THESIS

ABSTRACT

CONTRACTILE ACTIVITY OF THE RABBIT UTERUS
MONITORED IN VIVO WITH EXTRALUMINAL
FORCE TRANSDUCERS

by Jerome A. Dominic

The purpose of this study was to adapt the extraluminal strain gage transducer for the chronic recording of uterine contractile activity in the intact, unanesthetized rabbit during estrus and following ovulation. Modifying an existing method (Reinke _et al_., _Amer. J. Dig. Dis_. 12:113, 1967) "T" transducers were fabricated, calibrated, sterilized and sutured, under methoxyflurane anesthesia, in either a longitudinal or transverse axis along the extraluminal ventral aspect of one uterine horn. The lead wires, encased in silicone rubber tubing, were brought through the abdominal wall, then subcutaneously to a connector plug in the mid-scapular skin. Upon recovery, local and propagated contractile activity was monitored in the unanesthetized rabbit.

During estrus, the uterus exhibited high amplitude, rhythmic, coordinated contractile activity. Variations in contractile amplitude and frequency were common during estrus and changes in amplitude were inversely related to frequency. Evidence was presented in support of the proposal that a gradient of contractile activity exists in the uterus.

Several differences were found to exist between the circular and longitudinal muscle layers during estrus: (1) The average maximal contractile amplitude was greater for longitudinal muscle. (2) Contractions of the longitudinal layer were conducted at a more rapid rate. (3) The longitudinal muscle frequently exhibited a pattern of contraction bursts while single contractions were more characteristic of circular muscle.

During the initiation and termination of pseudopregnancy contractile activity patterns were similar and consisted of low amplitude, high frequency contractions of an irregular and uncoordinated nature. During the intervening period the uterus was relatively quiescent but was more active than during a similar period of pregnancy.

The immediate post-ovulatory contractile activity of pregnancy (one day post coitus), in contrast to that of pseudopregnancy, resembled that of estrus, but thereafter the activity declined rapidly and the uterus remained essentially inactive until several days before delivery.

As expected, during estrus, oxytocin increased tone, contractile amplitude and frequency, but elicited no response during pregnancy.

The extraluminal strain gage transducer was shown to be a satisfactory method for studying in vivo contractile activity of both the longitudinal and circular muscle layers of the rabbit uterus.

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MONITORED IN VIVO WITH EXTRALUMINAL
FORCE TRANSDUCERS

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The nature of uterine contractile activity associated with the estrous cycle in animals has been extensively investigated. A review of the recording methods will show that although they have provided an understanding of the basic physiological control of the uterus, they have been unsatisfactory for studying the contractile activity patterns associated with parturition, spermatozoa and ova transport and blastocyst implantation. These studies require a method that will permit the chronic extraluminal recording of uterine contractile activity in the intact, unanesthetized animal.

Review of Methods

Dale (1907) recorded contractile activity in the anesthetized cat by attaching a thread to both uterine horns and then passing it overhead on a pulley which was connected to an inscribing lever (18). In these earliest acute animal studies he found that the response of the uterus to drugs differed during pregnancy and non-pregnancy. In the same year Kehrer (as described by Corner and Csapo, 10) pioneered *in vitro* studies of uterine contractile activity. Rabbit uterine strips immersed in warm physiological salt solution were attached to a lever which recorded isotonic contractions on a kymograph. Unaware of the cyclic nature of uterine motility, Kehrer reported that the adult uterus was often less active and less predictable in its response to drugs than was the virgin uterus. Using Kehrer's in vitro method, other investigators as reviewed by Corner and Csapo (10) reported the absence of spontaneous contractile activity in the ovariectomized rabbit and guinea pig.

The cyclic nature of uterine motility was first defined by Keye in 1923 (26). He characterized the in vitro uterine contractile patterns of the twenty one day estrous cycle in the domestic sow. Prior to ovulation the contractions were of high amplitude (units unspecified) and low frequency (1.5-2 min. apart). Ovulation then occurred spontaneously and the contractions gradually diminished in amplitude and increased in frequency until at about ten days after ovulation the rate was six to eight per minute and the amplitude was one tenth of that observed prior to ovulation. Estrogen is now known to be the dominant hormone during the preovulatory follicular phase of the estrous cycle and progesterone dominates the two week corpora lutea phase (3). In the same year Blair (7) reported somewhat similar results with rat uteri in vitro. The mean contraction rate was lower during estrus than during the diestrus interval. Knaus (27) found high amplitude, low frequency contractions in vitro in the estrous rabbit. The characteristic post-ovulatory pattern resembled that reported by Keye for the domestic sow.

Reynolds in 1930 introduced a technique for chronic in vivo recording of intra-uterine pressure changes (31). A compressible rubber balloon (1.2 cm. maximum diameter and 2 cm. long) was inserted through a previously prepared vaginal fistula into the lumen of a rabbit uterine horn. The balloon was filled with water from a reservoir which was connected by an air column to a bellows attached to a writing lever. In these initial chronic experiments, the unanesthetized rabbit was tied to a board in a supine position for the 30 minute recording period. Reynolds recorded two types of intraluminal pressure patterns in the non-pregnant rabbit. Both high amplitude rhythmic contractions and low amplitude irregular activity were observed. He suggested that the variations were due to changes in the estrous cycle.

In 1930 crude ovarian extracts were prepared and the technique of replacement therapy in ovariectomized animals provided significant information regarding the role of the ovary in uterine motility. Reynolds (33) showed that the in vivo spontaneous motility abolished by ovariectomy was quickly restored by ovarian extracts and resembled the high amplitude rhythmic activity found in the estrous rabbit. Subsequent experiments (37) revealed that daily subcutaneous injections of corpora lutea extract significantly reduced or abolished postpartum estrous contractile activity within three days. Two to five days after withdrawal of the extract, estrous motility returned. Similar effects with pure crystalline progesterone were reported by Allen and Reynolds (1) for the estrogen treated ovariectomized rabbit.

Robson (41) in 1937 introduced an _in situ_ method for acute studies of intra-uterine pressure changes. Under ether anesthesia a rabbit uterine horn was cannulated through the vagina and a segment of the horn tied off and filled with saline. With the rabbit still anesthetized changes in intra-uterine pressure were recorded via an air tambour attached to a kymograph. Robson, too, found that progesterone diminished the spontaneous motility of an estrogen treated ovariectomized rabbit.

According to Bell (6) the contemporary recording techniques did not accurately monitor uterine contractile activity. He contended that the tension imposed _in vitro_ or the distension _in vivo_ distorted the true motility and presented evidence that the "unloaded" (free from any artificial tension) rabbit uterus was more active under the influence of progesterone. He inserted, under anesthesia, silver wire sutures along the uterine border of the mesometrium a short distance from the uterus. X-rays of the abdomen of the unanesthetized rabbit were taken at half minute intervals. The distances between the shadows of the stitches were measured and the sum of the distances taken as the total length of the uterus. The amount of activity was determined by variations in total length and was found to be greater when progesterone was the dominant hormone.

In contrast to Bell, most investigators have found that the high amplitude of the low frequency contractile activity of the rabbit uterus during estrus was diminished by progesterone both in vivo and in vitro.

Csapo and Corner (15) investigated the cellular mechanism of action of progesterone and observed different responses of the in vitro rabbit uterus to electrical stimulation. Following control stimulation (24 volts at 60 cps for 5 seconds), the estrogen dominated uterus responded to an increase in stimulation frequency with increases in contractile frequency and amplitude with no accompanying increase in tone (a "positive staircase" effect). The progesterone dominated uterus showed an increase in contractile frequency but a decrease in amplitude with no change in tone (a "negative staircase" effect). Schofield duplicated these results in situ (44, 45) with acute experiments on anesthetized rabbits. In her studies, a Plexiglas stimulating chamber was attached to the uterus and a hook passed through the center of the chamber and around a fixed segment of the uterine horn. The hook was attached by a thread to a lever which recorded contractions on a kymograph.

Chronic recordings of intra-uterine pressure changes in the unanesthetized rabbit were first carried out by Csapo et al. (17) in 1963. Under anesthesia one uterine horn of a parturient rabbit was emptied of fetuses and a balloon inserted into the horn through an incision in the ovarian end. The balloon was attached to polyethylene tubing which was passed subcutaneously to exit in the scapular area. The tubing extended through the top of the cage and was connected to a pressure transducer and intra-uterine pressure changes were recorded intermittently for several weeks. During the recording periods the rabbit was allowed to move freely in her cage. The amplitude of the pressure wave was maximal (35 mm. Hg.), when the volume of the water filled balloon was 70 ml., while little or no activity was recorded at a volume of 10 ml. or less. Fuchs (20,21) used the same method and recorded intra-uterine pressure changes before and during parturition in the rabbit. The balloon (the same volume as a fetus 3-4 days before term) was inserted into a horn emptied of fetuses. Although Fuchs had difficulty in distinguishing variations in abdominal pressure from intra-uterine pressure changes, she reported that the estrous motility pattern did not appear prior to parturition.

Use of the intra-uterine balloon has been a popular method for recording myometrial activity in humans (25). More recently recordings of intra-uterine pressure changes have been made with fluid filled open tip catheters inserted through the vagina. Hendricks (23) inserted three catheters of different lengths which permitted the recording of pressure changes in separate segments of the uterus. He found that post-ovulatory pressure waves were of higher amplitude and slower frequency than those observed prior to ovulation.

Bangham *et al*. (4) have used radio telemetry to monitor intra-uterine pressure changes in the rhesus monkey. Silicone rubber coated pressure-sensitive radio transmitters (1.5 cm. in diameter and 2.8 cm. long) were inserted into the uterine cavity. Variations in intra-uterine pressure resulted in frequency changes of the transmitter signal and were monitored by a radio receiver. They reported that the pregnant monkey uterus was generally quiescent except for a period between weeks 14 and 18 when spontaneous intra-uterine pressure changes were recorded.

Limitations of Existing Methods

The mechanical influence of *in situ* recording devices has been shown to induce spontaneous myometrial activity of variable amplitude and frequency. Setekleiv (47) using a method similar to Robson's (40) found that distension of a cannulated segment of an estrogen treated ovariectomized rabbit uterus with a 1 ml. Tyrode's solution induced rhythmic pressure waves of 15 cm. of H_2O every 1.5 min. As the volume of distending fluid was increased by 1 ml. increments to 7 ml. the amplitude increased to 35 cm. of H_2O then decreased. The frequency gradually increased over the entire range of distending volumes to a rate of 7 per minute. In addition to distension, the effect of acute surgical procedures and anesthesia alter *in situ* uterine contractile activity.

The *in situ* methods have demonstrated some of the properties and response characteristics of uterine smooth muscle but the methods previously discussed are not satisfactory for recording cyclic patterns of motility in chronic studies.

The introduction of a balloon into the uterine cavity for *in vivo* studies has produced several controversies. There seems to be no agreement regarding the proper size for the intra-uterine balloon and the level of distending pressure. Reynolds (31) showed that during estrus (but not pseudopregnancy) an increase in the intra-uterine balloon pressure resulted in an increase in amplitude of the recorded pressure wave. Cross (12) found similar changes in estrogen treated ovariectomized rabbits and also reported variable frequency responses to increased distension in two uterine horns of the same animal. The relationship between uterine volume and the amplitude of the pressure wave reported by Csapo *et al*. has been discussed (17). It is evident then that *in vivo* recording with the intra-uterine balloon does not accurately quantify uterine contractile activity.

Variations in intra-abdominal pressure have obscured the recording of true uterine motility by the balloon. In addition the balloon records net pressure changes within the uterine cavity and cannot monitor the tension exerted separately by the circular and longitudinal muscle layers in localized areas of the uterus.

Finally, the presence of the balloon *in utero* precludes the accurate recording of discrete contractile activity during parturition, spermatazoa and ova transport and blastocyst implantation.

Summarizing, the use of the intra-uterine balloon is unsatisfactory due to: (1) The apparent induction of spontaneous contractile activity by the balloon. (2) Recording artifacts created by intra-abdominal pressure changes. (3) The inability to monitor discrete contractile activity of the longitudinal and circular muscle layers. (4) The obstruction created by the presence of the balloon.

The Extraluminal Strain Gage Transducer

Jacoby *et al*. (24) have shown that the extraluminal strain gage transducer reliably monitors *in vivo* contractile activity of the duodenum in chronic studies on the unanesthetized dog. The transducer consisted of a strain gage bonded to the convex surface of an arched metal clip. Lead wires were attached and the unit was encapsulated with Silastic silicone rubber. The contractile force developed by the muscle beneath the two ends of the transducer resulted in a linear deformation along the longitudinal axis of the metal clip and a change in the electrical resistance of the strain gage. Two transducers were sutured to the serosa of the duodenum and depending on the axis of orientation, they monitored contractile activity of the circular and longitudinal muscle layers separately and simultaneously at the same level. Reinke *et al*. (30) increased the sensitivity of the transducer by bonding a second strain gage to the concave surface of the metal clip, changed the configuration (the lead wires were brought in at a right angle to the longitudinal axis of the strain gage) to "better approximate the transverse curvature of the gut," and investigated the patterns of dog gastrointestinal (stomach, duodenum, jejunum, ileum and colon) contractile activity. Bass and Callantine (5) implanted combination electrode-transducer units on the serosal surface of the dog uterus and monitored electrical and mechanical activity simultaneously in the unanesthetized dog. They were able to record for several months in the same animal but did not characterize any patterns of contractile activity. Feit and Freund (19) used the transducer to record uterine contractile activity in the intact, unanesthetized guinea pig. They reported that the force of contraction was greatest during estrus, but no relationship between frequency of contractile activity and the stage of the estrous cycle was apparent. Callantine *et al*. (8), have used the extraluminal strain gage transducer for *in vivo* drug studies on the rabbit uterus. The transducers were sutured to the serosal surface overlying placental sites on the twentieth day of pregnancy. In this manner control estrous motility of the post-partum rabbit was obtained.

Statement of the Problem

The purpose of this study is to investigate the patterns and parameters of *in vivo* rabbit uterine contractile activity during estrus, pregnancy and pseudopregnancy. It will attempt to show variations in contractile activity during estrus and compare the post-ovulatory contractile patterns of pregnancy and pseudopregnancy.

METHODS

Transducer Fabrication

Transducer fabrication is essentially the same as that described by Reinke *et al*. (30). Two etched-foil 350 ohm strain gages (No. SK-09-031-DE-350)[1] were bonded one to the convex and the other to the concave surface of a cleaned heat-treated 2.5% beryllium-copper shim stock (0.15 mm. thickness, 2 mm. width, 5.5 mm. length). The strain gages were bonded with GA-5, a filled epoxy cement,[2] and cured under pressure (approx. 1 psi) at 220°F for two hours. The lead wires (Teflon insulated, 36 gauge, 7 stranded, 3 conductor)[3] were soldered to the soldering tabs of the strain gages with special strain gage solder (Fig. 1). The transducer was waterproofed with a nitrile rubber solution (Gagekote No. 2).[4] The lead wires were drawn through a 16 in. length of medical grade Silastic silicone rubber tubing (0.062 in. I.D., 0.095 in. O.D.).[5] The transducer and initial soldered portions of the lead wires were encapsulated

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[1]Micro-Measurements, Inc., Romulus, Michigan.

[2]The Budd Company, Philadelphia, Pennsylvania.

[3]William T. Bean, Inc., Detroit, Michigan.

[4]*Ibid*.

[5]V. Mueller Co., Chicago, Illinois.

Figure 1.--Extraluminal contractile force transducer. Diagrammatic illustration of the transducer. A second strain gage is bonded to the concave surface of the metal shim stock. The unit is encapsulated with Silastic silicone rubber.

with Dacron mesh reinforced raw Silastic[6] (Fig. 1) and vulcanized under pressure (clamped in a vice) at 220°F for 24 hours.

The lead wires were soldered to an electrical connector plug (Cannon MD1-9SL1)[7] which was embedded in a mold fabricated from a plastic adhesive.[8] The bottom of the plug, along with the Silastic tubing ends, was covered with a medical grade Silastic silicone adhesive (Type A)[9] and a sheet of raw Silastic. The entire unit was cured for 24 hours at 220°F.

Transducer Circuit

The transducer, which contained the two active arms of a Wheatstone bridge circuit, was connected to the strain gage coupler (via a connector plug and the two 350 ohm inactive bridge arms) and chopper preamplifier of an ink-writing oscillograph (Dynograph Type R).[10] The DC activation voltage was five volts.

Transducer Calibration

The transducer was supported on a small rod (Fig. 2) and Mersilene 3-0 suture material (Ethicon R-552) attached to each end of the transducer. Each suture was passed over

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[6] Ibid.

[7] Radio Distributing Co. Inc., South Bend, Indiana.

[8] The Door Company, Houston, Texas.

[9] V. Mueller Co., Chicago, Illinois.

[10] Spinco Division, Beckman Instruments, Lincolnwood, Illinois.

Figure 2.--Transducer calibration. The transducer was secured to a supporting rod (sr). A suture was attached to each end and was led over the opposite pulley (p). Equal weights were attached to each suture and pen deflections obtained.

an opposite pulley. Weights were added to the free end of the Mersilene and pen deflections obtained for a 2 gm.-40 gm. range. The calibration curve shown in Figure 3 indicates that the transducer is linear for the range of weights used.

Experimental Design

New Zealand White female rabbits (9 lb.-11 lb.) were anesthetized with methoxyflurane administered with a nose cone. Initial amount of the fluid anesthetic delivered to the cone was 1.5 ml. and an additional 0.5 ml. increment was added approximately every 10 minutes during the 2 hour operation. Under sterile conditions a 0.75 in. diameter circular incision was made in the mid-scapular region and a 1 in. incision was made in the left flank. A 0.75 in. diameter stainless steel trocar was passed subcutaneously from the scapular to the flank incision. The transducers were brought through the trocar and it was withdrawn leaving the tubing subcutaneous. The scapular incision was closed securing the electrical connector plug.[11] A 3 in. abdominal midline incision was made and the transducers were brought through the abdominal wall into the abdominal cavity at the point of the flank incision. Two transducers were sutured (usually 3 cm.-4 cm. apart) with 3-0 Mersilene on one uterine horn in either a transverse axis when circular muscle was to be monitored or in a longitudinal axis when the longitudinal muscle was to be monitored. When in a logitudinal axis the

[11]Radio Distributing Co. Inc., South Bend, Indiana.

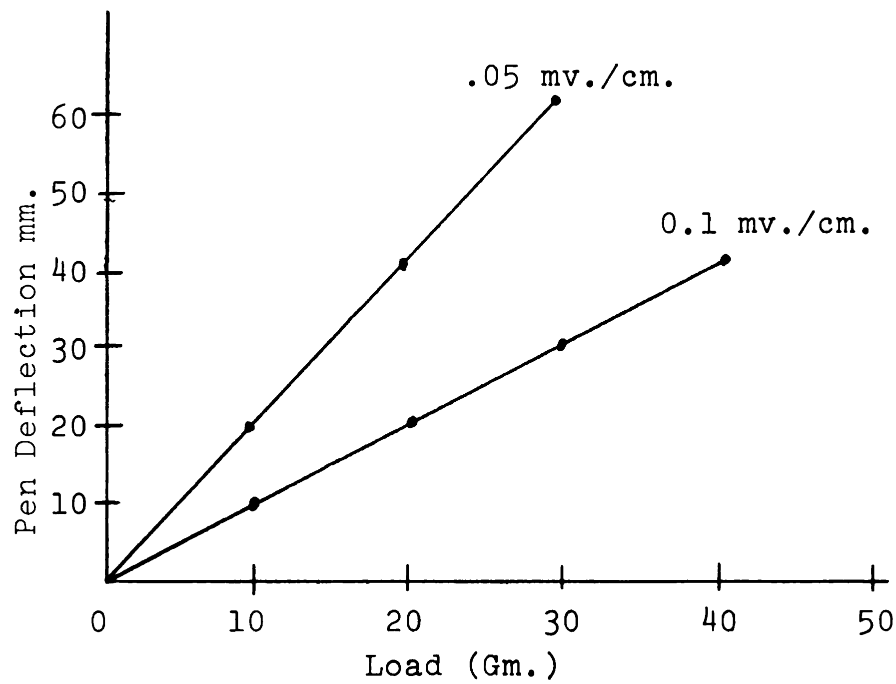


Figure 3.--Calibration Curve. A typical calibration curve for a single transducer. The usual recording sensitivity was 0.1 mv./cm. The abcissa represents the sum of the weights added.

transducer was sutured at the four corners and two sutures were used for placement in a transverse axis (30). The midline and flank incisions were closed. Penicillin (100,000 units) was administered and within an hour, the animal was upright and alert.

For recording purposes the connector plug from the Dynograph was brought through the top of the cage and connected to the scapular plug in the animal. During the 1-2 hour daily recording periods, the rabbit was confined to the rear of the cage by a board which spanned the width of the cage. Although the space was limited, the rabbit had freedom of movement and could turn end for end with little difficulty. Once accustomed to the procedure, the rabbit would lie quietly for most of the recording period.

The transducers were implanted during pregnancy, pseudopregnancy or in estrus after parturition. Of 25 rabbits used in this study, 6 were recorded at the termination of pseudopregnancy, 6 were recorded in early pseudopregnancy, 1 was recorded during pregnancy and 9 were recorded during estrus. When the rabbit came into the estrous state, control records were obtained for several days and the rabbit was then mated. Recordings were made daily for at least eight days following mating and in some cases were continued for the duration of pseudopregnancy or gestation.

Oxytocin[12] was injected subcutaneously during the recording period and the response during estrus and pregnancy was compared.

[12]Pitocin, Parke, Davis and Co., Detroit, Michigan.

RESULTS

Contractile activity patterns of the longitudinal and circular muscle layers were recorded during estrus and the parameters of amplitude, frequency and conduction velocity were determined. Post-ovulatory contractile activity changes associated with pregnancy and pseudopregnancy were also recorded.

Contractile Activity During Estrus

During estrus the uterus exhibited comparatively high amplitude, rhythmic, coordinated contractile activity. The frequency of contractile activity was generally higher in the more proximal portion of the horn and 70-85% of the contractions recorded near the junction with the Fallopian tube were propagated distally. The contractile amplitude differed at the two transducer sites but was not consistently higher at either.

Longitudinal Muscle

The contractions either occurred singly or as a burst (one or more contractions superimposed on an increased tone). The frequency as determined from the total number of contractions (not bursts) varied from 0.5 to 2 contractions per minute. The average distal conduction velocity calculated by determining the time interval between the initiation of the contractions at the two transducer sites, was 0.5-1.2 cm. per second. The maximal contractile force recorded from longitudinal muscle was 38 gm. Figure 4 shows typical estrous contractile activity. Both single contractions and contraction bursts are evident. Two days later (Fig. 4) the amplitude had decreased and the frequency had increased but the rhythmic coordinated nature of the contractile activity was maintained.

Circular Muscle

The contractions of the circular muscle layer usually occurred singly. The maximum recorded contractile force was 23 gm. and the frequency varied from 0.5-1 contraction per minute. The contractions were propagated distally at an average velocity of 0.09-0.11 cm. per second. The marked variation in the estrous contractile pattern of circular muscle is indicated in Figure 5, which shows the changes over a four day period. Note the variation in amplitude and comparative irregularity on day 5. Day 6 shows complete asynchrony between the two transducer sites but regular coordinated activity was again evident on day 7. A summary of the parameters for the two muscle layers is given in Table 1.

The Transition from Pseudopregnancy to Estrus

Near the termination of pseudopregnancy contractions of higher amplitude were recorded with increasing regularity but still lacking coordination. The number of contractions

Figure 4.--Estrous contractile activity patterns longitudinal axis. Two transducers were sutured on the left horn 3 cm. apart. The upper group of tracings was obtained 18 days after the induction of pseudopregnancy. The calibration bars represent 20 grams of force and apply to both pairs of tracings.

p.o.--Post-operation
L3 --Longitudinal axis; 3 cm. from utero-tubal junction.
L6 --Longitudinal axis; 6 cm. from utero-tubal junction.

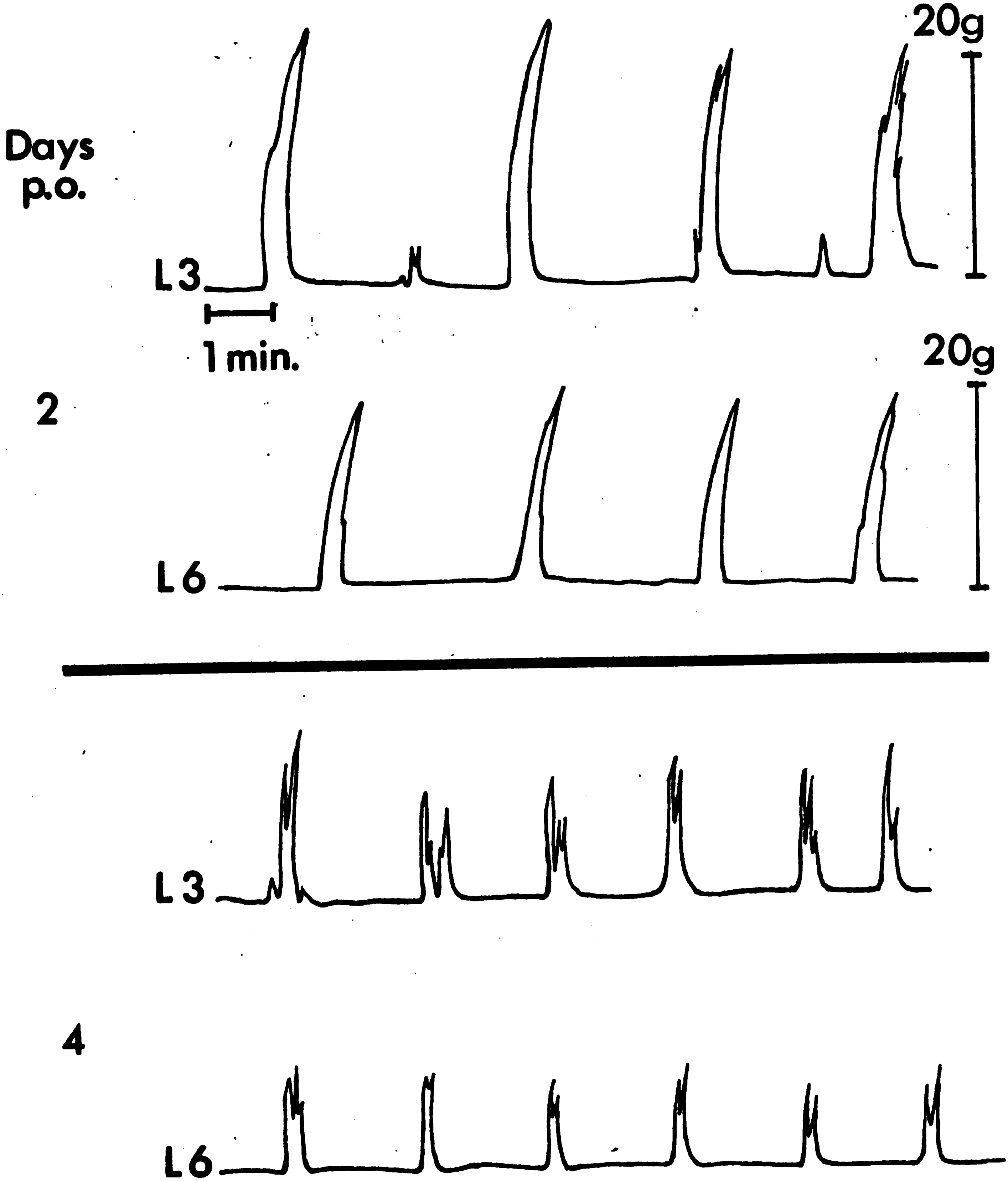

Days
p.o.
20g
L3
1 min.
20g
2
L6
L3
4
L6

Figure 5.--Estrous contractile activity patterns transverse axis. An example of variations in postpartum estrous activity. Both transducers were sutured on the right horn 3 cm. apart. The daily record consists of the pair of tracings. The height of the calibration bar represents 20 grams of contractile force and applies to all pairs of tracings.

DPP--Days postpartum.
T3 --Transverse axis; 3 cm. from utero-tubal junction.
T6 --Transverse axis; 6 cm. from utero-tubal junction.

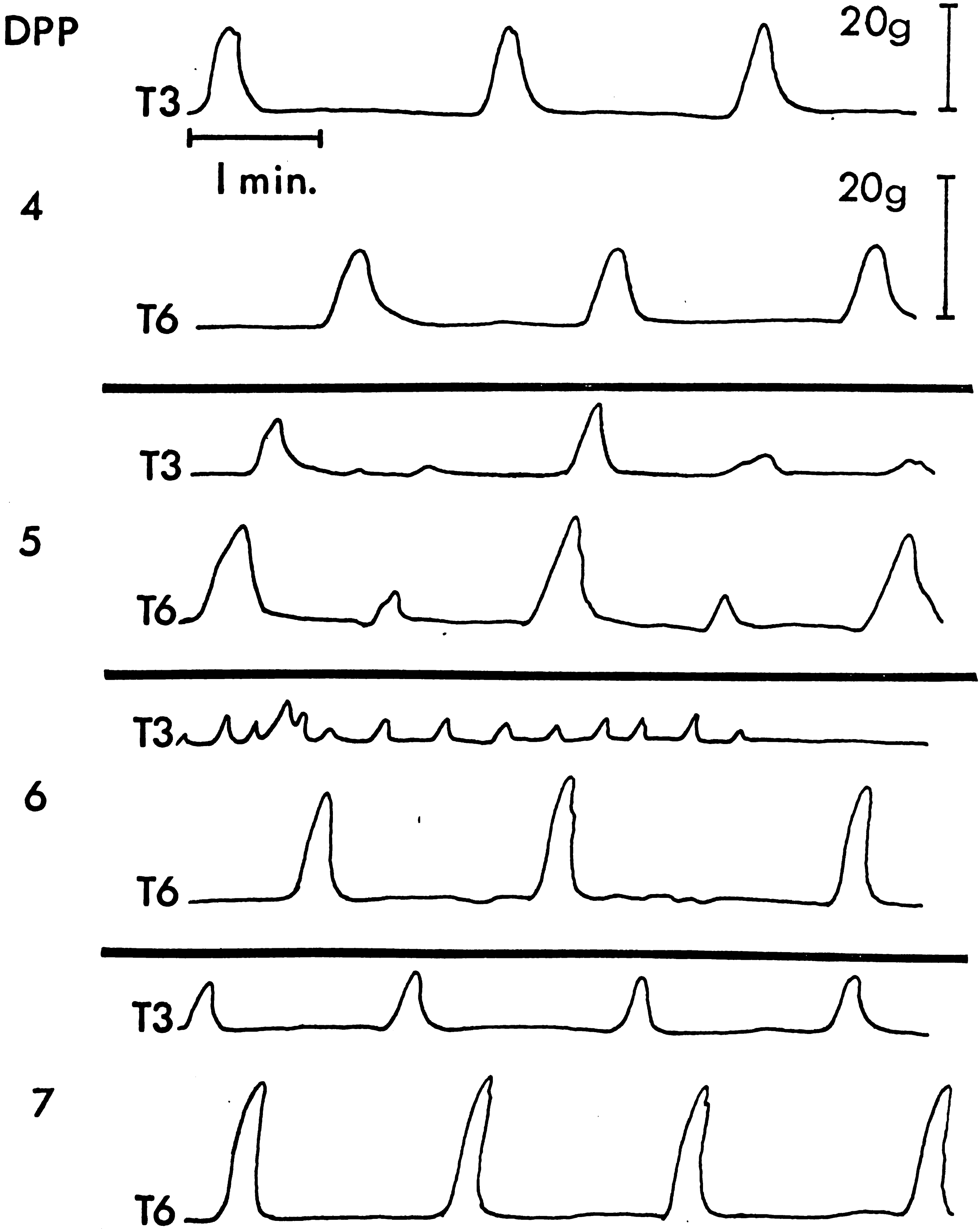

DPP
20g
T3
1 min.
4
20g
T6
T3
5
T6
T3
6
T6
T3
7
T6

TABLE 1.--Parameters of estrous contractile activity.

Transducer Axis	Average Maximum Amplitude gm./mm.^{2}	Frequency Range Contractions/min.	Conduction Velocity Range cm./sec.
Transverse	.41*	.5 - 1	.09 - .11
Longitudinal	.58**	.5 - 2	.55 -1.2

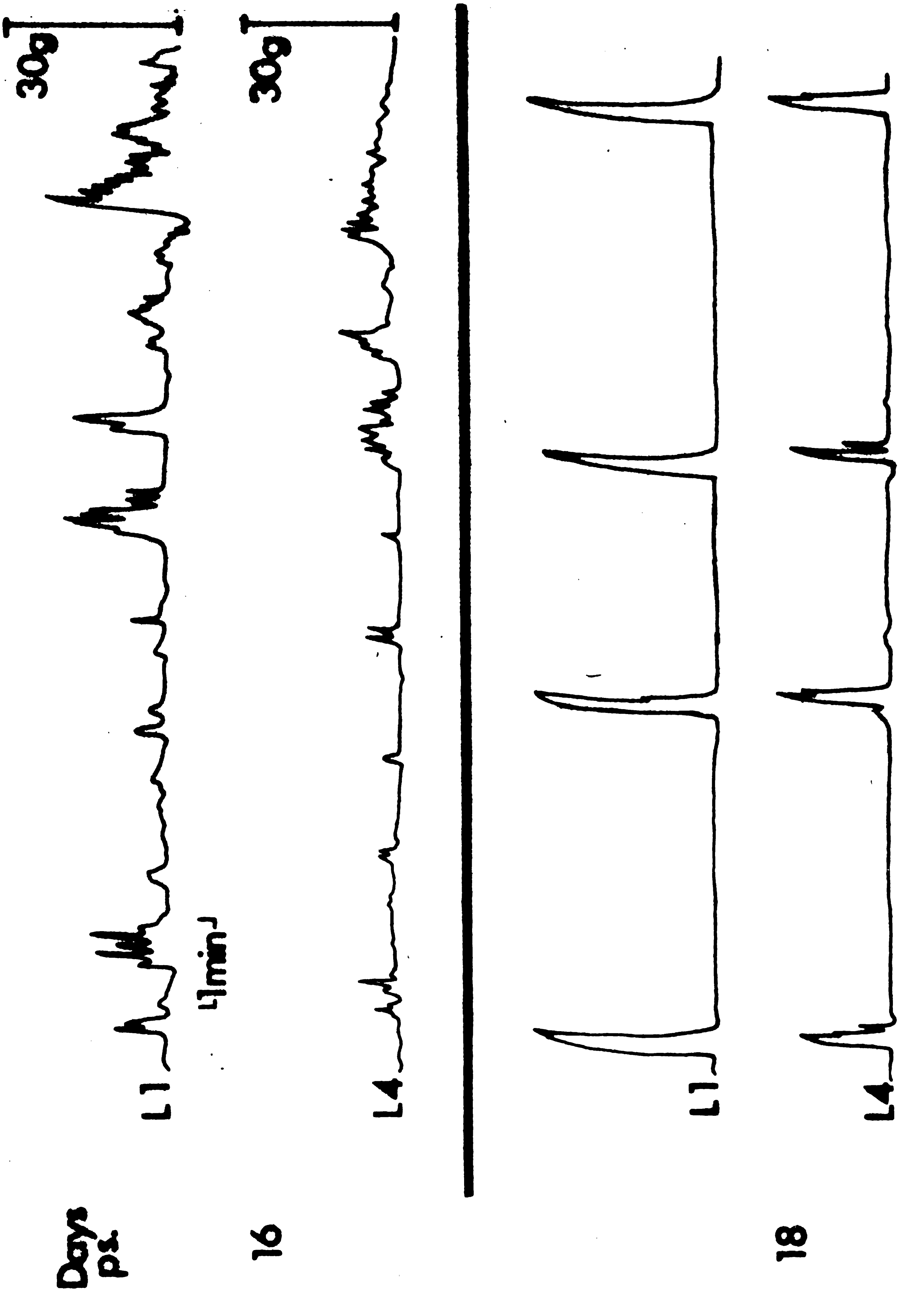
*Mean of 3 values.
**Mean of 6 values.

The difference between means of maxima for the two muscle layers was statistically significant with $0.01<p<0.02$, calculated by using a small sample method in Documenta Geigy, 5th ed., 1959, p. 39.

in a burst gradually decreased. By day 18 (approximately) the contractile activity pattern recorded was associated with estrus. Contractile activity of the longitudinal muscle layer on day 16 of pseudopregnancy is illustrated in Figure 6. At both transducer sites the contractile amplitude was vairable but discrete contractions of estrous proportions were becoming apparent. The frequency was irregular and relatively greater due to the large number of contractions in a burst. Two days later (Fig. 6) the amplitude had increased and the frequency had decreased as the number of contractions per group diminished, and the contractile activity pattern characteristic of the estrous state predominated.

Figure 6.--Transition from pseudopregnancy to estrus longitudinal axis. The upper pair of tracings is an example of contractile activity in the terminal days of pseudopregnancy. Both transducers are on the same horn 3 cm. apart. The height of the calibration bars represent 30 grams of force and applies to both pairs of tracings.

ps.--pseudopregnant
L1 --Longitudinal axis; 1 cm. from utero-tubal junction.
L4 --Longitudinal axis; 4 cm. from utero-tubal junction.


Days
p.s.
16
18
L1
L4
30g
1min

The Transition from Estrus to Pseudopregnancy

Longitudinal Muscle

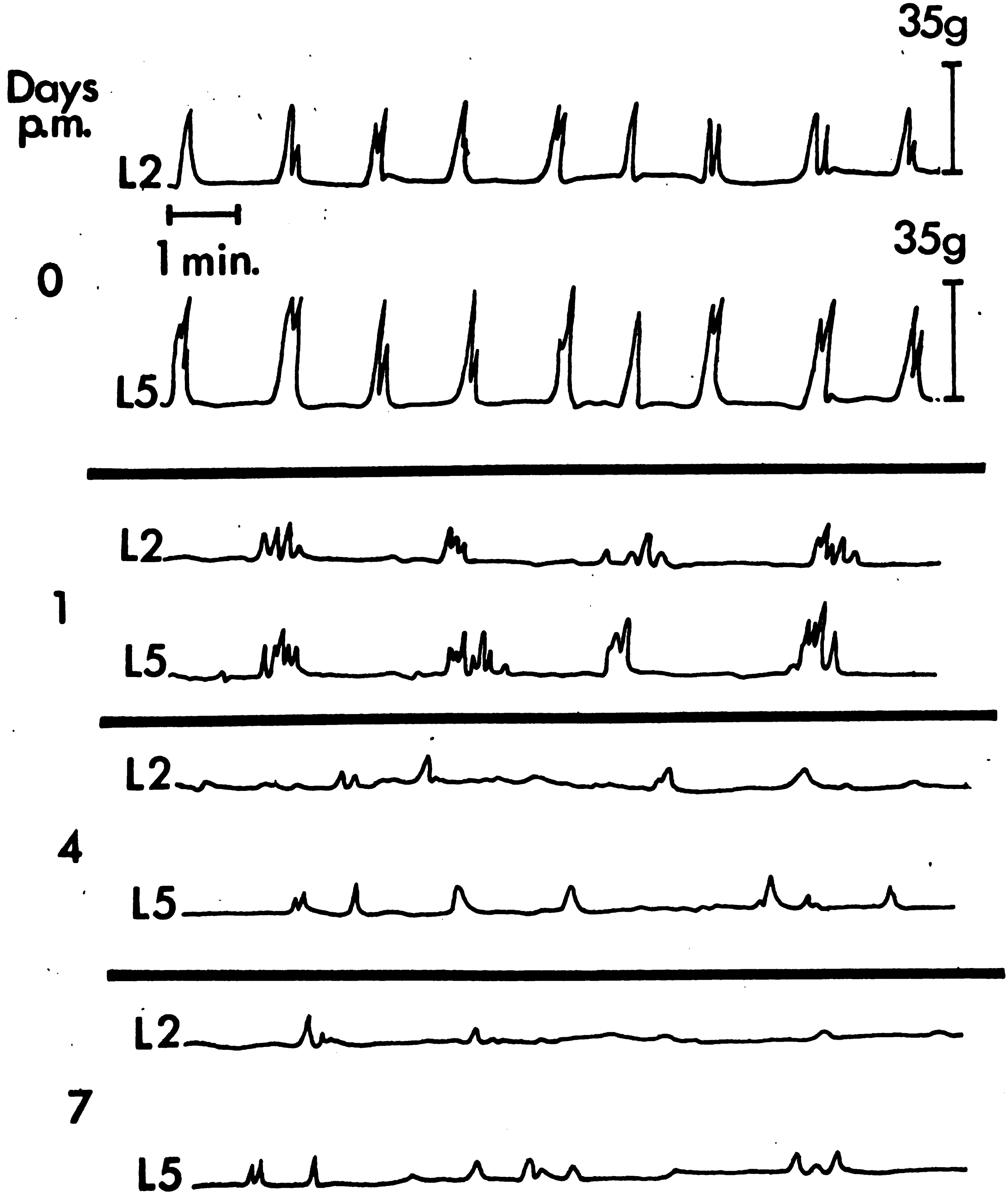
Following ovulation and subsequent pseudopregnancy, estrous contractile activity decreased in amplitude and lost its rhythmicity and coordination. During the first two days after ovulation the frequency initially increased due to the greater number of contractions per burst but thereafter gradually decreased. Typical estrous contractile activity and the changes that occurred through day 7 of pseudopregnancy are shown in Figure 7. The first day after ovulation the contractile amplitude decreased and the number of contractions per burst increased. By the seventh day the average amplitude was 25% of that recorded during estrus and the frequency was irregular.

Circular Muscle

Figure 8 shows estrous contractile activity of circular muscle and the changes that occurred through the first seven days of pseudopregnancy. The first day after mating the amplitude greatly decreased and the frequency increased. By the second day both transducer sites were relatively quiescent and little discrete contractile activity was evident. This pattern did not change significantly through day 7. It is apparent that the decrease in activity was more rapid in the more proximal transducer site and the contraction groups recorded in longitudinal muscle (Fig. 7) were absent.

Figure 7.--Transition from Estrus to Pseudopregnancy Longitudinal Axis. Typical estrous contractile activity is illustrated followed by the changes found during the first 7 days of pseudopregnancy. Pseudopregnancy followed an unfertile mating. Both transducers are on the same horn 3 cm. apart. The calibration bar represents 35 grams of force and apply to all pairs of tracings.

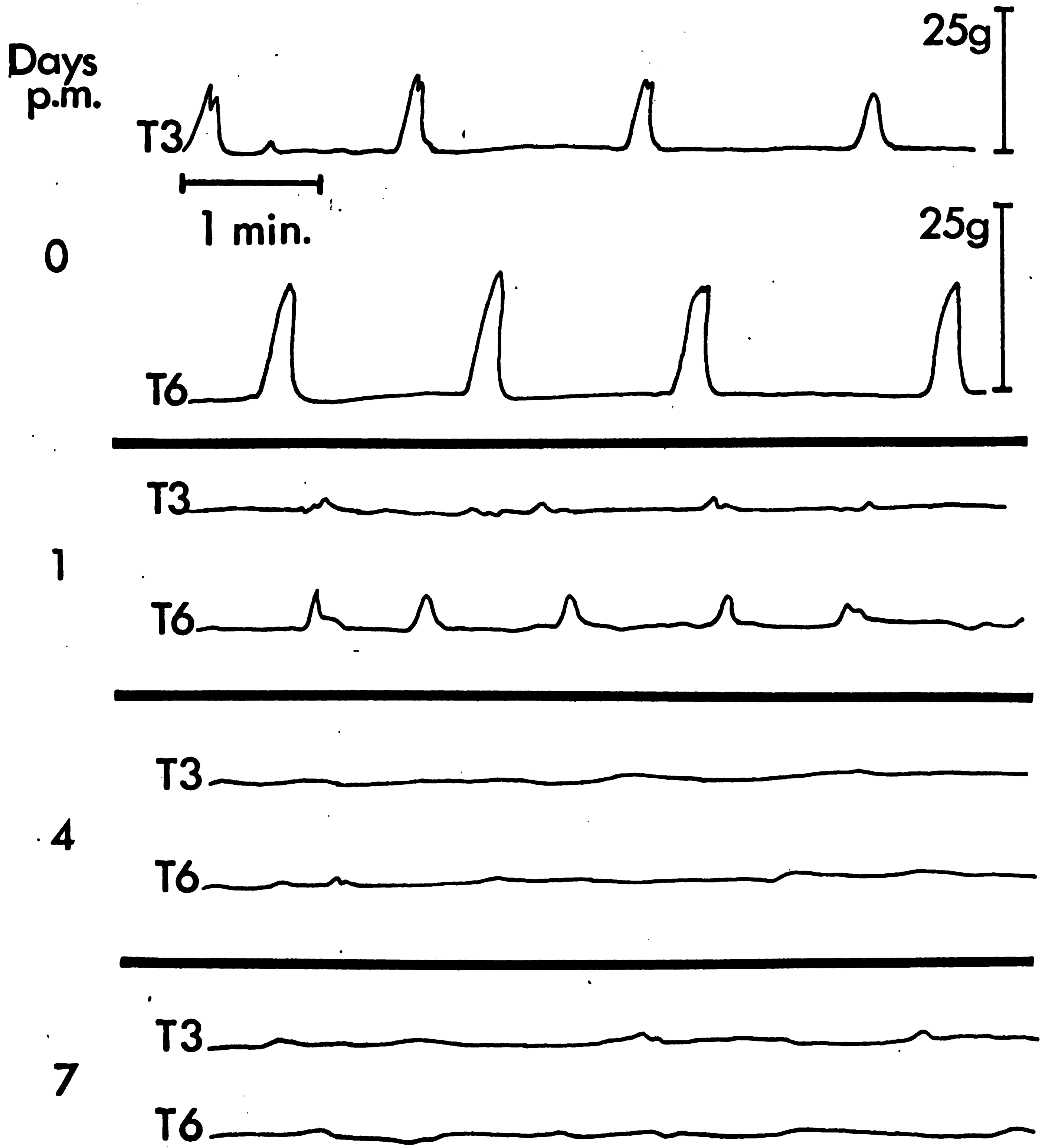
p.m.--Post-mating.
L2 --Longitudinal axis; 2 cm. from utero-tubal junction.
L5 --Longitudinal axis; 5 cm. from utero-tubal junction.



35g
Days
p.m.
L2
1 min.
0
35g
L5
L2
1
L5
L2
4
L5
L2
7
L5

Figure 8.--Transition from estrus to pseudopregnancy transverse axis. Typical estrous contractile activity is illustrated followed by the changes recorded during the first 7 days of pseudopregnancy, following an unfertile mating. Both transducers are on the same horn 3 cm. apart. The calibration bars represent 25 grams and apply to all pairs of tracings.

p.m.--Post-mating.
T3 --Transverse axis; 3 cm. from utero-tubal junction.
T6 --Transverse axis; 6 cm. from utero-tubal junction.


25g
Days
p.m.
T3
1 min.
25g
0
T6
T3
1
T6
T3
4
T6
T3
7
T6

The Contractile Activity of Early Pregnancy

The contractile activity pattern of the longitudinal muscle layer during the first seven days of pregnancy is shown in Figure 9. The recording was made from horn devoid of fetuses, a non-implanted horn. The first day after mating the decrease in amplitude was slight and the contractions became somewhat irregular. The second day the amplitude continued to decrease and the frequency increased as a result of a greater number contractions occurring in a burst. The amplitude declined more rapidly at the more proximal transducer site and this site was relatively quiescent three days after mating. Activity persisted somewhat longer at the distal site but by day 6 it was also inactive.

The Response to Oxytocin

The subcutaneous injection of 0.5 units of Pitocin as shown in Figure 10 increased the tone, amplitude and frequency of the estrous contractile activity. In the same rabbit an equivalent dose given on the ninth day of pregnancy elicited no response.

The Effect of Anesthesia

Figure 11 traces a recording made immediately after surgery while the rabbit was unconscious and four hours later when it was fully conscious. The record indicated that both the amplitude and frequency had decreased in the conscious animal.

Figure 9.--Transition from estrus to pregnancy longitudinal axis. Contractile activity during the first 6 days of pregnancy is illustrated. Both transducers are on the left horn which was devoid of implantation sites. The calibration bars represent 20 grams of contractile force and apply to all pairs of tracings.

p.m.--Post-mating.
L3 --Longitudinal axis; 3 cm. from utero-tubal junction.
L6 --Longitudinal axis; 6 cm. from utero-tubal junction.

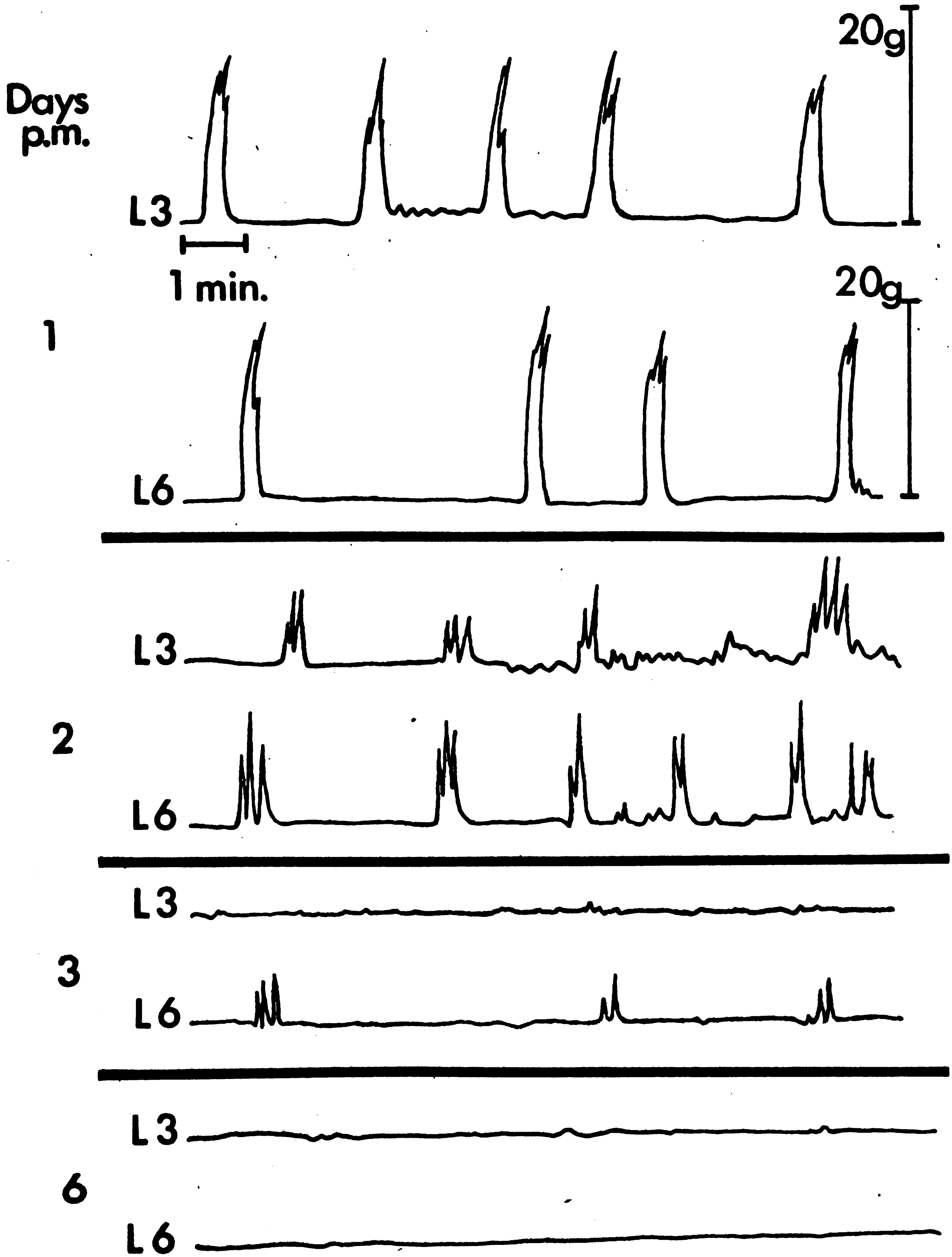

20g
Days
p.m.
L3
1 min.
20g
1
L6
L3
2
L6
L3
3
L6
L3
6
L6

Figure 10.--Response to Oxytocin longitudinal axis. The response of the uterus during estrus to 0.5 units of Pitocin is shown in the upper pair of tracings. The response in the same animal to an equal dose on the 9th day of pregnancy is illustrated in the lower pair of tracings. The hormone was given subcutaneously; the arrows indicate the point of injection. The calibration bars represent 20 grams of force and apply to both pairs of tracings.

Figure 11.--Effect of anesthesia transverse axis. The upper pair of recordings was made while the rabbit was still unconscious immediately after transducer implantation 4 days post-partum. The lower pair of tracings was obtained 4 hours later in the fully conscious animal. The calibration bars apply to both pairs of tracings.

p.o.--Post-operation
T3 --Transverse axis; 3 cm. from utero-tubal junction.
T6 --Transverse axis; 6 cm. from utero-tubal junction.

DISCUSSION

Validity of the Strain Gage Transducer

Jacoby et al. (24) presented evidence that: (1) The contractions recorded from the dog duodenum were not the result of respiration or other muscular activity. (2) The transducer was not sensitive to strain except along the longitudinal axis of the strain gage grid. (3) Physiological temperature variations were not a source of error. Reinke et al. (30) presented additional evidence that circular and longitudinal muscle activity could be recorded separately and simultaneously from all segments of the dog gastrointestinal tract.

Reliability of the Recorded Uterine Contractile Activity

The frequencies of respiratory movements and colonic contractile activity of rabbit colon (2) (possible recording artifacts) are greater than the frequencies recorded in this study. Bass and Callantine (5) demonstrated the correlation between the electrical and mechanical activity in the dog uterus and with similar transducers Callantine et al. (8) observed postpartum estrous motility of the type recorded in this study. Oxytocin which is known to stimulate the rabbit uterus during estrus but not in pregnancy, was shown to have the predicted response. Body movements did appear on the record but were easily recognized as artifacts and did not obscure the uterine contractile activity.

Post-Operative Condition of the Experimental Animal

There seemed to be no post-operative difficulties due to the presence of the transducers. Within 1-2 days the rabbit resumed normal dietary and excretory habits. Any respiratory difficulties could usually be eliminated with antibiotics. Post-operative infection presented a problem only when the rabbit aggravated the incision areas. Several animals were found to be paralyzed in the hind quarters immediately after surgery. This was likely the result of a spinal injury incurred while struggling in a restraining box during the induction of anesthesia. This injury did not occur when the rabbit was wrapped in a sheet for restraining purposes. At autopsy a fibrous connective tissue film (1-2 mm. in. thickness) was found covering the transducer.

Contractile Activity Patterns During Estrus

There has been no published data which quantifies the *in vivo* amplitude and frequency parameters of rabbit uterine contractile activity during the estrous state. Reynolds (31) described intra-uterine pressure waves of high amplitude and low frequency during estrus while other investigators using the *in vivo* balloon method have been even less precise in their description. Table 1 indicates that the contractile frequency for both muscle layers was essentially similar and varied from 0.5-2 contractions per minute. As mentioned, there is no in vivo data available for comparison although a similar frequency range has been reported in vitro (15). The table also shows that in this study the longitudinal muscle layer was capable of developing greater tension than circular. A possible explanation for this difference may involve the relative thickness of the two muscle layers. Although data are not available for the rabbit, a study in the mouse has shown the longitudinal muscle layer to have greater thickness than circular during estrus (36).

In view of the structural changes that occur in the myometrium during pregnancy the circular muscle layer might be expected to display greater contractile force than longitudinal. In the period between ovulation and implantation, rabbit myometrial cells increase in number, particularly in the circular layer (11). This hyperplasia has been attributed to mitoses of existing smooth muscle cells and to the metaplastic conversion of connective tissue cells which are also undergoing mitosis at this time. Estrogen, which is responsible for the contractile protein content of uterine smooth muscle cells, was shown to stimulate mitosis primarily of the connective tissue cells while progesterone was more effective in increasing the number of smooth muscle cells (11). Regardless of their source, new muscle cells are rarely formed after implantation has occurred (11). The subsequent increase in uterine weight following implantation is accomplished by hypertrophy of the smooth muscle and connective tissue cells which occurs in response to the distension created by the expanding fetuses (35). At term the circular muscle layer has a proportionately greater volume (11) which indicates the important function of this layer during labor.

Variations in both contractile amplitude and frequency occurred during estrus. In the longitudinal muscle layer, as contractile amplitude decreased, the frequency increased due to a greater number of contractions per burst and not to an increase in the number of bursts.

Multiple contraction bursts were not characteristic of circular muscle contractile activity, but the variations in amplitude and frequency of the single contractions were quite pronounced. From these studies it was evident that during estrus, as the amplitude decreased, the frequency increased. This was true for both muscle layers. This inverse correlation of frequency and force of contraction has also been described by Rosenbaum, et al. (42) for the longitudinal and circular muscle layers of the stomach and small intestine.

These variations in contractile activity can probably be accounted for by changes in the levels of estrogen and progesterone during estrus. This fluctuation in hormone levels has been reported in the rabbit (22) and this study indicates that it may influence contractile activity.

Propagation of Contractile Activity During Estrus

The frequency of contractile activity for both muscle layers was generally greater at the more proximal transducer site and 70-85% of the contractions recorded from this site were propagated distally. The average velocity of contraction propagation was calculated for the mechanical event and was found to be more rapid in the longitudinal muscle layer. The evidence presented here suggests that a gradient of activity exists during estrus from the Fallopian tube to the cervix and supports Reynold's proposal that the isthmus of the Fallopian tube is the normal pacemaker of uterine contractile activity during estrus (36). The correlation between contractile activity frequency and region of the gastrointestinal tract is well known and has been recorded *in vivo* by extraluminal transducers in the dog (42).

The Post-Ovulatory Contractile Activity of Pseudopregnancy and Pregnancy

In vivo uterine motility in the pseudopregnant rabbit has been reported by Reynolds and Friedman (38). They induced pseudopregnancy by mating. Ovulation occurred in response to coitus but fertilization was impossible due to the surgical interruption of the vagina. They maintained that the uterus became quiescent several hours before ovulation which is known to occur twelve hours after coitus (38).

The results of the present study are not in agreement with those of Reynolds. Following ovulation contractions became irregular and non-propagated, amplitude decreased and the frequency initially increased. This was true for both muscle layers. By mid-pseudopregnancy both amplitude and frequency had diminished to the extent that the uterus was relatively inactive. The circular muscle layer was less active during this time, but in both muscle layers the decline in contractile activity was more rapid at the more proximal transducer site. The rabbit was assumed to be pseudopregnant when, after eight to ten days following mating, a laparotomy revealed an enlarged uterus, new corpora lutea, no implantation enlargements; at the same time the contractile activity pattern could not be attributed to a variation in estrus.

The longitudinal muscle layer on the first day of pregnancy had a contractile activity pattern which resembled that during estrus. The second day after ovulation the amplitude had decreased and the frequency had increased as the number of contractions per burst increased. Thereafter, both amplitude and frequency rapidly diminished and the uterus became less active than during a similar period of pseudopregnancy. The initial persistance of high amplitude contractions may suggest that they have a function in the transport of spermatazoa but since implantation did not occur in the transducer monitored horn, the question remains unanswered as to whether this same contractile activity pattern is associated with blastocyst transport and implantation.

It was apparent from this study that the increasing level of progesterone following ovulation (3) is associated with a decrease in contractile amplitude, loss of coordination and contraction propagation, and an increase in contractile frequency. As progesterone becomes the dominant hormone, contractile activity is diminished and the uterus becomes relatively quiescent. Near parturition or the termination of pseudopregnancy when the hormone balance is progressing towards estrogen dominance (3), contractile activity similar to the post-ovulatory pattern is again observed. The high frequency activity described for the progesterone dominated uterus in vitro is evident in vivo only during the transition periods of changing hormone dominance.

Several criteria have been used to determine the onset of progesterone domination following ovulation. Reynolds (33) was able to overcome the post-ovulatory inhibitory effect of progesterone with estrogen treatment until the fourth day after ovulation. Schofield (44) employed the staircase effect elicited by electrical stimulation and found that the slope became negative 20-28 hours after mating. The "positive staircase" effect did not appear until 24 hours before delivery. Pituitrin, which stimulates the rabbit uterus during estrus, has been found to elicit no response 48 hours after coitus. The contractile activity patterns observed in this study suggest that progesterone becomes the dominant hormone 4-5 days after mating.

The rabbit uterus has been shown to be refractory to oxytocin during pregnancy until 1-2 days before delivery (45). The response to oxytocin during estrus in this study is similar to the *in vivo* motility patterns recorded by Fuchs during labor. She reported that typical estrous intrauterine pressure waves did not appear prior to labor and that the response to oxytocin was similar to the normal pressure waves of labor. In the present study contractile activity recorded (from a horn devoid of fetuses) one day prior to normal partuition date did not resemble that of estrus and suggests, as did Fuchs, that labor is initiated by a release of oxytocin.

SUMMARY

I. The extraluminal strain gage transducer has been shown to be a satisfactory method for studying *in vivo* contractile activity of both the longitudinal and circular muscle layers of the rabbit uterus.

II. The contractile activity patterns in several rabbit ovarian stages were recorded and analyzed.

1. During estrus, the uterus exhibited high amplitude, rhythmic coordinated contractile activity. Variations in contractile amplitude and frequency were common during estrus and changes in amplitude were inversely related to frequency. Evidence was presented in support of the proposal that a gradient of contractile activity exists in the uterus.

   Several differences were found to exist between the circular and longitudinal muscle layers during estrus: (a) The average maximal contractile amplitude was greater for longitudinal muscle. (b) Contractions of the longitudinal layer were conducted at a more rapid rate. (c) The longitudinal muscle frequently exhibited a pattern of contraction bursts while single contractions were more characteristic of circular muscle.

2. During the initiation and termination of pseudopregnancy contractile activity patterns were similar and consisted of low amplitude, high frequency contractions of an irregular and uncoordinated nature. During the intervening period the uterus was relatively quiescent but was more active than during a similar period of pregnancy.
3. The immediate post-ovulatory contractile activity of pregnancy (one day post coitus), in contrast to that of pseudopregnancy, resembled that of estrus, but thereafter the activity declined rapidly and the uterus remained essentially inactive until several days before delivery.
4. As expected, during estrus oxytocin increased tone, contractile amplitude and frequency, but elicited no response during pregnancy.

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