Experiment AM-4: Uterine Motility

Equipment Required

PC Computer

IXTA, USB cable, IXTA power supply

DT-475 Displacement transducer, or a FT-302 force transducer with 0-10 gram and 0-100 gram ranges

STB-125 Student tissue bath

Table-top water bath at 37^{o}C

Heating circulator, set at 37^{o} C (Optional)

Suture thread and needle

Pasteur pipets and bulbs

Non-toxic modeling clay

Air-tight chamber, and dry ice or CO_2 supply

Dissection pan and instruments

Cylinder with a mixture of 95% O_2 & 5% CO_2

Regulator, valve, and tubing for oxygenation setup

Tyrode's Physiological Saline (See appendix)

Various reagents in Tyrode's Physiological Saline (See appendix)

Transducer Setup

DT-475

1. Locate the DT-475 displacement transducer and plug it into Channel A5.

Figure AM-4-S1: The DT-475 displacement transducer.

Figure AM-4-S2: The DT-475 displacement transducer connected to an IXTA.

FT-302

The FT-302 dual range force transducer that can also be used in this experiment. This transducer can measure forces over two ranges, 0 to 10 grams and 0 to 100 grams.

1. Locate the FT-302 dual-range force transducer and a male DIN-DIN cable.

Figure AM-4-S3: The FT-302 dual-range force transducer.

2. Plug one end of the DIN-DIN cable into Channel A5. Plug the other end of this cable into the DIN connector on the FT-302 transducer.

Figure AM-4-S4: The FT-302 dual-range force transducer connected to an IXTA.

Tissue Bath Setup

1. Assemble the tissue bath. The components of the STB-125 student tissue bath are listed in Table 1. The completed tissue bath is pictured below.
2. Thread the 50cm stainless steel rod (502191) into the matching threaded hole on the back of the white non-magnetic base (502190). With the rod in this position, the base will provide a stable support for the components aligned above it.
3. Place a plastic parallel frame clamp (502193) on the stainless steel rod. This clamp should be about 20cm above the surface of the base and aligned as shown.
4. Place the arm of the glassware extension clamp (14016) in the clamping hole on the side of the parallel frame clamp. Position the extension clamp so its prongs can hold the tissue chamber vertically over the front half of the base.

Table AM-4-S1: Components of the STB-125 Student Tissue Bath Needed for this Experiment

Part Number	Part Description
502190	White Non-Magnetic Base
502191	50cm Stainless Steel Rod
47024	25ml Tissue Bath
502193	Plastic Parallel Frame Clamp
14016	Glassware Extension Clamp
4731	Nylon Tubing, 0.153”OD X 0.106”ID
14018	Oxygen Connector
4983	Silicone Tubing, 0.250”ID X 0.438”OD
7465	Polypropylene Pinch Clamp
160172	Glass Tissue Support
502198	Transducer Positioner

5. Place a second parallel frame clamp (502193) on the stainless steel rod. Align this clamp above the first clamp. The second clamp will eventually hold the glass tissue support (160172) that will hold the lower end of the uterus in the chamber.
6. If you need to hold another device above the tissue chamber, like an electrode or a pulley, place the third parallel frame clamp (502193) on the stainless steel rod. The third parallel frame clamp will eventually be a few centimeters above the second parallel frame clamp.
7. Place the transducer positioner (502198) on the stainless steel rod with the red adjustment knob of the positioner on top. Clamp the positioner on the rod so the bottom of the positioner is about 30cm above the base.

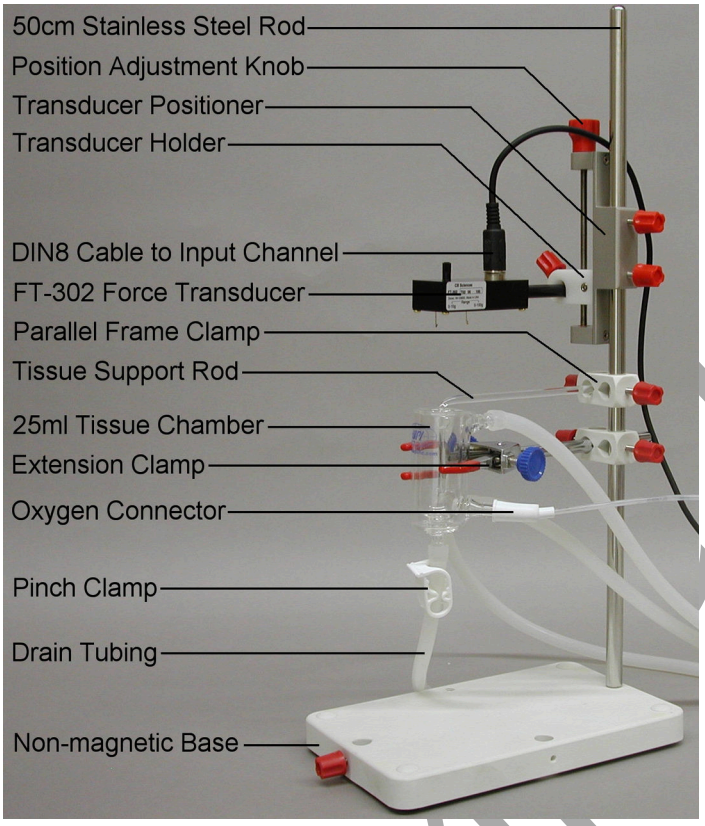

Figure AM-4-S5: STB-125 student tissue bath with a FT-302 force transducer.

8. Clamp the DT-475 displacement transducer or the FT-302 dual-range force transducer in the holder on the threaded rod of the positioner. Align the positioner and the transducer so the hook on the transducer is directly over the center of the tissue chamber:
    - When using the DT-475, tie one end of a suture thread, about 30cm long, to the upper eyelet of the rod on the transducer. Drape the thread over a pulley or rod clamped above the transducer. Place a small ball of clay on the end of the thread to be a counterweight to the transducer rod and as a tensioning device for the uterine muscle.
    - When using the FT-302, use the hook for the appropriate range of tension you expect in the experiment.
9. Turn the red adjustment knob on the positioner to move the transducer and its holder to the middle of the threaded rod.
10. Determine the best location on the lab bench for the tissue bath setup. The setup should be convenient to a sink or a drain flask, the mixed gas supply used for aeration, a water bath used for warming flasks of buffer used in the experiment, a warm water supply or a heating circulator used to maintain the temperature of the uterus.
11. Cut the silicone tubing into the lengths needed to make a drain line, an overflow line or a supply line to a buffer reservoir, and water lines to and from a warm water supply needed to maintain the temperature of the tissue bath.

12. Feed about 8cm of the drain line through the two circular holes on the plastic pinch clamp (7465). Carefully put the end of the drain line with the pinch clamp on the drain port of the tissue bath. Leave the drain open.
13. Carefully put the ends of the additional fluid lines on the appropriate ports of the tissue bath.
14. Put the oxygen connector (14018) on the aeration port of the tissue bath.
15. Carefully clamp the assembled tissue bath between the prongs of the glassware extension clamp already on the stand.
16. If the tubing needed on the tissue chamber has not been prepared, find the coils of silicone (4983) and nylon (4731) tubing.
17. Attach one end of the nylon tubing (4731) to the oxygen connector on the tissue chamber. Connect the other end of the nylon tubing to the valve on the cylinder containing 95% O_2 and 5% CO_2.

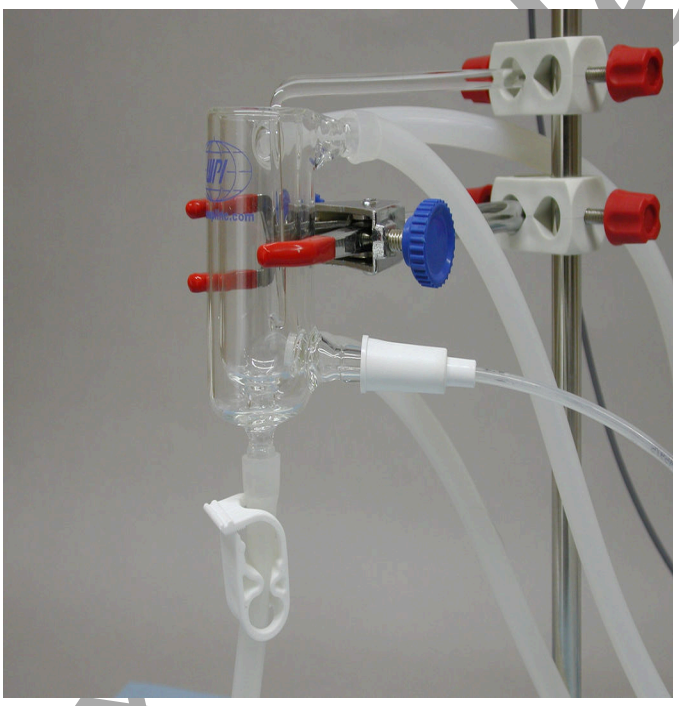

Figure AM-4-S6: 25 ml tissue bath in position for recording tissue tension.

18. Place the end of the drain line in the sink or a flask used to collect waste buffer. Connect the tubing on the water inlet, at the bottom of the tissue bath, to the warm water supply at the sink or to the outlet of a heating circulator. Place the end of the tubing on the water outlet, at the top of the tissue bath, in the sink or on the inlet of the heating circulator.

Calibration of FT-302 Force Transducer

Aim: To calibrate the force transducer used to measure uterine tension.

Procedure

1. Make sure that the IXTA is turned on and the FT-302 force transducer is connected to the DIN8 input for ten minutes before the calibration is performed.
2. Type **No Weight** in the Mark box. Click the Record button, and then click the Mark button. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
3. Type **5 grams** in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Click the Mark button. Record for ten more seconds.
4. Click Stop to halt the recording.
5. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Units Conversion

1. Scroll to the beginning of data when no weight was attached to the FT-302 force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the Display Time of the Main window to show the complete calibration data on the Main window. The required data can also be selected by:
    - Placing the cursors on either side of data required.
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the complete calibration data to the width of the Main window.
3. Click the Double Cursor icon so that two blue cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the FT-302, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.

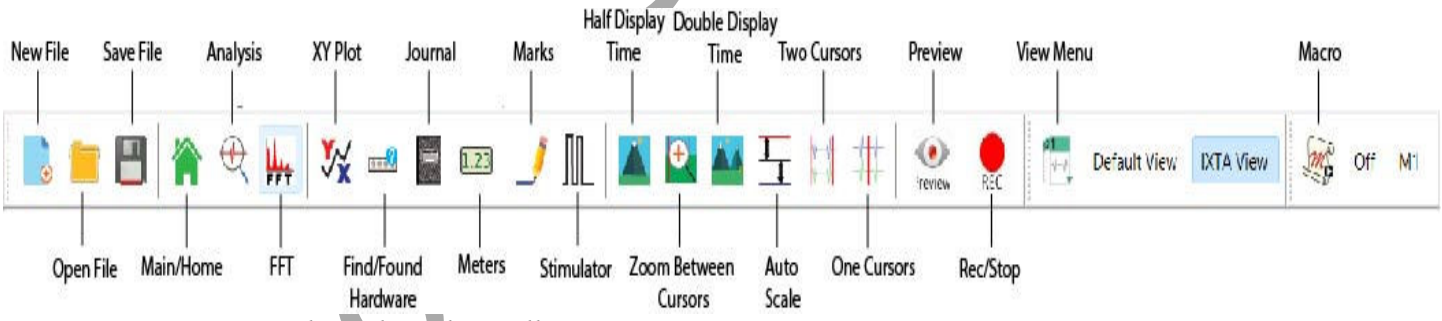

Figure AM-4-S7: The LabScribe toolbar.

4. To convert the voltages at the positions of the cursors to correct values, use the Simple Units Conversion dialogue window. Click V2-V1 on the Uterine Motility channel, then select Units, and select Simple.
    - Select 2 point calibration from the pull-down menu in the upper-left corner of the window.
    - Put a check mark in the box next to Apply units to all blocks.
    - Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter zero in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter 5 in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
    - Enter the name of the units, grams, in box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

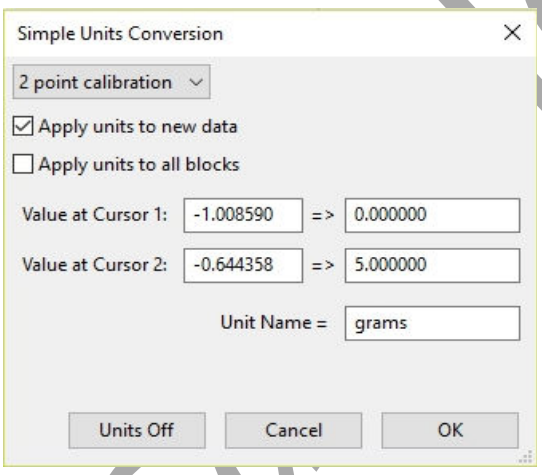

Figure AM-4-S8: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the weight used in calibration.

Calibration of DT-475 Displacement Transducer

Aim: To calibrate the displacement transducer used to measure uterine tension.

Procedure

1. Make sure that the IXTAis turned on and the DT-475 displacement transducer is connected to the DIN8 input for ten minutes before the calibration is performed.
2. Make sure the rod of the transducer is pushed all the way to one end of the track.
3. Type **Initial** in the Mark box. Click the Record button, and then click the Mark button. Record for ten seconds with the transducer at one end of its track.
4. Type **Final** in the Mark box. Push the rod of the transducer all the way to the other end. Click the Mark button. Record for ten more seconds.
5. Click Stop to halt the recording.

6. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder).
7. Measure the length of the tension rod of the transducer, it should be between 4-4.5 cm.
8. Follow the directions for the 2 point calibration of the FT-302:
    - Use the "Initial" position of the tension rod as "0" in the 1st right-hand box in the units conversion window.
    - Use the "Final" position of the rod – length of the movement – as the value to be entered in the 2nd right-hand box.
    - Change the units to cm.
    - Click OK.
9. Click on the Save button to save the data file.

Experiment AM-4: Uterine Motility

Precautions

1. Keep the uterus in well-oxygenated buffer at the experimental temperature at all times. This helps the uterus to function normally for the whole lab period.
2. Complete all the lab exercises before taking time to analyze any of the data. The functionality of the uterus is limited by time. Completing the exercises quickly improves the chances of completing the experiment with the same uterus.
3. The temperature of the fresh buffer used to rinse the uterus and replace the buffer in the chamber should be the same as the temperature of the uterus. Keep flasks of fresh buffer in the water bath at the same temperature as the uterus and the buffer in the chamber.
4. Start the experiment as quickly as possible after the isolation of the uterus. Designate members of the lab group to perform different parts of the equipment setup: opening and setting up the LabScribe software; assembling the tissue chamber, calibrating the transducer; and so on.

Note: Every university has its own rules regarding sacrificing mammals for research or teaching labs. Please be sure to follow the rules of your institution.

Tissue Preparation

Approximate Time: 15-30 minutes

Dissection

1. Sacrifice the female rat by placing it in the air-tight chamber with a piece of dry ice. Carbon dioxide is emitted as the dry ice warms quickly; this humanely kills the rat. Place the rat on its back in the dissection pan and make a mid-line incision along the lower half of the abdomen.
2. Displace the intestines to one side to expose the two horns of the uterus.
3. Tie a suture (15cm long) around the anterior end of each horn of the uterus. Carefully remove any fat and mesentery from the uterus. Tie another suture around each horn close to the point where the uterus bifurcates into the two horns.
4. Remove each horn from the rat. Avoid stretching the uterus. Place both horns in a beaker of aerating Tyrode's solution at 37^{o}C.

Placement of Tissue in Chamber

1. Use a clean beaker to obtain about 100 ml of Tyrode's solution at 37^{o}C from one of the large flasks in a water bath. Take only as much Tyrode's as you need for each rinse or buffer change. Reserve this beaker for transferring clean Tyrode's throughout the exercise.

Note: *Avoid contamination! Do not return any Tyrode's solution taken from the supply flask back to the supply flask!*

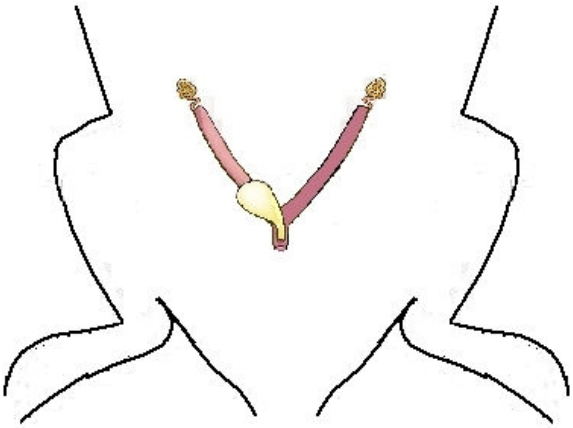

Figure AM-4-L3: Diagram of the position of the uterine horns in the lower abdomen of a female rat.

2. Rinse the tissue chamber thoroughly, three or four times, with Tyrode's solution.
3. Close the drain of the tissue chamber and fill the chamber with about 20ml of Tyrode's solution. Open the valve on the aeration line and adjust the flow of the oxygen/carbon dioxide mixture through the aeration frit to create a plume of small bubbles.
4. Obtain a uterine horn to use in the experiment. Keep the uterus in a beaker or dish of buffer at the desired temperature until you are ready to attach it to the support rod.
5. Work quickly and carefully when mounting the uterus in the chamber. Attach the one end of the uterus to the glass tissue support using a loop of suture thread securely tied to the end of the uterus and looped under the hook of the tissue support rod. Securely tie a piece of suture thread to the other end of the uterus. Make sure the suture thread is long enough to connect the uterus to the transducer. Tissue clips (501902, 501903) can also be used to attach the uterus to suture threads on the hooks of the tissue support and transducer. However, clips may slip off the uterus if the force developed by the uterus is greater than the grip strength of the clips.
6. Once the lower end of the uterus is attached to the hook of the tissue support rod (160172), lower the uterus and its support rod into the tissue chamber. Keep tension on the upper suture thread as the assembly is lowered into the chamber. This will prevent the uterus from coming off the hook on the support rod.
7. Attach the suture thread on the upper end of the uterus to the appropriate hook on the arm of the transducer. The length of the uterus should be no greater than its in situ length.
8. Align the transducer, the tissue bath, and the tissue support rod. The suture thread and the uterus should be vertical, and the uterus should not be touching the inside of the tissue bath.
9. Check the temperature of the tissue bath. Designate a member of your lab group to monitor the temperatures of the tissue bath and water bath holding the flasks of fresh buffer. It will take five to ten minutes for the uterus to recover normal function after it is placed in the warm tissue bath. Slow waves of contraction through the horn should be clearly visible once normal function has been restored.

10. Start recording the tension in the uterus. Click Record. Click the AutoScale button. Observe the position of the trace on the screen as you gradually raise the transducer by turning the adjustment knob on the positioner. Turn the knob until the trace on the screen visibly moves from its initial level. The amount of adjustment required depends on the initial slack in the uterus and the threads holding the uterus.
11. If necessary, adjust the flow of bubbles from the aeration frit to prevent the uterus from being moved around by the bubbles.

Note: *If contractions in the tissue are visible, but do not produce a noticeable movement in the recording, check the tension of the suture threads holding the tissue in place and the operation of the transducer and the recording system.*

Exercise 1: Spontaneous Contractile Activity

Aim: To measure the frequency and amplitude of spontaneous contractions in the rat uterus.

Approximate Time: 30 minutes

Procedure

1. Type **Normal** in the Mark box. Click the Record button, and click the Mark button.
2. Record until the contraction cycles are consistent and predictable. It may take as long as 30 minutes for the uterus to return to a consistent rhythm after it has been isolated from the rat.
3. Click Stop to halt the recording.
4. Select Save in the File menu.

Exercise 2: Effects of Various Agonists and Antagonists

Aim: To examine the effects of different drugs on amplitude and frequency of contractions in uterine tissue.

Approximate Time: 60-90 minutes

The following drugs will be used: Oxytocin, Acetylcholine, Atropine, and Epinephrine. Other drugs that cause changes in uterine tone or contractions are: Leucine encephaline, Naloxone, and Methergine.

Procedure

1. Type **Control 1** in the Mark box. Click Record button and then click the Mark button.
2. Before adding the first agonist to the uterine preparation, make sure the frequency and amplitude of the uterine contractions are consistent for two or three successive cycles. Continue recording.
3. Type **Oxytocin** in the Mark box.
4. Add one drop of the solution containing Oxytocin to the smooth muscle chamber. Click the Mark button at the same time the drug is added to the chamber.

5. If there is no effect in ten minutes, add a second drop of Oxytocin to the chamber and mark the recording.
6. Click Stop to halt the recording when the uterine response to the drug appears consistent and predictable.
7. Select Save in the File menu.
8. Remove the solution from the muscle bath chamber. Carefully rinse the uterine preparation and the muscle bath chamber with fresh Tyrode's solution that was kept at 37^{o}C. Repeat the rinsing of the tissue preparation and the chamber with fresh, warm saline to remove any remnants of the agonist from the tissue. Any residue of an agonist on the tissue or in the chamber could cause multiple drug effects.
9. Refill the chamber containing the uterine tissue with fresh Tyrode's solution at 37^{o}C.

Warning: After oxytocin is administered to the uterine preparation, the remaining drugs should be administered in the order listed. Epinephrine can have a permanent effect on the tissue, so it is the last drug to be tested.

9. Prepare to add the next drug to the uterine preparation. Apply the other agonists or antagonists to the muscle chamber in the order listed below:
    - Leucine Encephaline, an endogenous opiate with morphine-like effects on smooth muscle. Add one drop to the smooth muscle chamber. Do not remove the Leucine Encephaline before adding the next drug, Naloxone, the antagonist to Leucine Encephaline.
    - Naloxone, which reverses or prevents the effects of opioid drugs. Add one drop to the smooth muscle chamber while Leucine Encephaline is still present
    - Methergine, an obstetrical herb that is used to increase the force and frequency of contractions. Add one drop to the smooth muscle chamber.
    - Acetylcholine, a neurotransmitter that binds to muscarinic receptors to cause the contraction of smooth muscle. Add one drop to the smooth muscle chamber. If there is no effect in ten minutes, add another drop.
    - Atropine, a competitive antagonist of muscarinic cholinergic receptors that blocks the action of acetylcholine and causes relaxation of smooth muscles. To demonstrate the effect of Atropine, add one drop of Atropine to the smooth muscle chamber, wait a few minutes to see if there is any effect, then add a drop of Acetylcholine to the chamber while Atropine is still present in the chamber.
    - Epinephrine, a neurotransmitter that binds to beta-adrenergic receptors to cause the relaxation of smooth muscle. Add one drop of Epinephrine to the smooth muscle chamber. If there is no effect in ten minutes, add a second drop of Epinephrine to the chamber. Epinephrine is the last drug that should be tested since it can have a permanent effect on tissue.

10. Type **Control 2** in the Mark box. Click Record and record the contractions of the uterine preparation as it equilibrates to the fresh saline in the chamber. When the contractions of the uterus are consistent and predictable, press the Enter key on the keyboard to mark the recording.
11. Type the name of the next drug to be tested in the Mark box. Click Record and click the Mark button as the dose of the new drug is added to the smooth muscle chamber. Continue recording.
12. Click Stop to halt the recording when the uterine response to the drug appears to be consistent and predictable.
13. Select Save in the File menu.
14. Repeat Steps 7 through 13 for each new drug. Use the dosage and application method for each drug as listed in Step 9.
15. Remember to rinse the last drug from the uterine preparation and the smooth muscle chamber, using the same techniques explained in Steps 7 and 8.
16. Refill the chamber with fresh, warm Tyrode's solution.

Exercise 3: Length and Tension

Aim: To measure spontaneous contraction in the uterus stretched to different lengths. This treatment is analogous to preloading a muscle with different weights.

Approximate Time: 30 minutes

Procedure

1. Type **No Added Weight**, or **No Added Tension**, in the Mark box. Click the Record button.
2. When the contraction cycles are consistent and predictable, Click the Mark button. Continue recording.
3. Use a ruler to measure the length of the uterus (from ligature to ligature) when the uterus is fully relaxed. Type the length of the relaxed uterine tissue in the Mark box. Click the Mark button.
4. Click Stop to halt the recording.
5. Select Save in the File menu.

DT-475 Displacement Transducer

1. Add more clay to the counterweight that stretches the uterine tissue. More weight will increase the stretch or preload on the uterine tissue.
2. Repeat Steps 1 through 5 with the additional weight stretching the uterine tissue. Mark the recording appropriately.
3. Increase the weight of the counterweight by adding a small ball of clay (~ 5 mm in diameter) to it. Repeat Steps 1 through 5 until the relaxed length of the uterus stops increasing or the amplitudes of the spontaneous contractions decrease.

FT-302 Force Transducer

1. Turn the red adjustment knob on the transducer positioner to increase the length of the uterine tissue by a few millimeters.
2. Repeat Steps 1 through 5 with the additional stretching of the uterine tissue. Mark the recording appropriately.
3. Increase the length of the tissue and repeat Steps 1 through 5 until the amplitudes of the contractions decrease.

Data Analysis

Exercise 1-Spontaneous Contractile Activity

1. Scroll through the data file and locate a section near the end of the recording where the amplitude and period of the uterine contraction cycle is consistent.
2. Use the Display Time icons to adjust the Display Time of the Main window so that two uterine contraction cycles are displayed on the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. Values for V2-V1 and T2-T1 on each channel are seen in the table across the top margin of each channel, or to the right of each graph.
5. Maximize the height of the trace on the Uterine Tension Channel by clicking on the AutoScale All button on the toolbar.
6. Once the cursors are placed in the correct positions for determining the amplitude and period of each muscle twitch, the values of the parameters in the Function Table can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal, or on a separate data table.
7. The functions in the channel pull-down menus of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the amplitude and times of each muscle twitch.
    - Transfer the names of the mathematical functions used to determine the amplitude and times to the Journal using the Add Title to Journal function in the Uterine Tension Channel pull-down menu.
    - Transfer the values for the amplitude and times to the Journal using the Add Ch. Data to Journal function in the Uterine Tension Channel pull-down menu.

8. On the Uterine Tension Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - Contraction Amplitude is the active tension, or phasic response, developed in the uterus during its contraction. To measure this parameter, place one cursor at the beginning of the contraction, and the second cursor on its peak. The value for the V2-V1 function on the Uterine Tension Channel is the contraction amplitude.
    - Contraction Time is the time between the beginning and the peak of the contraction. To measure this parameter, keep the cursors in the same positions used to measure the contraction amplitude. The value for the T2-T1 function on the Uterine Tension Channel is the contraction time.
    - Relaxation Time is the time between the peak and the end of the contraction. To measure this parameter, keep the cursor on the peak of the contraction and place the other cursor at the end of the contraction. The value for the T2-T1 function on the Uterine Tension Channel is the relaxation time.
    - Contraction Period is the time between the beginnings of adjacent contractions. To measure this parameter, place one cursor at the beginning of one contraction and the other cursor at the beginning of the adjacent contraction. The value for the T2-T1 function on the Uterine Tension Channel is the contraction period.
    - Uterine Tone is the passive tension, or tonic response, present in the uterus before or after the contraction. To measure this parameter, keep the cursors in the same positions used to measure the contraction period. Value1 on the Uterine Tension Channel is the tone of the uterus at the beginning of a contraction, and Value2 is the uterine tone at the beginning of the adjacent contraction.
9. Record the values in the Journal using the one of the techniques described in Steps 6 or 7, and on Table 1.
10. Repeat Steps 2 through 9 to find the contraction amplitude, contraction time, relaxation time, contraction period, and uterine tone of two other uterine contractions recorded in this exercise. Record the values in the Journal and on Table 1.
11. Select Save in the File menu.
12. Determine the average contraction period of the three uterine contractions measured. Determine the average frequency of uterine contraction by finding the inverse of the contraction period.

Exercise 2-Effects of Various Agonists and Antagonists

1. Scroll to the section of data recorded for the control and the activity of each agonist or antagonist, and use the techniques explained in the data analysis section of Exercise 1 to measure the contraction amplitude, contraction time, relaxation time, contraction period, and uterine tone.
2. Enter the data in the Journal using one of the techniques explained in the data analysis section of Exercise 1, and on Table 1.

Questions-Effects of Various Agonists

1. What is the effect of each drug on the amplitude of the uterine contraction?
2. What is the effect of each drug on the frequency of uterine contractions?
3. What is the effect of each drug on tone of the uterine muscle?
4. For one of the drugs, hypothesize a mechanism by which the drug affects the contractility of the uterine muscle.

Exercise 3-Length and Tension

1. Scroll to the sections of data recorded for each of the relaxed lengths of the uterine tissue, and use the techniques explained in the data analysis section of Exercise 1 to measure the contraction amplitude, contraction time, relaxation time, contraction period, and uterine tone.
2. Enter the data in the Journal using one of the techniques explained in the data analysis section of Exercise 1, and on Table 2.

Questions-Length and Tension

1. How do the amplitudes of uterine contractions depend upon the tissue length?
2. How does the tone of uterine tissue depend upon the tissue length?
3. How does the frequency of uterine contractions depend upon tissue length?
4. Do your observations support the sliding filament theory for muscle contraction?
5. Do your observations supply evidence for plasticity?
6. How do your results for this smooth muscle compare to the length-tension relationship seen in skeletal muscle?

Table AM-4-L1: The Contraction Amplitude, Contraction Period, and Tone of Uterine Tissue Exposed to Different Agonists and Antagonists.

Treatment	Contraction Amplitude (g)	Contraction Time (sec)	Relaxation Time (sec)	Contraction Period (sec)	Uterine Tone (g) at Beginning	Uterine Tone (g) at End
Normal Contraction 1						
Normal Contraction 2						
Normal Contraction 3						
Normal Average						
Control 1						
Oxytocin						
Control 2						
Leu Encephaline						
Control 3						
Naloxone						
Control 4						
Methergine						
Control 5						
Acetylcholine						
Control 6						
Atropine-Acetylcholine						
Control 7						
Epinephrine						

Table AM-4-L2: The Contraction Amplitude, Contraction Period, and Tone of Uterine Tissue Stretched to Different Relaxed Lengths.

Relaxed Length (mm)	Contraction Amplitude (g)	Contraction Time (sec)	Relaxation Time (sec)	Contraction Period (sec)	Uterine Tone (g) at Beginning	Uterine Tone (g) at End