RABBIT UTERINE CONTRACTILE ACTIVITY AS MONITORED IN VITRO WITH EXTRALUMINAL CONTRACTILE FORCE TRANSDUCERS

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ABSTRACT

RABBIT UTERINE CONTRACTILE ACTIVITY AS MONITORED *IN VITRO* WITH EXTRALUMINAL CONTRACTILE FORCE TRANSDUCERS

by Carol A. Rowland

Although contractile activity patterns of the uterus for the ovarian stages have been established both *in vitro* and *in vivo*, no *in vitro* isometric technique exists for quantification of contractile activity of the longitudinal and circular muscle layers. The purpose of this study is to develop a reliable isometric method for quantification of rabbit uterine *in vitro* contractile activity.

An extraluminal contractile force transducer was waterproofed for *in vitro* use with consecutive coats of solvent-thinned nitrile rubber solution and subsequent coats of rubber-like epoxy resin. The resulting transducer was smaller in surface area and more convenient for *in vitro* use than those previously fabricated for *in vivo* use. The transducers were calibrated following quality control tests for hysteresis, waterproofing and temperature compensation.

The ovariectomized, estrogen-treated rabbits were killed and the uterus immediately removed and placed in

warmed, oxygenated Ringer-Locke solution for approximately one hour. At this time the extraluminal transducers were affixed to the uterine muscle segment with sutures fastened through holes drilled in the transducer shim stock. The transducers were arranged in a number of configurations. The segment was suspended in a tissue bath via a Grass transducer. A control segment was similarly suspended without attached extraluminal transducers. Recordings of contractile activity were obtained on an ink-writing oscillograph.

Control records were obtained; then drugs were added to the bath in an effort to differentiate the contractile activity of the two muscle layers. Paired comparison tests were used to determine whether the presence of the transducers on the muscle segment affected their in vitro contractile activity.

Analysis of control records showed that extraluminal transducers were measuring contractile activity of the longitudinal and circular muscle layers separately and simultaneously from the same segment. Oxytocin, vasopressin, and acetylcholine elicited differential responses from the two muscle layers.

The presence of the transducers on the muscle segment did not affect its contractile activity. Longitudinally oriented extraluminal transducers detected an average contractile force of 0.57 g./burst while the

circularly oriented extraluminal transducer detected an average contractile force of 0.90 g./burst. Average frequency of bursts for both muscle layers was 0.54 bursts/min. Analyzing simultaneous Grass transducer recordings, no statistically significant differences were found between the frequencies or contractile forces of the segments with the extraluminal transducers attached and the control segments without extraluminal transducers attached.

The longitudinally oriented extraluminal transducer recorded apparent decreased tone when a high tension was set with the attaching sutures.

Approved:_____

Date:_____

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By

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CHAPTER ONE

INTRODUCTION

Early investigators of uterine motility did not maintain a physiological environment; the uterus was exposed to air and no attempt was made to maintain body temperature (18). Subsequently, three classes of techniques have been devised for studying uterine contractile activity: *in vivo*, *in situ*, and *in vitro*.

Luduena (25) advocates use of the *in vitro* techniques for studying properties of mammalian smooth muscle because of the following advantages:

1. The simplicity of the *in vitro* method provides conditions which permit effective control of variables.
2. The effect of temperature, pH, and electrolyte concentrations on tonus, spontaneous contractile activity and drug responses can be studied *in vitro*, but these variables cannot be easily altered *in vivo*.
3. Several segments of a single tissue may be studied simultaneously. This allows accumulation of drug information in a shorter period of time and enables selection of the initial drug dosage to be administered to the intact animal.

A review of *in vitro* techniques will show the lack of a reliable isometric method for recording the discrete contractile activity of the different uterine muscle layers.

Review of In Vitro Methods

Reynolds (34,35) pointed out that many of the discrepancies found in the literature regarding the physiology and pharmacology of uterine muscle could be due to the variations in recording technique.

Csapo and Corner (16) were the first (1952) to use the isometric technique *in vitro* in the study of uterine muscle. Prior to this time, isotonic methods had been used.

Szent-Gyorgyi and Hadju (45), after investigating the properties of actomyosin, stated that isotonic recording may register only the percent of shortening of the muscle strip, while giving no indication of the amount of tension developed. Csapo (14) investigated the effects of temperature on the development of isometric tension and isotonic shortening. He concluded that isotonic shortening of an unloaded uterus is maximal at lower temperatures while tension is minimal. A similar effect was obtained by altering the strength of stimulation. Isotonic shortening may be maximal without having maximum muscle activation. Both investigations revealed that the isometric method of recording was a better quantitative estimate of the work the uterine muscle was capable of doing, and gave a better understanding of myometrial function.

Isotonic Recordings. Some of the first reliable isotonic recordings of rabbit uterine contractile activity

occurred in the laboratories of Cushny (18) and Dale (19) in 1906. After anesthetizing the animal with paraldehyde given by stomach tube, cannulas were inserted into the carotid arteries, the jugular vein, and the trachea. A midline abdominal incision was made to the peritoneum. The uterus was exposed after the animal was immersed in physiological saline preheated to body temperature. Two small stitches 1" apart were taken in the horn; the threads were connected to levers of an apparatus resembling a myocardiograph. The stitches were oriented in either the longitudinal or circular direction. In some pregnant does simultaneous recording of the activity of the two muscle layers was possible by taking four stitches and using a double system of levers. In most preparations the hypogastric nerve was severed prior to recording. Contractile activity was recorded with concurrent observation of the motility of the uterus. The contractile activity patterns of rabbits in estrus showed slow, rhythmical contractions which varied in frequency from one contraction every thirty seconds to every three to four minutes. The circular layer contractions were similar to the longitudinal layer contractions. When longitudinal and circular muscle contractions were recorded simultaneously, the contractions were not usually simultaneous. Sometimes the circular layer contracted without the longitudinal layer contracting. If circular and longitudinal contractions did

occur simultaneously, the longitudinal was usually masked by the contraction of the circular layer; Cushny stated that the longitudinal muscle showed relaxation on the record.

In 1907 Kehrer (24), (according to Csapo and Corner (10)), made pioneer in vitro drug studies on virgin rabbit uteri using the discovery of Rudolf Magnus (26) that strips of cat intestine contracted when put in warm oxygenated saline. Isotonic recordings of the excised myometrium could be made by attaching the muscle to a lever. Later Athias (1) used this method on the guinea pig uteri to demonstrate a definite relationship between the ovaries and uterine motility existed. He ovariectomized the animals to show the disappearance of uterine contractile activity; he transplanted ovarian tissue to the castrate and showed the return of contractile activity. In 1923 Corner (9) showed that different in vitro contractile activity patterns existed for each stage of the ovarian cycle in the sow. In estrus the contractions were slower (1.5-2.5 minutes apart) and of greater amplitude than after ovulation. Ten days after ovulation the rate was incrased to 6-8 contractions per minute with an amplitude only one-fifth that of estrus contractions. In pregnancy the sow uterus was quiescent.

Robson (37,38), using the Trendelenburg method (46), recorded both longitudinal and circular contractile

activity of the rabbit myometrium. This method involved recording of the longitudinal muscle contractile activity via an isotonic lever. Changes in intra-uterine pressure represented circular muscle contractile activity. Before suspending the muscle in the bath, a glass cannula was inserted into the uterine lumen through a small incision made in the horn. After the cannula was tied in place, warm Ringer-Locke solution was passed through the lumen. The appropriate segment was then tied off, removed, and placed in the bath. In ovariectomized rabbits, Robson (36) found that pretreatment with crystalline ketohydroxoestrone increased the spontaneous activity, and reactivity to oxytocin as compared with results obtained on nontreated castrates.

In 1935 Simeone (43) used cat uteri to demonstrate the effect of initial tension on spontaneous activity. The uterus was exposed by an abdominal midline incision, during Dial anesthesia, and one horn freed by ligation of the ovarian blood vessels and section of the hypogastric nerve. The vaginal end of the uterus was held in place by a dissecting needle while the ovarian end was attached to a light weight writing lever in apposition to a weight pan. The whole horn was then enclosed in a glass cylinder to insure adequate moisture and warmth. The uterus was raised to a vertical position by adjusting the weight in the pan. Care was taken not to stretch the muscle from

its resting length which was the optimum tension for maximum contractile amplitude. Further stretching of the muscle rendered it less excitable; an increase in tension caused a decrease in the amplitude of the spontaneous contractions.

Bonnycastle and Ferguson (5) used a different approach to the problem of studying the separate muscle layers in the rabbit myometrium. The segment to be studied was separated into its longitudinal and circular components before being suspended in the bath. The effects of oxytocin (Pitocin) and epinephrine (Adrenalin) on these muscle layers from different levels in the uterus were investigated. Three day prepartum and two day postpartum rabbits were used. These investigators found that in the postpartum rabbit (which is under estrogen dominance according to Schofield (42)) the longitudinal muscle layer was more sensitive to oxytocin than the circular muscle layer, while the circular muscle layer was more responsive to epinephrine.

Isometric Recordings. Csapo and Corner (16) studied ovarian hormones in relation to contraction cycle duration and to the "staircase" phenomenon, which had first been observed in cardiac muscle. An isometric method of recording was used for the first time in this in vitro study on contractile activity of the rabbit myometrium, as the isometric method had previously been shown by

Szent-Gyorgyi and Hadju to yield more quantitative results (45). Their study involved frog cardiac muscle. The uterine horns were placed in oxygenated Krebs' solution at 0°C. for ten minutes, because at this temperature the uterus reached a state of minimum energy expenditure and established a steady "equilibrium" length 20% shorter than the "resting" length. A muscle segment 25 mm. long was then suspended between two platinum electrodes in a vertical glass tube filled with warm, oxygenated Krebs' solution. The upper hook was attached by a silk thread to the short arm of a lever which was connected to a flat torsion spring. The long arm of the lever recorded the contraction. This conventional isometric tension recorder allowed less than 6% shortening of the muscle segment. These authors found that estrogen dominated uteri exhibited a "positive" staircase (a frequency of 1/min. electrical stimulation after a resting period of ten minutes caused an increase in the contractile amplitude developed without tone change), while progesterone dominated uteri exhibited a "negative" staircase (electrical stimulation at a frequency of 1/min. or greater than 1/min. caused a decrease in contractile amplitude developed without a change in tone). Estrogen dominance increased while progesterone dominance decreased the duration of the contraction-relaxation cycle (the time from beginning of contraction to the end of relaxation)

when compared to the contraction-relaxation cycle of the castrate rabbit's uterus.

In 1953 Csapo and Goodall (17), using the same in vitro isometric technique, determined that the muscle was most capable of developing maximum contraction amplitude at the "resting" length. In 1954 Csapo (14) discussed the adjustment of the muscle to obtain this "resting" length. The muscle was first put in Kreb's solution at 0°C. where it obtained its equilibrium length which was approximately 20% shorter than the "resting" length. It was then suspended between two hooks in Krebs' solution at body temperature, where it elongated after ten minutes to a length 25% longer than the equilibrium length. If the distance between the two hooks was increased, the muscle strip lengthened; it adjusted to the new length by an initial increase in the resting tension followed by a decrease (indicated by a slight rise in the tracing above the baseline and then a return to the baseline or below). If this limit of muscle adjustment was overstepped, the tracing did not return to the baseline. Subsequent increase in stretch of the muscle segment brought a still greater increase in amplitude above the baseline. The point at which the muscle fiber could not adjust to the new length without an accompanying increase in tension was designated as the "resting" length. This is the present standard for adjustment of "resting" tension in

in vitro work, although more recent investigators have omitted putting the muscle in 0°C. bathing solution before starting (20,21,28).

In 1965 Miller and Marshall (28) used the technique first developed by Varagic (47) for an isolated hypogastric nerve-uterine muscle preparation for studying the effects of stimulation on the rabbit myometrium. The animal was "bled out" to facilitate identification of the nerves. The nerves were tied 5-7 mm. below the bifurcation of the abdominal aorta. The bladder was then removed to make the dissection easier. The uterus and nerves, along with a piece of colon, were removed intact. The nerves were then carefully isolated. Miller and Marshall used only a segment of either horn while Varagic immersed the whole uterus in the bath. The segment, including the cervix, was tied by the cervical end to a hook in the organ bath. The other end was attached to a Grass force displacement transducer, FT-03, which measured isometric tension developed by the segment. The nerves were threaded through insulated platinum electrodes connected to a Grass stimulator. They concluded that the rabbit uterus contains both α-excitatory and β-inhibitory adrenergic receptors with the former predominating in the estrogen-dominated rabbit.

Diamond and Brody (20,21) investigated the relationship between *in vitro* contraction of rat uteri, the

biochemical changes occurring in the muscle, and the effect of catecholamines on its contractile activity. They used an *in vitro* isometric technique, but allowed the muscle to equilibrate in a warm, oxygenated bathing solution, rather than a 0° solution. Resting tension was adjusted according to the method of Csapo (14). They concluded that the estrogen-primed rat uterus contains both α-excitatory and β-inhibitory receptors with the latter predominating.

Statement of the Problem

Although accepted contractile activity patterns for the ovarian stages have been established both *in vitro* (4,9,10,16,18,28) and *in vivo* (2,8,32,33,41,42), this review has shown that no *in vitro* isometric technique exists for the quantification of the contractile activity of the uterine circular and longitudinal muscle layers.

The extraluminal contractile force transducer has been developed and used *in vivo* for recording contractile activity of the smooth muscle layers in the gut and uterus (2,3,8,23,30,31,39). This transducer is unidirectional (it measures only contractile activity of that muscle layer with which its longitudinal axis is aligned). *In vivo* in the uterus (2) and in the gut (3), this transducer with an attached electrode has been used to measure electrical and contractile activity; correlations have been made from the data obtained.

The transducer was modified by fabricating without the silicone rubber protection used *in vivo*. The resulting transducer was smaller in surface area and allowed convenient *in vitro* use. A combination of the Grass force displacement transducer with the extraluminal contractile force transducers measuring the contractile activity of the muscle layers on the same segment would allow a discrete monitoring of the separate muscle layers simultaneously and an analysis of the contractile activity which is measured by the Grass transducer.

The purpose of this thesis is to develop an *in vitro* isometric method for quantification of rabbit uterine muscle layer contractile activity. This will be attempted using the extraluminal contractile force transducer.

CHAPTER TWO

METHODS

Contractile Force Transducer

The extraluminal contractile force transducer monitors the contractile activity of that portion of the muscle layer which is beneath the two ends of the transducer. The transducer consists of two resistance strain gages bonded one to each side of an arched metal shim stock. The principle of this method of contractile activity measurement is based on the detection of linear deformation of the strain gage grid, which may result from muscle contraction or relaxation, either active or passive. The force of this contractile activity is detected by a change in the electrical resistance of the strain gage grid. Discrete activity of the muscle layer may be measured by orienting the longitudinal axis of the grid parallel to the muscle fibers of the muscle layer to be monitored.

A detailed description of the transducer fabrication used for *in vivo* recording, along with a list of materials required, has been given (23,30). A description of the modification of this fabrication for *in vitro* work follows.

Transducer Fabrication

Figure 1 shows a schematic drawing of the transducer. The 2.5 X 6.0 mm. shim stock was cut from 2-1/2% beryllium copper[1] alloy sheets, in the "quarter hard" condition. The shim stock was 0.15 mm. thick. A cylindrical mold was used to press the shim stock into an arch of 6 mm. diameter. The curved shim stocks were then heat cured for four hours at 600°F.

After removal from the oven, the heat treated shim stocks were immediately placed in room-temperature distilled water. They were inspected, polished with silicon carbide paper,[2] and cleaned with "metal conditioner"[2] and isopropyl alcohol to improve the surface for bonding. The square corners were rounded off with a file to eliminate the possibility of trauma to the muscle. A jeweler's drill (approximately 0.75 mm. diameter, #65) was used to drill one small hole in each end, to permit the transducer to be sewn directly on the muscle.

Two strain gages[3] were bonded, one to each side of the shim stock, using a filled epoxy cement.[4]

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[1]Compliments of the Beryllium Corp., Reading, Pa.

[2]Materials described may be obtained from William T. Bean, 18915 Grand River Ave., Detroit, Michigan.

[3]Catalogue #SK-09-031DE-350. Micro-measurements, Inc., Romulus, Michigan.

[4]Tatnall GA-5 epoxy cement. Instruments Division, Budd Co., Phoenixville, Pa.

Bonding pressure of one pound was applied via neg'ator clamps[5] while the unit was being heat cured at 220°F. for two hours.

A three-conductor lead wire system was used to compensate for the heat-induced lead wire resistance changes. The lead wire was Teflon-insulated, with seven strands per conductor.[5] The bared ends of the lead wires were soldered with special strain gage solder[5] to the four soldering tabs of the strain gage, as shown in Figure 1. After soldering, the solder joints were cleaned with rosin solvent[5] and the whole transducer was coated with three coats of Gagekote #2[5] (drying time of 15 minutes per coat), followed by two coats of Gagekote #5[5] (drying time of 6 hours per coat). Needles were placed in the holes in the shim stock to prevent filling the holes with the water-proofing materials. The combination of these two materials completely waterproofed the transducer for immersion in the in vitro bath.

Transducer Circuitry and Recording Instrument

The two-gage transducer was incorporated into a double active arm Wheatstone half-bridge circuit. The "free" end of the lead wire was soldered to a nine pin-position Cannon plug (MD1-9SL1),[6] which connected the

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[5]Materials described may be obtained from William T. Bean, 18915 Grand River Ave., Detroit 23, Michigan.

[6]ITT Cannon Electric, Inc., Los Angeles, California.

two resistance elements located within the transducer to two external resistors located outside and adjacent to the strain gage coupler of the ink-writing oscillograph[7] used in this study. Sensitivities of 0.05 and 0.02 millivolts per centimeter pen deflection were used in recording contractile activity via the extraluminal contractile force transducers. Sensitivities of 0.5, 0.2 and 0.1 millivolts per centimeter pen deflection were used in recording contractile activity via the Grass force displacement transducer.[8]

A paper speed of 12.5 mm./min., 25.0 mm./min., 0.1 cm./sec. or 0.5 cm./sec. was used to record contractile activity.

Transducer Calibration

The transducers were calibrated to determine the contractile force developed by each muscle layer. Initially calibration of the circularly oriented transducer was done according to the method of Reinke (30). This consisted of taping the transducer to a supporting rod to secure it in a horizontal position. A Mersilene 000 suture[9] was fastened to the transducer and passed through a small hole located

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[7]Type R Dynograph, Beckman Instruments, Inc., Spinco Division, Schiller Park, Illinois.

[8]Model FT-03 without springs. Grass Instrument Co., Quincy, Mass.

[9]Mersilene 000 suture, Ethicon #R-552, Ethicon, Inc., Somerville, N. J.

Figure 1. Extraluminal contractile force transducer

A diagrammatic illustration of the transducers and lead-wire orientation prior to waterproofing. Another strain gage is bonded to the concave side of each shim stock.

1-A. The lead wire orientation of the transducer to be used for recording contractile activity of the circular muscle layer.

1-B. The lead wire orientation of the transducer to be used for recording contractile activity of the longitudinally muscle layer.

in each end, under the transducer and over a pulley on the opposite side. Weights were hung from the sutures to simulate contractions of the muscle.

A different method of calibration of the longitudinally oriented transducer was necessary due to the orientation of its lead wire. The longitudinally oriented transducer was secured in a horizontal position by clamping the non-lead wire end of the transducer in a vise. Contraction was simulated by hanging weights from a Mersilene 000 suture passed through the hole and fastened to the lead wire end of the transducer, a modification of the calibration procedure of Jacoby (23).

A calibration curve obtained by Jacoby's method for the circularly oriented transducer showed that a lesser degree of deflection was developed per gram by this method than by Reinke's method. The orientation of the lead wires for the longitudinally oriented extraluminal transducers did not allow it to be calibrated according to Reinke's method. Calibration curves obtained using Jacoby's method were used to compare contractile force developed by the muscle layers. These curves are shown in Figure 2-A and B.

The Grass FT-03 transducers were calibrated by suspending weights from the muscle attachment point. A calibration curve obtained in this manner is shown in Figure 3.

Figure 2. Calibration curves

A. A typical calibration curve for a circularly oriented transducer. By knowing the deflection of the pen in mm., the grams of force developed by a particular contraction can be read directly from this graph.

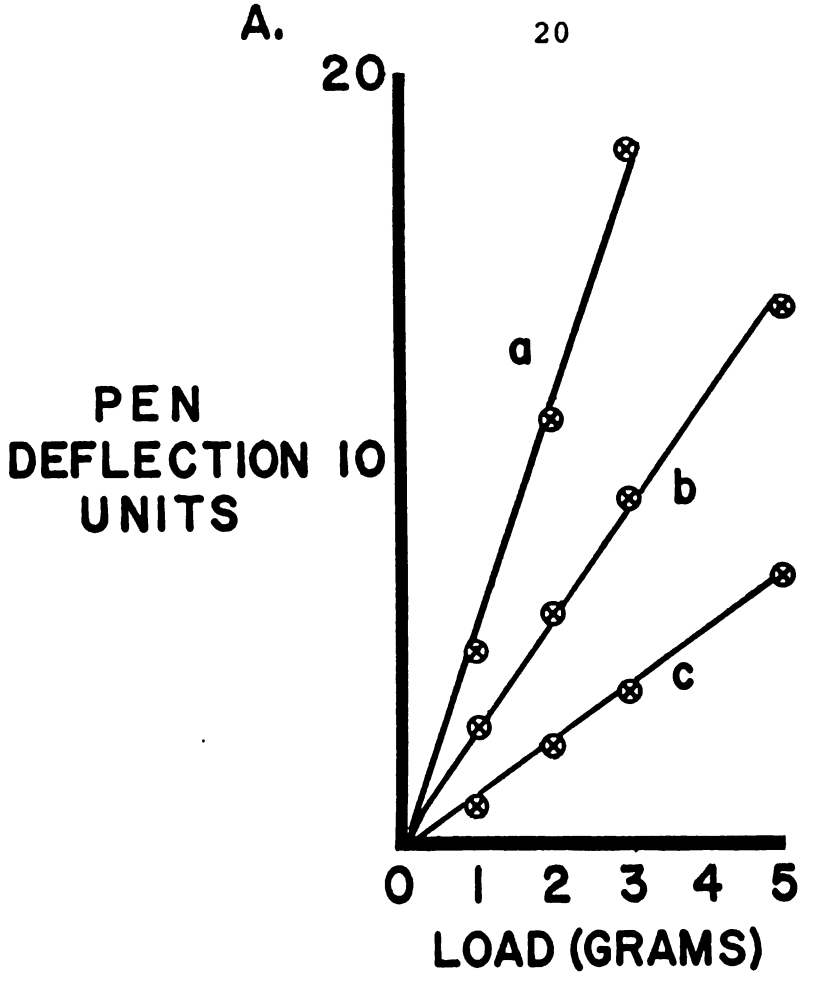
B. A typical calibration curve for a longitudinally oriented transducer. By knowing the deflection of the pen for a contraction recorded by this transducer, the force of contraction can be calculated from this graph.

   a. Sensitivity at 0.05 mv./cm.

   b. Sensitivity at 0.1 mv./cm.

   c. Sensitivity at 0.2 mv.cm.

   Units--millimeters.


A.
20
PEN
DEFLECTION
UNITS
10
a
b
c
0 1 2 3 4 5
LOAD (GRAMS)

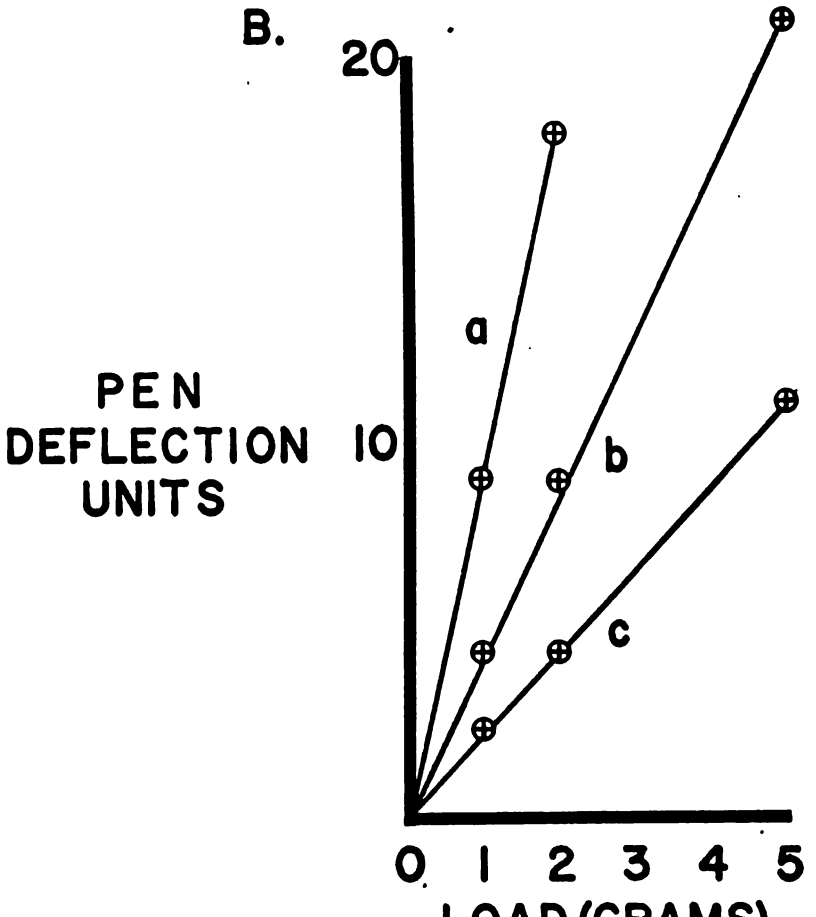
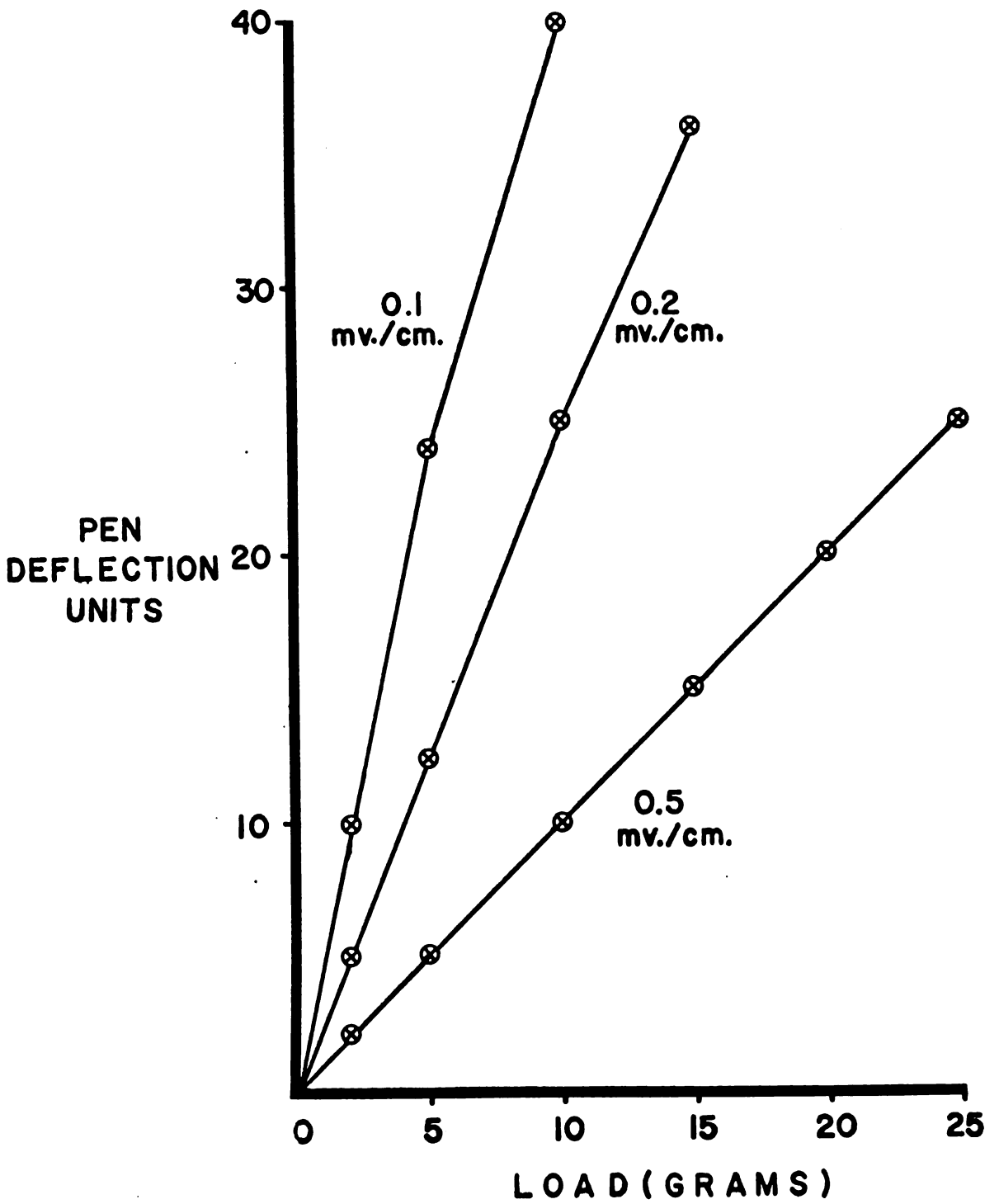

B.
20
PEN
DEFLECTION
UNITS
10
a
b
c
0 1 2 3 4 5

Figure 3. Calibration curve for Grass transducer

A typical calibration curve for a Grass FT-03 transducer. By knowing the deflection of the pen in mm., the grams of force developed by a particular contraction can be read directly from this graph.


40
30
20
10
PEN
DEFLECTION
UNITS
0.1
mv./cm.
0.2
mv./cm.
0.5
mv./cm.
0
5
10
15
20
25
LOAD (GRAMS)

The air around the transducers was warmed to 37-38°C. by a heat lamp and stabilized at that temperature for the entire calibration period, to eliminate the difference that might have occurred between calibration at room temperature and bath temperature.

The stability and waterproofing of the transducers was tested by completing the bridge curcuit to the Dynograph recorder and immersing them in water warmed to 37°C. for one-half hour. If any deviation from the baseline occurred, the transducers were discarded. They were tested for hysteresis during the calibration period, by rapid addition and removal of a weight. If the pen deflection did not return to its initial baseline position, the transducer was not used.

Experimental Procedure

It is well known that rabbits are induced ovulators; they may ovulate due to some stimulus other than coitus (mechanical stimulation to the cervix, play with other does). This produces a pseudopregnant condition in which progesterone is the dominant hormone and the uterus shows a contractile activity pattern which differs from the contractile activity pattern of the estrous state.

It was difficult to ascertain the ovarian state of the rabbit by external examination. In an effort to standardize experimental conditions 16 of the 24 rabbits tested were ovariectomized, then pretreated with 100 µg.

of estradiol cypionate[10] daily for five days prior to use. This treatment has been shown to simulate the estrous stage of the rabbit (17,22,41).

The rabbits were killed by a blow on the head. The uterine horns were immediately removed and placed in Ringer-Locke solution at 37°C. The composition of the medium was as follows: NaCl, 9.0 g.; KCl, 0.42 g.; $CaCl_2$, 0.24 g.; $MgCl_2$, 0.20 g.; $NaHCO_3$, 0.50 g.; and d-glucose, 0.50 g. (q.s. to 1000 ml. with distilled water). The solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide. The muscle was allowed to equilibrate under these conditions; during this time it relaxed and lengthened. It was necessary to allow the muscle to equilibrate for 45-60 minutes before sewing on the transducers; if they were sewn on before complete equilibration had occured, the muscle wrinkled under the transducer and reduced the ability of the transducer to register contractile activity.

Mersilene 000 suture was used to sew the transducers on the muscle. The tissue was kept immersed in Ringer-Locke solution during most of the procedure; care was taken to handle the tissue as little as possible. Two sutures were used to secure each transducer on the muscle. After the extraluminal transducers were sewn

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[10]Upjohn Company, Kalamazoo, Michigan.

on the muscle, a segment approximately 20 mm. long was cut and it, with the attached transducers, was suspended vertically in a double-walled, preheated bath which contained Ringer-Locke solution, which was continually being gassed with 95% oxygen and 5% carbon dioxide mixture. The segment was connected to a glass rod via a platinum hook on one end, and the other end was connected with thread to a Grass force displacement transducer (FT-03, without springs).

The transducer arrangement was as follows:

1. A muscle segment approximately 20 mm. long, with two extraluminal contractile force transducers attached, was connected to a Grass force displacement transducer. The extraluminal transducers were arranged in a vertical-inverted "T" formation on the antimesometrial side of the segment and in a direct line with the tension exerted on the segment by the attachment of the Grass force displacement transducer. The longitudinally oriented extraluminal transducer was positioned above and perpendicular to the circularly oriented extraluminal transducer. Another segment, cut from the opposite horn at approximately the same level, was suspended in a second bath and connected only to a Grass transducer as a control segment without extraluminal transducers attached. This arrangement shall be referred to as 3VTD (3 transducers in a vertically-inverted "T" formation in direct line with the tension exerted on the muscle segment by the Grass force displacment transducer). Refer to Figure 4-A.

Another group of recordings was obtained with the following transducer arrangements:

2. Two adjacent muscle segments, 15 mm. long, were suspended in separate baths. One extraluminal transducer measured the contractile activity of the circular muscle layer of one segment, while a Grass FT-03 transducer measured contractile

activity of the entire segment. A second extraluminal transducer measured the contractile activity of the longitudinal muscle layer in the second segment, while a second Grass FT-03 transducer measured contractile activity of this entire segment. This arrangement shall be referred to as 2D (2 transducers attached to one muscle segment; the extraluminal transducer in a direct line with the tension exerted on the muscle segment by the Grass FT-03 transducer).

3. To determine whether contractile activity of the muscle layers differed at the same level in the segment, the extraluminal transducers were placed in a horizontal "T" formation, with the circularly oriented transducer on the mesometrial side of the segment and the longitudinally oriented transducer on the antimesometrial side. The Grass FT-03 transducer was attached at a point midway between the two extraluminal transducers. A control segment was set up as in #1 above. This arrangement shall be referred to as 3HTI (3 transducers attached to one segment; the extraluminal transducers in a horizontal "T" formation and not in a direct line with the tension exerted on the segment by the attachment of the Grass FT-03 transducer). Refer to Figure 4-B.

Resting tension, as determined by recording from the Grass transducer, was adjusted according to the method of Csapo as explained on page 8 of this thesis. A slight increment in tension of the muscle under the extraluminal transducer was set at the time the transducer was sewn on. The distance between the attachment sutures was 3.5 mm.

Spontaneous contractile activity usually occurred within one hour after suspension of the uterine segment in the <u>in vitro</u> bath. Numerous control records were obtained; then drugs were used to show differential responses of the outer longitudinal and inner circular muscle layers, known to be present in rabbit uteri (11,27,29,33).

Figure 4. Orientation of extraluminal transducers in relation to the Grass FT-03 transducer

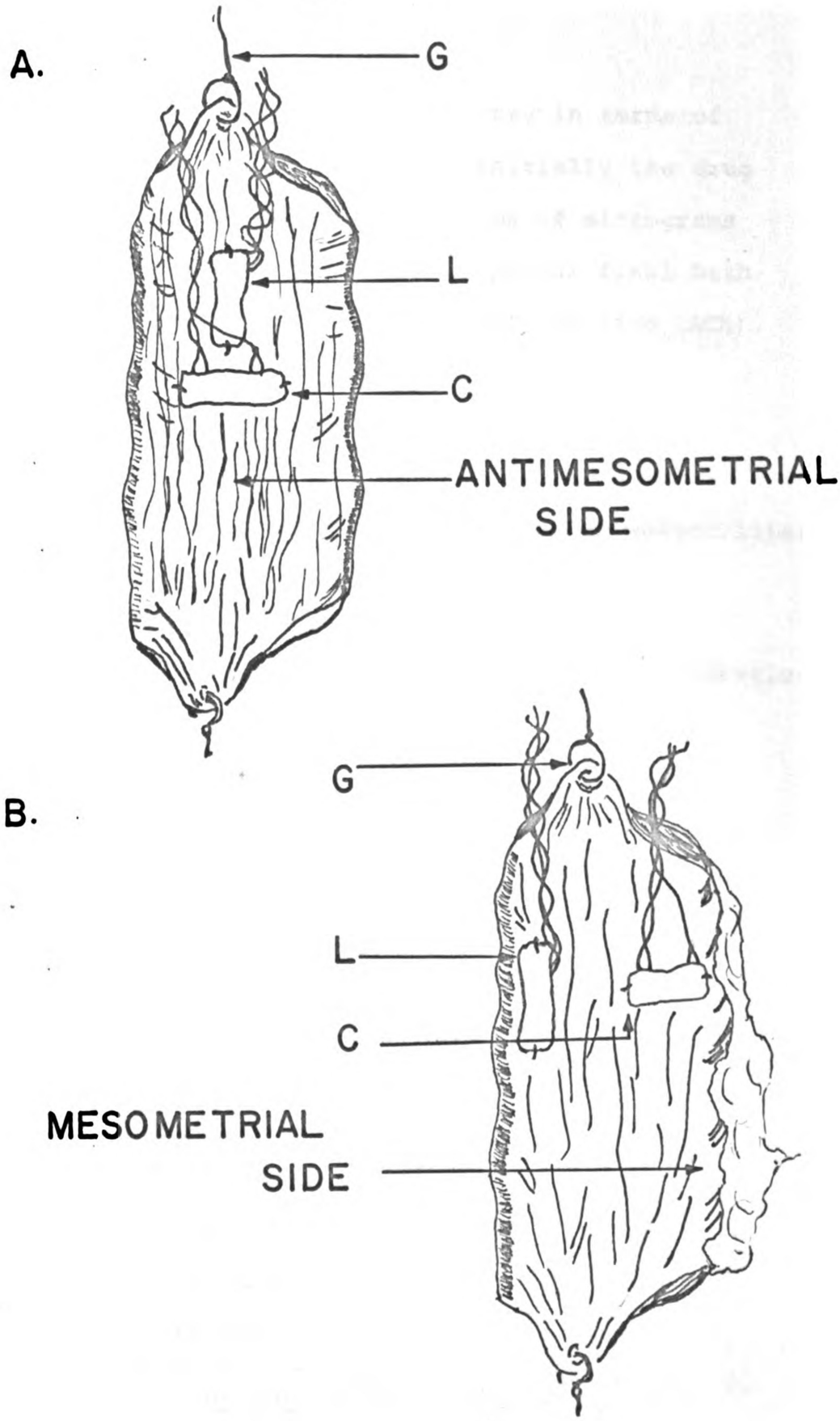
A. Orientation of the extraluminal transducer in a vertically-inverted "T" formation in a direct line with the resting tension exerted on the muscle segment by the Grass FT-03 transducer. This configuration shall be designated as 3VTD (3 transducers; two extraluminal transducers in a vertically-inverted "T" under direct tension).

B. Orientation of the extraluminal transducers in a horizontal "T" formation when the resting tension exerted on the muscle segment by the Grass FT-03 transducer is not in a direct line with the transducers. This configuration shall be designated as 3HTI (3 transducers; two extraluminal transducers in a horizontal "T" and not under direct tension).

G--Grass FT-03 transducer thread connection.

C--circularly oriented extraluminal transducer.

L--Longitudinally oriented extraluminal transducer.


A.
G
L
C
ANTIMESOMETRIAL
SIDE
B.
G
L
C
MESOMETRIAL
SIDE

Drug Concentration Calculations

Effective drug dosages are reported in terms of molarity of final bath concentration. Initially the drug dose administered to the bath was in terms of micrograms (μg.) or units/total bath capacity. A typical final bath concentration calculation is that for acetylcholine (ACh).

Bath capacity	80 ml.
Initial drug dose	40 μg.
Molecular Weight of Acetylcholine	163 g.

A one molar solution contains one gram molecular weight/liter.

1 molar ACh = 163 g./l.

Given:

40 μg. ACh/80 ml. = 0.5 μg./ml. bath concentrations.

Then:

$$0.5 \text{ μg. ACh/ml.} = 0.5 \text{ mg./l.} = 5 \times 10^{-4} \text{ g./l.}$$

The final bath concentration in molarity is calculated in the following manner:

$$\frac{163 \text{ g.ACh/l.}}{1 \text{ molar}} = \frac{5 \times 10^{-4} \text{ g. ACh/l.}}{X \text{ molar}}$$

$$X \text{ molar} = \frac{5 \times 10^{-4} \text{ g.}}{163 \text{ g.}} = 3.0 \times 10^{-6} \text{ molar.}$$

Statistical Analysis of Data

In an effort to detect a difference between contractile activity of the experimental and control muscle segments, a paired comparison[11] was made of data obtained.

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[11]Frederick E. Croxton, Elementary Statistics with Applications in Medicine and the Biological Sciences, Dover Publications, Inc., New York, 1953.

CHAPTER THREE

RESULTS

Characteristic activity patterns for both the longitudinal and circular muscle layers, recorded separately and/or simultaneously *in vitro*, were obtained for the estrogen dominated rabbit myometrium using the extraluminal contractile force transducers. Correlations between the contractile activity recorded by these transducers and the contractile activity recorded by the Grass FT-03 transducer were made.

Contractile Activity Patterns

Two types of contractile activity records were obtained using the three transducer arrangements. The record obtained depended on whether the extraluminal force transducers were in the 3VTD or the 3HTI configuration.

The first type of contractile activity pattern recorded by the extraluminal transducers and the Grass FT-03 transducer occurred when the 3VTD and the 2D configurations were used. Two typical examples of spontaneous contractile activity patterns obtained from a 3VTD transducer configuration are shown in Figures 5 and 6.

The results obtained when the extraluminal transducers were aligned with the tension exerted on the muscle

Figure 5. Spontaneous contractile activity patterns obtained under the 3VTD configuration

Spontaneous contractile activity is shown in this tracing of a recording, in which the transducers were oriented in a 3VTD configuration.

C--contractile activity recorded by circularly oriented extraluminal transducer.
L--contractile activity recorded by longitudinally oriented extraluminal transducer.
G--contractile activity recorded by the Grass FT-03 transducer.

Paper speed--12.5 mm./min.

Figure 6. Spontaneous contractile activity patterns obtained from the 3VTD configuration

This tracing of a recording shows apparent relaxation being recorded by the longitudinally oriented extraluminal transducer. Paper speed--0.1 cm./sec.

C--contractile activity measured by a circularly oriented extraluminal transducer.
L--contractile activity measured by a longitudinally oriented extraluminal transducer.
G--contractile activity measured by a Grass FT-03 transducer.

segment by the Grass FT-03 transducer may be summarized as follows:

1. The Grass FT-03 transducer and the transversely oriented extraluminal contractile force transducer registered contractions simultaneously, while the longitudinally oriented extraluminal contractile force transducer showed superimposed contractions on an apparent decrease in tone.

2. A contraction (superimposed on decreased tone) recorded by the longitudinally oriented extraluminal transducer was also recorded (without decreased tone) by the Grass FT-03 transducer, while the transversely oriented extraluminal transducer was recording an initial apparent relaxation followed by a contraction of the circular muscle.

3. The Grass FT-03 transducer, the transversely oriented extraluminal transducer, and the longitudinally oriented extraluminal transducer often registered contraction simultaneously.

Recordings from the transversely oriented extraluminal transducer and the Grass FT-03 transducer (Figure 7) which were attached to the same muscle segment showed the following results:

1. The Grass FT-03 transducer recorded simultaneous contractions with the transversely oriented extraluminal transducer.

2. The two transducers often recorded contraction peaks which did not coincide.

Recordings from the longitudinally oriented extraluminal transducer and the Grass FT-03 transducer (Figure 8) which monitored contractile activity of the same muscle segment showed the following results:

1. The Grass FT-03 transducer recorded simultaneous contractions with the longitudinally oriented extraluminal contractile force transducer.

Figure 7. Comparison of contractile activity recorded by a circularly oriented extraluminal transducer and a Grass FT-03 transducer under direct tension

These tracings show a recording of the contractile activity of a muscle segment recorded by the circularly oriented extraluminal transducer and a Grass FT-03 transducer. The transducer arrangement was the 2D configuration. Paper speed--upper record, 25 mm./min.; lower record, 5 mm./sec.

C--contractile activity recorded by the circularly oriented extraluminal transducer.

G--contractile activity recorded by the Grass FT-03 transducer.

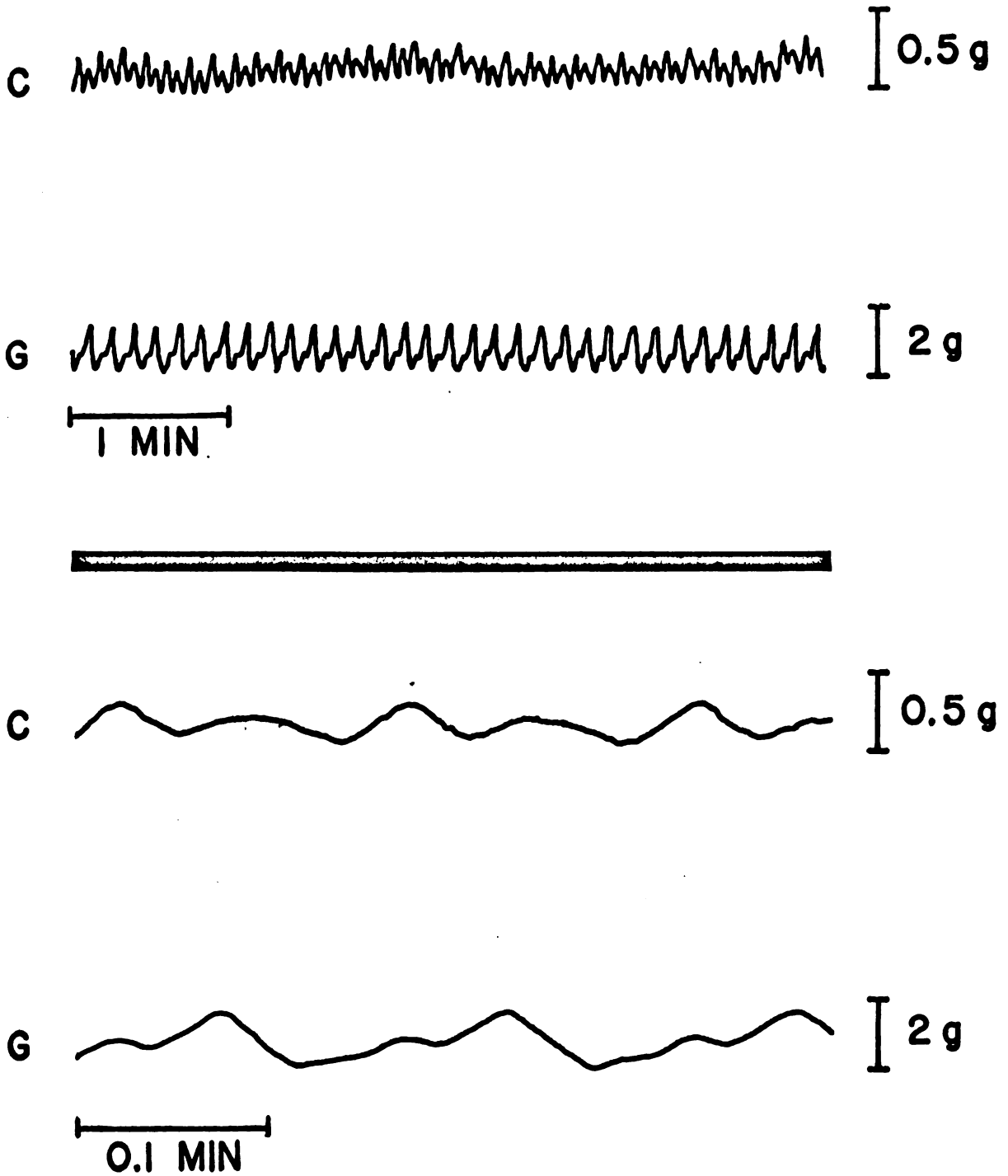
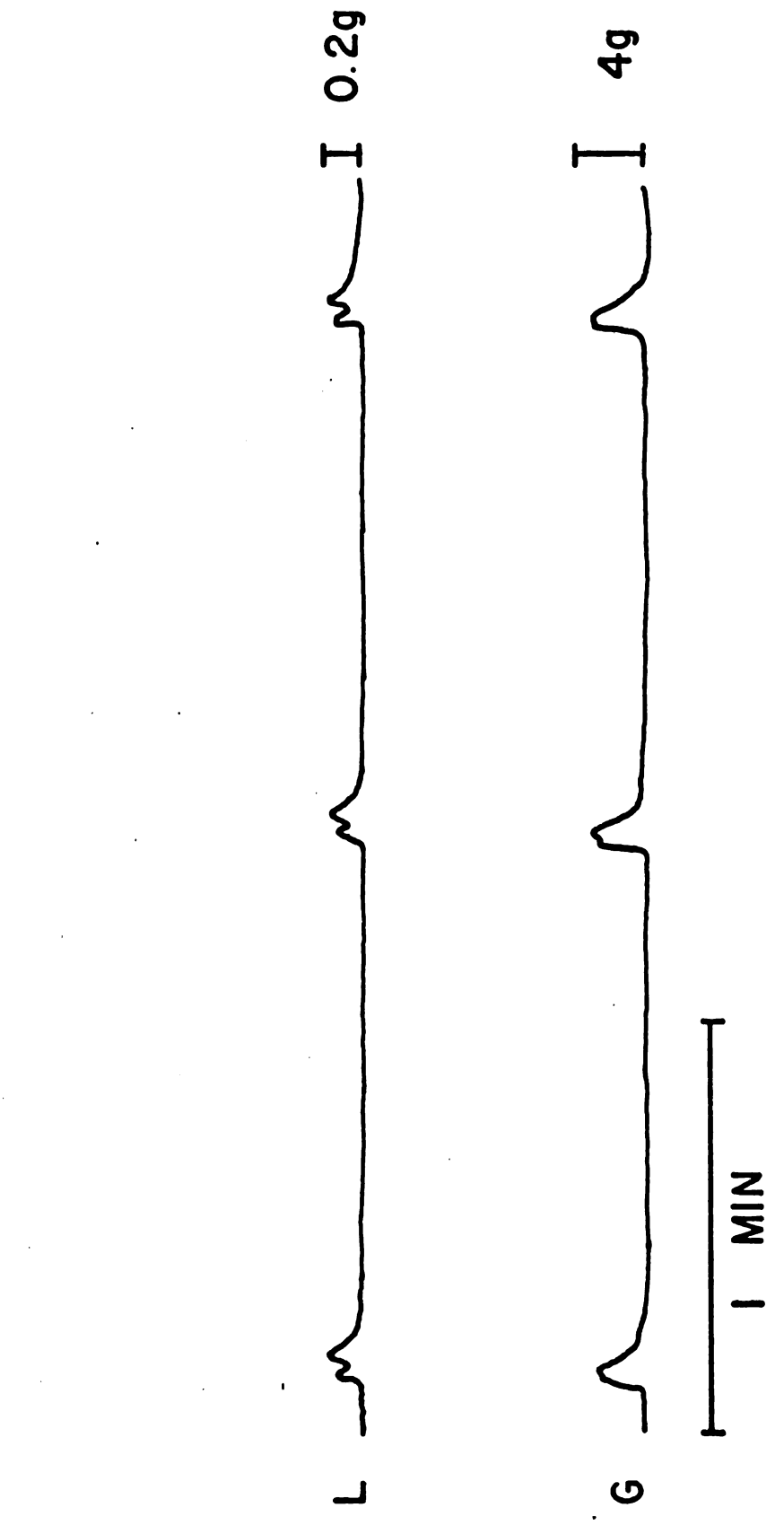

C
0.5 g
G
2 g
1 MIN
C
0.5 g
G
2 g
0.1 MIN

Figure 8. Comparison of contractile activity recorded by a longitudinally oriented extraluminal transducer and a Grass FT-03 transducer under direct tension

This tracing shows a recording of the contractile activity of a muscle segment recorded by the longitudinally oriented extraluminal transducer and a Grass FT-03 transducer. The transducers were arranged in the 2D configuration. Paper speed--0.1 cm./sec.

L--contractile activity recorded by the longitudinally oriented extraluminal transducer.

G--contractile activity recorded by the Grass FT-03 transducer.


0.2g
4g
L
G
1 MIN

2. The Grass FT-03 transducer showed a contraction peak while the longitudinally oriented extraluminal transducer showed an initial contraction peak, and then a slight relaxation followed by another contraction peak.

The second contractile activity pattern recorded by the extraluminal transducers and the Grass FT-03 transducer occurred when the 3HTI configuration was used. Tracings of recordings from this configuration are shown in Figures 9 and 10.

These two figures show the following results:

1. The characteristics of this contractile activity of the circular muscle layer does not differ from that obtained by the 3VTD or 2D configurations.

2. The contractile activity of the longitudinal muscle layer differs greatly from that obtained when the 3VTD or 2D configuration was used.

   a. The contractile activity of the longitudinal muscle shows a decreased tone with superimposed contractions. Close examination shows that this contractile activity approximates a mirror image of the contractile activity recorded by the Grass FT-03 transducer.

   b. Figure 10 (the polarity of the recording pen for the longitudinally oriented extraluminal transducer was reversed) shows that the contractile activity recorded by the longitudinally oriented extraluminal transducer is a mirror image of the contractile activity recorded by the Grass FT-03 transducer. Superimposed on this passive relaxation are contractions of the longitudinal muscle located under the extraluminal transducer.

Frequency of Contractile Activity

The frequency of bursts of contractile activity (number of bursts/minute) for both muscle layers in the isolated estrogen primed rabbit myometrium did not differ

Figure 9. Spontaneous contractile activity patterns obtained under indirect tension

The approximate "mirror image" recording by the longitudinally oriented extraluminal transducer of the contractile activity recorded by the Grass transducer is shown in this tracing. The Grass FT-03 transducer was recording from the mesometrial side of the muscle segment while the longitudinally oriented transducer was recording from the antimesometrial side. Similar recordings from the circular muscle layer were obtained regardless of the position of the circularly oriented transducer. The transducers were arranged in the 3HTI configuration. Paper speed--0.1 cm./sec.

C--contractile activity recorded by the circularly oriented extraluminal transducer.
L--contractile activity recorded by the lingitudinally oriented extraluminal transducer.
G--contractile activity recorded by the Grass FT-03 transducer.

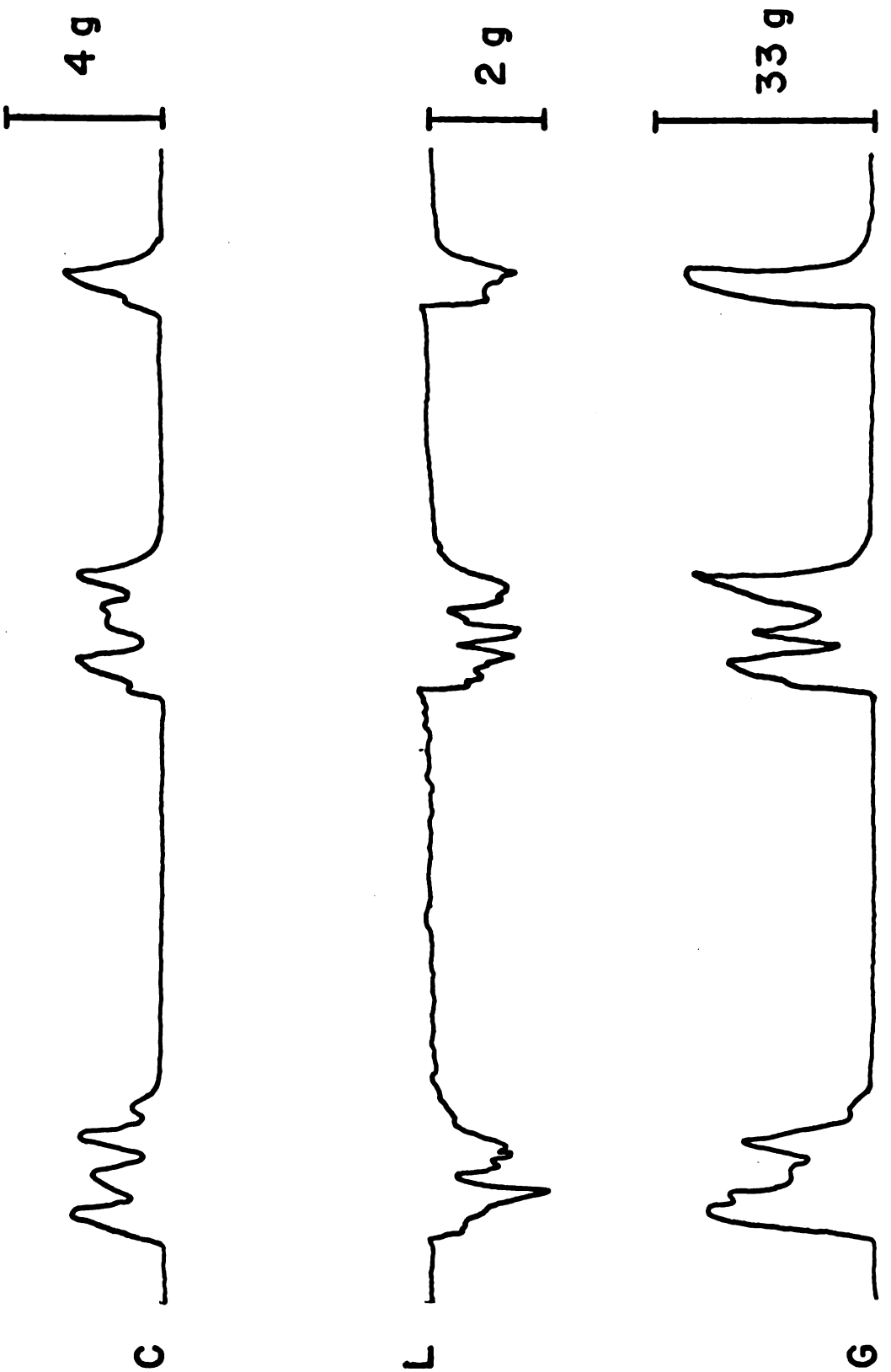
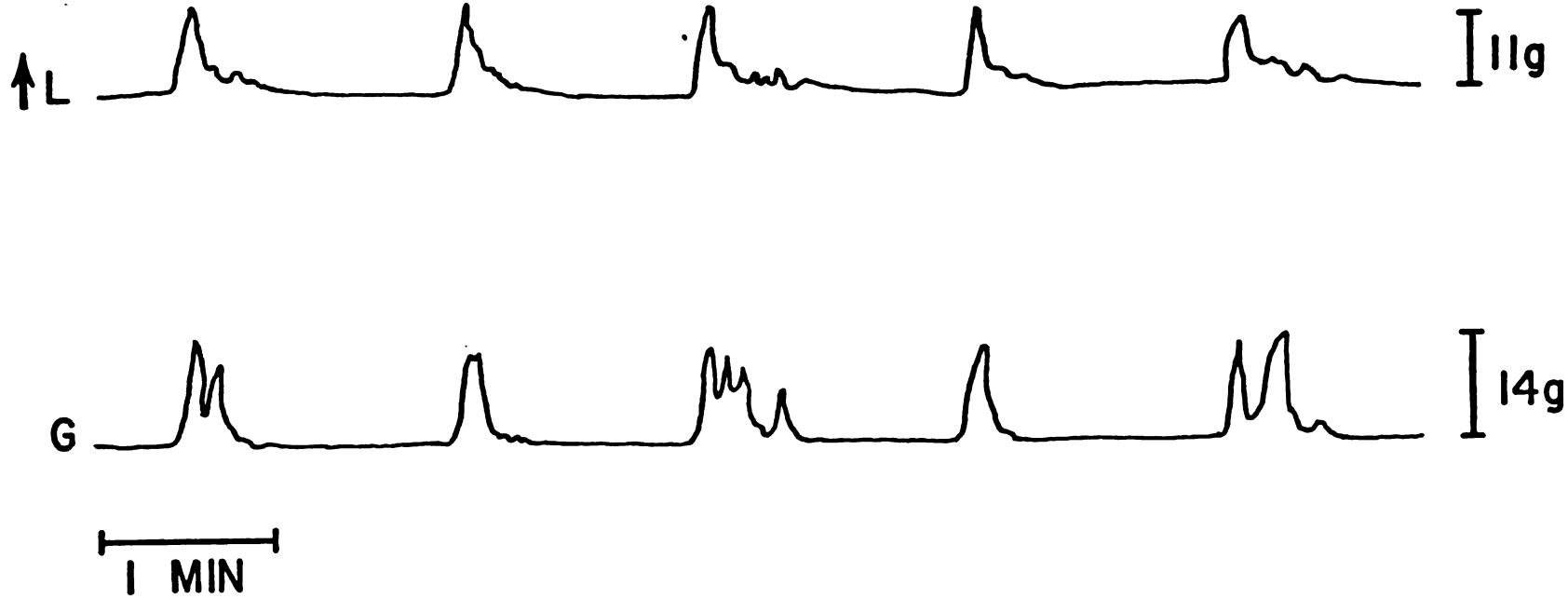

4g
2g
33g
C
L
G
1 MIN

Figure 10. "Mirror image" recording by the longitudinally oriented extraluminal transducer

The polarity of the pen recording the contractile activity monitored by the longitudinally oriented extraluminal transducer has been reversed. An upward deflection of the pen in the upper tracing indicates relaxation, while an upward deflection of the pen in the lower tracing indicates contraction. The extraluminal transducer was monitoring contractile activity of the longitudinal muscle layer on the mesometrial side, while the Grass FT-03 transducer was recording contractile activity from the antimesometrial side. Transducer configuration was 3HTI. Paper speed--25mm./min.

L--contractile activity recorded by the longitudinally oriented extraluminal transducer (reversed polarity).
G--contractile activity recorded by the Grass FT-03 transducer.


L
11g
G
14g
1 MIN

for any one segment. A burst is defined as an increase in contractile amplitude consisting of one or more contractions which may be superimposed on a tone increase. The range of frequencies of bursts for all rabbits tested was from 0.18 to 1.08/min., with an average frequency of 0.54 bursts/minute.

In those experiments which were designed to determine whether the presence of the extraluminal transducers affected frequency, no statistically significant difference in frequencies was found between the segments with attached transducers and the control segments. Data used in this comparison are shown in Table 1.

Force of Contractile Activity

The average force developed by the uterine muscle layers during spontaneous activity was determined for all segments with transducers arranged in the 3VTD or 3HTI configuration. The average contractile force, per burst as measured by the Grass FT-03 transducers, was 12.8 g. To determine whether the presence of the extraluminal transducers affected contractile force developed, a paired comparison was made between the force developed by the muscle segment with the attached extraluminal transducers and the control muscle segment without extraluminal transducers attached. No statistically significant difference was found, as shown in Table 2.

A paired comparison between the average contractile force per burst developed by the circular and longitudinal

Table 1. Comparison of the frequencies* of the experimental** and control muscle segments

Rabbit	Control X_1	Experimental X_2	D = $X_1 - S_2$	D^2
1	0.28	0.18	0.10	0.0100
2	0.42	0.42	0.00	0.0000
3	0.48	0.25	0.23	0.0529
4	1.0	1.08	-0.08	0.0064
5	0.83	0.33	0.50	0.2500
6	0.22	0.21	0.01	0.0001
7	1.0	0.44	0.66	0.4356
8	0.71	0.90	-0.19	0.0361
Total	4.94	3.81	1.23	0.7911

*Bursts/minute.

**The transducers were arranged in the 3VTD or 3HTI configuration.

$\overline{X}_D = 0.1537$; $\sigma_D = 0.2932$; $\sigma_{\overline{X}_D} = 0.1036$

$T = 1.48$

$p > 0.10$--At this level these differences in frequencies are not statistically significant.

Table 2. Comparison of contractile force* developed by the experimental** and control muscle segments as monitored by Grass FT-03 transducers

Rabbit	Control X_1	Experimental X_2	$D = X_1 - X_2$	D^2
1	9.5	18.0	-8.5	72.25
2	18.4	18.0	0.4	0.16
3	20.0	9.5	10.5	110.25
4	11.0	15.0	-4.0	16.0
5	5.5	6.2	-0.7	0.49
6	16.0	7.0	9.0	81.00
Total	80.4	73.7	6.7	280.05

*Grams; the summation of maximum amplitude of contractile activity of bursts divided by number of bursts.

**Transducers were arranged in the 3VTD or 3HTI configuration.

$\overline{X}_D = 1.11$; $\sigma_D = 7.38$; $\sigma_{\overline{X}_D} = 3.01$

$T = 0.368$

$p > 0.05$--At this level these differences in contractile force are not significant.

muscle layers indicated that there was no statistically significant difference (see Table 3).

Effects of Drug Stimulation on Contractile Activity

To establish further the differentiation between the contractile activity of the longitudinal and circular muscle layers, drugs were added to the *in vitro* baths. Results from the longitudinal and circular muscle layers of the same segment were compared at the same time.

Acetylcholine chloride, in a final bath concentration of 3.0×10^{-6} molar of base, caused a sustained increase in tone in the circular muscle layer which surpassed any tone measured in the control activity. The contractile amplitude was greater than any amplitude recorded in the control bursts. The longitudinal muscle did not show an increase in tone. There appeared to be numerous contractions superimposed on the decreased tone; the amplitude of these multiple contractions did not appear to differ from the control. A tracing from a recording of this drug's action on the muscle is shown in Figure 11-A.

Contractile activity, monitored by both the Grass FT-03 and the circularly oriented extraluminal transducers, in response to vasopressin in a bath concentration of 6.2×10^{-2} units/ml., was similar to the responses described previously to acetylcholine administration, although vasopressin allowed less complete relaxation initially. This response was recorded from the same muscle

Table 3. Comparison of contractile force* developed by the longitudinal and circular muscle layers of the the same muscle segment as monitored by extraluminal transducers

Rabbit	Circular muscle X_1	Longitudinal muscle X_2	$D = X_1 - X_2$	D^2
1	0.50	1.0	-0.50	0.2500
2	0.76	0.24	0.52	0.2704
3	1.50	1.25	0.25	0.0625
4	1.40	0.25	1.15	1.3225
5	0.35	0.10	0.25	0.0625
Total	4.51	2.84	1.67	1.9679

*Grams; the summation of the maximum amplitude of contractile activity of bursts divided by number of bursts.

$\bar{X}_D = 0.335$; $\sigma_D = 0.373$; $\sigma_{\bar{X}_D} = 0.166$

$T = 2.01$

$p > 0.10$--At this level these differences in contractile force are not significant.

Figure 11. Contractile responses to acetylcholine and vasopressin

A. This tracing of a recording shows the contractile responses of the muscle layers to acetylcholine (3.0×10^{-6} molar bath concentration). The circular muscle responded with a sustained increase in tone and greater contractile amplitude than any control contractile amplitude. The longitudinal muscle appeared to show an increased frequency of contractions superimposed on decreased tone.

B. This tendency from the same recording as the tracing described above shows the contractile response of the muscle layers to vasopressin (6.2×10^{-2} units/ml. bath concentration). The longitudinal muscle shows an increase in tone with increased frequency of contractile activity.

C--contractile activity as measured by the circularly oriented extraluminal transducer.

L--contractile activity as measured by the longitudinally oriented extraluminal transducer.

G--contractile activity as measured by the Grass FT-03 transducer.

Arrow indicates drug delivery.

segment as the previous description for response to acetylcholine. However, the contractile response of the longitudinal muscle layer was markedly different from that response recorded after the administration of acetylcholine. The longitudinally oriented extraluminal transducer measured contractile activity which coincided with that recorded by the Grass FT-03 and the extraluminal transducer. A tracing of this recording is shown in Figure 11-B.

Oxytocin in a bath concentration of 6.2×10^{-3} units/ml. elicited a similar response as that recorded from the longitudinal muscle after acetylcholine administration. Following initial tone increase in the circular muscle, more rhythmic, higher amplitude contractions were recorded as opposed to the control bursts recorded from this muscle layer. A tracing of the response of the muscle segment to this drug is shown in Figure 12.

Figure 12. Contractile response to oxytocin

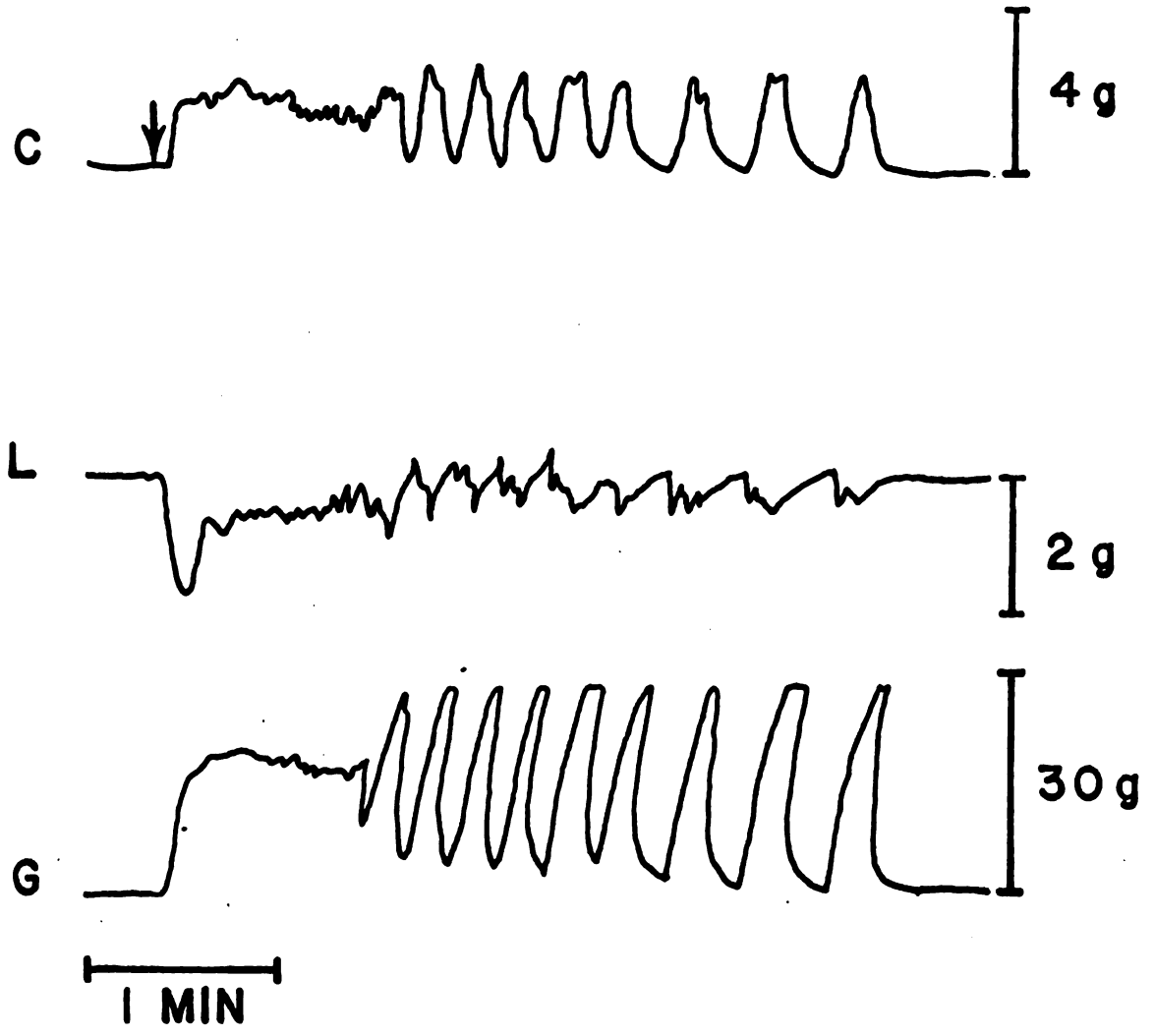
This tracing of a recording shows the contractile response of the muscle layers to oxytocin (bath concentration of 6.2×10^{-2} units/ml.). The circular muscle layer shows a sustained increase in tone followed by higher amplitude, more rhythmic concentrations than obtained in the control bursts. The longitudinal muscle layer shows a response similar to that obtained from the addition of acetylcholine to the bath--increased frequency of contractions superimposed on decreased tone.

C--contractile activity as measured by the circularly oriented extraluminal transducer.

L--contractile activity as measured by the longitudinally oriented extraluminal transducer.

G--contractile activity as measured by the Grass FT-03 transducer.

Arrow indicates drug delivery.


C
4 g
L
2 g
G
30 g
1 MIN

CHAPTER FOUR

DISCUSSION

Contractile Activity Patterns

Contractile activity patterns for the estrous stage of the ovarian cycle have been described for many species (4,9,18,34). This contractile activity is generally described as rhythmic, slow, and of greater amplitude than contractile activity found in a different stage in the cycle.

Reynolds (35) describes the motility of the intact uterus during estrus as being initiated by a pacemaker located in the isthmus of the Fallopian tube. These contractions begin in the circular fibers at intervals of 7-15 seconds; they cause first a lengthening of the tube, followed by a shortening which is due to contraction of the longitudinal fibers. These contraction waves spread to the utero-tubal junction where only every third or fourth contraction continues into the horn.

The above description pertains to the rabbit Fallopian tube. The results obtained in the present *in vitro* study monitoring contractile activity of the circular and longitudinal muscle layers of the rabbit uterine horn support the view that the two muscle layers contract simultaneously. Contraction of the circular fibers was recorded

by the circularly oriented extraluminal transducer and may have been recorded by the Grass FT-03 transducer. A simultaneous contraction of the longitudinal fibers was recorded by the Grass FT-03 transducer and the longitudinally oriented extraluminal transducer, if the tension set at the time of attaching the extraluminal transducer was minimal. If the tension set in attaching the extraluminal transducer was great, it recorded an apparent decrease in tone when the adjacent muscle contracted. Figure 6 illustrates the longitudinal muscle contractile activity with setting tension high, while Figure 8 shows it with setting tension low. With regard to smooth muscle layer contractile activity in the gastrointestinal tract, Reinke, et al. (30) state that in burst activity in the small intestine, the longitudinal muscle contracted before the circular muscle layer.

Frequency of Contractile Activity Patterns

Previous investigators have characterized estrous contractile activity as exhibiting one contraction every minute up to one contraction every four or five minutes (9,18,28,34). This quantification, when converted to frequency, gives an average frequency for estrous contractile activity which approximates that obtained from this investigation (0.55 bursts/minute). This rate of spontaneous activity has been attributed to the myogenic property of

uterine muscle fibers, which is modified by the dominant hormone, ionic concentrations, amount of actomyosin, and presence of calcium (17). The characteristic of this myogenic property is related to the resting membrane potential, which is under the control of hormones (17). Estrogen has been shown to control the synthesis of the contractile protein, actomyosin, and to raise the resting membrane potential to the critical level, that level at which rhythmic, spontaneous contractile activity occurs (12,13,15,17).

The role of the autonomic nervous system in the generation of spontaneous contractile activity has been considered (34,44). The presence of intrinsic innervation in the myometrium was debated; positive identification of its presence has been made (29), although exact morphology and extent is unknown. Recent in vivo and in vitro experiments on dogs, which involved nerve sections, electrical and pharmacological nerve stimulation, and acetycholine depletion were designed to discover other possible mechanisms which could contribute to spontaneous contractile activity (44). The investigators concluded from the results of their experiments that the cholinergic neurohormone, acetylcholine, was responsible for the generation and rhythmicity of contractile activity. It is possible that the intrinsic nervous system is acting in conjunction with the myogenic property of uterine smooth muscle cells in vitro.

This study showed that bursts of contractile activity from the longitudinal and circular muscle layers occur simultaneously. It is not known whether the cells in the longitudinal muscle layer and the circular muscle layer are alike. It is possible that the outer longitudinal muscle of the uterus is analogous to that in the small intestine and that it generates a BER (slow wave) which controls the rate at which the circular muscle layer contracts.

Effects of Drug Stimulation

A recent hypothesis set forth by Shabanah, Toth, and Maughan (44), suggests that the intrinsic nerve supply in the "autonomous" (excised) uterus consisting of postganglionic parasympathetic fibers and their endogenous acetylcholine, sets the pace for spontaneous contractility by acting on the longitudinal muscle fiber membranes. In this study acetylcholine appeared to differentially affect the longitudinal and circular muscle layers. The increase in frequency of contraction recorded from the high tension longitudinally oriented transducer following acetylcholine administration was likely due to increased tension in the muscle beneath this transducer resulting from: (1) contraction of adjacent longitudinal muscle above and below the extraluminal transducer, and (2) the direction action of acetylcholine on the muscle beneath the extraluminal transducer. The recorded apparent tone decrease was likely due

to the predominance of the adjacent longitudinal muscle contractile activity. Bortoff (6) used the cat small intestine *in vitro* to show that longitudinal muscle was stimulated to contract by small doses (2×10^{-9} molar) of acetylcholine while the circular muscle did not contract. Higher concentrations elicited contractions from both muscles. Burnstock and Holman (7) state that drugs which change the pattern of contractile activity in isolated preparations are probably acting directly on the smooth muscle membrane, which would indicate a mechanism for its action on the longitudinal muscle. Oxytocin elicited a response similar to that recorded after acetylcholine. Burnstock and Holman (7) state that oxytocin has an action similar to acetylcholine on smooth muscle.

In contrast to the longitudinal muscle response to acetylcholine, the vasopressin frequency and amplitude stimulation was superimposed on a sustained increase in tone.

The *in vitro* method developed for this study and the results obtained by it indicate that the extraluminal contractile force transducer is a reliable isometric *in vitro* method if adequate attention is given to the amount of tension applied at the time of attachment of the transducer to the muscle. The influence of this setting tension is more apparent in the longitudinal muscle layer due to the greater amount of adjacent muscle tissue.

Thus there is a possibility that the longitudinally oriented extraluminal contractile force transducer may record an apparent decrease in tone.

CHAPTER FIVE

SUMMARY

The extraluminal contractile force transducer has been modified for use *in vitro* by changing the orientation of the lead wires to facilitate immersion in the bath, and by fabricating with waterproofing materials which reduced the size of the transducer from that previously used *in vivo*.

1. Although a contraction of the circular muscle layer occurs in the vertically oriented segment, the contribution made by the circular muscle to the contraction recorded by the Grass FT-03 transducer cannot be quantified.

2. The contribution of the longitudinal muscle layer as recorded by the longitudinally oriented extraluminal transducer to the contraction recorded by the Grass FT-03 transducer depended on the degree of tension set at the time of attachment. If the force of contractile activity of the longitudinal muscle adjacent to the extraluminal transducer at each end was greater than the contractile force of the longitudinal fibers under the transducer, the longitudinally oriented transducer recorded apparent decrease in tone.

3. The presence of the extraluminal transducers on the muscle segment did not affect frequency or force of contractile activity, as shown statistically by paired comparisons.

4. The extraluminal transducers allowed recording of the contractile activity of the longitudinal and circular muscle layers separately and simultaneously as shown by the addition of drugs to the *in vitro* bath.

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