

Experiment AM-5: Intestinal Motility

by: Dayton Ford, Ph.D.; Margaret Weck, D.A.; R.J. Cooper, Ph.D.; at the St. Louis College of Pharmacy. Based on: "Experiment 13, Intestinal Motility" in *The Function of Life: A Laboratory Guide for Animal Physiology*, by J.D. Witherspoon, Addison-Wesley (1970).

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 38°C

Heating circulator, set at 38° C (Optional)

Suture thread and needle

Pasteur pipets and bulbs

Air-tight chamber, and dry ice or CO₂ supply

Dissection pan and instruments

Cylinder with a mixture of 95% O₂ & 5% CO₂

Regulator, valve, and tubing for oxygenation setup

Tyrodé's Physiological Saline Solution (See appendix)

Various reagents in Tyrodé's Physiological Saline Solution (See appendix)

DT-475

1. Locate the DT-475 displacement transducer.



Figure AM-5-S1: The DT-475 displacement transducer.

2. Insert the DIN connector on the cable of the DT-475 displacement transducer into Channel A5.



Figure AM-5-S2: The DT-475 displacement transducer connected to an iVortex TA-ROAM system.

FT-302

The FT-302 dual range force transducer is an optional device available from iVortex that can 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-5-S3: The FT-302 dual-range force transducer.

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



Figure AM-5-S4: The FT-302 dual-range force transducer connected to an IXTA with a male DIN-DIN cable.

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 in 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.

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 tissue in the chamber.
6. If you need to hold another device, like an electrode, on the tissue or above the tissue chamber, 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.

Table AM-5-29: 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

7. Place the transducer positioner (502198) on the stainless steel rod with 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.
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 intestine muscle.

- When using the FT-302, use the hook for the appropriate range of tension you expect in the experiment.

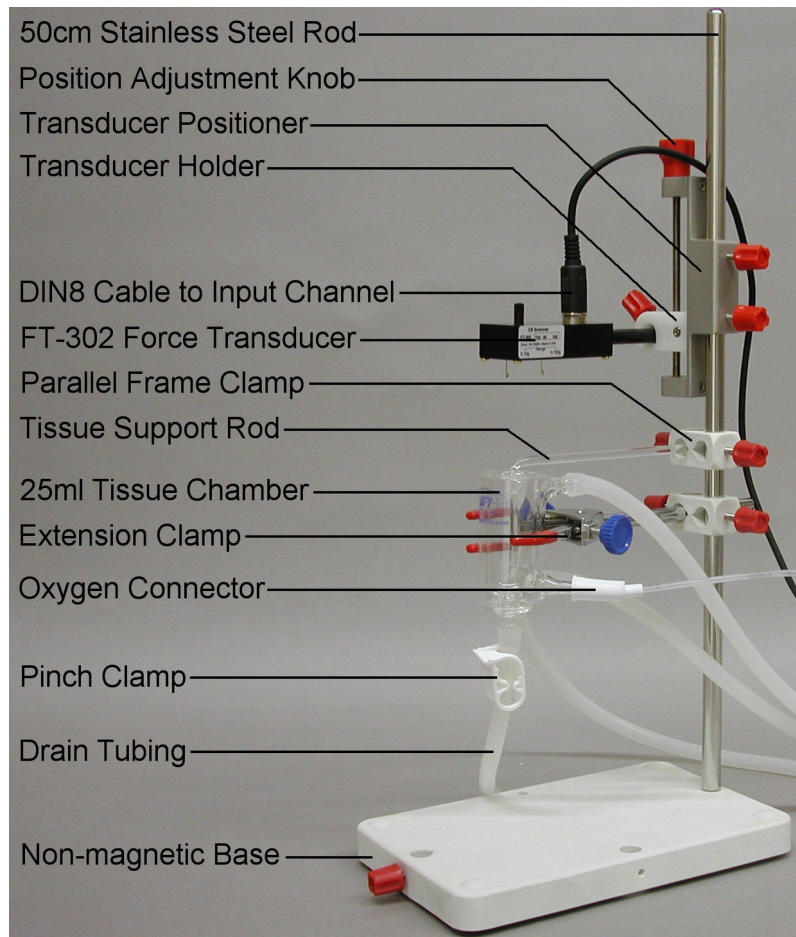


Figure AM-5-S5: STB-125 student tissue bath with a FT-302 force transducer.

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 tissue, and the data acquisition system used to record the responses of the tissue.
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% O₂ and 5% CO₂.



Figure AM-5-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 the FT-302 Force Transducer

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

Procedure

1. Make sure that the IXTA is turned on and the 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 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 force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.

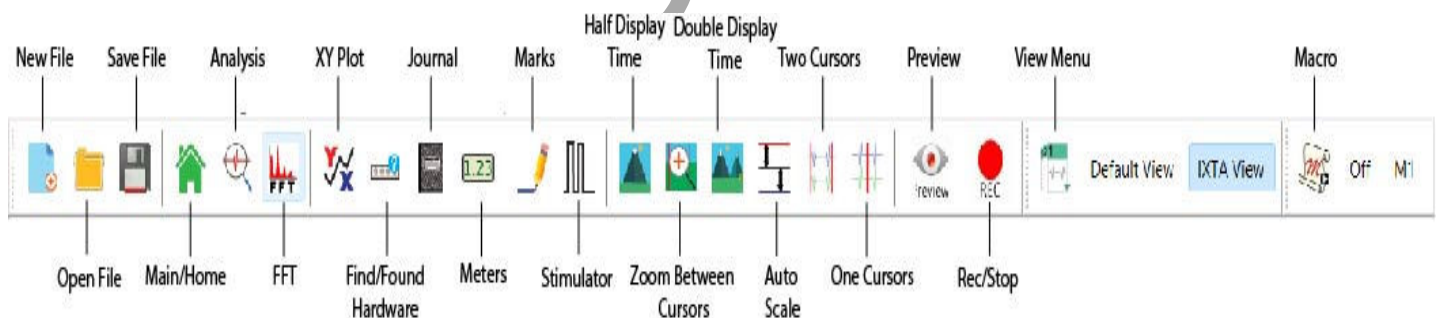


Figure AM-5-S7: The LabScribe toolbar.

4. To convert the output of the force transducer from a voltage to the grams of force:
 - Click V2-V1 to the right of the Intestinal Tension channel.
 - Select Simple.

5. The Simple Units Calibration window will appear. On this window:

- 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.

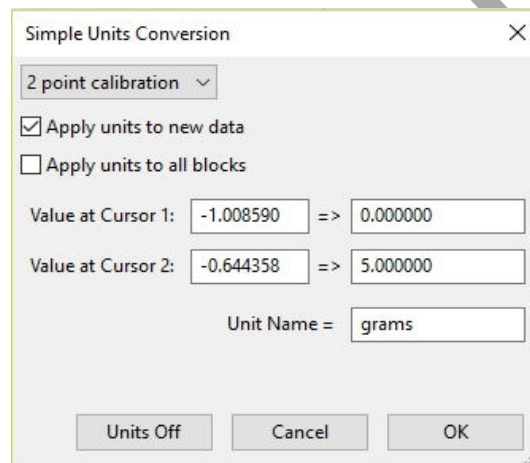


Figure AM-5-S8: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the weight used in calibration.

Calibration of DT-475 Displacement Transducer

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

Procedure

1. Make sure that the IXTA is 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. Click on the Save button to save the data file.
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.
9. Click OK.
10. Click on the Save button to save the data file.

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Precautions

1. Keep the tissue in well-oxygenated buffer at the experimental temperature at all times. This helps the tissue 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 tissue is limited by time. Completing the exercises quickly improves the chances of completing the experiment with the same tissue segment.
3. The temperature of the fresh buffer used to rinse the tissue and replace the buffer in the chamber should be the same as the temperature of the tissue. Keep flasks of fresh buffer in the water bath at the same temperature as the buffer in the tissue chamber.
4. Start the experiment as quickly as possible after the isolation of the tissue. 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.

Placement of Tissue in Chamber

1. Use a clean beaker to obtain about 100 ml of Tyrode's solution at 38°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 piece of intestinal muscle to use in the experiment. Keep the strip of muscle 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 muscle in the chamber. Attach the one end of the muscle to the glass tissue support using a loop of suture thread securely tied to the muscle and looped under the hook of the tissue support rod. Securely tie a piece of suture thread to the other end of the muscle. Make sure the suture thread is long enough to connect the muscle to the transducer. Tissue clips (501902, 501903) can also be used to attach the muscle to suture threads on the hooks of the tissue support and transducer. However, clips may slip off the muscle if the force developed by the muscle is greater than the grip strength of the clips.

6. Once the lower end of the muscle is attached to the hook of the tissue support rod (160172), lower the muscle 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 muscle from coming off the hook on the support rod.
7. Attach the suture thread on the upper end of the muscle to the appropriate hook on the arm of the transducer. The length of the muscle should be no greater than in situ length.
8. Align the transducer, the tissue bath, and the tissue support rod. The suture thread and the muscle should be vertical, and the muscle 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 three to five minutes for the muscle to recover normal function after it is placed in the warm tissue bath. Slow waves of contraction through the strip should be clearly visible once normal function has been restored.
10. Record recording the tension in the muscle. Click on the Record button. 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 muscle and the threads holding the muscle.
11. If necessary, adjust the flow of bubbles from the aeration frit to prevent the muscle from being moved around by the bubbles.

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

Warning: *Intestinal muscle is active for a limited period of time, so it is necessary to complete the exercises as quickly as possible, Postpone analysis of all the data until all the exercises are completed.*

Exercise 1: Spontaneous Contractile Activity

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

Approximate Time: 15 minutes

Procedure

1. Type **Normal** in the Mark box. Click the Record button and then click the Mark button..
2. Record until the contraction cycles are consistent and predictable. It may take a few minutes for the intestine 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: Effect of Stretch on Intestinal Smooth Muscle

Aim: To measure spontaneous contraction in the intestine stretched to different lengths; in this case, it is the same as being preloaded with different weights.

Approximate Time: 20 minutes

Procedure

1. Once a consistent and predictable contraction has been established in the muscle during Exercise 1, begin recording data for the first basal activity period. Type **Basal Activity 1** in the Mark box to the right of the Mark button. Click the Record button, and then click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer).
2. While the basal activity is recorded, use a ruler to measure the length of the intestine (in mm, from ligature to ligature) when the intestine is fully relaxed. Type the relaxed length of the intestinal muscle in the Mark box and press the Mark button.

If using the DT-475 Displacement Transducer

1. Increase the tension of the intestinal muscle by adding more clay to the counterweight that stretches the muscle. Each time you want to add weight to the counter weight add a small ball of clay (~ 5 mm in diameter) to it.
2. Type a comment in the Mark box and enter it on the recording to indicate the change in the tension. Record for another 30 seconds, or until the contractions are consistent. Again, it may take longer than 30 seconds for the contractions to become consistent. Continue recording.
3. Decrease the tension of the intestinal muscle by removing clay from the counterweight that stretches the muscle. Each time you want to remove weight from the counterweight remove a small ball of clay (~ 5 mm in diameter).
4. Type a comment in the Mark box and enter it on the recording to indicate the change in the tension. Keep enough tension on the muscle so that contractions still appear on the recording.

If using the FT-302 Force Transducer

1. Keep recording from the muscle as the tension on the muscle is increased by turning the red adjustment knob on the transducer positioner one full turn.
2. Type a comment in the Mark box and enter it on the recording (click the Mark button) to indicate the change in the tension. Record for another 30 seconds, or until the contractions are consistent. Again, it may take longer than 30 seconds for the contractions to become consistent. Continue recording.
3. Keep recording from the muscle as the tension on the muscle is decreased by turning the red adjustment knob on the transducer positioner one full turn in the opposite direction.
4. Type a comment in the Mark box and enter it on the recording to indicate the change in the tension. Keep enough tension on the muscle so that contractions still appear on the recording.

5. Click Stop to halt the recording.
6. Before proceeding to the next exercise, make sure the contraction amplitude, contraction frequency, and tone of the intestinal muscle can be easily identified on the recording.
7. Select Save in the File menu.

Note: Remember to complete all the exercises before taking measurements and analyzing the data.

Exercise 3: Effect of Acetylcholine

Aim: To examine the effect of acetylcholine on the tone, amplitude, and frequency of smooth muscle contractions.

Approximate Time: 20 minutes

Procedure

1. Remove the Tyrode's solution from the chamber holding the intestinal muscle. Rinse the intestinal muscle with fresh, warm solution. Replace the solution in the chamber with fresh, warm Tyrode's solution.
2. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.

Note: If the muscle shows no activity, try rinsing it again with fresh Tyrode's solution until visible contractions resume. It may be necessary to adjust the tension on the muscle so that the amplitudes of the individual contractions are clearly visible as separate events.

3. Type **Basal Activity-2** in the Mark box. Click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording
4. Type **One Drop-0.1% ACH** in the Mark box.
5. Add one drop of 0.1% acetylcholine solution to the Tyrode's solution in the bath chamber as the Mark button is clicked.

Note: The time it takes the intestinal muscle to respond to the drug depends on the rate of diffusion of the drug from the surface of the tissue to the muscles.

6. The effect of acetylcholine is usually dramatic. If the intestinal tissue does not respond to the drop of acetylcholine solution after one minute, add another drop of acetylcholine solution to the bath chamber. Mark the recording accordingly.
7. If no effect is seen within one minute of the addition of the second drop, add a third drop of acetylcholine solution to the bath chamber. Once an effect is noticed, continue to record the intestinal contractions until they are consistent.

8. Click Stop to halt the recording when the intestinal activity appears consistent.
9. Select Save in the File menu.
10. Remove the Tyrode's solution containing the acetylcholine from the bath chamber. Carefully rinse the intestinal tissue and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
11. Replace the solution in the chamber with fresh, warm Tyrode's solution.

Exercise 4: Blocking of Acetylcholine Receptors

Aim: To determine the effects of two different acetylcholine blocking agents, curare and atropine, on intestinal motility.

Approximate Time: 20 minutes per blocking agent

Procedure

1. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.
2. Type **Basal Activity-3** in the Mark box and click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording
3. Type **Two Drops-1.0% Curare** in the Mark box.
4. Add two drops of 1.0% curare (d-tubocurarine) to the Tyrode's solution in the bath chamber and then click the Mark button. Wait at least 45 seconds to see if the curare causes any change in the motility of the intestine. Continue recording.
5. Type **0.1% Acetylcholine** in the Mark box.
6. Add acetylcholine to the Tyrode's solution in the bath chamber as the Mark button is clicked. Use the smallest amount of acetylcholine that produced a visible effect in Exercise 3. If no effect is seen within thirty seconds of the addition of this dose of acetylcholine, add another drop or two of the drug. Mark the recording when each additional drop is added to the bath chamber. Wait thirty seconds after the addition of each drop before adding additional drops.
7. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording.
8. Select Save in the File menu.
9. Remove the Tyrode's solution containing the curare and acetylcholine from the bath chamber. Carefully rinse the intestinal tissue and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
10. Replace the solution in the chamber with fresh, warm Tyrode's solution.
11. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable proceeding with the exercise.

12. Type **Basal Activity-4** in the Mark box and click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording
13. Type **Two Drops, 0.5% Atropine** in the Mark box.
14. Add two drops of 0.5% atropine to the Tyrode's solution in the bath chamber as the Mark button is clicked. Wait at least 45 seconds to see if the atropine causes any change in the motility of the intestine. Continue recording.
15. Add acetylcholine to the Tyrode's solution in the bath chamber as the Mark button is pressed. Use the smallest amount of acetylcholine that produced a visible effect in Exercise 3. If no effect is seen within thirty seconds of the addition of this dose of acetylcholine, add another drop or two of the drug. Mark the recording when each additional drop is added to the bath chamber. Wait thirty seconds after the addition of each drop before adding additional drops.
16. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording.
17. Select Save in the File menu.
18. Remove the Tyrode's solution containing the atropine and acetylcholine from the bath chamber. Carefully rinse the intestinal muscle and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
19. Replace the solution in the chamber with fresh, warm Tyrode's solution.

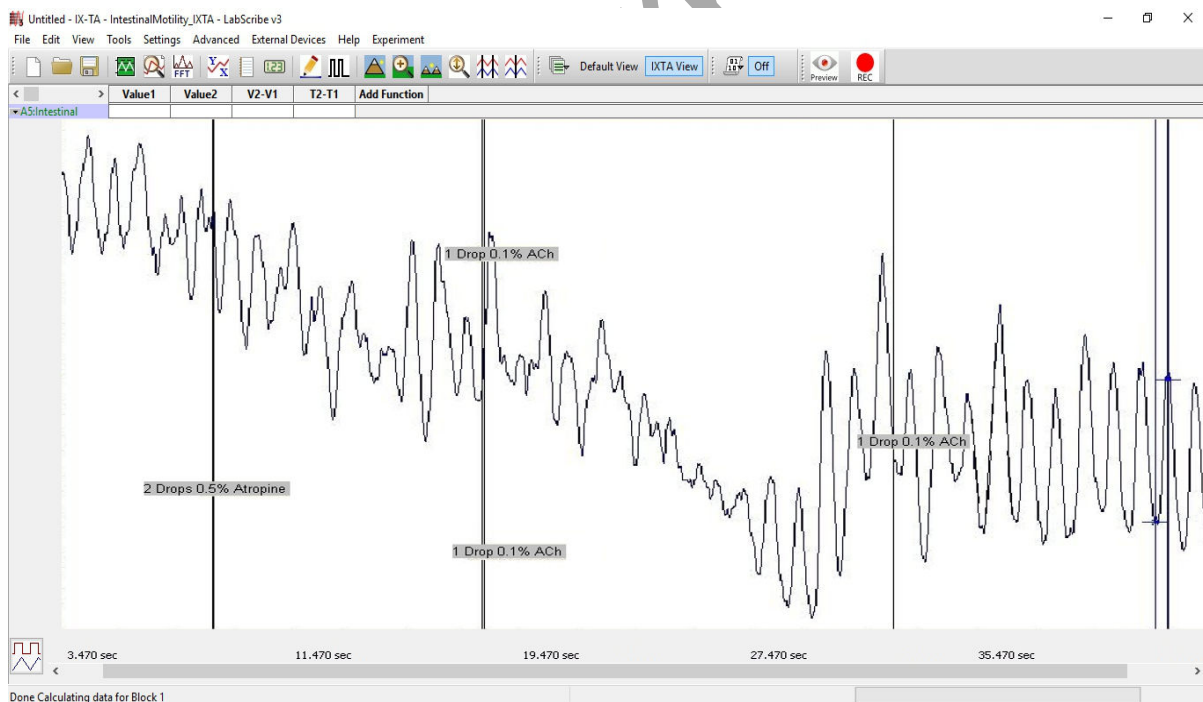


Figure AM-5-L1: The effect of atropine on intestinal muscle motility followed by the effect of acetylcholine in the presence of atropine.

Exercise 5: Effect of Epinephrine

Aim: To examine the effect of epinephrine on the tone, amplitude, and frequency of muscle contractions.

Approximate Time: 20 minutes

Procedure

1. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.
2. Type **Basal Activity-5** in the Mark box and then click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording.
3. Type **One Drop-0.05% Epinephrine** in the Mark box. Add one drop of 0.05% epinephrine to the Tyrode's solution in the bath chamber as the Mark button is clicked. Wait at least 45 seconds to see if the epinephrine causes any change in the motility of the intestine. Continue recording. Add another drop of 0.05% epinephrine to the bath chamber.
4. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording.
5. Select Save in the File menu.

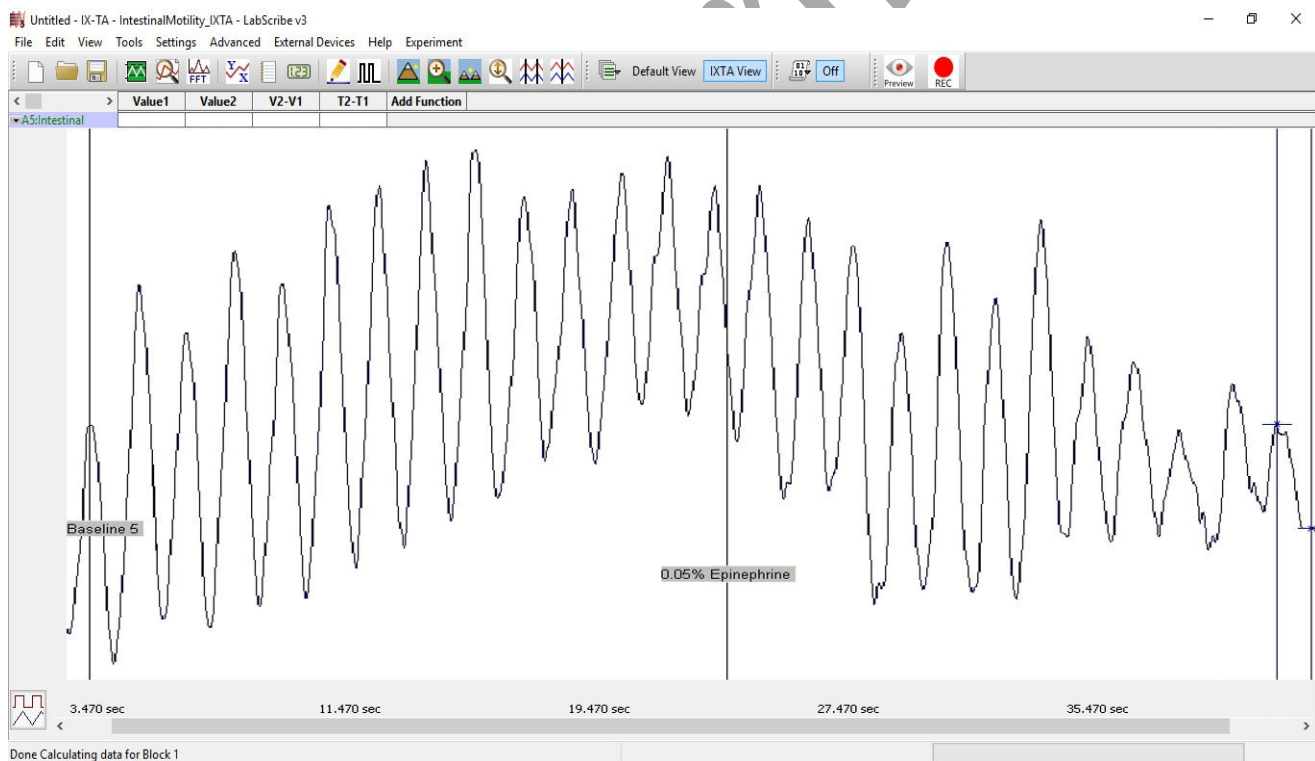


Figure AM-5-L2: The effect of epinephrine on intestinal muscle motility.

6. Remove the Tyrode's solution containing the epinephrine from the bath chamber. Carefully rinse the intestinal tissue and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
7. Replace the solution in the chamber with fresh, warm Tyrode's solution.

Exercise 6: Effect of Serotonin

Aim: To examine the effect of serotonin (5-Hydroxytryptamine) on the tone, amplitude, and frequency of smooth muscle contractions.

Approximate Time: 20 minutes

Procedure

1. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.
2. Type **Basal Activity-6** in the Mark box and then click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording
3. Type **One Drop-0.01% Serotonin** in the Mark box.
4. Add 1 drop of 0.01% serotonin (5-hydroxytryptamine) to the Tyrode's solution in the bath chamber as the Mark button is clicked. Wait at least 30 seconds to see if the serotonin causes any change in the motility of the intestine. Continue recording. Add another drop of 0.01% serotonin to the bath chamber.
5. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording.
6. Select Save in the File menu.
7. Remove the Tyrode's solution containing the serotonin from the bath chamber. Carefully rinse the intestinal tissue and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
8. Replace the solution in the chamber with fresh, warm Tyrode's solution.

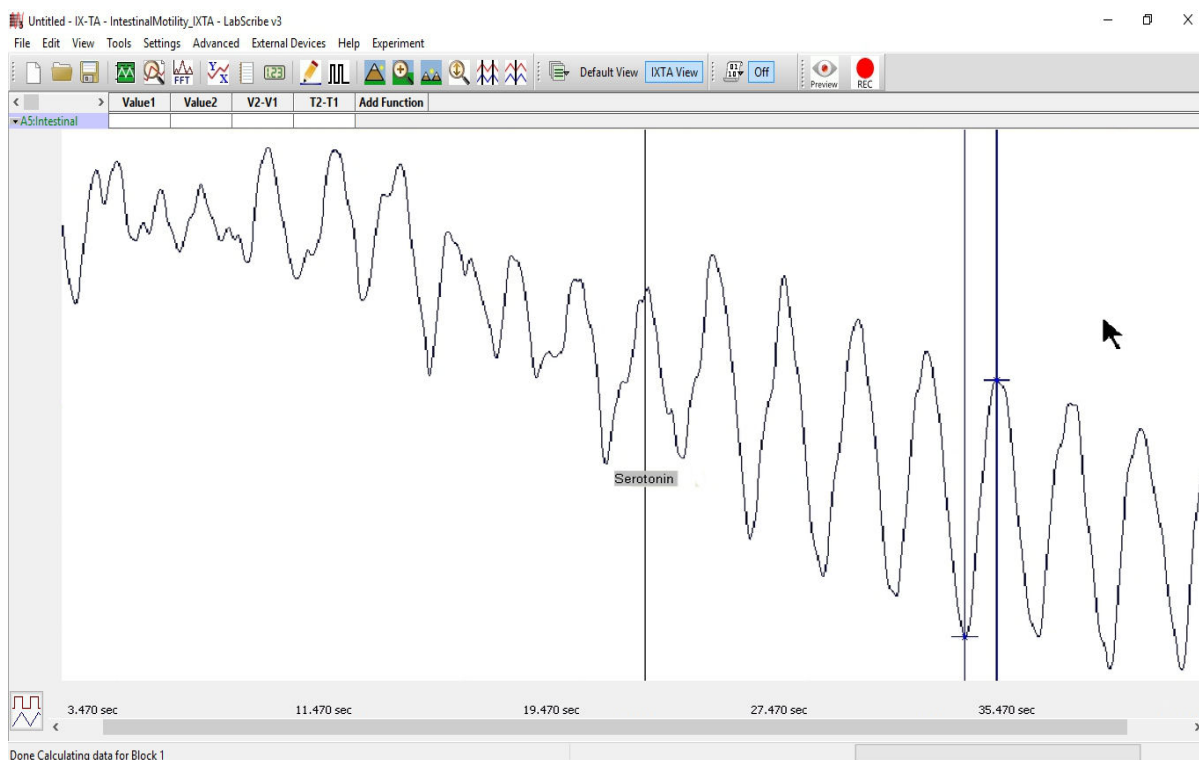


Figure AM-5-L3: Effects of serotonin on intestinal muscle motility.

Exercise 7: Effect of pH

Aim: To investigate the effects of lowering the pH of the fluid surrounding the tissue.

Approximate Time: 30 minutes

The gastric contents are acid (pH of 2 in mammals), but after entering the duodenum, the luminal contents become neutral, as bile from the liver and bicarbonate from the pancreas are added. The lining of the intestine also secretes its own alkaline fluid, so by the time food reaches the ileum, the pH is 7.5 to 8 (about the pH of Tyrode's solution). This raises the question of whether alkalinity helps or hinders intestinal motility. Because Tyrode's is already alkaline, we will lower the pH of the fluid and make it more acidic surrounding the tissue specimen.

Procedure

1. Begin this section of the exercise with slightly more Tyrode's solution in the apparatus than usual.
2. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced.
 - The contractions should be consistent and predictable before proceeding with the exercise.
3. Type **Basal Activity-7** in the Mark box and then click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording.

4. Use the pH paper provided to measure the acidity of the Tyrode's solution in the bath chamber. Record this value as the Initial pH.
5. Type **Five Drops-5% HCl** in the Mark box.
6. Add five drops of 5% hydrochloric acid (HCl) to the Tyrode's solution in the bath chamber as the Mark button is clicked. Wait 15 seconds to see if the hydrochloric acid causes any change in the motility of the intestine.
7. If there is no change in the muscle's motility, add more 5% HCl in aliquots of five drops each. Wait fifteen seconds between additions. Add aliquots of 5% HCl until a sustained change in the motility of the intestine is demonstrated.
8. Use the pH paper to measure the acidity of the Tyrode's solution in the bath chamber. Record this pH value. If the pH of the solution is still >5 , add an additional 8-10 drops of HCl and record the pH again.
9. To verify the effects of acidity of smooth muscle activity, add 5% sodium hydroxide (NaOH) to the Tyrode's solution in the bath chamber in aliquots of three drops each. Add aliquots of 5% NaOH until the motility of the intestine is the same as at the beginning of Basal Activity -7.
10. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording. Measure and record the pH of the solution in the bath chamber.
11. Select Save in the File menu.

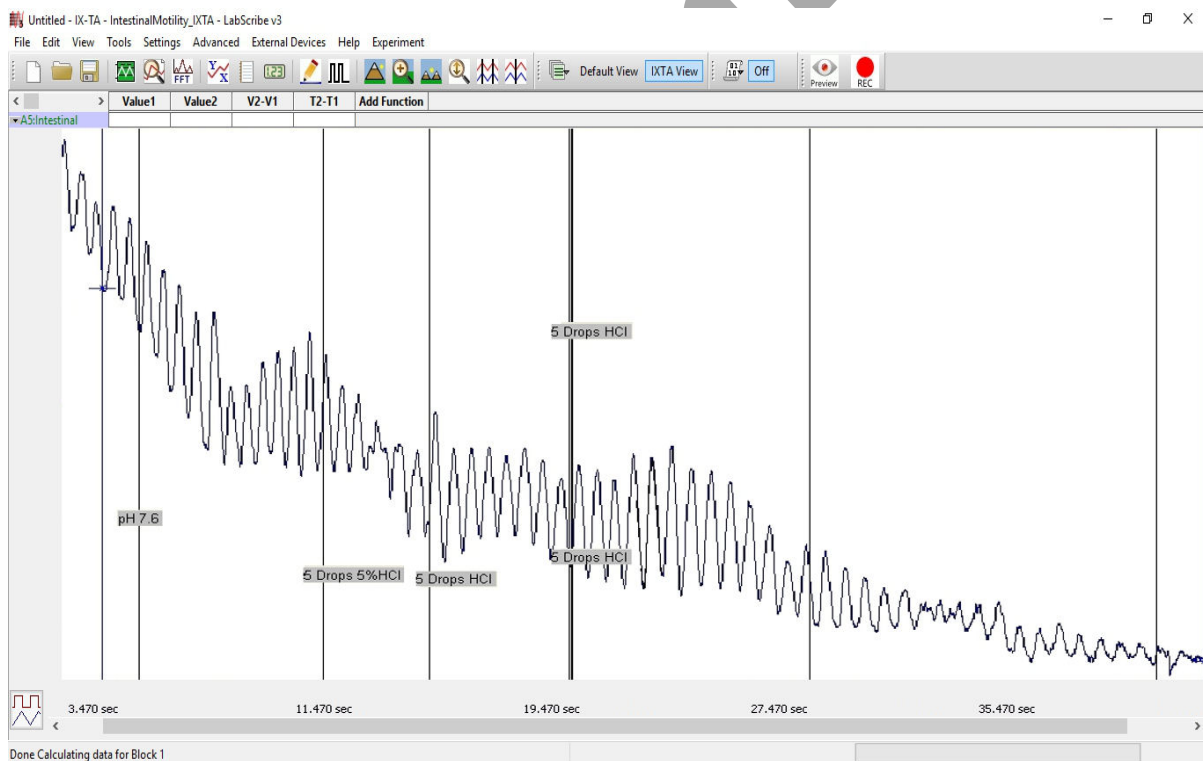


Figure AM-5-L4: The effect of increasing acidity on intestinal muscle motility.

12. Remove the Tyrode's solution containing acids and bases from the bath chamber. Carefully rinse the intestinal tissue and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the tissue and reduces the occurrence of multiple drug effects.
13. Replace the solution in the chamber with fresh, warm Tyrode's solution.

Exercise 8: Effects of Calcium Levels

Aim: To investigate the effects of calcium ion concentration in the extracellular fluid on smooth muscle contractions.

Approximate Time: 30 minutes

Calcium ions have what appear to be two contradictory actions on muscle cells, depending on the concentration of calcium ions in the extracellular fluid. This is especially true for those cells that rely most heavily on the extracellular fluid as their primary reservoir of calcium ions for contraction. In smooth muscle cells, sufficient calcium ions must be present to bind with calmodulin and activate the myosin kinase, initiating the latch bridge cycle of smooth muscle contraction. If, however, too many calcium ions are present, they can get "stuck" in sodium channels, thus decreasing the effective permeability of the cells to sodium ions. How would this action be expected to alter smooth muscle contractions? Would you expect the frequency or the amplitude of contraction to be altered to the greatest degree?

Procedure

1. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.
2. Type **Basal Activity-8** in the Mark box and click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording.
3. Type **Calcium-Deficient Saline** in the Mark box.
4. While recording, quickly replace the regular Tyrode's solution with a calcium-deficient solution as the Mark button is clicked. Continue recording the resulting contractions for at least 45 seconds.
5. Type **5 Drops-20% Calcium Solution** in the Mark box.

Warning: *Shake the dropper bottle containing the 20% calcium solution to be sure as much of the calcium is in solution as possible.*

6. Add five drops of 20% calcium solution to the calcium deficient saline in the bath chamber as the Mark button is clicked. If no effect is seen in 45 seconds, add an additional 5 drops of 20% calcium solution and mark the record. Record for another 45 seconds.
7. Type **High Calcium Saline** in the Mark box.

8. While recording, quickly replace the calcium-deficient Tyrode's solution with the high-calcium solution as the Mark button is clicked. As soon as the response of the intestinal muscle is stable and consistent, click Stop to halt the recording.
9. Select Save in the File menu.
10. Remove the Tyrode's solution containing the calcium from the bath chamber. Carefully rinse the intestinal muscle and the bath chamber with fresh, warm solution a couple of times. This removes excess drug from the muscle and reduces the occurrence of multiple drug effects.
11. Replace the solution in the chamber with fresh, warm Tyrode's solution.

Exercise 9: Effect of Cyanide

Aim: To investigate the effects of cyanide in the extracellular fluid on smooth muscle contractions.

Approximate Time: 20 minutes

Potassium cyanide is cytotoxic because it binds to the iron of cytochrome oxidase, thus preventing the transfer of electrons to oxygen and eventually halting all of aerobic respiration. Without the ability to generate ATP, cells cannot perform any cellular work like maintaining their osmotic balance or repair themselves. However, it is not uncommon to observe a short period of increased contractile activity in the smooth muscle preparation immediately following the initial application of cyanide. This paradoxical effect of cyanide appears to be the result of the increased release of neurotransmitter substances from the cells in the autonomic nervous system. The nerve endings of cells in the intrinsic intestinal nerve plexuses are stimulated by the presence of low levels of cyanide to release transmitters. Eventually, the cytotoxic effects of cyanide in all tissues overwhelms this effect.

Procedure

1. Click the Record button. Record the activity of the intestinal muscle after the Tyrode's solution in the bath chamber was replaced. The contractions should be consistent and predictable before proceeding with the exercise.
2. Type **Basal Activity-9** in the Mark box and click the Mark button. Record basal activity for at least four complete cycles or 30 seconds (whichever is longer). Continue recording.
3. Type **1 Drop-0.5% Sodium Cyanide** in the Mark box.
4. Add one drop of 0.5% sodium cyanide to the Tyrode's solution in the bath chamber as the Mark button is clicked. Wait 10 seconds to see if the sodium cyanide causes any change in the motility of the intestine. Add additional drops of 0.5% sodium cyanide to the solution in the bath chamber. Add one drop at a time, waiting at least ten seconds between drops. Mark the recording each time a drop is added to the bath.
5. Continue to record until no activity in the intestinal muscle is observed. Click Stop to halt the recording.
6. Select Save in the File menu.

Warning: When finished, be sure to follow the instructor's directions for cleaning your lab area and equipment.

Data Analysis

1. Scroll to the beginning of the data file and locate a section in the recording of normal intestinal contractions where the amplitude and period of the contraction cycle are consistent.
2. Use the Display Time icons to adjust the Display Time of the Main window so that two intestinal contraction cycles are displayed on the Main window. This section of data can also be selected by:
 - Placing the cursors on either side of the two contraction cycles of the recording, and
 - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand or contract the contraction cycle recording 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 Intestinal 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 Intestinal Tension channel.
5. Maximize the height of the trace on the Intestinal 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 intestinal contraction, 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 intestinal contraction.
 - 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 Intestinal 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 Intestinal Tension Channel pull-down menu.
8. On the Intestinal 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 intestine 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal Tension Channel is the contraction period.
 - Intestinal Tone is the passive tension, or tonic response, present in the intestine 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 Intestinal Tension Channel is the tone of the intestine at the beginning of a contraction, and Value2 is the intestinal 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 intestinal tone of all the other basal activity levels and muscle response to the treatments delivered to the tissue. Record the values in the Journal and on Table 1.
 11. Select Save in the File menu.

Questions

Exercise 2 - Effect of Stretch

1. How does stretching the intestinal strip affect intestinal motility (tone, amplitude, and frequency)?
2. By what mechanism does stretch influence the contraction of smooth muscle cells?
3. Is the response of smooth muscle to stretch different from the responses of skeletal and cardiac muscles to stretch? If so, in what way(s)?
4. Do the amplitudes of intestinal muscle contractions depend upon muscle length?
5. Does intestinal muscle tone depend upon muscle length?
6. Does the frequency of intestinal muscle contractions depend upon muscle length?
7. Do your observations support the sliding filament theory for muscle contraction?
8. Do your observations supply evidence for plasticity?
9. How do your results compare to the length-tension relationship that exists in skeletal muscle?

Exercise 3 - Effect of Acetylcholine

1. Acetylcholine acts by increasing the permeability of smooth muscle cells to ions such as sodium and potassium. Do your results correspond to the effects of the increased movement of these ions?
2. Which division of the autonomic nervous system primarily releases acetylcholine at synapses with effector cells?
3. Acetylcholine is also released from the intestinal wall itself. The amount released is proportional to the degree of gut tone as demonstrated by measuring the concentration of acetylcholine in the fluid surrounding isolated intestine stretched to various degrees. What effect is the irregular release of acetylcholine from axon terminals and intestinal walls going to have on the mixing of partially-digested food and digestive secretions, and the movement of the “food” bolus through the digestive tract of an intact animal? Is acetylcholine going to aid or inhibit the digestive process?

Exercise 4 - Blocking of Acetylcholine Receptors

1. Before beginning the experiment, find out which population of acetylcholine receptors is specifically blocked by the presence of curare and which receptors have their activity blocked by the presence of atropine.
2. What effect does curare have on intestinal motility (tone, amplitude, frequency)? What effect does curare have on intestinal motility in the presence of acetylcholine?
3. What effect does atropine have on intestinal motility (tone, amplitude, frequency)? What effect does atropine have on intestinal motility in the presence of acetylcholine?
4. Hypothesize mechanisms by which each drug affects the contractility of the intestinal muscle.
5. Based on your results and the known functions of curare and atropine, which type(s) of acetylcholine receptors are found on intestinal smooth muscle cells? How do your results confirm this?

Exercise 5 - Effect of Epinephrine

Consider the nature of the joint regulation of gut motility while examining the effects of epinephrine. Epinephrine stabilizes smooth muscle cell membranes so that sodium enters cells less readily and the rate of active transport of sodium out of the cell is doubled. Acetylcholine acts by increasing the permeability of smooth muscle cells to ions such as sodium and potassium; but less of it is secreted in the presence of epinephrine.

1. What is the normal source of epinephrine in the body?
2. How does the activity of norepinephrine usually compare to the activity of epinephrine at a target tissue?
3. What effect did epinephrine have on intestinal motility (tone, amplitude, frequency)?
4. Do your results fit this model of the mechanism of the action of epinephrine?

5. Would the presence of epinephrine increase or decrease the effectiveness of contractile stimuli (a food bolus, distention of the gut wall) to change intestinal motility?

Exercise 6 - Effect of Serotonin

The gut synthesizes large quantities of serotonin, especially after meals and during bursts of peristalsis. The intestine releases as much as eight times more serotonin when intraluminal pressures rise from 15 to 60 mmH₂O.

1. If serotonin lowers the threshold for mechanical excitation of intestinal stretch receptors and sensitizes the intestine to the action of acetylcholine, what is the expected effect of serotonin alone on smooth muscle activity? Why would this be a benefit?
2. What effect did serotonin alone have on intestinal motility (tone, amplitude, frequency)?
3. Hypothesize a mechanism by which serotonin affects the contractility of the intestinal muscle.

Exercise 7 - Effect of pH

1. What effect did lowering the pH of the Tyrode's solution have on intestinal motility (tone, amplitude, frequency)?
2. What effect did restoring the pH of the Tyrode's solution to its normal level have on intestinal motility?
3. How would an acidic food bolus affect intestinal motility?
4. Hypothesize a mechanism by which pH affects the contractility of the intestinal muscle.

Exercise 8 - Effect of Calcium Levels

1. What effect did the lack of calcium in Tyrode's solution have on intestinal motility (tone, amplitude, frequency)?
2. What effect did adding calcium to the calcium-deficient Tyrode's solution have on intestinal motility?
3. What effect did a high calcium concentration in Tyrode's solution have on intestinal motility?
4. Hypothesize a mechanism by which calcium affects the contractility of the intestinal muscle.

Exercise 9 - Effect of Cyanide

1. What effect did cyanide in Tyrode's solution initially have on intestinal motility?
2. What effect did cyanide in Tyrode's solution finally have on intestinal motility?
3. Hypothesize a mechanism by which cyanide affects the contractility of the intestinal muscle.

Table AM-5-L1: Tone, Amplitude, and Frequency of Intestinal Muscle Activity as a Function of Stretch, Transmitters, Drugs, Acidity, and Calcium.

Treatment	Contraction Amplitude (g)	Contraction Time (sec)	Relaxation Time (sec)	Contraction Period (sec)	Intestinal Resp.(g) at Beginning	Intestinal Resp. (g) at End
Basal Activity - 1						
Application of Stretch						
Basal Activity - 2						
Addition of Acetylcholine (ACH)						
Basal Activity - 3						
Addition of Curare						
Addition of ACH with Curare present						
Basal Activity - 4						
Addition of Atropine						
Addition of ACH with Atropine present						
Basal Activity - 5						

Addition of Epinephrine						
Basal Activity - 6						
Addition of Serotonin						
Basal Activity - 7 (Normal pH___)						
1° Activity Δ (new pH___)						
2° Activity Δ (Lowest pH___)						
Return to normal (Normal pH___)						
Basal Activity - 8						
Addition of low Calcium bath						
Addition of 20% CaCl_2 drops						
Addition of high Calcium bath						
Basal Activity - 9						
Addition of Sodium Cyanide						