

## Experiment AM-4: Uterine Motility

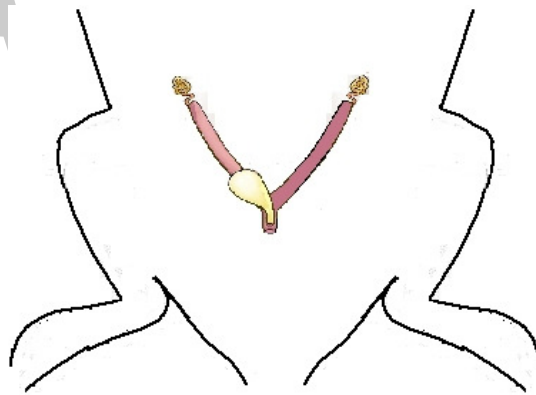
### Precautions

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.

### Tissue Preparation

#### 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 ([Figure AM-4-L3](#)).
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°C.



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

### ***Placement of Tissue in Chamber***

1. Use a clean beaker to obtain about 100 ml of Tyrode's solution at 37°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!*

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 on the Start button in the upper right corner of the LabScribe Main window. Click the AutoScale button on the upper margin of the recording channel. 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.

#### ***Procedure***

1. Type Normal in the Mark box to the right of the Mark button. Click the Record button, and press the Enter key to attach a comment to the recording.
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**

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

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 to the right of the Mark button. Click the Record button, and press the Enter key to attach a comment to the recording.
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. Press the Enter key on the keyboard to mark the recording at the same time the drug is added to the chamber. If there is no effect in ten minutes, add a second drop of Oxytocin to the chamber and mark the recording.
5. Click Stop to halt the recording when the uterine response to the drug appears consistent and predictable.
6. Select Save in the File menu.

7. 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°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.
8. Refill the chamber containing the uterine tissue with fresh Tyrode's solution at 37°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 Enkephalin, an endogenous opiate with morphine-like effects on smooth muscle. Add one drop to the smooth muscle chamber. Do not remove the Leucine Enkephalin before adding the next drug, Naloxone, the antagonist to Leucine Enkephalin.
  - Naloxone, which reverses or prevents the effects of opioid drugs. Add one drop to the smooth muscle chamber while Leucine Enkephalin 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 the Record button. 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 the Record button. Press the Enter key 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.

#### ***Procedure***

1. Type No Added Weight, or No Added Tension, in the Mark box to the right of the Mark button. Click the Record button.
2. When the contraction cycles are consistent and predictable, press the Enter key to attach a mark to the recording. 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. Press the Enter key to mark the recording.
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. This section of data can also be selected by:
  - Placing the cursors on either side of two uterine contraction cycles of the recording, and
  - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand or contract the two uterine contraction cycles to the width of the Main window.
3. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
4. Look at the Function Table that is above the Uterine Tension Channel in the Analysis window. The mathematical functions, Value1, Value2, V2-V1, and T2-T1, should appear in this table. The values for these parameters are displayed in the table across the top margin of the Uterine Tension channel.
5. Maximize the height of the trace on the Uterine Tension Channel by clicking on the arrow to the left of the channel's title to open the channel menu. Select Scale from the menu and AutoScale from the Scale submenu to increase the height of the data on that channel.
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 AM-4-L1](#).
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 AM-4-L1](#).
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***

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 AM-4-L1](#).

### ***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 AM-4-L2](#).

### ***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 Enkephaline						
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

iWorx Sample Lab