Laboratory Manual for

Vertebrate Physiology

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for

VERTEBRATE PHYSIOLOGY

by

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FOREWORD

The exercises included in this manual have been designed with two main purposes in mind: (1) to give the beginning student in Physiology a working knowledge of elementary laboratory procedures, and (2) to furnish the opportunity for students with more experience to apply techniques in more advanced study with a minimum requirement for special or expensive equipment. In the latter instance, many of the earlier investigations so important in development of physiological knowledge, and some so-called "classical" experiments are presented. In all instances the student is afforded an opportunity for independent work, since only a few of the exercises require special clarification by the instructor.

In addition to the aims already given, the student will also familiarize himself with a technical vocabulary essential to work in physiological and health studies and will learn to cooperate with other members of a group preparing a special experimental project and demonstrating it to the other students of the class.

The number of exercises furnished gives a wide range for selection to suit many situations and for courses at varying levels and of different durations. Thus the instructor will enjoy considerable freedom in choosing a program. In planning the work, it should be noted that some exercises will require more than one laboratory session of work; in fact, some may continue for several weeks with occasional observations.

While the manual includes space for answering questions, discussing results, and for kymograph records, it is recommended that the laboratory instructor follow a system best suited to local conditions in the manner of completing the exercises. With large classes, it may be found advisable to use the manual as a student guide and have the students hand in the exercises on separate sheets.

At the beginning of each exercise, there are listed materials and equipment required. These lists will be of invaluable aid in planning and preparing for the laboratory session. In the Appendix, formulae are given for various solutions and materials needed. Tables presenting essential and important physiological data will also be found in the Appendix.

The authors have been aided considerably in preparation of the manual. Others have contributed valuable comments and suggestions. We owe special thanks to Professor H. G. O. Holck for his suggestions regarding the manuscript, and to Mrs. Norma H. Pace for original preparation of most of the figures.

GENERAL INSTRUCTIONS

Students will be divided into groups of two each and will be equally responsible for the apparatus assigned to them. This is necessary, since few of the exercises can be carried out by one person. In exceptional cases and only with the instructor's permission may more than two students work together.

The work assigned is not intended to overload the student, but there are times when it may be necessary to work beyond the assigned period or it may be necessary to attend the laboratory at some other period (with the consent of the instructor) in order to finish an exercise. The student is expected to do this in good spirit. In fact, his laboratory mark will depend to a great extent on his attitude, diligence, ingenuity, etc., as well as on his laboratory experimentation and presentation.

Failures in exercises will be cut to a minimum if students acquaint themselves with the methods of the exercises before undertaking them. Therefore, each student will be expected to study thoroughly the assigned experiments before coming to the laboratory; textbooks and references should be consulted freely. Short tests may be given at any time during class hours to make certain that the students are properly prepared for the laboratory work.

APPARATUS

Procure from the stores department or from the laboratory assistant, keys or combinations for the following: a locker containing a compound microscope with substage condenser; a locker containing a kymograph with two ring stands and supports; and a drawer containing the following articles:

1 flat jaw clamp
1 light muscle level
1 tuning fork
4 clamp fasteners
1 tuning fork starter
1 inductorium
1 contact key or button
1 plate (porcelain)
1 evaporating dish
1 frog cage
1 platinum electrode
1 frog board
4 frog board clips
6 coils of wire
2 watch glasses (3")

Care of the apparatus provided for each group is the duty of that group. Any equipment broken or lost will be charged to the group or groups to whom it was assigned. In case any may be misplaced or damaged, call attention of the matter to the instructor immediately so that it can be remedied. Kymographs, ring stands, and blocks are to be returned to the lockers at the end of the laboratory period. All lockers, including microscope lockers are to be locked before leaving the laboratory. They will be checked after each period.

In some cases special equipment or apparatus may be required from the storeroom. This equipment must be returned to the storeroom at the end of the laboratory period. In no case should special equipment be left in the student's locker.

Each pair of students shall purchase one dissecting set and one non-returnable kit (at stores department) containing materials which will be used in most of the exercises during the course. Each student must have a 6" or 12" ruler and a 3H pencil.

Whenever experiments are to be performed on live animals these are to be treated kindly. Failure to observe the proper care and humane handling of experimental animals will exclude the offender from further participation in the course.

Where animals are to be subject to any operation beyond a simple injection, which is made on humans without anesthesia, a general anesthetic will be administered. One must keep in mind that deep anesthesia may obscure physiological functions, as, for example, when anesthetics depress certain reflexes; also that anesthetic agents have side actions of their own which may cause misleading results and thus may make the student draw false conclusions.

PRESENTATION OF RESULTS

1. Read the instructions carefully before an exercise is attempted and note the objective of the experiment. This is important as from time to time the instructor will question the students on the outline and procedure.

2. Records and description of results. Kymograph records may be held fast by means of small strips of scotch tape and neatly covered with tissue paper. Data obtained from the experiment should be tabulated whenever possible.

All answers to questions, notes, discussions, and conclusions are to be written in the space provided in the manual. All writing is to be neatly done in ink, drawings and sketches are to be done with a 3H pencil and shall be diagrammatic in character. Neatness in drawings should be stressed, but artistic drawing is not required.

3. At least thirty to forty-five minutes will be allowed at the end of each laboratory period to write up conclusions and make drawings. After completing the exercise, the notebook will be handed to the instructor who will lock it in the cabinet until the next period. Under no conditions will the student be allowed to take the notebook from the laboratory, except preceding an examination.

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* Exercises marked with an asterisk (*) are recommended for a one semester course.

** Exercises marked with a double asterisk (**) are recommended especially for advanced groups.

NERVE

RECEPTORS

BLOOD AND CIRCULATION

RESPIRATION

NUTRITION, DIGESTION AND ABSORPTION

ENDOCRINES AND REPRODUCTION

Exercise I
APPARATUS

Materials and Equipment

Kymograph and paper
Apparatus for smoking and shellacking kymograph paper
Film for writing points (or equipment for ink writing)
Vibrating interruptor
Signal magnet
Tuning fork and starter
Ring stand and clamps
Inductorium, key, wire, and battery
Platinum electrodes
Microscope, lamp, slides, and coverslips
Lens paper

THE INDUCTORIUM

Electrical, chemical, thermal and mechanical stimuli are the types which will elicit a response in muscle. However, the usual form of experimental stimulus in physiology is electrical, because (1) it is easily applied, (2) the intensity can be controlled, (3) it affects all fibers simultaneously, (4) it does not injure tissues permanently, and (5) it is nearest to the true physiological stimulus.

Learn how to set up the electrical stimulating apparatus according to the directions of the instructor (fig. 1). What is the meaning of the term "Primary circuit"?

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What is the meaning of the term "Secondary circuit"? .....

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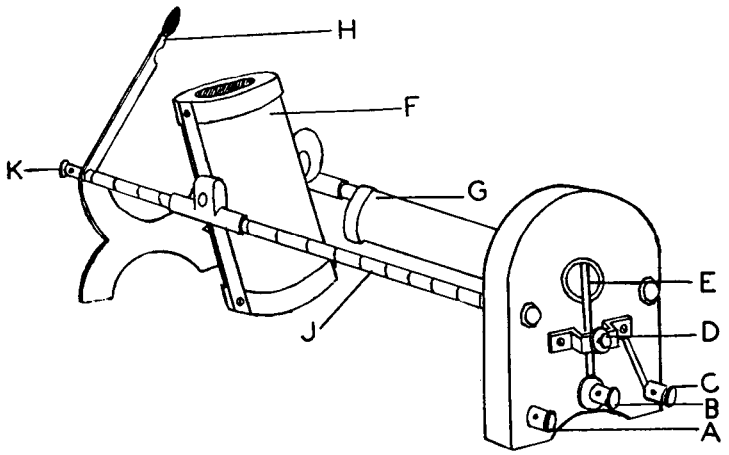

FIGURE 1

The Inductorium. Binding posts, A, B, and C, for the primary coil; A and B being used for single make and break induction shocks; A and C for tetanizing shocks. D, set screw for regulating the distance of the vibrator E, from G, the core of the primary coil. F, secondary coil; H, shunt or short circuiting key; J, metal bar slide of the secondary marked off in centimeters. K, one of the two terminals of the secondary coil. The intensity of the current is varied by the position of the secondary coil to the primary coil; the closer the secondary is to the primary, the stronger the current induced; the least current being produced when the secondary is at the far end of the inductorium in a vertical position.

Instructions will be given on how to connect the apparatus to a dry cell and how to adjust for simple make and break shocks and for tetanizing current. Learn how to use the (make and break) contact (key) and short-circuiting (shunt) keys in giving single make and break shocks.

Place electrodes from the secondary coil on the tip of the finger (moisten with saline solution) and find the position of the secondary necessary to obtain make, break, and tetanizing stimuli. What difference do you note between "make" and "break" shocks? .....

.....

How do you explain this? .....

.....

How does tetanizing differ from "make" and "break" current? .....

.....

THE KYMOGRAPH

Warning. The kymographs are not to be used until complete instructions have been given by the laboratory instructor. Care should be exercised in handling and carrying the kymograph.

Study the mechanism of the kymograph (first used by Ludwig, 1847). When you fully understand how to start and stop the revolving motion of the drum as well as the speed control, fasten a sheet of glazed kymograph paper around the drum and smoke it according to instructions.

Why is glazed paper best for this use?.....

Do not get the soot too thick. Why?.....

..... A dark brown color is preferable to black, since this signifies a rather thin layer of soot.

Learn how to shellac records. All kymograph records must be approved by the instructor before shellacking. The following information must be on each kymograph record before it will be approved: students' names, exercise number, date.

THE TUNING FORK, SIGNAL MAGNET, AND INTERRUPTOR

By means of a clamp, attach a tuning fork to a stand in such manner that when the prongs of the fork vibrate, a writing point attached to the inner edge of the uppermost prong will record a wavy line on the lower part of a moving smoked kymograph drum. This fork which vibrates at a rate of one-hundred vibrations per second is started by using a tuning fork starter.

The interruptor consists of a magnetized spring which is attached to a ring stand (fig. 2). A platinum wire protrudes from the tip of the spring. When the apparatus is adjusted properly, this wire (during the time the spring vibrates) dips down into the mercury cup of the interruptor. The circuit is arranged in such a way that this makes the current, the spring's recoil is reinforced by magnetic repulsion of like poles and the process is repeated. By connecting it with a signal magnet (fig. 2) which is fixed also to mark on the drum, the interruptor can be so adjusted that it will record fractions of a second.

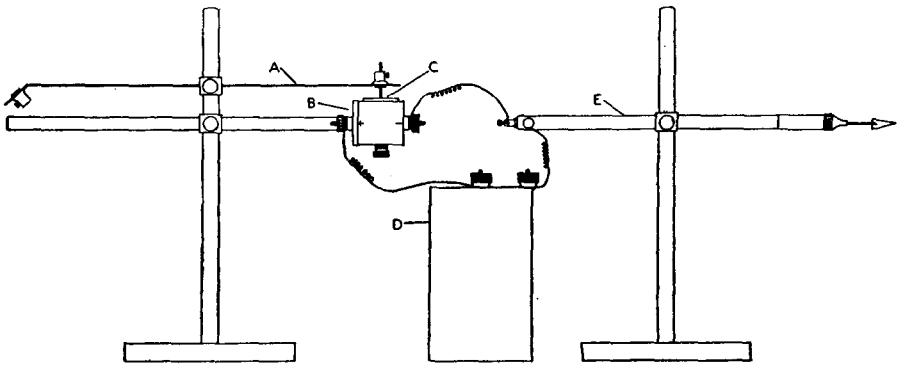

FIGURE 2

Diagram of the Vibrating Interruptor. A, vibrating steel spring; B, mercury cup and condenser box; C, mercury cup; D, battery with positive pole connecting to post of interruptor marked positive (+); E, signal magnet.

Calibrate the interruptor at the different file marks, or, if it is new, calibrate at approximately 4, 8, 12, and 20 vibrations per second, making file marks at these points. Make and keep a record of the time of vibration of the interruptor at the different marks with the number of the instrument in your notes for future reference.

THE MICROSCOPE

A. ILLUMINATION OF THE OBJECT.--Use the plane mirror for low powers and concave mirror for higher magnifications. Concave mirrors permit much more intense light than the plane mirror. (fig. 3)

B. TO FOCUS LOW POWER OBJECTIVE.--Attach the objective to the nosepiece. Lower the head to level of the stage, to be able to see the front of the objective and lower the tube by the coarse adjustment until the front of the objective is within one-quarter inch of the object. Look through the eye piece and slowly elevate by the coarse adjustment until the image is distinct, then use the fine adjustment.

C. TO FOCUS HIGH POWER OBJECTIVE.--Lower the head to the level of the stage and look between objective and cover glass. Slowly lower the objective with the coarse adjustment until the front of the objective is nearly in contact with the cover glass; look into the eyepiece, slowly elevate the tube by the coarse adjustment until the image appears. Use fine adjustment. Always Focus Upward! Never Down! Why?.....

.....

D. CLEANING THE LENS.--Use only lens paper in cleansing the objective or eye pieces. Breathe upon the surface and give a revolving motion with the lens paper.

E. CARRYING THE MICROSCOPE.--When carrying the microscope, grasp it by the handle arm with one hand and use the other for steadying and supporting the base. Always use both hands in carrying your microscope.

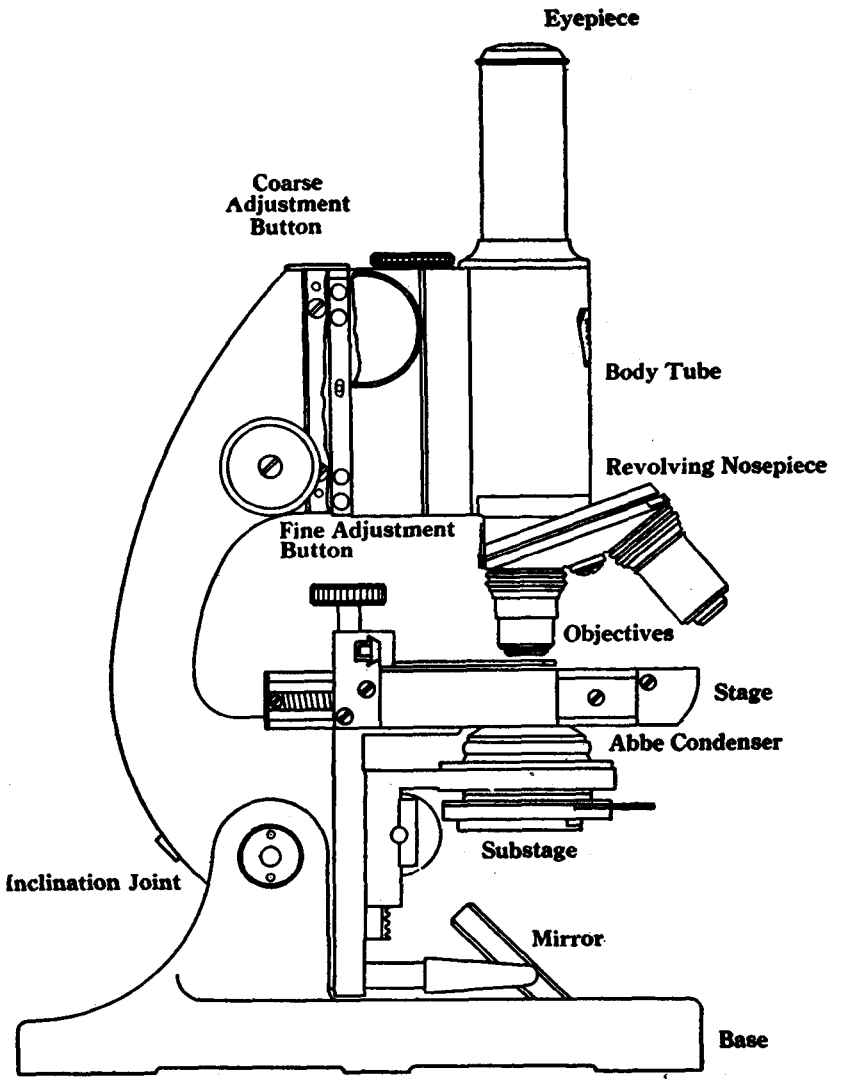

FIGURE 3

Mechanical Construction of the Microscope. (Bausch & Lomb Optical Co.)