# Muscle Layer Contractile Activity of the *in Vitro* Rabbit Uterus* (33682)

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Although rabbit uterine contractile activity patterns in various ovarian stages have been established *in vivo* (1–4) and *in vitro* (5, 6), no isometric, *in vitro* technique exists for quantifying differentially and simultaneously the contractile activity of the longitudinal and circular muscle layers. The extraluminal contractile force transducer (ECFT) has been used *in vivo* for monitoring contractile activity of the gut (7–9) and uterus (1, 2, 10), and *in vitro* for indirectly monitoring contractile activity of common bile duct longitudinal muscle (11). This paper describes a method of quantitative, isometric, *in vitro* sampling of contractile activity of either the outer longitudinal or inner circular muscle layer of the rabbit myometrium, a structure unique among mammalian uteri in possessing this distinct muscle layer arrangement (12).

*Materials and Methods.* The principle of sampling contractile activity with an ECFT is based on detecting linear deformation of a strain gage grid reflecting the contractile state of smooth muscle to which the uniaxially sensitive transducer is sutured, as originally described by Jacoby *et al.* (7).

A drawing of the transducer is shown in Fig. 1. Two etched-foil strain gages (Micro-Measurements, Romulus, Mich., Cat. No. SK-09-031DE-350) were bonded, one to each side of a prepared shim stock using a thin layer of Tatnall GA-5 epoxy cement (Budd Co., Phoenixville, Pa.) under pressure applied with a 1-lb. Neg'ator clamp[2] while being heated for 2 hr at 220°F.

Lead wires were soldered to the two strain gages using special strain gage solder[2]. The transducer was waterproofed for *in vitro* use

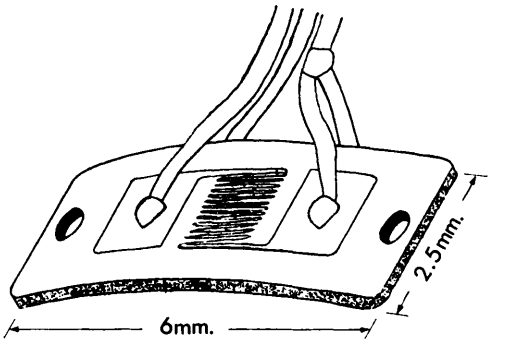


FIG. 1. Diagram of extraluminal contractile force transducer (ECFT). The 2.5 × 6 mm strips were cut from 0.15 mm thick, ¼ hard beryllium copper alloy shim stock (Berylco 25, Beryllium Corp., Reading, Pa.), formed into an arch and heat treated (4 hr at 600°F). A small hole (0.75 mm diam) was drilled (before heat hardening) in each end of the metal for suturing.

with four coats of solvent-thinned, nitrile rubber solution and two coats of epoxy resin.[2]

The two-gage transducer operated as a double active arm of a Wheatstone bridge circuit. Records were made on a curvilinear, ink-writing oscillograph (Type R Dynograph, Beckman Instr., Inc., Spinco Div., Schiller Park, Illinois).

Following quality control tests for waterproofing, temperature compensation and hysteresis, the ECFT's were calibrated with one end fixed (7); weights were hung from the free end (see Fig. 2A).

The Grass transducers (model FT-03, without springs, Grass Instrument Co., Quincy, Mass.), used in suspending muscle segments in the *in vitro* bath, were calibrated by suspending weights from the muscle attachment point (see Fig. 2B).

The 16 rabbits used in this study were ovariectomized and subsequently treated with estradiol cypionate[3] (100 μg/day for 5 days). After the rabbit was killed by concussion, the

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[1] Present address: National Institutes of Health (NIDR), Bethesda, Maryland.

[2] Fabrication materials are available from W. T. Bean, Inc., Detroit, Mich. 48223.

[3] Supplied gratuitously by the Upjohn Co., Kalamazoo, Mich., in a sterile cottonseed oil solution.

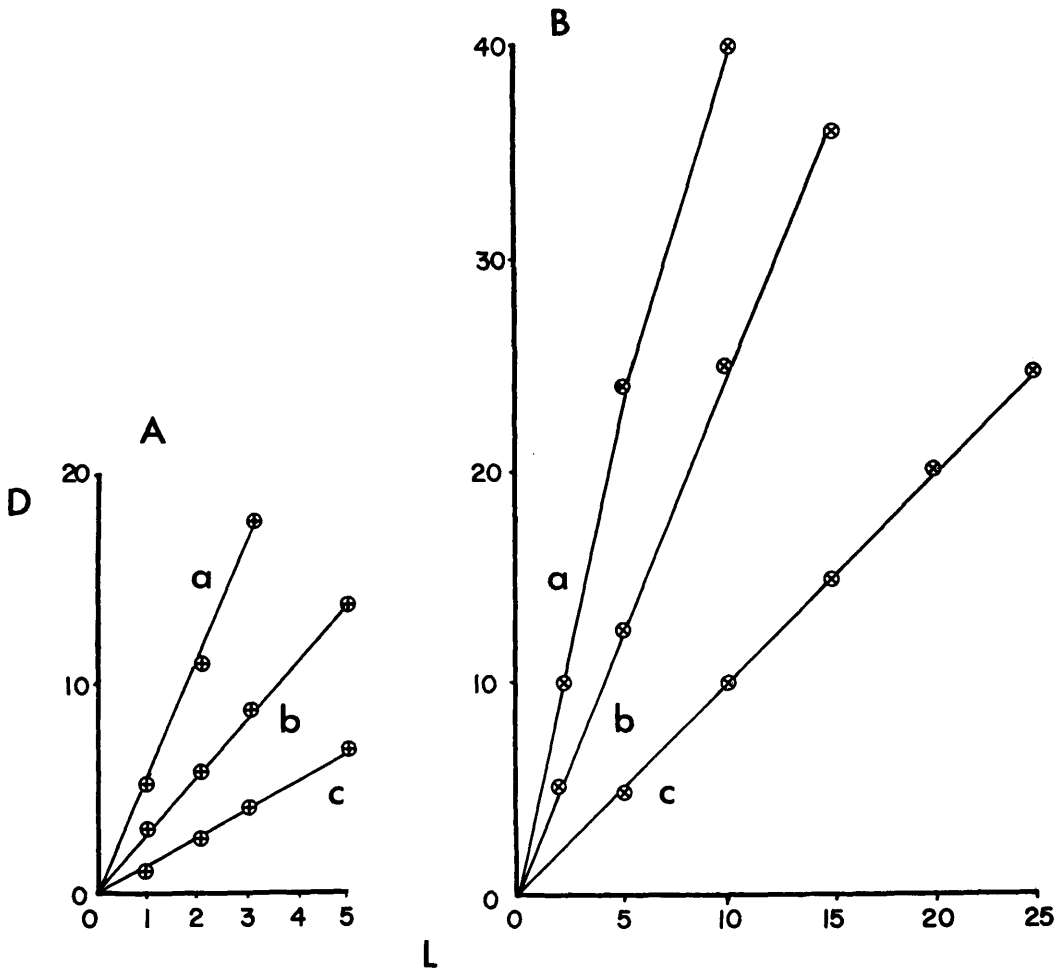


FIG. 2. Calibration curves for ECFT (A) and Grass transducer (B): recorder pen deflection, D (mm), as a function of calibration load, L (g); sensitivities are (a) 0.05 mV/cm, (b) 0.1 mV/cm, (c) 0.2 mV/cm.

uterine horns were removed and placed in an aerated (95% $O_2$, 5% $CO_2$) solution at 37°. The solution contained NaCl, 9.0; $CaCl_2$, 0.24; KCl, 0.42; $MgCl_2$, 0.2; $NaHCO_3$, 0.5; and *d*-glucose, 0.5 g/liter of distilled water.

The muscle was allowed to equilibrate for 45–60 min before attaching the two ECFT's. The ECFT's were affixed (Fig. 3) on a uterine segment (20 mm long) taken from mid-horn. A segment from one horn and a "control" segment without ECFT's from the opposite horn of the same rabbit were then suspended in separate *in vitro* baths. Resting tension of the total muscle segment was adjusted according to the method of Csapo (13) by raising the Grass transducer. Tension (0.5–1 g) was set on the muscle beneath the ECFT's at the time they were sewn on.

Acetylcholine hydrochloride (Merck) and vasopressin (Pitressin; Parke, Davis) were used attempting to show differential responses of the outer longitudinal and inner circular muscle layers of the segment.

*Results.* Two types of records were obtained depending upon the orientation of the ECFT's. Figures 4 and 6 show tracings of simultaneous contractile activity obtained when ECFT's were arranged vertically (see Fig. 3A). Contractions in bursts (defined

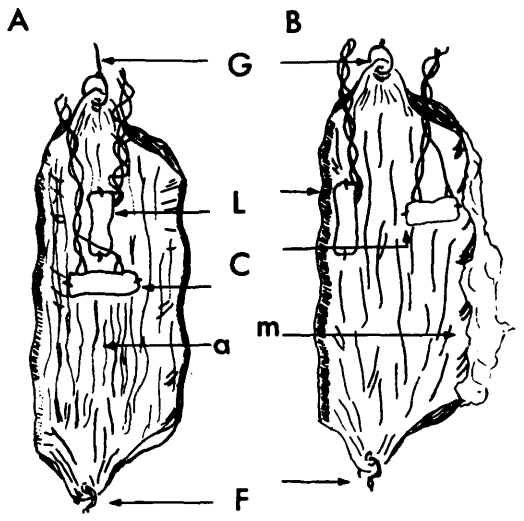


FIG. 3. ECFT placement configurations: "A" configuration consisted of Grass transducer attached via thread from G; longitudinal ECFT (L); circular ECFT (C); fixed attachment hook (F), with L and C in direct line of tension exerted between G and F. "B" configuration consisted of Grass transducer attached via thread from G: longitudinal ECFT (L); circular ECFT (C); fixed attachment hook (F), with L and C adjacent to the direct line of tension exerted between G and F; m indicates mesometrial border, a, the antimesometrial border.

here as an increase in contractile activity with one or more contractions during a period of apparent tone change) sampled from the longitudinal ECFT occurred with a recorded decrease in tone. Bursts occurring in the longitudinal muscle layer were sampled by the

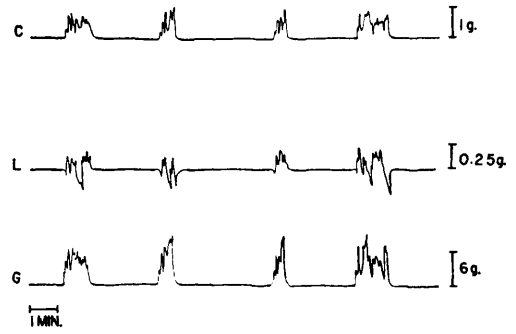


FIG. 4. Tracings of records of spontaneous *in vitro* uterine contractile activity (A configuration): Simultaneous recordings from circular ECFT (C), longitudinal ECFT (L), and Grass transducer (G). Vertical calibration bars indicate grams of tension in this and following figures.

longitudinal ECFT and by the Grass transducer; decreased tone was recorded during bursts measured by the longitudinal ECFT but not by the circular ECFT or the Grass transducer. Bursts occurring in the circular muscle layer were recorded by the circular ECFT. Tracings of contractile activity records obtained when ECFT's were arranged horizontally (see Fig. 3B) adjacent to the direct line of tension, are shown in Fig. 5. Contractile activity recorded from the circular ECFT in this orientation did not differ from that obtained in the vertical configuration. Likewise the contractile activity recorded by the longitudinal ECFT showed a decreased tone with superimposed contractions which approximated a mirror image of the contractile activity recorded by the Grass transducer.

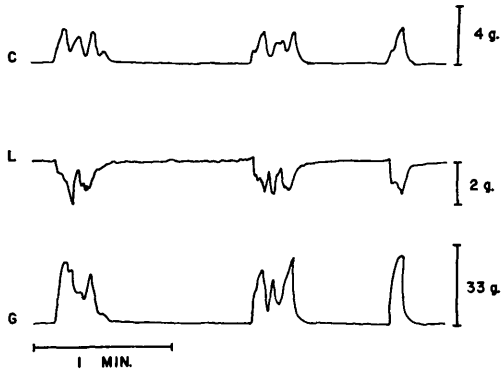


FIG. 5. Spontaneous contractile activity (B transducer configuration).

In those experiments designed to determine whether the presence of the extraluminal transducers affected frequency, no statistically significant difference in frequencies was found for the segments with attached ECFT's and the control segments (Table I). Comparison of average burst maximum forces also revealed no statistically significant difference for the segments with attached ECFT's and the control segments (Table I). There were no statistically significant differences (paired comparison, $p>0.05$) between ECFT longitudinal average maximum/burst (0.57 g) and ECFT circular average maximum/burst (0.90 g).

Activity of longitudinal and circular mus-

TABLE I. Frequency and Force Comparison.[a]

| | Segment[b] | |
|---|---|---|
| | Control | ECFT |
| Frequency (bursts/min) | | |
| *N* | 8 | 8 |
| Mean ± SE | 0.62 ± 0.10 | 0.48 ± 0.11 |
| *p* | >0.05 | |
| Force (g; mean of burst maxima) | | |
| *N* | 6 | 6 |
| Mean ± SE | 13.4 ± 2.0 | 12.3 ± 2.0 |
| *p* | >0.05 | |

[a] Contractile activity parameters obtained from Grass transducer recordings.

[b] Control segments had no ECFT's attached; ECFT segments had two ECFT's attached.

cle layers of the same segment in response to acetylcholine hydrochloride ($3 \times 10^{-6}$ *M*) is shown in Fig. 6A. Circular muscle layer developed an increase in contractile amplitude with a sustained increase in tone, but no change in average contraction duration; longitudinal muscle layer showed a decrease average contraction duration. These contractions occurred during an apparent decrease in tone.

Vasopressin ($1.2 \times 10^{-2}$ units/ml) elicited stimulation (Fig. 6B) similar to that of acetylcholine but had a longer duration of action which survived washing.

Contractile force developed per unit area of muscle monitored by both the Grass and longitudinal ECFT showed the latter was comparatively more effective in monitoring contractile activity of longitudinal muscle fibers. An average force per burst of 12.8 g for the Grass transducer for an approximate total muscle area of 439 mm$^2$ (based on a segment length of 20 mm and an uterine diam of 7 mm) yields a calculated unit area force of 0.029 g/mm$^2$. The longitudinal ECFT with an average force per burst of 0.57 g/15 mm$^2$ yields a calculated unit area force of 0.038 g/mm$^2$.

*Discussion and Summary.* Analyses of rabbit myometrial longitudinal and circular muscle activity indicate that these two muscle layers contract simultaneously *in vitro*. Simultaneous contractions of the longitudinal fibers were recorded by the Grass tranducer and the longitudinal ECFT (Fig. 4). Tone levels of bursts recorded by the longitudinal ECFT have been observed to be a function of the tension set on the underlying muscle at the time of ECFT attachment. If this tension was large, a decreased tone was recorded; if small, an increased tone was recorded. This apparent decreased tone was likely induced by contraction of adjacent longitudinal muscle above and below the ECFT.

Contractile patterns obtained for estrogen-dominated uteri correspond to similar estrous patterns recorded for other species (14–16). This contractile activity is generally described as rhythmic, slow, and of larger amplitude than for other stages of the ovarian cycle. Previous investigators have characterized estrous contractile activity as exhibiting

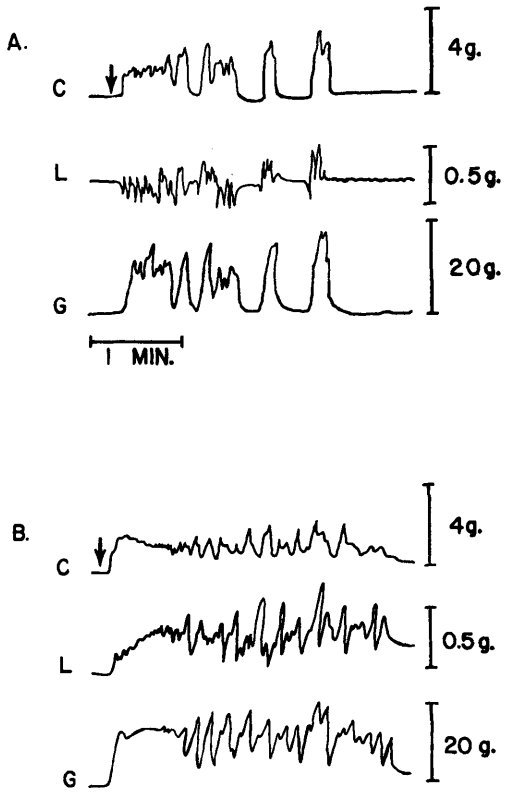


FIG. 6. Drug induced contractile activity (A transducer configuration): (A) $3 \times 10^{-6}$ *M* acetylcholine at arrow; (B) $6.2 \times 10^{-2}$ units/ml vasopressin at arrow.

a contraction every minute or every 4 or 5 min (14–16), corresponding to frequencies reported here (0.54 bursts/min).

The increase in frequency of contraction sampled by the longitudinal ECFT with a large setting tension in response to acetylcholine is attributed to the drug causing an increased tension in both the muscle beneath the ECFT and that in the remainder of the segment (principally the longitudinal muscle above and below the ECFT). The decreased tone recorded by the longitudinal ECFT during the acetylcholine response was likely due to the predominance of the comparatively large amount of longitudinal muscle in the rest of the segment (not beneath the ECFT).

The ECFT is a reliable isometric method for sampling muscle layer contractile activity if adequate attention is given to tension applied in attaching the ECFT. The ECFT does not influence uterine contractile activity monitored by the Grass transducer.

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