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## Introduction

A 2–3 hour laboratory exercise has been used annually in various revisions in the second semester of the introductory biology course at St. Lawrence University (BIO102: General Biology) since 1984. Approximately 100 students each spring semester perform this exercise during a 1-week period (10 students per laboratory time, 5 students per group). Students are required to sign up in groups for an available lab time and must bring a flow sheet to lab to be reviewed with their teaching assistant. This seems to confirm attendance and allows groups to communicate concerning the assignment and integration of chores prior to the performance of the lab.

The exercise is a standard muscle physiology laboratory which has been modified to include the consideration of the ethical concerns of dissection in the laboratory setting. This exercise reinforces and expands upon lecture content. As an introductory course satisfying university distribution requirements, our university Academic Affairs Committee requires that we address fundamental issues of our discipline. The fact that students will be involved with dissections is made known on the first day of classes. Experience has shown that students will understand and will be able to integrate large muscle movements with lecture material, which is presented primarily at the cellular level, through an experience utilizing living muscle tissue. After an introduction to the purpose, a presentation is made on ethics and concerns of dissection in which students are encouraged to discuss the methods and protocols to be used. They then examine the response of an excised frog gastrocnemius muscle to increments in stimulus voltage and frequency in order to observe minimal and maximal response, summation, and tetanus. Once the baseline values are established, they are blindly given an inhibitor or stimulant and asked to generate and test a hypothesis. Results are presented graphically and submitted, typed, in the format of a scientific report.

The level of preparation required of the students prior to lab reinforces the degree of seriousness with which the instructional staff approaches the subject of sacrificing an animal for educational purposes. Prior to the student lab, the instructional staff discusses and performs the exercise in their weekly seminar. In the discussion, the details of the purpose, equipment, ethical concerns, procedures for dispatching the frog, answering student questions, and the running of the lab are handled. Finally, individual TA reactions to aspects of the lab are explored and decisions and reassignments based on that discussion are finalized.

At no time are students or TAs mandated to perform this lab. Expression of hesitation at any point is met with empathy and with an interest in helping the individual define and clarify the level at which they wish to participate. Students who decline participation are encouraged and supported in finding alternative ways of accomplishing the same content. We find that this attitude of openness encourages students and TAs to challenge their own views in a more productive way than if the discussion never occurred or was ignored.

### **Fundamental Issues Concerning the Use of Animals in the Laboratory**

While the muscle physiology laboratory that is described in the chapter was conducted at the 14th Annual Workshop/Conference of the Association for Biology Laboratory Education (University of Nevada-Las Vegas, 1992) a discussion was generated concerning the ethical use of living organisms in the laboratory. The following are questions, concerns, and responses developed during the two workshops held on 3 June 1992:

#### *How to deal with questions on use of animals in the lab*

1. It is critical that an open, empathetic environment/atmosphere is fostered.
2. There is no such thing as an inappropriate question in this discussion.
3. Make clear we don't like killing animals but we feel it is necessary for this learning.
4. Make expectations and consequences of appropriate/inappropriate behavior and handling of animals clear.

#### *Questions students may ask and responses*

1. Why do we have to do the lab, why can't we read about it or do a simulation?
  - (a) Students learn the concepts better if actively involved in the experiment; retention is greater.
  - (b) Students learn how to be scientists by doing things a scientist does, not by reading about them.
  - (c) Demonstrations don't work because not all students are involved in the experiment.
  - (d) The reality is that mistakes are part of the learning process.
  - (e) Students gain respect for life that cannot be obtained by looking at a picture. This point is less frequently accepted by students but their extent of acceptance is a manifestation of their level of trust in their instructor.
  - (f) This is the only way to understand the complexity of living tissue.
2. Why do we do a lab in this intro course rather than waiting until upper-level courses?
  - (a) This may be the only opportunity for majors and non-majors to experience this phenomenon and function as scientists.
  - (b) It is important for majors to get a full spectrum of biological experience before they focus their interests.
3. Is this frog used for anything else? Things to consider:
  - (a) The lab and educational goals and objectives of this lab justify this experiment.
  - (b) Engage students in a nondirected exploration of the organism after the muscle lab.

4. What is the source of the frogs? Are they raised in the lab or collected in the wild? If wild-collected, are we contributing to a decrease in the wild population?
  - (a) You need to know your supplier and how/where they collect. (*Xenopus* is raised in the lab; *Rana* is collected in the wild.)
  - (b) Collection method(s) provide a good vehicle for a discussion of ecological principles.
5. Can we demonstrate muscle physiology with another organism?
  - (a) Small frogs are desirable because of large gastrocnemius development.
  - (b) Small frogs are desirable because being cold blooded, the excised muscle functions well at room temperature.
  - (c) *Bufo marinus* is not acceptable because they are difficult to kill and pith and they secrete a toxic, white substance.
  - (d) Mammals are undesirable because of a smaller gastrocnemius in relation to body size and an involved maintenance of normal body temperature for normal function.

*Unresolved topics of concern*

1. How do we advise pre-health and pre-veterinary students who do not want to get involved in dissection, vivisection, or experimentation?
2. How can we help students, who are opposed to animal dissection, vivisection, and/or experimentation, do well in professional school interviews?

*Questions posed by instructors for students to consider*

1. Are you consistent in your concern about use and treatment of animals in an educational setting and in your everyday activities?
2. How does the loss of a single individual affect the population? Do you value the life of an individual more than the health of the population (e.g., a deer exceeding carrying capacity of the population, or the impact of introduced species on native species).

## **Case Study: Muscle Physiology Laboratory**

### **Introduction**

The following muscle physiology laboratory has been adapted from several similar traditional laboratories. It requires the sacrifice of a frog to secure a gastrocnemius muscle for each student group of five. Every effort is made to have an equal number of student groups in order that the greatest use can be made of each sacrificed frog.

## Notes for the Instructor

### Materials

There are three standard recording systems available:

1. Kymograph recording system consists of: kymograph, simulator, electrode holder, femur clamp, recording pen, ink, and paper.
2. Harvard Modular Recording System: chartmover (#480), isotonic muscle module stimulator (#270), event time module (#280), hand electrode set and holder, and stimulator.
3. Narco Bio-Systems Physiograph: basic physiograph set up for single channel recording, pin electrodes, electrode cable, frog board, and pins,

Each group of five students is supplied with the following (2 groups per lab):

Kymograph recording apparatus (all discussion in this chapter will refer to this system)

Frogs (1 per lab; 1 leg/group)

Dissecting kit (including gloves, blunt stainless steel scissors, blunt stainless steel probe, tweezers, heavy scissors)

Frog Ringer's solution (1-liter squeeze bottle) (2)

Small dissecting pan

Flat-base stands

Double clamps

Anesthetizing solution, 25% urethane solution (1 unit = 0.6 g urethane/2.4 cc dH<sub>2</sub>O)

Inject 1 unit of urethane solution/100 g body weight intraperitoneally, 10 minutes prior to decerebration and pithing.

### Procedures

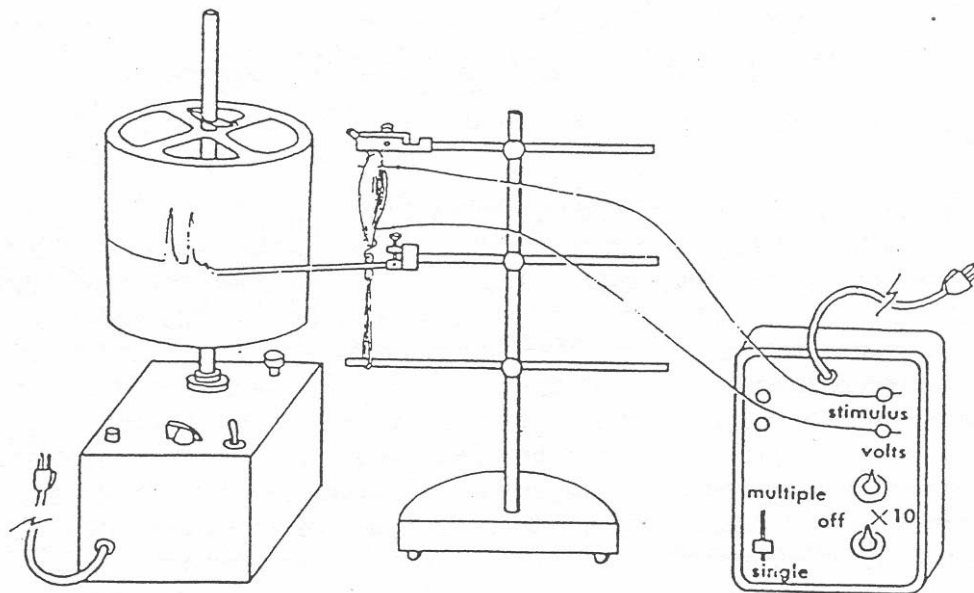
Once the groups are seated at their appropriate place, check to make sure that flow sheets are completed. Have students verbally describe their "roles" to be sure that they have prepared for their experiment.

The introduction to the lab can begin when the instructor feels confident that students have read and understood the sequence of the lab. The introduction includes:

1. Purpose: Identification of muscle phenomena at the whole muscle level as compared to the cellular level as presented in lecture.
2. Preparation of muscle: Dissection: use of dead organism for educational purposes.
  - (a) Why use dissection?
    - i. This experiment demonstrates an aspect of muscle physiology for which there is not an adequate substitute.

- ii. Serendipitous benefits: Academic Affairs Committee at SLU charges introductory courses with addressing fundamental issues of the discipline of biology (we feel this is a fundamental issue). Upper-level courses require dissection and vivisection (the department is served by the introduction we give these second-semester introductory students).
  - iii. Our concerns include: Maximizing use and benefit of one frog (two legs per frog, 10 people per frog). Most efficiently dispatching the frog (only done by instructor, urethane intraperitoneal injection to totally anesthetize as indicated by “no eye reaction,” decerebrate and pith to make sure the animal is dead and no ancillary reflex actions are present, and students are not required to observe but are allowed if they are so inclined. At this stage there may be snickering or other behavior not conducive to maintaining the serious attention of the students to the matter at hand. A simple non-judgemental statement employed to immediately stifle such a reaction and to allow constructive discussion to take place includes the facts that we often laugh at those things that embarrass us, that we know least about, or with which we are most uncomfortable. Lastly, consider our own students' feelings concerning the killing of an organism and answer any questions.
- (b) Methods for killing the frog: urethane, 25% solution; MS222 (a.k.a. TMS 0.3%), pH should be adjusted 7.0, see Sigma catalog under “MS222 dermal absorption”; other suggested methods utilized clove oil, tribromoethanol, and cooling with ice. Check with your Institutional Care and Animal Use Committee on the acceptable methods for your institution.
  - (c) Excision of muscle after the frog is dead: heavy scissor to remove leg from body; muscle preparation by student. Review student outline, care in contaminating exposed muscle surface, keeping bare muscle moist with saline.
3. Equipment identification (see Figure 9.1).
- (a) How the muscle is positioned to record contraction:
    - i. Femur clamp (distal on stand from desk surface): bared femur fits in notch and held horizontally, must be kept moist with saline.
    - ii. Gastrocnemius hangs vertically; must be kept moist with saline
    - iii. Thread tied around Achilles “knob” must be tight; attach other end of thread to muscle lever with pen.
    - iv. Moveable bar attached to muscle lever with pen which is muscle and thread length below femur clamp (moving muscle lever with pen support up stand to allow enough slack to tie thread. Once tied, return muscle lever with pen down stand to put some tension on muscle but not so much that it prevents muscle from contracting). Upon contraction, muscle shortens and muscle lever with pen moves up on kymograph drum.
    - v. Stationary bar attached to the muscle lever with pen above by a rubber band; tension in rubber band provides sufficient tension to return muscle lever with pen to baseline position after each muscle twitch.
    - vi. The entire muscle, thread, and rubber band must be perpendicular to table top.
  - (b) Kymograph recording apparatus:
    - i. Speed knob: Run by gear mechanism; must be on to change speed; direction drum rotates.
    - ii. Paper positioning: Rotation of drum and positions of pen determines overlap of paper.

- (c) Stimulator (electrical stimulation for muscle contraction):
- Note voltage knob: Range? Make sure off until ready to start. How to change voltage?
  - Note multiplier knob: Range? How to change voltage?
  - Note frequency knob: Range? How to change frequency?
  - Note single or multiple stimulus switch.
- (d) Electrode: Connection between stimulator and belly of muscle. Electrode tips must be cleaned of corrosion and inserted directly into muscle for best results. Positioning of electrode so it doesn't pull the muscle in any direction is important.



**Figure 9.1.** Frog gastrocnemius muscle set-up. The muscle is fixed in a muscle clamp so that when an electrical charge is delivered by a stimulator, the contraction causes a pen to write on a moving chart (kymograph).

### The Experiment

- Baseline experiments: Perform as per student outline, keeping the muscle moist with saline.
- Hypothesis testing: Before fatigue, use one of the chemicals provided to test your hypothesis. Consider how you will graphically present your data in your write-up.

3. To start experiment:
  - (a) The students need to familiarize themselves with the workings of the equipment for which they are responsible during the experiment.
  - (b) The surgeon needs to get prepared.
  - (c) All students need to determine the method of data collection and how to analyze.
  - (d) Instructor weighs frog and gives intraperitoneal injection of urethane (*warning*: urethane is a potent carcinogen – use in hood and wear gloves), waits for sedation, and performs decerebration, pithing, and excision of legs. Give one freshly excised leg to each group and the lab begins. (The instructor may wish to do the intraperitoneal injection prior to the beginning of the introduction.)

### **Student Outline: Action of Skeletal Muscle**

#### **Introduction**

The muscle cell, or fiber, is a cylindrical, elongated cell having many nuclei located at the periphery. Hundreds of myofibrils, proteinaceous contractile rods, are packed lengthwise in the cell. Each myofibril consists of repeating units called sarcomeres. The sarcomeres are the contractile units of the muscle cell. The sarcomere unit consists of two major sets of protein filaments called myofilaments. The thick myofilaments are composed of large fibrous molecules called myosin. The thin myofilaments consist of the protein actin associated with the smaller proteins, troponin and tropomyosin. Shortening of the sarcomeres results in the observable phenomenon that we call contraction.

One way to study the characteristics of muscle contraction is simply to stimulate a muscle and watch it. This procedure will tell you that muscles, like nerves, respond to many types of stimulation (touch, chemicals, heat, electricity, drying, etc.), and it can give you a crude idea of the relationship between the amount of stimulation and the extent to which the muscle responds.

Much more precise information can be obtained by using a recording apparatus. In this set-up, an electrically stimulated muscle is attached to a lever that amplifies the contraction and records it by writing on moving paper. Simultaneously, if an electromagnet, the signal marker, is available, it records the application of the stimulus, so that the relationship between the response of the muscle and the application of the stimulus can be determined. Very small responses can be detected because of the amplification through the lever system; in addition, the moving paper allows a detailed examination of the different phases of contraction and relaxation. The application and measurement of stimuli are much more readily controlled than in situ.

Electrical stimuli are used because they are easily applied and controlled for intensity, frequency and duration, because they do not damage the muscle, and because they are most like the muscle's natural stimulation.

Upon completion of this exercise, you should be able to:

1. Describe the kymograph recording apparatus and stimulator set-up used to record muscle contractions.
2. Describe the procedure used to prepare the frog gastrocnemius muscle.

3. Explain in words or by using a tracing what is meant by threshold stimulus, maximal stimulus, the components of the muscle twitch, summation, tetanus, and fatigue, and how these phenomena relate to shortening of the sarcomere.
4. Conduct your own investigation based upon a hypothesis you develop.

The following instructions pertain to the procedures you will follow while working with the kymograph set-up. Be sure that each step of the procedure is followed carefully if you expect to have a successful experience. Do not be afraid to check with the staff to be sure that your techniques are correct.

### Pre-Lab

*You must sign up in groups of five for this lab.* Sign up sheets are posted in the lab. A TA will assist you with this lab. *You must know* what you are doing before you will be allowed to proceed. Unprepared lab groups have spent up to 2 hours doing this with lousy results. *Prepared groups* have spent less than 1/2 hour completing this lab with excellent results. Cooperation and coordination is a must. Designate who in your group will be:

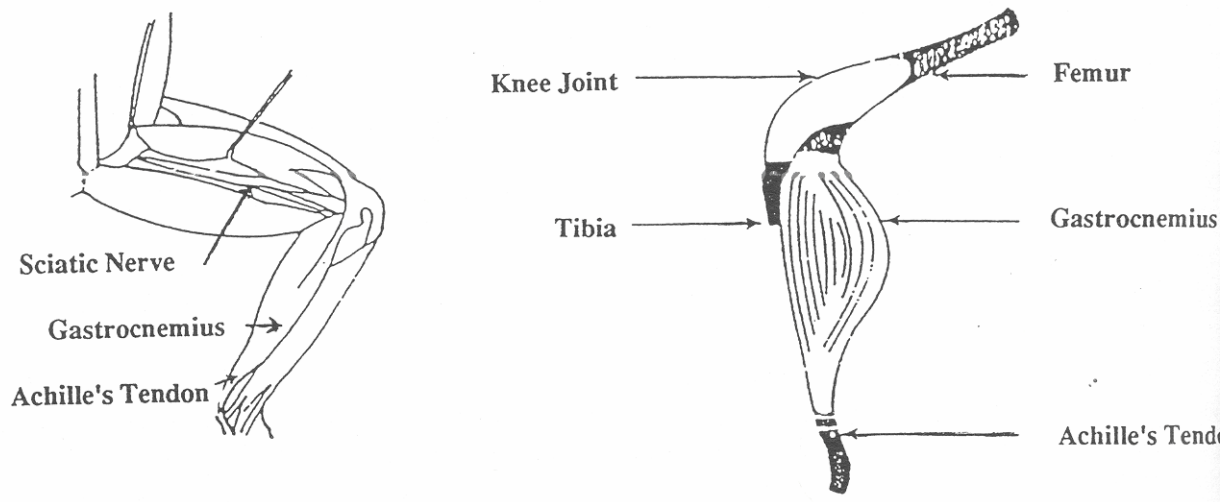
- reader: read instructions as experiment progresses/clean up,
- surgeon: prepare specimen/clean-up,
- recorder/paper adjuster/pen manipulator,
- keeper of the saline/clean-up, and
- set-up person: mount excised muscle while surgeon cleans up (*critical*).

### Recording Apparatus

The following materials are used to interpret muscle contractions as writing on the kymograph drum:

1. Femur clamp: holds the frog femur bone to which the gastrocnemius muscle is attached (Figure 9.2).
2. Muscle lever with pen: attached to the Achilles tendon of the gastrocnemius muscle by a strong thread. The lever is raised with each muscle contraction and the ink pen thereby records the activity on the muscle kymograph drum.
3. Signal marker (electromagnet, if used): the magnet is energized simultaneously with each stimulation to the muscle causing the pen attached to it to record on the kymograph drum the point of stimulation.
4. Electrodes: attached to the stimulator and passes an electrical current into the muscle to cause the contraction.
5. Ink bottle: ink supply for the recording pen.

Observe the proper set up of the recording apparatus (Figure 9.2). The TA will instruct you on its proper use. The writing tip of the pen must be positioned on the drum so that a fine line of ink is observed. Adjust the ink bottle to insure that the marking occurs properly by raising or lowering it or loosening or tightening the cap.



**Figure 9.2.** Frog gastrocnemius muscle preparation. After skinning the leg and removing other musculature, the gastrocnemius is left attached to the femur.

### Muscle Preparation

The frog will be anesthetized, decerebrated and pithed by a TA. They will then remove one leg for your experiment.

1. Remove the skin from one rear leg and prepare the muscle as follows: Make an incision in the skin around the thigh where it joins the body and peel the skin down off the toes with forceps as if you were turning a glove inside out as you were removing it. This will expose the muscles. If the outer surface of the skin touches the muscles, chemicals produced there will interfere with good experimental results. *Don't touch the muscle with fingers that have touched the frog's skin.* Wash your hands after pulling the leg skin down. You might want to wear a surgical glove.
2. Separate the gastrocnemius from the rest of the musculature of the shank by inserting a blunt probe and running it the length of the shank; reinsert the probe between the Achilles tendon and the bones of the foot; separate the Achilles tendon as it passes over the heel. Cut the Achilles tendon as close as possible to its attachment on the planter surface. See Figure 9.2.
3. Bare the femur by removing all musculature and connective tissue from the thigh. *Do not* cut the structures of the knee joint or damage the origin of the gastrocnemius.

4. Sever the tibia at the knee joint and cut away the remaining part of the lower leg. The preparation should now consist of the gastrocnemius muscle with severed Achilles tendon and the knee joint with parts of the femur and tibia. Refer to the diagram.
5. Place the femur in the femur clamp. Make certain that the jaws of the femur clamp are parallel with the laboratory table surface and that the muscle is suspended vertically directly above the point where it will be attached to the writing lever.
6. Tie a piece of thread tightly around the Achilles tendon immediately above the knob which was attached to the calcaneus and attach the other end of the thread to the writing lever. Adjust the writing lever on the stand so that you don't stretch the muscle.
7. Adjust the height of the femur clamp so that the muscle carries no weight but the thread is taut.
8. Position the electrodes against the surface of the muscle, close to the knee joint. The electrodes should not impede the shortening of the muscle. (*Note:* If the electrodes fall off the ring stand into the saline collecting pan, turn the stimulator off before picking up the electrode).
9. *Keep the muscle moist by constantly dripping room temperature ringer's solution on it.*

### Control Experiment

Can threshold and maximal responses, summation and tetanus be observed using the kymograph set-up?

#### *Threshold Stimulus*

1. Set the voltage at the lowest value (0.1 volts with multiplier set at 1X).
2. Set the frequency at 1.
3. *Do not* turn the recording apparatus on.
4. Stimulate with a *single* stimulus. Did you see a response? Record the voltage used and manually turn the drum about 1 cm. Stimulate another time. Record voltage used (from here on, record notes on your paper after you do anything, so that later you will have a reliable record of what was done).
5. Move the rotating drum manually just slightly so recordings won't overlap. Continue until contraction occurs. The voltage at the point of stimulation to contract is called the *threshold voltage* or *stimulus*. It is the least amount of voltage required to elicit a contraction.
6. If no contraction occurs, keep increasing voltage by 0.1 volts and stimulating again.

*Keep muscle moist with saline*

### *Maximal Stimulus*

After reaching the threshold voltage, continue increasing the voltage by 1.0 volt increments and note until you no longer perceive an increase in contraction (the excursion of the writing point does not go higher than the previous contraction). Don't forget to manually advance the drum between stimuli and record the voltages. The voltage used to get the first of these equally high contractions is the *maximal voltage stimulus*.

*Keep muscle moist with saline*

### *Single Muscle Twitch*

1. Set kymograph rheostat to 1 (high speed) for recording the muscle twitch.
2. With the voltage set just above the maximal stimulus, stimulate the muscle with a single stimulus while the drum is turning.
3. With the muscle relaxed, draw a base line with the muscle lever pen just beneath the muscle twitch tracing.
4. You will be unable to note the latent period (period after stimulation but before contraction) if you do not have a signal magnet. However, you can see the period of contraction, and that of relaxation.

*Keep muscle moist with saline*

### *Summation and Tetanus*

1. Set the voltage for maximal stimulus and the kymograph rheostat at a speed so that individual contractions will be isolated (you may have to experiment to determine which setting between high and low speed is best).
2. You will use the multiple stimuli setting.
3. With the drum revolving, stimulate the muscle with a maximal stimulus at a frequency of 3 stimuli per second for 2 or 3 seconds. For each successive set of stimuli: Increase the frequency of stimulation by one per second until summation and tetanus is observed. Make sure to mark your record every time you change the frequency. *Summation* will show individual contractions without complete relaxation while *tetanus* is a single prolonged contraction. Explain each phenomenon at the *cellular* level.

*Keep muscle moist with saline*

### **The Experiment** (Choose Your Variable)

1. Now that you have observed that aspects of muscle contraction can be monitored using the kymograph, look through the available chemicals and choose one. What effect will the chemical have on minimal and maximal stimuli? Single muscle twitch? Summation and tetanus?
2. Construct a hypothesis to test and set up your protocol.

3. How will you quantify your data? How will you convey differences or lack thereof?
4. Perform your experiment.

### **Fatigue**

1. Set the frequency just below that needed to obtain summation.
2. Voltage should be set for maximal contractions.
3. Allow the kymograph to run slowly while a multiple stimulus is applied.
4. What happens to the height of the contractions? Explain.
5. What happens when saline is applied to the muscle? Explain.

### **Clean Up**

1. The pens and ink bottles must be emptied. Return the ink to the main storage bottle. Rinse the ink bottle with distilled water and flush the pen by replacing the ink bottle top and squeezing water through the pen. This will prevent drying of the ink and clogging of the pen.
2. *All equipment touched by saline must be rinsed.* (Why?)
3. Tissue is to be disposed of in the appropriate container.

### **Write-Up**

1. A write-up consisting of your hypothesis, predictions, methods, results and conclusions for each section should be submitted. Make sure you *explain what is happening at the muscle cell level*. Direct your statements to facts that you have learned and not just ideas which might sound good but have no factual basis. You should include all data for threshold voltage, maximal stimulus, single muscle twitch, summation/tetanus, fatigue, and your designed experiment.
2. Each member of the experimental team should submit his/her *own* tracings based on the results of the team computed. You should photocopy the recordings. Only those areas of the paper most informative need be submitted. Cut out and paste on a report sheet of your design. Add additional sheets as necessary; staple *all* together. The names of *all* members of the team should be included on *all* reports and recordings.

### Literature Cited

- Ambrose, H. W., and K. P. Ambrose. 1987. A handbook of biological investigation. Fourth edition. Hunter Textbooks, North Carolina, 204 pages.
- Pflanzer, R. G. 1979. Contractility of skeletal muscle in experimental and applied physiology. Wm. C. Brown, Dubuque, Iowa. [pages 59–80]

### APPENDIX A *Discussion Material*

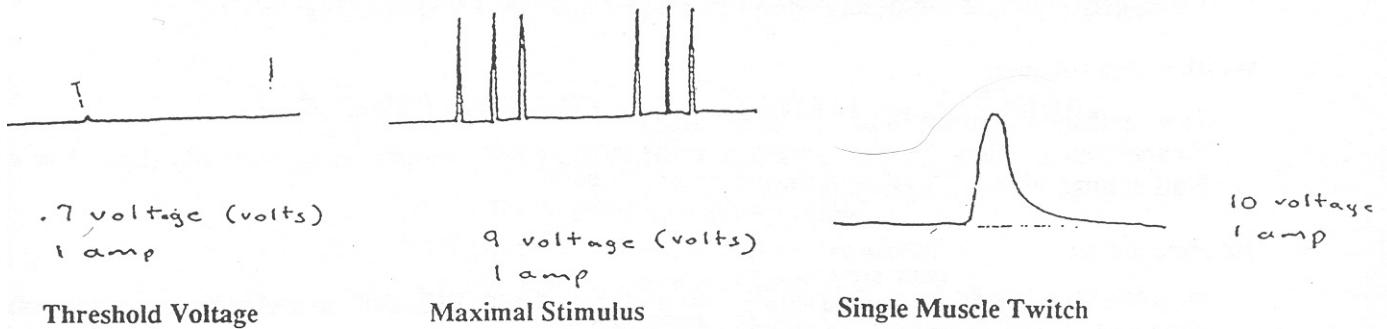
The following statement was presented at the workshop to stimulate discussion about animal use in the laboratory and to begin the process of obtaining the opinion of ABLE members on this subject:

“Biology is the study of life, and biologists through their study gain an appreciation for and an understanding of the many forms of life. The sacrificing of a life, whether it be for sustenance, protection, or understanding, should be taken most seriously and should be accomplished in the most humane way. Society does not question the need to consume living organisms for the purpose of its sustenance or protection, but the taking of a life to further understanding is contested. We believe that no life should be sacrificed indiscriminantly. However, it is our belief that it is not only appropriate but also at times necessary to sacrifice an organism when it is the only means to obtain the understanding requisite for continued study of a principle or concept of life.”

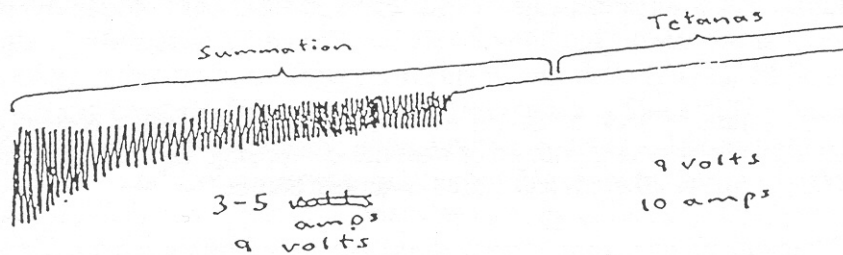
APPENDIX B  
The Laboratory

**Expected Results**

The following are student-generated tracings of expected results for the control experiment:



Summation, Tetanus



To quantify differences between the control values and the experimental values, students in the past have graphed differences in voltage as a function of control vs. experimental chemical or some have actually measured difference height of tracings from baseline upon exposure to control vs. experimental chemicals. Sample data are provided in Table 9.1.

**Table 9.1.** Sample data.

Chemical variable	Saline	Acetylcholine
Threshold	40	100
Maximal stimulus	4	13
Frequency at tetanus	25	17.5

## Preparation of Chemicals

1. Frog saline:

- 0.14 g potassium chloride
- 6.50 g sodium chloride
- 0.12 g calcium chloride
- 0.20 g sodium bicarbonate
- 1 liter dH<sub>2</sub>O (for the lab described in this chapter, prepare 2 liters)

2. Anesthetizing solution:

- 25 g urethane dissolved in up to 100 ml dH<sub>2</sub>O
- To anesthetize, inject 0.6 g urethane/2.4 cc dH<sub>2</sub>O/100 g body weight intraperitoneally (i.p.).
- Use a 5-ml syringe with a 20-gauge needle.

3. Norepinephrine:

- Dissolve 50 mg in 50 ml normal saline, adjust pH to 2.5 with HCl, store in amber bottle. If solution turns pink, it has degraded, and should be remade.

4. Acetylcholine chloride:

- Dissolve 50 mg in 50 ml saline, store in an amber bottle.

5. Hydralazine:

- 50 mg in 50 ml normal saline. Check bottle for exact composition of tablet; normally two tablets each containing 25 mg hydralazine (along with other material comprising the “pill” part) need to be ground, dissolved in 50 ml saline, filtered and stored in an amber bottle in the refrigerator.

6. Inderal:

- 80 mg in 80 ml saline. Check bottle for exact composition of tablet; normally two tablets each containing 40 mg inderal (along with other material comprising the “pill” part) need to be ground, dissolved in 50 ml saline, filtered, and stored in amber bottles.

7. Neostigmine:

- 50 mg in 50 ml normal saline. Store in amber bottle.

## Addresses of Suppliers

- Connecticut Valley Biological Supply Co., Inc., P.O. Box 326, 82 Valley Rd., Southampton, MA 01073
- H. L. Moore Medical Corp., 389 John Downey Dr., New Britain, CT 06050
- Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178