OUTLINE OF DIRECTIONS FOR THE LABORATORY COURSE IN PHARMACOLOGY AND TOXICOLOGY

By

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UNIVERSITY OF LOUISVILLE
SCHOOLOF MEDICINE

1950

Revised

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Πάντα δοκιμάζετε
τὸ καλὸν κατέχετε

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CORRECTIONS

Exp. 1. Elements, iodine. Be sure to allow the chloroform layer to separate.
Iron compounds. Wash the coating off the pill of ferrous carbonate before dissolving.

Exp. 2. Salicylates. The solution of acetylsalicylic acid must be just acid to litmus, to avoid precipitation of ferric hydroxide.

Exp. 7, p. 2. Substitute strong silver protein for the silver nitrate.

Exp. 10. Irritant. Remove the ointment with alcohol after the observations.

Exp. 16. The dose of ouabain should be 0.5 mgm. per dog.

Exp. 17, p. 2. The dose of acetyl-choline bromide should be 0.01 mgm., instead of 0.1 mgm. Nitroglycerine should be injected after the acetyl-choline, instead of after the posterior pituitary.

Exp. 21, p. 1, II, A. Add solution #4, 1% para-aminophenol hydrochloride.

Exp. 22. It is necessary to allow the cadmium sulfate-blood mixture to stand several minutes before adding the NaOH. The filtrate must be colorless.

Exp. 24. Omit the dinitrophenol. Alternate groups will give either vaccine alone, or vaccine and acetylsalicylic acid.

Exp. 25, p. 1. A syphon tube is supplied with the apparatus to permit changing the solution. The first six solutions are added on top of each other without changing the contents of the bath.

Exp. 28. Instead of rabbit uteri, rat uteri will be supplied. The rats will have been injected with diethyl stilbestrol. The contents of the bath should be changed between each drug.

The chief purpose of the laboratory course is to give the student an opportunity to demonstrate to his own satisfaction that bodily functions can be modified by foreign agents, that "drugs do work." As it is hoped that the student will develop the faculty of judging critically the value of drugs, he should begin to apply it in these experiments. He may be confident that they will yield results.

The student is already familiar with the use of pharmacological methods; he has used drugs to study the innervations of skeletal muscle (Laboratory Outline for Physiology, Exp. 27), the nerve synapse (Exp. 42), and the innervation of the heart (Exp. 6). These observations the student should reexamine, indeed throughout the course in pharmacology, he is referred to his experiments in physiology and physiological chemistry, He will find that he is but studying the response of mechanisms with which he is already familiar to agents that are foreign to the normal environment. In this way he will gain information about the organisms and the drug. Inasmuch as the student will wish to know the practical possibilities of the use of drugs, he will proceed from pharmacology as a pure science to its applications, and will be concerned with materials for the prevention, diagnosis, and treatment of disease. As much as possible, drugs of use and value in medicine have been included, in the hope that the student will find the course to supply him with a basis for <u>therapy</u>.

The conduct of the laboratory will in general be the same as that of the course in physiology. To insure contemplation of the experimental results and search for their significance, some reporting on the experiments is required. One set of complete data must be turned in by each group; each student will write a brief statement of his conclusions. These must be handed in at the end of the period; they will serve for discussion of the laboratory work at a weekly conference. Students wishing to compose permanent notebook recording procedures, data, and conclusions may find it profitable to do so.

Let not the student be misled by the apparent simplicity of the directions; they are so to require some intellectual effort on his part to grasp the meaning of the experiment and its possible outcome by putting questions to himself. The results obtained will often be far from simple, but they should be interpreted to the best of his ability. Needless to say, the outline should be examined before the laboratory is entered, and the phenomena should be observed, if not with a comprehending eye, at least an apprehending one. Louis Pasteur remarked that in the field of experimentation, chance favored only the prepared mind.

It is imperative that an accurate and complete "protocol" or chronological record be kept during the performance of the experiment. Each procedure should be stated in detail and the results noted and written down at the time. All too frequently experiments are valueless simply because of negligence in recording data. In certain instances the student will be required to consider not only his own data but those of the rest of the class as well. He is urged to do this whenever it is possible, also to consult freely with the instructor. Whenever possible the use of graphic representation of data is recommended. Depiction of results in this way renders them more readily comprehensible.

Near the end of the course, the students will perform experiments of their own devising. It is hoped that some enterprise and ingenuity will be exercised. If a source of material needs to be consulted, the standard laboratory manuals of Sollmann and Hanzlik, Barbour, and Jackson are recommended, or the current literature may supply a subject. The nature of the experiments will of course be restricted by the facilities of the laboratory, as well as the abilities of the student.

Technique of Injection.

This course should supply practice in the use of the "hypodermic syringe." Each group will be provided with three syringes and two each of three sizes of needles. Good care must be taken of these, and they should be sensibly used. In general, for accuracy one uses the smallest size syringe compatible with the procedure. The size of needle taken depends upon the use; for injections into an exposed vein, or the rabbit's ear, the smallest size is indicated The others serve for subcutaneous and intraperitoneal injections. Wash with water syringe and needle immediately after using them. Dry them at the end of the period, and keep the wires in the needles

For some experiments, a burette system is used for intravenous injections. A straight glass cannula is tied in the vein and connected by a rubber tube with a burette. The entire system must be filled with saline, to the exclusion of air, before unclamping the vein. Injections are made with syringe and needle into the rubber tube, then washed in with saline, or large volumes can be injected via the burette itself. It is advisable to allow small amounts of saline to run in from time to time to prevent clotting.

Refer to the Outline for Physiology on laboratory technique.

OUTLINE OF PHARMACOLOGY AND TOXICOLOGY 102

Lectures	Laboratory Experiments	Demonstrations
A. Historical Development of Modern Pharmacology Chemistry of Drugs Relation of Chemical Constitution to Pharmacological Action The Nature of the Action of Drugs. Quantitative Aspects of Drug Action (1) Administration, Excretion, Detoxification (2) Response to Drugs, Animal Variation, Constitutional Factors, Drug Allergy 8 Lectures	1.(Chemistry of 2.(Drugs 3. Determination of Toxicity and Bioassay 4. Absorption of Drugs 5. Excretion of Drugs 6. Acidifying and Alkalinizing Substances	Chemistry of Drugs
B. Drugs Used in Diagnosis of Disease 2 Lectures		Diagnostic Drugs
C. Drugs Used in Prevention of Disease Vitamines. Antiseptics. 6 Lectures	7.) 8.) Antiseptics	
D. Drugs Used in Treatment of Disease Etiotropic Agents Chemotherapy of Malaria, Syphilis, Amebiasis, Helminthiasis, Leprosy, Tuberculosis	9. Anthelmintics and Trypanocides	
Locally Acting Drugs 6 Lectures	10. Locally Acting Drugs	
E. Drugs Used in Treatment of Disease Organotropic Agents		
Pharmacology of the Nervous System Alcohol. General Anesthesia. Hypnotics, Stimulants. Local Anesthetics. Analgesics. Opium Alkaloids. 14 Lectures	11. General Anesthesia 12. Alcohol and Caffeine 13. Central Nervous and Peripheral Neuromuscular Systems	Absorption of Inhalation Anesthetics Cardiac Syncope in Anesthesia

OUTLINE OF PHARMACOLOGY AND TOXICOLOGY 102

	14. Local Anesthetics	Toxicity of Local Anesthetics
		Action of Morphine and Related Drugs
Pharmacology of the Somatic Neuromuscular System.		
Introduction to the Pharmacology of the Visceral (Autonomic) Nervous System.		Types of "Autonomic" Drugs
Pharmacology of the Circulation. Digitalis. Xanthine Derivatives.	15. Analgesics. Nasal Mucosa 16. Digitalis in Cardiac Disease.	
8 Lectures	17. Drugs Affecting the Heart.	
	18. Pupil Drugs. Secretion of Sweat and Saliva.	
Pharmacology of Respiration, External and Internal, Gaseous Metabolism. Carbohydrate Metabolism. Heat Formation and Transfer. Blood Formation Hemoglobin	19. Respiratory Drugs. 20. Bronchial Drugs.	Bronchial Asthma; Anaphylaxis
	21. Blood Cells and Hemoglobin 22. Carbohydrate Metabolism 23. Oxygen Consumption 24. Antipyretics	
6 Lectures		
Pharmacology of the Digestive System.	25. Smooth Muscle of the Intestine. 26. Diuresis.	
4 Lectures		
Pharmacology of Excretory Systems: Skin, Kidney, Water Balance. Maintenance of Blood Volume. Edema, Cerebrospinal Fluid.	27. Edema. Antidiuresis.	Pressure of Cerebrospinal Fluid.
3 Lectures		
Pharmacology of the Sex Organs. Hormones.	28. Uterine Drugs.	
2 Lectures		

Exp. 1. Elements, iodine. Be sure to allow the chloroform layer to separate.
Iron compounds. Wash the coating off the pill of ferrous carbonate before dissolving.

Experiment 1

THE CHEMISTRY OF DRUGS

4 Students

The many chemical compounds used in medicine fall into several distinct chemical groups which are determined by the presence of an important common chemical structure. In this experiment the characteristics of each of these groups will be determined so that they may be applied to any new compound that may later be encountered. Another object of this experiment is to emphasize the importance of correlating chemical structure with chemical and pharmacological action. For this reason the student should write out the structural formulas for the reactions which take place. Each student should perform as many of the experiments as possible, and must see the essential step in those he does not do.

Elements.

While such elements as iron, iodine, and mercury are administered as such, they are changed in the body before they are effective.

1. Examine a thin smear of mild mercurial ointment, U.S.P. XI under the microscope.

2. Add a few drops of a solution of iodine in alcohol to 5 c.c. of artificial gastric juice and allow to stand for 30 minutes. Add 5 c.c. of chloroform and shake until all the iodine is in the chloroform layer. Decant some of the supernatant fluid into a test tube, add a few drops of hydrogen peroxide and a few drops of starch solution. A blue color shows the presence of iodide.

3. Add a small amount of reduced iron U.S.P. XI to artificial gastric juice, mix and let stand for 10 minutes. Test for ferrous and ferric iron as follows. Divide the supernatant fluid into two portions. To one add a few drops of 1% potassium ferrocyanide and to the other a few drops of 1% potassium ferricyanide. Potassium ferrocyanide gives a deep Prussian blue precipitate with ferric iron and a white precipitate with ferrous iron. Potassium ferricyanide gives a Prussian blue precipitate with ferrous iron and a clear brown solution with ferric iron.

Inorganic Compounds.

Compounds of the alkali and alkaline earth metals.

Compounds of this type are used either for their osmotic effect, such as shown by sodium sulfate, or to neutralize gastric acidity. In the latter group, the neutralizing power and possibility of absorption of the base (with corresponding danger of alkalosis) are the most significant considerations.

1. Titrate with N/1 HCl, using methyl orange as an indicator, 1 Gm. samples of each of the following: (a) MgO (b) $NaHCO_3$ (c) $CaCO_3$

2. Refer to the Outline for Physiology for the absorption of soluble magnesium and calcium salts.

3. Shake artificial gastric juice with MgO, $CaCO_3$, and $NaHCO_3$, enough of each to give a solid excess, and determine the reaction of the fluid to litmus, phenolphthalein, and congo red.

Iron Compounds.

There is considerable evidence that ferrous iron is the only effective form of iron in anemia. Ferrous compounds are easily oxidized to ferric compounds in the air, so that keeping an effective iron preparation is difficult. Due to their tendency to react with protein and their acidity, many iron compounds are irritating.

1. Refer to the experiment on reduced iron under elements.

2. Dissolve a pill of ferrous carbonate U.S.P. XI in dilute HCl and test the solution for ferrous and ferric iron.

3. Test the effect on egg albumin of: (a) 1% ferric chloride (b) 10% ferric ammonium citrate

4. Add a drop of ferric chloride to 5 c.c. of water and determine the reaction to congo red and litmus. Repeat using iron ammonium citrate.

Mercury.

Mercury is a typical heavy metal which forms two series of compounds, mercurous and mercuric. There are essential differences between the two, such as seen in the relative solubility of the chlorides. Both types are readily reduced to mercury by reducing agents, precipitate proteins, and form oxides with dilute alkali. However, the insoluble mercurous chloride in an alkaline medium is partly reduced to metallic mercury and partly oxidized to mercuric oxide. The latter compound is soluble enough to give sufficient mercuric ions for absorption.

1. Add to a solution of egg albumin some saturated solution of mercuric chloride.

2. Test the relative solubility of mercuric and mercurous chloride in water by adding to a saturated solution of each a few drops of solution of sodium sulfide.

3. Add a few cc. of saturated mercuric chloride to (a) 10% solution of sodium thiosulfate; (b) 10% solution of sodium sulfoxalate formaldehyde.

Exp. 2. Salicylates. The solution of acetylsalicylic acid must be just acid to litmus, to avoid precipitation of ferric hydroxide.

Experiment 2

THE CHEMISTRY OF DRUGS (Continued)

Organic Compounds.

Alcohols.

The toxicity of a compound may not be due to the compound itself, but to substances formed when it is oxidized in the body. The hydroxyl group of the alcohols is the easiest part of the molecule to oxidize so that they are converted into the corresponding aldehyde and acid in the body. In this way the polyhydroxy alcohol, ethylene glycol, is oxidized to the toxic oxalic acid. The oxidation products of alcohols also serve to identify them.

1. Plunge a hot copper wire into a test tube of 10% methyl alcohol and smell the vapor given off.

2. Add a few cc. of dilute sulfuric acid to 5 cc. of 10% ethyl alcohol and 2-3 cc. of sodium dichromate solution. Heat to boiling and note the odor of acetaldehyde.

3. To 1 cc. of 10% ethylene glycol add 3 cc. of conc. nitric acid. Boil gently for five minutes. Cool, make alkaline with ammonium hydroxide and add a few drops of calcium chloride solution. Note the precipitation of calcium oxalate.

Alkyl Halides.

The toxicity of some alkyl halides is concerned with the ease with which the halogen is split off.

1. To 1 cc. of carbon tetrachloride add 5 cc. of N/1 NaOH and boil for one minute. Acidify with conc. nitric acid and add a few drops of solution of silver nitrate.

Phenols.

The activity of phenols, both chemical and pharmacological, is greatly modified by replacing the hydrogen with an aliphatic radical. A simple example is the methyl ether of phenol; for others see the salicylates, below.

1. Add ferric chloride solution to:
   (a) aqueous phenol (b) phenol methyl ether in alcohol
   (c) resorcinol (d) epinephrine

2. Determine the approximate pH of an aqueous solution of phenol, with litmus and congo red.

Salicylates

In acetylsalicylic acid, the phenolic OH is muzzled by acetylation. It is important to know if the sodium bicarbonate which is often given with acetylsalicylic acid might hydrolyze this compound.

Test an aqueous solution of salicylic acid with ferric chloride.

Dissolve 1 Gm. of acetylsalicylic acid in 20 cc. of 10% solution of sodium bicarbonate. Take half of this, make just acid to litmus with dilute HCl and add solution of ferric chloride. Boil the other half for three minutes, cool, make just acid to litmus, and add ferric chloride.

Tannins

The tannins are organic acids occurring in the bark and leaves of trees. While they are of uncertain composition, they have the common property of being strong reducing agents, forming insoluble compounds with protein and forming dark blue precipitates with iron compounds. They precipitate some but not all, heavy metals, glucosides and alkaloids. One product of acid hydrolysis is tri-hydroxy benzoic acid, which explains its strong reducing power and reaction with iron.

The following tests illustrate these properties:

Add 2 cc. of 1% tannic acid to the following:

(a) egg albumin
(b) 10% ferric chloride
(c) Benedict's solution (heat to boiling)
(d) 5% mercuric chloride
(e) 5% lead acetate

Glucosides

Glucosides are complex compounds occurring in nature which yield glucose on hydrolysis. This hydrolysis can occur by heating with dilute acid, alkali, or by the action of a specific enzyme.

Test tincture of digitalis with Benedict's solution.

Alkaloids.

The alkaloids are basic substances occurring in plants. Many are very active pharmacologically. They have the chemical property in common of being precipitated by certain agents, but have widely different chemical structures, some being aliphatic and others aromatic compounds. Some being extremely potent poisons, are of great toxicological importance, and the student should look up the specific tests for individual members of this group when they are studied in more detail in later experiments. Only their general chemical properties will be considered in this experiment.

1.

	Solubility in		
Substance	Water	$CHCl_3$	Alcohol
Procaine Base			
Procaine Hydrochloride			

Determine solubility by placing a few crystals on a watch glass, and add the solvent drop by drop until solution occurs. Record the number of drops.

2. To 2 cc. of 1% procaine hydrochloride solution add a few cc. of:

(a) Lugol's solution
(b) Mayer's reagent
(c) Phosphotungstic acid
(d) Picric acid
(e) Tannin
(f) Potassium permanganate

Experiment 3

BIOASSAY: DETERMINATION OF TOXICITY

The toxicity of a substance refers to its power to endanger or destroy life; it is a feature of importance forensically and in therapy. In either case, it is necessary to have knowledge of the qualitative and quantitative aspects of this toxicity. We must be able to recognize the symptoms of the intoxication, and we should know the amount which is likely to produce it, or cause death. The expression "minimal lethal dose" is often used as a single index of toxicity, but this has long been a poorly defined entity, and is manifestly only a partial index of toxicity. A basic cause for this is the variation among individuals of a species in the resistance to a toxic agent. The student should refer to a later experiment (No. 15) for mention of the importance of this factor, and sources of information on it.

For the purposes of this experiment, the class will determine experimentally the dosage range in which a potent alkaloid, strychnine, will cause death. What has been called a "characteristic curve" of the quantitative reaction of mice to varying amounts of strychnine will be determined. Groups of animals will be injected with a range of doses, and the percentage mortality at each dose observed. From these values the LD_{50} or median lethal dose can be arrived at, but the student will also be interested in the "spread" of the curve, as this indicates at what fraction of a certain lethal dose will toxic reactions possibly fatal be encountered. The mice used in this experiment should be injected intraperitoneally, using the tuberculin syringe. The range of dosage should be 1 to 5 mgm. per kilo. Systems of intoxication should be carefully identified.

To Isolate, Identify, and Quantify a Potent Toxic Agent.
This exercise makes use of the preceding experiment for forensic purposes. Suppose an instance of a serious intoxication, with symptoms resembling those of strychnine poisoning, resulting from ingestion of a prepared powder. Our problem is to isolate the toxic agent, if any, to identify it, and measure its quantity. As we suspect strychnine, we may proceed on the assumption. Each group of four students obtains one of the powders, consisting of about 1/2 gram of crystalline material. This is dissolved in 60 cc. of water. Take off 10 cc. and test part of this with Mayer's reagent (potassium mercuric iodide, useful for alkaloids as a group). This will suggest the presence or absence of an alkaloid. Carefully taste some of this solution.

Put the remaining 50 cc. in a separatory funnel and add 1 cc. of concentrated ammonium hydroxide, and test with litmus paper to make sure the solution is alkaline. (This step will liberate an alkaloidal base from its salt). Add 5 cc. of chloroform, shake, allow to settle, and separate off the chloroform layer. Repeat the extraction with 4 cc. more of chloroform, combine the chloroform extracts and make up to 10 cc. with chloroform. This should contain nearly all of any alkaloid that was present.

Take 5 cc. of this and set aside. The remaining 5 cc. is evaporated just to dryness on a water bath. Dissolve the residue in a few drops of dilute hydrochloric acid. (This converts an alkaloidal base to the HCl salt.) Evaporate this to dryness. Ordinarily the weight of the contents would now be determined. Accept this as done and the weight to be 5 mgm. The contents of the dish are now carefully dissolved in water (or 0.9% NaCl), the dish being rinsed several times. Make up this volume to 25 cc. If the weight was 5 mgm., then each cubic centimeter = 0.2 milligram (of strychnine hydrochloride?).

In the meantime the other 5 cc. portion of chloroform extract should have been evaporated to dryness on the water bath. Mix the contents with a few drops of concentrated sulfuric acid, then drag across the dish a small crystal of potassium dichromate. Is the "fading purple test" positive?

The unknown potent substance has been tentatively chemically identified as an alkaloid, strychnine. We now resort to a "bioassay" to give us pharmacological evidence of the nature of the substance, and the amount of it present. The material isolated is likely to be the pure substance. Its weight is known, so we may attempt immediately to determine quantitatively its toxicity. As the point of the previously found toxicity curve that is determined with greatest accuracy is the LD_{50}, we now inject a group of mice with an amount of our solution expected to produce a 50% mortality. If this confirms our chemical assay and shows us the syndrome of intoxication that we have recently studied, our evidence will be complete.

To calculate the amount of substance in the original sample, it is necessary to multiply the determined content of the final 25 cc. sample by two (the chloroform extract was divided in two), and to multiply this figure by 1.2 (the original sample was in 60 cc., of which we took only 50).

What was the dose taken? What relation to the usual therapeutic dose had it? Is this likely a fatal dose?

Experiment 4

ABSORPTION AND DISTRIBUTION OF DRUGS;

DETERMINATION OF VOLUME OF THE FLUID COMPARTMENTS OF THE BODY

In a barbitalized dog, insert a tracheal cannula. Isolate an external jugular vein and cannulate the central end. Connect this with a burette containing enough saline to fill the cannula and tubing. Isolate a carotid artery, ligate it twice peripherally, and cut between the ligatures. Insert a straight bleeding cannula, and allow blood to flow directly from the end of the cannula into a 50 cc. centrifuge tube containing dried oxalate to prevent clotting. Take approximately 30 cc. of blood, then mix this with the oxalate by placing the hand over the end of the tube and slowly inverting the tube until all the oxalate is dissolved and mixed with the blood. It is of the utmost importance to avoid hemolysis. The tube should not be shaken vigorously. The bleeding cannula should be rinsed by means of a syringe and needle with saline solution, so that it is completely free from blood. Measure in a graduated cylinder exactly 10 cc. per kgm. of body weight of a solution containing 10 mgm. of T-1824, 2 gm. of sodium bromide, and 0.5 gm. of sulfanilamide per 100 cc. Inject this intravenously by means of the burette, rinsing the cylinder and burette with a small volume of saline solution. Thirty minutes after this injection, take another 30 cc. sample of blood in similar fashion, but wasting the first few drops of blood that flow from the cannula. The two blood samples are centrifuged (Instructor) and about 10 cc. of the plasmas are drawn by mouth suction into the small flasks provided. Avoid drawing any cells into the plasma.

Two students proceed with the analyses as described below, while the others arrange for the recording of arterial pressure on a kymograph, and make injections of epinephrine HCl, U.S.P., 0.005 mgm. per kgm.

1. Into the jugular vein, injecting it into the rubber tubing with syringe and needle, washing it in with saline solution.
2. By local application to the sublingual mucosa.
3. Into the exposed stomach with syringe and needle.
4. Into the exposed small intestine with syringe and needle.
5. Into the splenic vein, with syringe and needle.
6. Into the urinary bladder with syringe and needle.

Now, apply locally to the sublingual mucosa,

7. Spirit of nitroglycerine, U.S.P., 5 drops
8. Nicotine alkaloid, 10% solution, 5 drops

Determination of T-1824. With a transfer pipette, put 5 cc. of the undyed plasma into a photometer tube, and add 5 cc. of 0.9% NaCl. This serves as a blank. Pipette 5 cc. of the dyed plasma into another photometer tube, and add 5 cc. of 0.9% NaCl. Mix. Compare photometrically at 620 millimicra and read the dye concentration from the standard curve.

Determination of Sodium Bromide. It is necessary to prepare a protein-free filtrate, which is used for the determination of both bromide and sulfanilamide. Of the second, post-injection sample of plasma, 2 cc. are added with a transfer pipette to 18 cc. of 5% trichloracetic acid in a small flask. Mix, let stand 10 minutes, and filter through paper.

Of this filtrate, put 5 cc. with a transfer pipette into a photometer tube, and add 1 cc. of a 0.5% solution of gold chloride. Mix, and read in the photometer. Determine the concentration from the standard curve.

Determination of Sulfanilamide. With a transfer pipette, put 5 cc. of the above-mentioned trichloracetic acid filtrate in a photometer tube. Add 1 cc. of 2N HCl, and mix. Add 1 cc. 0.1% sodium nitrite, mix, let stand 5 minutes. Add 1 cc. sulfamate solution, mix, let stand 2 minutes. Add 1 cc. Naphthyl-ethylene diamine solution, mix, let stand 10 minutes, and read in the photometer at 540 millimicra. Determine the concentration from the standard curve.

Calculations.

$$\text{Volume occupied by substance} = \frac{\text{milligrams injected}}{\text{milligrams per cc. of plasma}}$$

Volume occupied by T-1824 = plasma volume = cc.

Volume occupied by NaBr = extracellular fluid = cc.

Volume occupied by sulfanilamide = body water = cc.

Interstitial fluid = extracellular fluid - plasma volume = cc.

Intracellular fluid = body water - extracellular fluid = cc.

Experiment 5

EXCRETION OF DRUGS

Ascorbic Acid. The percentage of a test dose of this substance that appears in the urine is claimed to give an indication of the subject's saturation, and thus the adequacy of his intake of ascorbic acid. Since a saturated individual shows most rapid excretion within four hours, this period is taken here.

Procedure. At a suitable time during the morning, the urinary bladder is emptied, and 10 milligrams per kilogram body weight of ascorbic acid are taken by mouth, with some water. If possible, no urine is passed until four hours after the ingestion of the ascorbic acid. When the urine is collected, 15 cc. of it are diluted with 15 cc. of 10% phosphoric acid, which serves as a preservative. The total volume of urine is also measured, in order to calculate the total amount of ascorbic acid excreted.

Determination. This depends on the fact that dichlorobenzeneone-indophenol, which has a red color in its acid form in a xylene solution, is extracted from this solution (and reduced to the colorless form) by solutions containing ascorbic acid.

Pipette 1 cc. of the diluted urine into the large centrifuge tube provided, and add 15 cc. of 0.03N HCl. Pipette in exactly 10 cc. of the xylene-dye solution, insert a rubber stopper, and shake vigorously for exactly 15 seconds. Remove stopper, centrifuge (instructor), pour some of the xylene layer into a photocomparator tube, and read in the comparator at 540 millimicra. This instrument has been adjusted to zero with xylene, and the dye solution has been adjusted so that its reading is 150. Therefore, if ascorbic acid is present, a reading between 0 and 150 will be obtained, as seen in the standard curve. The determination is most satisfactory if the sample contains 0.01-0.06 mgm. of ascorbic acid, therefore, the 50-50 urine-phosphoric acid mixture should be diluted with 5% phosphoric acid so that the concentration of the 1 cc. sample used in analysis is in that range. From the concentration in the finally satisfactory sample, the various dilutions, and the volume of urine, the amount of ascorbic acid excreted in four hours is calculated. The percentage which this is of the test dose is entered in the table provided.

Sulfanilamide. This and related compounds are excreted in the urine in the free form and in the form of conjugates.

At 7 A.M. on the day preceding the determination, the subject empties the urinary bladder, and takes 1 gm. of sulfanilamide by mouth. Urine is collected for 12 hours subsequently, to form the first sample, and for the next 12 hours, to form the second sample. The volumes of the two samples are measured.

Determination. Free. Pipette 2 cc. of urine into 98 cc. of distilled water and mix thoroughly. Pipette 5 cc. of the diluted urine into a photometer tube, and follow the method as outlined in Experiment 4.

Total. Pipette 2 cc. of urine into a test tube. Add 1 cc. of 2 N HCl and about 7 cc. distilled water. Heat in a boiling water bath for 1 hour. Transfer contents of tube quantitatively to a graduated cylinder, and dilute to a volume of 100 cc. Mix thoroughly. Pipette 5 cc. into a photometer tube and proceed as for "Free."

Calculation. 1. Free sulfanilamide, mgm. per 100 cc. urine.

2. Total sulfanilamide, mgm. per 12 hour period.

3. Per cent free, per cent conjugated in each 12 hour period.

Iodide and Bromide. The rate of excretion of these in the urine differs markedly. At 7 A.M. on the preceding day, the subject takes 0.3 gm. of potassium iodide or potassium bromide. All of the urine passed in the following 24 hours is collected and saved, and its volume measured.

Procedure. The total urinary halides are precipitated as silver salts. The iodide (or bromide) is then oxidized to iodate (or bromate) by sodium hypochlorite. After the excess hypochlorite is reduced by sodium formate, potassium iodide is added and is oxidized to iodine by the iodate (or bromate). The iodine is then titrated with sodium thiosulfate.

Pipette 5 cc. of urine into a flask, and add 25 cc. of 10% nitric acid. Add drop by drop, with gentle shaking, 7.5 % silver nitrate until no further precipitation occurs (not more than 3 cc. will be needed). Let stand for 30 minutes, then filter off the fluid with the filter stick and suction. Wash the precipitate with 25 cc. 1% nitric acid and filter this off. To the precipitate, add 5 cc. of 40% sodium acid phosphate, and 10 cc. of sodium hypochlorite solution. Bring to a boil over a flame, shaking occasionally. Add 10 cc. of 50% sodium formate, running it down the sides of the flask, and mixing thoroughly. Bring to a boil again, with occasional shaking. Rinse down the sides of the flask with 10 cc. of water and bring to a boil again. There must not be an odor of chlorine at this stage. Filter the contents through paper into another flask, rinsing the first flask and the filter with two 10 cc. portions of water. Cool. Add 50 cc. of 6N sulfuric acid and 5 cc. of 20% potassium iodide. If a brown color does not appear immediately, add starch solution as indicator and titrate immediately with 0.02N sodium thiosulfate. If a brown color appears, titrate immediately with sodium thiosulfate until the brown color has almost disappeared, then add starch solution and finish the titration.

It is necessary to run a blank determination on the reagents, beginning at the point of adding the sodium acid phosphate. This value is subtracted from the other determinations. It is suggested that this blank be done first.

Calculation. The amount of potassium iodide in mgm. in the 5 cc. sample is obtained by multiplying the cc. of 0.02N sodium thiosulfate by 0.55. For potassium bromide, multiply by 0.40.

Report as mgm. excreted in 24 hours.

Experiment 6

ACIDIFYING AND ALKALINIZING AGENTS

One student serves as the subject. The total output of urine is collected for two successive 24-hour periods, as follows. On the morning of the second day preceding the laboratory period, the morning urine is discarded, and urine passed during the following 24 hours is collected, including the morning urine of the first day before the laboratory period. This constitutes the control sample. The urine passed during the following 24 hours, including the morning urine of the day of the laboratory period, constitutes the experimental sample. Each should be kept in brown glass bottles containing some toluene as preservative, with the bottles kept tightly stoppered and refrigerated when possible. The food intake during these days should be as constant as possible. The subject takes one of the following substances on the second day of urine collection.

1. Ammonium chloride, NH_4Cl, mol. wt. 53.5.
   Take eight tablets, 0.325 gm. each, after each (3) meal and at bedtime. Total = 10.4 gm. NH_4Cl or 194 milliequivalents Cl^-.

2. Calcium chloride, $CaCl_2 \cdot 2H_2O$, mol. wt. 147.
   Take 10 cc. of solution containing 3.7 gm. in a glass of milk at each (3) meal and at bedtime. Total = 14.7 gm. = 200 milliequivalents Cl^-.

3. Sodium chloride, NaCl, mol. wt. 58.5.
   Take five tablets, 0.65 gm. each, after each (3) meal and at bedtime. Total = 13 gm. or 222 milliequivalents.

4. Sodium bicarbonate, $NaHCO_3$, mol. wt. 84.
   Take two tablets of 2.1 gm. each, after each meal and at bedtime. Total = 16.8 gm. = 200 milliequivalents Na^+.

5. Sodium citrate, $Na_3C_6H_5O_7 \cdot 2H_2O$, mol. wt. 294.
   Take eight tablets, 0.65 gm. each, after each (3) meal and at bedtime. Total = 20.8 gm. = 211 milliequivalents Na^+.

Determinations. The volume of each 24-hour sample is measured, and the following determinations and calculations made.

Hydrogen Ions. The pH of the urine is measured with the glass electrode, with the assistance of the instructor.

Total Base. For this determination, advantage is taken of the fact that a synthetic resin, when treated with a salt solution, exchanges hydrogen ions for the cations present. Therefore, the difference in hydrogen ions before and after such treatment is a measure of the total base.

Procedure. To exactly 1 cc. of urine, add 10 cc. of water, 1 drop of phenolphthalein indicator, and titrate with 0.02 N NaOH. This value = A.

Pipette exactly 1 cc. of urine into the top of the tube containing the resin, arranged so that the fluid running through will drip into a small flask. Follow with 10 cc. of water, washing all of the urine sample through the resin into the flask. The small amount of fluid remaining in the resin tube after drainage has ceased is ignored. Add 1 drop of phenolphthalein indicator to the contents of the flask and titrate with 0.02 N NaOH. This value = B.

Calculation. 20 x (B cc. - A cc.) = milliequivalents per liter.

Ammonia. Procedure. The ammonia is aerated into standard acid, and the remaining acid is titrated. Inspect the aerator and connect it so that the air bubbles first through the tube marked "urine" and then through the tube marked "acid." It is necessary that these tubes not be interchanged. There must also be no leaks. Into the tube marked "urine" pipette exactly 5 cc. of urine, and add a drop of caprylic alcohol. Into the tube marked "acid" pipette exactly 25 cc. of 0.02 N hydrochloric acid, and add 2 drops of methyl red indicator. Add to the urine about 10 cc. of saturated solution of potassium carbonate, stopper immediately, and turn on the air slowly at first, for two minutes, then more rapidly, but not rapidly enough to splash liquid out of the tubes. Continue the aeration for 15 minutes. Disconnect the tubes, pour the contents of the acid tube into a flask, and rinse this tube and its bubbler into the flask with water. If the color of the indicator is now yellow, instead of red, the determination must be repeated with 2 cc. of urine instead of 5 cc. If the color is red, titrate with 0.02 N NaOH to a yellow color. This titration value = A. Wash the tubes and bubblers thoroughly before the next determination. It is desirable to do this determination in duplicate.

Calculation. B = value of blank determination on the reagents alone, reported by the instructor.

4 x (25 - A - B) = milliequivalents of ammonia per liter of urine.

Chloride. Procedure. This determination rests on the fact that mercuric chloride ionizes poorly and mercuric nitrate ionizes well. Diluted urine is titrated with mercuric nitrate, in the presence of an indicator which develops a violet color in the presence of mercuric ions.

Pipette exactly 1 cc. of urine into a small flask and add exactly 9 cc. of water. Mix, and pipette exactly 2 cc. of this diluted urine into another flask, and add 2 drops of 0.2 N HNO_3, 2 drops of 30% H_2O_2, and 2 drops of diphenylcarbazone indicator. Titrate with mercuric nitrate solution from a burette until a permanent violet color appears. This value in cc. = A. This determination should be done in duplicate.

Calculation. A x factor of mercuric nitrate solution = milliequivalents Cl^- per liter of urine.

HCO_3. This value is not determined, but is calculated from the pH. The H_2CO_3 content of urine is closely equivalent to the H_2CO_3 content of plasma. This is rather closely regulated by the body, and we shall assume that it does not change during this experiment, and that the H_2CO_3 content of urine is normally 1.8 milliequivalents per liter. The Henderson-Hasselbach formula states that

$$pH = pK + \log \frac{BHCO_3}{H_2CO_3}$$

Substituting and solving for $BHCO_3$,

$$BHCO_3 \text{ (or } HCO_3^-) = 1.8 \times 10^{pH - 6.1} \text{ milliequivalents per liter}$$

By inspection, it will be seen that this will be very small in acid urine.

Treatment of Data. Diagram the electrolyte composition of the control and experimental urines, with adjacent rectangles for positive and negative ions. Make two such sets, one for the concentration in milliequivalents per liter of urine, and one for total milliequivalents in 24 hours. Subtract the total output for the control period from the total output for the experimental period, and compare this difference with the amounts ingested.

In the construction of the diagrams, the total concentration of cations (and also of anions) is fixed by the value for total base. The difference between this and the NH_4^+ is the concentration of fixed base (Na, K, Mg, Ca, etc.). The total for anions is composed of Cl^- (determined), HCO_3^- (calculated) and the remainder, of organic acids, SO_4^-, PO_4^-, etc.

Exp. 7, p. 2. Substitute strong silver protein for the silver nitrate.

Experiment 7

ANTISEPTICS

Skin Disinfection

The ideal skin disinfectant is one which is penetrating, rapid in its action, and non-irritating. In the experiment the efficiency of some procedures used to disinfect the skin before surgery will be compared. The method is that of Novak and Hall, Surgery 5: 560, 1939.

The experiment is performed by pairs of students, one serving as the subject. Two corresponding areas of skin, preferably devoid of hair are selected in the right and left hypochondrium. One area serves as a control, the other is treated as below. To the control area, and to the other after preparation, a small plate of blood agar is applied for 15 minutes, being held on by adhesive tape. The plates are then returned to the large Petri dishes and incubated at body temperature for 48 hours, at which time the colonies on each are counted. The two plates should be the same size and should be handled carefully to avoid contamination. The procedures are:

A. 1. Control area.
   2. Wash three times with a sterile gauze thumb sponge and sterile physiological saline solution, taking a new sponge each time. Dry with a new sponge.

B. 1. Control area.
   2. Scrub one minute with Liniment of Soft Soap U.S.P. on a sterile sponge, and follow with three washings with sterile physiological saline, taking a new sponge each time. Dry with a new sponge.

C. 1. Control area.
   2. Apply the solution of alcohol 50%, acetone 10%, three times with sterile sponges, allowing to dry each time. Follow with three washings with sterile saline, taking a new sponge each time. Dry with a new sponge.

D. 1. Control area.
   2. Apply three times with sterile sponges the following antiseptic solution, allowing to dry each time. Follow with three washings with sterile saline, taking a new sponge each time. Dry with a new sponge.

Alcohol, 95%	525 cc.
Acetone	100 cc.
Water	375 cc.
Tricresol	5 cc.
Mercuric chloride	0.7 gm.
Acid fuchsin	0.08 gm.

The results of all experiments should be recorded in the tables provided.

The Disinfection of Mucous Membranes

This experiment determines the effectiveness of various agents used for this purpose. The four parts are divided among the four members of each group.

Place a plug of absorbent cotton above the upper lateral incisor between the gum and the upper lip to keep the gum dry during the experiment. Dry the gum with a cotton swab. Paint the dried area of the gum and well down on the teeth with the following agents and allow to dry for five minutes. Take a flamed wire loop and insert it well under the gingival margin, and inoculate this into a tube of peptone broth. Incubate at body temperature for 48 hours and examine for growth. Record all results in the tables provided.

A. Solution of Gentian Violet (Methylrosaniline chloride, U.S.P.) 1%.

B. Mild Tincture of Iodine, U.S.P.

C. Solution of Silver Nitrate, U.S.P. 5% (!! NOT ON PORCELAIN INLAY!!)

D. Saturated solution of Sodium Perborate, U.S.P.

Experiment 8

ANTISEPTICS, II

The four parts of the experiment are divided among the four members of the group.

A. Mandelic acid. The subject is preferably on a ketogenic diet, and should limit his fluid intake on the day preceding the experiment and the day of the experiment to one liter. At 6:00 P.M. on the preceding day, and at 8:00 and 10:00 A.M. and at noon of the day of the experiment, he will take 2 gm. of ammonium chloride. At 1:00 P.M. he will take 2 gm. of mandelic acid and empty his bladder into a sterile flask, the urine to serve as a control. The flask is made sterile by boiling water in it, and pouring it out. At 4:00 P.M. he will empty his bladder into a sterile flask. Approximately 9 cc. of this urine and the control urine are poured into sterile test tubes, and inoculated with 1 cc. of diluted broth culture of E. coli. The tubes are incubated for 24 hours. They are then examined for growth. The contents of the tubes are mixed by gentle swirling, and a loopful from each is streaked on an agar plate, marked off into halves. The plates are incubated for 24 hours, and examined for growth.

B. Methenamine. The procedure of part A is followed, except that 1 gm. of methenamine is taken instead of the mandelic acid.

C. Methenamine alone. The course of acidification of the urine is omitted. At 1:00 P.M., a course as in part B is followed.

D. Sulfanilamide. The procedure is as in part C, except that 1 gm. of sulfanilamide is taken instead of the methenamine.

Experiment 9

ANTHELMINTICS

From tests *in vitro* of ascaricidal activity, one can predict with some accuracy the effectiveness *in vivo*, but an equally important consideration is the action of the agent on the host. The student should obtain this information and compare it with the results of this experiment. In ascariasis the *therapeutic hazard* may be greater than the *disease hazard*.

Examine the ascaris lumbricoides of the pig, as provided. It is indistinguishable from the human variety. Test for activity by dropping singly into a beaker of water at 40° C. Use only those which respond to this treatment by moving. Put one into each of the beakers of 0.7% sodium chloride at 37° C. containing the following in saturated solution.

1. Santonin, U.S.P.
2. Oil of chonopodium, U.S.P.
3. Oleorosin of aspidium, U.S.P.
4. Thymol, U.S.P.
5. Hexylresorcinol
6. Tetrachlorethylene, N.N.R.

Observe the responses. At fifteen minutes, remove the worms and test for activity as before. Return to the beakers and test again at one hour.

The results of this experiment should be compared with the reported efficacy of these agents on other parasites. *It is inexcusable to treat for helminthiasis without determining the identity of the parasite.*

Chemotherapy of Helminthiasis. H. W. Brown. J.A.M.A., 103: 651, 1854. 1934.

ARSENICAL DRUGS IN RAT TRYPANOSOMIASIS

The effect of arsenicals on rat trypanosomiasis can readily be studied, using methods that are essentially those developed by Paul Ehrlich which led to the discovery of the usefulness of arsphenamine in human syphilis.

A. Action in vitro. On a clean glass slide, put a drop of Ringer's solution at one end, and a drop of Ringer's solution containing the drug at the other end. This latter solution is prepared so that its concentration is approximately that reached in the body following injection. The rat, which has been infected previously with Trypanosoma equiperdum, and which would die shortly, in untreated, is obtained, and its tail is immersed in water at 45 degrees for 3 minutes. The tail is then dried, and the very tip of the tail is cut off with a sharp knife. A very small amount of blood is then added to each drop of solution on the slide. Too much blood will give an opaque preparation; avoid this. At this time, blood is taken as below for a count. Cover slips are put on the slide, and microscopic examination continued until the trypanosomes are no longer motile, which time is noted.

B. Action in vivo. When blood is taken for part A, some is also taken into a pipette, as in making a red cell count. Ringer's solution is used as the diluting fluid. With the counting chamber, determine the number of trypanosomes per cu. mm. of blood. This determination is repeated every 15 minutes after the injection of the drug.

The rat is weighed, and is given an intraperitoneal injection of either:

A. Tryparsamide. 250 mgm. per kg. intraperitoneally.

B. Mapharsen. 5 mgm. per kg. intraperitoneally.

C. "BAL" 25 mgm. per kg. intramuscularly followed in 15 minutes by mapharsen 5 mgm. per kg. intraperitoneally.

D. Potassium antimony tartrate. 5 mgm. per kg. intraperitoneally.

E. Penicillin 40,000 units per kg. intraperitoneally.

References. H. H. Dale, Physiol. Rev. 3: 359, 1923.
C. Voegtlin, Physiol. Rev. 5: 63, 1925.
C. C. Pfeiffer and A. L. Tatum, J. Pharmacol. 53: 358, 1935.

Exp. 10. Irritant. Remove the ointment with alcohol after the observations.

Experiment 10

DRUGS ACTING LOCALLY

1. Rubefacients.

(a) Soak a small piece of mustard plaster in tepid water for several minutes, then apply to the volar surface of the forearm, holding it on with a small strip of adhesive plaster. Remove in fifteen minutes. Note carefully the local sensations and the appearance of the skin.

(b) Soak a very small piece of cotton in chloroform and apply this to the concave surface of a watch glass. Strap this to the volar surface of the forearm with adhesive plaster; do not apply liquid chloroform to the skin. In fifteen minutes remove and note the appearance and sensation.

2. Irritant and "Counterirritant."

Rub the capsicum-turpentine ointment into an area about 3 cm. in diameter on the volar surface of the arm. Observe the development of any painful sensation at this area over a period of thirty minutes. At that time, the subject notes the effect on the intensity of this pain during and after the application to this area for two minutes of

(a) an Erlenmeyer flask containing water at 40-50 degrees Centigrade

(b) an Erlenmeyer flask containing ice-water at 4-10 degrees Contigrade

3. Protein Precipitants.

Put in test tubes 2 cc. of solution of egg albumen and add drop by drop the following agents:

Mercuric chloride	5%	Concentrated sulfuric acid	
Copper sulfate	1%	Sodium hydroxide	20%
Zinc chloride	2%	Liquefied phenol	
Ferric chloride	1%	Alcohol	
Lead acetate	5%	Tannic acid	10%

Record the results. The tubes should be saved for comparison with Part 6.

4. Astringent Action of Silver Compounds.

To egg albumen in test tubes as in Part 3, add:

Strong silver protein	5%
Mild silver protein	5%
Silver nitrate	1%

5. Local Action of Phenol.

Arrange four beakers so that four fingers of one hand can be inserted into them. Put in the beakers:

1. 5% phenol in water
2. 5% phenol in a mixture of alcohol 1/4 water 3/4
3. 5% phenol in a mixture of glycerine 1/4 water 3/4
4. 5% phenol in olive oil

Immerse the fingers for five minutes, then withdraw, examine, and compare. Rinse the finger from the water solution in a large amount of water; does any change occur? Now rinse in alcohol.

6. Corrosive Action.

(a) A stomach tube is passed in a rabbit anesthetized with pentobarbital U.S.P. Each group then administers 10 cc. of one of the agents listed below, and removes the stomach tube, washing it immediately.

Sulfuric acid	10%
Sodium hydroxide	10%
Liquefied phenol and water,	equal parts
Mercuric chloride	5%

Fifteen minutes later, the animals should be killed, the abdomen opened, and the stomach removed, carefully cut open, washed, and examined. The results with the different agents should be closely compared.

(b) Take from the rabbit two pieces of small intestine about one foot long, and slit open lengthwise. Wash and lay on a board mucosal side up. Apply the agents of Section 3 to one inch segments, leaving a space between each. Leave for ten minutes, and wash off and examine.

Experiment 11

GENERAL ANESTHESIA

Handling the dog gently, avoiding excitement, put it on the board and when it has become quiet, observe cardiac and respiratory rates, size of pupil, eye movements, swallowing, corneal and flexion reflexes, knee jerk, tone of abdominal and leg muscles, rectal temperature, color of mucous membranes of mouth and conjunctiva.

Now administer ether, U.S.P. by cone and drop method, slowly and carefully. The struggling seen if one jams the cone on the dog's nose is not the stage of delirium. Avoid such a procedure, and pass through this stage to light surgical anesthesia, making the observations above as often as possible. Insert tracheal and carotid arterial cannulas. Apply a pneumograph on the thorax to record its movement. Connect ether bottle and blood pressure apparatus and record on a slow drum.

Allow the anesthesia to become very light, then while recording, turn the ether on full. Make observations of eye movements, reflexes, and muscle tone continuously, and indicate on the drum the time at which changes occur. Make the remaining observations of Paragraph 1 when the dog is in deep surgical anesthesia. Continue giving ether until respiration stops, then immediately remove the ether and revive the dog with artificial respiration.

Let the dog recover to very light anesthesia, and repeat the observations of the first paragraph. Replace the ether in the bottle with chloroform, U.S.P.; repeat the same procedure as followed with ether, particularly comparing the results with the two agents. When respiration stops, try to revive the dog with artificial respiration. If this fails try cardiac massage through the diaphragm, inserting the hand through an abdominal incision. If this fails, try intracardiac injection of epinephrine hydrochloride, U.S.P., 1 cc. of 1:10:000.

Label the kymograph records from this experiment carefully, noting all procedures and the data obtained.

Experiment 12

CENTRAL NERVOUS FUNCTIONS IN MAN

Modification of the function of the human central nervous system is difficult to study, in a purely objective fashion, and the results are often difficult of interpretation. In this experiment, the four tests that are used are frequently employed in psychometry.

1. Repetition of Digits. This is a simple memory test. For "digits forward," the examiner says, "I am going to say some numbers. Listen carefully, and when I am through, say them right after me. Repeat them as fast or as slow as you like." (Because the ability to remember digits is a function of the time interval between presentations, it is necessary to standardize this time. The optimal time interval between digits has been found to be about 3/4 of a second. A little practice with a watch will enable the tester to carry this out automatically.) If the subject repeats a series correctly, mark plus and continue with the next higher series. If the subject fails, give a second trial on series of equal length. Discontinue after subject has failed on both trials of a given series. Score is the highest number of digits repeated without error on either of two trials. Thus, if the subject repeats correctly 5 digits forward, he gets a score of 5.

"Digits backward." Say, "Now I am going to say some more numbers, but this time when I stop, I want you to say them backwards. For example, if I say 7-1-9, you say (pause) 9-1-7." If subject gives them correctly, say, "That's right." Begin with the three digit series, continuing until subject fails both trials of the series. Score is the highest number of digits correctly reversed.

The Total Score on the test is the sum of the number of digits repeated forward and backward. Thus, if a subject repeats 7 forward and 4 backward, his total score is 11.

2. Digit-Symbol Test. The tester says, "Look at these divided boxes or squares (pointing to key); notice that each has a number on the upper part and a little mark on the lower part. Also that with every number there is a different mark. Now, look here (pointing to sample) where the boxes have only numbers, and the squares underneath are empty. I want you to put in each of these squares (pointing) the little mark that should go there, like this." (The tester points alternately to key and to the digit.) "Here is a (1), so you would put in this mark. This is a (3), therefore you would put in this mark," etc. After doing five of the samples, pause and ask, "Now what should I put in here?" (Indicating the next empty square.) After finishing this demonstration, tester says, "Now when I say time you begin here and fill in as many squares as you can before I call time again."

Scoring. The score is the total number of symbols correctly entered, in 1½ minutes time. Precision and neatness are disregarded, but symbols must be identifiable. Entries must be made successively, not in heterogeneous fashion.

3. Hand Steadiness. The stylus and the plate of the apparatus are connected with a battery and signal magnet so that every contact will be recorded on a smoked drum. According to the normal individual steadiness, a hole is chosen which will give about five contacts per minute, and this is used throughout. The test is run for two one-minute periods with a one-minute wait between. It is scored as the average number of contacts per minute. The contacts made when the stylus is introduced or removed are not to be counted, so the drum should not be started until after the stylus is inserted, and should be stopped before the stylus is removed.

4. Rate of Tapping. The device consists of two plates and a stylus, for the tapping, an atuomatic one-minute timer, and a counter. The timer is reset to starting position by momentarily pushing the button; if the pilot light is lit, the timer is in the starting position. The tapping is done by the subject, tapping alternately on the two plates as rapidly as possible. The first contact starts the timer, which will open the circuit exactly one minute later. Therefore, the subject continues to tap until the pilot light goes on, and the counter ceases to advance. The counter is read to give the number of taps per minute, and the push-button is pushed to reset the apparatus. The test is run for two trials, with a one-minute wait between. The score is the average number of taps per minute.

Performance of Experiment. One member of each group serves as subject, and should omit lunch, to insure rapid absorption of the drug. A control set of observations is made, the subject takes one of the substances listed below, and observations are repeated for four times, at 1/2 hour intervals. The results are recorded in the table provided.

Drugs. 1. Ethyl alcohol, 250 cc. of 25%

2. A suitable dose of a short-acting barbiturate

3. Caffeine alkaloid, 0.3 gm.

4. Benzedrine sulfate, 10 mgm.

5. Substance "X"

Experiment 13

RESPONSES OF THE CENTRAL NERVOUS AND PERIPHERAL NEURO-MUSCULAR SYSTEMS

This experiment demonstrates the augmentation and depression of a spinal reflex, and the production and removal of a block between motor nerve and skeletal muscle.

In a dog anesthetized with barbital, a tracheal cannula is inserted. Additional anesthesia with ether may be required for this and subsequent surgical procedures. On the medial aspect of the right hind foot, the tubercle on the first metatarsal is located, and a button of skin over it is removed with scissors. The tubercle and attached tendon are exposed, and the tubercle is detached from the bone with bone forceps; take care not to damage the attachment of the tendon while doing this. One blade of a scissors is inserted under the skin, and the skin is cut through on a line over the tibialis tendon to a point above the ankle joint. Considerable bleeding may be met at this time, as a result of cutting the large veins on the anterior surface of the foot. These should be clamped and ligated. The tendon of the tibialis is again identified, and the tough ligament across the front of the foot, under which this tendon runs, is cut through so that the distal end of the tibialis muscle is freed. A length of fishing line is tied to the tendon for later connection of the myograph. The bony tubercle on the end of the tendon will prevent this line from slipping off. On the lateral aspect of the leg, a button of skin is removed over the lower end of the fibula, and a small area of the distal end of this bone is exposed. A hole is made a small distance into the bone with a hand awl, then a drill is put transversely through the bones of the leg, so that the base of the drill is close to the leg. The lateral condyle of the femur is located, a button of skin is removed over it, proximal and somewhat posterior to the patella, the bone is exposed, and a drill put transversely through the femoral condyles. On the medial surface of the leg, the plantar (posterior) branch of the saphenous artery is palpated as it runs under the gastrocnemius tendon. An incision is made over it, running about five centimeters proximal from the ankle. The artery and the tibial nerve which runs with it are carefully exposed. With careful blunt dissection the nerve and artery are separated the length of the incision, the nerve is ligated distally and cut, and freed from the artery. In the isolation of this nerve, and the sciatic nerve later on, great care must be taken not to pinch the nerve or pull on it, as these procedures are severely damaging.

A tubular electrode is placed on the nerve, and the skin is closed with a suture over the electrode to hold it in place and to keep the nerve from cooling and drying. The left femoral vein is now exposed in the femoral triangle, so that injections may be made into it.

The animal is brought to a stand, clamped to the table, that has two adjustable clamps to receive the heads of the drills and a torsion wire isometric myograph. The line from the tibialis tendon is connected to the myograph with a turnbuckle, which is adjusted so that the muscle is under slight resting tension. The muscle should have a straight pull, from its origin to the myograph. The wires from the electrode are connected with a stimulator which is controlled through a relay by the timing circuit, so that every four seconds a brief period of stimuli at the rate of sixty per second is applied to the nerve. The stimulator is also connected with a signal magnet, which indicates on the drum the moment of stimulation, and time in four second intervals. The instructor will assist in connecting the apparatus. The drum is run at its slowest speed.

First determine the threshold intensity of stimulus at which the flexion reflex appears. Demonstrate the effect of increasing the intensity of the stimuli, and find the intensity at which the response is maximal. Thereafter use supramaximal stimuli. Administer the following substances, making the injections into the femoral vein with syringe and needle, and waiting until the effect of each is fully developed before giving the next.

1. Metrazol, N.N.R. 10 mgm./Kgm., repeated until an effect is apparent.

2. Pentobarbital sodium, U.S.P. 10 mgm./Kgm.

3. Picrotoxin, U.S.P. 0.5 mgm./Kgm. Its action develops slowly.

4. Paraldehyde, U.S.P. 0.2 cc./Kgm.

Now remove the electrode from the tibial nerve. By palpation on the posterior surface of the thigh, the separation of the medial and lateral groups of muscles is located, and an incision is made through the skin in this line, from the popliteal space about halfway up the thigh. With blunt dissection, the sciatic nerve is exposed as it lies between these groups of muscles. The division of the sciatic nerve into the tibialis and common peroneal nerves is identified. The common peroneal nerve, sweeping laterally around the knee, carries the motor fibers to the tibialis anticus muscle that is being used. This nerve is ligated and cut as close to its origin from the sciatic as possible, and the tubular electrode is slipped onto it. The skin is closed over the electrode with a suture.

Determine the threshold intensity as before, and notice that the tension developed by a maximal contraction from stimulation of the motor nerve is greater than that developed in a maximal reflex response. Use submaximal stimuli for the following.

Before making the following injections, the artificial respiration machine should be prepared, and should be connected as soon as there is any obvious interference with breathing.

Administer by intravenous injection, the following substances:

1. Beta-erythroidine hydrochloride 2 mgm./Kgm. If no effect appears, repeat.

2. Neostigmine methylsulfate, U.S.P. 0.1 mgm./Kgm.

3. Dihydro-beta-erythroidine 0.1 mgm./Kgm. If no effect appears, repeat.

4. Potassium chloride, U.S.P. 15 mgm./Kgm., injected in 15 seconds. If no effect appears, repeat, while palpating the cardiac impulse.

Experiment 14

LOCAL ANESTHESIA

This experiment demonstrates three clinical applications of local anesthesia - on a mucous membrane, by subcutaneous infiltration, and by subarachnoid injection.

Corneal Anesthesia. In a normal rabbit, test the corneal and lid reflexes, and observe the size of the pupils and the appearance of the conjunctival blood vessels. Holding the rabbit firmly to prevent jerking away, instill the solutions into the sac formed by gently pulling the lower lid away from the eye. Drop the solution slowly into this sac for one minute, then squeeze out the solution and test the corneal and lid reflexes every minute, until they appear to be normal again. The local anesthesia is estimated to be light or deep, depending on the degree of pressure on the cornea required to elicit the reflex. Observe and record any changes in size of pupil or appearance of conjunctival vessels.

Right eye	cocaine hydrochloride USP	1%
Left eye	procaine hydrochloride USP	1%

Infiltration Anesthesia. In the rabbit, test the lid reflexes of both eyes. Take up 0.75 cc. of the desired solution in a syringe, and insert the needle subcutaneously halfway between the root of the ear and the external canthus of the eye. Inject 0.25 cc. Withdraw the needle slightly and then infiltrate the area both above and below the external canthus of the eye so as to anesthetize a crescent-shaped area, 0.25cc. of the solution being injected above and 0.25cc. below. The infiltrating solution should make the palpebral conjunctive bulge slightly. Determine onset and duration of anesthesia by testing the lid reflex. Use 1% procaine hydrochloride in one eye, 1% procaine hydrochloride in 1:50,000 epinephrine hydrochloride in the other.

Intradermal Anesthesia. Sterilize a syringe and needle by boiling. Wipe with alcohol the skin of the flexor surface of the forearm and the top of the bottle of sterile 0.25% procaine hydrochloride. Make an intradermal injection (raising a wheal) and determine the time of onset, duration, and extent of anesthesia.

Spinal Anesthesia. After the rabbit has recovered from the infiltration anesthesia, it is placed in the canvas hammock supplied, and the spinal length is measured, from the occiput with the head flexed, to the first and most prominent sacral spinous process. Into a 1 cc. tuberculin syringe with a 22 gauge needle, take 1% procaine hydrochloride USP, in the amount of 0.02 cc. per centimeter of spinal length. The assistant now takes the four legs of the rabbit in one hand, and holds the rabbit's head with the other hand. The operator stands with the rabbit's head at his left, and places his left forearm on the animal's back. The thumb and middle finger of the left hand are placed on the iliac crests, and the index finger is placed on the spinous process of the last lumbar vertebra, which is almost

in line between the crests. Now, by moving the index finger backward over the animal, the first sacral spinous process is found. Between these two spinous processes lies the space at the lumbosacral union.

The filled syringe is held in the right hand, and the needle placed just to one side of the cephalad tip of the first sacral process with its bevel pointing downward. During the time of its insertion and the injection of the fluid it is held rigidly at an angle of about 45 degrees to the axis of the spinal column, so that the needle will enter the subarachnoidal space pointing slightly toward the head. Because the first sacral spinous process is curved cephalad, the needle is inserted off the midline, with the intent of having it reach the midline when it pierces the dura.

The needle traverses the following tissues: the skin which is frequently tough, a layer of muscle which is soft, the ligamentum flava which is dense and resistant, and lastly, the dura and arachnoid, which are rather soft. If the needle is carried too far, it may touch the bony wall ventral to the cord and may enter a venous channel. This latter occurrence is to be avoided; it can be demonstrated by aspirating with the syringe to see if blood is obtained. With the needle properly in place, the needle and syringe are held firmly, and the solution is injected slowly during thirty seconds.

The rabbit is removed immediately from the hammock and placed on the table. The presence of any motor weakness or paralysis is observed. Reflexes of the hind legs are tested for. Anesthesia is tested for by pinching the skin. The extent of the anesthesia over the body is determined. The duration of the changes are noted, and the time at which the animal returns completely to normal. Intercostal paralysis is determined by observation of thoracic movements during respiration.

References. A. D. Hirschfelder and R. N. Bieter. Physiol. Rev. 12: 190, 1932.
R. N. Bieter. Am. J. Surg. 34: 500, 1936.
R. N. Bieter et al. J. Pharmacol. 57: 221, 1936.

Experiment 15

ANALGESICS

Three members of each group serve as subjects, one member makes the determinations. With the subject lying on his back with eyes closed, two spots on the mucocutaneous junction of the upper lip are marked with ink. The threshold for painful stimulation of these spots is determined with the algesimeter, the use of which will be explained by the instructor. Each subject then takes by mouth, with water, a substance expected to have analgesic action. These will be identified only by code letter, and their composition will be revealed after the observations are completed. The determination of threshold is repeated ninety minutes after the ingestion of the drugs.

Seevers, M. H. and C. C. Pfeiffer. J. Pharmacol. 56: 166, 1936.
Wolf, H. G., J. D. Hardy and H. Goodell. J. Clin. Invest. 19: 659, 1940, and 20: 63, 1941. Harvey Lectures, 39: 39, 1944.
Pain, Assoc. for res. in nervous and mental diseases, 23: 1943.

EFFECT OF DRUGS ON THE NASAL MUCOSA

The apparatus measures the resistance to flow of air through the nasal passages. The flow of air should be adjusted first so that the flowmeter reads at the point indicated, which is about 7 liters per minute. This adjustment should be checked frequently. Then the fluid reservoir of the draft gauge is adjusted so that the level on the scale is at zero. The subject then inserts the glass tip into one nostril, watching the procedure in the mirror and attempting to insert it in the same way for each observation. The subject then holds his breath and closes the side arm on the nasal tube, so that the air will now flow in one nostril, through the nasal passages, and out the other nostril. At this time, the pressure reading on the draft gauge is observed by another member of the group. This constitutes the determination of nasal resistance to flow, an index of the dimensions of the airway. The observation is repeated fifteen minutes after the application of the drug, using the same nostril as before.

The student not ingesting a drug for the first part of the experiment serves as subject here. The substances used are (1) Benzedrine, two inhalations through each nostril, (2) menthol, from an inhaler as with (1), (3) ephedrine sulfate, 3% solution, dropped into the nose with the head turned laterally while bending over.

Butler, D. S. and A. C. Ivy. Arch. Otolaryngology 38: 309, 1943.

Exp. 16. The dose of ouabain should be 0.5 mgm. per dog.

Experiment 16

DIGITALIS IN CARDIAC DISEASE

In a normal animal, the demonstration of the action of digitalis which makes it so useful in cardiac disease is difficult. Professor Hirschfelder designed this experiment, which produces cardiac disorder which can be modified by digitalis. This is auricular fibrillation, which is produced first in the normal dog, then against a background of the therapeutic action of digitalis.

In a dog anesthetized with barbital, insert tracheal, carotid arterial, and femoral venous cannulas. Arrange for artificial respiration, connect apparatus for arterial pressure, and burette device for intravenous injections. Make an incision through the skin and down to the sternum along its entire length, in the midline. Now saw through the middle of the sternum, applying artificial respiration as soon as the pleural space is opened. Tie the chest open and excise carefully the pericardium to allow access to the right auricle, and a clear view of the ventricles. Keep the heart moist with warm saline. Apply the special electrodes to the right auricle, arranging them not to drag on it, and connect them with the secondary of an induction coil set for tetanizing stimulation. Now call the instructor who will make sure that the stimuli are not so intense as to throw the heart into complete terminal fibrillation.

Throughout the experiment observe the heart and relate its changes with those seen on the drum; use a drum speed rapid enough to show the pulse waves.

Stimulate briefly to produce a short period of auricular fibrillation. Observe the heart and the record of arterial pressure. What are the results of auricular fibrillation?

Now inject intravenously 0.2 mgm./Kgm. of ouabain (g-Strophanthin) N.N.R., or other cardiac glycoside suitable for intravenous administration. Repeat the auricular stimulation 10 minutes later, and every 10 minutes thereafter, observing the changes in the response of the heart and in the arterial pressure, until the effects of auricular fibrillation on the ventricles and arterial pressure are abolished. Do the auricles still fibrillate as before? This should not be prevented by digitalis; if the fibrillation ceased to occur, this is probably due to local changes at the electrodes. Digitalis does not stop auricular fibrillation, but it prevents the deleterious effects of such fibrillation on ventricular function.

After the therapeutic action has been observed, either (a) continue to observe the progressive action of the drug, or (b) when a definite irregularity of the beat occurs with stimulation, begin the repeated intravenous injection of 5 mgm./Kgm. doses of quinidine sulfate, U.S.P. After each dose, observe the nature of the heart beat, and redetermine the threshold stimulus for auricular fibrillation.

Digitalis. A. R. Cushny. J. Pharmacol. 11: 103, 1918.

Digitalis and Its Allies. A. R. Cushny, London, 1925.

Use of Digitalis. G. C. Robinson. Medicine 1: 1, 1922.

Action of Digitalis, in Failure of the Circulation. T. R. Harrison, Chap. 28, Baltimore, 1935.

Exp. 17, p. 2. The dose of acetyl-choline bromide should be 0.01 mgm., instead of 0.1 mgm. Nitroglycerine should be injected after the acetyl-choline, instead of after the posterior pituitary.

Experiment 17

ACTION OF DRUGS ON THE HEART

4 Students

This experiment permits observation of the action of drugs on the heart beat and also on the coronary circulation. The apparatus consists of a vertical glass tube into which Ringer solution with glucose is run at a constant rate. The bottom of the tube leads through a warming bath at 38-40° to a cannula which is tied into the aorta. The pressure of the column of fluid closes the aortic valve, and the fluid enters the coronary arteries and escapes from the right auricle. The resistance to flow in the coronary circulation determines the height of the column of fluid, which is recorded by connecting a volume recorder to the top of the vertical tube. The chambers of the heart are empty, and it does not function as a pump, although beating.

The apparatus is assembled, the water bath is heated, the system is filled with Ringer, and the Mariotte tube is inserted in the reservoir. The removal of the heart is not difficult, but speed is essential. A young rabbit is killed with a blow on the head and placed back down on the table. The skin is reflected from the front of the thorax, and with strong scissors all the ribs are cut through on either side and the diaphragm cut through, so that the entire front of the thorax is turned over the head. The pericardium is excised and the aorta quickly located. It is cut through just where it first branches; it is desirable to have as long a segment as possible, to avoid pushing the cannula through the aortic valve. The heart is then lifted up and the remaining vessels cut through, not too close to the heart. It is then placed in a dish of Ringer and gently massaged a few seconds to empty it of blood, so that clots will not form in the aorta. By this time another member of the group should have made certain that the bath is at the proper temperature, that there are no air bubbles in the perfusing system; he should open the screw clamp on the tubing above the cannula so that Ringer is slowly dropping from it, and should place a loose ligature, with half a square knot, around the cannula. The operator now lifts the heart in his left hand, and bringing it up to the cannula, carries a tissue forceps in his right hand through the ligature, picks up the and of the aorta, and leads it onto the cannula, where it is securely tied. Precautions must be taken that no air bubble enters the aorta. The screw clamp is now removed and immediately a cut is made into the left ventricle to allow escape of the small amount of fluid which leaks past the aortic valve. A thread is stitched to the tip of the heart and tied to a heart lever, and the amount of air in the volume recorder is adjusted. The heart should now be beating. The escaping fluid is caught in a pan or funnel and led away.

The following drugs are injected with a tuberculin syringe and needle into the rubber tubing above the cannula.

1. Epinephrine HCl, U.S.P. 0.00025 mgm.
2. Acetyl-choline bromide, 0.1 mgm.
3. Solution of posterior pituitary, U.S.P., 1/20 unit.
4. Nitroglycerin, U.S.P., 0.05 mgm.
5. Theophylline ethylene diamine, U.S.P., 1.0 mgm.
6. Add to the reservoir through the Mariotte tube, 2.0 cc. of Tincture of Digitalis, U.S.P.

Experiment 18

EFFECT OF DRUGS ON THE PUPIL (Rabbit)

4 Students

Follow the changes in size of pupil, measuring with dividers the size in millimeters, and the reaction to light, with the administration of the following drugs. Be sure to measure the same diameter of the pupil each time. The transverse diameter is preferable. Have the conditions of illumination the same each time the size of the pupil is observed. The drugs should be dropped into the sac made by pulling the lower lid gently away from the eye. This should be just filled with the drug (about 10 drops) and held for one minute. Both eyes should be observed for about ten minutes, then the drugs are applied and the observations made every five minutes.

1. Use in one eye, cocaine hydrochloride, U.S.P., 5%, and in the other, epedrine sulfate, U.S.P., 10%.

2. Sixty minutes later, put in one eye, physostigmine salicylate, U.S.P., 0.2%. When the effect is maximal (10-15 min.), put atropine sulfate, U.S.P., 1%, in the same eye. Physostigmine poisoning may occur unless the lacrimal duct is compressed.

3. In the other eye, use pilocarpine nitrate, U.S.P., 1%, when the effect is maximal, apply to the same eye, homatropine hydrobromide, U.S.P., 1%.

Present the results in graphic form, and explain the mechanisms.

The action of pilocarpine nitrate, U. S. P. and atropine sulfate, U. S. P. on the secretion of sweat and saliva.

The apparatus consists of a chamber in which the finger is inserted. Air which has been dried by passage through concentrated sulfuric acid and through a tube immersed in a solution of solid carbon dioxide in alcohol is passed through this chamber and picks up water from the skin. The air then passes through a second tube immersed in the above-mentioned freezing mixture, in which the water is held as ice. The tube is weighed before and after, to give the weight of water evaporated from the skin.

The determination of sweat-secretion is made for ten minutes. For five minutes during this period, the subject chews paraffin and expectorates the saliva as it is secreted into a graduated cylinder. The volume is measured.

After the control observation, each subject takes by mouth with water, either 1 mgm. of atropine sulfate or 10 mgm. of pilocarpine nitrate. The determinations are repeated at thirty minutes after the pilocarpine, and two hours after the atropine.

Record any objective or subjective changes that occur in the subject, such as a feeling of warmth, flushing, dryness of the mouth.

Experiment 19

EFFECTS OF DRUGS ON RESPIRATION

4 Students

The apparatus provided separates expired air from inspired air, and measures it in terms of water displaced. The box is filled with tap water, through the short tube on the top, while the outlet tube on the side is kept closed with the finger. When the box is full, the short tube is stoppered and on opening the outlet only a small amount of water will run out. The long tube on top is then connected with the inspiratory valve.

A rabbit is weighed and put on a board. Try to avoid excitement and struggling on the part of the rabbit. If it is constantly attended, it should lie quietly on the board and continue to breathe from the apparatus throughout the experiment. The mask is put on the rabbit and made tight around its nose. An observation is made by closing the side arm on the mask with the finger for exactly one minute. The water displaced is collected and measured; this is the minute volume. The number of respirations during this minute are counted, and from these figures the tidal air is calculated. Enough observations should be made to establish a steady state, and the box should be refilled with water after not more than three or four observations.

The response to carbon dioxide is now determined. The student's alveolar air is used for this purpose. This is collected in a six-liter bag as follows. The stopper is removed from the large end of the bag. The student takes a maximal inspiration, then makes a maximal expiration into the bag. The contents of the bag are now rebreathed for five breaths, with each inspiration emptying the bag as near as possible. The stopper is then replaced. This supplies gas that is more or less in equilibrium with venous blood. This is supplied to the rabbit by connecting the rubber tube from the bag with the entrance to the inspiratory valve. A determination for one minute is made as before, the bag is disconnected, and after the rabbit has respired atmospheric air for a few minutes, the determination is repeated to show return to normal. The collection of this alveolar air should be done immediately before it is to be used, and it must be repeated for the determination after the morphine.

The rabbit is now given a subcutaneous injection of morphine sulfate U.S.P., 2 mgm. per Kgm. The determination is repeated at fifteen minute intervals. After the third, at forty-five minutes after the morphine, the response to CO_2 is determined as before. After another determination with the animal breathing room air, an intravenous injection of caffeine U.S.P., metrazol N.N.R, or niketHamide N.N.R., 10 mgm. per Kgm. is made, and the respiratory volume measured immediately and five minutes later. If no effect is observed, the injection and observations are repeated.

Experiment 20

REACTIONS OF THE BRONCHIOLES

In this experiment the "bronchoconstrictor" and "bronchodilator" action of substances is measured by observing the resistance which the air passages of the lungs offer to the passage of a saline solution through them. The experiment shows the local nature of the phenomenon of anaphylaxis, and also the production of desensitization to the sensitizing substance.

The apparatus used for perfusing the isolated heart is used here for perfusing the lung. The apparatus is arranged according to the example, and is filled with warm Ringer's solution made especially for this experiment. Care is taken to exclude all air bubbles, and screw clamps are applied to the two rubber tubes with which the cannulas will be connected. A burette with rubber tubing attached, and a large syringe and needle are also filled with the Ringer's solution.

A guinea pig, that has received previously a sensitizing injection of egg-white, is killed by a blow on the head, The anterior chest wall is rapidly removed, with care to avoid injuring the lungs. The heart is exposed, the pericardium removed, and an incision made with scissors into the right ventricle on its anterior surface near the base and near the root of the pulmonary artery. The left auricle and left ventricle are also opened with small scissors cuts. A ligature is passed around the pulmonary artery, and the special cannula, which has been filled with Ringer's solution with the syringe, is inserted into the pulmonary artery through the incision in the right ventricle, and tied there. The cannula is refilled with the syringe, the burette is connected with the cannula, and about 10 cc. of solution are run through the pulmonary circulation to wash out the blood. From now on, no particular speed is necessary. The trachea is exposed high in the neck and cannulated with a small straight glass cannula. The trachea, lungs, and heart, with cannulas, are now <u>very carefully</u> dissected free, and carried to the apparatus. The cannulas are again filled with Ringer's solution with the syringe, and are connected with the apparatus; <u>take great care</u> that air is excluded from the system.

The screw clamp on the tube to the pulmonary arterial cannula is now opened, and the escaping saline solution caught in a pan. This clamp is adjusted so that the pulmonary circulation is perfused at a rate of about 40 cc. per minute. With a fine needle, about twenty punctures are made lightly into the surface of the lung, and the screw clamp on the tube leading to the vertical glass tube is adjusted so that saline solution runs through the air passages at a pressure of about 40 centimeters. The volume recorder is adjusted so that it has a small amount of air in it and writes near the bottom of the drum.

The following injections are made with syringe and needle into the rubber tubing above the cannula in the pulmonary artery.

1. Acetyl choline bromide, 0.02 mgm. (0.1 cc. of 0.02%).
   When the pressure in the tracheal system has risen, inject
2. Atropine sulfate, U.S.P., 0.02 mgm., (0.1 cc. of 0.02%),
   When the pressure has become constant, inject
3. Egg-white, 2.0 mgm. (0.1 cc. of 2%)
   When the pressure has risen appreciably, inject
4. Epinephrine hydrochloride, U.S.P., 0.2 mgm. (0.2 cc. of 0.1%).
   When the pressure has become constant, inject
5. Egg-white, 2.0 mgm.
   Follow by
6. Histamine phosphate, U.S.P., 0.1 mgm. (0.1 cc. of 0.1%),
   When the pressure has risen appreciably, inject
7. Atropine sulfate, U.S.P., 0.2 mgm. (1 cc. of 0.02%).
   Follow shortly by
8. Epinephrine hydrochloride, U.S.P., 0.2 mgm.

References:

Bronchioles. Thornton, J. W. Quart. J. Exper. Physiol. 21: 305, 1932.

Dixon, W. E. et al. J. Physiol. 29: 97, 1903; 45: 413, 1914.

Jackson, D. E. J. Pharmacol. 4: 291, 1913; 5: 470, 1914.

Histamine. Best, C. H. et al. Physiol. Rev. 11: 371, 1931.

Anaphylaxis. Dragstedt, C. A. Physiol. Rev. 21: 563, 1941.

Exp. 21, p. 1, II, A. Add solution #4, 1% para-aminophenol hydrochloride.

Experiment 21

BLOOD CELLS AND HEMOGLOBIN

4 Students

The important therapeutic measures in man concerned with blood cells and hemoglobin unfortunately are not suitably demonstrated in short time experiments, so that this experiment dwells more largely on toxic influences.

I. The blood cells. Earlier experiments, which should be reviewed (Outline for Physiology, Exp. 1), showed various means of hemolysis in vitro. These seldom operate similarly in vivo, but there are certain agents which diminish the number of circulating erythrocytes, some of them having some vogue as actual therapeutic agents. Phenylhydrazine has been used in human polycythemia. Benzene affects both erythrocytes and leucocytes, and has been used in leukemia. In the past, when benzene had a wide technical use as a cleaner, it was of considerable toxicological interest. Nucleoproteins have been claimed to be stimulant to leucocyte formation. Their value is inadequately demonstrated, but one preparation has been used recently for agranulocytosis. The actions of these substances are not readily shown in acute experiments, so a consultation of the literature is recommended.

II. Hemoglobin. The forms in which this pigment may exist should be recalled from earlier experiments (Outline for Physiological Chemistry, pp. 31-33). Each group should demonstrate to itself the appearance of oxyhemoglobin and reduced hemoglobin, using whole blood and the dilute solution of hemoglobin as provided. Stoke's reagent is used as in Physiological Chemistry.

A. Methemoglobin. Formation of methemoglobin is blamed for the toxic action of several substances, but some claims are made that sulfhemoglobin, a less well characterized material, is implicated. The first experiment shows the formation of methemoglobin in vitro.

To about 5 cc. of dilute hemoglobin, add about 2 cc, of the following, and observe the change of color indicating formation of methemoglobin. Note especially differences in rate of change. If no change occurs, immerse the tubes in water at 40° C. and continue observation.

1. Saturated potassium chlorate
2. Saturated aniline
3. 10% sodium nitrite

To a tube showing methemoglobin, add some Stoke's reagent and compare with the effects of the same treatment on oxyhemoglobin.

Formation of methemoglobin in vivo is more difficult to observe. Each group takes one rat and does one of the following:

1. Sodium nitrite, 150 mgm./kg., is injected intraperitoneally. Observe the rat, noting appearance of cyanosis and signs of anoxia, and at death, expose the heart, take blood with a syringe and needle, dilute properly with water, and examine grossly and with the spectroscope.

2. Potassium chlorate, 1 gm./kg., is injected intraperitoneally. Observe, and at death, or at the end of the period, take heart blood and examine as above.

3. Aniline. Apply a few cubic centimeters to the skin of the back. Observe until death occurs, then examine the blood. Absorption of aniline through the skin is rapid; it and nitrobenzene have been responsible for numerous cases of "shoe-dye poisoning."

B. Carbon monoxide hemoglobin, carbonyl hemoglobin, HbCO. The interesting and important aspects of carbon monoxide poisoning are too numerous to mention here, but the phenomena we can readily demonstrate.

1. Characteristics of Carbon Monoxide Hemoglobin. The distinctive color of this substance is most helpful in detection of its presence. Add a few drops of blood to each of two test tubes containing water, and pass illuminating gas through one of them. Observe the change in color, and when it seems to be complete, examine both tubes spectroscopically. The two spectra are difficult to identify as different, but now add a little Stoke's reagent to both tubes and reexamine. The stability of carbonyl hemoglobin is another distinguishing feature. This is the basis for the colorimetric method used in Part 2.

2. Carbon Monoxide Poisoning. Each group does one of the following:

a. Put a rat in the gassing chamber. Observe the results closely, and when death has surely occurred, remove. Observe the characteristic color, seen especially around the lips. Take blood from the heart, determine the percentage saturation of hemoglobin with carbon monoxide as described below, and examine the blood spectroscopically.

b. Put a rat in the chamber, observe, and when severely intoxicated, near death, remove. Observe the color. Leave in open air, and time carefully the appearance of varying stages of recovery. When the rat has recovered to what would be considered a mild degree of intoxication, kill it, take heart blood, determine the percentage saturation of the hemoglobin with carbon monoxide, and examine spectroscopically.

Operation of the Gassing Chambers. For the CO treatment, set the air flowmeter (carefully) to 1000 cc. per minute, the gas flowmeter to 142 cc. per minute. This will give a concentration of 0.5% of carbon monoxide, as Louisville gas contains 5% CO. Since this mixture will contain 18.4% oxygen, there will be nocchance of external anoxia. The percentage saturation of hemoglobin with CO at this concentration should be calculated from the formula:

$$\% \text{ HbCO} = \frac{\% \text{ CO} \times 300}{\% \text{ O}_2 + (\% \text{ CO} \times 300)} \times 100$$

This expresses the situation at equilibrium, which probably will not be reached in this actual experiment.

Determination of Percentage Saturation of Hemoglobin with Carbon Monoxide. A sample of 0.1 cc. of blood is taken and diluted to 2.0 cc. with water. To the diluted blood add approximately 40 mgm. of the pyrogallol-tannic acid mixture. Mix by inverting the tube twice and allow to stand for thirty minutes. Compare with the standards, and arrive at the value by interpolation. The tubes are viewed with reflected light, and carefully. Care must be taken in this method that the dilution of the blood and addition of the reagent are rapid to prevent escape of carbon monoxide from the blood.

Industrial Poisons in the U.S. A. Hamilton, N.Y. 1925.
Noxious Gases. Y. Henderson and H. W. Haggard, N.Y. 1927.
Determination of HbCO in Blood. Quantitative Clinical Chemistry. J. P. Peters and D. D. Van Slyke, vol. 2, p. 674. Balt. 1932.

Exp. 22. It is necessary to allow the cadmium sulfate-blood mixture to stand several minutes before adding the NaOH. The filtrate must be colorless.

Experiment 22

CARBOHYDRATE METABOLISM

In the rabbits, blood is obtained for the determination by cutting into (not through) the marginal ear vein, and allowing the blood to drop into a small crucible containing a pinch of oxalate, stirring it thoroughly. The rabbits should not be excited, as this readily alters the blood sugar level. The blood should flow freely, but cotton should be applied to the ear with pressure as soon as enough blood is obtained. The determination of blood sugar is described below. Each group does one of the following procedures.

(1) Epinephrine. After obtaining the control sample, inject subcutaneously, epinephrine hydrochloride, U.S.P., 0.1 mgm. per kgm. body weight. Determine the blood sugar level one hour later.

(2) Insulin. After obtaining the control sample, inject intravenously insulin, 1 unit per kgm., into the ear vein of the opposite ear to the one from which blood is drawn. Determine the blood sugar level one hour later.

(3) Ether Anesthesia. After obtaining the control sample, induce ether anesthesia with a small mask, and maintain in second plane surgical anesthesia for thirty minutes, at which time another sample of blood is taken for determination of the blood sugar level. Ether anesthesia in the rabbit requires the closest attention.

(4) Pentothal Anesthesia. After obtaining the control sample, inject in the vein of the opposite ear Pentothal, 20 mgm. per kgm. This should be enough to produce surgical anesthesia for about twenty minutes. More may be required to produce anesthesia of sufficient depth, and to prolong the anesthesia for thirty minutes, at which time a second sample of blood for sugar determination is taken.

(5) Epinephrine after Liver Damage. The procedure here is exactly the same as in (1), except that the animal has received, twenty-four hours previously, 4 cc. per kgm. of carbon tetrachloride by stomach tube.

Determination of Blood Sugar. Pipette into a test tube 8 cc. of cadmium sulfate reagent. Pipette into this 1 cc. of blood, mix and let stand until it turns brown. Then pipette in 1 cc. of 1.1 N NaOH Stopper and mix by shaking until the precipitate appears to separate from the fluid. Filter through paper into a clean dry test tube.

Take three sugar tubes marked at 25 cc. Pipette 2 cc. of the blood filtrates into two of these, 2 cc. containing 0.2 mgm. dextrose into the third. Add to each tube 2 cc. of the copper reagent, mix by lateral shaking, and put the tubes in a bath of actively boiling

water. Six minutes later, remove the tubes and cool them by immersion in cold water. Add to each tube 2 cc. of the phosphomolybdic acid reagent, and mix, making sure that the precipitate dissolves. After one or two minutes, dilute the solutions to the mark with distilled water. They should be compared photometrically within ten minutes.

Calculation. Mgm. dextrose per 100 cc. blood = 500 x mgm. in sample.

C. F. and G. Cori. Physiol. Rev. 11: 143, 1931.
S. Soskin. Physiol. Rev. 21: 140, 1941.
W. C. Stadie. Harvey Lectures, 37: 129, 1942.

Experiment 23

OXYGEN CONSUMPTION IN THE DOG

The students will find the spirometer and accessory apparatus set up. HANDLE WITH CARE. The time marker and stationary writing point have been adjusted so that the lever traversing the space between them marks 500 cc.

A dog anesthetized with barbital is provided; its weight should be known. Insert tracheal, carotid arterial, and femoral venous cannulas. Connect the burette for intravenous injection, and put some physiological saline in it, allowing some to run in from time to time to prevent formation of a clot. Connect the blood pressure apparatus, arranging it so that the upper writing point is at some convenient level, indicating, say, 50 mm. of mercury. Have kymograph at its slowest speed. Connect two short rubber tubes with the large outlet of the spirometer, and connect one of these with the tracheal cannula. The small spirometer tube is connected with the oxygen cylinder, and the spirometer is washed out with oxygen. Handle the oxygen cylinder with care; do not explode the spirometer. Fill the spirometer with oxygen so that the lever is below the base line (the time marker); clamp with a large hemostat the tube to the atmosphere, so that the dog breathes from the spirometer; clamp the small tube to the oxygen cylinder. Now start the drum.

The spirometer contains soda lime which absorbs excreted carbon dioxide. Thus the individual excursions of the lever indicate the tidal air, and the progressive rise in the lever indicates oxygen consumption. This is timed between the passage of the lever at its downstroke over the horizontal lines. When the upper line has been passed, stop the drum and refill the spirometer so that the lever is below the bottom line. This constitutes the normal reading.

Repeat the observation of oxygen consumption during the continuous intravenous injection from the burette of 0.02% sodium cyanide. Inject at the maximum rate that causes stimulation of respiration but stops short of depressing the respiration. This will be about 4-6 cc. per minute, but close observation and adjustment are necessary.

After this observation, inject intravenously methylene blue, 10 mgm./kg., wait ten minutes, refilling the spirometer as necessary, then take another reading.

While taking a reading, inject sodium cyanide at the same rate in cubic centimeters per minute as before.

Inject very slowly intravenously sodium dinitrophenol, 10 mgm./kg., wait ten minutes, and take another reading.

When taking a reading, inject intravenously very slowly a solution of sodium arsenite, 1 cc. = 5 mgm., until the blood pressure has fallen about 50 mm. and continue the recording.

Exp. 24. Omit the dinitrophenol. Alternate groups will give either vaccine alone, or vaccine and acetylsalicylic acid.

Experiment 24

THE PRODUCTION AND RELIEF OF HYPERPYREXIA

4 Students

A rise in the internal temperature of the body is the result of the production of more heat than is lost. In the fever produced by typhoid vaccine, the low skin temperature suggests that heat loss is reduced. Since the fall of internal temperature following the administration of acetyl-salicylic acid to such a fevered individual is accompanied by a rise in skin temperature, it seems that this antipyretic action is a result of increased heat loss, rather than reduction in heat production. In this experiment, the action of acetylsalicylic acid is examined in two conditions of hyperpyrexia; one in which heat loss is decreased, one in which it is increased.

Rectal temperature is measured in the rabbit by inserting a vaselined clinical thermometer into the rectum for 4-5 centimeters and leaving it there until the mercury has stopped rising. Skin temperature is measured with a clinical thermometer to which a segment of cork has been attached. A spot on the external surface of the rabbit's ear, towards the margin along which the large vein runs, but itself comparatively free from large blood vessels, is marked with ink. The thermometer is applied to this spot, the cork and the internal surface of the ear being held between two fingers. The thermometer is held there until the reading is constant for thirty seconds; this may take several minutes. Throughout this experiment it is of the utmost importance to avoid exciting the rabbits, as this will result in struggling and rise in temperature above normal.

In two rabbits, rectal temperature and skin temperature are measured at fifteen minute intervals throughout the experiment. After two sets of normal, control observations are made, the rabbits receive (a) typhoid vaccine, U.S.P., 0.1 cc./kgm. intravenously, into the marginal ear vein on the ear not used for temperature readings, or (b) sodium dinitrophenol, 20 mgm./kgm. subcutaneously. After the temperatures have been measured fifteen minutes after these administartions, each rabbit receives by stomach tube 0.2 gm./kgm. acetyl-salicylic acid freshly made into solution with sodium bicarbonate. Temperatures are measured at fifteen minute intervals until they have returned to normal.

References:

Temperature, Its Measurement and Control. Chap. 5, 6. New York, 1941.
Basal Metabolism in Health and Disease. E.F. DuBois. Phila., 1936.
The Mechanism of Heat Loss and Temperature Regulation. Lane Medical Lectures, 1937. E.F.DuBois.
H.G. Barbour. Physiol. Rev. 1: 295, 1921.
H.C. Bazett. Physiol. Rev. 7: 531, 1927.
T. Deighton. Physiol. Rev. 13: 427, 1933.

 A syphon tube is supplied with the apparatus to permit changing the solution. The first six solutions are added on top of each other without changing the contents of the bath.

Experiment 25

ISOLATED INTESTINAL STRIP

Segments of the intestine of the rabbit when removed and suspended in a suitable medium do not function identically as in situ, but they show characteristic rhythmical activity and respond vigorously to drugs. The student should refer to his experiments according to the Outline for Physiology, pp. 64-65, and compare.

The necessary apparatus must be set up first. This consists of a 100 cc. beaker supported by a ring in a large beaker which serves as a water bath, with its temperature kept at 35° C. The inner bath contains freshly made Locke's solution. A glass hook for attaching the strip and for bubbling air through the solution is in the inner beaker. The air should bubble at a slow constant rate. The lever is adjusted so that the strip is stretched slightly, and to give a record of the rhythmic contractions of the longitudinal muscle of an amplitude of one-half inch or more. The kymograph should move slowly. When all the apparatus is ready, each group secures, with the assistance of the instructor, a segment of duodenum, jejeunem, ileum, or colon, about 3 cm. long and ligated at both ends. The strip is set up in the bath, and preferably left for about 30 minutes to permit motility to develop. The following drugs are then applied:

		Final Concentration in the Bath
Epinephrine HCl, U.S.P.	0.1 cc. of 1:100,000	1:100,000,000
" " "	1.0 cc. " "	1:10,000,000
Acetyl choline bromide	0.1 cc. " "	1:100,000,000
" " "	1.0 cc. " "	1:10,000,000
Atropine sulfate, U.S.P.	0.1 cc. " "	1:100,000,000
Acetyl choline bromide	10 cc. " "	1:1,000,000

Now replace the contents of the inner beaker with fresh Locke's solution, wait until the correct temperature is reached and motility appears normal, and add to the inner beaker:

Morphine sulfate, U.S.P., 2%	2 cc.
Papaverine hydrochloride, 0.05%	2 cc.

Experiment 26

DETERMINATION OF RENAL FUNCTION. DIURESIS.

In a barbitalized male dog, insert tracheal, carotid arterial, and external jugular venous cannulas, as described in Exp. 4. The venous cannula is connected with a 100 cc. burette, in which has been placed 5 cc. per kgm. body weight of a solution of sodium ferrocyanide 5%, sodium aminohippurate 0.5%. This constitutes a "priming" dose of these substances, to develop an adequate blood concentration. This is injected in about one minute. The urinary bladder is now catheterized with assistance from the instructor.

Renal Clearance. The data needed for this determination include the volume of urine formed in a certain period of time, and the concentration of a substance in this urine and in the blood plasma. The procedure is as follows. A solution containing 1% sodium ferrocyanide and 0.02% sodium aminohippurate and other substances whose effect on renal function is to be studied is injected intravenously at a constant rate of 1 cc. per kgm. per minute throughout the clearance period. After this injection has been started, the urinary bladder is completely emptied of urine with a syringe, and washed out with 10 cc. of 0.9% NaCl on top of which is added 10 cc. of air. The air assists in determining when the bladder is emptied. This washing is repeated once. The catheter tip is then placed in a small flask to collect the urine, and the time is noted. Exactly 5 minutes after the start of urine collection, about 10 cc. of blood are taken directly from the arterial cannula into a calibrated 15 cc. centrifuge tube containing dried oxalate to prevent clotting. The cannula is then rinsed with saline solution and dried with a cotton swab. The blood is mixed with the oxalate thoroughly by gentle inversion of the tube. The period of urine collection is 10 minutes, so that, about one minute before this time, the urinary bladder is emptied and washed twice, as before. The second washing is timed so that it is withdrawn from the bladder exactly at the end of the 10 minutes. The washings are added to the urine in the flask. This constitutes one clearance period. Each group does two clearance periods, as listed below.

All of the following solutions contain 1% sodium ferrocyanide and 0.02% sodium aminohippurate, which will maintain constant blood concentrations.

A. First period: 0.9% sodium chloride
   Second period: 1.89% sodium sulfate

B. First period: 0.9% sodium chloride
   Second period: 5% dextrose

C. First period: 1.89% sodium sulfate
   Second period: 1.89% sodium sulfate, epinephrine hydrochloride 0.001%

D. First period: 1.89% sodium sulfate
Second period: 1.89% sodium sulfate, epinephrine HCl 0.01%

E. First period: 1.89% sodium sulfate
Second period: 1.89% sodium sulfate Between the first and second periods, the left kidney is exposed with a dorsal approach, its vessels are ligated, and it is returned to the abdomen.

Determinations. Blood. The two samples are centrifuged (Instructor) and the volumes of cell and plasma portions are read. Of each plasma, 2 cc. are pipetted into 18 cc. of 5% trichloracetic acid, mixed, let stand for five minutes, and filtered. This makes a 1-10 dilution of the plasma.

Urine. The volumes of urine and washings in the flasks are measured with a graduated cylinder. To obtain the actual volume of urine, subtract 20 cc. (volume of washings) from the observed volume. To prepare a 1-500 dilution, pipette 1 cc. of the contents of the flask into a large graduated cylinder, and dilute with distilled water, according to the following formula:

volume to which 1 cc. is diluted =

$$\frac{500 \times (\text{cc. of urine and washings} - 20)}{\text{cc. of urine and washings}}$$

Take 8 photometer tubes, and number them from 1 to 8, putting the appropriate sample in each tube, as listed in the following chart.

Ferrocyanide. For plasma take 1 cc. of plasma filtrate plus 4 cc. of water. For urine, take 5 cc. of diluted urine. To each tube, add 2 cc. of ferric sulfate reagent and mix thoroughly. Five minutes later, add 2 cc. of water to each tube. Read in photometer.

Aminohippurate. Take 5 cc. of plasma filtrate or 5 cc. of diluted urine. Proceed exactly as for determination of sulfanilamide as described in Experiment 4. Read in photometer.

Tube #	Contents	Photometer Reading	mgm. in sample	mgm. per 100 cc.
-	Ferrocyanide	620 mu	-	-
1	Urine 1			
2	Urine 2			
3	Plasma 1			
4	Plasma 2			
-	Aminohippurate	540 mu	-	-
5	Urine 1			
6	Urine 2			
7	Plasma 1			
8	Plasma 2			

Calculation.

Urine ferrocyanide. mgm. in sample x 10,000 = mgm. per 100 cc.
Plasma ferrocyanide. mgm. in sample x 1,000 = mgm. per 100 cc.
Urine aminohippurate. mgm. in sample x 10,000 = mgm. per 100 cc.
Plasma aminohippurate. mgm. in sample x 200 = mgm. per 100 cc.

Urine volume. $\frac{\text{measured volume} - 20}{10}$ = urine cc. per min.

Clearance = $\frac{U \times V}{P} = \frac{\text{mgm. per 100 cc. urine x urine cc. per min.}}{\text{mgm. per 100 cc. plasma}}$

Glomerular filtrate, cc. per min. = clearance of ferrocyanide.

Renal plasma flow, cc. per min. = $\frac{\text{clearance of aminohippurate}}{0.85}$

Renal blood flow, cc. per min. = $\frac{\text{renal plasma flow, cc./min. x 100}}{\text{percentage plasma in hematocrit}}$

Renal vascular resistance, arbitrary units = $\frac{\text{arterial pressure (assume 100 mm. Hg)}}{\text{renal blood flow, cc. per second}}$

Filtrate fraction, % = $\frac{\text{glomerular filtrate cc. per min. x 100}}{\text{renal plasma flow, cc. per min.}}$

Tubular reabsorption, cc. per min. = glomerular filtrate, cc./min. - urine vol., cc./min.

Body surface, square meters, = 0.112 x (weight, kgm.)$^{2/3}$

For comparative purposes, the urine flow, glomerular filtrate, tubular reabsorption, renal plasma flow, and renal blood flow should all be calculated as cc. per minute per square meter of body surface.

	Period 1 Treatment =	Period 2 Treatment =
Urine Flow		
Glomerular Filtrate		
Tubular Absorption		
Renal Plasma Flow		
Renal Blood Flow		
Renal Vascular Resistance		
Filtrate Fraction %		

Experiment 27

EXPERIMENTAL EDEMA

Weigh a rabbit, observe normal cardiac and respiratory rates. Inject subcutaneously in the groin 0.2 gm./kgm. of paraphenylenediamine hydrochloride. AVOID CONTRACT WITH THIS AGENT. One hour later, repeat the above observations, and look for the appearance of any edema. Now inject intravenously 50 mgm./kgm. of trypan blue. Repeat the observations 1½ hours after the injection of the paraphenylenediamine, and when severe respiratory difficulty appears, kill the rabbit with chloroform and perform an autopsy, examining especially the site of injection and the tissues of the head and neck, noting the presence and nature of edema, and staining of the tissues of these regions with the dye, in comparison with other tissues.

M. L. Tainter & F. J. Hanzlik. J. Pharmacol. 24: 179, 1924.

ANTIDIURESIS

One student in each group will serve as subject, either as control, or receiving the drug. Each should take no lunch. At the beginning of the laboratory period, each subject empties his bladder, then collects and measures urine secretion at fifteen minute intervals thereafter. At the time of the second fifteen minute collection, each subject drinks one liter of tap water of a temperature not less than 25° C. At the same time the subject receiving the posterior pituitary extract is given a subcutaneous injection of 0.2 cc. of a sterile 1:10 dilution of solution of posterior pituitary, U.S.P. The subjects should remain as quiet as possible during the experiment, and should not smoke or take food or water. The results should be presented graphically.

J. H. Burn. Methods of Bioassay. pp. 50-52. Oxford, 1928.

Exp. 28. Instead of rabbit uteri, rat uteri will be supplied. The rats will have been injected with diethyl stilbestrol. The contents of the bath should be changed between each drug.

Experiment 28

THE ACTION OF DRUGS ON THE ISOLATED UTERUS

The apparatus should be set up with water in the large beaker and Locke's solution in the muscle bath. The solution in the muscle bath must be maintained between 37°-38° C. It is necessary to have a separate beaker of Locke's solution at the above temperature for washing out the muscle bath after each drug is given.

Two rats will be provided for each two groups. One of the rats serves as the normal, the other has been treated with diethylstilbestrol. Kill the rats with a sharp blow on the head, remove the uteri, divide the horns, and place in warmed (37°-38° C.) Locke's solution. Each group should have a uterine horn from a treated and non-treated rat. These should both be attached to the glass hook provided so that longitudinal activity is recorded. Be sure that air is bubbling through the Locke's solution at a moderate, constant rate. Each strip is connected to a heart lever so that it is stretched slightly. The slowest speed on the kymograph will probably be sufficient.

To obtain the maximum information from this experiment the following procedures should be closely followed:

1. Make certain that a constant temperature is maintained in the water bath.
2. A good normal record should be obtained. This usually means a record of 20-30 minutes in length after the kymograph is started.
3. After a response to the drug is obtained the muscle bath should be washed out with Locke's solution at least once. In some cases two or more washings may be necessary.
4. After washing, a period of time must be allowed for the strips to recover. Sometimes the rhythm will not revert to the type obtained in (2). If it does not, time should be allowed for a proper recording of the new level of activity. Probably a time period of 5-10 minutes should be allowed from washing until the next drug is given.
5. After the last assigned drug is given repeat the first drug and compare with the original record for that drug.

The following drugs will be used:

Drug	Amount	Calculate Final Concentration in Bath
Epinephrine	1 cc. of 1:10,000	
Acetylcholine	1 cc. of 1:10,000	
Posterior pituitary extract	1 cc. of 1:200	
Pitressin	1 cc. of 1:400	
Pitocin	1 cc. of 1:200	
Ergonovine	1 cc. of 1:10,000	