UNIVERSITY OF CALIFORNIA.SYLLABUS SERIES NO.25.LABORATORY INSTRUCTIONS IN INTRODUCTORY PHYSIOLOGY

Published @ 2017 Trieste Publishing Pty Ltd

ISBN 9780649274185

University of California.Syllabus series No.25.Laboratory instructions in introductory physiology by Various

Edited by Trieste Publishing Pty Ltd.
Cover @ 2017

www.triestepublishing.com

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Trieste

UNIVERSITY OF CALIFORNIA

SYLLABUS SERIES

NO. 25

PHYSIOLOGY

LABORATORY INSTRUCTIONS IN INTRODUCTORY PHYSIOLOGY

REVISED EDITION

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY
January, 1918

PRICE 25 CENTS

LABORATORY INSTRUCTIONS IN INTRODUCTORY PHYSIOLOGY

GENERAL DIRECTIONS

Apparatus must be returned clean and in good order. The lockers will be inspected from time to time to see that the apparatus is in good condition, and this condition will influence the marks given during the course. Apparatus not in use between laboratory periods must be cleaned and put away.

Full notes of the experiments must be written in the laboratory in connection with the actual work, and never from memory afterwards. The notebooks will be called for for inspection from time to time without previous notice, and also at the end of the course. Be concise and brief, expressing results in figures, diagrams and tables where possible. Graphic records are not to be handed in loose, but must be pasted in the notebook and accompanied by suitable explanation.

CARBOHYDRATES

1. Add as much powdered dry starch ($C_6H_{10}O_5$) as you can put on a ten-cent piece, to 50 c.c. cold water. Filter 5 c.c. of this suspension and compare its appearance with the original. Add to the filtrate one or two drops (not more) of a weak solution of iodine in iodide of potassium. If starch is present, the solution will turn blue upon addition of the iodine solution.

2. Take the starch water remaining from (1) and boil for two or three minutes. Cool, filter 5 c.c. as in (1), and test for starch with the iodine solution. If the blue reaction is present, heat the test tube. Does the blue color disappear? Allow the solution to cool. What happens?

3. Test the starch solution with Fehling's solution, as follows: take 1 c.c. copper solution (No. 1), and 1 c.c. alkaline solution (No. 2), mix, and add distilled water to make 10 c.c. Boil, then add a few drops of the starch solution. Boil again. If sugar is present there will be a red precipitate and the color of the Fehling's will change.

NOTE: Whenever this test is made, the Fehling's should be boiled before the solution to be tested is added.

4. Put some starch solution in a parchment tube and place the tube in a vessel of distilled water. Allow it to stand until the next laboratory period and then test the water for starch.

5. Add a very small quantity of dextrine $(C_6H_{10}O_5)n$ to 10 c.c. of water. Why is it cloudy? Boil. Why does it become clear? To 2 c.c. of this solution carefully add alcohol. It may be necessary to add several times its volume. Result?

6. Take 5 c.c. of the dextrine solution and add one or two drops (not more) of iodine solution. Is starch present?

7. In the same way test a solution of glucose (dextrose or grape sugar, $C_6H_{12}O_6$) with iodine solution. Result?

8. Test a solution of glucose with Fehling's solution, using the same method as in (3). Result?

9. Test a solution of cane sugar $(C_{12}H_{22}O_{11})$ with iodine; with Fehling's solution. Result?

10. Take 10 c.c. of cane sugar solution, add 1 c.c. of 10% sulphuric acid (H_2SO_4) and boil 5 minutes. Add m/10 NaOH to make up the original volume, and test with Fehling's solution. Result?

11. Put some glucose solution in a parchment tube and dialyse it as in experiment (4). Test for glucose. Result?

FATS

1. Use olive oil, and prove that fats are soluble in ether and chloroform, but insoluble in water.

2. Add to olive oil some caustic potash (KOH) and boil. A soap is formed and glycerin is set free. Saponification.

3. Shake some rancid oil with a few drops of a solution of sodium carbonate (Na_2CO_3). The whole becomes white. This is an emulsion. Examine it under the microscope and describe its appearance.

PROTEINS

1. Add strong nitric acid (HNO_3) one drop at a time to an albumin solution. A white precipitate is thrown down, which turns yellow on boiling. After cooling, add ammonia (NH_4); the yellow precipitate becomes orange.

2. Precipitate albumin with lead acetate $((C_2H_3O_2)_2Pb)$; with mercuric chloride $(HgCl_2)$; with alcohol (C_2H_5OH).

3. Heat a small quantity of albumin solution to boiling. Any coagulation? Add one or two drops of 2% acetic acid $(C_2H_4O_2)$ and boil if necessary. Result?

4. To a solution of albumin add an excess of caustic soda (NaOH), m/1, and then a few drops of very dilute solution of copper sulphate $(CuSO_4)$, m/30. Result? This is the "Biuret Reaction."

5. Take 10 c.c. of albumin solution and add 1 c.c. of a 25% sulphuric acid solution. Boil for three to five minutes, and allow it to cool. Add

water sufficient to make up the original volume. Taking a small quantity of the solution and repeat the Biuret test as above.

6. Put some albumin solution in a parchment tube and dialyse against distilled water as in the case of starch and sugar. Test the water for albumin at the next laboratory period.

PHYSIOLOGY OF NERVE AND MUSCLE

The tissues employed should be kept on a clean glass plate and should not be allowed to change the concentration of their contents through drying. The secretion from the frog's skin is injurious and contact with it is to be avoided. The nerve should be handled in such a way as to avoid mechanical injury, or chemical action, preferably by means of a clean glass rod or a camel's hair brush, wet with physiological salt solution.

The Nerve-Muscle Preparation.

"Wrap a frog in a moist cloth, the head out. Place one blade of a stout scissors in the mouth against the angle of the jaw and the other just back of the foramen magnum. Cut off the head with a single, firm movement. Pith the spinal cord by thrusting a coarse wire down the neural canal. Cut the body across transversely just back of the forelegs. Draw off the skin from the thunk and hind legs. Remove the viscera. The large sciatic plexuses will now be exposed. Lay the frog back uppermost on a clean glass plate. Push apart the semimembranosus and triceps femoris muscles and expose the sciatic nerve. Seize the tip of the urostyle with forceps and excise it up to the last vertebra, taking care not to injure the sciatic plexuses. With the scissors split the spinal column between the points of exit of the nerves. Grasp the half from which the prepared nerve comes and lift it gently, freeing the nerve with the scissors down to the knee."

"Cut through the tendons of Achilles below the thickening of the heel. Free the muscle up to its attachment to the femur, avoiding injury to the branch of the nerve which enters its posterior surface, and cut away the remainder of the lower leg. Clear away the muscles from the femur, taking care not to injure the sciatic nerve. Cut the femur across at about the middle of its length. In the whole operation avoid stretching or pinching the nerve."

1. Mechanical Stimulation—

Make a nerve-muscle preparation. Fasten the bone to the L of the muscle lever so that the muscle hangs vertically. Tie a thread to the tendon and attach it to the muscle lever. Adjust the nerve holder so that the nerve can rest upon a piece of filter paper kept moist with physiological salt solution. Tap the nerve near its end with the handle of a slight scalpel;

the muscle contracts. Pinch the end of the nerve with the forceps. With sharp scissors snip off the end of the nerve which has presumably been injured by the above treatment.

2. Chemical and Osmotic Stimulation—

a. Immerse the nerve in M/8 sodium chloride. After 15 minutes replace the M/8 solution by M/1. As soon as the muscle begins to twitch wash off from the nerve the concentrated solution with distilled water. When the twitching has ceased immerse in physiological salt solution. Test the irritability by mechanical stimulation.

b. Arrange the lever to write on the smoked paper of a kymograph set for slow speed. Fill the nerve holder with M/8 sodium citrate solution, but keep the muscle moist with physiological salt solution and be careful not to get any of the citrate solution on it. Mark on the drum the position of the writing point at the moment when the nerve is immersed in the citrate, and record the time. Record also the time when the muscle begins to twitch.

c. Wipe out the nerve holder and allow the nerve to dry. If twitching begins see if you can stop it by moistening with water.

3. Electrical Stimulation—

a. Galvani's Experiment—Twist one end of a piece of copper wire around the head of an iron nail. Place the nerve of a nerve-muscle preparation across the wire and touch the muscle or nerve with the point of the nail.

b. Induced Currents—Arrange the inductorium for single shocks. Mount the nerve-muscle preparation on a muscle lever adjusted to write on the kymograph. Record each contraction with the drum stationary; after each record move the drum about 5 mm. Obtain records to show: (1) The relative efficiency of make-and-break shocks as stimuli; (2) Relation between strength of stimulus and height of contraction. (Meaning of subminimal, minimal, and maximal stimuli.)

4. Opening and Closing Contraction—

Connect a dry cell with a pair of non-polarizable electrodes, placing a simple key in the circuit.

a. Place the nerve of a n-m-p in contact with the electrodes. Note that a contraction occurs on closing and on opening the key, but not during the passage of the current.

b. Place a rheocord in the circuit. Connect electrodes with the slider and one pole of the rheocord. Find a position of the slider which gives a current just below threshold and another just above. Placing the slider in the former position it may be moved smoothly to the latter without causing a contraction.

5. The Muscle Curve—

Mount a gastrocnemius without the nerve on the muscle lever and load it with a ten-gram weight. Connect the ends of the muscle to the poles of the secondary coil of an inductorium by fine copper wires. Put an electric signal and a key in the primary circuit. Arrange a writing point on a tuning fork to record hundredths of a second. Bring the three writing points into the same vertical line, and as close together as possible. It will be most convenient to have the muscle lever above and the tuning fork and signal, below.

Set the drum going at its most rapid speed, or turn by hand, and record the curve obtained from a maximal shock. Remove the tuning fork, stop the drum, but *carefully avoid disturbance of the relative position of the other two points.* The tuning fork must be allowed to mark only during the revolution in which the curve is actually made. Turn the drum cautiously until the signal is exactly at the point where the shock was given and mark the corresponding position of the muscle lever (Simultaneous ordinates). Calculate in fractions of a second (1) the latent period, (2) the period of shortening, (3) the period of relaxation.

6. Tetanus—

With the same arrangement, but moderately slow speed of drum, give a succession of stimuli by tapping the key at first slowly, then more rapidly. The record should show incomplete tetanus passing over into complete tetanus.

7. Effect of Repeated Excitation—

Inject the arterial system of a frog with a 5% solution of fuchsin S. Make two n-m-preparations.

a. Leave one gastrocnemius at rest. Mount the other in the moist chamber with the nerve on the n. p. electrodes. Load it with ten grams. Arrange the lever to write on a very slowly moving drum. Move the secondary coil outwards until the single shocks are just below "threshold value." If a succession of shocks be given a contraction occurs. Does the number of repetitions of the stimuli bear any relation to their frequency?

b. Using maximal make shocks, record a muscle curve as in (5), then stimulate rhythmically once a second. Do you get "staircase" effect? Now increase the load to forty or fifty grams. Continue the rhythmic excitation until the contractions diminish to half their height. At this stage record a second muscle curve. How does the curve differ from the first? Quickly resume the rhythmic stimulation and continue it until the contractions cease. Now stimulate the muscle directly. Were nerve, nerve-ending, and muscle equally affected by fatigue?