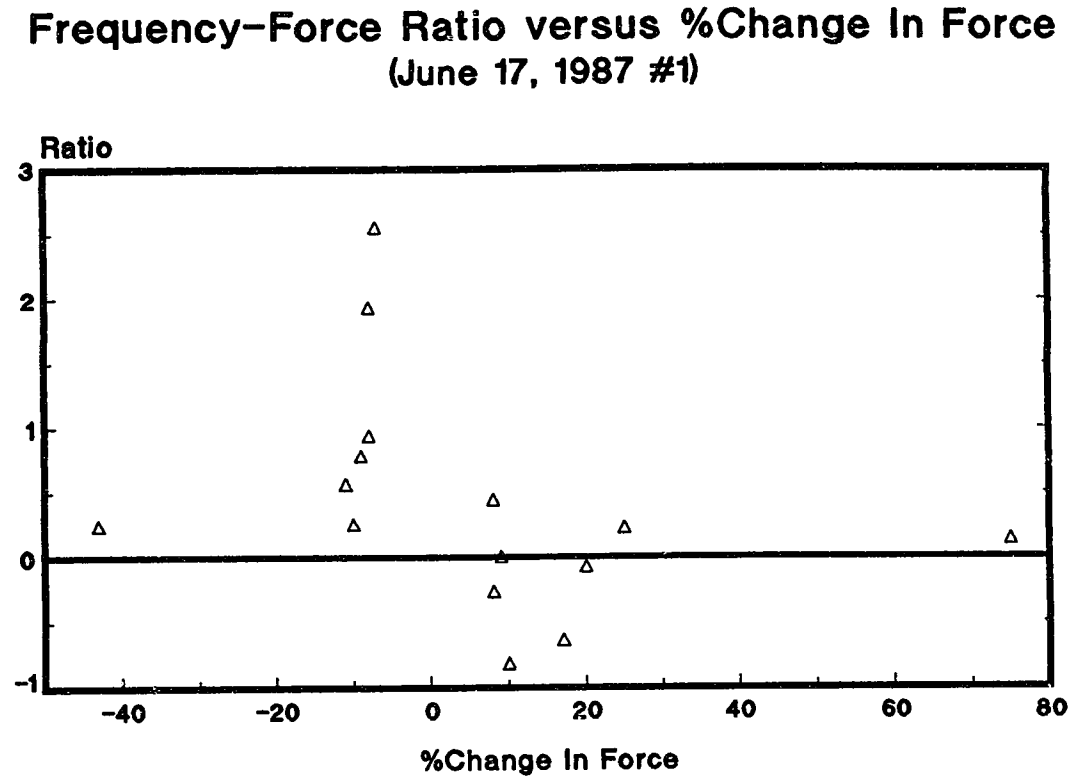

Figure 16. Ratio different for -'ve and +'ve changes in force

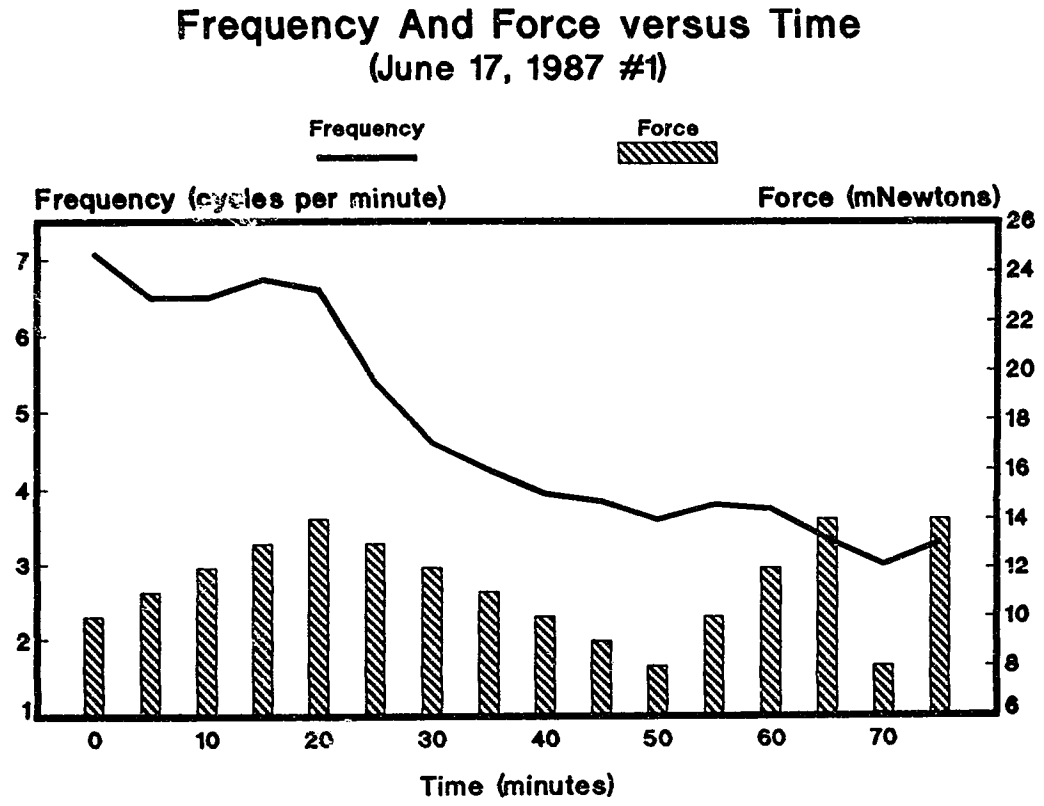

Figure 17. Spontaneous decrease in frequency

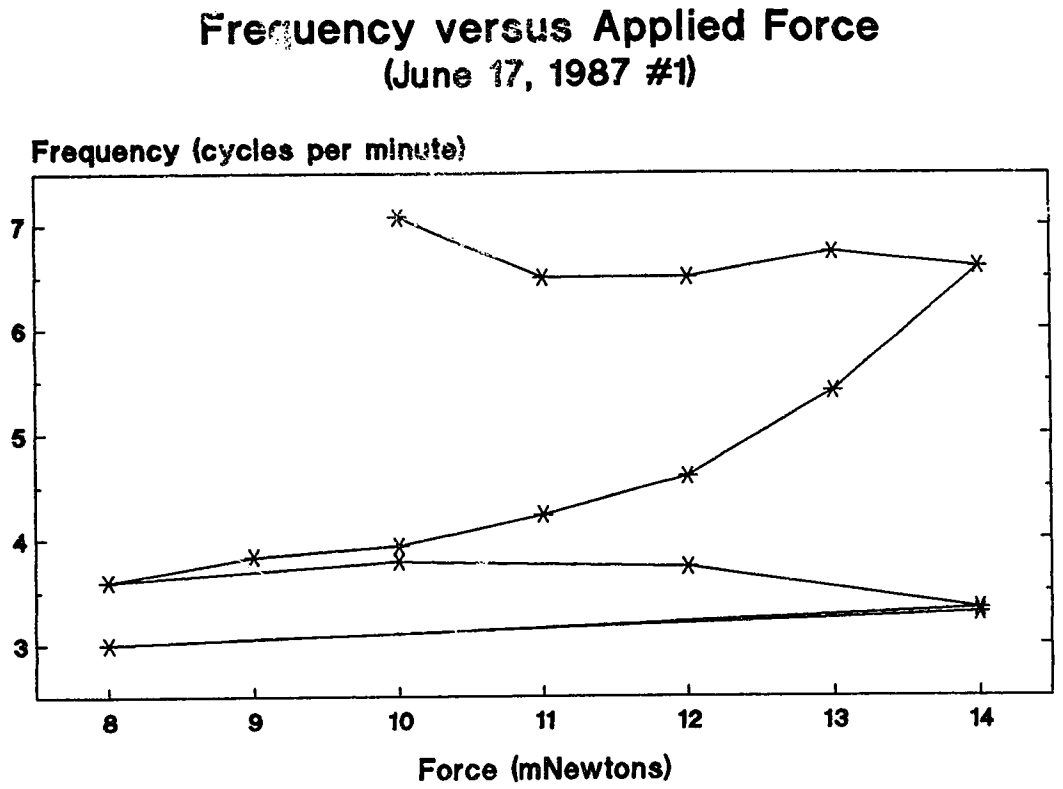

Figure 18. Spontaneous decrease in frequency

Frequency is plotted against force and therefore the slope of the line is related to the frequency-force ratio (they have the same sign, if the ratio is relatively large the slope will be relatively large, but the magnitude is not the same, note that a continuous slope of magnitude 1 would have a varying 'ratio' along its length). The asterisks indicate the frequency and force at a particular time interval and the lines indicate the progression with time. From this graph we can again clearly see that frequency decreased with time. With this graph one can study simultaneously the relationship of the slope (ratio) with time and also with the direction of force adjustment. In this specimen we note that the slope is close to zero during the first series of force increases. Apparently, the spontaneous decrease in frequency was overcome by the increase in frequency that resulted from the force increases. Following this, the force was decreased several times and the slope became noticeably more positive since the spontaneous and force induced decreases in frequency were combined.

In Table 2 the data are presented for a specimen with nearly the same average ratio as the previous specimen. Figure 19, which is a graph of ratio versus percent change in force, shows that in contrast to the previous specimen this specimen had similar ratios of frequency to force for negative and for positive changes in force.

The data are presented in Table 3 for a specimen in which a spontaneous decrease in frequency was not observed. This is seen in

Force (mNewtons)	Frequency (cycles per minute)	% Change In Force	% Change In Frequency	Freq.-Force Ratio
17.5	6.84			
19	6.8	9%	-1%	-0.07
20.5	5.82	8%	-14%	-1.83
19	5.08	-7%	-13%	1.74
17.5	4.86	-8%	-4%	0.55
19	4.86	9%	0%	0.00
17.5	4.52	-8%	-7%	0.89
19	4.5	9%	0%	-0.05
17.5	4	-8%	-11%	1.41
19	4.2	9%	5%	0.58
17	3.94	-11%	-6%	0.59
19	4.44	12%	13%	1.08
16.5	4.08	-13%	-8%	0.62
19	4.24	15%	4%	0.26
16	4.2	-16%	-1%	0.06
19	4.48	19%	7%	0.36
16	4.12	-16%	-8%	0.51
			Average Ratio =	0.42

Table 2. Frequency and force data, Specimen #1, June 18, 1987

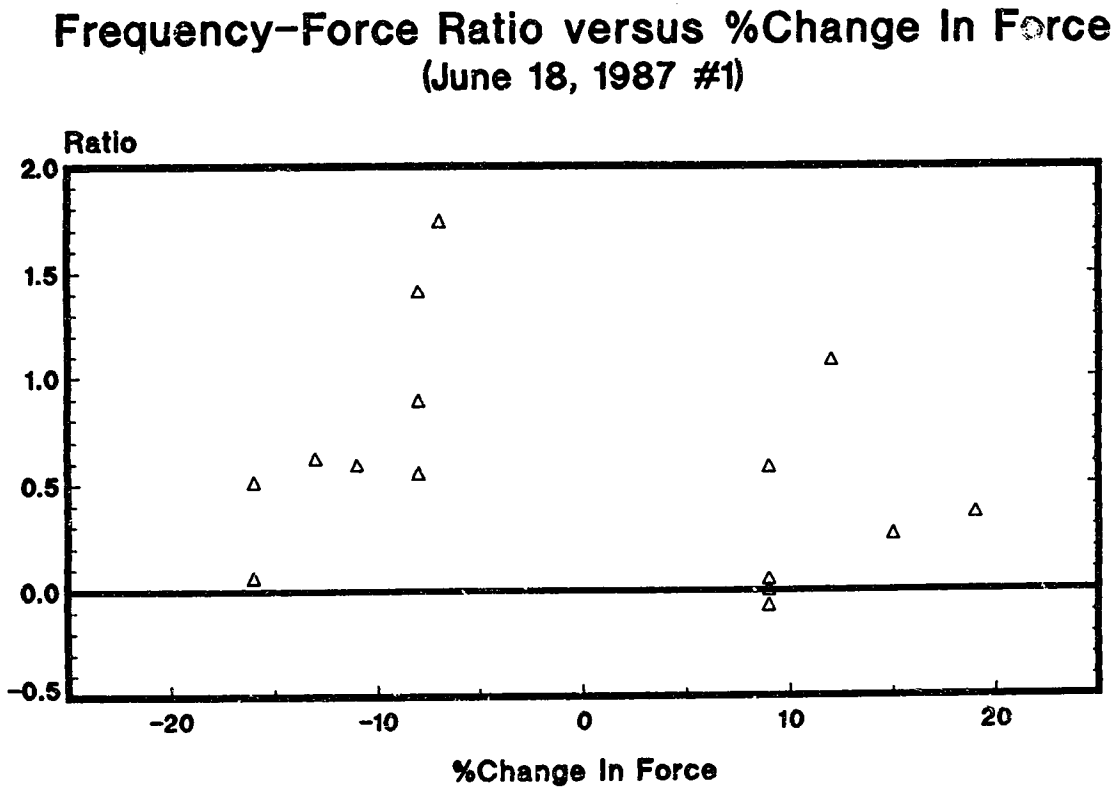

Figure 19. Ratio similar for -'ve and +'ve changes in force

Force (mNewtons)	Frequency (cycles per minute)	% Change In Force	% Change In Frequency	Freq.-Force Ratio
18	4.92			
20	4.68	11%	-5%	-0.44
22	4.94	10%	6%	0.56
24	5.2	9%	5%	0.58
22	5.28	-8%	2%	-0.18
20	4.72	-9%	-11%	1.17
18	4.68	-10%	-1%	0.08
21	4.66	17%	0%	-0.03
24	4.8	14%	3%	0.21
21	4.72	-13%	-2%	0.13
18	4.38	-14%	-7%	0.50
24	4.8	33%	10%	0.29
18	4.52	-25%	-6%	0.23
24	4.96	33%	10%	0.29
18	4.92	-25%	-1%	0.03
24	5.08	33%	3%	0.10
18	4.92	-25%	-3%	0.13
24	5.04	33%	2%	0.07
			Average Ratio = 0.22	

Table 3. Frequency and force data, Specimen #3, June 19, 1987

Figure 20 where the oscillation frequency varied in the five cycle per minute range in response to force changes. The force bars in Figure 20 demonstrate how the force adjustment protocol was varied from small increases and decreases in force to larger increases and decreases. Figure 21 is a graph of frequency versus applied force from the same sample. Since the frequency did not decrease spontaneously the lines are all overlapping. From this grouping of overlapping lines it is immediately apparent that the average slope (ratio) is positive. A few of the lines, however, have a negative slope which seemed to be the case in all samples studied.

Table 4 shows a different arrangement of data from a sample that was treated with tetrodotoxin. The average ratio was calculated before and after TTX administration. This sample was chosen as typical since we see a positive average ratio both before and after TTX. Note, however, that the ratio after has been decreased, by about 50 % in this case. This is illustrated in Figure 22 where the grouping of lines in the top half of the graph have a smaller slope and represent the response of the tissue after TTX. The lines towards the bottom half of the graph represent data measured before TTX administration and have a larger average positive slope.

A summary of the average ratios obtained from all circular muscle specimens (n=20) is shown in Table 5. If the Student T test is used to assess the effect of applied force on oscillation frequency compared to no effect then the 'before TTX' group is found

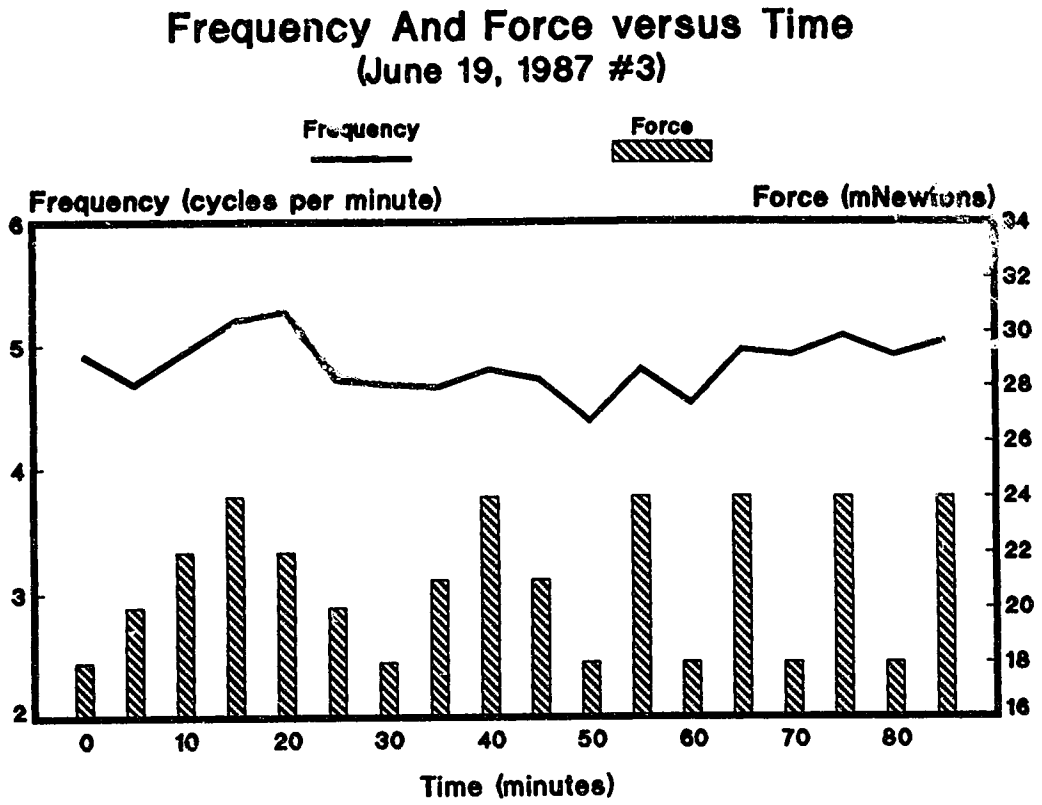

Figure 20. No spontaneous frequency decrease

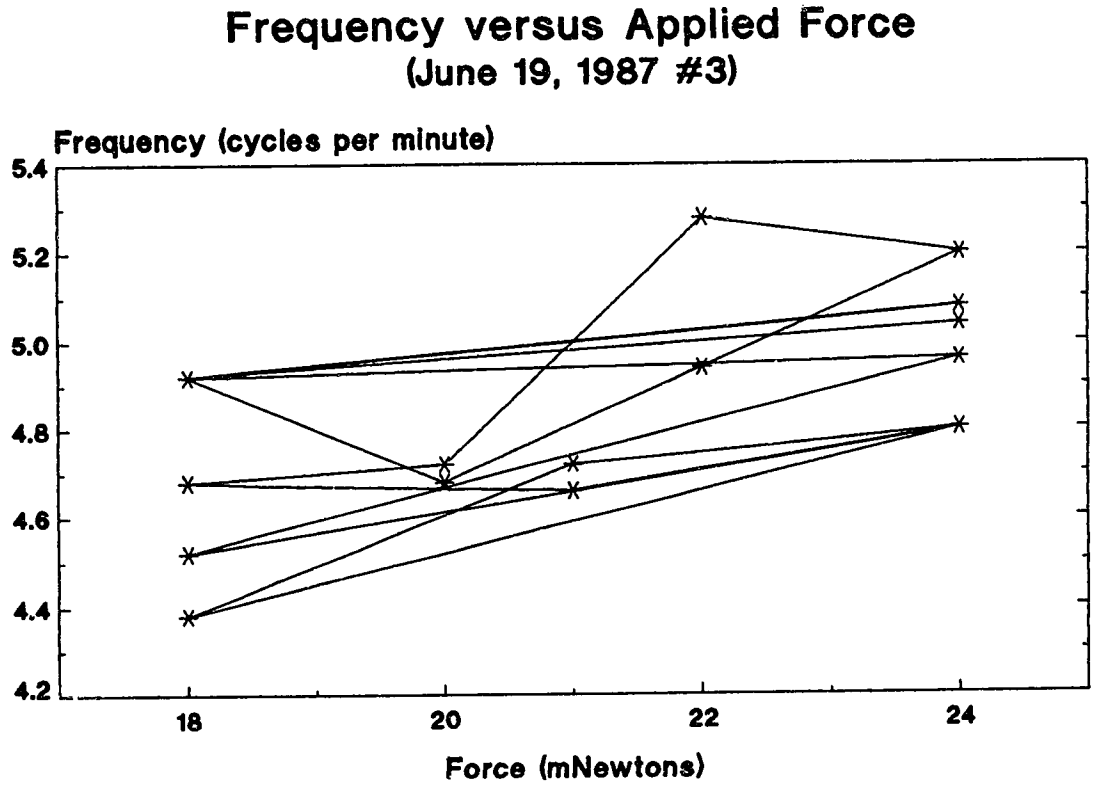

Figure 21. A positive frequency-force ratio

Force (mNewtons)	Frequency (cycles per minute)	% Change In Force	% Change In Frequency	Freq.-Force Ratio
30	23			
21	20.4	-30%	-11%	0.38
32.5	24.6	55%	21%	0.38
22.5	21.5	-31%	-13%	0.41
33	23.6	47%	10%	0.21
			Average Ratio = 0.34	

Administer Tetrodotoxin

32.5	21.4			
22.5	20.3	-31%	-5%	0.17
32.5	25.2	44%	24%	0.54
22.5	24.2	-31%	-4%	0.13
32.5	26.6	44%	10%	0.22
22.5	25.3	-31%	-5%	0.16
32.5	24.4	44%	-4%	-0.08
22.5	24.2	-31%	-1%	0.03
32.5	25.8	44%	7%	0.15
22.5	25	-31%	-3%	0.10
32.5	25.9	44%	4%	0.08
22.5	24.8	-31%	-4%	0.14
			Average Ratio = 0.15	

Table 4. Frequency and force data, Specimen #1, July 3, 1987

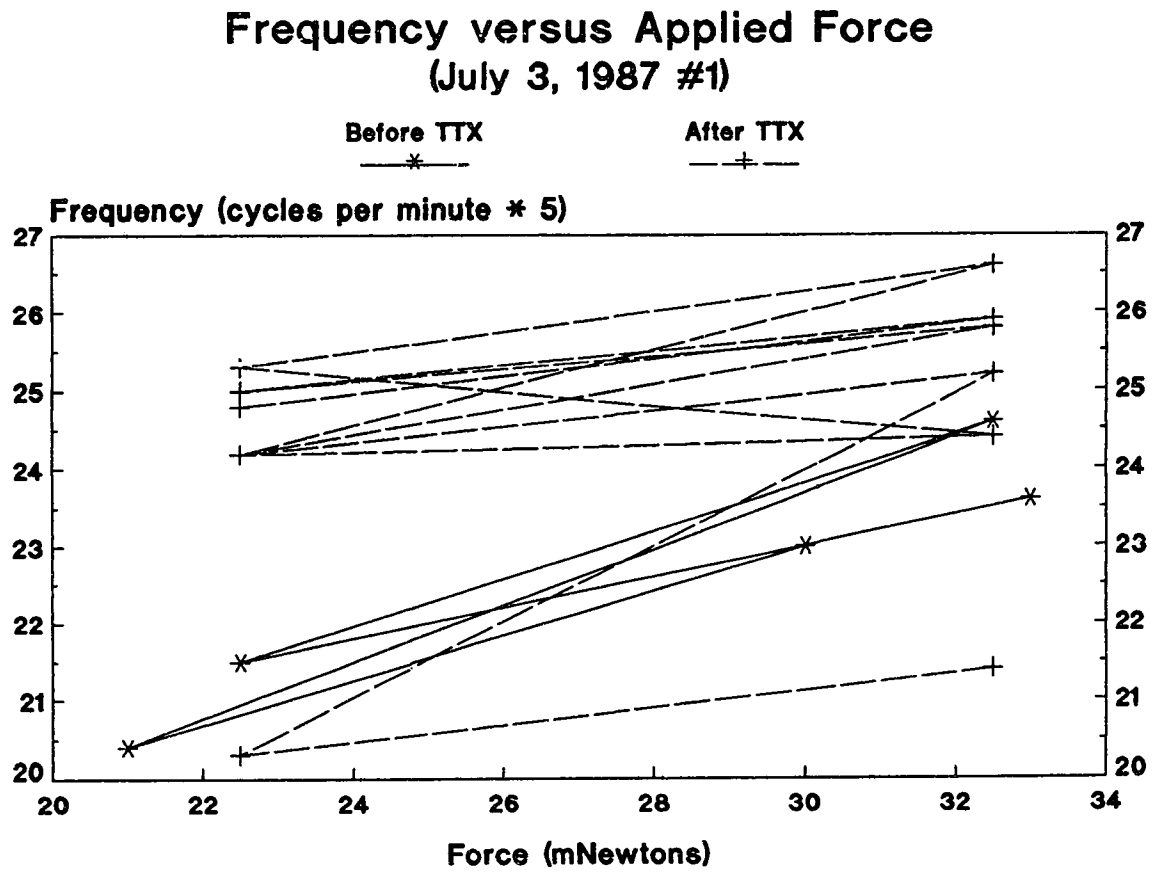

Figure 22. Frequency-force ratio different after tetrodotoxin

Before Tetrodotoxin Administration	After Tetrodotoxin Administration
0.28	0.08
0.34	0.15
0.01	-0.01
0.45	0.07
0.33	0.15
0.02	-0.06
0.15	0.08
0.06	0.04
0.16	-0.04
0.12	0.27
-0.04	-0.02
0.08	0.16
0.02	-0.09
0.45	
-0.03	
0.52	
0.02	
0.07	
0.12	
0.22	
Mean	0.17	0.06
SDev	0.17	0.10

Compare 'Before TTX' To 'No Effect'

p=0.0001

Compare 'After TTX' To 'No Effect'

p=0.05

Compare 'Before TTX' To 'After TTX'

p=0.05

Table 5. Summary of frequency-force ratios, circular muscle

to be significantly different from no effect (p=0.0001). Applied force has a positive effect on electrical and contractile oscillation frequency. Comparing the 'after TTX' group to no effect a significant positive correlation (p=0.05) is noted. Although both before and after TTX there is a positive correlation of frequency to force, a comparison of 'before TTX' to 'after TTX' reveals that TTX causes a significant reduction in this correlation (p=0.05).

Longitudinal Muscle

A series of stretch release protocols were also applied to 10 specimens of longitudinal muscle from 5 dogs. In contrast to the circular muscle, the longitudinal specimens did not have a pronounced background decrease in oscillation frequency. The longitudinal muscle also demonstrated a positive correlation between applied force and oscillation frequency. The data are summarized in Table 6.

Frequency-Force Ratios From 10 Specimens				
0.63	0.23	0.26	0.26	0. 4
0.35	0.19	0.1	0.29	0..
Mean = 0.27		Standard Deviation = 0.15		
Is Frequency-Force Ratio Different From Zero? Yes, p = 0.0004				

Table 6. Summary of frequency-force ratios, longitudinal muscle

Analysis of the oscillation frequency before and after changes in force was difficult with longitudinal muscle. The longitudinal layer does not always generate continuous oscillations which can be readily compared before and after force changes. Membrane potential oscillations often appear in bursts only and there are often irregular periods of quiescence. Further, in this apparatus the longitudinal layer is often quiescent for an hour or more at the start of experiments before regular membrane potential oscillations result. The 10 samples from 5 dogs mentioned previously are actually a subset from 43 samples from 17 dogs that were attempted. The remainder were eliminated from the results since the activity of the specimens was too irregular and could not be reliably analyzed. Tetrodotoxin was not applied to longitudinal muscle samples since

the expected number of experiments required to achieve an acceptable level of significance for the results was not considered worthwhile.

E. DISCUSSION

A goal of this thesis is to better understand how the two colonic muscle layers coordinate their contractile activities. The presence of a bolus within the colonic lumen provides a distension stimulus to both layers simultaneously. One can hypothesize that the motility patterns associated with the movement of this bolus are the result of contractile events coordinated between the two muscle layers. Further, as one layer contracts it may be that this is 'sensed' in the other layer and that this other layer's activities are altered as a result, which is a form of coordination.

The data presented in this chapter show that electrical oscillations and contractile frequency are affected by the degree of distension or force applied to the colonic wall. This could provide a basis for contractile coordination. For example, contraction of the longitudinal layer could cause an increase in the force applied to the circular layer near the region of contraction. The circular layer would then begin to contract more frequently in the vicinity of the longitudinal contraction.

Contractile frequency alone may not be so important, but in combination with other excitatory effects that have been reported to occur in smooth muscles in response to stretch, the alteration in motility could be significant. For example, stretching of the

guinea-pig taenia coli decreased the resting membrane potential making the muscle more excitable (Bülbring 1955,1963, Gillespie, Kosterlitz et al.). Spiking activity, and hence more powerful contractions, has been elicited by stretch in a number of species (Bülbring 1955, Burnstock et al. 1960, El-Sharkawy 1983, Huizinga et al. 1983). In human colon circular and longitudinal muscle (Gill et al. 1986) the force of contractions was noted to increase (although after sufficient stretching the force decreases again) with increases in the length of a stretched muscle preparation. Patch clamp experiments have shown that stretch-activated channels are present in smooth muscle (Kirber et al.). These channels can apparently conduct substantial ionic current into the cell, both sodium and calcium, which could lead to more powerful contractions. These results provide a basis by which the contractile activity of one smooth muscle layer is in part coordinated with the activity of the other layer as a result of stretch mediated interaction that leads to excitation and oscillation frequency increases. Coordination of electrical activities in pig colon were noted to occur consistently only after stretch or cholinergic stimulation (Huizinga et al. 1987a). This supports the hypothesis that contraction induced stretch could also enable coordination.

In the present study tetrodotoxin reduced but did not block the correlation between force and oscillation frequency. This suggests that both a myogenic and a neurogenic component may be involved. Experiments on opossum esophagus in which a balloon was

inflated (on response) then deflated (off response) also suggested a composite response. The off response appeared to be a noncholinergic neural excitation while the on response was suggested to be a direct response to stretch (Christensen 1970). Numerous responses to stretch have been reported for different preparations and different organs. These included excitatory and inhibitory, neurogenic and myogenic. Some even report a variety of responses within a single type of preparation (Huizinga et al. 1983). It appears that stretch induced responses are complex and apparently act through many pathways.

F. CONCLUSION

Electrical and contractile oscillation frequency can be increased by increased distention of the colonic wall. This response may play a role in the coordination of electrical and contractile activities between the circular and longitudinal muscle layers of the canine colon.

CHAPTER V. SIMULTANEOUS MEASUREMENT OF ELECTRICAL ACTIVITY FROM TWO COLONIC SMOOTH MUSCLE LAYERS USING A DUAL SUCROSE GAP APPARATUS

A. ABSTRACT

An apparatus using the sucrose gap technique is presented. With this apparatus, simultaneous measurements of contractile and intracellular electrical activity from the two smooth muscle layers of the colon are made. An 'L-shaped' muscle preparation consisting of a leg from the circular muscle layer and a leg from the longitudinal muscle layer is used. A theoretical discussion of the device's operation is presented. Finally, experimental results that validate the theory are included.

B. INTRODUCTION

The colon is responsible for the storage, transport and evacuation of fecal matter. This activity is accomplished by two perpendicularly oriented smooth muscle layers that form the colonic wall. The smooth muscle mass is made up of spindle shaped smooth muscle cells. In one muscle layer, the longitudinal layer, the long axis of these cells is oriented along the long axis of the colonic tube. In the other smooth muscle layer, the circular layer, these cells are oriented transverse to the long axis of the colonic tube.

The cells of the two smooth muscle layers demonstrate periodic electrical oscillations that can be accompanied by contractile activity. In canines, the two muscle layers demonstrate rather different electrical and contractile activity when studied

separately in vitro. Despite these differences in activities various types of interaction and coordination have been reported (El-Sharkawy 1983, Huizinga et al. 1987a, Sabourin et al. 1988, Smith et al. 1987b). These reports have usually focussed on the electrical activity while the contractile activity has been recorded from only one muscle layer at a time (El-Sharkawy 1983, Huizinga et al. 1987a), or not at all (Smith et al. 1987b). In order to elucidate the mechanisms of interaction and coordination that occur in the colon an apparatus was devised based on the sucrose gap technique (Sabourin et al. 1988, Coburn et al.) with which simultaneous measurement can be made of the electrical and mechanical activity of both smooth muscle layers.

C. THEORETICAL CONSIDERATIONS

Basic Sucrose Gap

The sucrose gap technique was first developed to measure the intracellular activity of nerve fibres using extracellular electrodes (Stämpfli). These fibers behaved as conducting cables and one dimensional cable equations were used to explain the operation of the sucrose gap apparatus (Jirounek et al., Katz). Later, the sucrose gap technique was also applied to smooth muscle samples since smooth muscle was noted to demonstrate cable like properties (Tomita 1966, 1970, Burnstock et al. 1958). These multi-cellular preparations were assumed to be a syncytium in which the cells were coupled to one another and had the same electrical

activity in all active smooth muscle cells that made up the sample.

In a basic sucrose gap apparatus a tissue sample is exposed to three different solutions in three separate compartments as shown in Figure 23. The potassium chloride solution (KCl) depolarizes and inactivates all muscle cells in that compartment. The sucrose compartment provides a high impedance extracellular path owing to the high specific resistance of sucrose solution. The cells in this region are also inactivated since sucrose solution does not contain the ions required to generate electrical oscillations in membrane potential. The Krebs compartment contains a Krebs-Ringer solution that maintains the muscle cells in an active 'normal' state. All solutions should be adequately oxygenated. The electrical potential that exists across the sucrose gap is measured using a high input impedance amplifier and is related to the intracellular activity of the cells in the Krebs compartment.

To understand this, consider the distribution of currents and voltages as shown in Figure 24. By Ohm's law the current along the outside is,

$$i_0(x) = -1/r_0(dv_0(x)/dx) \tag{1}$$

where r_0 is the resistance per unit length of the outside medium. Similarly the current along the inside is,

$$i_i(x) = -1/r_i(dv_i(x)/dx) \tag{2}$$

We have tacitly assumed that the internal and external media

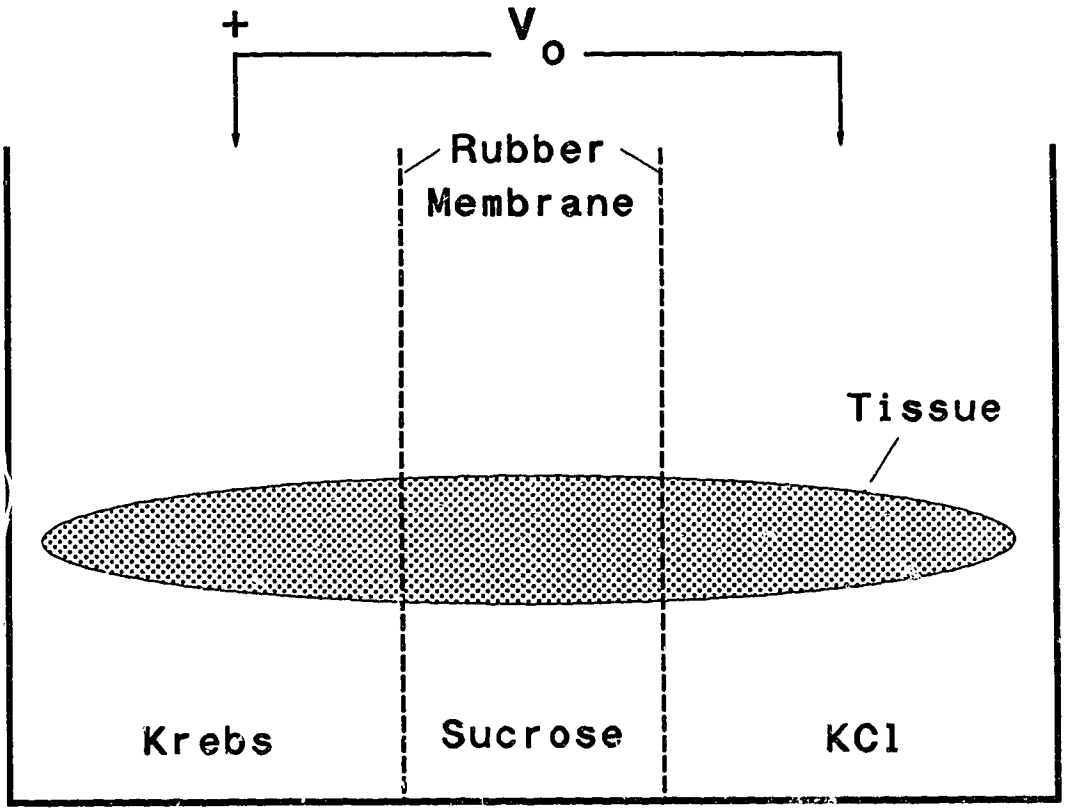

Figure 23. Smooth muscle tissue sample mounted in a sucrose gap apparatus

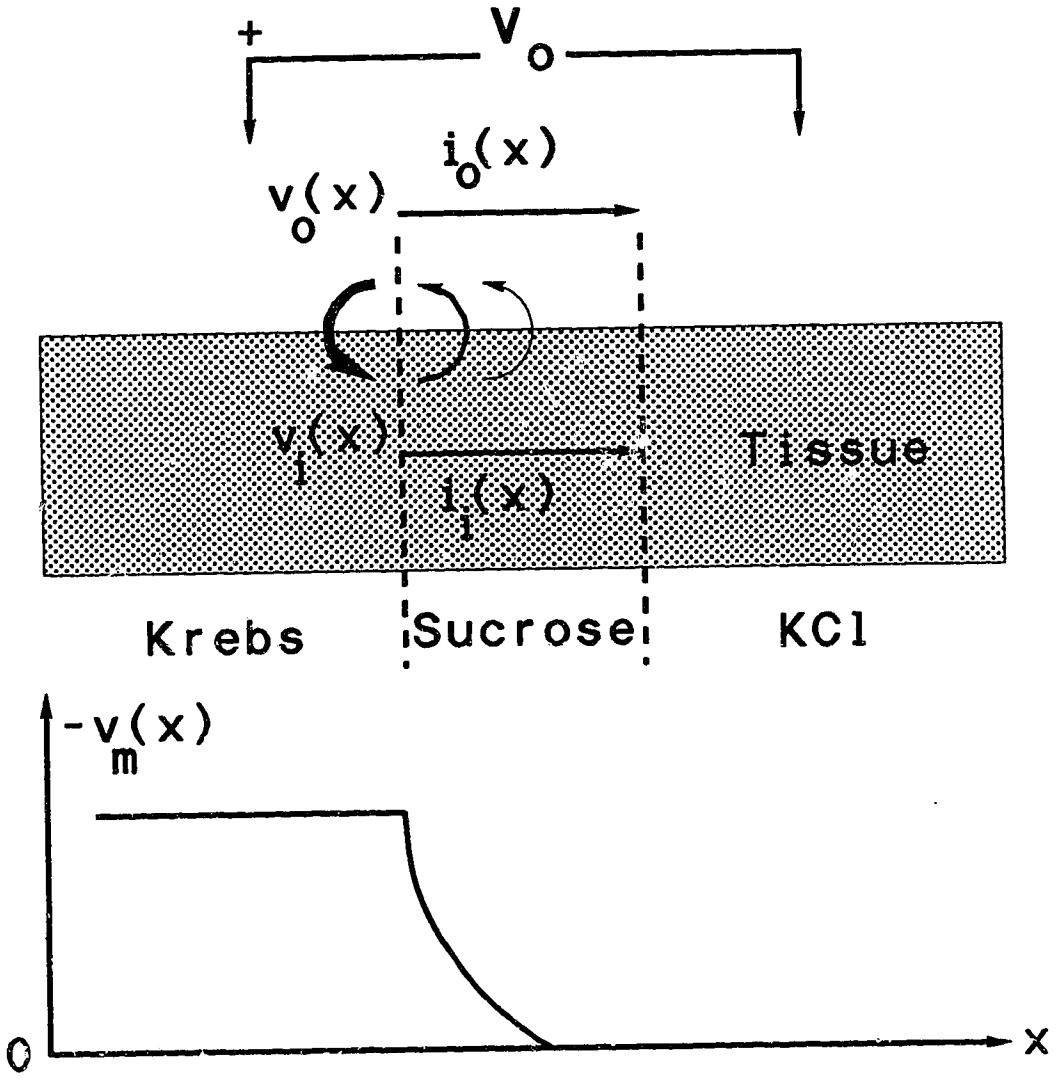

Figure 24. Distribution of currents and voltages in a smooth muscle tissue specimen mounted in a sucrose gap apparatus

behave as pure Ohmic resistances. As well, we are solving for steady state conditions. Note that $i_i(x)$ is equal in magnitude but opposite in direction and sign to $i_0(x)$ for any given x, or,

$$i_i(x)=-i_0(x) \tag{3}$$

since the net current flowing through any infinite cross section must be zero. The smooth muscle cells of the tissue in the KCl compartment are depolarized so that $v_{iKCl}=v_{oKCl}=0$. The voltage that exists between the Krebs and the KCl compartment can then be calculated by integrating i_0 between the two compartments which yields,

$$V_0=-r_0\int i_0(x)dx \tag{4}$$

If we now substitute 2) and 3) into 4) we obtain,

$$V_0=-(r_0/r_i)\int dv_i(x)=-(r_0/r_i)(v_{iKrebs}) \tag{5}$$

This result requires that the membrane potential $v_m(x)$ has decayed to zero within the sucrose compartment. $v_m(x)$ decays with distance since the tissue acts like a resistive cable (Coburn et al., Jirounek et al.) which has an associated space constant (λ). The space constant of canine colonic smooth muscle is 1-2 mm in Krebs solution (Huizinga et al. 1988). In sucrose solution the space constant is much shorter since,

$$V_m(x)=V_{mKrebs}\exp(-x/\lambda) \text{ and } \lambda=[r_m/(r_0+r_i)]^{0.5} \tag{6}$$

and also, $r_{0sucrose}>>r_{0Krebs}$ (r_m is the membrane resistance which is

assumed to be unchanged). We have used a gap width of 5 mm. which ensures that $v_m(x)$ has decayed to zero.

Further, if we wish to relate V_0 to the membrane potential,

$$v_m=v_{iKrebs}-V_0 \tag{7}$$

we obtain,

$$V_0=-v_m(r_0/(r_i+r_0)) \tag{8}$$

which indicates that the potential measured across the sucrose gap is the membrane potential attenuated by a resistive voltage divider circuit. As mentioned previously, sucrose has a high resistance and therefore r_0 is large with respect to r_i and V_0 can approach the value of $-v_m$. A factor ignored thus far, however, is that liquid junction potentials exist between the different solutions which add a constant offset voltage to the measured value of V_0 (Jirounek et al.). Since an attenuation factor and a dc offset voltage are present, the sucrose gap is commonly used to measure the shape of the intracellular waveform rather than its absolute voltage levels.

Inhomogeneity Through The Depth

Canine colonic smooth muscle has been shown to produce different electrical signals at various points through the depth of the colonic wall (Barajas-López et al. 1988, 1989, Chow et al., Debski et al., Smith et al. 1987b). Since this violates one of the assumptions made in the previous derivation, a second derivation

will be made for this different case. Consider the inhomogeneous smooth muscle specimen depicted in Figure 25. We can now write,

$$i_{i1}(x)=-1/r_{i1}(dv_{i1}(x)/dx) \text{ and } i_{i2}(x)=-1/r_{i2}(dv_{i2}(x)/dx) \qquad (9)$$

where r_{i1} and r_{i2} are the resistances per unit length of region 1 and region 2 respectively. Moreover, the relationship for $i_o(x)$ can now be written as,

$$i_o(x)=-i_{i1}(x)-i_{i2}(x) \qquad (10)$$

Note that the coupling current $i_c(x)$ between the two layers would lead to a more complex equation of decay for $v_{i1}(x)$ and $v_{i2}(x)$ than equation 6). However, if we assume that the sucrose compartment is wide enough so that $v_{i1}(x)$ and $v_{i2}(x)$ have decayed to zero within the sucrose compartment, as previously, we can substitute 9) and 10) into 1) to obtain,

$$V_o=-r_o/r_{i1}(v_{i1Krebs})-r_o/r_{i2}(v_{i2Krebs}) \qquad (11)$$

Once again, substituting and rearranging to obtain V_o in terms of the membrane potentials yields,

$$V_o=-r_o(r_{i2}v_{m1}+r_{i1}v_{m2})/[r_{i1}r_{i2}+r_o(r_{i1}+r_{i2})] \qquad (12)$$

which is a weighted average of the two different populations of cells. With r_o made large with respect to r_{i1} and r_{i2} this equation simplifies to,

$$V_o=-(r_{i2}v_{m1}+r_{i1}v_{m2})/(r_{i1}+r_{i2}) \qquad (13)$$

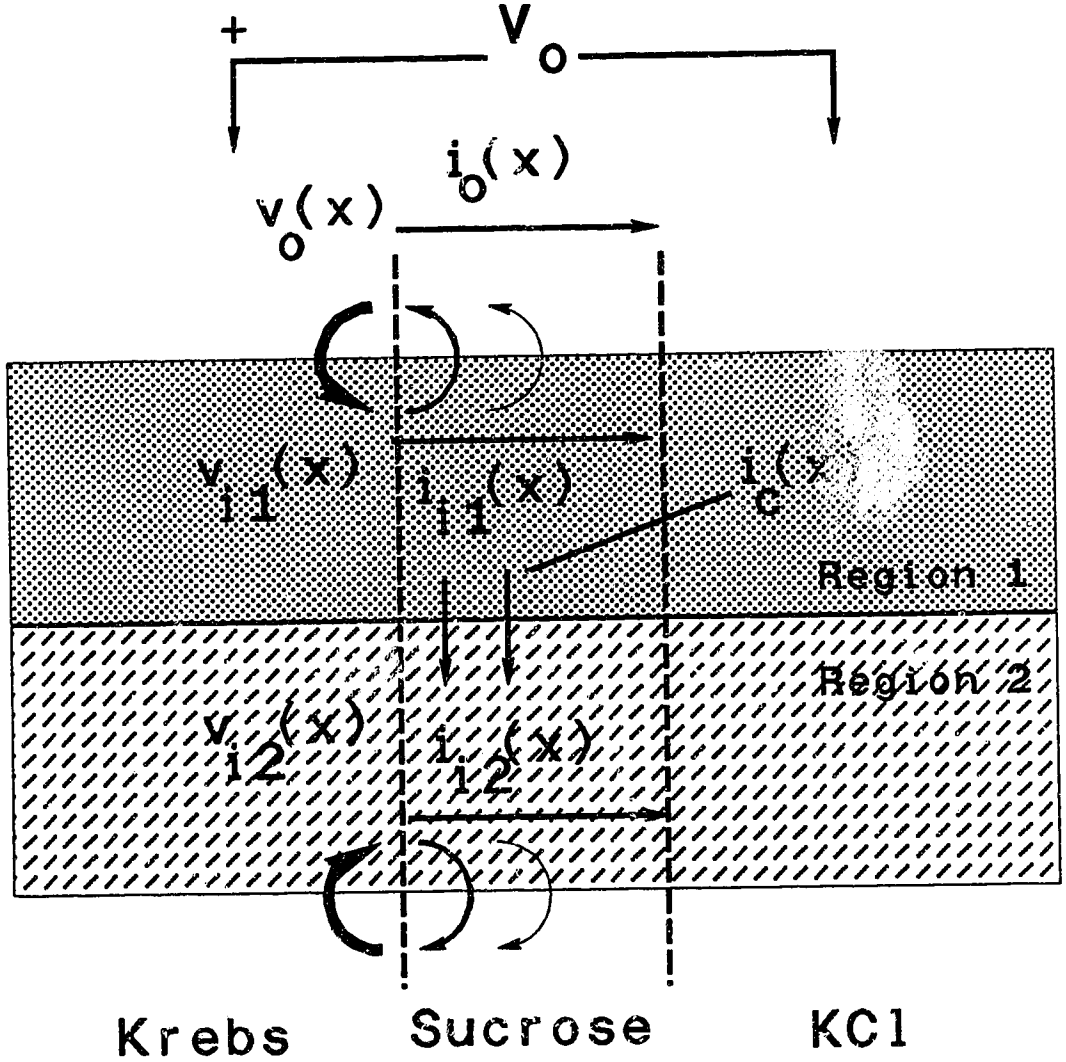

Figure 25. Inhomogeneous smooth muscle specimen with two regions of activity occurring through the depth

Equation 11) can be generalized for n different active cellular regions and it becomes,

$$V_o=-r_o/r_{i1}(v_{i1})-r_o/r_{i2}(v_{i2})-\ldots-r_o/r_{in}(v_{in}) \qquad (14)$$

The general form of 12) contains a weighted average of all the different v_{ik}, k=1..n. A result of 11) through 14) is that a quiescent portion of the tissue attenuates the voltage measured from an active portion. Also, if for example a larger and a smaller region of a tissue sample exhibit different electrical activities, then the larger region will have a larger effect on the voltage measured across the sucrose gap (provided r_i is similar for both regions).

Longitudinal Inhomogeneity

Another possible departure from the assumptions made in the original derivation is one in which two different regions of electrical activity are present along the axis of the muscle preparation within the Krebs compartment. The distribution of currents and voltages for this case is shown in Figure 26. Using 1) through 7) in a similar manner as previously and calculating the voltage changes in the extracellular space as a result of current flow we obtain an additional contribution to $v_o(x)$ of,

$$-r_o/r_i(v_{ix2}-v_{ix1}) \text{ or } -r_o/r_i(v_{mx2}-v_{mx1}) \qquad (15)$$

where v_{mx2} and v_{mx1} are the membrane potentials at the points x_2 and x_1. This indicates that the voltage measured across the sucrose gap

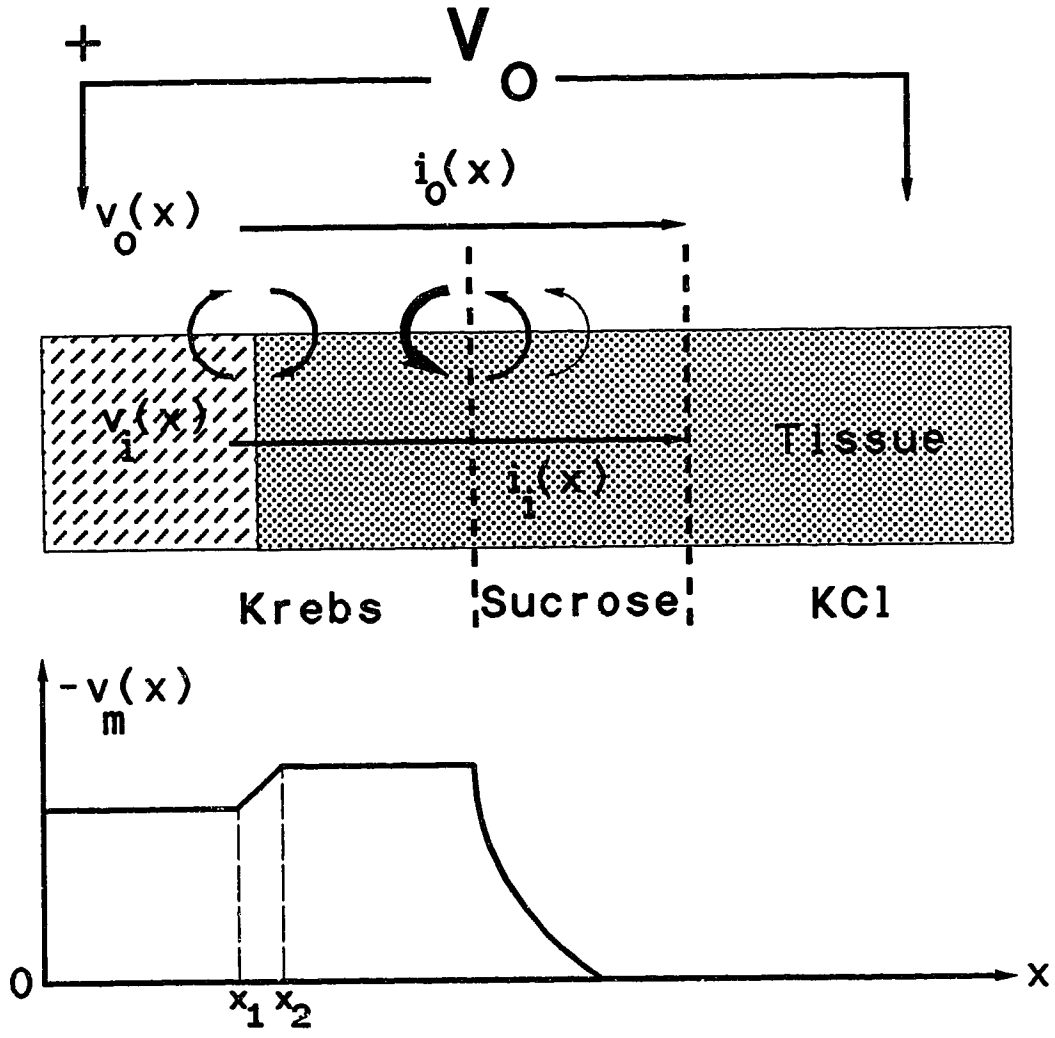

Figure 26. Inhomogeneous smooth muscle specimen with two regions of activity occurring along the length

placed anywhere convenient in the Krebs compartment rather than at a point directly adjacent to the sucrose gap.

We conclude that the second region of activity will only contribute significantly to the recorded potential across the sucrose gap if it actually couples into the intracellular activity of the cells at the sucrose-Krebs border. Therefore, if a second oscillator is detected in the electrical record of the sucrose gap, this should not be considered as a spurious artifact but rather as an indication that a second oscillator is coupling into cells in the region of the sucrose-Krebs border.

D. THE DUAL SUCROSE GAP

Two sucrose gaps were combined with a common Krebs compartment to form a 'dual' sucrose gap to record from 'L' shaped tissue samples. Tissue samples were obtained as described in the methods section of Chapter 4. 'L' shaped tissue specimens were prepared. The legs of the 'L' were cut 10 to 15 mm. long by 1.5 to 3 mm. wide. One leg was cut parallel to the circular muscle direction while the other was cut parallel to the longitudinal muscle direction. The longitudinal muscle layer was removed from the circular leg by blunt

dissection, and the bulk of the circular muscle layer (including the submucosa) was removed from the longitudinal leg by sharp dissection. This resulted in an L-shaped preparation where only the hub contained the full thickness (excluding mucosa) of the colonic muscle wall. The tissue samples were then mounted in the dual sucrose gap apparatus. The legs of the 'L' were attached to force transducers with silk suture thread and the bend of the 'L' was fixed to the Sylgard bottom of the organ bath with insect pins. The sucrose compartment was bounded by dental rubber dam material. The tissue sample was pulled through small holes in the rubber dam. Since the rubber dam is pliable it offers little resistance to contractions while maintaining proper isolation of the solutions.

The dual sucrose gap was designed to have a single common Krebs compartment and separate KCl and sucrose compartments. With one electrode placed in the Krebs compartment and one each in the KCl compartments it was possible to measure simultaneously the electrical events occurring in both legs of the tissue sample. From the previous derivations it is clear that the use of the single electrode in the Krebs compartment will not lead to spurious crosstalk between the electrical events from the two legs of the tissue sample. With a force transducer attached to each leg of the 'L' simultaneous measurement of contractile activity from both legs was possible. As a result of the dissection described previously, the activity recorded from one leg was activity from the circular muscle layer, while activity recorded from the other leg was

activity from the longitudinal muscle layer.

Electrical activity was measured with Ag/AgCl electrodes. Contractile activity was measured using isometric force transducers (Grass FT.03C). All signals were recorded using a six-channel Beckman Type R Dynograph (Beckman Instrument Inc.). The signals were also digitized and stored on a PC-AT (IBM Corp.) using a Lab Master input/output board (Scientific Solutions). The signals could then be analyzed and plots produced on the computer screen or on a printer (see Figures 27-29).

E. EXPERIMENTS

Several experiments were performed with the dual sucrose gap apparatus in order to verify its correct operation. Where possible, intracellular electrical recordings were obtained using microelectrodes since this revealed the precise relationship between intracellular activity at various points in the tissue sample and the activity recorded by the sucrose gap apparatus (Kuriyama and Tomita 1970).

In the first experiment, the hypothesis that the sucrose gap apparatus selectively records from the cells directly adjacent to the sucrose-Krebs boundary was tested. Slow waves from the circular layer of canine colon were useful for comparison purposes in this test. While the sucrose gap was recording these slow waves, a smooth muscle cell four millimetres from the sucrose compartment was impaled with a microelectrode. The two waveforms, although

synchronized, were out of phase and showed significant differences in shape as shown in Figure 27A. In this figure the vertical scale has arbitrary units since the waveforms were scaled for comparison purposes. However, when the microelectrode was moved to within one millimetre of the sucrose gap the intracellular and sucrose gap records were very similar and no phase lag was present as seen in Figure 27B. This confirms the theoretical result that the sucrose gap apparatus records selectively from the region directly adjacent to the sucrose-Krebs boundary.

In the second experiment, the hypothesis that the sucrose gap apparatus averages together different electrical activities that occur through the thickness of a tissue sample at the sucrose-Krebs border was tested. When the circular muscle layer is sufficiently stimulated, the slow waves can become prolonged and high frequency membrane potential oscillations with superimposed spike potentials develop, usually superimposed on the slow wave. Recent evidence has shown that these oscillations and spike potentials are occurring only on one side of the circular muscle layer, the myenteric plexus side (Barajas-López et al. 1988, 1989, Smith et al. 1987b). Moreover, the slow wave is generated on the opposite side, the submucosal side, of the circular muscle layer and its amplitude decreases from the submucosal to the myenteric plexus side (Caprilli et al., Durdle et al. 1983a, Smith et al. 1987a, Barajas-López et al. 1989). Two different experiments were carried out while the sucrose gap apparatus recorded: slow waves, superimposed membrane

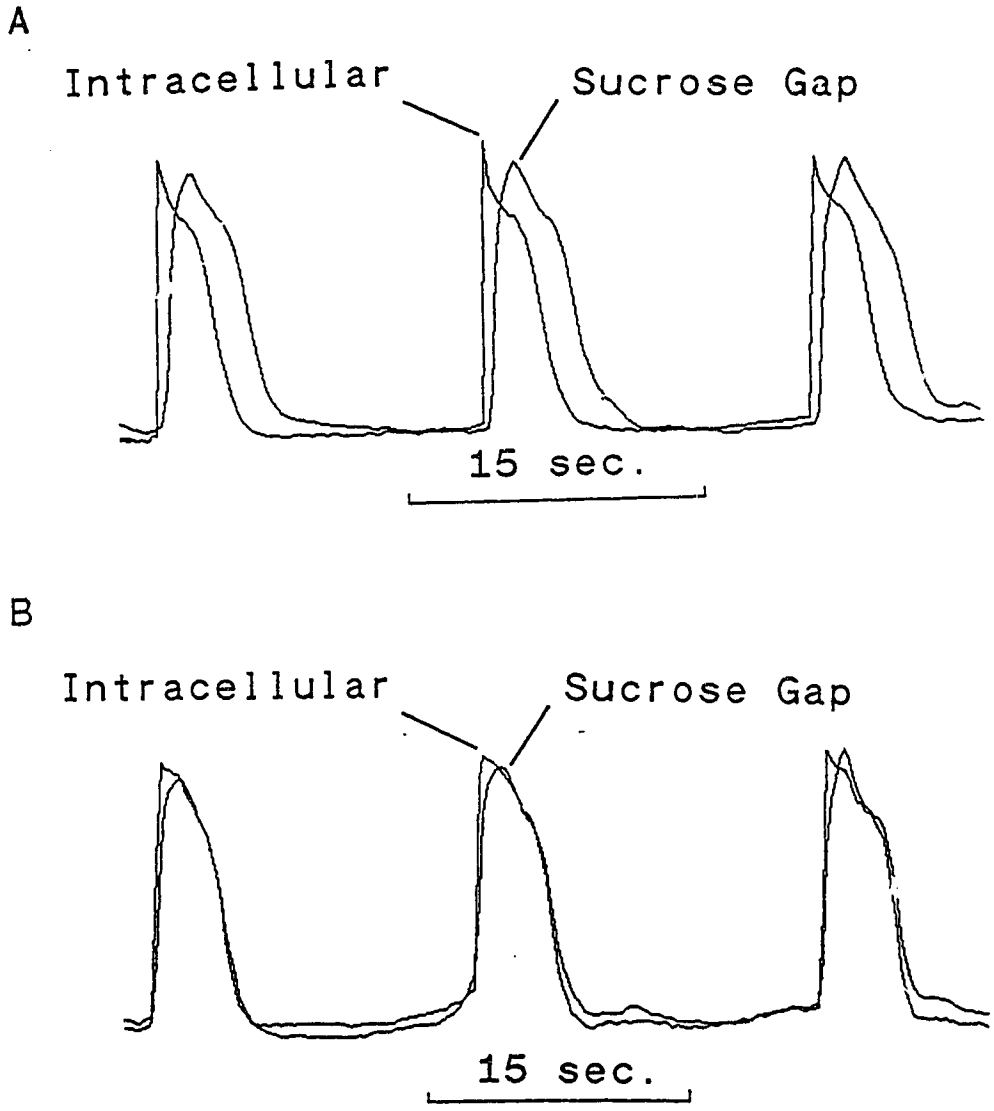

Figure 27. Comparison of sucrose gap and intracellular electrical activity at a point distant (A) and near (B) the sucrose-Krebs boundary

potential oscillations, and spike potentials. Simultaneous microelectrode recordings from the submucosal side of circular muscle showed slow waves only without superimposed oscillations and without spike potentials, as seen in Figure 28A. Simultaneous microelectrode recordings from the myenteric plexus side of circular muscle showed predominant oscillations and spike potentials and no, or small amplitude, slow waves as seen in Figure 28B. Thus, the sucrose gap apparatus averages these activities together as postulated.

It should be noted that although microelectrodes can be used to delineate this inhomogeneous activity, they are also prone to breakage, especially during contractile activity. Moreover, they are not suited to providing long duration uninterrupted recordings which are possible with the sucrose gap apparatus (see Figure 28A). This is especially true when two simultaneous electrical records are required (as is the case for this apparatus).

In the third and last experiment the hypothesis that the apparatus could simultaneously and selectively record the electrical activity of both the circular and the longitudinal layer was tested. A tissue sample was prepared as described above and mounted in the sucrose gap apparatus. The electrical activity from the two legs was completely distinct and did not show any interaction or crosstalk as shown in Figure 29.

Electrical activity within the longitudinal muscle layer has

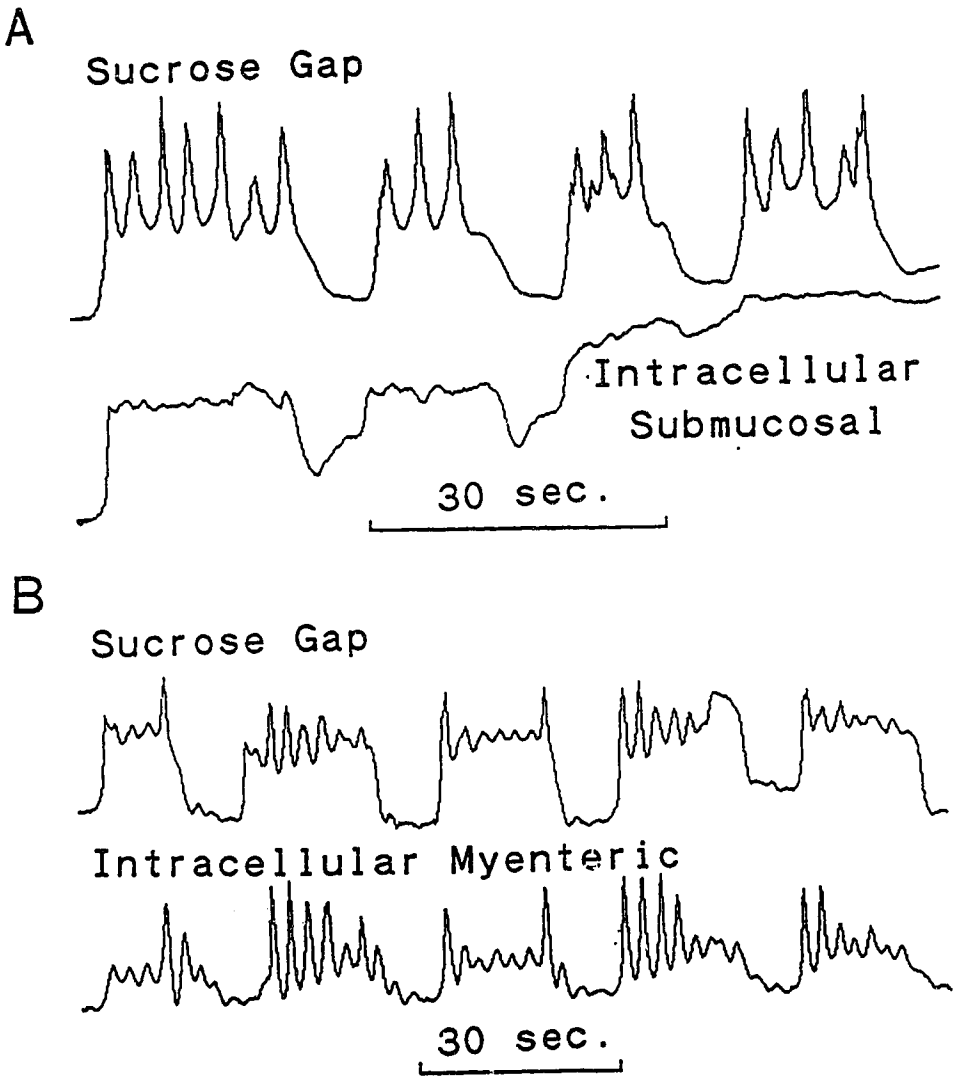

Figure 28. Comparison of sucrose gap and intracellular electrical activity on the submucosal (A) and myenteric plexus side (B) of circular muscle

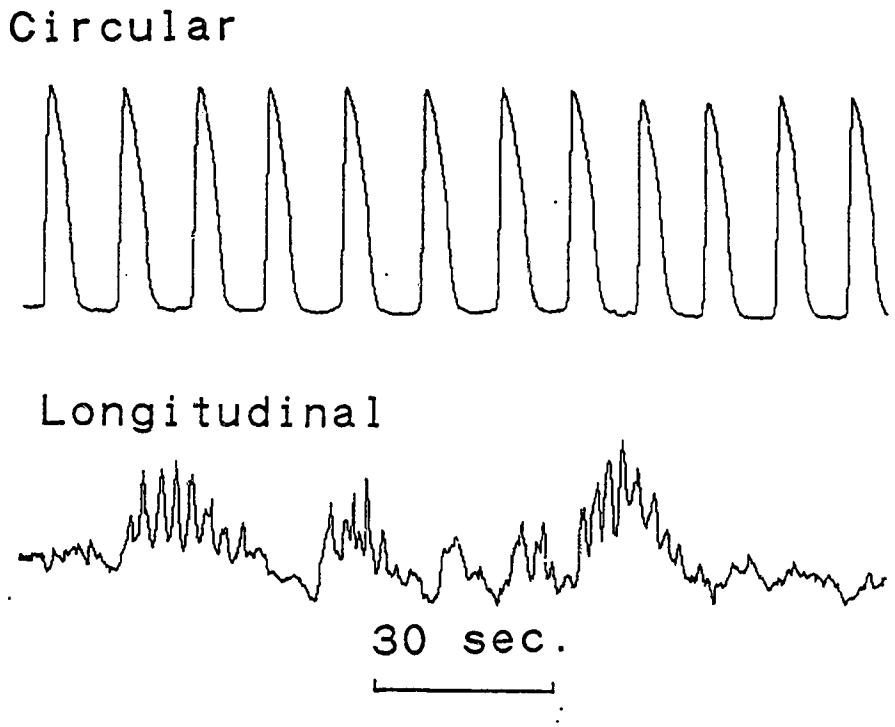

Figure 29. Simultaneous recording of electrical activity from circular and longitudinal legs of 'L'-shaped tissue specimen

been shown not to be tightly coupled through the entire layer (Smith et al. 1987b). The theoretical discussion above suggests that the sucrose gap apparatus would average this activity to produce a composite record as appears to be the case judging from Figure 29 (longitudinal). As well, the bursts of membrane potential oscillations appear superimposed on a depolarization (Figure 29, longitudinal). This appears to be the result of averaging of uncoupled regions of electrical oscillations since intracellular records do not generally show this depolarization (Chow et al., Smith et al. 1987b).

F. CONCLUSIONS

By combining two single sucrose gap apparatus into a dual sucrose gap apparatus electrical activity from two smooth muscle layers can be measured simultaneously. This electrical activity is a weighted average of intracellular electrical activity from two rather narrow regions of an 'L-shaped' multicellular tissue sample. Proper dissection and pinning of the tissue sample facilitates simultaneous measurement of the contractile activity of the two muscle layers. With these characteristics this apparatus is ideally suited to elucidate mechanisms of coordination and interaction between the muscle layers of colon.

CHAPTER VI. ELECTRICAL AND MECHANICAL INTERACTIONS BETWEEN THE MUSCLE LAYERS OF CANINE PROXIMAL COLON

A. ABSTRACT

Electrical and mechanical interactions between the two smooth muscle layers of canine colon have been studied using a dual sucrose gap apparatus. Muscle samples were dissected into an 'L-shape' with one leg cut in the circular direction and the other cut in the longitudinal direction. Longitudinal muscle was removed from the circular leg and circular muscle was removed from the longitudinal leg. The bend of the 'L' contained both layers. The activity of the two layers was studied simultaneously under basal conditions, after stimulation by neostigmine and carbachol, and in the presence of tetrodotoxin. Interactions were more common after stimulation and were marked by modification of one layer's mechanical and electrical activity during increased activity in the other layer. Two patterns were commonly observed. First, during a burst of membrane potential oscillations and spike potentials in the longitudinal layer, slow waves in the circular layer developed spike potentials and some slow waves were also prolonged. Second, during a slow wave cycle in the circular layer, the amplitude of membrane potential oscillations in the longitudinal layer was increased with an associated increase in the incidence of spike potentials. These interactions were associated with contractions of increased strength which were similar in both layers. All interactions continued after nerve conduction blockade by tetrodotoxin.

B. INTRODUCTION

The circular and longitudinal muscle layers of the colon demonstrate different electrical and mechanical activities (Chow et al., Christensen et al. 1969, Debski et al., El-Sharkawy 1983, Huizinga et al. 1984, 1987a, Smith et al. 1987b). This is in contrast to the small bowel where similar activities in the two layers can be observed (Bortoff et al. 1965, 1970, Connor et al., Kobayashi et al.).

In canine colon, the circular muscle layer typically demonstrates repetitive regular electrical slow waves in a frequency range of 4 to 7 cycles per minute which often have associated repetitive phasic contractions. The longitudinal layer typically demonstrates higher frequency membrane potential oscillations in the range of 15-25 cycles per minute which may have superimposed spike potentials. In the longitudinal layer, spike potentials occur in bursts, with associated prolonged contractions or continuously with an associated tonic contraction. The circular muscle slow wave is generated at the submucosal border in the circular muscle layer (Durdle et al. 1983a, Smith et al. 1987a) and propagates away from the lumen (Barajas-López et al. 1989, Smith et al. 1987a, 1987b). The higher frequency membrane potential oscillations seen in the longitudinal layer may be generated in the myenteric plexus region (Smith et al. 1987b).

The correlation of contractile activity between the two muscle layers of the canine colon is not well understood. There are no

reported studies of contractile activity recorded simultaneously from both muscle layers of canine colon. Simultaneous electrical recordings from the two muscle layers have given conflicting results. In studies using suction electrodes El-Sharkawy found the circular layer to have an increased incidence of spike potentials superimposed on the slow wave during a burst of spike potentials in the longitudinal layer (El-Sharkawy 1983). However, the same study could not confirm this finding with microelectrodes and the suction electrode used may not have measured selectively from a confined region. A later microelectrode study did not report an interrelationship of spike potentials between the layers (Smith et al. 1987b).

The purpose of the present study was to elucidate the interactions that take place between the smooth muscle layers of canine colon. An apparatus and tissue preparation were designed to study electrical and mechanical activity simultaneously from both muscle layers. The involvement of spike potentials in interactions between the two muscle layers was clarified. The relationship of contractile activity in the two layers was determined. The effect on interactions of cholinergic stimulation and nerve conduction blockade with TTX was determined.

C. METHODS

Tissue samples were obtained as described in the Methods section of Chapter 4. Tissue specimens were prepared as described

in the 'The Dual Sucrose Gap' section of Chapter 5. The longitudinal muscle layer was removed from the circular leg by blunt dissection, and more than 50% of the circular muscle layer (including the submucosa) was removed from the longitudinal leg by sharp dissection as shown in Figure 30. This resulted in an L-shaped preparation where only the hub contained the full thickness of the colonic muscle wall (excluding the mucosa). All dissection was performed with the aid of a dissection microscope. Histological sections confirmed that the circular leg did not have attached longitudinal muscle in the isolated portion and only small remnants of the myenteric plexus. The longitudinal leg had an intact myenteric plexus throughout. The preparations were then mounted in a dual sucrose gap apparatus that is shown schematically in Figure 31 (the device is essentially two sucrose gaps with a common compartment). The legs of the 'L' were attached to force transducers with silk suture thread and the bend of the 'L' was fixed to the Sylgard bottom of the organ bath with insect pins. The sucrose compartment was bounded by dental rubber dam material. The tissue sample was pulled through small holes in the rubber dam. Since the rubber dam is pliable it offers little resistance to contractions while maintaining proper isolation of the solutions. Temperature was maintained at 37°C and recording was begun immediately.

Recording Conditions

The dual sucrose gap is described in detail in Chapter 5.

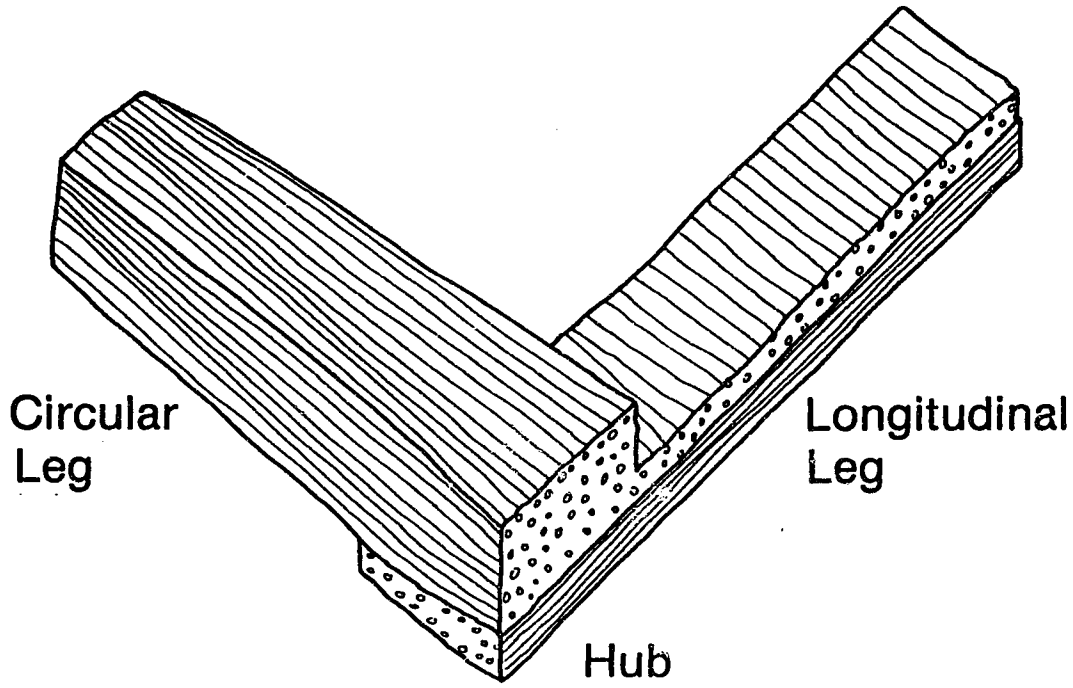

Figure 30. Isolated muscle preparation. Longitudinal muscle was fully removed from the circular leg. Circular muscle was partially (>50%) removed from the longitudinal leg. Hub contains the full colonic muscle wall excluding mucosa.

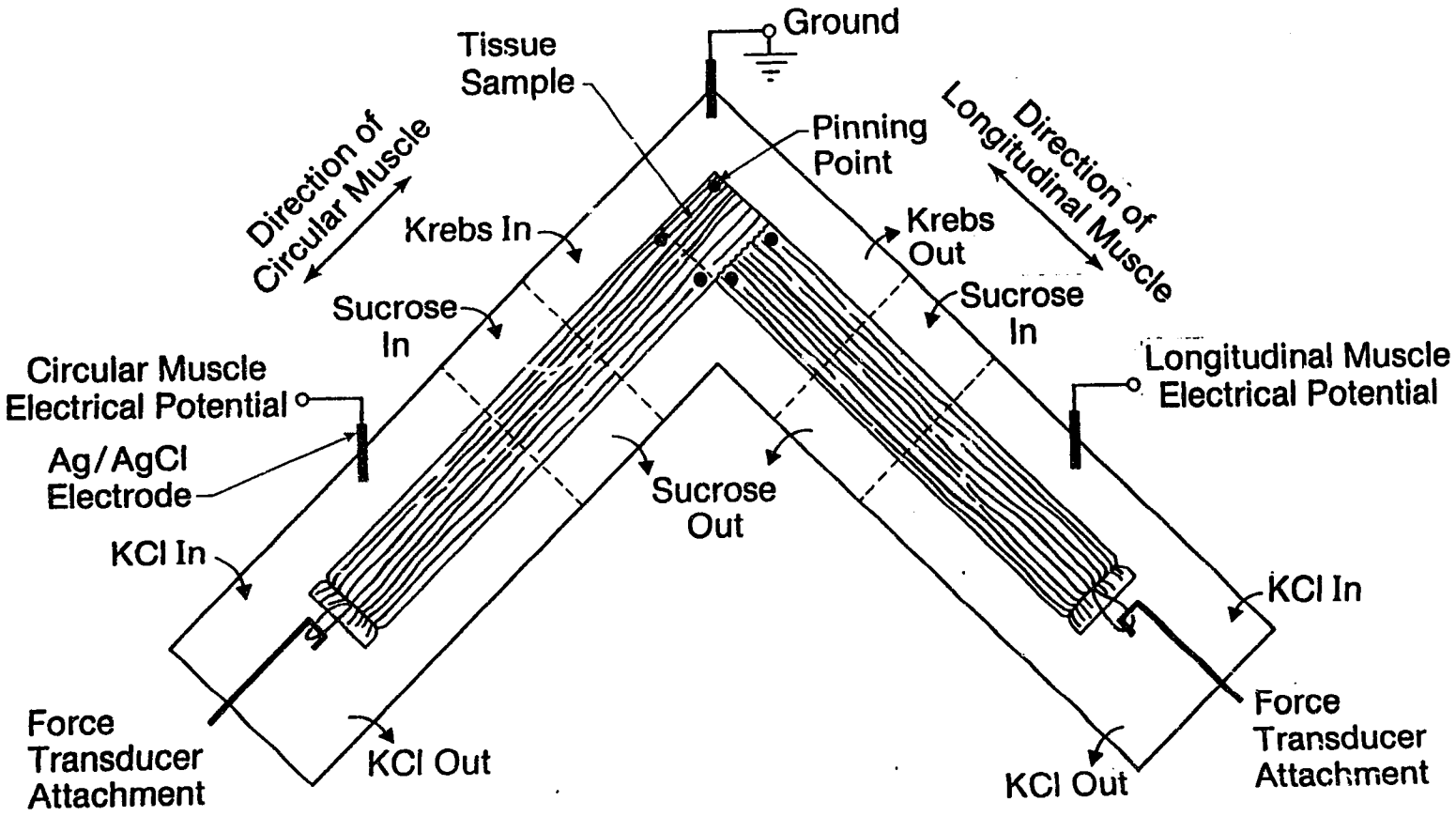

Figure 31. Schematic diagram of dual sucrose gap apparatus. The device is essentially two sucrose gaps with a common compartment. Myoelectrical and contractile activity can be recorded simultaneously from both circular and longitudinal muscle.

Electrical activity in the sucrose gap was measured with Ag/AgCl electrodes. Mechanical activity was measured using isometric force transducers (Grass FT.03C).

Microelectrodes were pulled from glass capillaries and filled with 3 M KCl then mounted in a microelectrode holder (W-P Instruments, Inc.). Microelectrode signals were amplified using a Dual Microprobe System (WPI).

All signals were recorded using a six-channel Beckman Type R Dynograph (Beckman Instrument Inc.). The signals were also digitized and stored on a PC-AT (IBM Corp.) using a Lab Master input/output board (Scientific Solutions).

Solutions and Drugs

The Krebs solution contained the following (mM): NaCl, 116; Glucose, 10.1; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2 and $NaHCO_3$, 22. The other solutions were 8% sucrose and 117 mM KCl.

The drugs used were: TTX 10^{-6} M (tetrodotoxin, Sigma Chemical), neostigmine 10^{-6} M (Prostigmin, Roche) and carbamylcholine 10^{-7}-10^{-6} M (Carbachol, Allen and Hanburys). All solutions were equilibrated with 95% O_2-5% CO_2. Drugs were administered in Krebs solution to the center compartment which contained the hub of the 'L-shape' preparation.

Data Analysis

Various patterns of interaction between the two muscle layers were observed. These 'patterns' were classified into two types each associated with a characteristic electrical activity of one of the layers. Interaction between the two layers was only accepted when an interaction in contractile activity was also present. 'Type C' interaction refers to electrical and contractile events observed in the longitudinal layer that were temporally related to the slow wave in the circular layer. 'Type L' interaction refers to increased electrical and contractile activity in the circular layer that coincided with a burst of spike potentials in the longitudinal layer. 'Type B' refers to Type C and Type L occurring simultaneously.

The oscillation frequency during simultaneous bursts of high frequency membrane potential oscillations in the two muscle layers was compared using a paired Student's t-test. All other P values comparing the incidence of interactions were calculated using a Chi-Squared test of independence for dichotomous variables. Individual values are expressed as mean ± standard deviation.

Experimental Protocol

Muscle samples were prepared and mounted in the dual sucrose gap as shown in Figure 31. The hub of the 'L' was pinned so that contractions of one layer did not appear in the mechanical record of the other layer. This was routinely tested by applying traction to

one leg and observing the effect on the other. Any subsequently observed mechanical coordination between the two legs was considered not to be an artifact. After pinning the hub the legs of the muscle preparation were stretched to 110% of the length where tension first developed and recording was started. The tissue preparation was transected at the hub on several occasions during periods of supposed interaction, with the assumption that genuine interactions would cease. Electric field nerve stimulation was applied with platinum wire electrodes placed parallel to a strip of tissue to verify the effect of TTX. Pulse trains of 5 to 10 seconds, 0.5 millisecond pulse width, 10 Hertz rate at 60 to 80 Volts were used.

In one group of experiments, microelectrodes were used to record the circular muscle slow wave from the submucosal side of the tissue preparation at several distances from the sucrose compartment. Electrical activity was recorded simultaneously using the sucrose gap apparatus to allow comparison of the two methods.

D. RESULTS

Validation Of The Methods

The ability of the technique to record selectively from individual legs was determined by simultaneous measurement using the dual sucrose gap and microelectrodes. When a cell within one millimeter of the sucrose compartment was impaled by a microelectrode the waveshape of the sucrose gap recording and the

microelectrode recording were very similar and no phase lag was seen (Figure 32A). When the microelectrode was moved more distant from the sucrose compartment, up to four millimeters, the two waveforms, although synchronized, were out of phase and showed significant differences in shape (Figure 32B). This indicates that the sucrose gap records from a population of cells directly adjacent to the sucrose compartment, apparently within one millimeter.

Forty six specimens from sixteen dogs were studied. When interactions were not present the circular and longitudinal legs developed activity consistent with previous reports (Barajas-López et al. 1988, El-Sharkawy 1983, Huizinga et al. 1984, Sanders et al., Smith et al. 1987a, 1987b) for the two layers. This activity consisted of 4 to 7 cycle per minute circular muscle slow waves with associated phasic contractions and higher frequency longitudinal muscle membrane potential oscillations that occurred in bursts with superimposed spike potentials and had associated prolonged contractions. Basal activity is shown in Figure 33. The basal activity of the two legs was independent.

Patterns Of Interaction

The most prevalent pattern of interaction, Type L, was a change in the circular muscle layer associated with a burst of spike potentials in the longitudinal layer. Slow waves in the circular layer changed from having no superimposed spike potentials to having one or several superimposed spike potentials (See Figures 34-38). Associated contractions in the circular layer were of greatly

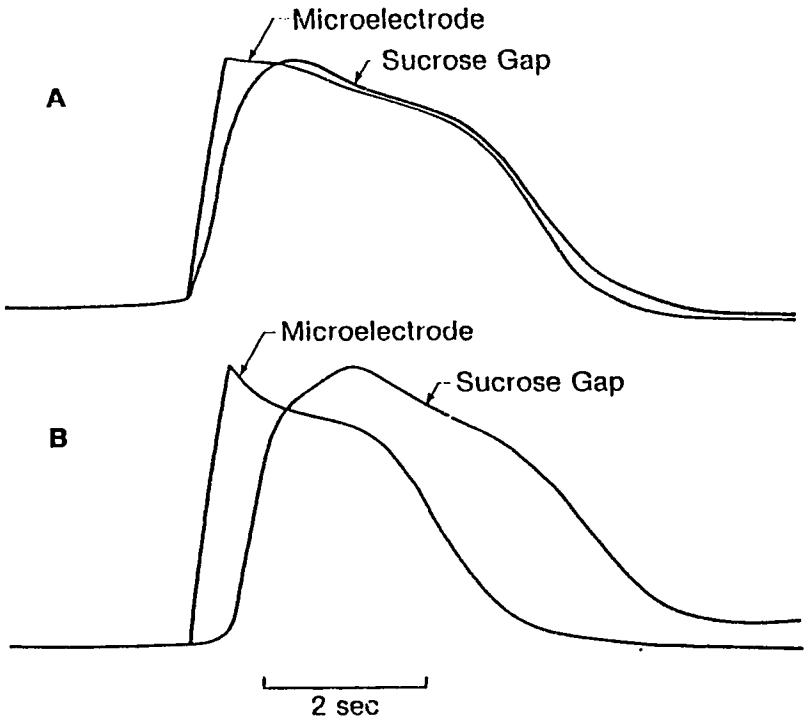

Figure 32. *Comparison of slow wave recorded with microelectrode and the sucrose gap. In trace A the microelectrode impaled a cell on the submucosal surface of the circular muscle in the Krebs compartment within one millimeter of the sucrose compartment. The waveforms are in phase and very similar. Although the microelectrode recorded slow wave has a faster upstroke both slow waves begin simultaneously. In trace B the microelectrode was four millimeters from the sucrose compartment. The waveforms are out of phase and have more differences in shape. The sucrose gap recording shows no deflection during the initial microelectrode recorded slow wave upstroke. The sucrose gap apparently records from a region directly adjacent to the sucrose compartment. Traces A and B are from the same sample and ten minutes apart.*

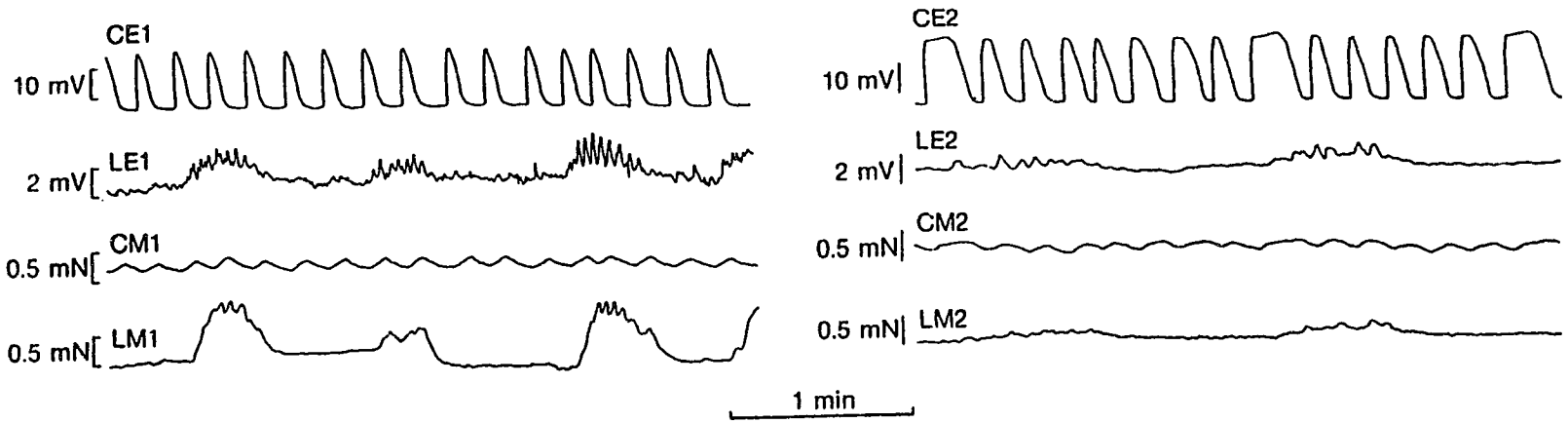

Figure 33. *Basal activity without interactions; effect of tetrodotoxin on basal activity. C tracings are from the circular leg. L tracings are from the longitudinal leg. E tracings are electrical activity and M tracings are mechanical activity (the same convention is used in the following figures). Tracings on left show basal activity with no manifested interactions. Tracings on right were after addition of TTX 10^{-6} M, still with no interactions. Slow waves in trace CE2 were prolonged after addition of TTX. The strength of contractions in the longitudinal layer were reduced in trace LM2 after addition of TTX.*

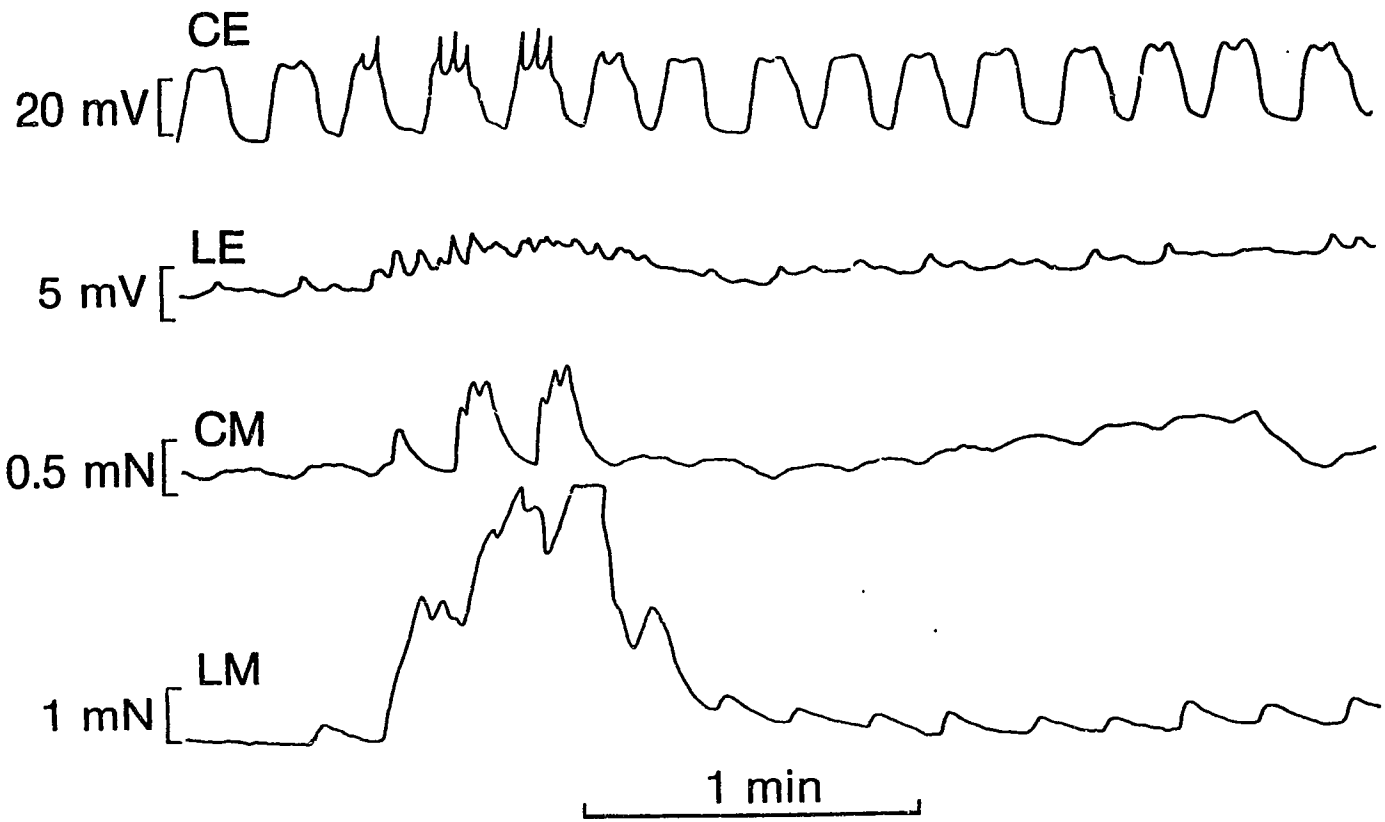

Figure 34. Basal activity with interactions. Circular muscle slow waves bore spike potentials during the burst of membrane potential oscillations in the longitudinal layer. Associated contractions in both layers were of increased strength. Contractions at the slow wave frequency appeared in the longitudinal layer (Trace LM).

increased strength or appeared where none existed previously. The slow wave in the circular layer was prolonged up to 6 times longer in 9 of 24 (39.1%) experiments that demonstrated Type L interactions. This occurred during an associated burst of spike potentials in the longitudinal layer (see Figure 37) and associated contractions in the circular layer were prolonged and similar in shape to those observed in the longitudinal layer. Although an association was noted between prolonged slow waves and a burst in the longitudinal layer, they also occurred spontaneously at other times both basally and more so after stimulation.

In Type C interactions membrane potential oscillations in the longitudinal layer appeared or were amplified during circular slow wave cycles (see Figures 35, 36, 37 and 39). These were associated with the appearance of or increase in amplitude of longitudinal contractions. In 50% of the experiments where Type C interaction was observed it occurred both during and between bursts of longitudinal membrane potential oscillations (see Figures 36 and 39). In the remaining 50% Type C interaction only occurred during the longitudinal burst episode (Figure 37). When Type C interaction occurred while the slow waves in circular muscle were of short duration, one or two longitudinal membrane potential oscillations were amplified and the contraction in the longitudinal layer was short and phasic as in the circular layer (Figures 36, 37 and 39). When interactions occurred during a prolonged slow wave a burst of membrane potential oscillations in the longitudinal layer was

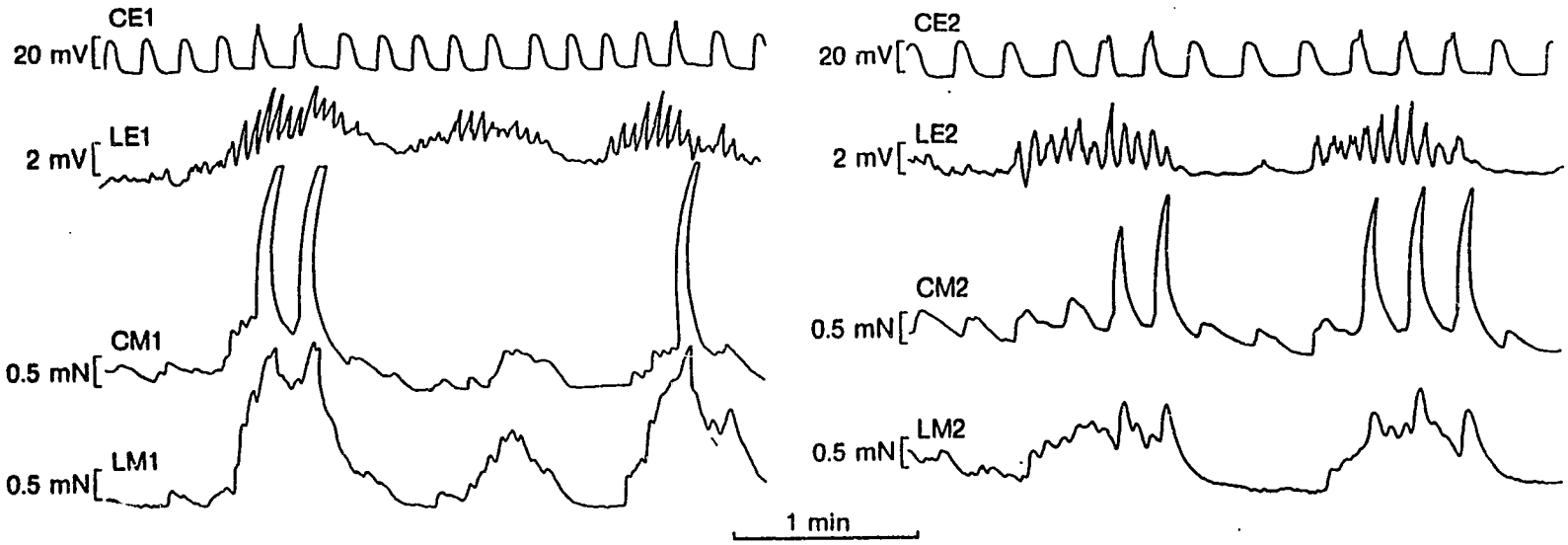

Figure 35. *Interaction after administration of carbachol and TTX. Tracings on the left were after addition of carbachol 10^{-7} M. Tracings on the right are after addition of carbachol 10^{-7} M and TTX 10^{-6} M. Circular muscle slow waves bore a single spike potential during a burst of membrane potential oscillations and spike potentials in the longitudinal layer. Associated contractions were of greatly increased strength. Interactions continued unaltered after addition of TTX.*

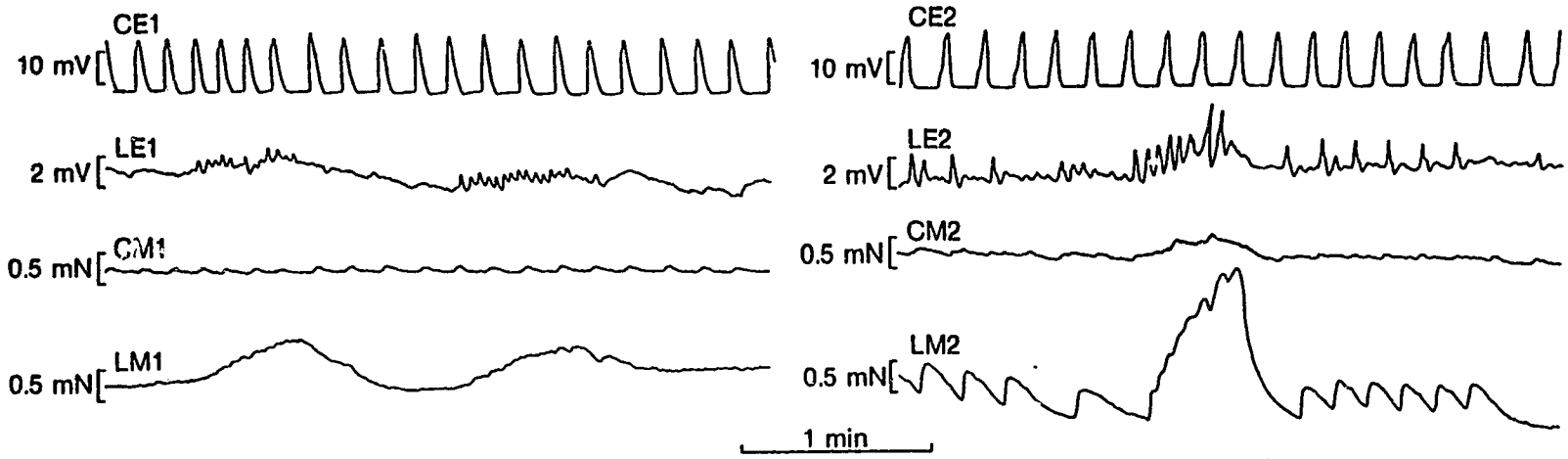

Figure 36. Interaction after administration of carbachol and TTX. Tracings on the left are after addition of carbachol 10^{-7} M. Tracings on the right are after addition of carbachol 10^{-7} M and TTX 10^{-6} M. No interaction is seen before addition of TTX. A pronounced influence on the longitudinal layer at the slow wave frequency is seen after addition of TTX.

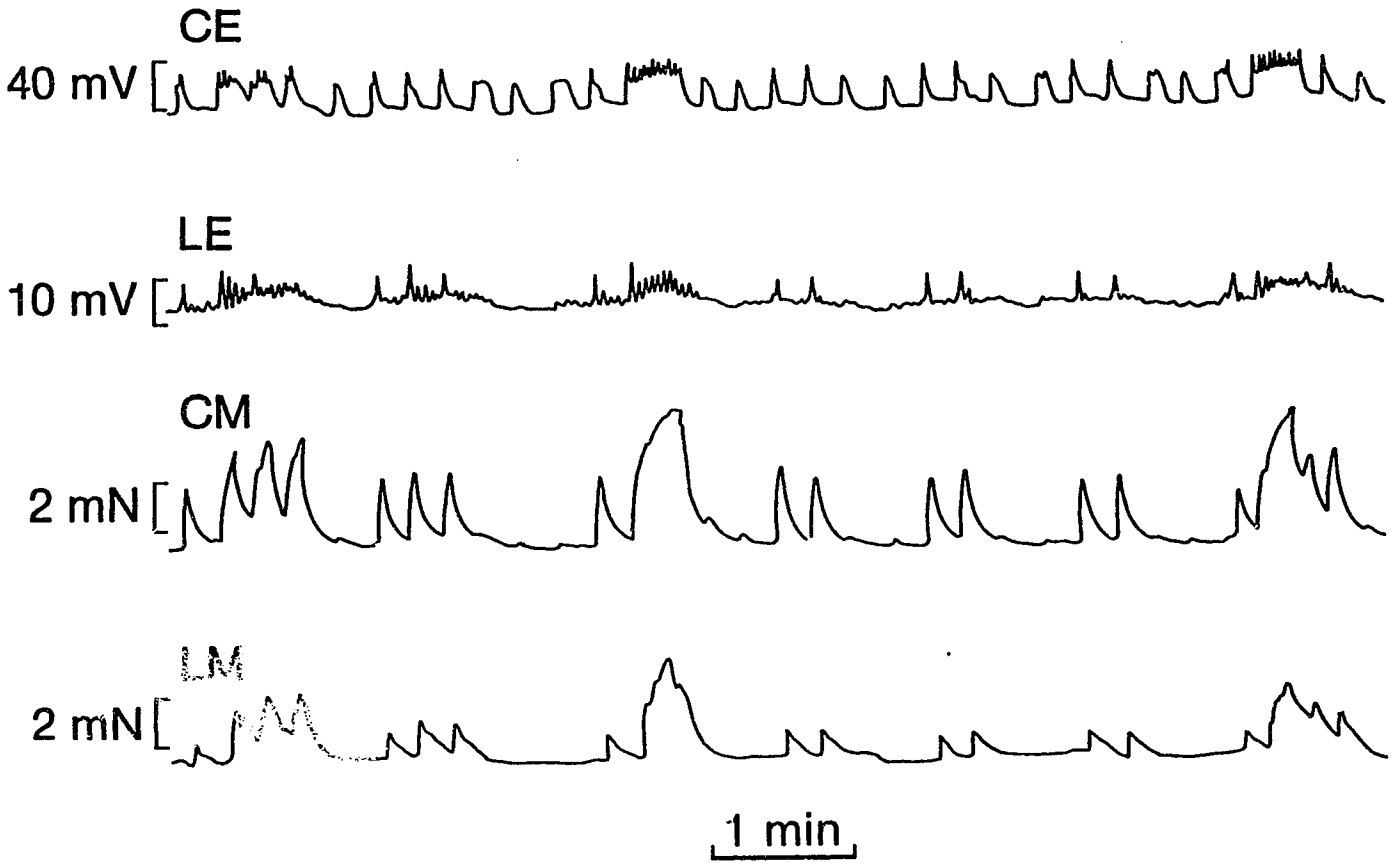

Figure 37. Interaction after administration of carbachol 10^{-6} M to the sucrose compartments. This experiment showed interactions which were synchronized to the periodic burst of membrane potential oscillations and spike potentials in the longitudinal layer. Note the occurrence of prolonged slow waves with superimposed spike potentials. Both muscle layers had very similar contractions, both short and prolonged.

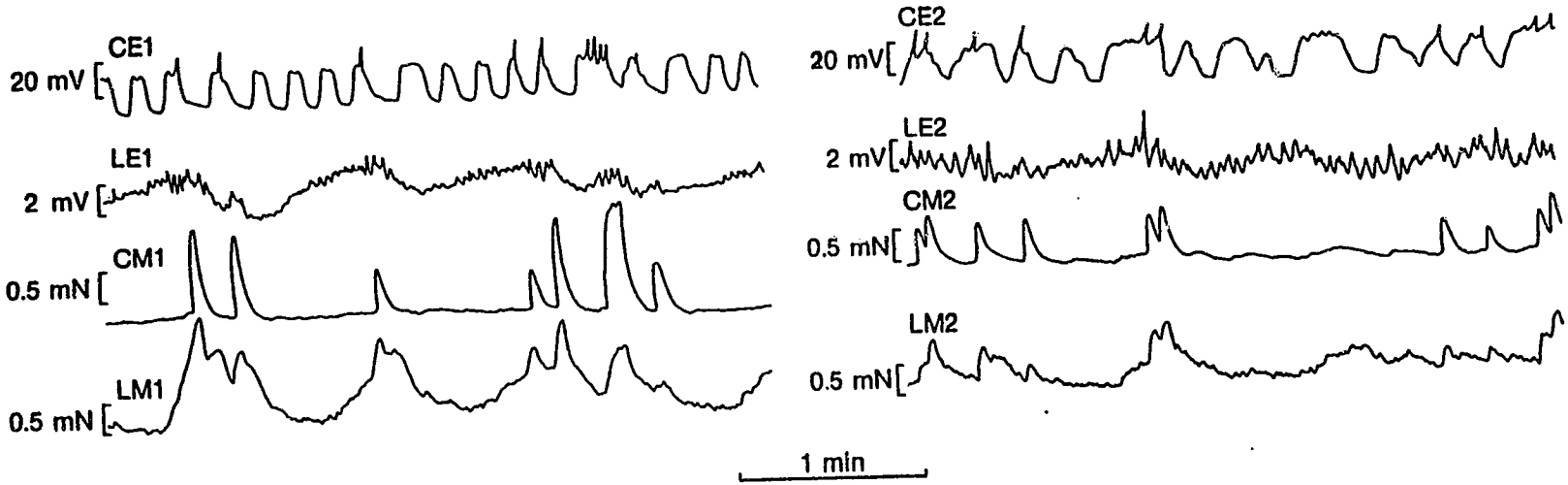

Figure 38. Interaction after administration of neostigmine and TTX. Tracings on the left are after addition of neostigmine 10^{-6} M. Tracings on the right are after addition of neostigmine 10^{-6} M and TTX 10^{-6} M. Circular muscle slow waves bore one or several spike potentials during associated activity periods in the longitudinal layer. Associated contractions in the circular layer were both short and prolonged. Similar activity was observed after addition of TTX.

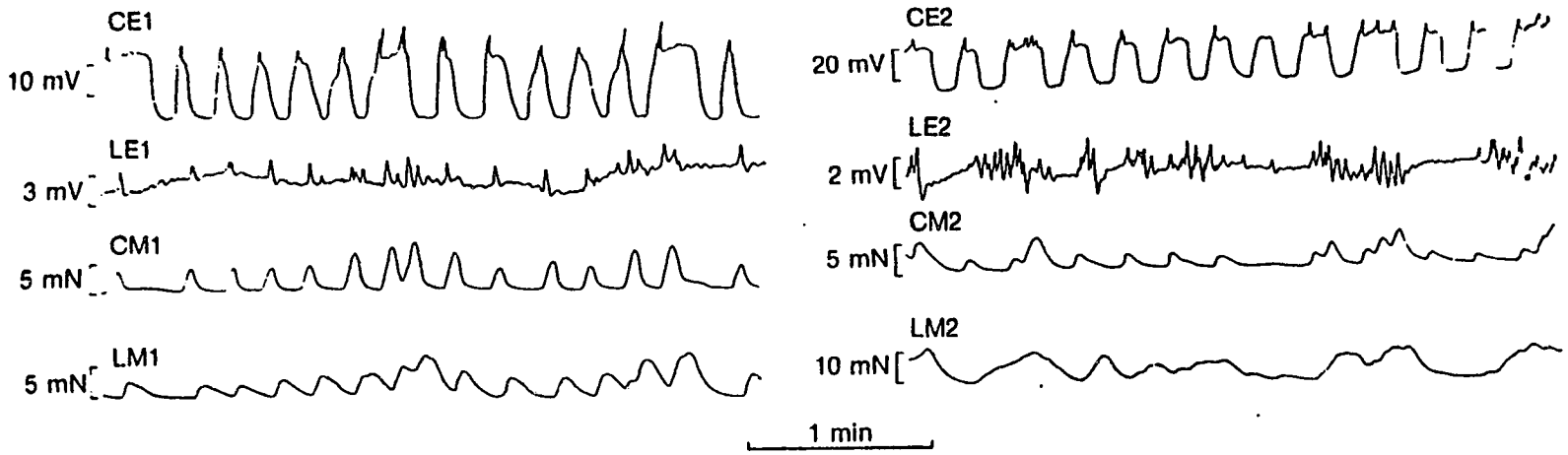

Figure 39. Interaction after administration of neostigmine and TTX. Tracings on the left are after addition of neostigmine 10^{-6} M. Tracings on the right are after addition of neostigmine 10^{-6} M and TTX 10^{-6} M. Before addition of TTX, events at the slow wave frequency were dominant in all traces. Events at the slow wave frequency were continuously observable in the longitudinal layer. Addition of TTX decreased this slow wave dominance and the activity changed.

amplified, and a prolonged contraction resulted (Figure 37).

Interactions also occurred in episodes separated by periods of relative quiescence (Figure 40), even during a continuous stimulus. Episodes of interaction ranged from a duration of a few minutes to several hours. Transection at the hub (n=11) caused interactions to cease immediately in all cases indicating that they were not an artifact of the apparatus or the tissue preparation.

Spontaneous Activity Of The Muscle Layers

46 samples were initially studied in the basal state for a total recording time of 1214 minutes. Of these 46 samples, 5(10.9%) demonstrated interactions: 1(2.2%) Type C only, 1(2.2%) Type L only, and 3(6.5%) Type B. The total time of the three types of interaction during the 46 experiments were: Type C alone 90(7.4%) minutes, Type L alone 5(0.4%) minutes, and Type B 130(10.7%) minutes.

Cholinergic Stimulation

Interaction was more common after cholinergic stimulation.

Effect Of Carbachol. Doses of carbachol ranging from 10^{-7} M to 10^{-6} M were administered to 21 muscle preparations for a total stimulation time of 825 minutes (this excludes a 10 minute equilibration period after administration of carbachol for each sample). Of these 21 samples, 13(61.9%) demonstrated interactions: 1(4.8%) Type C only, 6(28.6%) Type L only, and 6(28.6%) Type B. The

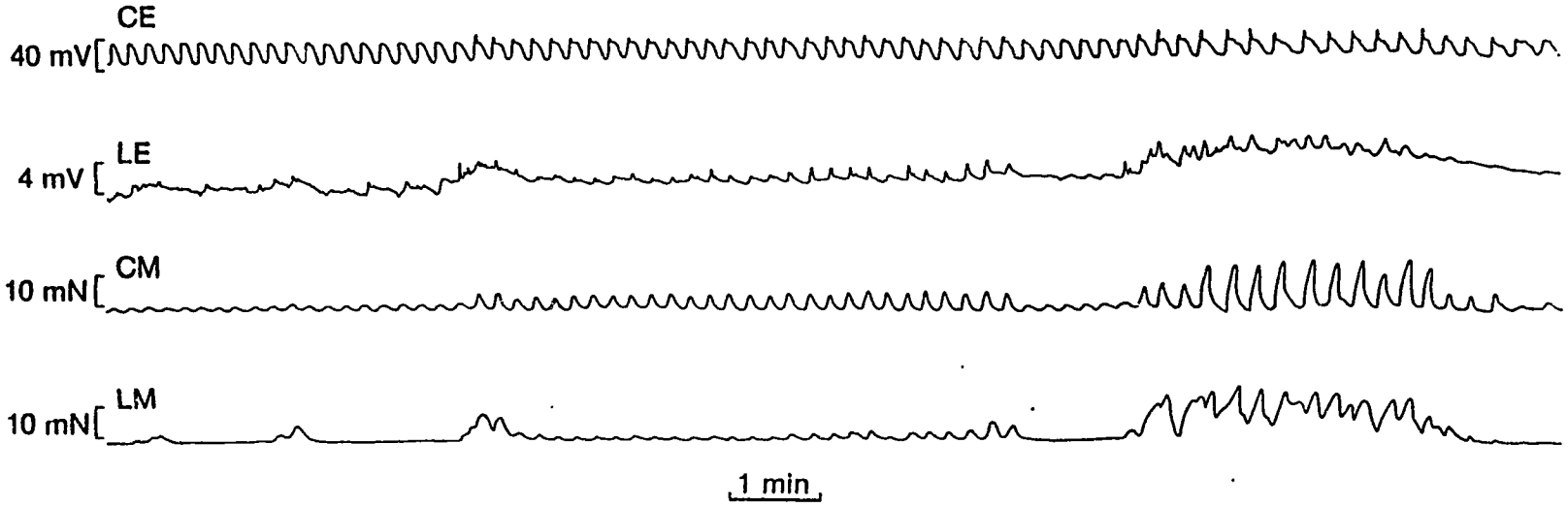

Figure 40. Phases of interaction and quiescence after administration of neostigmine. All traces were after the addition of neostigmine 10^{-6} M. The figure illustrates that interactions can occur in episodes separated by periods of relative quiescence. Note that this occurred over several minutes.

total time of the three types of interaction during the 21 experiments was: Type C alone 55(6.7%) minutes, Type L alone 240(29.1%) minutes, and Type B 210(25.5%) minutes. Compared to the basal state carbachol increased the occurrence of all three types of interactions (Type C $p=.030$, Type L $p<.0001$, Type B $p=.039$).

Carbachol increased contractile activity in both muscle layers. Carbachol also induced membrane potential oscillations and spike potentials on the slow waves and prolonged the slow wave plateau period. When the slow wave was prolonged and bore a burst of membrane potential oscillations during a similar burst in the longitudinal layer, the frequency of oscillation was suggestively similar in both layers. The frequency was analyzed in four experiments that demonstrated this event multiple times. The mean frequencies in circular muscle were: 25.3±1.2, 20.6±1.0, 28.9±1.5, and 27.1±1.7 cycles per minute. The mean frequency in longitudinal muscle was, respectively: 20.9±2.0, 18.1±1.2, 22.6±1.4, and 23.6±3.3 cycles per minute. The frequency in the circular layer was significantly higher in all cases, the p values were, respectively: .001, <.00001, <.000001, and .005.

Effect Of Neostigmine. Neostigmine at a concentration of 10^{-6} M was administered to 20 muscle preparations for a total stimulation time of 1201 minutes (this excludes a 10 minute equilibration period after administration of neostigmine for each sample). Neostigmine elicited the same patterns of interaction as were seen after the use of carbachol. Of the 20 samples 13(65.0%) demonstrated

interactions: 2(10.0%) Type C only, 4(20.0%) Type L only, and 7(35.0%) Type B. The total time of the three types of interaction during the 20 experiments was: Type C alone 45(3.7%) minutes, Type L alone 265(22.1%) minutes, and Type B 326(27.1%) minutes. The effect of carbachol and neostigmine were not significantly different.

Effect of Tetrodotoxin

Tetrodotoxin 10^{-6} M was applied both under basal conditions (n=2) and during cholinergic stimulation (carbachol n=8, neostigmine n=8). TTX caused a substantial reduction in the strength of the longitudinal layer's contractions in 10 of 18 (55.6%) experiments and in 6 of 18 (33.3%) in the circular layer ($p > 0.05$). A brief temporary block of interactions was seen for 5 to 15 minutes in 6 of 6 neostigmine stimulated interacting specimens immediately after TTX was introduced into the tissue bath. Interactions, however, rapidly returned in spite of continued TTX perfusion in all experiments. TTX had no effect on two neostigmine stimulated specimens that were not interacting before TTX was introduced. During carbachol stimulation TTX did not abolish interactions in 6 of 6 interacting specimens. In two carbachol stimulated specimens that were not interacting before addition of TTX, interactions developed while TTX and the carbachol stimulus were maintained. TTX applied under basal conditions when the specimen was not interacting had no effect on the lack of interactions. TTX also caused the slow wave in circular muscle to become prolonged in 2 of 18 (11.1%) specimens.

TTX was effective in abolishing the response of the tissue preparation in our apparatus to electric field nerve stimulation. The blocking effect was complete within five minutes and was checked every five minutes up to 35 minutes (n=6).

E. DISCUSSION

This study shows that the muscle layers of canine colon can develop coordinated electrical and contractile events. In the absence of stimulation the two layers generally act independently but coordinated activity is common after cholinergic stimulation. This coordination is not through known neural pathways as TTX did not abolish it.

The technique adequately separates activity in the two legs such that activity in one leg is not recorded from the other leg. In the basal state, independent activity was observed in both legs and coordinated activity was rare. After proper pinning traction applied to one leg had no effect on the other leg. The microelectrode experiments showed that the sucrose gap recorded from a region apparently less than one millimeter wide in the Krebs compartment adjacent to the sucrose compartment. The 'recording region' in the circular leg consisted of isolated circular muscle and therefore recorded from the circular layer. The 'recording region' in the longitudinal leg consisted of the longitudinal layer and a remnant of circular muscle not including the slow wave generating region (Barajas-López et al. 1988, Durdle et al. 1983a,

Smith et al. 1987a, 1987b). Others have shown that the slow wave was not generated in a similar remnant of circular muscle (Durdle et al. 1983a, Smith et al. 1987a), even after stimulation by acetylcholine (Smith et al. 1987a). The influence of circular muscle slow waves on the longitudinal layer could not therefore be due to the remnant of circular muscle attached to the longitudinal leg. A remnant of circular muscle was left in order to preserve the myenteric plexus since this may be the pacemaker region of the longitudinal muscle layer (Smith et al. 1987b). Since interactions ceased when the tissue sample was divided at the hub they were not due to a mechanical artifact.

Spontaneous interactions were infrequent in the present study. Cholinergic stimulation increased significantly the incidence of interactions and two patterns were observed. In Type L interactions the circular layer slow wave displayed superimposed high frequency membrane potential oscillations and spike potentials during a burst of membrane potential oscillations and spike potentials in the longitudinal layer. This clarifies the ambiguity of a previous study (El-Sharkawy 1983) regarding spike potentials in the circular layer during the longitudinal 'burst' episode. The frequency of the simultaneous high frequency membrane potential oscillations in the two layers was significantly different which suggests that they do not arise from the same pacemaker. The 'burst' event appears to pace Type L interaction yet the mechanisms responsible for the 'burst' are unknown. High frequency membrane potential oscillations

may be generated at the myenteric border in the longitudinal (Smith et al. 1987b) and the circular muscle (Barajas-López et al. 1988, 1989, Smith et al. 1987b). It is possible that during Type L interactions both muscle layers, with different pacemakers, are influenced by a common source in the myenteric plexus region associated with the 'burst'.

In Type C interactions membrane potential oscillations and spike potentials were observed in the longitudinal layer at the slow wave frequency during circular slow wave cycles. Since the slow wave is generated at the submucosal border of the circular muscle layer (Durdle et al. 1983a, Smith et al. 1987a), and this layer was absent from the longitudinal leg, these events are due to the circular layer influencing the longitudinal layer in the hub. Could the propagation of slow waves into the longitudinal layer be responsible? Slow wave propagation into the longitudinal layer has not been clearly established by previous studies. Although slow waves have been recorded from the longitudinal muscle of intact preparations using extracellular electrodes (Caprilli et al., Durdle et al. 1983a, El-Sharkawy 1983) they have not been observed in intracellular recordings from longitudinal muscle (Chow et al., Durdle et al 1983a, Smith et al. 1987b). A recent intracellular study of the circular layer reported that the slow wave does at least reach the myenteric region (Barajas-López et al. 1988). It is possible that the appearance of the slow wave in the myenteric plexus region influences a longitudinal muscle pacemaker.

Interactions were associated with contractions of increased strength that were coordinated and often similar in both layers. Interactions often resulted in spike potentials and these caused contractions of increased strength. Contractions were coordinated and similar since one or several spike potentials were elicited in both layers simultaneously. Therefore, the different and independent basal electrical activity of the two layers leads to coordinated contractile activity by eliciting coordinated spike potentials.

The circular muscle in vivo undergoes prolonged contractions much like those seen in the longitudinal layer (Kingma et al. 1980, Kocylowski et al., Sarna 1986). This may be related to periods of interaction between the two layers since prolonged contractions in the circular layer during interaction were often observed (Figures 34, 37, 38, and 39). In this study periods of interaction were often separated by quiescent periods, similar to the periodic bursts of contractile activity reported in vivo (Sarna 1986).

In the present study both Type C and Type L interactions were noted to occur primarily during a 'burst episode' in the longitudinal layer. Coordination of electrical activities in the pig colon, as recorded by suction electrodes, also occurred coincident with the 'burst' in the longitudinal layer (Huizinga et al. 1987a). "Each oscillation in the circular muscle layer occurred at the same time as the onset of a burst of oscillations in the longitudinal muscle". A previous study of the dog colon also

suggested an influence on the circular layer during the 'burst' in the longitudinal layer (El-Sharkawy 1983). The association of this 'burst' of activity with interactions and contractions of increased force suggests an importance to this mechanism which is contrasted by a lack of understanding about it.

Cholinergic stimulation significantly increased the incidence of interactions. This might be caused by partial depolarization of the muscle cells of both layers making them more susceptible to external stimuli. Since both layers have increased electrical and contractile activity after cholinergic stimulation they may be more likely to provide this stimulus to the other layer. Acetylcholine and carbachol have been shown to activate channels that pass mostly sodium, which would cause membrane depolarization (Inoue et al.).

The present study has shown that interactions continue in the presence of TTX suggesting that neural conduction does not form an essential part of the interaction pathway. This is in contrast to a previous report (El-Sharkawy 1983) that periodically induced spike potentials on the circular slow wave during a burst in the longitudinal layer were mediated by cholinergic neurones. This earlier finding was based on the blocking effect of atropine. Periodic interaction was not abolished by TTX in the present study. In the present study, TTX abolished interactions for a short period in neostigmine stimulated specimens but not in carbachol stimulated specimens. It is therefore possible that cholinergic neurons modulate or enable interactions but are not part of the

communication pathway. The TTX-resistant pathway has not been identified in this study. The interstitial cells of Cajal, which are TTX resistant, could be involved as they have been reported to play an intermediary role between smooth muscle and neurones (Berezin et al., Daniel et al.). TTX also occasionally caused a reduction in the strength of contractions in both the circular and longitudinal layers. TTX may have blocked interactions transiently by reducing activity below the threshold required for interactions. This would also explain why under basal conditions, when the muscle layers were not as active as after cholinergic stimulation, interactions occur infrequently. A modulatory effect of nerves was also reported in a study using pig colon where TTX enhanced some aspects of coordination; this effect was attributed to the presence of tonically active inhibitory nerves (Huizinga et al. 1987a).

In summary, interactions occur during periods of excitation in the tissue and not during quiescent periods. Interactions led to forceful contractions of similar shape and duration in both muscle layers. These interactions occurred in the presence of TTX suggesting that they are not directly coordinated by nerves.

CHAPTER VII. PATTERNS OF CONTRACTILE COORDINATION BETWEEN THE TWO MUSCLE LAYERS IN CANINE PROXIMAL COLON

A. ABSTRACT

Coordinated contractions of the two muscle layers of canine proximal colon have been studied using a dual sucrose gap apparatus. 'L-shaped' preparations with isolated circular and longitudinal muscle legs connected by a full thickness hub were used. Two types of interaction between the muscle layers were observed: contractions in both muscle layers coordinated with a) the circular muscle slow wave (Type C), and b), the longitudinal muscle electrical spike burst (Type L). Coordination in the basal state was rare. The effect of the excitatory agent $BaCl_2$ varied with concentration: a lower concentration, $2X10^{-4}$ M, increased the incidence of Type L interaction only whereas a higher concentration, $2X10^{-3}$ M, increased the incidence of Type C only. The incidence of Type L was increased by selective carbachol 10^{-6} M stimulation of the circular layer, the longitudinal layer or both. The incidence of Type C was increased only when both layers were stimulated. The effects caused by transecting the legs of the tissue preparation free from the hub during periods of interaction suggested that both layers influenced each other through the hub and activity in the legs was strengthened when attached to the hub. We conclude that at least two patterns of coordinated contractions occur in the canine proximal colon and both occur significantly more often after an excitatory stimulus. Contractions coordinated to the longitudinal

muscle electrical spike burst are induced with less excitation than contractions coordinated to the circular muscle slow wave.

B. INTRODUCTION

The circular and longitudinal muscle layers of canine colon display very different electrical and contractile activities when studied in isolated in vitro strips (Barajas-López et al. 1988, Chow et al., Debski et al., Durdle et al. 1983a, El-Sharkawy 1983, Sabourin et al. 1990b, Smith et al. 1987b). Yet in vivo (Kingma et al. 1980, Sarna 1986) and ex vivo (Kocylowski et al.) studies usually demonstrate coordinated contractions working in concert. The mechanisms underlying this coordination are not known.

In the previous chapters, it was reported that coordinated electrical and contractile activity in the two muscle layers could be observed in vitro. Two patterns of coordination were observed: contractions occurring in both layers synchronized to the circular muscle slow wave (Type C), and contractions of increased force occurring in both layers during a burst of membrane potential oscillations, with or without spike potentials, in the longitudinal layer (Type L). The term membrane potential oscillations is used to refer to rapid oscillations in membrane potential that are usually associated with longitudinal contractions and upon which spikes often occur. The incidence of these interactions was increased by cholinergic stimulation.

The purpose of the present study was to further elucidate the

two patterns of interaction. Specifically, were the two patterns of interaction associated with different levels of excitation? Further, did a particular muscle layer have a dominant role in either pattern of interaction? A second goal was to determine the origin of the membrane potential oscillations that commonly accompany interactions. Membrane potential oscillations and spike potentials have been reported in the circular muscle layer (Huizinga 1984). This activity has been recorded from the myenteric plexus area (Barajas-López et al. 1988, Smith et al. 1987b) and in the longitudinal muscle (Smith et al. 1987b) and was reported to originate at the myenteric border (Smith et al. 1987b). The specific question was, are the myenteric plexus region and the longitudinal layer required to generate this activity in the circular layer?

In the present study barium chloride was used as an excitatory agent. Barium blocks potassium channels which depolarizes the smooth muscle cells (Hille) and makes them more excitable. Barium also enhances calcium spike potentials which are often present during interactions.

C. METHODS

Tissue Preparation

'L' shaped tissue specimens were obtained as described in the previous chapters. The longitudinal muscle layer was removed from the circular leg by blunt dissection, and more than 50% of the

circular muscle layer (including the submucosa, see Figure 41) was removed from the longitudinal leg by sharp dissection. A second tissue preparation was cut in the circular direction 15 mm. long and 1.5 to 3 mm. wide. The attached longitudinal muscle, the myenteric plexus, and approximately 25% of the circular muscle adjacent to the myenteric plexus were removed by sharp dissection yielding a 'pure' circular muscle preparation. Histological sections confirmed that the circular muscle strip had no longitudinal muscle or myenteric plexus attached. The strip was mounted in one half of the dual sucrose gap apparatus.

Solutions and Drugs

The Krebs solution contained the following (mM): NaCl, 116; Glucose, 10.1; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2 and $NaHCO_3$, 22. The other solutions were 8% sucrose and 117 mM KCl.

The drugs used were carbamylcholine 10^{-6} M (Carbachol, Allen and Hanburys) and barium chloride ($BaCl_2$, Fisher Scientific) $2X10^{-4}$-$2X10^{-3}$ M. All solutions were equilibrated with 95% O_2-5% CO_2. $BaCl_2$ was administered in Krebs solution to the center compartment which contained the hub of the 'L-shape' preparation. Carbachol was administered in sucrose solution to the sucrose compartments.

Data Analysis

P values comparing the incidence of interactions were calculated using a Chi-Squared test of independence for dichotomous

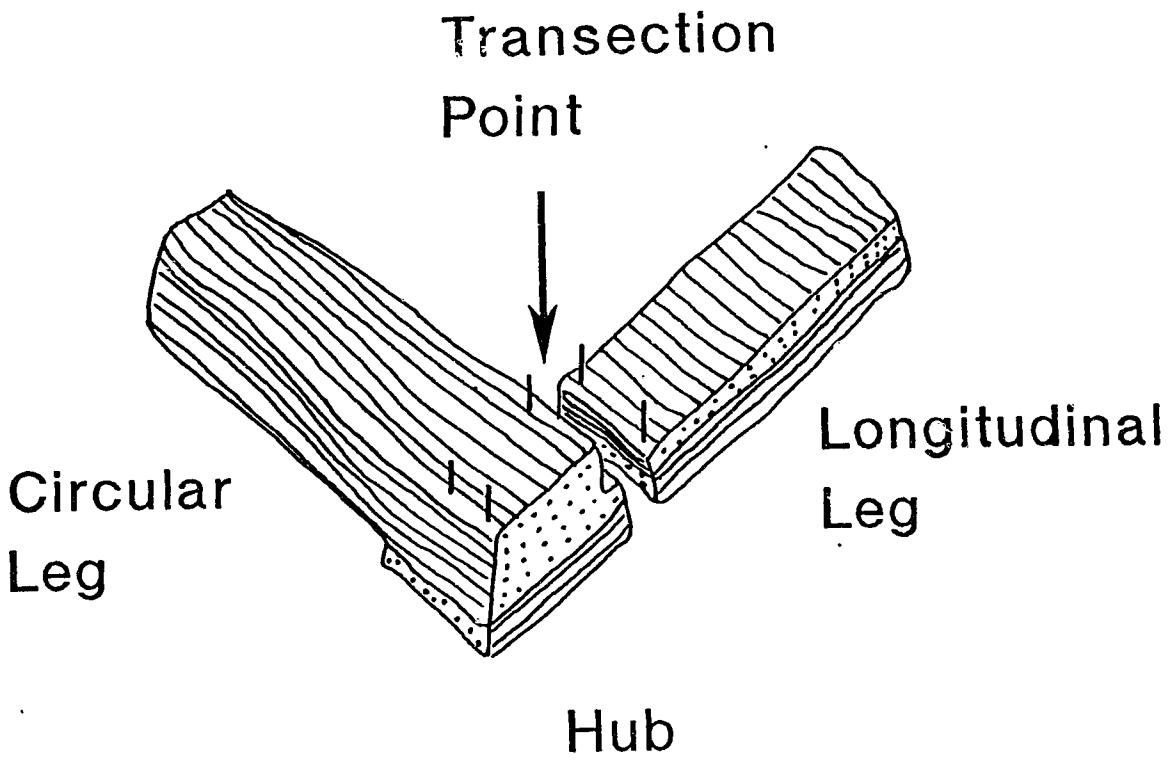

Figure 41. *Diagram of the 'L-shoped' tissue preparation. Diagram shows how the preparation was transected in one set of experiments. The transection was made between pinning points.*

variables. The frequency of membrane potential oscillations superimposed on the slow wave was compared using the Student's T test. Linear regression was applied to membrane potential oscillation frequency versus time. The Student's T test was used to test if the slope was significantly different from zero. Individual values are expressed as mean ± standard deviation.

Experimental Protocol

Tissue preparations were mounted in a dual sucrose gap apparatus which has been described previously. The legs of the 'L-shaped' preparation were attached to force transducers with silk suture thread and the bend of the 'L' was fixed to the Sylgard bottom of the organ bath with insect pins. In the case of the 'pure' circular muscle strip one end was pinned and the other was attached to a force transducer. The sucrose compartment was bounded by dental rubber dam material (Dental Dam, HCC Corp.). The tissue specimens were pulled through small holes in the rubber dam. Since the rubber dam was pliable it offered little resistance to contractions while maintaining proper isolation of the solutions. The isolated circular strip used only half of the dual sucrose gap apparatus. Temperature was maintained at 37°C and recording was begun immediately.

Several experiments were performed with the 'L-shaped' tissue preparations. The circular or longitudinal leg was selectively stimulated by applying carbachol in sucrose solution through the

sucrose compartment of the sucrose gap apparatus. Leaks out of the sucrose compartment which compromised the selective stimulation were detected by injecting blue food coloring (Liquid Color, Scott-Bathgate Ltd.). When a leak was detected the results were discarded. The effects of various levels of stimulation on interactions were investigated by applying $BaCl_2$ $2X10^{-4}$ M to $2X10^{-3}$ M in Krebs solution to the center compartment of the sucrose gap apparatus.

To study what effect interactions in the full thickness hub were having on the individual muscle layers a leg of the specimen was transected close to the hub in the center compartment during periods of interaction (Figure 41). The transection was made between pinning points and yielded a leg separated from the hub that did not have the full thickness of both muscle layers. This allowed a comparison of activity in an isolated sample (after the transection) to activity in the same sample which was attached to the full thickness hub (before transection). A drawback of this method is that transection caused a transient stimulus followed by weakening of activity in most samples.

It was investigated whether 'pure' circular muscle, devoid of an attached myenteric plexus and longitudinal muscle could generate membrane potential oscillations and spike potentials during slow wave cycles. The 'pure' circular muscle strip was mounted in one half of the dual sucrose gap apparatus and $BaCl_2$ was applied in Krebs solution to the center compartment.

To aid in the interpretation of recordings made with the double sucrose gap apparatus, intracellular microelectrode recordings were made at the submucosal and the myenteric side of the circular muscle of 'L-shaped' specimens mounted in the dual sucrose gap. These specimens were stimulated with $BaCl_2$ in Krebs solution in the center compartment to elicit spike potentials and high frequency oscillations on the slow wave. With tissue preparations mounted submucosal or serosal side up impalements were made in the isolated leg of circular muscle which extended from the full thickness hub in the center compartment.

Recording Conditions

Recording conditions were as described in Chapter 6.

D. RESULTS

Barium Chloride

Twelve specimens from nine dogs were used. The incidence of Type C or Type L interaction was noted during the basal period, during $BaCl_2$ stimulation and during the washout period. Times given exclude ten minutes equilibration at the start of each period.

Twelve specimens were studied for a total of 518 minutes in the basal state. No interactions were noted and the activity of the two legs was independent (Figure 42). This activity consisted of 4 to 7 cycles per minute (CPM) circular muscle slow waves with associated phasic contractions and 15 to 25 CPM longitudinal muscle

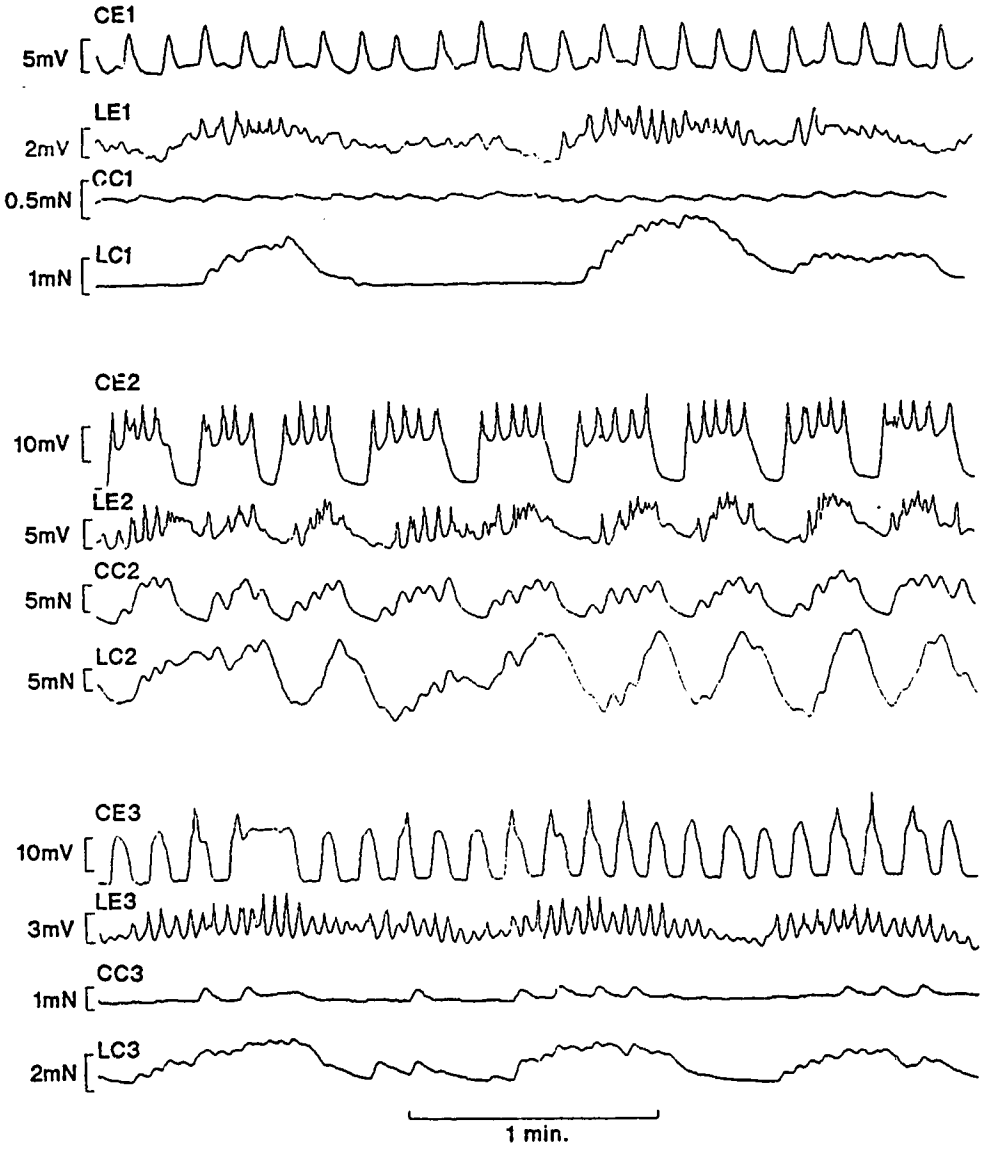

Figure 42. Activity at various levels of $BaCl_2$ stimulation. In this and the following figures: CE=circular electrical, LE=longitudinal electrical, CC=circular contractile, LC=longitudinal contractile. Panel 1 shows basal activity without interactions. Panel 2 is after $BaCl_2$ $1X10^{-3}$ M and shows coordination of activity to the circular muscle slow wave, Type C interaction. Panel 3 is during the washout period and contractions are coordinated to periodic electrical activity in the longitudinal muscle, Type L interaction.

membrane potential oscillations that occurred in bursts with superimposed spike potentials and were associated with prolonged contractions.

$BaCl_2$ $2X10^{-4}$ M was applied to 7 specimens for a total time of 208 minutes (Figure 43). Type L and Type C occurred simultaneously in 2 of 7 for 50 minutes total. Type L occurred alone in 4 of 7 specimens for 128 minutes total. Type C interaction did not occur alone in any specimen. Combining these results, during $BaCl_2$ $2X10^{-4}$ M Type L occurred in 6 of 7 specimens, significantly ($p<0.0008$) more often than basal while Type C occurred in 2 of 7 specimens, which was not different from basal ($p>0.05$). $BaCl_2$ $2X10^{-4}$ M induced membrane potential oscillations and spike potentials on the circular muscle slow wave and prolonged the slow wave. Spike potentials were likewise seen in the longitudinal muscle.

$BaCl_2$ $2X10^{-3}$ M was applied to 10 specimens for a total time of 182 minutes (Figures 43,44). Type L and Type C occurred simultaneously in 2 of 10 specimens for 24 minutes total. Type L interaction did not occur alone in any specimen. Type C interaction occurred alone in 7 of 10 specimens for 140 minutes total. Combining these results, during $BaCl_2$ $2X10^{-3}$ M application, Type C occurred in 9 of 10 specimens, significantly ($p<0.0002$) more often than in the basal state while Type L occurred in 2 of 10 specimens, which was not different from basal ($p>0.05$). $BaCl_2$ $2X10^{-3}$ M also prolonged the slow wave, induced membrane potential oscillations on the slow wave, and induced spike potentials in both layers (Figure

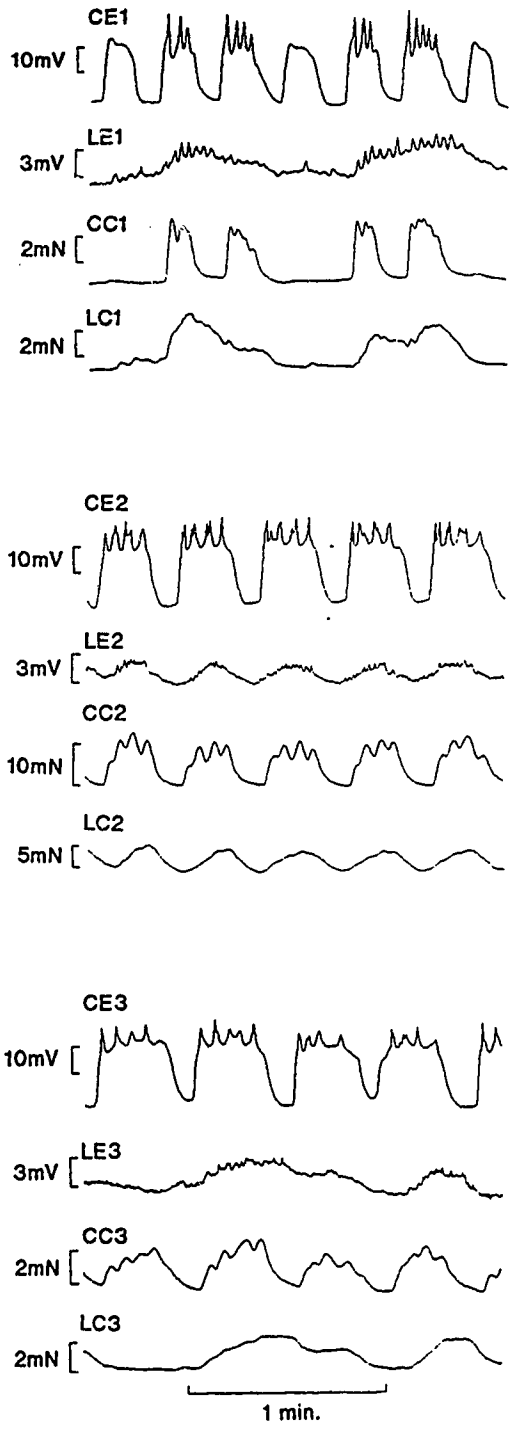

Figure 43. Activity at various levels of $BaCl_2$ stimulation. Panel 1 shows Type L interaction after $BaCl_2$ $2*10^{-4}$ M. Panel 2 shows Type C interaction after $BaCl_2$ $2*10^{-3}$ M. Panel 3 shows loss of coordination after prolonged $BaCl_2$ $2*10^{-3}$ M stimulation.

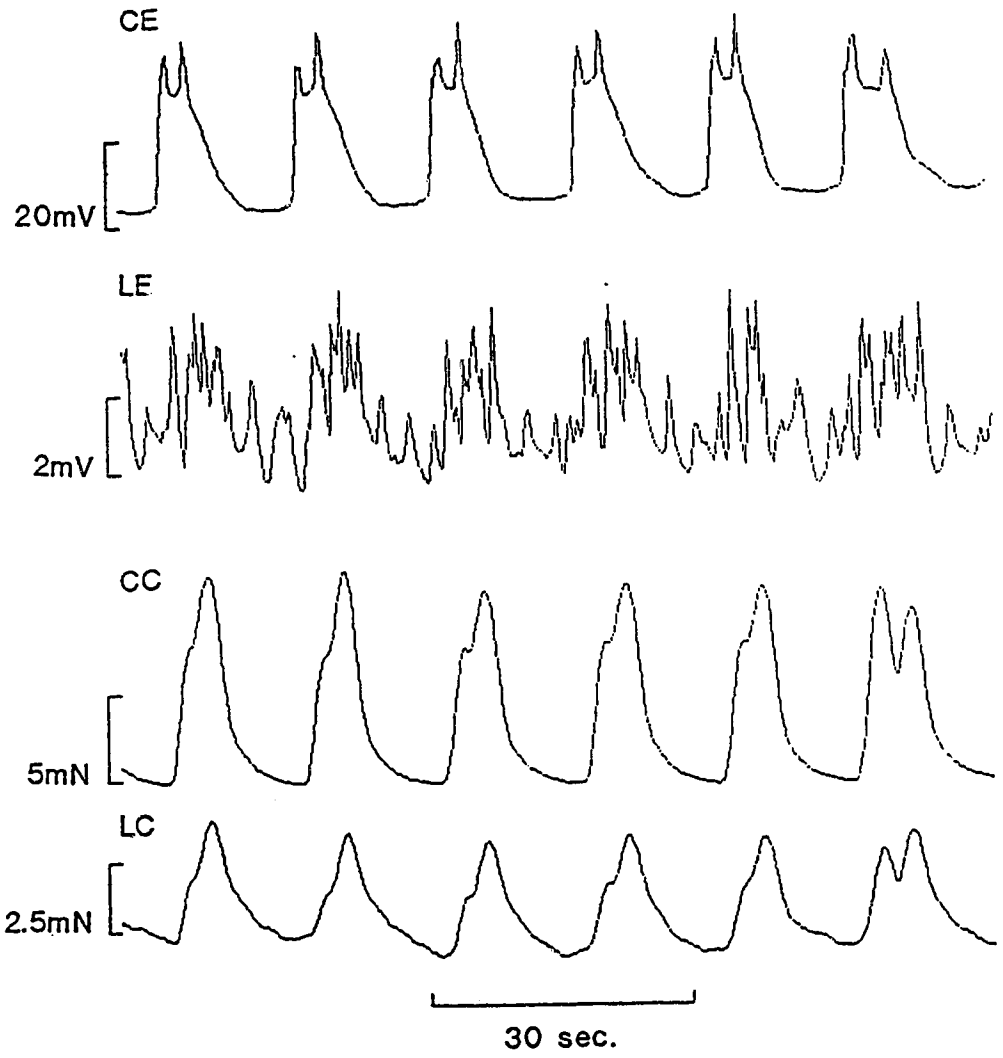

Figure 44. Activity after $BaCl_2$ $2X10^{-3}$ M. Activities in all traces were coordinated to the circular muscle slow wave.

45).

Type C interaction occurred significantly more often during $BaCl_2$ $2X10^{-3}$ M stimulation compared to $BaCl_2$ $2X10^{-4}$ M stimulation ($p=0.036$) while Type L interaction occurred significantly more often during $BaCl_2$ $2X10^{-4}$ M stimulation compared to $BaCl_2$ $2X10^{-3}$ M stimulation ($p=0.029$).

During the washout period following $BaCl_2$ $2X10^{-4}$ M and $2X10^{-3}$ M stimulation, interactions would decrease and after sufficient time, depending on the duration and concentration of the prior stimulation period, cease completely. Interestingly, the specimen would pass through certain phases of interaction, always in the same order (Figure 42). This order was as follows: Type C, followed by Type C and Type L together, followed by Type L, followed by no interactions. A particular specimen did not always pass through all these stages and some could be skipped, but in 10 of 10 washout periods this particular order was always followed.

The oscillations superimposed on the slow wave had different responses to different concentrations of $BaCl_2$. In 6 of 9 $BaCl_2$ $2X10^{-3}$ M stimulated specimens, and the 1 $BaCl_2$ 10^{-3} M specimen, a significant ($p<.05$) decrease with time in the frequency of oscillations was noted (Figure 45). In 3 of 9 these oscillations became irregular. In contrast, in 3 of 3 episodes of $BaCl_2$ $2X10^{-4}$ M stimulated specimens the oscillations did not become irregular and they did not decrease in frequency.

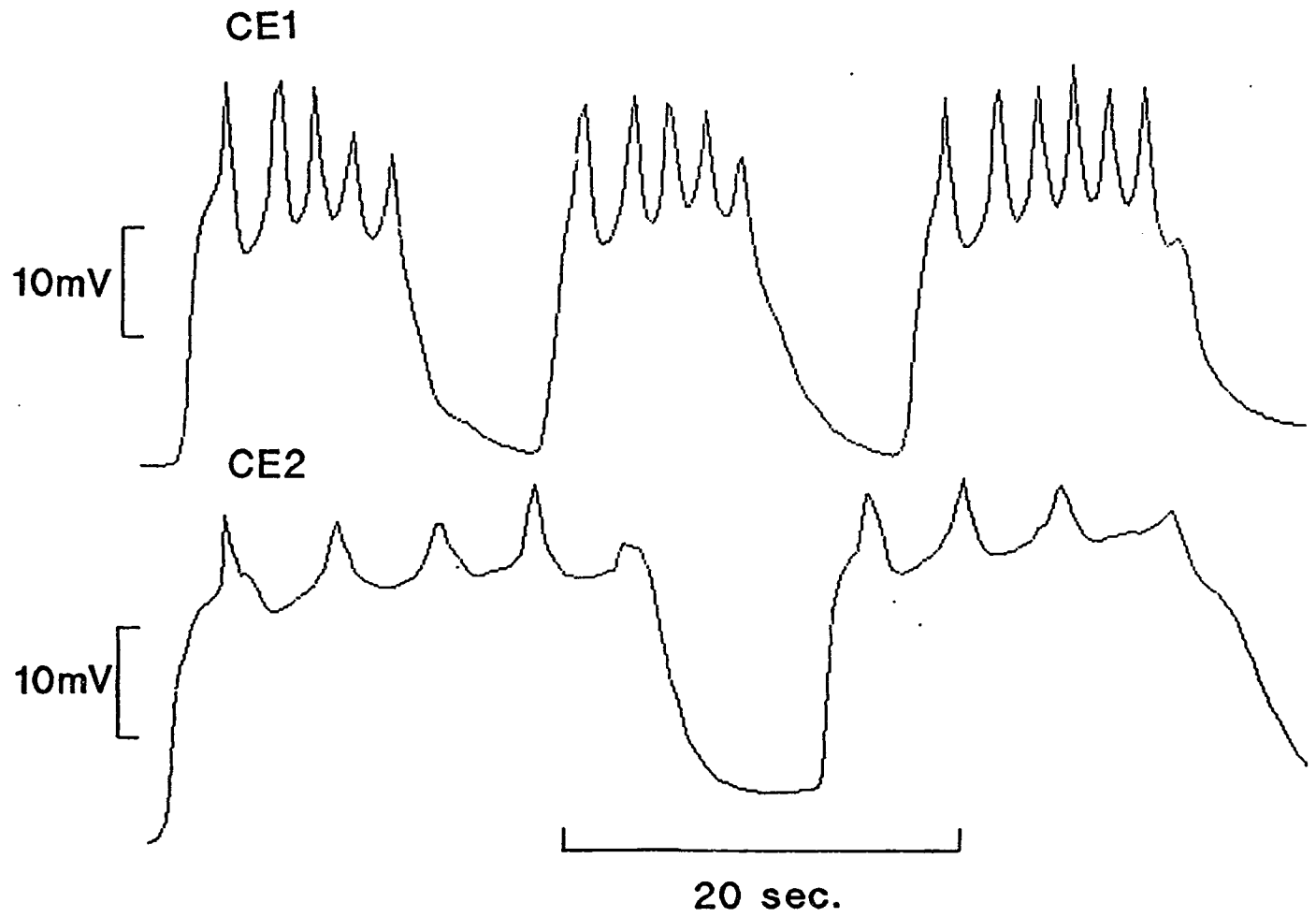

Figure 45. *Decrease in the membrane potential oscillation frequency on the slow wave during prolonged exposure to $BaCl_2$ $2*10^{-3}$ M. Second tracing is 25 minutes after the first.*

The involvement of the myenteric plexus border in the generation of spike potentials and membrane potential oscillations superimposed on the circular muscle slow wave was investigated. $BaCl_2$ was applied to 10 specimens of 'pure' circular muscle from 3 dogs as described in the methods. The 'purity' of the circular muscle was verified histologically. Specimens exhibited regular slow waves in the basal state. $BaCl_2$ $2X10^{-4}$ M was applied to 5 specimens for a total of 106 minutes. $BaCl_2$ $2X10^{-3}$ M was applied to 6 specimens for a total of 221 minutes. $BaCl_2$ caused prolonged slow waves and also oscillations and spike potentials superimposed on the slow wave as in the 'L-shape' preparation (Figures 46,47). Overall, the frequency of the membrane potential oscillations superimposed on the slow wave was significantly ($p<.01$) lower in the 'pure' circular muscle preparations, frequency=19.7 ± 2.5, than the regular L-shaped preparation, frequency=22.1 ± 4.0.

Microelectrode Recordings

The regions of smooth muscle that responded to $BaCl_2$ and the functioning of the dual sucrose gap were investigated with microelectrodes. 6 L-shape specimens from 3 dogs were mounted in the dual sucrose gap and stimulated by $BaCl_2$ $2X10^{-4}$ M to $2X10^{-3}$ M in the center compartment while microelectrode recordings were made alternately at the surface of the submucosal and the myenteric plexus side of the circular muscle. When the sucrose gap recorded slow waves with superimposed oscillations and spike potentials, a microelectrode at the submucosal surface recorded slow waves without

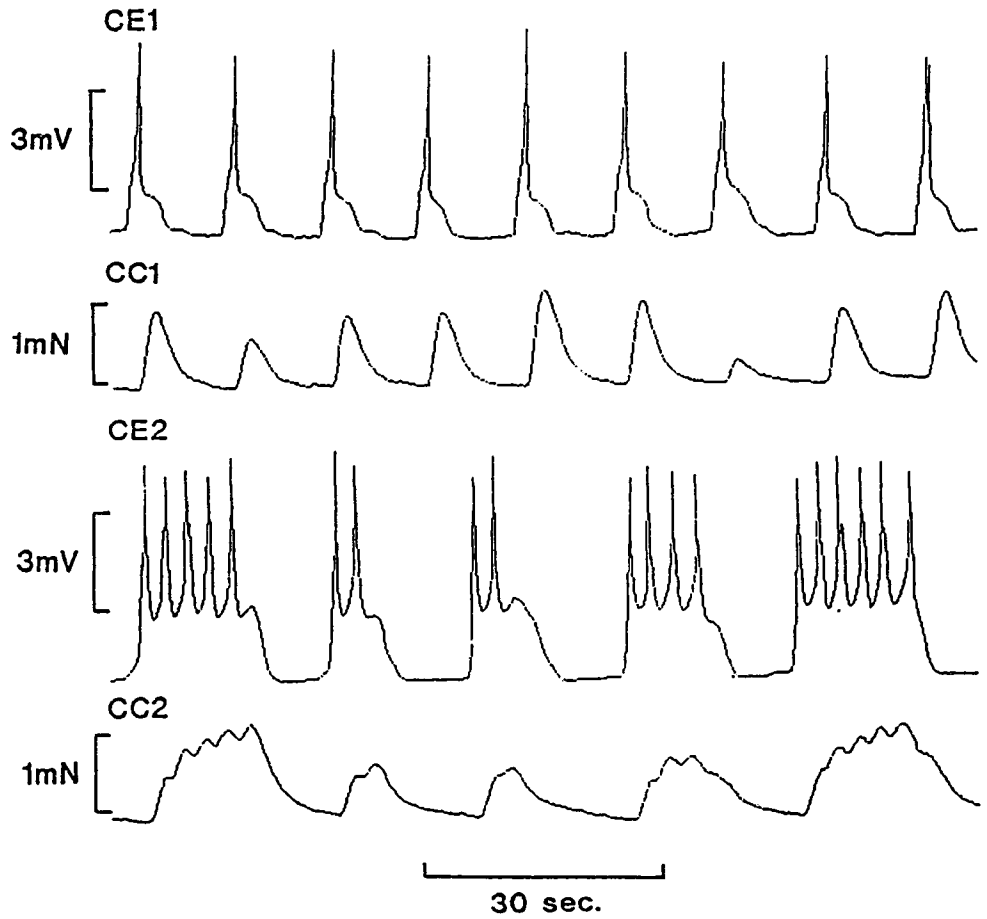

Figure 46. Activity from circular muscle devoid of longitudinal muscle and the myenteric plexus. Recordings are after $BaCl_2$ $2*10^{-3}$ M. Spike potentials and bursts of membrane potential oscillations are still observed on the slow wave.

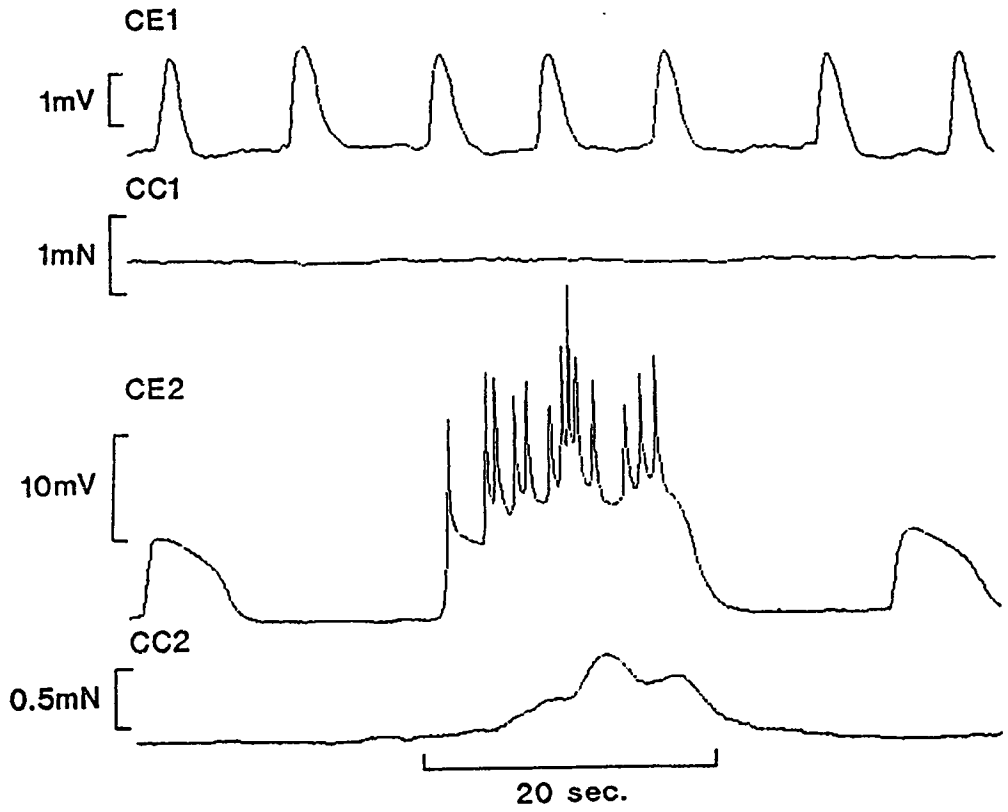

Figure 47. Activity from circular muscle devoid of longitudinal muscle and the myenteric plexus. Traces from 1 are basal, traces from 2 are after $BaCl_2$ $2*10^{-3}$ M. Basal slow waves are similar to those recorded from full thickness circular muscle. $BaCl_2$ can elicit elaborate oscillations on the slow wave between 'normal' slow waves.

oscillations or spike potentials (Figure 48). A microelectrode at the myenteric plexus surface recorded oscillations and spike potentials with low amplitude slow waves and in some cases no slow wave was observed (Figure 49).

Selective Carbachol Stimulation

The circular and the longitudinal leg were selectively stimulated by administering carbachol 10^{-6} M in sucrose solution to the bounded sucrose compartments of the dual sucrose gap. 30 specimens from 12 dogs were used and the incidence of interactions was noted and compared. Carbachol increased the strength of contractions in both layers.

27 specimens were studied in the basal state for a total of 726 minutes. Type C occurred in one specimen for 20 minutes and Type L occurred in a different specimen for 30 minutes (Figure 50). The circular leg of 14 specimens was selectively stimulated for a total of 550 minutes. Of these 14 specimens none demonstrated Type C only, 4 demonstrated Type L only for a total of 90 minutes, and one demonstrated Type L and Type C simultaneously for 30 minutes. With 16 specimens the longitudinal leg was selectively stimulated for a total of 539 minutes (Figures 50,51). None demonstrated Type C only, 6 Type L only for a total of 190 minutes, and one Type L and Type C simultaneously for 15 minutes. With 22 specimens both legs were stimulated for a total of 996 minutes. 2 demonstrated Type C only for 115 minutes, 5 Type L for 225 minutes, and 8 Type L and

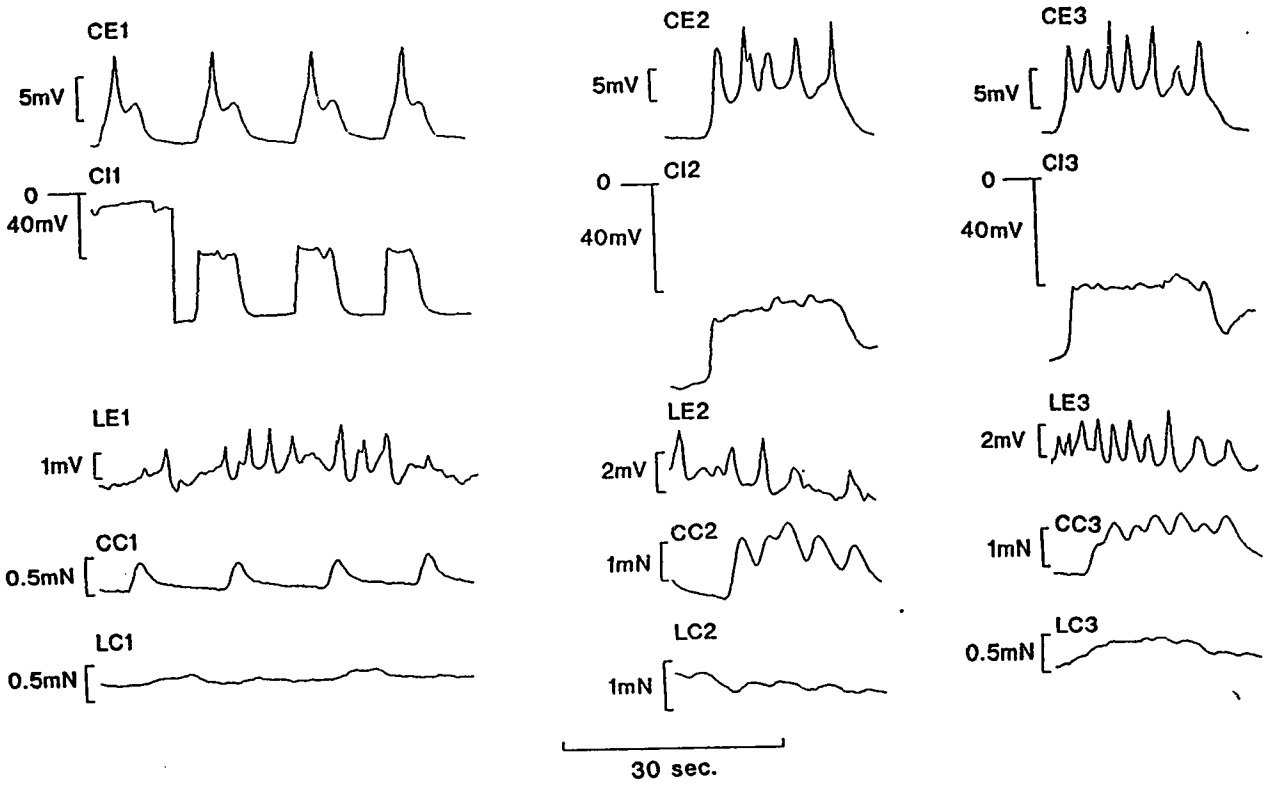

Figure 48. Intracellular recording at the submucosal border of circular muscle and simultaneous recordings from the sucrose gap apparatus. Traces from 1 are after $BaCl_2$ $2*10^{-4}$ M, traces from 2 and 3 are after $BaCl_2$ $2*10^{-3}$ M. Sucrose gap apparatus records slow waves, oscillations and spike potentials. Intracellular record from submucosal border shows only slow waves.

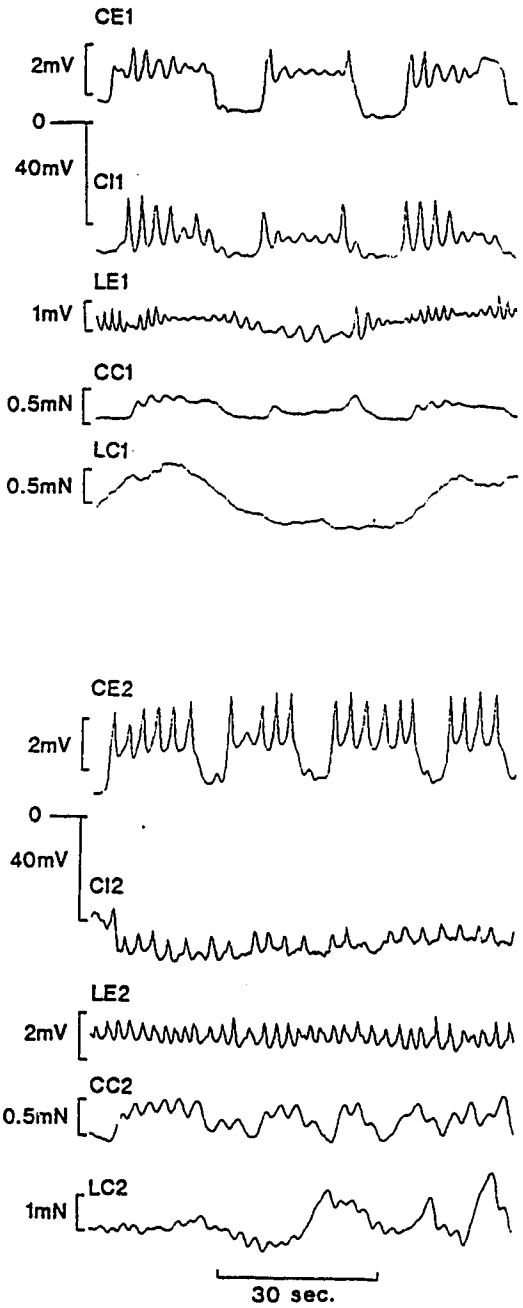

Figure 49. *Intracellular recording at the myenteric border of circular muscle and simultaneous recordings from the sucrose gap apparatus. Traces from 1 are after $BaCl_2$ $2*10^{-4}$ M, traces from 2 are after $BaCl_2$ $2*10^{-3}$ M. Sucrose gap apparatus records slow waves, oscillations and spike potentials. Intracellular record from the myenteric border shows oscillations and spike potentials with no or low amplitude slow waves.*

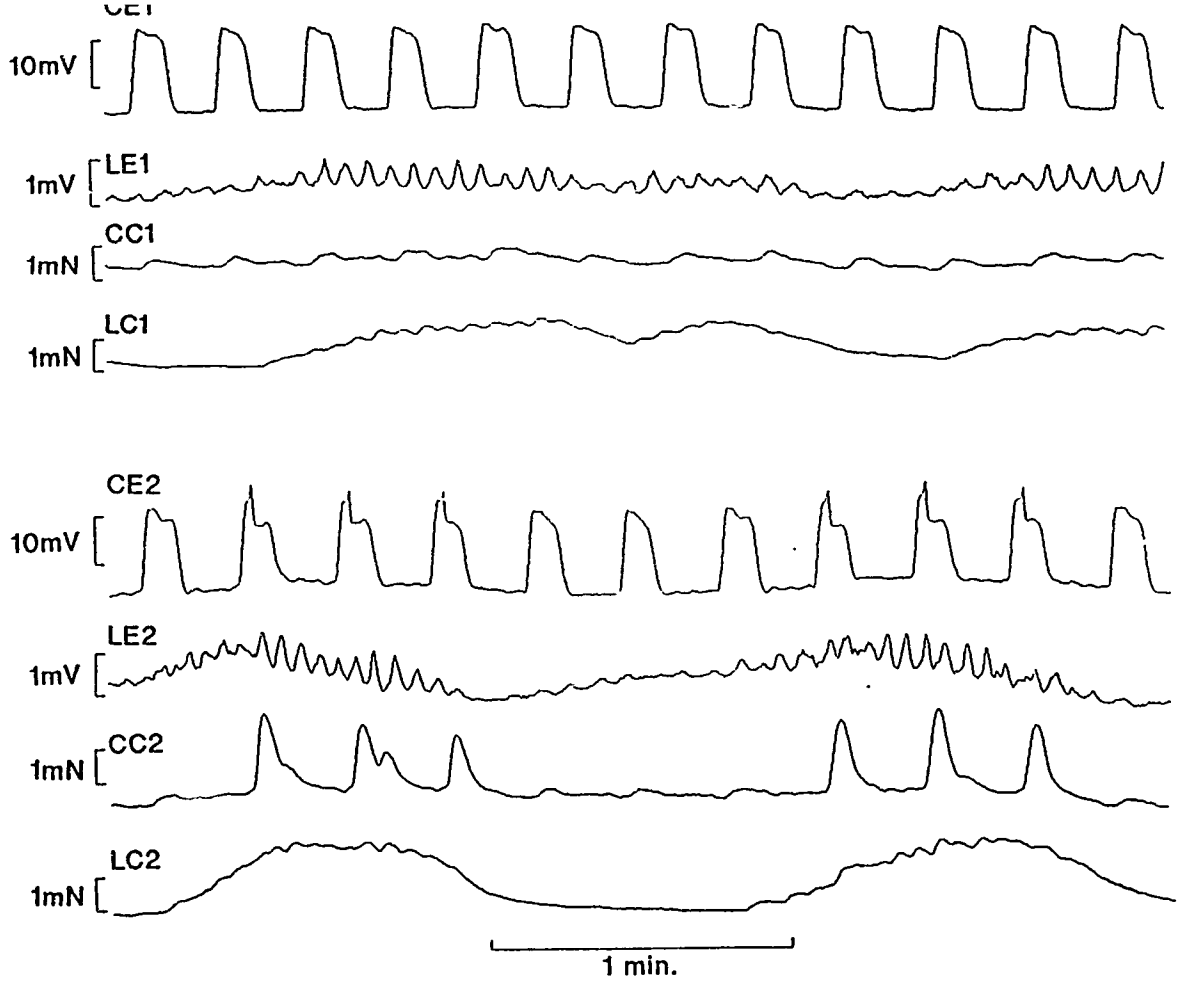

Figure 50. Activity after selective stimulation by carbachol 10^{-6} M in sucrose solution. Traces from 1 are basal. No interactions observed. Traces from 2 are after stimulation of the longitudinal layer. Type L interaction observed.

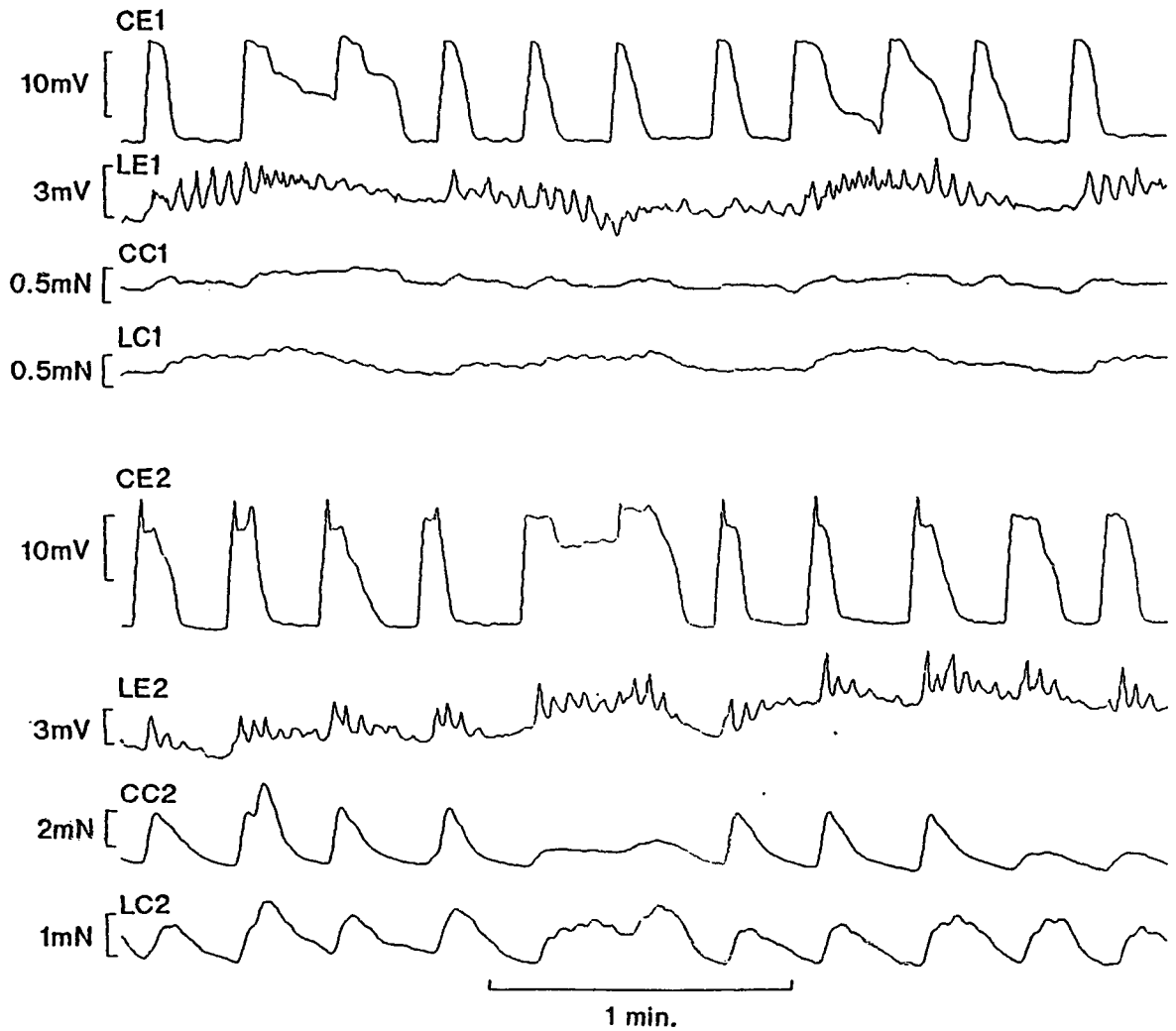

Figure 51. *Activity after selective stimulation by carbachol 10^{-6} M in sucrose solution. Traces from 1 are after stimulation of the longitudinal layer. No interactions observed. Traces from 2 are after stimulation of both layers. Type C interaction observed.*

Type C simultaneously for 289 minutes. The incidence of interactions during selective stimulation is compared in Table 7.

Transection During Periods Of Interaction

The circular or longitudinal leg of 14 specimens from 9 dogs was transected during periods of interaction. Transection caused a transient stimulus to 10 of 14 specimens followed by weakening of activity in 12 of 14 specimens.

The longitudinal leg was transected in 10 specimens (Figure 52). A Type C influence on the longitudinal leg ceased in 3 of 3 specimens. Normal activity continued in the longitudinal leg in 5 specimens and became irregular in the other 5. Longitudinal contractions were weakened in 7 specimens. An apparent Type L influence on the circular leg continued in one of 8 specimens, although no longer coordinated to the activity in the longitudinal leg, and ceased in the other 7 specimens. Circular contractions were weakened in 8 specimens.

The circular leg was transected in 4 specimens. A Type L influence, spike potentials and powerful contractions, in the circular leg ceased in all 4 specimens. The circular muscle slow wave continued or recovered in 3 of 4 specimens. A Type C slow wave influence on the longitudinal leg continued in one of 3 specimens, although no longer coordinated to the slow wave recorded from the circular leg, and ceased in the other two specimens. 'Burst' activity in the longitudinal leg continued unaltered in all 4

	Type L Interaction	Type C Interaction
Stimulate Circ. (n=14)*	5	1
	(p=0.022)§	(NS)
Basal (n=27)	1	1
	(p=0.004)	(NS)
Stimulate Long. (n=16)	7	1

	Type L Interaction		Type C Interaction
Stimulate Circ. (n=14)	5	(NS)	1
	(NS)		(p=0.039)
Stimulate Both (n=22)	13	(NS)	10
	(NS)		(p=0.023)
Stimulate Long. (n=16)	7	(p=0.04)	1
Overall (n=52)	25	(p=0.014)	12

* Stimulate Circ. means the circular leg was selectively stimulated, Stimulate Long. the longitudinal leg, and Stimulate Both both legs.

§ The significance values appear between the two values being compared either above and below or on either side. (NS) means not significant, $p>0.05$.

Table 7. A comparison of the incidence of interactions when the muscle layers were selectively stimulated by carbachol.

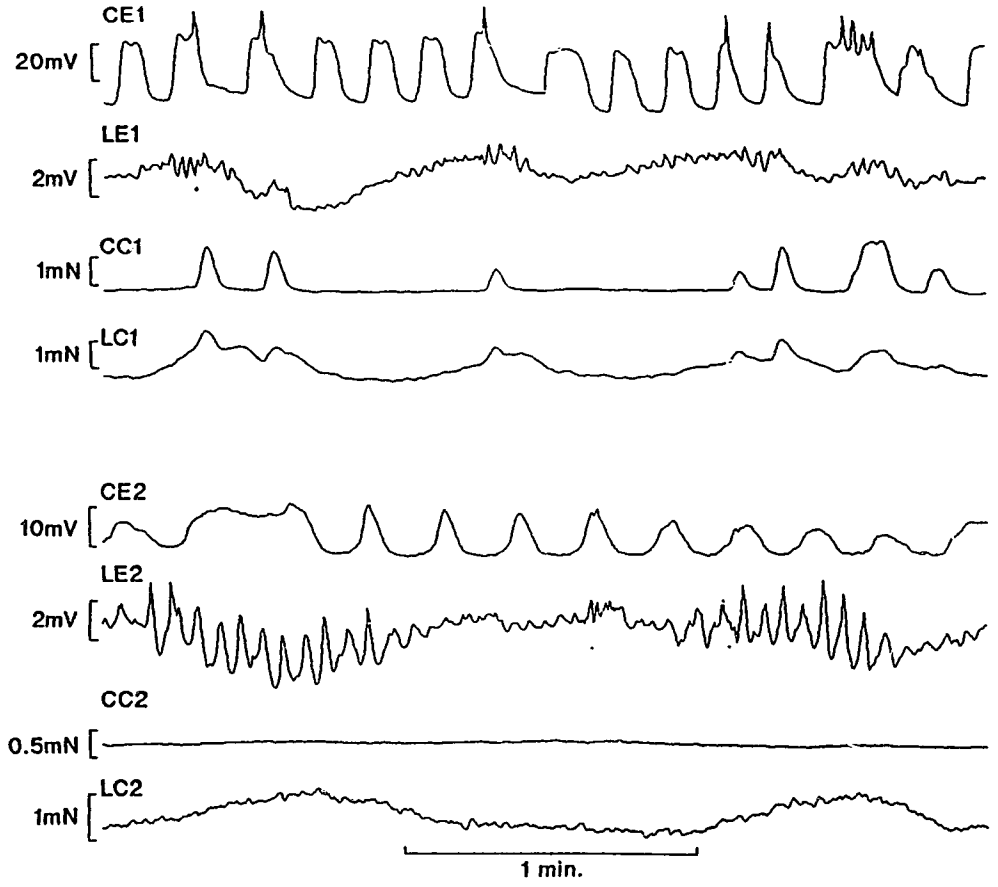

Figure 52. Activity before and after transection of the circular leg. Traces from 1 show Type C and Type L interaction before transection. Traces from 2 show distinct uncoordinated activity in both layers after transection.

specimens although contractions were weakened in one specimen.

To determine whether the interactions were directional (orad or aborad) the specimens were oriented in two different ways. 17 L-shape specimens were dissected such that the longitudinal leg was orad (in the original colon segment) to the bend of the 'L' while 11 were dissected with the longitudinal leg aborad to the bend. The incidence of interaction was not significantly different ($p>0.05$) for the two groups for any of the types of interaction.

E. DISCUSSION

The canine proximal colon has two basic electrical rhythms, the circular muscle slow wave and the longitudinal muscle electrical spike burst, which have been studied in some detail (Barajas-López et al. 1988, Chow et al., Debski et al., Durdle et al. 1983a, El-Sharkawy 1983, Sabourin et al. 1990b, Smith et al. 1987a,b). Yet the manner in which these electrical rhythms work in concert to generate coordinated patterns of motility is poorly understood. The present study showed that both electrical rhythms could generate unique patterns of contractions that were coordinated in the circular and longitudinal muscle layers.

Coordination is dependent upon an intact myenteric plexus region where the two muscle layers influence each other. Our transection experiments showed that electrical and contractile activity in the isolated legs of the tissue preparation were coordinated and strengthened due to attachment to the full thickness

hub. Is this spread of coordinated activity directional? Dissection of the longitudinal leg either orad or aborad to the hub did not affect the incidence of interactions so no directionality is suggested. In a previous study of the dog (Sabourin et al. 1990b) and of the pig (Huizinga et al. 1987a) coordination did not cease in the presence of the nerve conduction blocker TTX. Those findings and the finding of a lack of directionality in the present study suggest that coordination results from a spread of electrical events through electrically connected structures and not as a result of neural conduction.

Coordinated contractions result from controlling electrical oscillators and controlled regions of smooth muscle. Type C interaction occurs when the circular muscle slow wave is able to control the longitudinal muscle as well as the circular muscle. Type L interaction occurs when both the circular and longitudinal muscle layers contract in response to an electrical spike burst. Stimulation appears to increase the incidence of coordination in two ways. First, the controlling electrical oscillator is strengthened. For example, the circular muscle slow wave is often prolonged and oscillations appear on the slow wave plateau with superimposed spike potentials. Alternately, the intensity of the longitudinal muscle spike burst is increased. Second, the controlled regions of smooth muscle are more susceptible to external influences since they are depolarized. Any external oscillator that then couples into the controlled cells will be more likely to reach the mechanical

threshold and cause a contraction. The resulting contraction is coordinated with any contraction that occurs in the region of the external oscillator.

The barium chloride experiments suggested that the 'coordination threshold' was lower for Type L interaction than Type C since the incidence of Type L interaction is increased at a lower concentration of barium chloride stimulation. The selective carbachol stimulation experiments also suggested a lower threshold for Type L interaction since stimulation of either muscle layer increased the incidence of Type L interaction in contrast to Type C which required stimulation of both layers.

The concept of a controller and a controlled also explains why selective stimulation of the circular muscle increased the incidence of Type L interaction. The controlling electrical oscillator is the 0.5 to 2 CPM electrical spike burst which is not reported to occur in isolated circular muscle. Therefore, it appears that the incidence of Type L interaction increased due to an increased susceptibility of the stimulated circular muscle to be controlled.

The apparent difference in the threshold that must be reached to achieve Type C and Type L interactions may in part result from the location of the controlling electrical oscillator. During Type L interaction both muscle layers demonstrate 15 to 30 CPM membrane potential oscillations and spike potentials. This activity is intrinsic to the longitudinal muscle layer (Chow et al. 1987, Debski

et al. 1984, Smith et al. 1987b) and our experiments with 'pure' circular muscle suggest that it is also an intrinsic activity for circular muscle. Our microelectrode experiments and others (Barajas-Lopez et al. 1988, Smith et al 1987b) have shown that this activity occurs predominantly at the myenteric plexus border of circular muscle. Smith et al. (Smith et al. 1987b) have also suggested that myenteric potential oscillations originate at the myenteric plexus border. Therefore, Type L interaction may be the result of a controlling electrical oscillator that is in close proximity to the two muscle layers being controlled, which explains the lower coordination threshold. In contrast, Type C interaction is clearly controlled by the circular muscle slow wave which is known to originate at the submucosal border of the circular muscle (Barajas-Lopez et al. 1988, Durdle et al. 1983a, Smith et al. 1987a). Our study and others (Barajas-Lopez et al. 1988, Durdle et al. 1983a, Smith et al. 1987a,b) have shown that the amplitude of the slow wave is reduced, sometimes to zero, at the myenteric plexus border of the circular muscle. Therefore, Type C interaction results from a relatively distant controlling electrical oscillator that must overcome barriers of decay through the thickness of the circular muscle and communication across the myenteric plexus. This situation is consistent with the higher threshold found for Type C interaction.

Barium chloride increased the incidence of interactions by depolarizing the smooth muscle cells and increasing the excitability

of the tissue. This is the result of barium's ability to block K^+-channels that are open at the resting membrane potential (Benham et al., Hille). The strength and incidence of coordinated contractions are also facilitated by other effects of barium, namely: 1) barium is known to unmask the response of voltage sensitive Ca^{2+}-channels by blocking the antagonist K^+-channels (Hille) thereby increasing the occurrence of spike potentials, and 2) barium can also enter through calcium channels (Gilmore et al., Hille) and may cause contractions by mobilizing intracellular calcium (Hai et al.) or by triggering the contractile mechanism directly (Peiper et al., Satoh et al.).

In summary, at least two distinct patterns of coordinated contractions occur in canine proximal colon and both occur significantly more often after an excitatory stimulus. Contractions coordinated to the longitudinal muscle electrical spike burst are induced with less excitation than contractions coordinated to the circular muscle slow wave. We also conclude that membrane potential oscillations and spike potentials are generated in circular muscle devoid of an attached myenteric plexus or longitudinal muscle layer.

F. APPENDIX

Carbachol In Sucrose Solution

Traditionally the sucrose gap apparatus was assumed to require a sucrose solution with a high specific resistance (Coburn et al.). In this study this assumption was violated by introducing carbachol

into the sucrose solution, although without any apparent attenuation of recorded electrical activity. Perhaps the rubber membranes used in this study provided the required high impedance extracellular path. However, since sucrose solution does not contain the ions required to maintain periodic electrical events, the cells in the sucrose compartment will be inactivated. It is suggested that this is an important factor required for the functioning of the sucrose gap (Sabourin et al. in 1990a). This would explain why carbachol in the sucrose solution did not compromise the recorded electrical activity. Finally, in the experimental design it is assumed that sufficient carbachol diffuses to an active region of tissue in the Krebs compartment to elicit a response, as was observed.

CHAPTER VIII. A MODEL OF ELECTRICAL AND CONTRACTILE INTERACTIONS IN THE CANINE COLON

A. A MODEL

The sum of observations made in this study, together with those reported in the literature, have led to the formulation of a model that attempts to explain electrical and contractile events in the colon. At the present time this model is incomplete and undoubtedly contains errors, but it is useful to have a framework within which hypotheses can be formulated. The various electrical oscillating regions were identified, interrelationships were outlined between them, and a few external influences were considered. This model considers relationships within a cross section of the colonic wall consisting of the circular layer, the myenteric plexus area and the longitudinal layer. Coordination that occurs around the colon's circumference and along its length is ignored in this model.

The following elements are considered (the terminology is in part my own):

1) Slow wave oscillator (SWO), a 4 to 7 cycles per minute electrical oscillator generated at the submucosal border of circular muscle.
2) Longitudinal membrane potential oscillations (L-MPO), representing 15 to 40 cycles per minute electrical oscillations. These oscillations may originate from a

pacemaking site or they may be an intrinsic property of all longitudinal muscle cells.

3) Circular membrane potential oscillations (C-MPO), representing 15 to 40 cycles per minute electrical oscillations. These oscillations may originate from a pacemaking site or they may be an intrinsic property of all circular muscle cells.

4) Spike potentials (SP), rapid depolarizations in membrane potential caused mainly by calcium influx into the cell. These occur superimposed on other electrical events and cause powerful contractions in both circular and longitudinal muscle.

5) Spike burst control wave (SBCW), a 0.5 to 2 cycles per minute electrical control wave that enables the L-MPO and C-MPO oscillations. This event may not be an actual wave of depolarization. It encapsulates the periodicity of the longitudinal muscle spike burst and the periodicity of Type L interactions.

6) Cholinergic excitatory innervation that provides an excitatory and enabling (as far as interactions are concerned) influence in the region of the myenteric plexus.

7) Distension which provides an excitatory and enabling influence to all regions of the colonic wall.

The relationships between these elements are shown in Figure

53. The arrows indicate that an element is able to influence another. The influence is a depolarization of the target cells' membrane leading to spike potentials and/or contractions of increased strength. These influences can also act to increase the amplitude, or in some sense the 'strength', of a particular pattern of electrical oscillation.

Distension is shown as having a general excitatory influence in all regions of the colonic wall. This encompasses the fact that electrical and contractile activities in colonic smooth muscle preparations are generally strengthened by applied stretch and it also embodies the relationship between applied force and oscillation frequency described in Chapter 4 of this thesis.

Cholinergic innervation is shown to affect the C-MPO, L-MPO, and SBCW oscillations in the myenteric plexus region. This incorporates observations that cholinergic stimulation increased the incidence of interactions between the two muscle layers. This effect was localized to the myenteric plexus region since this is where interaction between the two muscle layers must be occurring (for simplicity cholinergic effects are ignored that occur elsewhere).

Type L interaction was defined as coordinated contractions in both muscle layers that occurred during spike burst episodes. Traditionally the spike burst was associated with the longitudinal muscle since isolated longitudinal muscle demonstrated this type of

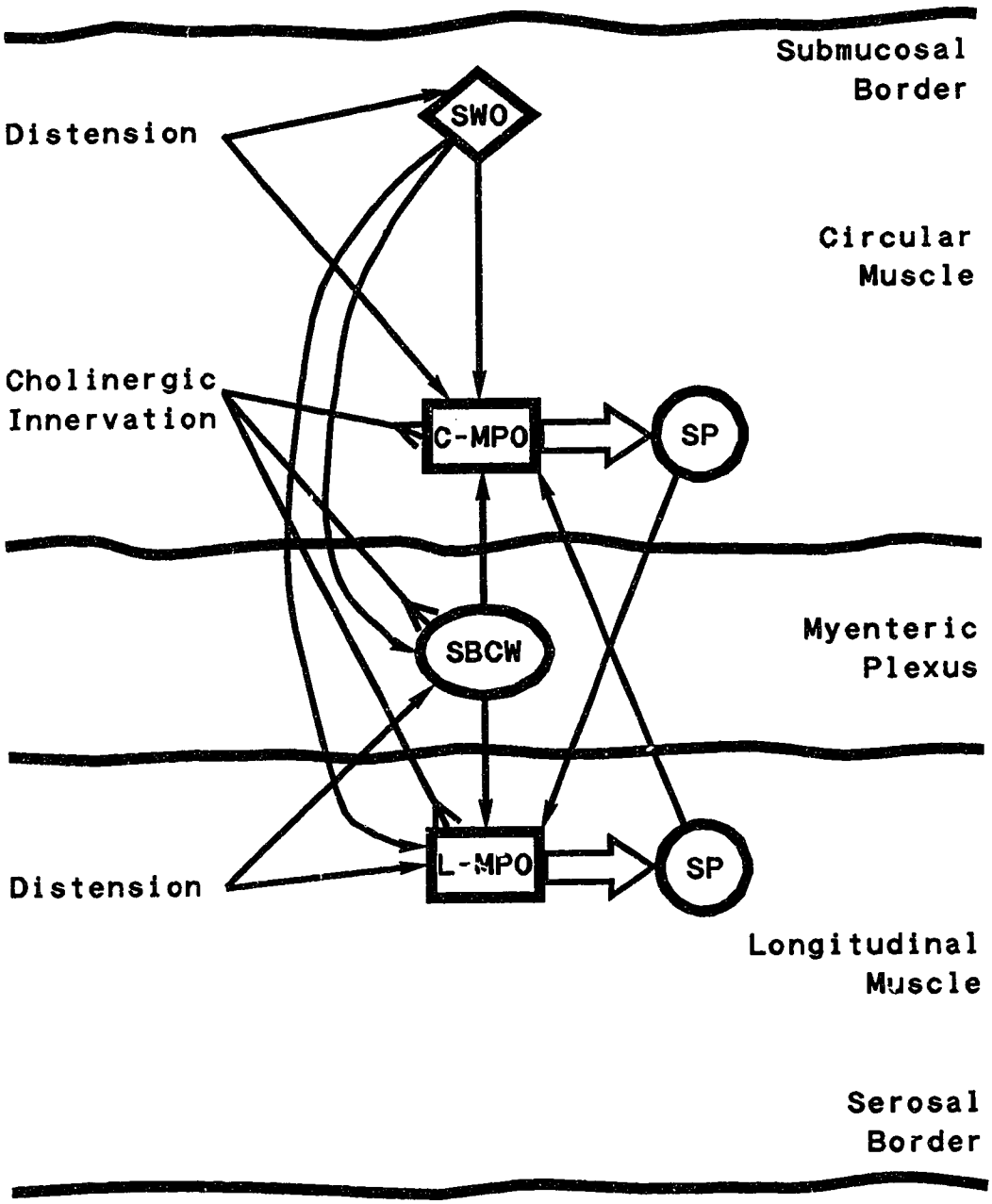

Figure 53. A model

activity but isolated circular muscle did not. However, as the model suggests, and as the observations show, events in the circular muscle can also be influenced during the spike burst. For this reason the spike burst is considered as an event that is controlled by a spike burst control wave (SBCW). The SBCW enables the C-MPO and the L-MPO oscillations which in turn can generate spike potentials. When this happens in both muscle layers simultaneously contractions coordinated in both layers and synchronized to the spike burst occur, Type L interaction. In the basal state the longitudinal layer's contractions are synchronized to the SBCW while the circular muscle usually is not. This suggests that a higher threshold has to be overcome before the SBCW can influence the circular muscle layer. Figure 53 shows that C-MPO oscillations are also influenced by the SWO. During Type L interaction the circular muscle often generated contractions synchronized with the slow wave but they were of greatly increased force during the spike burst. This supports the hypothesis that influences from the SWO and the SBCW combine to overcome the C-MPO oscillations' higher threshold. An arrow of influence was included between the SP symbol and the L-MPO oscillations in the opposite muscle layer. It is difficult to be sure with the apparatus used but it sometimes appeared that spike potentials were synchronized in both layers.

Type C interaction was defined as coordinated contractions in both muscle layers that occurred during circular muscle slow wave cycles. The SWO has arrows of influence that reach the C-MPO and

the L-MPO oscillations. Type C interaction would therefore occur when the slow wave influenced both oscillations simultaneously, leading to coordinated contractions. Type C interaction often occurred during a spike burst episode which suggests that the SWO and the SBCW worked together to influence the C-MPO and L-MPO oscillations as indicated in Figure 53. The author is not aware of direct evidence that the circular muscle slow wave can reach the longitudinal muscle. It is possible that during Type C interaction the SWO does not influence the longitudinal layer directly and for this reason an arrow of influence was included between the SWO and the SBCW. The hypothesis is that the slow wave may influence the longitudinal layer indirectly through a pathway in the myenteric plexus region, perhaps involving the SBCW mechanism.

The experiments in which barium chloride was used showed that Type L interaction occurred more readily that Type C. This suggests that the thresholds which must be overcome before the SBCW can influence C-MPO oscillations are smaller than those which must be overcome before the SWO can influence L-MPO oscillations. Contractile patterns seen in vivo include both short and prolonged contractions (Sarna 1986) but prolonged contractions appear to be more common (Kingma et al. 1980, Kocylowski et al., Sarna 1986). This is interesting since Type L interaction which is easier to elicit in vitro is associated with prolonged contractions while Type C, which is more difficult to elicit, is associated with short contractions.

B. FUTURE RESEARCH AVENUES

The techniques and apparatus are now proven and they could therefore be readily applied to studies of human colon. Initially these studies would have to establish whether or not interactions can be observed in human colon and to describe the patterns of interaction. Further studies could elucidate the factors that contribute to or enable coordination between the muscle layers.

On the microscopic scale, more work is required to understand many of the basic mechanisms involved in the generation and propagation of electrical events in the human and animal colons. The location of pacemaking elements (if they exist), their structure and function, are not well understood. Whether or not the various patterns of oscillation are an intrinsic property of all cells that manifest those patterns, or, result from pacing is not known. Coordination of contractile events requires communication across the myenteric plexus. In human colon regions of the circular and longitudinal muscle pass into each other providing close contact between the two layers. In dog, the two muscle layers are not known to contact each other in the same manner. The experiments with tetrodotoxin suggest that known neural elements are not responsible for the communication either. More work is required to discover what structures or mechanisms in the myenteric plexus area might be responsible for the communication and pacemaking functions that appear to underlie the observed interactions.

On the macroscopic scale, many questions remain unanswered as

to how coordination is organized in the colon as a whole. This study has shown that the muscle layers can coordinate their activities. However, the contractile patterns responsible for the colon's functions of storage, mixing and propulsion are not fully known. Knowledge in the field of gastrointestinal motility is in need of integration. We understand in part at the microscopic level and the macroscopic level. We understand in part electrical activity and contractile activity. We understand in part the effects of various foods, drugs and even diseases on colonic transit and motility. This thesis has sought to bridge a specific gap between some in vivo and in vitro results. More studies are required that integrate the 'in part's into a coherent and unified whole.

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