FOR MEDICAL STUDENTS

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EXPERIMENTAL PHYSIOLOGY

FOR MEDICAL STUDENTS

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PREFACE TO THE FIFTH EDITION

It has been the aim in earlier editions of this practical handbook to maintain a representative balance between the various branches of experimental physiology. The happy combination of authorship in this new edition will not only preserve this balance but also broaden the base of the subject matter.

The experiments are designed to cover the whole field evenly except in so far as this is limited by the costliness of apparatus or by the time which can be spared from an exacting curriculum. Difficulty is no obstacle to the average student to-day since the excellence of the scientific training in our schools enables all the experiments in this book to be classed as students' exercises. It only remains, therefore, for any particular laboratory to make the selection of experiments which coincides with the apparatus available and afterwards to correlate the results and discuss the variability found in large classes. Working instructions are framed so that any experiment can be tackled by the new student ; the book may thus be used with advantage in laboratories equipped with only one set of each type of apparatus.

We have enjoyed the valued help of our many colleagues ; in particular, we are indebted to Dr. L. Bernstein for several contributions.

D. T. H.
H. P. G.
W. A. M. S.

INTRODUCTION

INSTRUCTIONS TO STUDENTS

Apparatus :

Each medical student should provide himself with a note-book and dissecting instruments : *coarse and fine scissors and forceps, scalpel for cutting skin only, and a seeker.* In order to accustom himself to the physical signs of the normal subject, and especially to their variations in health, he is strongly recommended to procure for himself the following instruments which will be essential later in clinical work : *Stethoscope (binaural), ophthalmoscope, head mirror, laryngoscope* and *ear speculum.*

Course :

This will usually be circulated on a typed sheet or printed record card.

In the *Junior Course*, each experiment is performed by the whole class with general laboratory apparatus or special apparatus of an inexpensive character.

In the *Senior Course*, only a few sets of apparatus for each experiment are available on account of their special nature and cost, so that thirty different experiments may be running at the same time. Each pair of students is given a different number and begins the course at the experiment with this number ; thereafter, he progresses arithmetically.

Procedure :

(1) *Economy* must be exercised with frogs. Muscle experiments may be repeated with frogs left over from heart experiments and *vice versâ.*

(2) *Observations* and *conclusions* must be carefully and legibly recorded in a large note-book. *Diagrams* and *graphs* are essential. Procedure need not be written up unless it differs from the text. Where results are outside the range of normal variation, the student should offer an explanation for the discrepancy.

(3) All apparatus must be left in a *clean and orderly* condition before the experiment can be credited as completed.

(4) Not only must any *breakage* be reported to the steward, but also the student responsible must get the repairs effected himself at his own cost if negligence is apparent.

(5) Time will be saved if, after the completion of the physiological experiment on an organ, the *pharmacological* tests (Chap. XVIII.) be carried out before taking down the apparatus.

(6) Present your record card to the demonstrator before dissembling the apparatus. The *awards* on this card take the place of examinations and have the following values :—

α. A good experiment embodying an original method or technique (any set experiment may be partly or wholly replaced by one devised by the student).

β. A satisfactory experiment.

γ. An experiment which must be repeated.

Physiological Dissection:

The brain of the cat, only, is destroyed, so the remainder of the animal is alive, with living tissues, therefore :—

(1) do not obstruct respiration, as by leaning on the animal or allowing the tongue to sag back into the throat ;
(2) do not ill-treat the tissues, as by tearing or dragging ;
(3) do not pick up delicate structures in forceps, otherwise, *e.g.*, blood vessels suffer intravascular clotting and nerves suffer block of conducting power ;
(4) keep the whole animal warm and prevent drying and chilling of wounds with swabs wrung out in warm Ringer's solution (40° C.).

Speed:

Prepare all electrical and recording apparatus before operating, and work as speedily as possible to minimise shock.

Example:

A practical session is set out briefly in order to guide the student in the order of performance of the experiments with the object of making the fullest use of a single animal. Lethal procedures are reserved to the end.

It is not an economy, however, when several bad experiments take the place of one really good one.

B.P. IN THE DECEREBRATE CAT

Decerebration (by the demonstrator). The cat is deeply anæsthetised with chloroform and ether, artificial respiration applied, carotid arteries tied, the skull trephined, the brain stem cut completely across and the cerebral hemispheres destroyed.

Two Students A and B will carry out the following (watching the breathing incessantly) :—

Record and Measure the Arterial B.P. in the Carotid. A passes a ligature under the right carotid artery and clips the artery in preparation for the introduction of the cannula.

Observe the respiratory and cardiac undulations on the tracing and note the difference in artificial and natural respiration. What is the effect of: (i.) pressure on the abdomen and (ii.) posture ?

Excitation of the Vagus. B applies 2 ligatures to the left vagus and cuts between. Excite the central end and the peripheral end with a Faradic current just perceptible on the tongue. Note pulse and B.P.

Excitation of the Sympathetic. A does this as in 2. Note the pupil.

Excitation of a Sensory Nerve. B exposes the sciatic or median nerve, applies a ligature and cuts the nerve distally. Excite the central end and note B.P.

Asphyxia and Recovery. A clamps the trachea for 2–3 minutes.

Hæmorrhage and Intravenous Infusion of Gum Saline. B introduces a venous cannula into the external jugular vein, and A a similar cannula into the femoral artery. A withdraws 40 c.c. of blood and after an interval B replaces it with 40 c.c. of warmed gum saline or reconstituted serum (p. 35) (avoid air bubbles). Note B.P.

The ideal method of measuring the **blood volume** in an animal is to draw off a small sample of blood to supply the 100% Hb standard ; bleed and infuse reconstituted serum *simultaneously* at the *same rate* ; wait $\frac{1}{4}$–$\frac{1}{2}$ hour for a steady state to be established and draw off a final sample of blood to give the final %Hb. Measure the volume and %Hb of the mixed bleedings. You now possess all the data for calculating the blood volume.

Excitation of the Splanchnic Nerve. B passes a ligature under the left Splanchnic Nerve, cuts it and excites the peripheral end.

Intravenous Injection of Adrenaline. A injects 1 c.c. of 10^{-5} solution of adrenaline into the external jugular vein.

Open the Chest. Note the collapse of the lungs, use artificial respiration.

Feel the heart and listen to it through a stethoscope.

Cut out the heart, again auscultate, and observe the sequence of death in the heart chambers. Stimulate it electrically.

CONTENTS

EXPERIMENTAL PHYSIOLOGY

CHAPTER I

EXCITATION OF TISSUES

EXPERIMENTAL PHYSIOLOGY examines the way in which living organs work. These organs may be set into activity by the application of suitable stimuli—the most easily controlled type of stimulus being electrical.

ELECTRICAL ENERGY may be obtained from—

(i.) **Primary Cells,** like the Leclanché cell. The Carsak modification of this cell has a lower internal resistance than the original form ; both

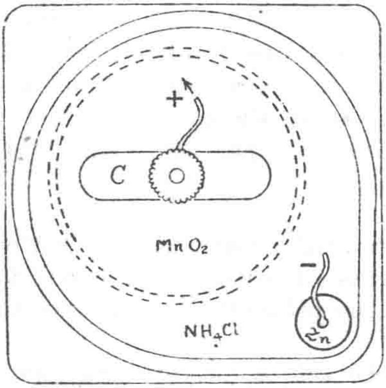


FIG. 1. Leclanché cell in plan.

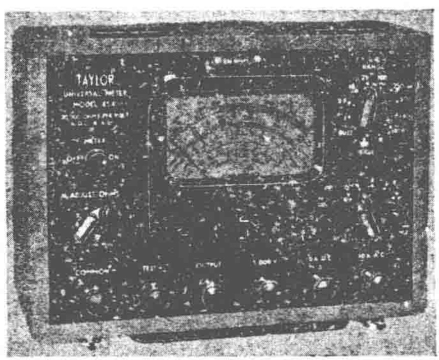


FIG. 2. A 90-range meter (*Taylor*).

forms are recommended for general laboratory use because they show good recovery in virtue of the depolarising action of the MnO_2 in the porous pot (Fig. 1). The ordinary dry cell is also a form of Leclanché cell, but the electrolyte and amalgamated zinc cannot be renewed.

(ii.) **Secondary Batteries.** The Nife accumulator possesses a long life of service and suffers no injury from short circuiting ; it consists of a negative plate of iron oxide and a positive plate of nickel oxide immersed in an alkaline solution.

(iii.) **The Dynamo** supplying the current to the electric lighting mains. Its use will be studied in Chapter XV.

Electrical Measurements :

There should be at least one instrument for electrical measurements in every physiological laboratory ; if only one can be supplied, it should be of the universal type (Fig. 2).

Observation 1. Test the **voltage** of the Leclanché or other cell with a pocket type or other form of voltmeter. If the e.m.f. is much below $1\frac{1}{2}$ volts, re-amalgamate the zinc plate by cleaning it with dilute H_2SO_4 and rubbing mercury into it, clean up the terminals, and renew the saturated sal-ammoniac solution. Test the voltage across the terminals with the cell free and also "on load," *i.e.* connected to primary coil (Fig. 3).

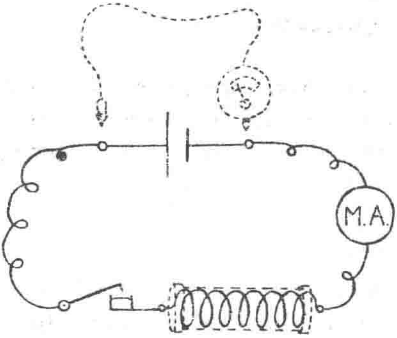


FIG. 3. Milliampère-meter, Resistance and Key in series — Voltmeter in parallel—with cell.

Observation 2. Verify the **polarity** of the cell with a piece of moistened blue litmus paper (pole paper is used in Fig. 219). The wire connected to the carbon pole turns the litmus red (red paint is the recognised marking for the positive terminal or anode), while the kathode makes the litmus blue because the $\overset{+}{NH_4}$ kation travels with the current to the negative terminal. The current flows *outside* from the carbon, the positive pole, to the zinc, and completes the circuit *inside* the cell from the electro-positive metal Zn to the electro-negative C. Make a wiring diagram showing with arrows the passage of the current and the transport of the ions.

A KEY or SWITCH should always be inserted in series with the battery to prevent exhaustion of the cells, otherwise the e.m.f. would soon fall with the increase of back e.m.f. arising from polarisation at the anode.

Observation 3. Connect the Leclanché cell, a mercury key, and a milliampère-meter in series with an external resistance such as the secondary winding of an induction coil (Fig. 3). Switch on the current and observe its strength. Notice how it dies away as the H_2 accumulates at the C; record the voltage of the cell (see Observation 1), as indicated in Fig. 3. Open the key and note the recovery of the cell by occasionally testing it with a voltmeter; recovery is brought about by the removal of H_2 from the carbon anode by MnO_2.

Write equations expressing the chemical changes occurring during polarisation and recovery of the cell.

Calculate R, the **resistance** of the secondary coil, from Ohm's law (neglecting the relatively small resistance of the milliampère-meter) :—

E.M.F. = current × resistance.

1·4 volts = ... ampères × R ohms.

Further, calculate the **power** (work done per second) of this external circuit—

. . . . ampères × . . . volts = . . . watts.

ELECTRODES are used for applying an e.m.f. to a living tissue. Make a simple pair from ½ metre of silk-covered twin-flex bell wire by soldering a pair of brass pins to the wires at one end, using resin as a flux. The pins may be pushed through a cork or piece of rubber to insulate them, or they may be fixed with plasticene (Fig. 5). A universal joint, such as that used for supporting the cat's whisker in crystal detectors, makes an elegant electrode holder.

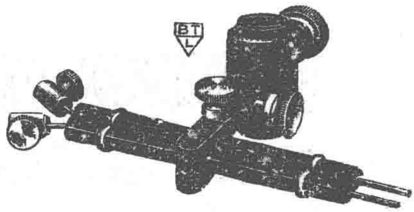


FIG. 4. Mounted electrodes.

Observation 4. Connect the electrodes in series with the battery and its key. Apply the pin electrodes to the tongue and test the effect of

- M : making the circuit (closure of key);
- G : continuous galvanic current; and
- B : breaking the circuit (opening the key).

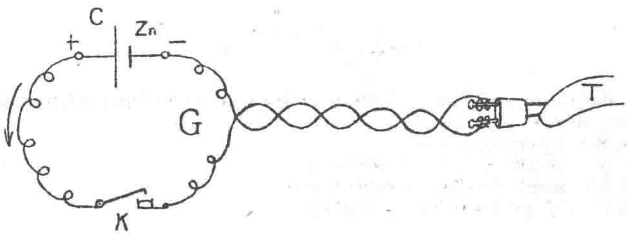
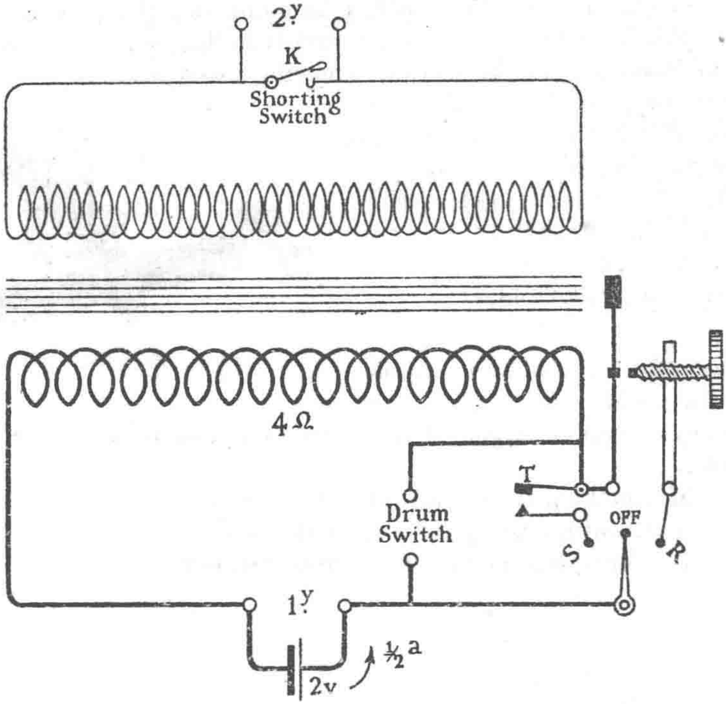


FIG. 5. Galvanic circuit.

No shock is felt during G, merely a metallic taste due to electrolysis. Give a diagram of the migration of the $\overset{+}{Na}$ and $\overset{-}{Cl}$ ions in the tongue. Which is the more exciting, M or B?

For exciting living tissues the single cell alone is not very effective; much higher voltages (forty upwards) are usually employed, but in this case the current can be correspondingly small (less than a milliampère) and of much shorter duration. This fortunately minimises electrolysis in the tissue, and since it exhibits a more rapid growth or decline, is relatively more effective.

An INDUCTION COIL is the simplest apparatus for transforming the electrical energy from the Leclanché cell into an exciting form: pulses of smaller flow, but rising to high pressure and falling to low pressure with extreme speed. The induction coil is an open-cored variety of step-up, static, high-tension transformer which depends for its action on the interruption of the primary current by an independent break or interrupter (Fig. 6). It yields a "peaky" potential wave.



K : short-circuiting key, closed during preparation, open for recording.
2-way switch
on R : repeated shocks.
on S : single shocks by tapping T.
on S : single shocks for drum switch.
OFF : off position, saving battery.

FIG. 6. Induction coil incorporating all switching.

Observation 5. **Self-Induction : Break Extra Current of Faraday.** For this experiment *only*, remove the secondary coil. Arrange the alternative circuit shown in Fig. 7, using the top pair of terminals on

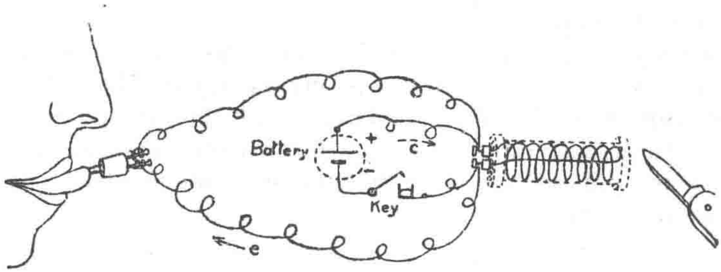


FIG. 7. Illustrating the break extra current and electro-magnetic induction.

the primary bobbin to which the ends of the primary coil are directly attached. Test the magnetism induced in the soft-iron core with a penknife before and during the passage of the current in the primary coil. Place the electrodes on the tongue, and open the key ; a shock will be felt owing to the passage of the induced extra current from the coil through the tongue ; this is the way in which the electromagnetic energy evidenced with the penknife dissipates itself from the primary coil, viz., as a self-induced current of very small size, but at a very high voltage. Make separate diagrams showing (*c*) the course of the exciting current, and (*e*) the path of the break extra current.

The break extra current flows in the same direction as the battery current and thus tends to maintain the magnetic field in the coil ; it thus prevents the *sudden* fall of the primary current to zero. If, however, the alternative path through the tongue were not open to the break extra current, the electromagnetic energy would be dissipated in the form of heat and light as a big spark on opening the key. Test this. The fall in the primary current is here practically instantaneous. Replace the secondary coil.

A SHORT-CIRCUITING KEY is a useful form of switch for preventing the accidental passage of induced voltages through the tissue under investigation ; this should always be placed *across* the electrodes to provide a safety path of no resistance when closed, thus preventing disturbance of the recording apparatus by voltages induced during manipulation of the primary circuit. If, however, the key does not make perfect closure, it is better to use it as a series switch in one of the leads from the coil to the tissue ; it is then used to break unwanted currents during the preparation.

Observation 6. **Currents Induced in the Secondary Circuit by Make and Break of the Primary.** The primary circuit is always set up with cell, key and primary coil in series. The secondary circuit (which is nowhere in electrical contact with the primary) consists of the secondary

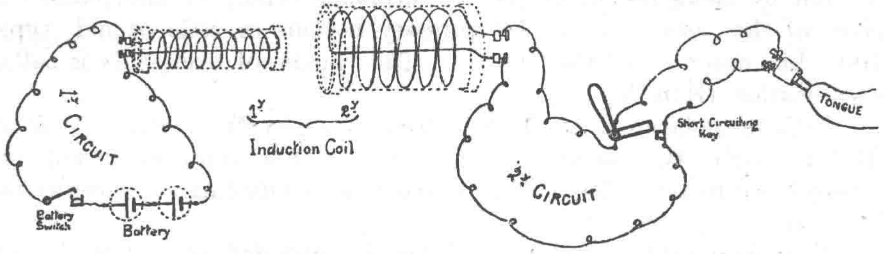


FIG. 8. Single induction shocks.

coil and the stimulating electrodes bridged across with the short-circuiting key (Fig. 8).

Starting with the secondary coil withdrawn some distance from the primary, advance it until a shock is felt when the electrodes are applied to the tongue.

M_F : **Make Shock.** Open the secondary short-circuiting key and close the battery key ; slide up the secondary coil and note the scale reading when shocks are first definitely appreciated on making the primary circuit.

N: **No Shock during Passage of Primary Current** since there is no variation in the magnetic field. Test this while the battery key is closed.

B_F: **Break Shock.** With the secondary key still open and the coil in the final position of M_F, open the battery key. The secondary induced current is much stronger than in M_F because the sudden breaking of the primary circuit by the battery key does not permit of a gradual decline in the primary current owing to the fact that the self-induced extra current is unable to flow; whereas at make, the key, being closed, allows the backward make extra current to pass and to retard the growth of the primary current. There is thus a *gradual* development of the primary current at make, but a sudden drop at break. The induced current, depending as it does upon the rate of change in the magnetic field, will therefore be greater at break than at make, and it will be zero during the passage of a constant current.

Very powerful shocks may be obtained from the secondary coil on account of the summated e.m.f. of so many turns of wire.

Observation 7. **Single break Induction Shocks.** Using the standard arrangement of Fig. 8 for single induction shocks, withdraw the secondary coil until the M_F shocks just become imperceptible on the tongue. This is approximately the position in which the coil should be set for the excitation of a motor nerve.

Observation 8. **Repeated Shocks: Faradisation.** Using the standard arrangement of Fig. 8, successive break shocks may be given at a slow rate by repeatedly opening the key.

To produce a very rapid succession of shocks the trembler interrupter—Neef's hammer—must be included in the primary circuit. This is effected by using the lower pair of terminals—those on the pillars—in place of the terminals on the primary bobbin in coils of old type. Test this current on the tongue. This mode of excitation is called Faradisation (R in Fig. 6).

If the secondary coil be moved up so as to give M_F and B_F shocks we then get double the number of shocks, and as these induced currents are in opposite directions ($M_F < B_F$, however), we are dealing with alternating currents.

Make diagrams showing the course of the secondary current in the two positions of Neef's hammer—opening and closing.

Exercises. (1) Make a working diagram of an arrangement (derived from that in Fig. 8) for cutting out either M or B shock. Test it on yourself.

(2) Block either M or B shock by interposing a copper/copper oxide rectifier in series in the secondary circuit. Test it on yourself and on a milliammeter.

(3) Equalise M and B shocks by using a primary circuit in which the current is never completely broken, and which, therefore, allows the passage of the break extra current. (A side wire from the vibrating reed to the adjustable contact screw.)

(4) Place the secondary coil (i.) at right angles, and (ii.) at 45° to the axis of the primary, and find the point at which a B shock is first felt, as in Observation 6.

(5) The student who possesses among his wireless components a

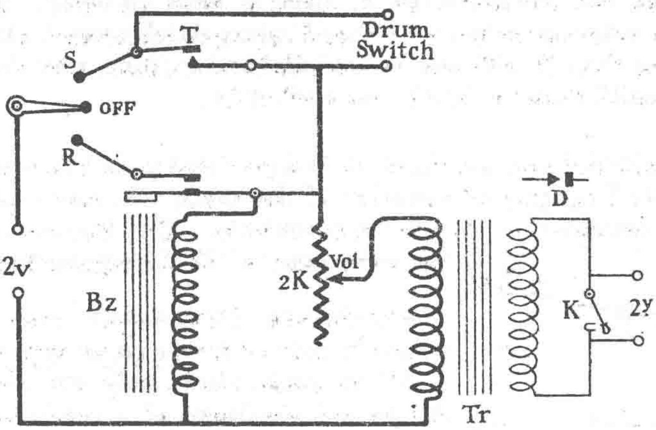


FIG. 9. Student's stimulator built from low-pitched buzzer, Morse key, filament resistor, bell transformer. For D.C. shocks, insert rectifier (Westector) at D.

buzzer, transformer and a crystal detector or any form of rectifier can assemble an excellent stimulator on the lines of the circuit of Fig. 9.

Observation* 9. **Wave-form of Faradic Voltage. With the trembler in circuit, connect the terminals of the primary coil to the Y_1 plates and those of the secondary to the Y_2 plates of a double-beam oscilloscope; if a Cossor is being used, set the amplifier switch to the $2Y_1$ position. The traces show Y_1 the primary voltage in the form of a square wave, and Y_2 the secondary voltage as a damped train of oscillations (Fig. 10).

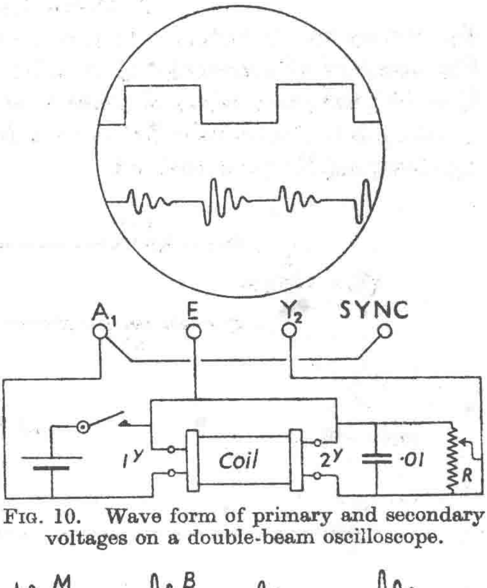


FIG. 10. Wave form of primary and secondary voltages on a double-beam oscilloscope.

The insertion of a $0{\cdot}01\mu F$ condenser across the 2^y tunes the coil to a suitably low frequency so that individual cycles can be observed. Note

(i) oscillation: B > M, and

(ii) initial swing: reversed at M and B as shown in the diagram (Fig. 11).

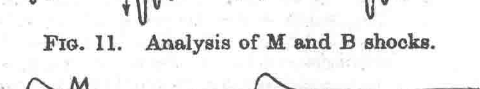


FIG. 11. Analysis of M and B shocks.

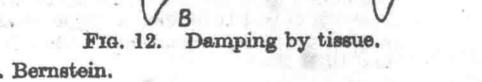


FIG. 12. Damping by tissue.

The variable resistance R rep-

* L. Bernstein.

resents the tissue to be stimulated. When R is reduced to a value (usually below 10 K Ω), the train of oscillations becomes critically damped (Fig. 12) giving the usual single stimulus at M and at B as with tissues. In Fig. 12, B is not only bigger than M but is also kathodal, so that in this position of the electrodes the B shock is most effective; if now the secondary connections be reversed and appropriately spaced, the M shock, though less than B, will now be kathodal and explains why the M shock is occasionally found to be the more effective.

The induction coil is unfortunately a poor instrument for quantitative work. The frequency of vibration of the hammer is very variable, and so is the resistance of the platinum contacts. Rectification occurs and the wave-form is full of irregular harmonics.

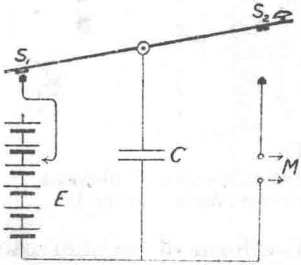


FIG. 13. Condenser shocks.

CONDENSER DISCHARGES may be conveniently derived from a paper-type condenser. When *single shocks* only are required the charge and discharge of a condenser C may be utilised (Fig. 13). The current from a 100-volt high-tension battery is used to charge the condenser C through the contact S_1 and to discharge C *viâ* the muscle M by closing S_2. A Morse key is useful for manually repeating the shocks up to 10/sec. which is an ideal rate for frog muscle. Since the quantity of electricity Q to fully charge the condenser is given by $Q = EC$, vary the values of either E or C. Thus a strong shock would be given by $E = 100$ volts and $C = 0{\cdot}1$ microfarad; and it may be diminished by dropping E *or* decreasing C.

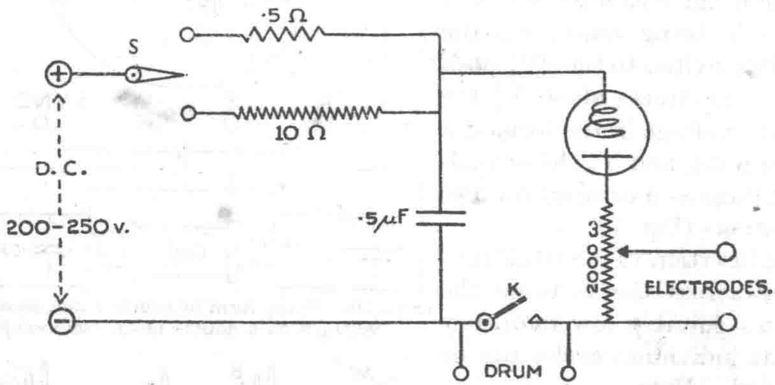


FIG. 14. Neon tube stimulator. For repeated shocks, *e.g.*, for tetanus, throw the switch S over to the smaller feed resistance ½ megohm, while for single shocks bring in the high resistance 10 megohms. Single shocks may be administered to the tissue by closing key K *or* by the drum key when K is open and leads are attached to the terminals to both sides of K. Any student building a neon stimulator for his own use may replace the three components S, 0·5 M Ω and 10 M Ω by a single variable resistance of 10 megohms. The key K may also be omitted. Alter ·5 Ω and 10 Ω in the diagram to ·5 M Ω and 10 M Ω.

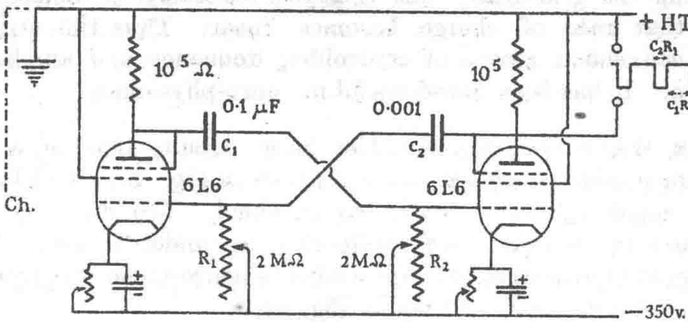


FIG. 15. Square-wave circuit.

When *repeated shocks* are required the condenser is slowly charged through a grid-leak form of resistance up to the striking voltage of a neon lamp. The condenser then discharges through the neon lamp—indicated by a red flash—and through a potentiometer from which

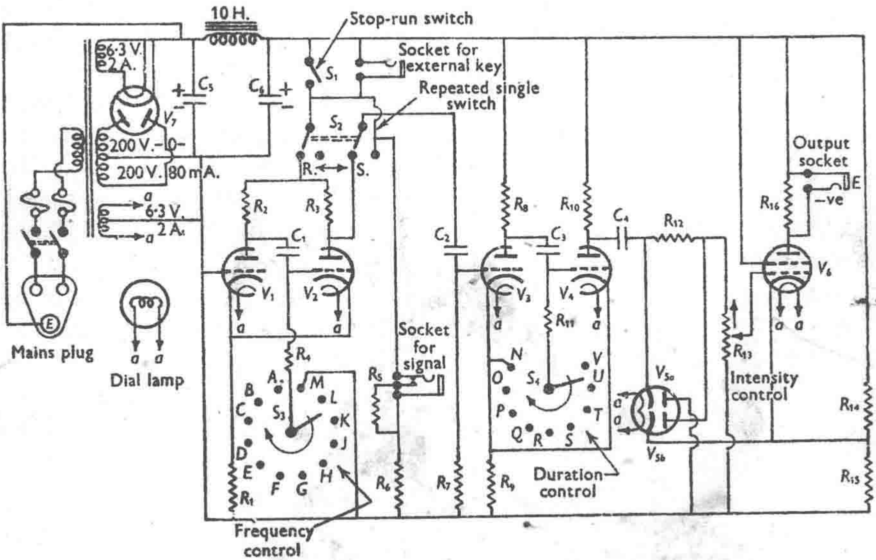


Fig. 16. Square-wave stimulator (*Bernstein*).*

R_1, 800 Ω., ½ W. ; R_2, 32 kΩ., ½ W. ; R_3, 32 kΩ., ½ W. ; R_4, 10 kΩ., ¼ W. ; R_5, 22 kΩ., ½ W.; R_6, 39 kΩ., ½ W.; R_7, 10 kΩ., ¼ W.; R_8, 100 kΩ., 1 W.; R_9, 10 kΩ., 1 W.; R_{10}, 47 kΩ.,1W; R_{11}, 18 kΩ., ¼ W.; R_{12}, 32 kΩ., ½ W.; R_{13}, 0·5 MΩ., carbon. var.; R_{14}, 6 kΩ., 12 W., w.w.; R_{15}, 1 kΩ., 2 W., w.w.; R_{16}, 68 kΩ., 2 W.; C_1, 0·25 μF., 350 V., wkg.; C_2, C_3, 0·002 μF., 350 V., wkg.; C_4, 0·25 μF., 350 V., wkg.; C_5, C_6, 8 μF., 450 V., wkg., elect.; $V_1 + V_2$, $V_3 + V_4$, 6SN7, G.T.; $V_{5a} + V_{5b}$ 6H6; V_6, 6V6, G.T.; V_7, 6X5, G.T. *A–B*, 9·5 MΩ.; *B–C*, 3·3 MΩ.; *C–D*, 1·7 MΩ.; *D–E*, 1 MΩ.; *E–F*, 330 kΩ.; *F–G*, 180 kΩ.; *G–H*, 120 kΩ.; *H–J*, 33 kΩ.; *J–K*, 15 kΩ.; *K–L*, 6·8 kΩ.; *L–M*, 1·5 kΩ.; *N–O*, 82 kΩ.; *O–P*, 330 kΩ.; *P–Q*, 220 kΩ.; *Q–R*, 330 kΩ.; *R–S*, 1 MΩ.; *S–T*, 2·2 MΩ.; *T–U*, 4·2 MΩ.; *U–V*, 12·6 MΩ.

variable fractions of the " kick " may be tapped and led off to the nerve (Fig. 14). If D.C. mains are not available, use two radio H.T. batteries in series.

THYRATRON STIMULATOR. In this assembly, the neon tube is replaced by a gas-filled relay or thyratron whose discharge voltage may be altered

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