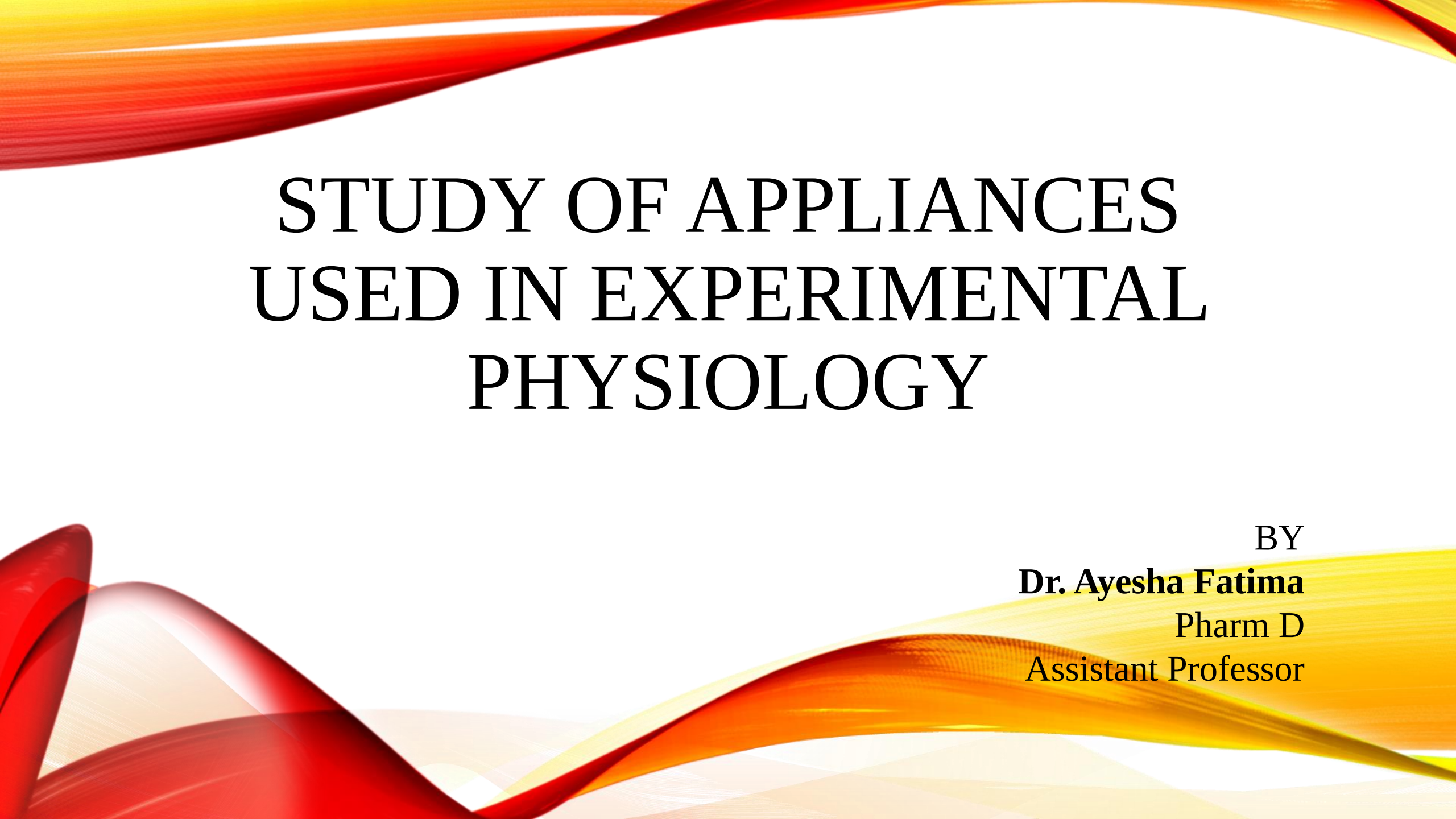




STUDY OF APPLIANCES USED IN EXPERIMENTAL PHYSIOLOGY

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Advantages of using Frog in Experimental Physiology

1. Frogs are cold blooded animals. Therefore, their nerve muscle preparation survival under average experimental conditions is superior to that of warm-blooded animals.
2. They are easily available round the year and create no nuisance during storage or while handling (absolutely harmless).
3. Their tissues can survive for quite some time in vitro (outside the body) without oxygenation and temperature control. Their muscles can absorb oxygen directly from the atmosphere.
4. They are easy to handle during experiment and can easily be disposed of afterwards.

Gastrocnemius Muscle-Sciatic Nerve Preparation

1. First used by Jan Swammerdam in the 17th century.
2. Simple and useful for studying:
 - Skeletal muscle contractions and Nerve functions.
3. **Gastrocnemius muscle** is preferred over **sartorius** because:
 - It is thicker and It generates stronger force during contractions.
4. **Stimulation of Muscles**
 - In the body, muscles contract due to nerve impulses (action potentials).
 - In isolated setups, muscles can be made to contract by:
 - Stimulating the nerve and by Directly stimulating the muscle.

Frog Heart

1. The frog heart can work both inside and outside the body.
2. It keeps beating on its own even when isolated, because the heartbeat starts within the heart itself (it's myogenic).

Mammalian Experiments

1. Common animals: **dog, rabbit, and rat.**
2. Used to demonstrate various physiological functions in students' experiments.

DISSECTION OF GRASTROCNMIUS MUSCLE SCIATIC NERVE PREPARATION

1. Stunning the Frog

- Hold the frog gently with a cloth.
- Use a wooden mallet to give **1–2 firm blows on the head** to make it unconscious.
- Don't hit too many times — it's inhumane and unnecessary.

2. Pithing (Destroying the Brain and Spinal Cord)

- Purpose: To ensure the frog doesn't feel pain or show reflexes during dissection.
- Flex the frog's head and insert a sharp **pithing needle** into the back of the skull.
- Move the needle forward to destroy the brain.
- Then move the needle backward into the spine to destroy the spinal cord.
- Signs of successful pithing:
 - Initial limb jerks (convulsions).
 - Limbs go limp.
 - No reflexes when toes are pinched.

3. Removing the Skin

- Cut around the trunk just below the front legs.
- Pull off the skin (like trousers) down to the toes.
- Discard the skin in a waste tray.

5. Isolating the Sciatic Nerves

- You now have a piece of the spine with sciatic nerves attached.
- Split the spinal piece in two.
- Carefully remove small side nerves without injuring the sciatic nerve (which leads into the thigh).

4. Exposing the Nerves

- Place the frog belly-down.
- Lift the **urostyle** (tailbone) and cut below it.
- Cut muscle on both sides carefully to avoid damaging nerves.
- Extend the cuts and cut through the **hip bones** (girdle).
- Lift the urostyle to reveal **sciatic nerves** running parallel to it.
- Cut above and below the sciatic nerve exit.

6. Separating Thigh Muscles

- Cut the fascia (covering) of the thigh.
- Gently separate muscles with a blunt tool.
- The **sciatic nerve** is now visible.
- Free the nerve up to about 2 cm above the knee.

7. Isolating the Gastrocnemius Muscle

- Cut and separate the **tendon of the gastrocnemius** muscle.
- Tie a strong thread around the tendon above the **sesamoid bone** (a small bone inside the tendon).

9. Final Setup of the Nerve-Muscle Preparation

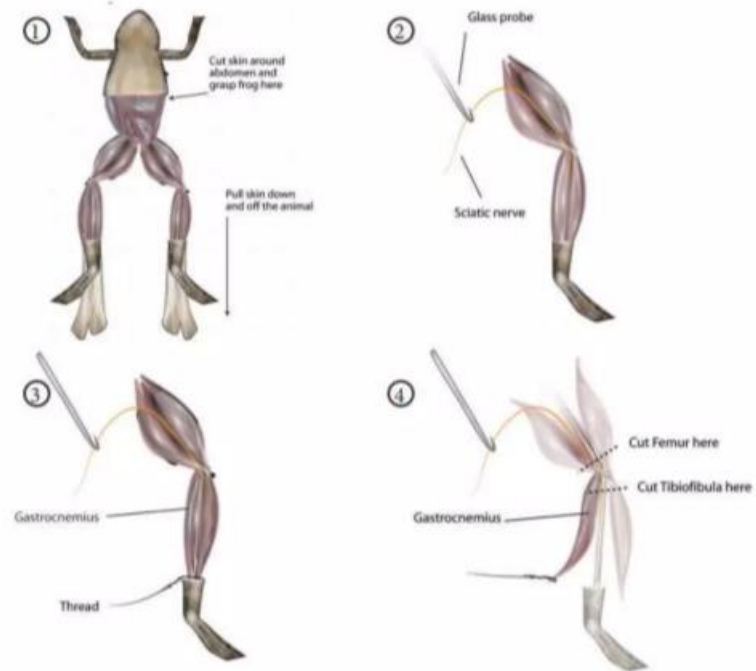
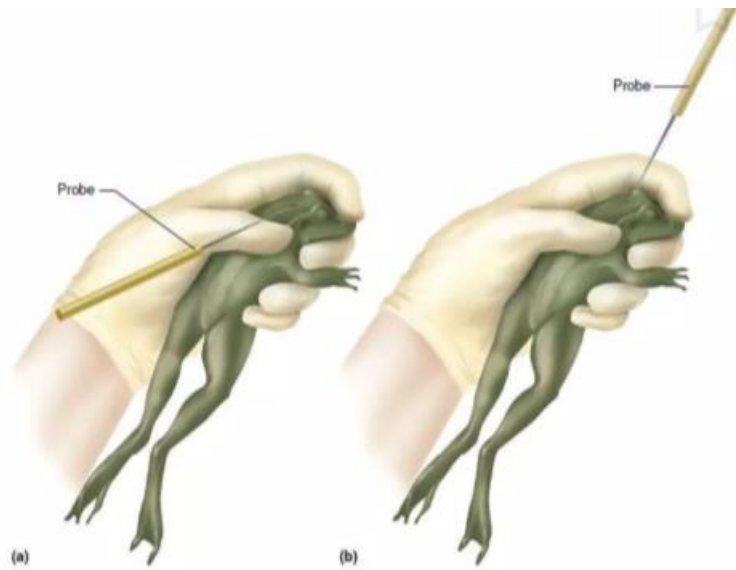
- You now have **two nerve-muscle preparations**.
- Push a **strong pin through the knee joint** (not through muscle).
- This pin will hold one end of the muscle during experiments.
- Place the setup in **Ringer solution** to keep tissues alive.
- Be careful not to let the nerve dangle.

8. Removing Bone Parts

- Cut off the **lower leg bones (tibia-fibula)** below the knee.
- Cut the **thigh bone** above where the nerve was freed.
- Clean off any extra tissue.

Mounting in the Muscle Chamber

- Push the pin into **cork at the bottom of the chamber**.
- Tie the thread to the **writing lever**.
- Make sure the lever is **horizontal** and the thread is tight.
- Add a **10 g weight** about 3 cm from the lever's center to measure muscle force.



Ringer's Solution

- This solution is isotonic with the frog's tissues and has optimal ionic constituents. The isotonic saline for frog is 0.6% NaCl, while for mammals it is 0.9% NaCl solution.
- The composition of Ringer is as follows:
 - Sodium chloride = 0.6 g
 - Calcium chloride = 0.01 g
 - Potassium chloride = 0.0075 g
 - Sodium bicarbonate = 0.01 g
 - Distilled water to 100 ml.

1. Excitability:

- **What it is:** The ability of living cells to respond to stimuli.
- **Where it's most important:** In nerve and muscle cells (called excitable tissues).
 - **Muscle cells:** Contract when stimulated.
 - **Nerve cells (neurons):** Generate and transmit electrical signals (action potentials).
 - **Gland cells:** Produce secretions when stimulated.

2. Stimulus:

- **What it is:** A change in the environment that causes a response in the tissue.
- **Example:** Touching something hot causes the skin to feel pain.

3. Types of Stimuli:

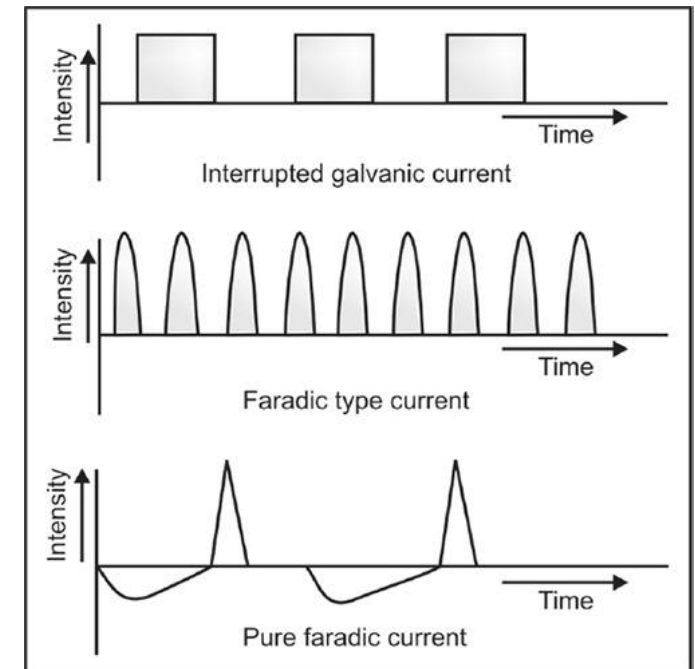
- **Physical:** Changes in temperature or dryness (e.g., cooling or warming).
- **Mechanical:** Physical actions like tapping or pinching.
- **Chemical:** Chemicals like acids or alkalis.
- **Osmotic:** Changes due to water or salt concentration (e.g., salt solutions).
- **Electrical:** Electric currents, such as DC (direct current), AC (alternating current), and other types.

4. Why Electrical Stimuli Are Used in Experiments:

- **Controlled easily:** You can adjust the strength (volts) and duration (milliseconds) of electrical stimuli easily.
- **Safe:** They are short and don't cause damage to tissues, so they can be used repeatedly.
- **Similar to natural stimulation:** Nerve cells and muscles naturally respond to electrical signals, so this method mimics that.

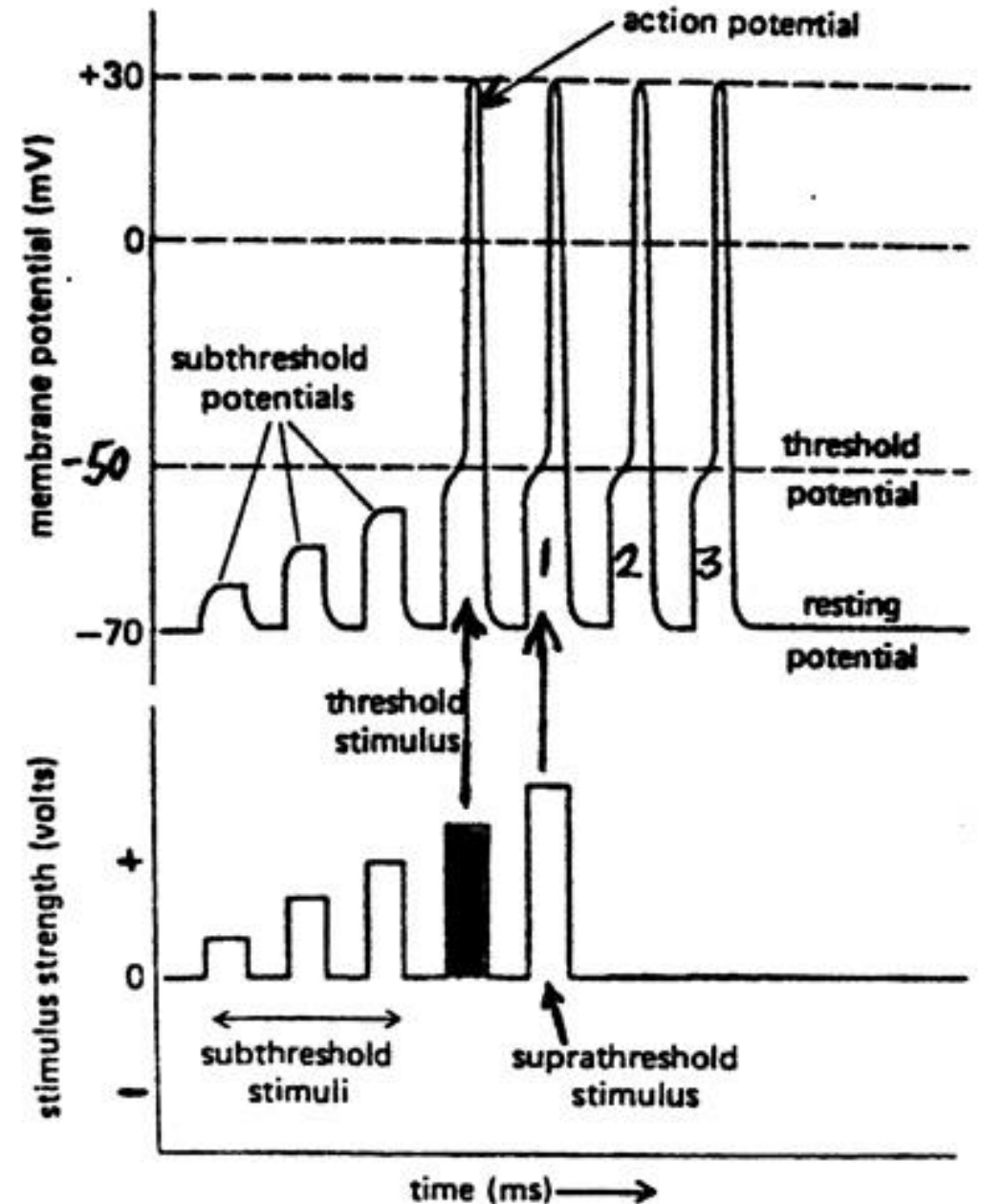
5. Types of Electrical Stimuli:

- **Faradic or Induced Current:** Short-lived and safe, commonly used in experiments.
- **Rectilinear (Rectangular) Current:** Controlled by an electronic stimulator; the voltage rises immediately to a set level and stays there for a set time.
- **Galvanic Current:** Used in neurophysiological studies, like testing nerve degeneration.
- **Household AC (220V, 50Hz):** Dangerous in its original form and never used directly; it's modified for safety.



6. Degrees of Stimuli:

- **Threshold Stimulus:** The minimum strength of stimulus that will trigger a response.
- **Subthreshold Stimulus:** A stimulus too weak to cause a response.
- **Maximal Stimulus:** The strongest stimulus that produces the maximum response.
- **Submaximal Stimulus:** A weaker stimulus than the maximal one.
- **Supramaximal Stimulus:** A stronger stimulus than the maximal one.



1. Source of Current:

- **Purpose:** Provides a low, constant current for the induction coil.
- **Low Voltage (3–6V):** The current needed is low voltage, supplied by:
 - **Dry Battery Cells:** Multiple 1.5V batteries connected in series.
 - **Central Low-Voltage Unit:** A unit that:
 - Converts mains AC current to DC (via a rectifier).
 - Reduces the voltage to the required low level (few volts).
- This constant current is then supplied to workstations for experiments.

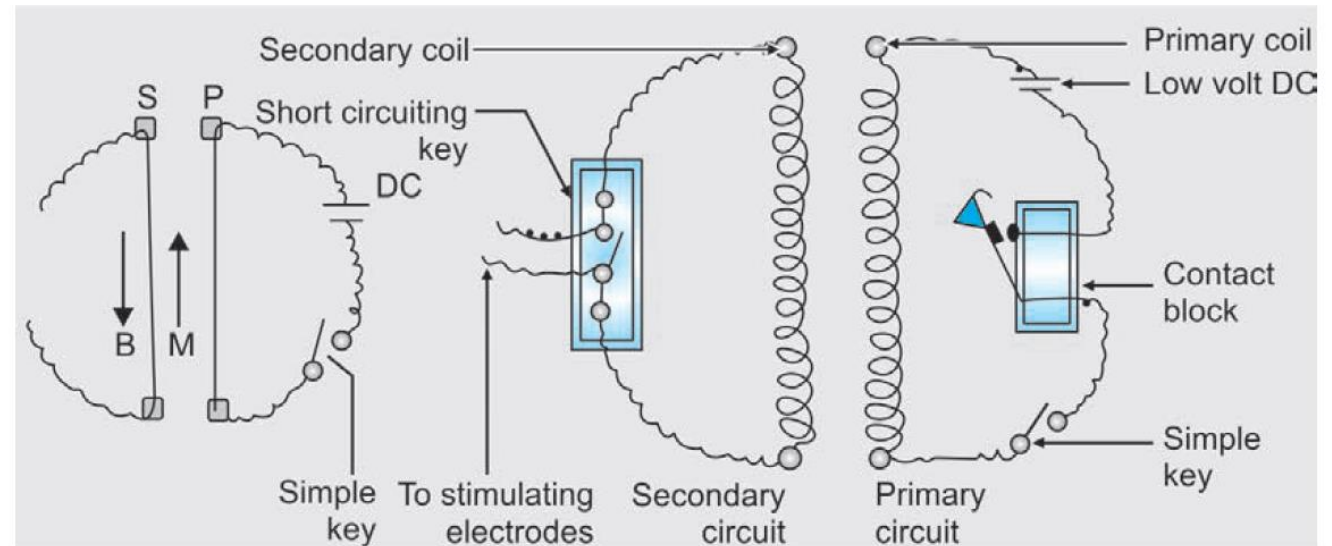


Figure 4-1: **Left:** Genesis of induced current. Flow of direct current (DC) in wire P causes induced (Faradic) current in a nearby wire (S, secondary) only at make or break of DC. Arrows show the direction of induced current at make (M) and break (B) of direct current. **Right:** Circuit for electrical stimulation of frog's nerve or muscle. The contact block makes and breaks the primary circuit by the striker of the rotating drum. The circuit can also be completed by tapping the spring with a finger

2. The Keys:

- **Purpose:** Switches that control the electrical circuit (either making or breaking it).

Types of Keys:

- **Simple Key:**

- A basic "on-off" switch, usually mounted on a wooden or vulcanite base.
- It connects to two adjustable screw terminals.
- **Other Variants:** Metal blocks, Morse key, and unspillable mercury key.
- **Location:** The simple key is used in the primary circuit to control current.

- **Short-Circuiting Key:**

- **Purpose:** Prevents accidental current from passing into tissues and stops unipolar induction.
- **Location:** Located in the secondary circuit.
- **How It Works:**
 - It has 4 terminals: two left and two right that are always connected.
 - The two poles of the switch connect to the two inner terminals.
 - When the switch is **closed**, it short-circuits the induced current.
 - When the switch is **open**, the current flows to the stimulating electrodes.
- **Types Available:** Du-Bois Reymond, Sherrington, and switch type.

3. Induction Coil (Du-Bois Reymond Inductorium, 1849):

- **Purpose:** Converts **Galvanic current** (DC) into **Faradic (induced) current** (AC).
- **Principle:**
 - A flow of current in a wire creates a magnetic field.
 - A changing magnetic field can induce a current in a nearby wire or coil.

How It Works:

- Two coils (primary and secondary) are placed near each other.
- **Primary coil ("P"):** Receives Galvanic current (e.g., from a battery).
- **Secondary coil ("S"):** Gets an induced current when the current in the primary coil starts or stops.
 - When the primary current **starts** (make), the induced current in the secondary coil flows in the **opposite direction**.
 - When the primary current **stops** (break), the induced current in the secondary coil flows in the **same direction**.
- **Steady current** in the primary coil causes **no induced current** in the secondary coil.

Construction:

•Primary Coil:

- 250-300 turns of thick, cotton-covered copper wire.
- Wound around a wooden reel with a soft iron core to enhance induction.

•Secondary Coil:

- 7000-8000 turns of thin enamelled copper wire.
- Wound around a hollow wooden reel.
- **No electrical connection** between the primary and secondary coils.
- The distance between the two coils can be adjusted.

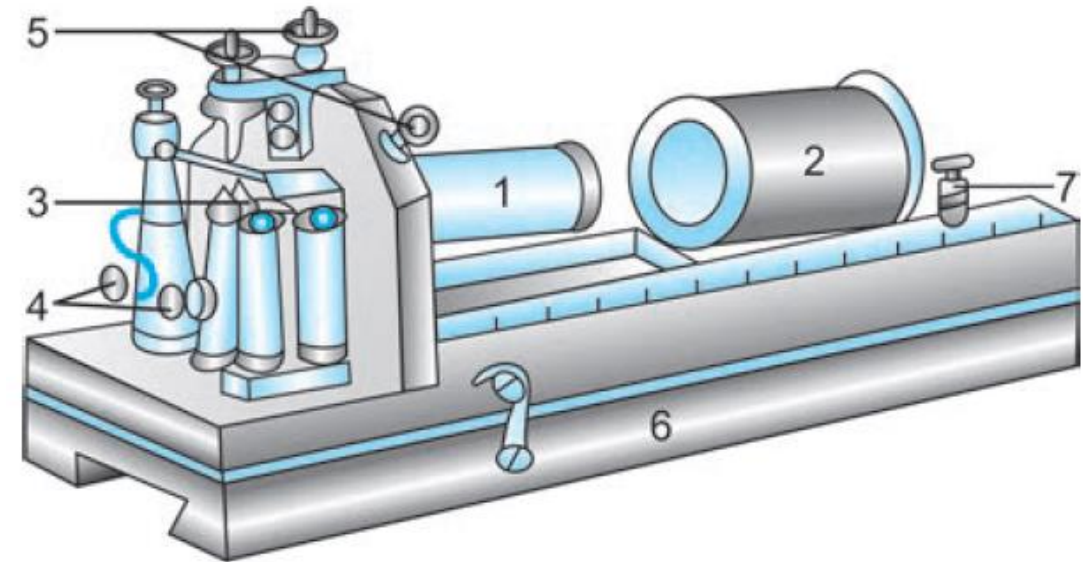


Figure 4-2: The Du Bois–Reymond induction coil. (1) Primary coil, (2) Secondary coil, (3) Neef's hammer assembly, (4) Terminals for Neef's hammer, (5) Input terminals for primary coil (the output terminals of the secondary coil are not shown), (6) Wooden base with scale, (7) Pointer, for indicating distance between primary and secondary coils

Factors Affecting Induced Current:

- 1. Number of Turns:** More turns in both coils result in a stronger induced current.
- 2. Strength of DC in Primary Coil:** Increasing or decreasing the current affects the induced current.
- 3. Distance Between Coils:** The closer the coils are, the stronger the induced current.
- 4. Angle Between Coils:** If the coils are at a right angle to each other, no induced current. As the angle decreases, the strength of the induced current increases.

Break-induced vs Make-induced Current:

- **Break-induced current** is stronger than **make-induced current**.
- This happens because:
 - When the current **starts** in the primary coil (make), it is resisted by **self-induction** (opposite current in the primary coil).
 - When the current **stops** (break), the change in magnetic field is more sudden, leading to a stronger induced current in the secondary coil.

Neef's Hammer:

- **Purpose:** Used when multiple, repeated stimuli are required.
- It's part of the primary circuit and includes:
 - **Electromagnet:** Causes a T-shaped iron bar to vibrate.
 - **Iron Bar:** Moves up and down, rapidly **making** and **breaking** the primary circuit.
 - **Speed:** The vibration occurs at 30-40 to over 100 times per second.
- **Platinum Points:** Used on the iron bar and contact screw to avoid sparking.

4. Electric Kymograph (“Drum”):

- **Purpose:** A device used to graphically record the time course of events in tissues, especially muscle contractions.
- **Power Source:** It runs on mains 220V AC current.

Main Features:

1. Speed Control:

1. **Speed Setting Lever:** This lever adjusts the speed of the drum.
2. **Calibrated Speed:** The lever has different speed slots, including a **neutral (N)** slot where the gears disengage from the motor.
3. **Variable Speed Lever:** Allows the drum to move at speeds between **0.12/0.25 mm/sec** and **320/640 mm/sec** (not RPM).

2. ON/OFF Switches:

1. **Mains Switch:** This switch is labeled **ON/OFF** and should always be left “ON”.
2. **Clutch Lever:** Engages/disengages the gears and acts as an ON/OFF switch to protect the gears from damage.
3. The cylinder can be manually rotated when the clutch lever is in the **OFF** position (horizontal).

3. Spindle and Cylinder:

1. A **spindle** projects from the top of the kymograph, holding the cylinder where the graph is recorded.
2. The cylinder is **6” × 6”** in size and is wrapped in **glazed paper**. It must be securely fixed on the spindle to avoid shifting, which would affect the results.
3. **Locking Lever:** Used to firmly attach the cylinder to the spindle.
4. **Leveling Screws:** Located at the base of the drum, these screws adjust the tilt of the cylinder.

Striker and Contact System:

- **Dual Electric Contact Arms** (called **the striker**): Fitted one above the other at the base of the spindle, these arms rotate with the cylinder.
- **Striker Adjustment**: The arms can be separated to allow two separate stimuli.
- **Contact Block**: Mounted on top of the kymograph at the striker's level, it includes:
 - A **spring contact** with a plastic button.
 - Two **electrical terminals** that complete the circuit when the striker pushes the spring contact. This marks the stimulation point on the recording surface.

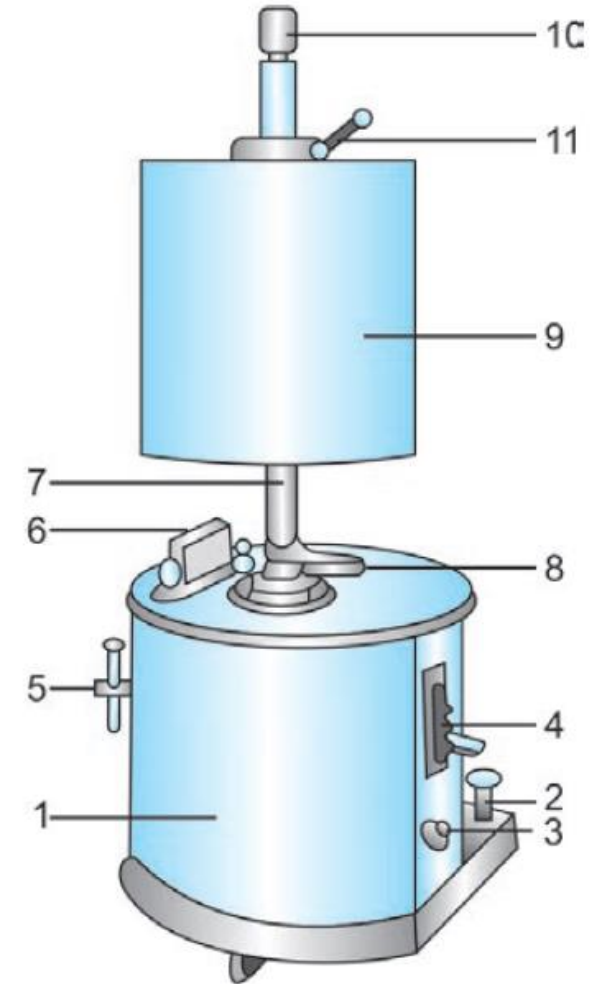


Figure 4-3: Electric kymograph: (1) Kymograph body containing electric motor and gear system, (2) Leveling screw, (3) On-off switch for mains current, (4) Calibrated speed setting system, (5) Clutch lever, (6) Electric contact block, (7) Spindle, (8) Dual electric contact arms, (9) Cylinder, (10) Screw lift for cylinder, (11) Lever for fixing cylinder on the spindle (The variable speed setting lever is not visible in this view)

Stimulus and Circuit:

- When the striker touches the spring contact, it completes the primary circuit, creating an electrical “make” (current starts) and then a “break” (current stops).
- These two shocks (at **make** and **break**) create a single stimulus, where the “**make-induced**” **current** is the effective stimulus.
 - “**Make-induced**” **Current**: This is the stimulating shock.
 - “**Break-induced**” **Current**: This second shock occurs during the refractory period of the first shock and does not contribute to stimulation.

Effect of Speed:

- At **faster speeds**, both the **make** and **break** currents are generated in quick succession, acting as a single stimulus.
- At **slower speeds**, the **make-induced current** can be isolated by adjusting the distance of the secondary coil, making the **break-induced current** ineffective.

Clockwork and Mechanical Kymographs:

- In some labs, **clockwork** or **mechanical kymographs** (pulley-driven) are also used.

5. Frog Muscle Chamber (Luca's Moist Chamber):

- **Purpose:** Used to immerse the frog muscle in **Ringer's solution** for experiments.
- **Design:**
 - **Size:** 6" × 4" × 2".
 - **Material:** Transparent perspex.
 - It has a **clamp** and **drain pipe** for controlling and draining the solution.
 - The chamber sits on an upright stand (called **QTVT stand**), which has a quick stop at the base for stability.
 - **Alternative:** Ordinary adjustable stands can also be used.



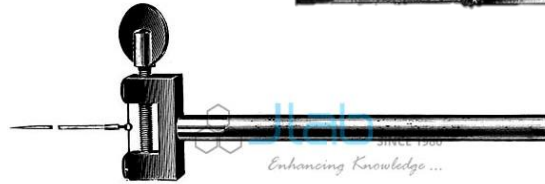
6. Writing Lever:

- **Purpose:** Used to magnify and record muscle contraction.
- **Design:**
 - **L-shaped lever** with a **horizontal arm** (has a writing point) and a **vertical arm** (for attaching the muscle tendon).
 - A weight can be hung near the fulcrum of the horizontal arm to adjust leverage.
 - The vertical arm of the lever connects to the muscle tendon through a thread.
 - The thread must have no slack to ensure accurate readings.
- **Adjustments:**
 - An **after-load screw** supports the vertical arm for after-loading, or it can be withdrawn for **free-loading**.
- **Ink-writing Lever:** An ink stylus can be attached to the writing lever to directly record on the glazed paper, eliminating the need to “smoke” the paper.



8. Isometric Lever:

- **Purpose:** Used to record **isometric contractions** (contractions without muscle length change).
- **Design:**
 - The lever consists of a **steel tension spring** and a **flat writing lever**.



7. Starling Heart Lever:

- **Purpose:** Records the mechanical events of the frog's heart.
- **Design:**
 - The lever has a **light, flat arm** supported by a finely adjustable spring, keeping it horizontal.
 - A thread is tied to the writing lever, with a **bent pin** attached to the thread. This pin hooks to the **apex of the ventricle** of the frog's heart.

9. Variable Interrupter:

- **Purpose:** Delivers a variable number of **induction shocks**.
- **Principle:** Works like **Neef's hammer**.
- **Adjustment:** The **arc of vibration** of the pendulum can be set using a **stop screw**, giving a range of **6 to 80 shocks per second**.



on:

When the heart contracts, it pulls the lever down.

When the heart relaxes, the spring pulls the lever back to its horizontal position.

10. Time Marking:

- **Purpose:** Adds a time marker or trace to the recording surface in experiments.

Types of Time Markers:

•Tuning Fork:

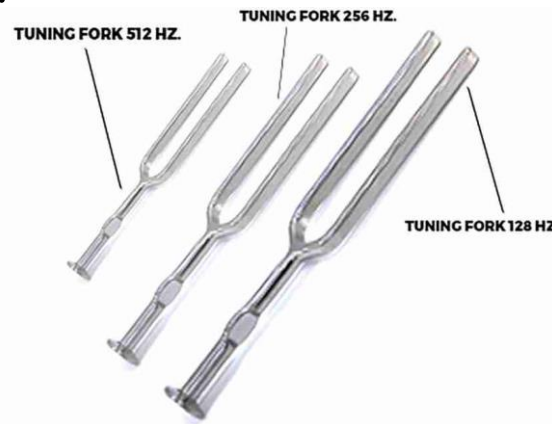
- **Frequency:** 100 Hz or 256 Hz for fast events (e.g., muscle contractions).
- The tuning fork has a stylus on one prong, which is gently touched to the smoked surface below the graph.
- The time between two crests or troughs represents a set time interval:
 - **100 Hz** = 0.01 sec.
 - **256 Hz** = 0.0039 sec.
- **Electrically vibrated tuning forks** are also available.

•Spring Time Marker:

- A **weighted vibrating spring** that can be tapped with a finger to maintain vibration.
- It provides two adjustment intervals: **1/2 sec** and **1/5 sec** per complete cycle.

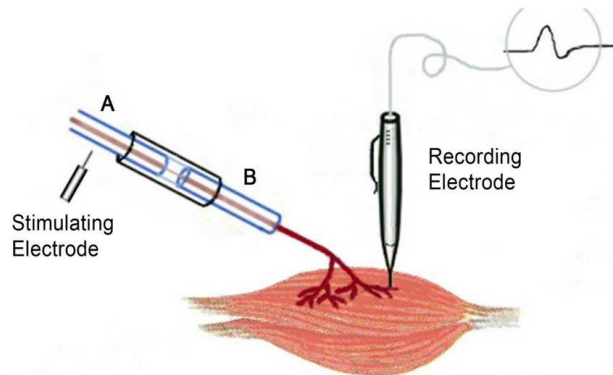
•Electromagnetic Time Marker:

- Works with the **central low-voltage unit**.
- Provides “**make-break**” contacts at time intervals of **1, 2, 5, and 10 seconds**.



11. Stimulating Electrodes:

- **Purpose:** Used to deliver electrical stimuli to tissues.
- **Types:**
 - **Ball-and-socket-mounted silver electrodes:** These are typically used with the muscle chamber and placed on one side of the chamber.
 - **Wooden electrodes:** Made of two copper wires fitted into a small wooden or vulcanite block. These are simple to use.



12. Student Stimulator:

- **Purpose:** Electronic device used to generate electrical stimuli.
- **Specifications:**
 - **DC Output:** Adjustable between **0 to 15 volts**.
 - The stimulator controls the following stimulus parameters:
 - **Voltage.**
 - **Pulse duration** (how long the pulse lasts).
 - **Pulse frequency** (how often the pulse occurs).
 - **Mode of operation:**
 - **Single:** One pulse.
 - **Repetitive:** 1 to 100 pulses per second.
 - **External trigger:** For use with other equipment like the contact block of the kymograph.



13. Smoking:

- **Purpose:** To prepare the glazed paper on the cylinder for recording by coating it with soot (smoke).
- **Method:**
 - **Smoked paper** is achieved by passing coal gas through benzene or kerosene, creating an intensely black flame.
 - **Rotating the cylinder** while smoking ensures an even, thin layer of soot on the paper.
 - If the paper is not tightly fitted around the cylinder, it might burn or not smoke evenly.



14. Varnishing:

- **Purpose:** To preserve and fix the recorded graph on the smoked paper, making it permanent.
- **Method:**
 - **Varnishing solution:** A 2% solution of **shellac** or **resin** in **methyalted spirit**.
 - After the experiment, the paper with the recorded graph is cut and dipped into the varnish solution.
 - The paper is then drained and placed on a drying board to dry at room temperature.
 - Once dried, the paper has a fine protective coat, preserving the record permanently.

Electrical Connections

a. Circuit for Single “Make” and “Break” Stimuli:

- **Primary Circuit:**
 - **Power source** (low voltage, such as a battery).
 - **Simple key** (used as a switch to complete or break the circuit).
 - **Primary coil** (part of the induction coil).
 - These components are connected in series (one after the other).
- **Secondary Circuit:**
 - **Secondary coil** (the second coil in the induction coil setup).
 - **Short-circuiting key** (prevents current from flowing into tissues when needed).
 - **Stimulating electrodes** (delivers electrical stimuli to the tissues).
 - These components are also connected in series.

b. Taking Kymograph in Circuit:

- **Primary Circuit:**
 - **Power source** (low voltage).
 - **Simple key** (switch to complete or break the circuit).
 - **Primary coil** (part of the induction coil).
 - **Contact block of drum** (used to mark the point of stimulation or record successive stimuli).
 - These are connected in series.
- **Secondary Circuit:** Same as in the previous setup (secondary coil, short-circuiting key, and stimulating electrodes).
- The **contact block** is included in the primary circuit to mark stimulation points or when two successive stimuli are needed for recording.

c. Taking Neef's Hammer, Variable Interrupter, or Vibrating Reed in Circuit:

- **Primary Circuit:**
 - **Power source** (low voltage).
 - **Simple key** (switch).
 - **Neef's hammer, vibrating reed, or variable interrupter** (devices for generating repeated stimuli).
 - **Primary coil** (part of the induction coil).
 - These components are connected in series.
- **Secondary Circuit:** Same as in the previous setups.
 - This circuit is used for generating **repeated stimuli** (e.g., tetanus or stimulating the vagus nerve).
 - These connections allow for different types of electrical stimuli, ranging from single "make" and "break" stimuli to repeated stimuli for advanced experiments.

Arranging the Apparatus:

1. **Induction Coil:** Place it directly in front of you.
2. **Kymograph:** Place it just beyond the induction coil.
3. **Simple Key:** Place it on the **right side**.
4. **Short-circuiting Key:** Place it on the **left side**.
5. **Muscle Trough:** Place it to the **left** of the induction coil so that the writing lever is **tangent** to the surface of the cylinder. This allows you to clearly see the writing point and the graph without twisting or bending.

Troubleshooting :

1. Check the Galvanic Current:

1. Hold one end of a wire on one terminal and brush the free end on the other terminal.
2. If you see sparking, the current is good. You can also use a **voltmeter** to check the voltage.

2. Check the Simple Key and Contact Block:

2. Switch on the drum. Every time the striker touches the contact spring, it should cause sparking.
3. This checks if the primary circuit is working.

3. Check the Secondary Circuit:

1. Place the severed leg of a frog on the secondary coil terminals.
2. If the leg twitches with each shock as the spindle rotates, the fault is further along the circuit.
3. Switch off the short-circuiting key and place the leg on the **stimulating electrodes**. If the leg twitches, the stimuli are reaching the electrodes.
4. If the nerve doesn't respond, place the electrodes directly on the muscle belly. If the muscle contracts, the nerve is damaged.
5. If the nerve is damaged, replace the preparation with a backup.

4. If the issue persists, consult an **electrician** or your **tutor** for assistance.