EXPERIMENTAL PHARMACOLOGY. A LABORATORY GUIDE FOR THE STUDY OF THE PHYSIOLOGICAL ACTION OF DRUGS

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NOTE.

The instruction in Pharmacology in the University of Missouri from the date of its establishment in the fall of 1900 has been based on a rigid course of required laboratory experiments. The student in Pharmacology no less than in Physiology should be given every opportunity to observe for himself the changes produced by a drug in the activities of a tissue, of an organ, and in the entire organism. It is only on such intimate personal experience with the facts that one can base a rational discussion of the principles of Pharmacology. The directions presented here have been formulated during the growth of the course as presented in this University and are now printed for the first time. Valuable aid and suggestion have been rendered by my coworkers, Dr. Waldemar Koch, and Mr. Omar Ray Gullion, now of Cornell University.

Charles W. Greene.

THE ACTION OF DRUGS.

ALCOHOL.

The effects of alcohol.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart.**
4. **On muscle work.**
5. **On voluntary work of human muscle. Demonstration.**
6. **On the circulatory and respiratory systems of the mammal.**
7. **On the reaction time of the reflex frog.**

1. Alcohol on the frog. Inject into the dorsal lymph sacs of two frogs doses of **0.3cc** (5 minims) and **0.6cc** respectively of **95 percent alcohol.** Strong alcohol is quickly removed from the lymph sac. The larger dose above is sufficient to produce complete loss of reflexes including all respiratory movements. A dose of **1cc** is toxic for a 40 gram frog. Since the smaller dose above is equivalent to 525cc for a 70 kilo man, it is evident that the frog is the more tolerant of alcohol.

2. Alcohol on the heart muscle. Mount a strip of the ventricle of a terrapin in 0.7 percent sodium chloride and when it is contracting with an even and regular rhythm change to a solution of **2 percent alcohol** in physiological saline. Renew the saline after two to five minutes. Record the contractions on a drum moving 1 mm a second. Repeat with strengths of alcohol of **5 and 10 percent.** The alcohol effect will be demonstrated rather better on a ventricular strip that is contracting in the weaker Ringer's solution, page 45, but the alcohol must be dissolved in the same physiological solution.

3. Alcohol on the frog's heart. Destroy the brain and ~~only~~ of a frog, expose the heart by cutting away the body wall from directly over the ventricle, using care not to lose

blood. Take a record of the movements of the ventricle, using a light straw lever of the fulcrum-power-weight order. Give **1.5cc of 95 percent alcohol** in the lymph sac. Take a continuous record during the time of absorption. When much blood is lost and the circulation is poor it is better to apply the alcohol directly to the heart from an irrigating bottle, page 52. Irrigate the surface of the heart with **50 percent alcohol** in physiological saline.

Still more satisfactory results are obtained by perfusing the heart through a cannula in the ascending vena cava. Perfuse the alcohol from supply flasks provided with constant level tubes. The perfusion strength of **alcohol** to use is **2 to 5 percent** for 2 to 4 minutes at a time. Record the contractions of the ventricle by a thread from its tip to the vertical arm of a balanced lever, page 48. In this experiment, as in all frog's heart perfusions, it is better to use the weaker Ringer for the normal solution.

4. Alcohol on muscle work. Ligate one leg of a frog near the thigh to exclude its circulation. Inject **0.3cc** (5 minims) of **95 percent alcohol** in the dorsal lymph sac. In exactly 20 minutes pith the frog, and quickly prepare its alcoholized muscle covered with its skin, mount and determine the work it can do by stimulating the muscle directly with a single induction shock once every two seconds until it is completely exhausted. Record the contractions on a drum with a speed of 1 mm a second. Prepare the second or normal muscle, mount and stimulate in the same manner. Record the second experiment on the same smoked paper and parallel with the first. Repeat this experiment using a dose of **0.6cc** of **95 percent alcohol.**

5. Alcohol on voluntary work of human muscle. Demonstration. Measure the voluntary power of the flexors of the middle finger using Mosso's ergograph with a load of 3 kilos. Take normals at intervals of 15 or 20 minutes. Now take a dose of **20** to **40cc** of **20 percent alcohol.** Remeasure the muscular power after 60, 90, and 120 minutes respectively. Compute the work done in kilogrammeters.

References: Lombard; Jour. Physiol., Vol. 13, p. 49; Hellsten; Skand. Arch. f. Physiol., Bd. 16, S. 139.

6. Alcohol on the circulatory and respiratory systems of the mammal. Anaesthetize a dog with morphine and chloroform, p. 46. Take the blood pressure from the carotid artery, p. 49, and the respiration from a side branch from a tracheal cannula. Expose the saphenous vein and insert a cannula for intravenous injections from a 50cc burette. Open the abdominal cavity, remove the outer sheath from the left kidney and enclose that organ in a renal onkometer. Record the kidney volume changes by means of a Brodie's bellows or Roy's piston recorder. The anaesthetic must be given with perfect regularity, 2 to 6 drops of chloroform every 30 seconds.

Take a record on the continuous kymograph and when all is in good working condition slowly inject **20 percent warmed alcohol** from the burette into the vein until some decided effect on the blood pressure is noted, i. e. after a dose of **20** to **40cc.** Extreme caution must be observed lest the heart be paralyzed and the animal die at once. The experiment should be repeated with different doses.

7. Alcohol on the reaction time of the reflex frog. Destroy the brain only of a frog and when it has recovered from the shock test the normal reaction time to electrical stimuli applied to the toe of the foot, page 51. Measure the time of the reaction with a watch or record with a writing point attached to the foot or leg of the suspended frog. Give a dose of **0.3cc** (5 minims) of **95 percent alcohol** in the dorsal lymph sac. Retest the reaction time at exactly 20 and 40 minutes after the injection. Compare the results with experiments 1 and 4 above.

ETHER.

The effects of ether.

1. **On the frog.**
2. **On the ventricular muscle.**
3. **On the frog's heart.**
4. **On the mammalian heart.**
5. **On the irritability of voluntary muscle.**
6. **On nerve tissue.**
7. **On the blood pressure and respiration of a mammal.**
8. **On the germination of seeds.**
9. **On the growth of yeast.**

1. **Ether on the frog.** Inject into the dorsal lymph sac of a frog **0.2cc** (3 1-2 minims) of **ether.** Give **0.3cc** to a second frog. The first dose will produce anaesthesia in 8 to 10 minutes. Slight power of reflex response is retained. The voluntary motions will be regained in from 60 to 90 minutes if the animal is kept moist, and complete recovery in two hours. The larger dose will be recovered from in 20 to 24 hours or may even prove fatal.

2. **Ether on the ventricular muscle.** Mount a strip of terrapin's ventricle and establish rhythmic contractions in a bath of 0.7 percent saline. Record on a drum moving 1 to 2 mm per second. Immerse the strip in a bath of **1 percent ether** in saline for 2 to 3 minutes, then return to a physiological saline bath. The sharp decrease in both amplitude and rate of contractions is recovered quickly in the saline bath.

Repeat using **2, 4,** and **6 percent ether** solution. The weaker solutions occasionally produce an increase in rate, the initial excitation stage.

3. **Ether on the frog's heart.** A frog is pithed, its heart exposed, and a record taken by a simple lever on the ventricle. Record on a slow drum. Irrigate the surface of the heart with **0.7 percent saline solution** from an irrigating bottle, method page 48, and consider this record as the normal. Substitute a **saturated ether** solution in 0.7 percent saline for the irrigating liquid (about 8 percent by volume).

Return to physiological saline irrigation after 3 to 5 minutes. Repeat after complete recovery. Solutions of ether applied to the surface of the heart rarely produce complete anaesthesia. The saline quickly restores contractions to twice and even thrice the original amplitude.

A second and more effective method is to insert a cannula in the inferior vena cava and perfuse the heart in place. Feed the heart first with 0.7 percent saline and follow with **1 percent ether** in saline. Repeat using **2 percent ether.**

4. Ether on the mammalian heart. Demonstration experiment. Use method as presented by Cushny, Jour. Exp. Medicine, Vol. II, page 233.

5. Ether on the irritability of voluntary muscle. Mount a gastrocnemius of the frog in the moist chamber, arrange to stimulate the muscle directly with a current of medium intensity but which produces a maximal contraction. Connect a vapor apparatus containing **1** or **2 percent ether** water with the gas tube of the moist chamber. Stimulate the muscle with single induction currents two times in quick succession, every 30 seconds through the entire experiment. Record on a drum having a rate of 2 mm per second. Take three or four normals then turn on the **ether vapor** for 5 minutes. Remove the vapor quickly with fresh air. The muscle's irritability will decrease to a point at which the stimulus is submaximal or even subminimal, but when the ether vapor is removed the contractions quickly reappear and attain their former amplitude.

One may readily demonstrate on this preparation that the muscle has a diminished power to do work when etherized.

6. Ether on nerve tissue. Prepare a muscle nerve of the frog isolating the entire sciatic with a piece of the cord and with the skin covering the muscle. Mount the preparation with the nerve in the moist chamber and on the electrodes but with the muscles hanging through the hole in the floor of the moist chamber and on the outside. Close the hole with moist filter paper. Proceed exactly as in