AVIAN GUT EXPERIMENTS: AN ALTERNATIVE APPROACH FOR TEACHING THE PROPERTIES OF INTESTINAL SMOOTH MUSCLES

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Pant J, Mohan L, S S. Avian gut experiments: an alternative approach for teaching the properties of intestinal smooth muscles. Adv Physiol Educ 44: 295–304, 2020; doi:10.1152/advan.00195.2019.—Experiments on isolated mammalian gut are essential components of the physiology curriculum worldwide. Over the years, these routine experiments have been largely replaced by simulation modules, to reduce the euthanization of animals for understanding established facts and mechanisms in gut physiology. However, a medical undergraduate needs hands-on training to handle a living tissue to have a better understanding of physiology. The present sourcebook update describes the use of avian gut, which is usually discarded in abattoirs, as an effective replacement of mammalian gut to understand basic gut smooth muscle physiology. The avian gut can be used to study the effect of various drugs and ions as used in mammalian gut experiments. The experiment protocol described in the update can be performed by students of basic sciences and medical students using minimal laboratory set up and at low cost, producing results comparable to mammalian gut experiments. Ethical permissions may not be necessary; however, the disposal of tissue waste has to follow proper guidelines.

Introduction

Objectives and Overview

Smooth muscles constitute an important component of various organs, like blood vessels, gastrointestinal tract, urinary bladder, and bronchi. These muscles contract involuntarily and maintain their tone for a prolonged duration (9, 10). Contraction of smooth muscles in these organs alter lumen diameter, affecting various physiological functions. Smooth muscles are small and spindle-shaped nonstriated muscles. These muscles show a wide range of functional specialization in different organs. In certain diseases like asthma, gastrointestinal dysmotility, and vascular disorders, smooth muscle functioning may be altered. The gut is composed of mostly smooth muscles with autonomic innervation and is a suitable study tissue to understand its properties. The motility in these muscles is initiated by spike potentials and is also under influence of various chemical mediators and local factors (5, 12, 15, 16). Functional study of gut and the effect of drugs and their antagonists on the tissue helps us to understand the mechanism of motility better in health and disease and also provides concept of receptors, agonists, and antagonists. Mammalian gut, therefore, has been the preferred tissue type to teach the students about smooth muscle physiology in medical schools over the years. With the implementation of stringent regulations on animal experiments, performing these experiments as a part of a regular physiology curriculum has become a rarity. Advanced simulation software is available; however, it cannot replace the hands-on handling of animal tissue in the experimental laboratory.

The present sourcebook update describes a method to perform avian gut experiments as a replacement for mammalian gut experiments. Furthermore, effects of various drugs and ions on in vitro avian gut tissue are also described.

The effect of the drugs and ions used in the present experiment protocol is well established for mammalian gut (7), the present practical demonstrates comparable results of drugs and ions on avian gut motility with previously documented mammalian gut experiments. Moreover, the practical emphasizes the usage of discarded avian gut as an alternative for mammalian gut tissue to understand smooth muscle physiology and can be performed by students of medical sciences at the undergraduate level. The use of discarded avian gut is not only useful for teaching experimental skills to the students, but is also cost effective, as there is no need of procurement and maintenance of animals. Furthermore, these experiments can be performed in a minimal laboratory setup with basic instruments, which are available in physiology departments across the globe.

Background

Animal experiments have been an essential element for training students in basic medical sciences for many years. These experiments have played a pivotal role in biomedical research (7). These experiments served as the basis of understanding the mechanisms of action of different organs of the human body and have led to the discovery of uncountable principles responsible for understanding various body functions (7). Teaching animal experimentation helps to develop a scientific temperament in medical students.

At present, these experiments have been largely replaced by computer simulation (2). The use of animal experiments for teaching undergraduate and postgraduate students has been a matter of debate for the last few years across the world (6). Despite its relevance in medical science, there has been a lot of opposition from various lobbies that experimentation on animals is a form of cruelty and it does not impart much to medical science (6). Notwithstanding such arguments, stu-
Prerequisite Student Knowledge or Skills

The student must have a prior knowledge of the following:
1. Smooth muscle: mechanism of contraction and relaxation
2. Innervations of gut smooth muscle
3. Concept of receptors, agonists, antagonists
4. Handling an analytic balance
5. Working principles of physiograph

Learning Objectives

After completing this lesson, the student shall be able to perform the following:
1. Content knowledge: Describe the action of various drugs on smooth muscles of the avian gut.
2. Process skills: Prepare Tyrode solution and learn the basis of using this physiological solution.
3. Process skills: Prepare segments of gut tissue for mounting and recording.
4. Process skills: Mount the tissue properly in an isolated organ bath.
5. Process skills: Record baseline contractility of the tissue and the effect of drugs on the rate and tone of contractility and compare these results to prepare an observation table to show the effects before and after the administration of drugs on the rate and tone of gut contractility.

Activity Level

The experiment may be used to train postgraduate students who are pursuing masters in the discipline of physiology and pharmacology, in addition to undergraduate medical students and basic science students. It may serve as an alternative to the mammalian model in preliminary stages for the researchers and basic science students. It may further discourage the teaching of these experiments to medical students. To prevent the art of performing animal experiments from becoming nonexistent from our medical schools and to inculcate student’s interest in handling animal tissue, some experiments may be redesigned in a way that can be performed with minimal setup and can overcome these inhibitory factors.

For example, the discarded organs of poultry or other animals that are slaughtered for meat in abattoirs in nearly every part of the world can be used as an efficient alternative to traditional mammalian experiments described in physiology. Ethical approval may not be required for these experiments; however, those interested in performing these experiments must check with their institutional guidelines to meet the legal/ethical requirements and proceed accordingly.

The present article is a step-by-step description of in vitro animal experimentation using discarded avian gut that can be performed by basic sciences students.

Time and Resources Required

The entire experiment needs a dedicated time of ~2 h. This time is utilized in the preparation of Tyrode solution, preparation of the gut tissue, mounting, and recording. This time does not include the time of transportation of the tissue from the local abattoir to the laboratory, as it is dependent on the distance between them. It would be better to acquire the tissue and bring it to laboratory at least 0.5 h before beginning the practical, to reduce time lapse.

A dedicated team consisting of a medical physiology teacher who is experienced in experimental skills, along with postgraduate residents, tutors/demonstrators to teach the students, and a technical staff for the management of the laboratory is required for training of undergraduate students in one practical class.

METHODS

The experimental protocol described in the present sourcebook update was performed and standardized on chick ileum (n = 12) in our laboratory by the postgraduate students under the supervision of faculty. Throughout the process of collecting the gut sample, dissecting, mounting, and recording, the tissue was handled very gently, and care was taken to avoid any injury to the segments while using various instruments for the experiments. This holds relevance since a medical student must learn to handle tissues gently. This comes with experience, and doing animal experiments helps them acquire this skill, which simulation software can never teach.

Equipment

The essential equipment for the experiments is as follows:

- An isolated organ bath with temperature control thermostat. The organ bath must have a bath chamber, organ tube, tissue holder, a tube to connect the tissue holder for aeration, and a heater.
- A laboratory thermometer to check the temperature of the water if the temperature display is not available in the organ bath.
- An aerator, which bubbles atmospheric air into the Tyrode solution, and the gut tissue is aerated during the experimental procedure.
- An analytic balance, to weigh the salts for preparing the Tyrode solution.
- Laboratory glassware: beakers (50–1,000 mL), measuring flasks (50–100 mL), and test tubes.
- Dissecting instruments: blunt and fine scissors, blunt and toothed forceps, and artery forceps.
- Fine silk sutures to secure the tissue to the tissue holder.
- A micropipette to administer the required quantity of the drug to tissue.
- A physiograph.
- Computerized data recording using laboratory chart (AD Instruments), if available, can be used to record motility.

Solutions

The solutions to be prepared on the day of the experiment are as follows:

1. Tyrode solution (Table 1) is to be prepared on the day of the experiment.
2. Drugs: stock solutions of acetylcholine (ACh) and atropine sulfate. Adrenaline, CaCl₂, and KCl are to be prepared (Table 2).
3. All of the solutions are to be prepared using distilled water.
Animal Subjects

Gut tissue obtained from a freshly slaughtered chicken from a butcher’s shop must be used for the experiment. Care must be taken that the chicken is not killed more than 3 h before the practical.

In the experiments performed in our laboratory, the gut tissue was obtained from chicks that were slaughtered by decapitating the head by a butcher’s knife or cutting through the neck blood vessels, followed by exsanguination and death of the bird (as practiced in India). This was followed by removal of feathers, claws, gut, and other waste material by pulling them out with the hands. No chemical treatment was used for slaughtering the birds at any stage.

The discarded gut was generally soiled with blood and feces; it did not interfere with the results. During the process of initial standardization of these experiments, we collected the gut from freshly slaughtered birds; however, later on we could successfully perform the experiments on the gut obtained from chicken slaughtered 3 h before experimentation. In the laboratory, the chicken ileum was harvested for the experiment.

Chemical treatments, exposure to extreme environmental temperature, and >3-h time lapse may influence the results, and precaution must be taken during the collection of samples, that they are not subjected to any of the aforesaid factors/conditions.

Instructions

Preparation before practical. The checklist before beginning the experiment is as follows:

1. Tyrode solution must be prepared (Table 1).
2. Stock solutions of drugs must be prepared (Table 2).
3. An observation table must be prepared (Table 3).
4. Functioning of physiograph/ laboratory chart must be checked.

Transportation of chicken gut. The steps of chick gut collection and transportation on the day of the experiment are as follows:

1. Freshly prepared Tyrode solution is poured in a beaker (1,000 mL), and atmospheric air is bubbled by an aerator for 15 min (3–5 bubbles/s). The solution is maintained at room temperature (20–25°C) throughout the period of transportation. (The tissue was collected during both summer and winter months, with temperature of 35–40°C and 5–10°C, respectively, in the butcher’s shop, but it was transported and the experiment was performed in a laboratory with temperature maintained at 20–25°C.)
2. The mouth of this aerated Tyrode solution-filled beaker is closed to prevent the escape of air and prevent contamination.
3. This beaker is placed inside a closed box and taken to the abattoir.
4. The avian gut tissue obtained from the butcher’s shop is immediately placed inside this beaker.
5. Care is taken that the gut remains immersed in this solution and is transported to the laboratory.
6. Once the gut is brought into the laboratory, it is transferred into another beaker filled with freshly prepared Tyrode solution, and aeration is maintained continuously (3–5 bubbles/s) by the help of an aerator.
7. This is followed by preparation of the gut segments and securing them to the tissue holder.

Preparation of ileum segment. The method of identification of ileo-cecal junction to prepare ileum segments is as follows:

1. Identify the ileo-cecal junction. To identify the junction, place the entire gut in the dissecting tray, and, starting from the oral end, trace the esophagus, crop, small intestine, ceca (2 in number), and finally the large intestine. The junction of ceca and small intestine is part of the ileo-cecal junction. Trace the gut toward the oral end from this junction, and cut 6–8 cm of the oral segment from the ileo-cecal junction.
2. Transfer ileum into a petri dish filled with Tyrode solution and aerate continuously (3–5 bubbles/s).
3. Clear the entire adjoining fascia with the help of forceps and scissors.
4. Once the gut is cleaned, prepare small segments of ~2- to 2.5-cm lengths.
5. Cleanse the inner lumen by flushing with Tyrode solution by a 2-mL syringe so that the segment is free from any fecal matter present inside the lumen.
6. Tie threads at the oral and caudal ends of the segment separately.
Mounting the gut segments in organ bath. The steps to be followed for mounting the gut segments to the tissue holder in the organ bath are as follows:

1. Fill the inner chamber of the organ bath with freshly prepared Tyrode solution.
2. Maintain the temperature of the water in the outer chamber of the organ bath at ~37°C. The chick’s body temperature is 40–42°C. However, keeping the bath temperatures a few degrees cooler than the animal core temperature will reduce the force of spontaneous motility, ensuring that it does not “mask” the change produced following the treatment application. (When standardizing experiments were performed at 40–42°C temperature, an irregular baseline was obtained. Optimum baseline was recorded at 37°C. See Appendix A).
3. Secure the oral end of the segment to the tissue holder.
4. Thereafter, fasten the thread of the caudad end of the segment to a force transducer. This helps in recording contractility of the longitudinal muscles of the gut. Furthermore, tying a string at the caudal end of the tissue will help to stabilize the tissue so that, during a contraction, the produced force can be monitored by changes in the string tension picked up by the force transducer.
5. The flow of air into the inner chamber must be controlled by the aerator, so that the bubbling rate is maintained at 1–2 bubbles/s.
6. Adjust the tension of the thread so that it is neither too loose nor too tight and tissue is at the optimum length. To confirm whether the thread is tied at the correct tension to the force transducer, record the spontaneous contractions. Recording of spontaneous contractions marks that thread tension is appropriate and also confirms the viability of tissue. The adjustment of thread tension is important because, if it is tied too tight or too loose, the spontaneous contractility of the gut cannot be obtained. Tying the thread too tight would lead to excessive stretching of the gut, and, if the thread is tied too loose, the thread tension will not be sufficient enough, and contractility cannot be recorded by a force transducer. In either case, the muscle does not achieve its optimum length. (Baseline tension was 4.9 N in the recording depicted in Fig. 1).
7. Allow the mounted segment to stabilize for 30 min. The equipment setup is shown in Fig. 2.

Recording. The steps to be followed while recording gut contractility by physiograph are as follows:

1. Once the tissue is stabilized, record the contractility of the tissue on a physiograph paper after adjusting speed, sensitivity, and gain of amplification.
2. Record the baseline activity for 30 s after the tissue is stabilized. Baseline recording is the recording of tissue’s spontaneous contractility, after it is mounted in the organ bath.
3. Then add 0.1 mL of ACh into the inner chamber (Table 2).
4. Record the changes in contractility in terms of rate, amplitude, and shift in baseline. These changes start appearing soon (2–3 s) after the drug is administered. In case no change in contractility is recorded, the drug may be repeated, or a next higher concentration of the drug may be administered. Smooth muscles follow the latch bridge mechanism of contraction and need a longer time to relax and lose tension spontaneously. Smooth muscle relaxation occurs when the stimulus causing the contraction is removed or by directly stimulating inhibition of the contraction (23). If the contraction persists, as can be seen by a plateau, the tissue may be washed after waiting for 5 min. Washing of tissue leads to removal of drug/ion, and baseline tension can be achieved after restabilization after wash.
5. After 5 min, wash the gut segment thrice with Tyrode solution.
6. Allow the tissue to stabilize again. Immediately after washing, no contractions are recorded; however, after ~10–15 min, the tissue’s spontaneous contractions reappear and attain the baseline tension. The time of stabilization depends on the previous drug used.
7. Record baseline activity again.
8. Thereafter, add 0.1 mL of adrenaline into the inner chamber.
9. Record the changes in contractility in a similar way as mentioned for ACh in step 4.
10. After 5 min, wash the gut segment three times with Tyrode solution. Allow the tissue to stabilize again and record baseline activity.
11. Thereafter, add CaCl2, KCl individually (Table 2) and record the findings as described in steps 4, 5, 6, and 7.
12. Wash the tissue three times and allow it to stabilize, before adding each drug.
13. Add atropine in the inner chamber (Table 2).
14. Record the contractility of tissue, as mentioned in step 4.
15. After 30 s of administration of atropine, add 0.1 mL of ACh to the tissue.
16. Record the changes in contractility.
17. If computerized software is used for recording, the data must be saved from time to time.

Troubleshooting

The precaution to be taken while preparing gut segments for experiment is as follows:

1. Care has to be taken during flushing and cleaning of the lumen of the gut segments using a 2-mL syringe, as these segments are quite delicate, and they may get damaged while introducing the mouth of the syringe into the segments.
2. Initially, the baseline recording might need adjustment of gain and sensitivity in the physiograph so that spontaneous gut contractions are recorded. It is better to keep the sensitivity high so that most feeble contractions can be recorded. Later on, with the addition of certain drugs, like ACh, the tone will increase, and then sensitivity has to be decreased. Furthermore, if the contractility decreases, as in the case of adrenaline, the sensitivity may be increased.
3. If no spontaneous contractions are obtained, then check for tension in the thread and readjust to get recording of contractions. In case you still fail to get a response, check for sensitivity, as explained in step 2 of Troubleshooting.
4. If you still do not get a recording, wash the tissue with Tyrode solution and again allow it to stabilize, check tension, and record contractility.
5. During washing of the mounted tissue in between drug administration, the thread tension often gets altered and the tension has to be readjusted, as mentioned in step 6 in Mounting the gut segments in organ bath, to get a baseline recording.
6. Sometimes there is no response on adding the drug. In that case, repeat the drug or else increase the concentration of the drug to obtain the response.
7. It is important to note that pretreatment of the gut with atropine is expected to prevent the response of ACh; hence you have to add increased strength of ACh to displace the effect of atropine. Hence it is preferred that experimentation with atropine be performed last.
8. In situations where you are not left with much time to wash the segment before adding every drug, you may start the experiment with adding KCl, followed by CaCl2, followed by adrenaline, ACh, and atropine followed by ACh, one after the other. A time gap of ~5 min may be required for stabilization before adding individual drugs (Appendix B).

Safety Considerations

The tissue must be handled gently during preparation and mounting so that it is not damaged.
Fig. 1. Effect of drugs on chick ileum. A: addition of 0.003 mM acetylcholine (ACh) increased the tone of contractions. B: addition of 0.002 mM adrenaline (Adr) decreased rate, tone, and amplitude of contractions. C: addition of 1.8 mM CaCl$_2$ increased tone and amplitude of contractions. D: addition of 0.2 mM KCl increased tone of contractions. E: pretreatment with 0.0009 mM atropine (Atr) and thereafter adding 0.003 mM ACh decreased rate, tone, and amplitude of contractions.
RESULTS

Expected Results

Gut motility in mammals is governed by the presence of the enteric nervous system and autonomic innervations throughout the gut. Rhythmic contractions take place in the intestine, which is called phasic contractions (4, 10, 13). These contractions are initiated by the interstitial cells of Cajal, which are specialized mesenchymal cells located within the muscle layer of the intestine (4). They generate basic electrical rhythm for phasic contractions (9, 10, 13). Spike potentials are required to be generated for the active contraction of gut muscles (5, 12). Several chemical mediators are also involved in the regulation of the gut motility (15, 16). Similarly, the avian gut is also expected to show its intrinsic contractions and is regulated by various factors. The expected effects of various drugs used in the present experiment are depicted in Table 4.

Effect of adding ACh on the chick ileum contractions. ACh acts through G protein-coupled muscarinic receptors (17–22). It causes activation of phospholipase C, which increases inositol triphosphate (IP₃) and diacylglycerol (DAG). This leads to increased mobilization of intracellular calcium, which binds to calmodulin and activates myosin light-chain kinase (MLCK) to produce smooth muscle contraction. Hence, on adding ACh, the tone will increase, which can be appreciated as an upward shift in the baseline.

Effect of adding atropine sulfate on the chick ileum contractions. Atropine is an antagonist of ACh and blocks the muscarinic receptors (17–22). Atropine per se produces variable effects on the gut, but mostly there is a decrease in tone and motility of the gut. Hence, pretreatment of the gut with atropine and addition of ACh thereafter is expected to show reduced responses.

Effect of adding adrenaline on the chick ileum contractions. Adrenaline acts through adrenergic receptors (17, 18, 20). It reduces cAMP and IP₃-DAG and causes increased activation of potassium channels. Administration of adrenaline is expected to decrease contractility, and the tone can be recorded as a downward shift in the baseline. The rate of contractions is expected to decrease, which may be recorded as a decrease in the number of contractions over a while compared with the initial baseline before the drug addition. The force of contrac-

Fig. 2. A–C: photographs of tissue mounted in the tissue holder of isolated organ bath. D: schematic representation of tissue mounted in the tissue holder of isolated organ bath.
Effect of adding CaCl₂ on the chick ileum contractions. Adding CaCl₂ increases the Ca²⁺ levels in the surrounding solution of the gut in the inner chamber. Hence, increasing its level in the extracellular solution favors entry of calcium inside the cell through ion channels. This entry of Ca²⁺ does not need any special receptor. Ca²⁺ is a second messenger and binds to calmodulin and activates MLCK to produce smooth muscle contraction (8, 16, 17). Hence, CaCl₂ is expected to increase the force of gut contraction, like ACh.

Effect of adding KCl on the chick ileum contractions. Potassium increases intestinal motility, like ACh (11, 19). Potassium causes depolarization of smooth muscle membrane, which leads to the opening of the calcium channels, and there is an increased influx of calcium. This leads to activation of contractile elements in smooth muscles.

Evaluation of Student Work

The students are expected to record the motility in the gut and thereafter identify the effect of the drugs or ions according to the response obtained. The students can use the recording to find the change in rate and tension of the gut contractility before and after administering the drugs or ions. The data may be pooled from individual recordings of students (minimum 6 students) in the same practical session, or in different practical sessions by the same student, to calculate mean value, and Student’s t test for paired observation can be applied for statistical analysis of data.

Grading can be based on segment preparation, mounting, recording, preparation of solutions, demonstration of the effect of drugs on the tissue, and their response to related questions asked orally (Table 5).

Plotting of the dose-response curve can be worked up for postgraduate evaluation; however, the present update is an undergraduate-level practical and is beyond the scope of this update.

DISCUSSION

Inquiry Applications

The experimental set required for this experiment is simple and inexpensive. An organ bath along with its accessories, a physiograph to record the contractions, and analytic balance to weigh salts for preparing Tyrode solution were used in the experiment. Recording on the physiograph was obtained by an ink pen recording system. For recording of gut contractions, a strain-gauge coupler and force transducer were used. The speed of paper selected was 0.5 mm/s, sensitivity was maintained at 50 –100 V/cm, and filter was 50 Hz. AC current with input – 220 V was used as the main supply; earthing cord was connected to the ground.

Table 4. Expected results of drugs used on chick ileum contractions

| Solution No. | Drugs                          | Receptors                        | Expected Effects                                      |
|------------|--------------------------------|----------------------------------|-------------------------------------------------------|
| 1          | Acetylcholine                  | Muscarinic receptors             | Increase in tone of contractions                      |
| 2          | Atropine                       | Blocks muscarinic receptors      | Decrease in rate, tone, and amplitude of contractions |
| 3          | Pretreatment with atropine and then addition of acetylcholine | Blocks muscarinic receptors | Decrease in rate, tone, and amplitude of contractions |
| 4          | Adrenaline                     | Adrenergic receptors             | Decrease in rate, tone, and amplitude of contractions |
| 5          | CaCl₂                          | Binds to calmodulin              | Increase in tone and amplitude of contractions        |
| 6          | KCl                             | Causes depolarization of membrane and opening of calcium channels | Increase in tone of contractions                      |

Table 5. Suggested objective structured practical examination evaluation

| Skill Assessed                     | Evaluation Checklist                                                                 |
|------------------------------------|----------------------------------------------------------------------------------------|
| Preparation of solutions           | • Did the student make correct use of analytic balance?                                   |
|                                    | • Were the salts measured appropriately?                                                 |
|                                    | • Were the solutions prepared appropriately?                                             |
| Segment preparation                | • Did the student identify the ileo-cecal junction?                                      |
|                                    | • Did the student cut ileum segments measuring 2–2.5 cm in length?                        |
|                                    | • Was cleaning of tissue performed gently?                                               |
|                                    | • Were the threads tied properly to both ends of the segment?                            |
| Mounting of segment                | • Did the student take care to check the initial tension in thread while mounting?       |
|                                    | • Was the tissue kept away from touching the inner wall chambers?                         |
|                                    | • Was the transducer placement proper?                                                   |
| Overall recording of effect of drugs| • Did the student able to obtain an initial baseline contraction?                       |
|                                    | • Was the student able to show the effect of different drugs in terms of changes in rate, tone, and force of contractions? | |
|                                    | • Was the tissue rinsed in between administration of drugs?                              |
|                                    | • Was the student able to perform statistical analysis of his/her data?                  |
| Response to oral questions         | • Did the student able to explain his/her recordings?                                     |
|                                    | • Was he/she able to explain the gut smooth muscle properties?                           |
|                                    | • Was the student able to answer questions related to gut physiology?                     |
The main benefit in performing these experiments is that offal, which is discarded as waste from abattoirs, is used for the experiment. A large number of animals are euthanized for meat in nearly every part of the world; hence using the discarded organs for experiment provides a good alternative for experimental studies. These experiments hence prove to be a cost-effective solution to expose medical students to animal experimentation. Furthermore, animal cruelty is out of question while conducting these experiments, and special ethical approvals are not envisaged as an issue.

The experiments help undergraduate students to develop deeper insight into physiological phenomena and help develop a better understanding of what they study in lectures and books. The postgraduate students, on understanding the basic principles, may incorporate the model into future research. Computer simulation modules just give an overview to a topic but cannot replicate the actual experiments’ benefits (2). Handling a tissue, preparing physiological solutions and drug dosages, recording the activity, and understanding and learning through failures can never be possible by a simulated module. Cell culture studies may also be used to study the behavior of smooth muscles in a well-regulated environment. New intestinal smooth muscle cell culture model using rat neonates can be used for research purposes (3). Some studies also report generation of functional smooth muscle with spontaneous contraction through culture bioengineering techniques (14). These culture cells may be used to delineate the molecular mechanisms underlying a functional smooth muscle. However, the technique of using cell culture studies at an undergraduate level for teaching routine experiments is prohibitively expensive and needs proper infrastructure and trained personnel.

Fig. A1. Baseline contractility recorded when the organ bath was maintained at different temperatures (40, 37, and 42°C) is shown.
Fig. B1. Effect is shown of drugs/ions on chick gut contractility without washing the tissue in between administration of different drugs.

Pretreatment with Atropine followed by Acetylcholine administered in different concentrations to wear off Atropine effect
Certain limitations of these experiments can be that some of the properties that are applicable for mammalian tissue may not hold true for avian tissues, although none are known to the authors. Transportation of the samples of avian gut from the abattoir to the laboratory may pose some difficulty if located far away. A dedicated person needs to be deputed to manage the transportation of the samples to the laboratory.

There are a number of pros and cons associated with these experiments; however, every experimental model has its own pitfalls and advantages. Notwithstanding, we have found that these difficulties are easy to overcome and should not deter us from taking up these experiments, which are possible in a most simple and minimal setup, providing an easy alternative to using experimental animals.

Wider Educational Applications

The experimental setup can be used to record a number of parameters other than that described in the update, such as:

- Dose-response curve of different drugs.
- Research purposes to observe the effect of any chemical/toxin and work out for possible underlying mechanism of action. The receptor antagonism mechanism (as suggested in the Ach/atropine protocol) will be suitable for determining the receptor(s) with which the treatment agonist may be interacting to determine the possible mechanism.

Additional Information

For additional information, the students can refer to any book on experimental physiology or pharmacology.

APPENDIX A: RECORDED BASLINE CONTRACTILITY

Figure A1 shows the baseline contractility recorded when the organ bath was maintained at different temperatures (40, 37, and 42°C). At 37°C, stable baseline activity was recorded.

APPENDIX B: DRUG/ION EFFECT WITHOUT WASHING

Figure B1 shows the effect of drugs/ions on chick gut contractility without washing the tissue in between administration of different drugs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.P. and L.M. conceived and designed research; S.S. performed experiments; J.P. analyzed data; J.P. interpreted results of experiments; J.P. and S.S. prepared figures; J.P. drafted manuscript; J.P. and L.M. edited and revised manuscript; J.P., L.M., and S.S. approved final version of manuscript.

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