APPENDIX 5

The Pharmacology Medical Courses

A. Manual of Experimental Pharmacology

B. Lecture outlines for Medical Pharmacology Course, 2004 and 2018-19

C. Pharmacology teaching in transition year, 2020-2021

D. Lecture outline for current Medical Pharmacology Courses (PCOL812 & PCOL820)

WEST VIRGINIA UNIVERSITY

Manual of EXPERIMENTAL PHARMACOLOGY

Department of Pharmacology

West Virginia University Medical Center

MANUAL OF

EXPERIMENTAL PHARMACOLOGY

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INTRODUCTION

This laboratory course in pharmacology is designed to acquaint the student with the physical and chemical properties of some of the more important therapeutic agents, their effect on living tissue and, when possible, their mechanism of action. The scope is necessarily limited and emphasis is placed on pharmacological principles.

The experiments are designed for a three-hour laboratory period and there should be no difficulty in completing the assignment in the time allotted. It cannot be emphasized too strongly, however, that, before coming to the laboratory students should be thoroughly familiar with the procedure to be carried out. This preparation should involve a review of the directions for the experiment and the appendices related thereto.

General Information:

In most experiments students will perform their laboratory work in pairs or groups of four. Cleanliness and order are essential for good work. For the proper appearance of the laboratory, containers are provided for all wastes except animal carcasses. It is especially important to keep the sinks clean; pieces of paper, animal hair and other debris can soon clog a sink and render it useless. Before you leave the laboratory your table top must be clean. All equipment and apparatus must be clean and put in its appropriate place. Breakage or mechanical failure should be promptly reported.

In dealing with drugs and poisons, chemical cleanliness is doubly important. It protects you and prevents contamination of the experiment. The following points should be observed.

1. Do not keep drugs in an unlabeled container.
2. Check the concentration of the solution provided.
3. Calculate the amount of solution needed and obtain just this amount.
4. Avoid contamination of all stocks of drugs and reagents.

Equipment:

Each student at a desk will be assigned a drawer of equipment. In addition, a drawer of special equipment will be assigned jointly to each group of four students. The equipment in the desk drawers must be shared by the students at that desk, by a group of four students when the students from two desks work together and (in the fall term) by student groups from two laboratory sections. Those using shared equipment are responsible for it. The individual who damages a piece of equipment must obtain a replacement. Damage or shortage must be reported when detected. Replacement equipment will be issued by the stockroom attendant in exchange for a receipt.

Equipment or apparatus needed for a particular experiment, in addition to that assigned to students and routinely kept in the desk, will be placed on the desk before the laboratory period and should be left on the desk, clean and in good order, at the conclusion of the laboratory.

The apparatus and equipment provided in or on each desk has been checked and put in working order. If something does not appear to work, please see an instructor before making adjustments.

Clean and dry each piece of equipment before leaving or storing it at the end of the period. Blood smears and salt solution are easy to remove immediately after the equipment is used but become practically impossible to remove in a few days. This is particularly true of glass syringes. Clean, or at least rinse, a syringe immediately after use. If a syringe cannot be cleaned immediately, separate barrel and plunger before laying it aside. The washed barrel and plunger should always be left apart to dry. Hypodermic needles should always be flushed immediately after use.

Solutions:

Drugs used in this course, almost always, will be supplied as solutions. To help insure proper use of drug solutions, students should be thoroughly familiar with the material presented in Appendix I.

Most of the solutions are specially prepared for each experiment and will be found on the side table. In most instances the entire class will use common supplies and extreme care must be taken in order that this procedure be satisfactory (see "GENERAL INFORMATION"). In order that common stocks of drugs may be available at all times for use by others, no student should carry the stock supply to his desk. Drugs may be supplied in vials or ampoules exactly as used in clinical practice. Because of the expense involved you may have to share an ampoule with someone else. Before using ampoules please check for any special instructions.

For individual experiments no sizable excess of drug solutions will be available. Thus if someone is wasteful, or takes 10 ml. when 1 ml. is sufficient, others will be inconvenienced.

Observations:

Before starting an experiment a group should go over the entire exercise, discuss it, and plan their procedure. Proper "controls" are essential. Make observations of the normal animal or secure a normal record of the tissue involved before any drug is added. Unless the normal responses are accurately known, drug action cannot be evaluated.

Normal physiological actions are seldom identical in any two animals or in any two human beings: some actions may be nearly alike while others differ widely. Responses to drugs may also show wide deviations in different animals or even in the same animal under different conditions. Do not expect exact agreement with your neighbor's experimental results but rather look to see how your results may deviate from his. Deviations do follow certain patterns and normally fall within a definite range which varies with conditions. All experiments illustrate this principle but certain ones are designed to emphasize and measure this animal variation.

Check dose calculations carefully in order to avoid killing your animal with a fatal dose of drug. Do not administer a drug until the full effect of the preceding one has been observed, unless otherwise instructed.

Laboratory Conference:

When time permits, a laboratory period will be concluded by a brief discussion of the experiment just completed.

Reports:

After the laboratory work is finished, the data should be thoroughly studied, organized, interpreted with the aid of statistical analysis if necessary, compared to that of the rest of the class, and compared to statements in the textbooks and reference material. However, it is not expected that the written laboratory report will include passages copied from the textbook or other reference sources.

Reports should be original and as concise as possible while making the results clear and understandable. Emphasis should be placed upon the logical interpretation of results. Efforts to be neat are essential. Reports need not be typewritten but must be legible. The general pattern of the report should cover the points specified on the report form.

One laboratory report will be required from each student. These reports will be due at an announced time. All reports should be made on the standard departmental report form (available at the Medical Center Book Store). Individual reports should not be submitted weekly in a bulky binder. Reports will be received and returned in the laboratory through the use of a file cabinet in which students will use slots bearing their names.

Reports will be graded by a system announced by the instructor responsible for the conduct of the laboratory. Reports submitted late may receive reduced credit. Students may be asked to revise unacceptable reports.

At the end of the semester, all reports must be bound in loose-leaf form and the entire series of laboratory reports submitted for final appraisal. The exact date will be announced well in advance. The pages should be numbered consecutively and an index prepared. The earlier in the semester this task is started, the easier it will be.

Tables and Graphs:

In every possible instance data should be tabulated. In many experiments you will be required to complete and hand in data sheets provided in this manual. Tabulated class data will be duplicated and distributed for inclusion in reports. You are expected to compare your individual results with the average and also the average with the expected results.

Graphic representation of data is frequently desirable. The simplest graph shows the relationship between two variables, one of which is non-controllable (put this on the ordinate, vertical axis), the other being systematically controllable (put this on the abscissa, horizontal axis). While a line graph is usually better for time-or dose-response curves, a bar graph may be preferable for an all-or-none type of response. Use the type of graph most suitable for your data.

Polygraph Records:

In experiments that employ the Grass Polygraph make a record of the "normal" contractions, blood pressure, respiratory rate, or whatever is being measured. Continue this record, at appropriate speed, without interruption while the procedure is being carried out and until conditions have returned to "normal" or have reached a new steady state. If the change is slow, occasional time interruptions (clearly marked) are permissible. Sufficient time should always be allowed between procedures for arrival at a constant condition.

From polygraph records quantitative data can and should be obtained and tabulated; then, interpreted and discussed in the report. Polygraph records must be shared by students of a laboratory team. The complete polygraph record should be submitted with the report of a single student. For purposes of record and review, it is desirable that each student's report include reproductions of the significant parts of the polygraph record.

Demonstrations:

From time to time, members of the staff will present demonstrations in the laboratory or the lecture room. The student should know the object of the demonstration and the significance of the observations made and results obtained. The results of such demonstrations are to be included in the written reports because each student is responsible for all the experiments done in the laboratory whether he has actually done them himself or not. The laboratory examination at the end of the semester may cover the demonstration.

Displays:

Physicians and dentists prescribe many compounds without ever seeing them. This course in pharmacology is one of your best opportunities to learn something about the dosage forms of drugs. A number of drug manufacturing firms have cooperated with this department in presenting displays of the most important drugs. The drugs are arranged in groups in the display cabinets.

At intervals a special group of drugs is placed in trays in the laboratory. The purpose of this smaller display of drugs is to permit closer examination of the more important preparations. Usually it will supplement the lectures given earlier. Note the forms in which a drug is available. At the same time recall the origin of the drug, whether it has been crystallized or

concentrated, its stability at room temperature, how it is assayed, and other features of the drug you have studied.

Other supplementary material related to the course will be displayed in the laboratory or on the bulletin board at the entrance to the laboratory.

Experiment 1

Introductory Procedures

The purpose of this experiment is to introduce the general laboratory procedures and to standardize the handling of rats for injection in this laboratory course. This experiment will also illustrate several factors which can modify the response of the organism to drugs. The first objective will be accomplished by reading the complete Introduction. This must be done before this experiment is undertaken.

1. Handling of the rat for injection (also applicable to mice)

There are several techniques for holding rats but the following one has proved satisfactory for experimentors who have had little previous experience with rats. It will be demonstrated by the instructors. With the left hand, pick a rat up by the tail. Avoid stripping the flesh from the tail by holding the tail well up from the tip. Place the animal on the cage top or table and grasp it firmly by the nape of the neck with the thumb and index finger of the right hand, the other fingers being closed. Pull the scruff back tightly so the animal cannot turn its head, but be careful not to choke the animal. Still holding the tail, stretch the rat out with its back against the closed fingers of the right hand. It may be convenient to grasp the rat's hind legs also with the left hand. The rat is now belly up and ready for the other partner to inject.

For intraperitoneal injection, the needle should be inserted near, or a little posterior to the umbilical region. Draw back on the syringe to see that no intestinal loops or blood vessels have been punctured. The No. 20 needle is about right for use on rats. For mice. a somewhat smaller needle should be used. Practice in the handling and injection of rats will be obtained in part 2.

2. Dose-Response Curve in Rats with Hexobarbital Na

It is a fundamental principle of pharmacology that the magnitude of the response produced by a given drug is, within limits, related to the quantity of drug administered. This is referred to as the dose-response relationship. It is possible using hexobarbital, for example, to produce responses varying from mild sedation to surgical anesthesia (even death) by varying the dose from a small one to a larger one.

This experiment will also illustrate that the response to any given dose may vary according to the route of adrninistration of the drug and the functional capacity of the organ that metabolizes the drug.

Finally, the experiment will illustrate that there may be sex differences in the response of a given species of animal to a drug. Refer to Tables I, II and III on page 1/4.

Method: (Work in group of 4) Obtain six (6) male rats and one (1) female rat. One of the male rats is marked with dye to indicate that on each of 2 days prior to this experiment it received carbon tetrachloride (0.50 ml/kg) i.p. Weigh each rat and distinctively mark each with dye. Observe the general behavior; rate and character of respiration; response to stimuli, such as

gentle probing with a blunt instrument, and blowing on the fur; and such reflexes as the righting and corneal reflexes

Using the method described above, inject each rat with hexobarbital Na (50 mg/ml) according to the following table, noting that rat No. 5 is a female rat and that rat No. 6 is the rat pretreated with carbon tetrachloride.

Rat No.	Dose	Route	Sex
1	50 mg/kg	intraperitoneally	male
2	100 mg/kg	intraperitoneally	male
3	200 mg/kg	intraperitoneally	male
4	400 mg/kg	intraperitoneally	male
5	100 mg/kg	intraperitoneally	female
6 (CCl_4)	100 mg/kg	intraperitoneally	male
7	400 mg/kg	subcutaneously	male

The above order of injection is to be followed, and all injections should be made within a few minutes of one another.

Determine the length of time required for any effect to become evident, making observations comparing the rat's general behavior, respiration, and reflexes after drug administration with the same observations before dosage.

Accept the loss of righting reflex as the time for onset of anesthesia.

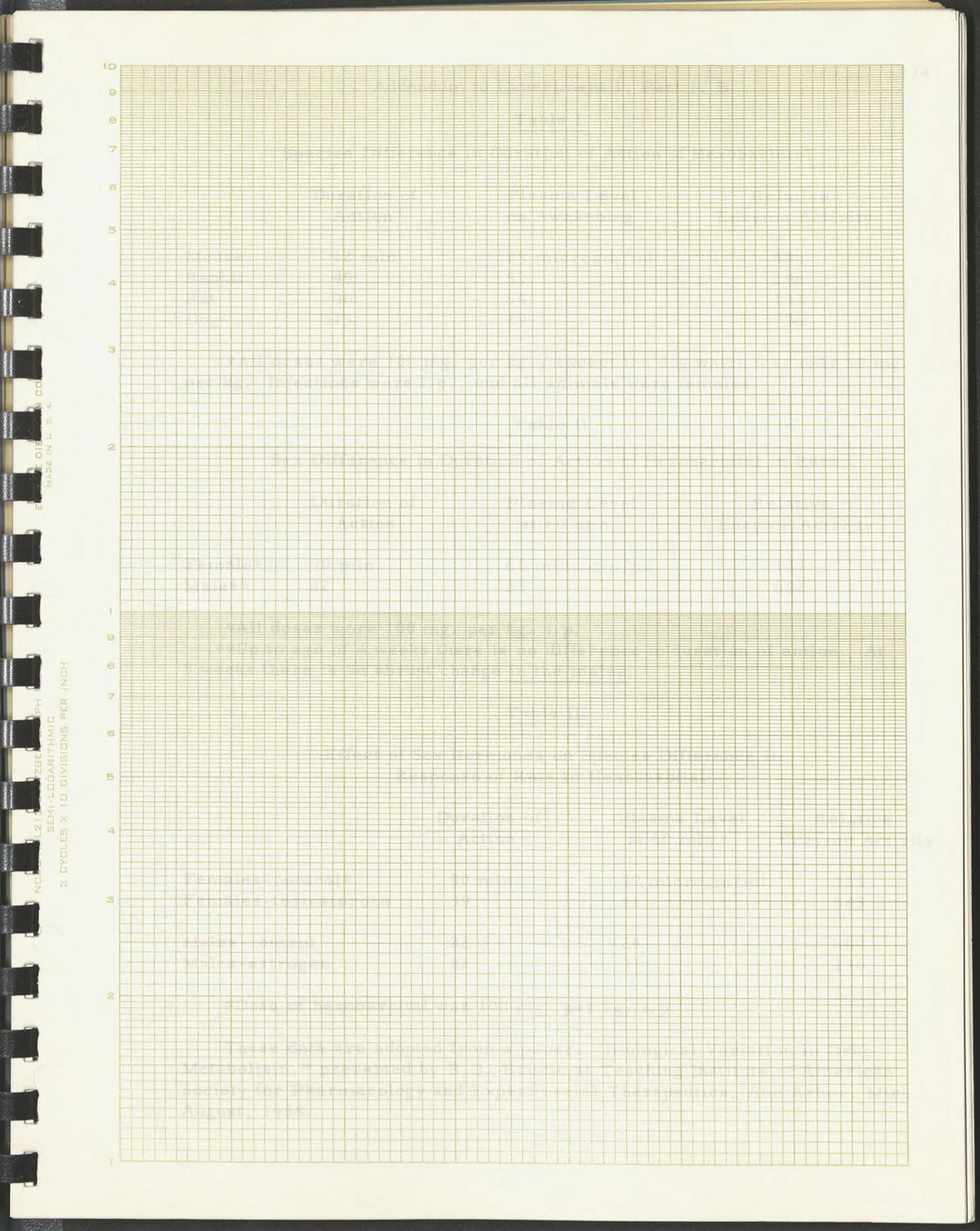
Determine and record the duration of action of each dose, taking the return of the righting reflex as the time of recovery, or respiratory arrest as the time of death.

Tabulate the results on the blackboard or on data sheets provided and include the combined data of the class in your report. Critically evaluate the results and account for the variability. Using class data from rats 1, 2, 3 and 4 plot a dose-response curve of dose (on the abscissa) against % mortality (on the ordinate). Estimate the LD_{50} for hexobarbital.

Animals: 6 male rats and 1 female rat in each cage

Drugs: Hexobarbital Na - 50 mg/ml

Equipment: Available in desks
Plastic animal weighing containers
Animal balances (spring type)
Dye to mark rats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Addendum to Experiment 1, Part 1. B

Table I

Species Difference in Duration of Action of Hexobarbital

	Duration of Action	Plasma Level on Awakening	Relative Enzyme Activity
Mouse	12 min	89 microgm/ml	598
Rabbit	49	57	196
Rat	90	64	134
Dog*	315	19	36

*All doses were 100 mg. per kg., except the dog which was only 50 mg. per kg. Injections were i.p., and all animals were females.

Table II

Sex Difference in Duration of Action of Hexobarbital in Rats

	Duration of Action	Plasma Level at 60 min.	Relative Enzyme Activity
Female*	90 min.	65 microgm/ml	134
Male**	22	23	682

*All doses were 100 mg. per kg. i.p.

**Up to age of 4 weeks there is no difference in duration of action. At 5 weeks there is an abrupt change in the males.

Table III

Effect of Sex Hormones on Species Difference in Response of Rats to Hexobarbital

	Duration of Action	Plasma Level at 60 min.	Relative Enzyme Activity
Females-control*	90 min.	70 microgm/ml	123
Females-testosterone	39	41	545
Males-control	22	25	660
Males-estrogen	82	71	170

*Dose of hexobarbital was 100 mg. per kg. i.p.

These data are adopted from a paper, "Biological Variation in Drug Metabolism," presented by B.B. Brodie at Teaching Institute of American Society for Pharmacology and Experimental Therapeutics, Ann Arbor, Mich., August, 1958.

Experiment 2
General Anesthesia I
Volatile Agents in Mice
(No Smoking or Open Flame)

I. Introduction

Mice are observed in known volumes of air containing known amounts of anesthetic gas. Use a fresh animal for each experiment. Wash the jars out with a stream of air before they are used again. After noting the normal behavior of the animal in the jar, measure the desired amount of anesthetic in a syringe, squirt it through the hole in the lid onto the filter paper and stopper the jar tightly. Tip the jar from side to side so as to mix the anesthetic vapor thoroughly with the air. Observe each mouse for 30 minutes after the anesthetic has been added, following the time schedule suggested below.

Estimate the depths of anesthesia in terms of the following signs and record the times at which each is attained.

1. Ataxia
2. Side position (move the jar gently about to test for inability to right)
3. Rate of respiration less than 100 per minute
4. Death

Remove the mouse from the jar, follow the sequence of events during recovery, and note the time from the point of removal from the jar to the return of righting reflexes.

II. Experimental Procedure (groups of 2 students)

A. Concentration of Anesthetic in Inspired Air

Add Ether as follows:

1. 1.24 ml in the 8.6 l. jar
2. 0.55 ml in the 3.8 l. jar
3. 1.00 ml in the 3.8 l. jar
4. 1.50 ml in the 3.8 l. jar

B. The Respiratory Volume. Effect of Carbon Dioxide

Place a mouse in a 3.8 l. jar and note the behavior and respiratory rate. Wash out jar with a mixture of 95% O_2, 5% CO_2 from the tank supplied. When a change in respiratory rate is evident, introduce 0.55 ml of Ether and record the time at which the various signs of depression appear. Compare the speed of induction with that in experiment A above.

C. The Effective Partial Pressure of Other Inhalation Anesthetics

Study the general anesthetic effect of the following drugs, making the same observations and using the same technique as with Ether in part A.

1. 0.22 ml of Chloroform in the 8.6 l. jar
2. 0.88 ml of Vinyl Ether in the 3.8 l. jar

Continue your observations on each mouse for at least 30 minutes and then observe recovery noting the time from the point of removal from the jar to the return of righting reflexes.

D. Calculations

1. With the additional information given in section F calculate the partial pressures of Vinyl Ether, Chloroform, and the various concentrations of Ether used in sections A, B and C. Enter the results in Table 1.

2. Complete the calculations required for Table 2 by assuming that at equilibrium the following anesthetic concentrations would give similar depths of anesthesia:

Ether	1.24 ml in 8.6 l.
Chloroform	0.22 ml in 8.6 l.
Vinyl Ether	0.88 ml in 3.8 l.

E. Questions

1. Does the total amount of anesthetic agent added determine the depth of anesthesia?

2. How is the speed of induction to a particular stage of anesthesia influenced by anesthetic concentration?

3. What is the influence of a change in rate and depth of respiration on the speed of induction and the rate of recovery from ether anesthesia?

4. Does the presence of CO_2 alter the degree of depression attained at equilibrium?

5. What are the dangers of increased pCO_2?

6. How do Vinyl Ether and Chloroform compare with Ether with respect to the speed of induction and recovery?

F. Relevant Data for Calculations

	MOLECULAR WEIGHT	SPECIFIC GRAVITY	SOLUBILITY IN BLOOD AT 37.0°C
Ether	74	0.71	14.9
Vinyl Ether	70	0.77	1.5
Chloroform	119	1.47	7.3

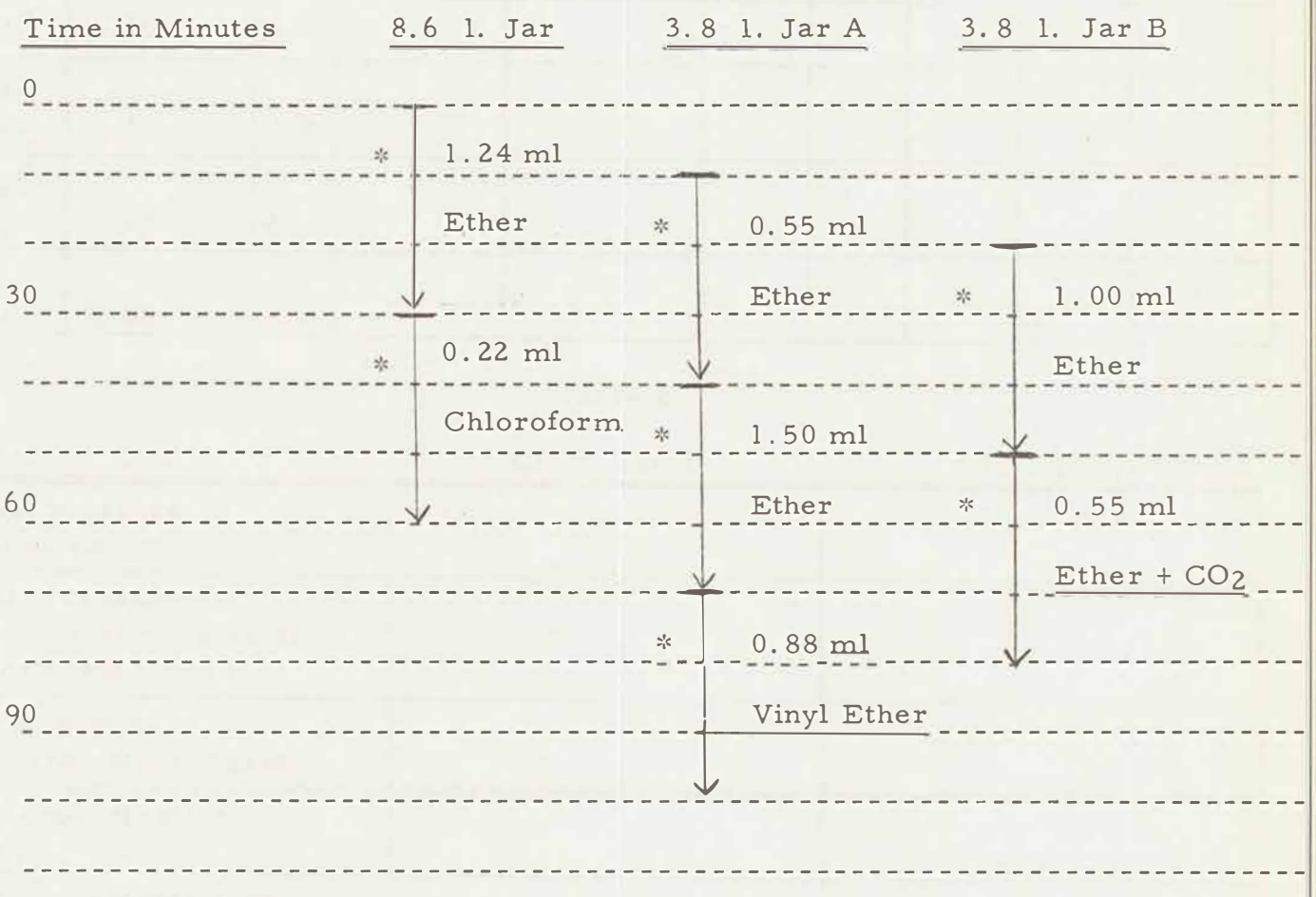
$$\text{Solubility} = \frac{\text{Concentration of anesthetic in blood, moles/l}}{\text{Concentration of anesthetic in air, moles/l}}$$

This relationship is based on Henry's Law.

The ideal gas law: $PV = nRT$

P = the pressure exerted by the gas (i.e., the partial pressure)
V = the volume occupied by the gas (jar volume)
n = the number of moles of gas
T = the temperature in degrees absolute; assume 300°A (27°C)
R = the gas constant, is the product of P x V per mole per degree temperature absolute, and has the dimensions of work. If P is in millimeters of mercury and V is in liters, R = 62

Suggested Time Schedule



*Observe the mouse closely during the first 10 minutes.

References:

Haggard, W.W. The absorption, distribution and elimination of ethyl ether. II. Analysis of the mechanism of absorption and elimination of such a gas or vapor as ethyl ether. J. Biol. Chem. 59: 753 (1924).

Kety, S.S. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol. Rev. 3: 1 (1951).

Animals: Mice

Drugs: Carbon dioxide, Ether, Chloroform, Vinyl ether

Equipment: Jars for anesthesia (8.6 l = AHT 2211C)
Other available in desks

Table 1

	Anesthetic	Vol of Liquid Anesthetic Added (ml.)	Vol of Jar (l.)	Partial Pressure (mm Hg)	Anesthetic Added at (Time)	Minutes from start to: 1. Ataxia	2. Side Position	3. Slow Resp.	4. Death	Time for recovery of righting
1.	Ether	1.24	8.6							
2.	Ether	0.55	3.8							
3.	Ether	1.00	3.8							
4.	Ether	1.50	3.8							
5.	Ether + 5% CO_2	0.55	3.8							
6.	Chloroform	0.22	8.6							
7.	Vinyl Ether	0.88	3.8							

Table 2

	Chloroform	Vinyl Ether
Partial pressure in inspired air, mm Hg		
Partial pressure of Ether* for similar depth of anesthesia, mm Hg		
Ratio of partial pressures, ether/agent		
Blood concentration, moles/l.		
Blood concentration of Ether* for similar depth of anesthesia, moles/l.		
Ratio of blood concentrations, ether/agent		

*Assume that ether at 1.24 ml/8.6 l. meets this criterion. This assumption is necessary for ether has a very slow rate of attaining equilibrium, taking over 90 minutes (Haggard, W.W., 1924). Hence the final depth of anesthesia at equilibrium was not determined for ether in this experiment.

Experiment 3

General Anesthesia II
Volatile Agents in the Dog

(Ether or Chloroform and Preanesthetic Medication)

In this experiment the signs and effects of anesthesia with a volatile gas will be observed in the dog. Experience will be gained in preparing the animal for the recording of blood pressure, respiration and heart rate on the Grass Polygraph Recorder.

Premedication agents, used as adjuvants of anesthesia, dry up mucous and salivary secretion (atropine) and render the patient less apprehensive without significantly depressing the central nervous system (morphine). Opiates and their substitutes may produce some respiratory depression, cardiovascular depression, nausea, and dysphoria. They should be used only when pain is present.

Students will work in groups of four. Alternate groups will be assigned to carry out one of the two procedures below. Groups working without premedication will be expected to make observations with an adjacent group working with premedication and vice versa.

Obtain a dog and make the observations and examinations required on the data sheet. Enter your control observations and proceed with either Procedure I and II.

Procedure I: Ether (or Chloroform) alone

Muzzle the dog, hold the animal on the floor and induce anesthesia with ether or chloroform by the open drop method.

As soon as the animal is unconscious place it on a dog board, remove the muzzle, cannulate the trachea (see Appendix II) and rapidly attach an ether bottle so that administration of the anesthetic may be continued. Cannulate a carotid artery and arrange to record blood pressure, heart rate and respiration as directed in Appendices II and III.

Discontinue the administration of anesthetic and allow the animal to recover until the onset of struggling (Light Anesthesia). Make observations and entries required on data sheet. Note lapse of time required for recovery.

Readminister anesthetic until a level satisfactory for most surgery is reached (Deep Anesthesia). Maintain the animal at this level - UNIFORMLY- for 15 to 20 minutes. Make observations and entries required on data sheet. If at any time respiratory arrest should occur, immediately administer artificial respiration (see Appendix II).

After the period of uniform deep anesthesia, administer sufficient anesthetic to produce respiratory arrest (Very Deep Anesthesia). To accomplish this it may be necessary to immerse the ether bottle in hot water to increase the volatilization of the anesthetic. Attempt to revive the animal by artificial

respiration. Note cardiac activity during period of respiratory paralysis. Make observations and entries required on data sheet.

Terminate the experiment by producing respiratory arrest without subsequently administering artificial respiration. Note the lapse of time between onset of respiratory arrest and cardiac arrest.

Procedure II: Preanesthetic Medication with Morphine and Atropine followed by Ether (or Chloroform)

After making control observations on the unanesthetized dog, record data and then administer the following drugs:

(a) morphine sulfate - 5 mg/kg - s.c.

(b) atropine sulfate - 0.1 mg/kg - s.c.

Fifteen minutes later repeat control observations and follow the instructions given in Procedure I for the induction and maintenance of anesthesia, and the termination of the experiment.

Animals:	Dogs
Drugs:	Ether
	Pentobarbital sodium - 25 mg/ml
	Morphine sulfate - 25 mg/ml
	Atropine sulfate - 1 mg/ml
Equipment:	Grass Polygraph
	Dog boards
	Muzzles
	Newspapers
	Animal clippers
	Gauze sponges
	Cannulae: tracheal; arterial
	Ether cones
	Ether bottles
	Artificial respiration hoses
	Other: available in desk

Experiment 3
Data Sheet

Weight:	I WITHOUT PREMEDICATION				II WITH PREMEDICATION				
	Control Values	Light Anesth.	Deep Anesth.	Very Deep Anesthesia	Control Values*	Control Values**	Light Anesth.	Deep Anesth.	Very Deep Anesthesia
Respiration: Rate									
Amplitude (0-4+)	++				++				
Intercostal Activity (0-4+)	+++				+++				
Diaphragmatic Activity (0-4+)	+++				+++				
Cardiovascular: Heart Rate									
Mean Blood Pressure									
Pupil Size (0-4+)	++				++				
Involuntary Eyeball Activity (0-4+)	0				0				
Salivation (0-5+)	++				++				
Skeletal Muscle Tone (0-4+)	++++				++++				
Eyelid Reflex (0-4+)	++++				++++				
Patellar Reflex (0-4+)	++++				++++				
Pain Reflex - Superficial (0-4+)	++++				++++				

*Before premedication
**After premedication

On separate sheet compare non premedicated and premedicated animals as to amount of struggling, time required for induction, amount of salivation and any other differences observed.

What stage and plane of anesthesia does your data indicate the animal was in during light, deep and very deep anesthesia? (See Goodman and Gilman, 2nd ed., page 29)

Experiment 4

General Anesthesia III

(Barbiturates and Muscular Relaxants in the Dog)

Thiopental Na will be used as an example of the barbiturates. It is short acting and like other barbiturates it does not cause adequate muscle relaxation for many surgical procedures. In practice, therefore, it is frequently used in conjunction with a muscle relaxant such as d-tubocurarine or succinylcholine. The greatest danger in the use of barbiturates is overdosage, so be cautious.

Obtain a healthy dog and make control observations as in Experiment 3. For the initial anesthetization, use a drug solution with a concentration of 40 mg/ml and into the saphenous vein administer SLOWLY (at least one minute) 20 mg/kg of thiopental Na. Without delay, fasten the dog to the animal board, expose a femoral vein and cannulate it with polyethylene tubing. Administer a dilute solution (4 mg/ml) of the anesthetic by i.v. drip from a burette (see Appendix II). In preparing the animal for the i.v. drip, use ether as a supplemental anesthetic only if necessary. Maintain the animal in surgical anesthesia by cautious administration of drug; avoid depressing the animal to the point of respiratory paralysis. Cannulate the trachea and be prepared to administer artificial respiration by means of an air hose (see Appendix II). Cannulate a carotid artery and arrange to record blood pressure, heart rate and respiration as directed in appendices II and III. Expose the opposite femoral vein for subsequent i.v. injections (see Appendix II). Injections should be made directly into the vein.

Maintain the dog at a uniform level of anesthesia, satisfactory for most surgery, for 15-20 minutes.

Withhold the i.v. drip of the anesthetic for 3 to 5 minutes prior to, and during, the following i.v. injection via the opposite femoral vein.

A. Inject succinylcholine chloride SLOWLY (2 to 3 min.) until a perceptible effect is obtained. The dose should NOT exceed 0.8 mg/kg. Discontinue the injection and note the short duration of action. Be prepared to administer artificial respiration.

Administer additional anesthetic before B, below, ONLY if necessary (e.g., struggling).

B. After the dog's respiratory rate and depth have returned to the level observed prior to the injection of succinylcholine chloride, proceed with the injection of d-tubocurarine chloride. Administer SLOWLY 0.1 mg/kg of d-tubocurarine chloride i.v. Repeat this injection at three-minute intervals until a perceptible effect is obtained.

If the dog is still surviving, terminate the experiment with either succinylcholine chloride or d-tubocurarine chloride.

Would you routinely use muscle relaxants with barbiturate anesthesia? If not, under what conditions would they be used?

Animals: Dogs

Drugs: Thiopental Na - 40 mg/ml
4 mg/ml - in 0.9% saline, containing Na heparin (3 mg/100 ml)
Ether
Succinylcholine Cl - 2.5 mg/ml
d-tubocurarine Cl - 0.5 mg/ml

Equipment:
Grass Polygraphs
Dog boards
Muzzles
Newspapers
Animal clippers
Gauze sponges
Polyethylene tubing
Drip indicators
Burettes
Burette clamps
Clamp stands
Ether cones
Cannulae: tracheal; arterial
Artificial respiration tubing
Other: available in desk

Experiment 5

Central Nervous System Stimulants and Depressants in Man

This experiment is designed to discover any differences in mental alertness and reaction time which may follow the oral administration of certain drugs. Visual reaction time will be measured, as well as mental alertness. Also a qualitative test for the presence of alcohol in the expired air will be employed. Each student will determine his control visual reaction time according to the posted time schedule for use of the reaction times. Each will then complete the first three code substitution tests before taking the assigned drug. Then repeat all tests approximately 60 minutes AFTER taking the drug.

A drink, containing a drug mixed in grapefruit juice, will be provided for each student. The drugs to be studied are ethyl alcohol (25 ml of 95%), seco-barbital Na (100 mg), amphetamine SO_4 (15 mg), and a placebo. Any student preferring not to take a particular drug should consult the instructor before the start of the experiment so that he may receive an alternative drug. Otherwise, all students will participate in the experiment. The consumption of the drink containing the experimental drug should be completed within a few minutes.

1. Code substitution:

This test appears on the following two pages. Please do not look at it until you are prepared to complete it under the experimental conditions. The test consists of a key, a square array of letters, and a corresponding array of spaces. The letters in the square array are found in the upper (alphabetical) line; below them are located the substitute letters to be placed in the blank spaces. For example, if in the first test the first row of letters in the first array were

C B Z L S D Y K P E

the letters to appear in the first row of spaces would be

N I V T G S O F K B

(it is necessary to look at the key at the top of page 5/2 to check this result).

The three tests on page 5/2 are to be done before taking the drug; the three tests on page 5/3 are to be done immediately following the reaction time tests. Time each test to the nearest second.

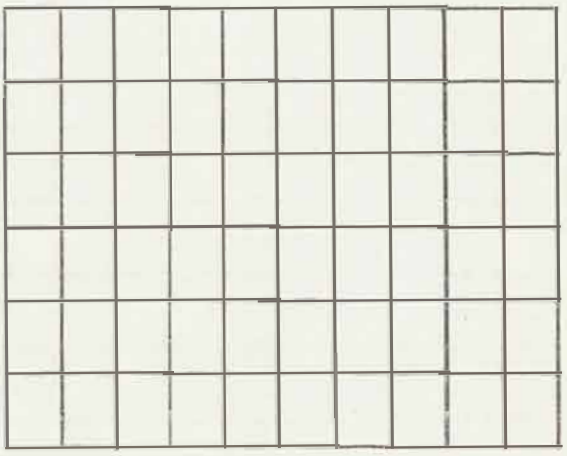
For each error add 5 seconds to the total test time.

In the appropriate spaces on the blackboard, record (in seconds) only the time taken for the third test (before and after drug). Draw your conclusions from the class means.

1. Code substitution test (complete these tests BEFORE taking drug)

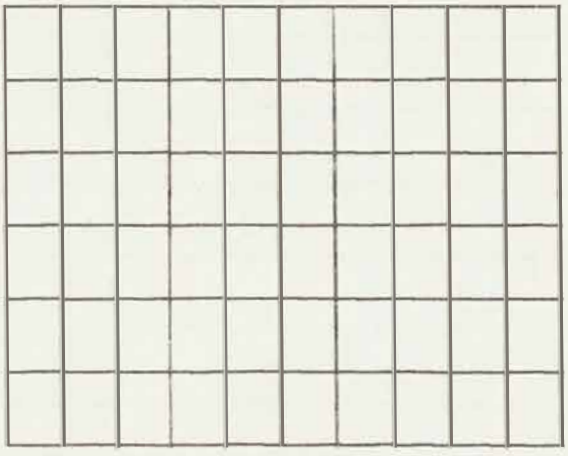
(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
KEY (
(D I N S B W Z A Y Q F T X R M K H U G E C J K P O V

D M Y N P C U G R D
K B W Q J E T D I O
G S C I N X S D E E
W P S E J L E Y E Z
X P X U L P I M O R
T S Z W A H Y G S B



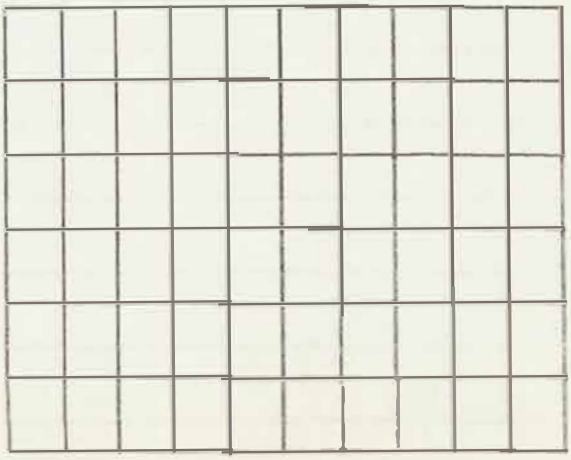
(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
KEY (
(C R F Y G B H P M L X Q V E K I N T U A S O Z D J W

A N O I S E R P E T
N I X O T W S B Q Y
R C V P Z D L J M F
H T A U G M P H B R
D K E O Y U X D J B
E S D R J S G S I H



(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
KEY (
(V H Y N O W Q Z X T U D R I K G N F B E C A L S P J

Y C N E I F H G E Z
C I T L A N R T X E
D A G I M P F L W Y
S U A J O E L N F B
E J P S L C A Z X T
M Y W Q I K R D C A



(Complete these tests immediately following reaction time tests)

(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

KEY (

(Q X E V T N U A O S W Z D R C F Y G B I H M J P L K

U J G O R O D W M A

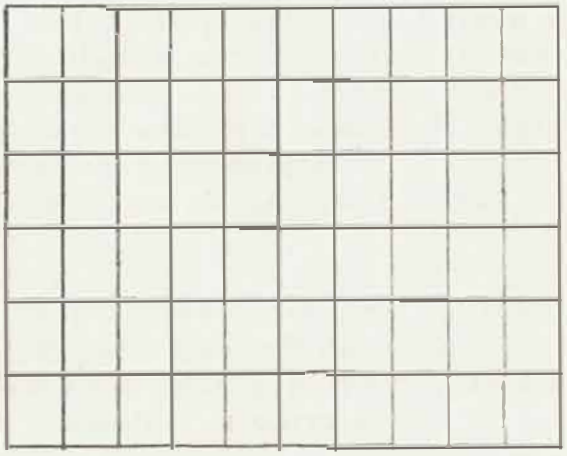
H T L P F W C K S F

Z E V J Q I D L C P

V S H W O H K B M E

B J D X U Y O E K D

H A O R D U P L H S



(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

KEY (

(N K P B J O C G U Z D T V H I M L X F Y A W S Q E R

D U W I N W E X O A

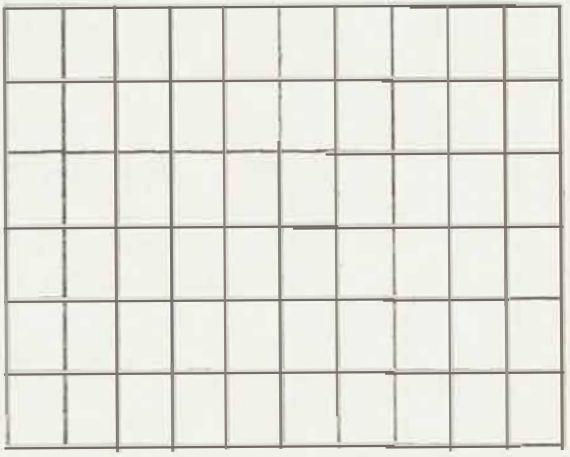
M G Q O X P Z U L S

Y R J G S S E I T H

N D E W D E Y P R Y

P K T C E J W I Z G

C B D I E J P L W S



(A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

KEY (

(M J T I N B A C E D F X R Z U Y V G H W P Q L O S K

U H Z V B H J R E S

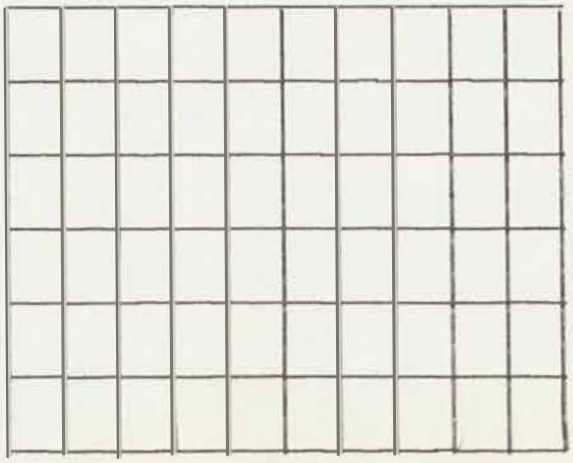
J A G L V H D O O P

W J X R O W I H Y U

D C D K L P W A L E

L M S C M K H A F P

E D S R F Q O U D Z



2. The "Drunkometer" test

This test is to be done before taking drug and again, with fresh solutions, just before reaction time tests are repeated (that is, approximately 57 minutes after taking a drug).

Large test tubes each containing 10 ml of 16 N sulfuric acid and glass beads to prevent "bumping" will be provided. Each student should pipette into his own tube 1.0 ml of potassium permanganate immediately prior to making the test. Mix well. Blow the breath through the pipette for exactly 2 minutes. Characterize the degree of decolorization as no change (-), moderate (+), or complete decolorization (++). Report degree of decolorization as the result on the blackboard in the appropriate space.

Students must be especially careful to keep the very corrosive sulfuric acid-potassium permanganate solution off themselves and off desk tops. Also, do not pour this solution into the sinks. Leave the solution in the tubes which will be collected by the technician after the laboratory is completed.

At the end of the period the composition of drinks will be announced by the instructor. Note advantages of an experiment in which the subject does not know what reaction to expect. Note advantages of having an adequate experimental control, and of drawing conclusions from the means of several observations rather than from individual observations.

Animals:	None
Drugs and Reagents:	Amphetamine SO_4 - 15 mg/25 ml water
	Ethyl alcohol (95%) - 25 ml
	Secobarbital Na - 100 mg/25 ml water
	Potassium permanganate - 0.8% solution
	Sulfuric acid - 16 N
	Gratefruit juice - 46 oz. cans
	Crushed ice
	Glass beads - 2 lbs
Equipment:	Large test tubes - 25 x 200 mm
	Beverage containers
	Visual reaction timer
	Other: available in desk

Experiment 6

Drugs Affecting Sleep in Man

The experiment is designed with the following intentions:

(1) To provide a direct demonstration in the human subject of the action of two safe, widely used drugs.

(2) To bring the student face to face with the difficulties of evaluating drug effects in humans.

In the clinical evaluation of a drug it is preferable to compare its response with that of a placebo, which is a substance considered to be inactive with respect to the response being observed. Certain agents such as barbiturates and caffeine are widely accepted as being able to affect sleep. An attempt will be made to confirm these impressions in this experiment in which students will take either pentobarbital, caffeine or lactose (placebo).

Students are urged to participate, unless there are special reasons for not doing so, to make observations as objective as possible, and not to exchange impressions or speculations about the drugs until the experiment is concluded.

Double Blind Technique:

In order to eliminate bias in the clinical evaluation of drugs, it is absolutely essential that the subject and the observer are both "blind" (double blind technique) to the identity of the particular drug administered. A third person, otherwise unassociated with the experiment, is responsible for the code to the drugs and, particularly with potentially hazardous drugs, is responsible for the safety of the subjects.

In this experiment in which only subjective observations will be made, the student will serve as both the subject and the observer. The drugs have been prepared as identically appearing capsules. Do not attempt to identify the capsule contents by means of taste or other methods; this will destroy the aim of the experiment by introducing bias. The capsules will be distributed in randomly numbered envelopes.

Method:

(1) Do not begin the experiment until you are near to your bed and ready to retire.

(2) One hour (60 minutes) before your usual bedtime:

a. Record the number of your capsule on the data sheet.
b. Swallow the capsule with some water.

(3) If, during the next 60 minutes, you feel sleepy, go to bed. If not, go to bed anyway 60 minutes after taking the capsule.

(4) When you wake up in the morning, try, as best you can, to record on the data sheet the number of minutes it took you to fall asleep from the time you took the capsule.

(5) Answer the other questions on the data sheet and return the completed data sheet to the Pharmacology Secretary's Office (Room 3152) by noon on the day or weekend after taking the drug. The data will be summarized for you so that the results may be analyzed statistically during the assigned laboratory period.

Drugs: Pentobarbital Sodium, U.S.P. - 150 mg
Caffeine Citrated, U.S.P. - 200 mg
Lactose Powder, U.S.P. - 300 mg

Equipment: Drug envelopes

DATA SHEET FOR EXPERIMENT 6

Name: _____
Capsule No.: _____
Date Taken: _____

Estimated time to fall asleep (minutes after capsule) _____

Answer all questions by either Yes or No. Circle Correct Answers

Answer these questions upon waking:

Did you sleep more soundly than usual? Yes No

Did you sleep less soundly than usual? Yes No

Answer this question at least 4 hours after rising:

Did you have hangover drowsiness? Yes No

Please add any remarks you consider significant:

Please return this data sheet completed to the Pharmacology Secretary's Office (Room 3152) by noon on the day after taking the capsule.

Formulae

t Test (measurement data)

Standard deviation: $$S = \sqrt{\frac{\sum (\bar{x} - x)^2}{(N-1)}}$$

Standard error: $$SE = \frac{S}{\sqrt{N}}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}$$

Degrees of freedom: $n = N_1 + N_2 - 2$

Example: $t = 2.301$ $n = 30$

From table: $p < 0.05$ and $p > 0.02$

Conventionally we write: $0.05 > p > 0.02$ (significant)

Chi Square Test (enumeration, Yes-No, data)

$$X^2 = \sum \frac{[\ (\text{Observed-Expected})^* - 0.5^+\ ]^2}{\text{Expected}}$$

*This difference between the observed and expected values is the absolute difference (ignore the sign). When this absolute difference is 0.5 or less, the whole term may be taken as zero.

+Yates' correction factor, used when small numbers are involved.

Legend:

- $\bar{x}$ = arithmetic mean
- x = individual value
- N = number in group
- n = degrees of freedom
- S = standard deviation
- SE = standard error of the mean
- p = probability

Student's t Distribution

Probability

n	.1	.05	.02	.01	.001
1	6.314	12.706	31.821	63.657	636.619
2	2.920	4.303	6.965	9.925	31.498
3	2.353	3.182	4.541	5.841	12.941
4	2.132	2.776	3.747	4.604	8.610
5	2.015	2.571	3.365	4.032	6.859
6	1.943	2.447	3.143	3.707	5.950
7	1.895	2.365	2.998	3.499	5.405
8	1.860	2.306	2.896	3.355	5.041
9	1.833	2.262	2.821	3.250	4.781
10	1.812	2.228	2.764	3.169	4.587
11	1.796	2.201	2.718	3.106	4.437
12	1.782	2.179	2.681	3.055	4.318
13	1.771	2.160	2.650	3.012	4.221
14	1.761	2.145	2.624	2.977	4.140
15	1.753	2.131	2.602	2.947	4.073
16	1.746	2.120	2.583	2.921	4.015
17	1.740	2.110	2.567	2.898	3.965
18	1.734	2.101	2.552	2.878	3.922
19	1.729	2.093	2.539	2.861	3.883
20	1.725	2.086	2.528	2.845	3.850
21	1.721	2.080	2.518	2.831	3.819
22	1.717	2.074	2.508	2.819	3.792
23	1.714	2.069	2.500	2.807	3.767
24	1.711	2.064	2.492	2.797	3.745
25	1.708	2.060	2.485	2.787	3.725
26	1.706	2.056	2.479	2.779	3.707
27	1.703	2.052	2.473	2.771	3.690
28	1.701	2.048	2.467	2.763	3.674
29	1.699	2.045	2.462	2.756	3.659
30	1.697	2.042	2.457	2.750	3.646
40	1.684	2.021	2.423	2.704	3.551
60	1.671	2.000	2.390	2.660	3.460
∞	1.645	1.960	2.326	2.576	3.291

The X^2 Distribution

1	2.706	3.841	5.412	6.635	10.827

n = degrees of freedom

Experiment 7

Morphine and Synthetic Narcotics

Species and individual variation to the effects of morphine is very marked, but the rabbit and rat exemplify the usual human response to morphine. Just as some species (cats, horses) become stimulated or excited instead of depressed following morphine injection, and others (dogs) show initial vomiting, so certain human beings show comparable individual idiosyncrasies, which parallel the species idiosyncrasies to be demonstrated.

One-half of the class will perform Method 1, below, while the other half will perform Method 2. All students will familiarize themselves with both portions of the experiment. Ample opportunity will be available for one group of students to observe the progress of an opposite group.

1. Morphine (or Methadone) Narcosis: Students at odd-numbered desks will perform this experiment.

Record observations on the rate and depth of respiration, heart rate, response to pain, reflexes, muscle tone, posture, size of pupils and general behavior of a rabbit. Test its respiratory and pupillary response to carbon dioxide.

A. Inject morphine SO_4 - 20 mg/kg - s.c. (or alternatively, if so instructed: inject methadone - 8 mg/kg - s.c.). The drug solution used to induce narcosis in the rabbit should be in a concentration of 50 mg/ml

Omitting the administration of carbon dioxide, repeat your observations at 15-minute intervals, and if at the end of 45 minutes marked depression is not observed, inject an additional one-half of the original dose. Note particularly the animal's indifference to stimuli of uniform strength, even though painful, and its quite normal response to sudden changes in intensity of stimulation (sudden shocks).

B. When the respiration shows marked depression, administer the 95% O_2 - 5% CO_2 mixture, and compare its effect on the rate and depth of respiration and pupillary size with its effect on the animal before treatment.

C. Inject caffeine sodium benzoate - 10 mg/kg - s.c.

Observe the effects of the caffeine on morphine (or methadone) narcosis, repeating all the above observations. If necessary, repeat the injection of caffeine sodium benzoate.

D. If the rabbit is not already severely depressed, it should now be given an overdose of the narcotic in order to demonstrate more clearly the analeptic action of nalorphine. Therefore, cautiously injection morphine SO_4 - 20 mg/kg - i.v. (or methadone - 8 mg/kg - i.v.). Be prepared to administer nalorphine HCl - 1 mg/kg - i.v. at once if respiration should stop or if the animal should convulse. If not faced with an emergency, repeat observations

of pulse and respiration before injecting nalorphine (dose and route indicated above). Note the dramatic analeptic effect which this drug has as soon as it is injected. Repeat the above observations and compare with the data after caffeine.

E. A table will be provided on the blackboard for recording the following data on respiration and pulse: rates before morphine and at 15, 30 and 45 minutes after morphine; rates before and after CO_2, caffeine, and nalorphine.

2. Analgesics in Rats: Students at even-numbered desks will perform this experiment.

The principle pharmacologic response to morphine is the relief of pain. Morphine relieves pain without blocking motor activity and may not seriously interfere with consciousness.

In man, morphine seems to increase the threshold to pain. Other compounds which equal or surpass the analgesic activity of morphine and do not have the addictive property of morphine have been sought, but have not been found.

Each group will obtain 8 rats, weigh each one, and place each rat in the restraining device, leaving the tail extended. Using the electrical apparatus (wire coil and rheostat), determine what rheostat setting (usually not more than 15) will provide a voltage that produces a response (voluntary removal of the tail from the wire) within 3-5 seconds. In no case should the rat's tail be exposed to the hot wire for longer than 10 seconds. Between determinations the wire should be cooled by applying moist toweling. When this optimal voltage is established, do not change the rheostat setting.

Now determine the reaction time to the thermal stimulus for each of the 8 rats by making 3 separate tests on each rat. Report the average of the 3 tests as the "control" value.

Administer the drugs according to the following protocol:

Rat No.	Drug	Dose	Route
1 & 2	Morphine*	3 mg/kg	s.c.
3 & 4	Methadone*	3 mg/kg	s.c.
5 & 6	Meperidine	30 mg/kg	s.c.
7 & 8	Codeine	18 mg/kg	s.c.

*Use drug concentration = 10 mg/ml

Without changing the rheostat setting, determine at intervals of 20 minutes the time required to elicit a response after the analgesics. Follow the effects of these analgesics for at least 2 hours, making 3 tests (report averages) on each rat at each time interval.

Each group will tabulate its data on the blackboard. In the laboratory report each student will use the data pooled from the entire class.

Compare the relative effectiveness and potency of these 4 analgesics.

3. Demonstration: Certain laboratory animals will be injected as follows:

cat	- morphine	-	20 mg/kg - s.c.
mouse	- morphine	-	0.5 mg (total dose) s.c.
mouse	- meperidine	-	5.0 mg (total dose) s.c.

When the effects of the morphine in the cat have been clearly demonstrated, nalorphine will be injected 1 mg/kg - s.c.

Observe the responses of these animals to the administered drugs.

Animals:
- Cats
- Mice
- Rabbits
- Rats

Drugs:
- Codeine - 40 mg/ml
- Meperidine - 50 mg/ml
- Methadone - 10 mg/ml
- Morphine - 10 mg/ml
- Nalorphine - 5 mg/ml
- Caffeine Na benzoate - 20 mg/ml
- Morphine - 50 mg/ml
- Methadone - 50 mg/ml
- Cylinder 95% O_2-5% CO_2

Equipment:
- Rat holder
- Thermal stimulating apparatus
- Stopwatch
- Tubing and funnel for gas cylinder
- Scales for rats and rabbits
- Other in desk

Experiment 8

The Laboratory Evaluation of Anticonvulsant Drugs

Introduction

The bromides and barbiturates were the most widely used antiepileptics until about 1940. These drugs were effective but they produced undesirable sedation and drowsiness. In the late 1930's Putnam and Merritt (1, 2) published results of experiments which not only introduced diphenylhydantoin as a new anticonvulsant with little or no sedative properties, but also established electroshock induced convulsion in cats as a means of screening anticonvulsant drugs. This method modified for use in mice and rats (3, 4) has been used to develop the clinically effective drugs used for the treatment of epilepsy today. A second method for testing the anticonvulsant properties of drugs is to determine their effectiveness in protecting rats against pentylenetetrazol, 93 mg/kg. This chemshock method (4) will be demonstrated (see Part 2, A&B).

Table 1, based on data from Swinyard et al. (4) shows the relative effectiveness of the more useful antiepileptics. In general, the compounds which are most effective against electroshock are useful for "grand mal" epilepsy whereas those that are selectively effective against chemshock are potentially good for "petit mal" seizures. The data for diphenylhydantoin and trimethadione illustrate this point. Diphenylhydantoin which affords excellent protection against electroshock, and essentially no protection against chemshock is the drug of choice for "grand mal" epilepsy. Trimethadione selectively protects rats against chemshock and is the drug of choice for "petit mal" epilepsy. It should be noted that the higher the protective index (PI) the more effective the compounds as an anticonvulsant.

Table 1

Anticonvulsant and Sedative Properties of Clinically Effective Antiepileptics in Rats

Drug	SD_{50}-mg/kg	Electroshock		Chemshock	
		PD_{50}-mg/kg	PI	PD_{50}-mg/kg	PI
Phenobarbital	48	10	5	35	1.4
Diphenylhydantoin	125	10	12	--	<1
Trimethadione	450	375	1	300	1.5
Mephobarbital	80	18	4	40	2.0

SD_{50} = Sedative Dose
PD_{50} = Protective Dose
PI = Protective Index
PI = SD_{50}/PD_{50}

1. Protection Against Electroshock Convulsions in Rats

Weigh six rats, mark and place in a cage. An anticonvulsant will be assigned for testing by your group. Using an oral needle, give to each of five rats one of the doses from the graded series listed for one of the following drugs. The instructor will demonstrate the use of the oral needle.

Phenobarbital:	0, 3, 10, 30, 100, 150 mg/kg
Diphenylhydantoin:	0, 5, 10, 50, 100, 500 mg/kg
Trimethadione:	0, 100, 200, 400, 700, 1000 mg/kg
Mephobarbital:	0, 3, 10, 30, 100, 150 mg/kg

Allow an hour for absorption of the drugs. During this interval an instructor will carry out the demonstrations described in Parts 2 and 3 of this experiment.

After one hour start observations to determine if the animal is sedated. First observe the control rat followed by the rat receiving the highest dose; sedation can be detected by the following tests:
Positional sense test: Gently lower a hind leg over the edge of the table. The normal rat will quickly pull the leg up to a normal position; the sedated rat returns the leg more slowly.
Righting test: Place the rat on its back; the control quickly assumes a normal posture; a sedated animal more slowly so.
Gait and stance test: The normal rat moves about a table top in a purposeful manner; a sedated animal will exhibit ataxia, circular and zigzag gait, quiescence, abnormal body posture and lack of exploratory activity.
Muscle tone test: Normal rats have a characteristic skeletal muscle tone whereas neurological depression results in flaccidity.
Equilibrium: If a rat is placed on a narrow edge such as the rim of a cage, it can maintain equilibrium and even move along the rim. A sedated animal will usually fall from a narrow edge. Report any observed neurological deficiency as sedation.

Using these criteria, determine if the rat at each dose is normal (o) or sedated (+).

One and one-half hours after the administration of the anticonvulsant drug, proceed to shock the animals using the Hans Electroshock Apparatus, 150 milliamps for 0.2 seconds. In carrying out this procedure, it is important that the foot switch be held for at least 0.2 seconds and that the rat be held securely in order that an initial lurch does not break contact prematurely. Dip the electrodes in physiological saline before applying to the cornea. This shock will produce convulsions with the extensor component in 100% of the unprotected rats. The following components of the seizure are rather uniform in the rat:
(a) Latent period of about 2 seconds. (b) Flexor component of tonic phase with flexion of all limbs, about 2 seconds. (c) Extensor component of tonic phase with extreme extension of hind leg lasting about 5-10 seconds, ending with abrupt relaxation. It is this extensor component of the tonic phase which is abolished by the anticonvulsant drugs. (d) Clonic phase which is one or more extensor thrusts and is often absent. The seizure lasts about 12-15 seconds in rats and is followed by (e) Post-seizure depression of about 4 minutes.

Proceed to shock each rat and record whether the animal is normal (o) or protected (+) by the drug. Remember that the absence of the extensor component of the tonic phase indicates protection.

Record in the table at the end of this experiment the data obtained for the control animals and for each dose of drug used. Hand this in at the end of the exercise. It will be tabulated and returned at the next class period.

The data in Table 1 was obtained by experienced observers. It is likely that your values will be somewhat higher. Estimate the sedation dose, the PD_{50} and calculate the PI for each compound tested by the class.

The therapeutic dose of these drugs for adults are as follows: phenobarbital, 30 mg; diphenylhydantoin, 100 mg; trimethadione, 300 mg; and mephobarbital, 60 mg. Can you relate this to the sedative dose in rats?

2. Protective Actions of Anticonvulsants vs Drug-Induced Convulsions

A. Trimethadione vs Pentylenetetrazol: Observe the normal activity and responsiveness of 2 untreated rats and 4 rats treated 30 minutes previously with trimethadione, 300 mg/kg, p.o. All 6 rats will be injected with pentylenetetrazol, 100 mg/kg, s.c.

Follow the animals' behavior for 30 minutes. In the case of severe convulsions, animals will be protected with pentobarbital Na, 40 mg/kg, i.p.

B. Phenobarbital vs Pentylenetetrazol: As above, observe 4 rats treated 30 minutes previously with phenobarbital Na, 25 mg/kg, p.o. All 4 rats will be injected with pentylenetetrazol, 100 mg/kg, s.c.

Follow the animals' behavior for 30 minutes and compare with that of the rats receiving only pentylenetetrazol in "A" above. Animals that convulse severely will be protected as above.

3. Barbiturates as Antidotes for Drug-Induced Convulsions

A. Observe a rat before and after the administration of picrotoxin, 8 mg/kg, i.p. When a convulsion occurs, pentobarbital Na, 40 mg/kg, will be given intraperitoneally. Does the barbiturate effectively control the convulsion?

B. Following the procedure above, a rat will be given cocaine HCl, 75 mg/kg, i.p. and treated with pentobarbital Na, 40 mg/kg, i.p. should a convulsion occur? Does the barbiturate effectively control the convulsion?

Examine the clinical preparations on display. List them in your laboratory report indicating a specific use and principle side effect of each.

REFERENCES: These are classical experiments which led to the development of clinically useful anticonvulsants. Please examine the copies of these papers displayed on the shelves near the stock room.

1. Putnam, T.J. and Merritt, H.H. Experimental determination of the anticonvulsant properties of some phenyl derivatives. Science, 85:525, 1937.

2. Merritt, H.H. and Putnam, T.J. A new series of anticonvulsant drugs tested by experiments on animals. Arch. Neurol. Psychiat. 39: 1003, 1939.

3. Toman, J.E.P., Swinyard, E.A. and Goodman, L.S. Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents. J. Neurophysiol. 9: 231, 1946.

4. Swinyard, E.A., Brown, W.C. and Goodman, L.S. Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. 106: 319, 1952.

Animals: Rats

Drugs:
Cocaine - 30 mg/ml
Diphenylhydantoin - 10 mg/ml
Pentobarbital Na - 25 mg/ml
Pentylenetetrazol - 100 mg/ml
Mephobarbital Na - 10 mg/ml
Phenobarbital Na - 10 mg/ml
Picrotoxin - 2.5 mg/ml
Trimethadione - 50 mg/ml

Equipment: Electroshock apparatus, model 2-C, Hans Technical Associates, Palo Alto, California
Oral needles

Display: Clinical package of each of above anticonvulsants

Table # _____ Names _____

Anticonvulsant Drug _____ Date _____

Dose mg/kg							
Sedation							
Electroshock Protection							

+ Sedation or Protection

0 No Sedation or Protection

Experiment 9

Antagonism of Barbiturate Poisoning

There are certain drugs which act as stimulants of the central nervous system, and find clinical applications in the treatment of respiratory depression. The drugs which are of special value in antagonizing drug-induced CNS depression are referred to as "analeptics."

Obtain and weigh a dog, observing the normal respiratory and cardiac rates and conditions. Anesthetize the animal by the i.v. administration of pentobarbital Na, 30 mg/kg. Fasten the anesthetized animal to the dog board and arrange to record mean arterial blood pressure, cardiac rate (tachograph) and respiratory activity on the Grass Polygraph. Cannulate a femoral vein with a piece of polyethylene tubing attached to a stopcock for intravenous injection of drugs. Refer to Appendices II and III for directions on cannulations and the use of the Grass Polygraph.

In order to reduce the respiratory rate to an adequately depressed level (25 respirations/min. or less), cautiously supplement the initial dose of the anesthetic with additional doses of 5 mg/kg each. The analeptics are most effective when the respiration is in a depressed state. When a regular control period has been achieved, and observations indicated in the table have been made, inject intravenously the analeptics indicated. The animal may be returned to a state of depression by injecting more pentobarbital as needed after the maximum effect of an agent is obtained.

Following observation of the effects of picrotoxin, administer supplemental doses of pentobarbital until the respiration stops and the mean blood pressure falls to about 50 mm Hg. Immediately begin artificial ventilation in an effort to restore the blood pressure to normal values. Record your observations as part 5 of the table.

While maintaining artificial ventilation, administer further doses of pentobarbital until the blood pressure again falls to about 50 mm Hg. This signifies that the total dose of barbiturate has become great enough to cause direct myocardial depression. Set up an intravenous drip of norepinephrine (4 μg of norepinephrine base/ml of 5% glucose) and by adjusting the rate of infusion, attempt to restore the blood pressure to the normal range and maintain it there for 15 minutes. Record your observations as part 6 of the table.

Animals: Dogs

Drugs:
- Amphetamine SO_4 - 50 mg/ml
- Caffeine Na benzoate - 500 mg/ml
- Nikethamide - 250 mg/ml
- Norepinephrine bitartrate - 8 μg/ml in 5% glucose
- Pentobarbital Na - 60 mg/ml
- Picrotoxin - 1.5 mg/ml

Equipment:

Grass Instruments	Newspapers
Dog boards	Animal clippers
Muzzles	Gauze sponges
Intravenous drip bottles	Tracheal cannulae
Intravenous drip indicators	Artificial respiration hoses

ANTAGONISM OF BARBITURATE POISONING

Analeptic Drug	Dose mgm/kg	Respiratory Rate		Respiratory Amplitude (mm)		Cardiac Rate		Blood Pressure mm/Hg	
		Control	After Drug	Control	After Drug	Control	After Drug	Control	After Drug
1. Nikethamide	75								
2. Caffeine Na Benzoate	50								
3. Amphetamine Sulfate	20								
4. Picrotoxin	0.6								
5. Artificial Ventilation									
6. Norepinephrine after Myocardial Depression									

EXPERIMENT 10

ACTION OF LOCAL ANESTHETIC AGENTS I

Anesthesia means absence of sensation. General anesthesia is produced by agents which depress the central nervous system and is accompanied by loss of consciousness. In this type of anesthesia all tissues of the organism are exposed to approximately equal concentrations of the anesthetic agent and are affected by the toxic properties of the drug. In local anesthesia a high concentration of the anesthetic agent with complete nerve block is obtained at the point where it is needed. Other tissues of the body are exposed to much lower concentrations of the drug. Depending upon the site of injection or type of action, local anesthesia is often subdivided into "surface," "spinal," "block," or "infiltration" anesthesia.

1. Infiltration Anesthesia: (groups of 4 students)

Students with known sensitivity to any of these agents should refrain from participation in this portion of the experiment.

Each student in the group will receive two injections, one control (upper wheal) and one experimental (lower wheal). All will receive the same control injection: 0.1 ml of 2% procaine hydrochloride. Each student in the group will receive a different experimental drug injection: 0.1 ml of one of the following:

(a) 2% procaine hydrochloride + 0.002% epinephrine hydrochloride
(b) 2% procaine hydrochloride + 0.002% histamine diphosphate
(c) 0.25% dibucaine hydrochloride
(d) 2% lidocaine hydrochloride

Sterile syringes and needles will be provided by the instructor who will also make the injections or supervise students wishing to make injections. Preparation for the injection will be done by the students. Scrub the inner surface of the forearm with soap and water, rinse with water, then with 70% ethyl alcohol and allow to dry. The injections should be intracutaneous, not subcutaneous, and the two wheals should be about three inches apart.

Using a hypodermic needle, determine the duration of anesthesia by testing the wheal every few minutes. The end of anesthesia is indicated by a sensation of pain (not pressure) when the wheal is stimulated with the needle. Note the distance away from the injection site that shows any anesthesia. Also note whether there is any hyperemia or ischemia, and what effects they may have on the duration and extent of anesthesia, in comparison with the control injection of procaine. Record the duration (to the nearest minute) and extent (diameter to the nearest millimeter) of the anesthesia in the appropriate space of the data sheet. Draw your conclusions from the class averages.

2. Surface Anesthesia (groups of 4 students)

Students with known sensitivity to any of these agents should refrain from participation in this portion of the experiment.

Each student in the group will test one of four local anesthetics. The local anesthetics to be tested are:

(a) 2% cocaine hydrochloride
(b) 1% dibucaine hydrochloride
(c) 10% procaine hydrochloride
(d) 5% lidocaine hydrochloride

Grasp your lower lip with the left hand and pull it away from the gum. Place the tongue in the concavity formed so as to form a trough with the teeth and lips. Place 1.0 ml of the solution to be tested in this trough for exactly one minute, at which time it is expectorated and the time noted to the nearest ten seconds. The tester then waits for anesthesia which usually appears successively in the tongue, lips and gums.

Be sure to distinguish between true anesthesia and paresthesia (abnormal sensation, as prickling, itching). Anesthesia is indicated by the absence of feeling when a hypodermic needle is pressed against the tissue or when no pain occurs when the fingernail is pressed against the gum below the alveolar ridge. Using this method, determine (1) the length of time it takes for the anesthesia to become effective and (2) the length of time the anesthetic is effective. Record the two times to the nearest minute in the appropriate spaces on the data sheet.

3. Spinal Anesthesia: (groups of 2 students)

Cut off the head of a frog at the level of the angle of the mouth and suspend by the lower jaw. Use a silver electrode stimulator and inductorium (See Appendix IV). Determine the minimal tetanizing current which will produce contraction in the hind limb. Now insert a No. 25 hypodermic needle as close to the spinal cord as possible without injuring the cord, entering at the cut end, and inject 0.1 ml of 1% procaine hydrochloride. Determine the rate of onset and depth of anesthesia by electrical stimulation of the hind limb (starting with the current which just produced contraction before the injections).

N.B. Local stimulation of the leg muscles may occur if current of sufficient intensity is used; this should not be confused with reflex stimulation.

4. Blocking Anesthesia: (groups of 2 students)

From the frog used above and with attention to the suggestions that follow, make a muscle-nerve preparation. Strip the skin from the back and leg of the frog in such a manner as to avoid contact of the external skin surfaces with the muscles and nerves to be used. Use glass needles to isolate as long a piece of sciatic nerve as possible. Cut the femur and tibiofibula close to the knee joint and remove from the frog as a unit the sciatic nerve, the knee joint and the gastrocnemius muscle. Fasten the proximal end of the muscle to the wax of a dissecting pan by means of pins passed through the tissue of the knee joint. Tie a thread to the tendon of Achilles. Attach the free end of this thread to the strain gauge that is connected with channel #3 of the Grass Polygraph (See Appendix III). Lay the nerve in a shallow groove in the wax. Cover the nerve with a small amount of Ringer's solution. Use masking

tape or pins to fasten a silver electrode stimulator to the surface of the dissecting tray in such a way that the electrodes extend over the groove containing the nerve. Lift the free end of the nerve from the groove and lay it across the electrodes. The portion of the nerve between the electrodes and the muscle should remain immersed in Ringer's solution and the free portion of the nerve held above the groove should be kept moist by the occasional application of a drop of Ringer's solution. The electrodes should not be immersed nor should a drop of Ringer's solution be permitted to bridge the gap between the electrodes. Connect the stimulator with the secondary coil of an inductorium. Wire the inductorium to deliver "make and break" stimulation (Appendix IV). Connect the primary coil of the inductorium (with a key in circuit) to direct current (3.6 volts). Set "Sensitivity MV/CM" of polygraph channel #3 at "2" (this setting should prove satisfactory for most preparations).

A. Determine the minimal "make and break" current that will produce a recordable contraction when applied to the free end of the nerve as described above. Adjust "Sensitivity MV/CM" if necessary.

B. Operate the chart driver of the polygraph at its slowest speed (0.25 mm/sec) and obtain a record of the muscle's responses to "make and break" stimulation applied at one minute intervals.

C. While continuing stimulation, replace the Ringer's solution bathing the nerve with 1% procaine hydrochloride and record the onset of anesthesia.

D. When the nerve has been blocked, replace the procaine solution with Ringer's solution. Continue stimulation, as above, and change the Ringer's solution in the groove at intervals of one to two minutes until the nerve recovers. Try to avoid disturbing the contact of nerve and electrodes.

E. If the nerve recovers, record for several minutes as a control and repeat the above procedure using 0.2% dibucaine hydrochloride.

F. If the nerve fails to recover from blockade first determine the effect of a slight change in the position of the nerve upon the electrodes. If this measure fails to elicit a response, remove the electrodes from the tray and attempt direct stimulation of the muscle.

Animals: Frogs

Drugs: Ringer's solution (frog)

Cocaine HCl	-	2% in water
Dibucaine HCl	-	1% in water
Dibucaine HCl	-	0.2% in Ringer's solution
Lidocaine HCl	-	5% in water
Procaine HCl	-	1% in Ringer's solution
Procaine HCl	-	10% in water
Ethyl alcohol	-	70%

Sterile solutions:

Dibucaine HCl	-	0.25%
Lidocaine HCl	-	2%
Procaine HCl	-	2%

Procaine HCl (2%) + Epinephrine 0.002% (1:50,000)
Procaine HCl (2%) + Histamine diphosphate 0.002%
Distilled water

Equipment:

Tuberculin syringes (sterile)	
Hypodermic needles (25-gauge; 5/8 in.; sterile)	
Gauze swabs	Grass Polygraph
Dissecting pans	Glass needles
Stimulators	Pins
Other: available in desks	

Data Sheet

Experiment 10

Name _____

Date _____

Part 1 Infiltration Anesthesia

	Control	Experimental Drug
	2% procaine HCl	_____
Duration of Anesthesia in Minutes	_____	_____
Extent of Anesthesia in millimeters	_____	_____

Part 2 Surface Anesthesia

Experimental Drug _____

Time for anesthesia to become effective: _____ minutes

Duration of anesthesia: _____ minutes

This sheet is to be submitted at the conclusion of the laboratory period. Class data will be summarized and distributed.

Experiment 11

Action of Local Anesthetic Agents II

This experiment, which extends observations of local anesthetic action as exemplified by Experiment 10, will be done with students working in groups of four in the sense that all students should be familiar with the procedures and results of all parts of the experiment. In practice, students at odd numbered desks will perform parts A and B, and students at even numbered desks will carry out part C.

A. Blocking Anesthesia (in vivo)

1. Carefully and closely clip the hair from the outer surface of both ears of a rabbit.

2. In Appendix IV review the operation of the inductorium. Connect a silver electrode stimulator to the secondary coil of an inductorium set up to deliver tetanizing current and adjust the secondary coil to provide a current that is moderately stimulating when tested on the inner surface of your wrist and a clipped ear of the rabbit. Fasten the secondary coil in the location determined by these tests.

3. About one inch from the base of the rabbit's right ear on the outer surface directly over the median blood vessels inject, subcutaneously, with a 25 gauge needle, 0.1 ml of 2% lidocaine HCl. Into the same ear inject 0.1 ml 2% lidocaine HCl, subcutaneously, on the inner side of the ear at a point directly opposite the first injection. Do these treatments result in any change in the appearance of the ear as compared with the untreated ear?

4. Into the left (untreated) ear, in an area free from major blood vessels, inject 0.1 ml of 2% lidocaine HCl, subcutaneously.

5. Five minutes after the injections use tetanizing current and with the stimulator determine on the outer surface of both ears the approximate dimensions, in millimeters, of the anesthetized surfaces. Test the ears again 15 minutes after anesthetic injections. Can you detect any change in the dimensions of the anesthetized surfaces?

B. Surface Anesthesia

1. Open the mouth of a frog and move the blade of a pair of scissors back to the angle of the jaw. Cut off the top of the frog's head by cutting through the head at the level of the trympanic membranes. Rinse the body of the frog with tap water. Place the jaw of the frog in a flat jaw clamp and fasten the clamp to a stand in such position that the frog is suspended with its feet about six inches above the table.

2. Test one hind foot at a time and determine, in seconds, the time for reflex withdrawal following immersion of the foot in 0.5% HCl. For each foot, at intervals of at least one minute, carry out three or more tests and record the average time for withdrawal. After each test rinse with tap water the leg, legs or even body posterior to the forearms, by dipping into a beaker raised from beneath the suspended frog.

3. Hold the shank of the right leg and immerse the right foot for 10 seconds in a 2% aqueous solution of phenol. Restrain the leg to minimize splashing of the phenol solution. After 60 seconds rinse the foot briefly in tap water. Discard the rinse water and thoroughly rinse the vessel.

4. Repeat step 2 (above) testing first the right foot. If no response is obtained within 60 seconds exposure to the acid, record withdrawal time as > 60 seconds, give the foot a brief rinse with tap water, discontinue further tests with the right foot and complete tests with the left foot.

5. Suspend the frog knee deep in fresh tap water for 15 minutes, change water once or twice during this interval. Then repeat step 2 as directed in step 4 (above).

6. Obtain another frog and repeat steps 1 and 2.

7. Punch a hole in a paper towel and insert right foot of the frog through this hole. Use the towel to protect the rest of the frog and with an atomizer lightly spray the frog's foot with oil of clove. Blot the foot to remove any excess of clove oil, discard the towel, restrain the treated leg to avoid contamination of other skin surfaces with clove oil and after 60 seconds briefly rinse the treated foot in tap water. Discard this rinse water and thoroughly wash the container.

8. Repeat step 2 as directed in step 4 (above).

9. Repeat step 5 (above).

What clinical uses are made of the two agents tested? What is the principal constituent of clove oil?

C. Systemic Toxicity

Aside from depressing the activity of nerve endings or nerve fibers at or near the site of administration, local anesthetics in overdose may have more generalized and often disconcerting systemic effects. For example, they may cause convulsions, respiratory depression and even death.

Each group of students (at even numbered desks) will be provided with 12 mice and 4 jars. Number the jars I---IV and place 3 mice, distinctively marked with dye, in each jar. Observe the normal activity of the mice.

Each group will be assigned one of three drugs for use in the experiment. Administer the assigned drug subcutaneously to each of the 3 mice contained in a jar at mg/kg dosage levels indicated in the following table:

Jar / Drug	Dosage in mg/kg			
	I	II	III	IV
Procaine HCl	300	600	900	1200
Lidocaine HCl	160	320	480	640
Dibucaine HCl	10	20	30	40

Two concentrations of each drug will be available. For a particular dosage use a concentration that will keep the drug volume within the range of 0.1 - 0.5 ml.

Use the record sheet provided (page 11/4). During the experiment keep records in terms of clock time (eg. 2:27). Later, convert to minutes time from injection to convulsion and/or death. During the experiment watch the mice closely. Note any abnormal activity or behavior, eg., hyperactivity, ataxia, convulsions, opisthotonus; depression, unconsciousness; respiratory depression, Cheyne-Stokes respiration, respiratory paralysis; cardiac failure. In your own written report describe in more detail the responses of the mice you observed. Have your mice exhibited any of the symptoms seen clinically in acute poisoning with local anesthetic agents? Do any of the mice remain normal or return to normal?

Plot the class data for each drug on the logarithmic-probability graph paper provided. A plotting of the logarithm of the dosages on the ordinate (vertical axis) against the percentage of mortality (as probits) on the abscissa (horizontal axis) should produce a straight line (instead of the sigmoid curve that results from a semilogarithmic plot).* From these plots read and report, for each drug, the LD-50 doses, ie., the points at which the dosage-mortality "curves" intersect the line of probit 5 (or the 50% mortality line).

*Litchfield, J.T. and F. Wilcoxon, 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. and Exper. Therap. 96: 99-113.

Animals: Rabbits, frogs, mice

Drugs:

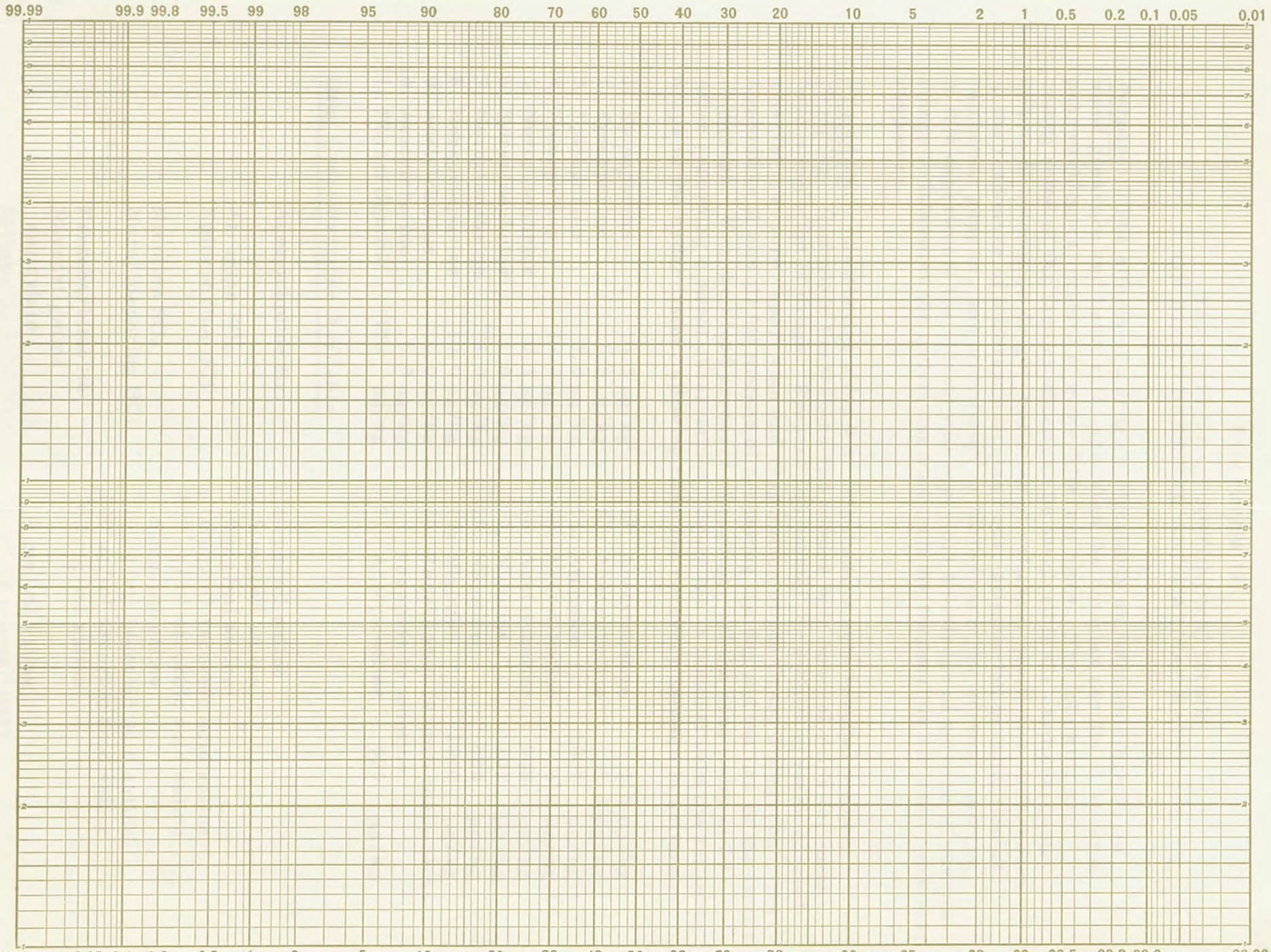
Clove oil USP	
Dibucaine HCl	0.30%
	0.15%
HCl	0.5%
Lidocaine HCl	5%
	2.5%
Phenol	2%
Procaine HCl	10%
	5%

Equipment:
Animal clippers
Silver Electrode Stimulator
Inductorium
Flat jaw clamp
Atomizer (De Vilbiss No. 127)
Jars
Other available in desks

Students _____;_____ Desk#_____ Date_____

Drug_____

Jar#	Dosage	Mouse	Clock Time			Minutes		Observations and Remarks
			Injection	Convulsion	Death	to convulsion	to death	
I		A						
		B						
		C						
II		A						
		B						
		C						
III		A						
		B						
		C						
IV		A						
		B						
		C						



Experiment 12

THE EFFECT OF AUTONOMIC DRUGS ON BLOOD PRESSURE AND CARDIAC RATE

Purpose: The effects of a drug, particularly an autonomic agent, on blood pressure and heart rate, are often the complex results of several pharmacological actions at many different sites. The following are among some of the factors which can be involved in the cardiovascular effects of autonomic drugs: initiation of reflexes, qualitative differences in the responses of different vascular beds, stimulation or inhibition of autonomic ganglia, and stimulation or inhibition of the adrenal medulla. In this experiment the student will apply his knowledge of autonomic pharmacology to interpret the underlying mechanisms responsible for cardiovascular responses.

Procedure: Anesthetize a dog with intravenous pentobarbital sodium, 30 mg/kg. Cannulate the trachea and expose both femoral veins for intravenous injection of drugs. Prepare the animal for recording mean carotid arterial pressure, heart rate and respiration on the Grass Polygraph. See Appendices II and III for method of preparing the dog and the use of the Grass Polygraph. For the blood pressure recording, set the sensitivity at 20. After recording control observations, inject into a femoral vein the following drugs in the order listed, allowing time for recovery between injections.

1. Epinephrine	0.3 µg/kg	12. Acetylcholine	1.0 µg/kg
2. Epinephrine	1.0 µg/kg	13. Acetylcholine	5.0 µg/kg
3. Epinephrine	3.0 µg/kg	14. Atropine	1000 µg/kg
4. Epinephrine	10.0 µg/kg	15. Acetylcholine	5.0 µg/kg
5. Norepinephrine	0.3 µg/kg	16. Acetylcholine	500 µg/kg
6. Norepinephrine	1.0 µg/kg	17. Nicotine	100 µg/kg
7. Norepinephrine	3.0 µg/kg	18. Nicotine	500 µg/kg
8. Norepinephrine	10.0 µg/kg	19. Nicotine	1000 µg/kg
9. Acetylcholine	1.0 µg/kg	20. Repeat step 19 until there is no response to nicotine	
10. Acetylcholine	5.0 µg/kg		
11. Physostigmine	100 µg/kg	21. Acetylcholine	500 µg/kg

Drug Concentrations Available:

Acetylcholine Br.	50 µg/ml saline
	5 mg/ml saline
Atropine SO_4	10 mg/ml saline
Epinephrine HCl	10 µg/ml ascorbic acid saline
	100 µg/ml ascorbic acid saline
Nicotine SO_4	5 mg/ml saline
Norepinephrine HCl	10 µg/ml ascorbic acid saline
	100 µg/ml ascorbic acid saline
Pentobarbital Na	60 mg/ml saline
Physostigmine salicylate	2 mg/ml saline
Saline	0.9% (9 grams/liter)
Ascorbic saline	100 µg/ml

Report: Plot the blood pressure dose-response curves for norepinephrine and epinephrine on the sheet of semi-log paper provided. The dose should be plotted along the semi-log scale of the abscissa and the response on the ordinate. For each dose, plot the increase in mean arterial pressure above the preinjection pressure. Near each point on the curves, write in parenthesis the change in heart rate associated with that particular blood pressure response. Indicate increases in rate by + signs and decreases by - signs. In your report record the heart and blood pressure responses to acetylcholine, physostigmine, atropine, and nicotine, indicating the alterations in the response to acetylcholine produced by the other drugs. Discuss the results in terms of your knowledge of autonomic pharmacology.

Equipment:

- Dog boards
- Muzzles
- Newspapers
- Animal clippers
- Gauze sponges
- Tracheal cannulae
- Grass polygraphs
- Other: available in desk

Experiment 13

Autonomic Drugs In Vitro and In Vivo

1. Cholinesterase Inhibition in vitro (work in pairs)

This portion of the experiment is designed to demonstrate the cholinesterase activity of serum in vitro, and to show how it can be affected by certain drugs. The method employed here involves the change in pH brought about by the enzyme as it converts acetylcholine to acetic acid and choline. As the color change proceeds in the tube, the decrease in pH becomes increasingly evident.

Method: Obtain 10 test tubes and label them 1 through 10. In the following order ($NaHCO_3$, acetylcholine, drug or buffer, indicator, and serum) add each reagent to the tubes as indicated in the protocol below:

Tube	$NaHCO_3$ ml	ACh ml	ml	Drug	Universal Indicator ml	Serum ml
1	1.6	1.0	---		0.1	0.5
2	1.5	1.0	0.1	physostigmine	0.1	0.5
3	1.3	1.0	0.3	physostigmine	0.1	0.5
4	1.0	1.0	0.6	physostigmine	0.1	0.5
5	1.5	1.0	0.1	neostigmine	0.1	0.5
6	1.3	1.0	0.3	neostigmine	0.1	0.5
7	1.0	1.0	0.6	neostigmine	0.1	0.5
8	---	1.0	1.6	neostigmine	0.1	0.5
9	---	---	2.6	buffer-pH 6.5	0.1	0.5
10	---	---	2.6	buffer-pH 5.5	0.1	0.5

Note the time at which the 0.5 ml of serum is added to each tube. The reaction starts as soon as the serum is added.

Tube number 8 will serve as a reference standard for the original color before any enzyme activity occurs because the amount of neostigmine contained therein is sufficient to prevent ACh hydrolysis.

Tubes numbers 9 and 10 will serve as reference standards for color endpoints. These particular endpoints are chosen merely as convenient ones for the laboratory period, since the majority of the tubes can be expected to show the color in tube 9 within a reasonable period of time. In no case should even the lower pH color value be taken as the absolute endpoint of cholinesterase activity.

Record in the appropriate spaces on the table provided the times required for the color in each tube to match the colors shown in tubes 9 and 10. For those tubes not reaching the lower pH value (tube 10) it will be sufficient to record an approximate pH value (e.g. lower than 6.5, but higher than 5.5). Describe the significance of and interpret the events taking place in each tube.

Approximate colors at representative pH values as seen with the Universal Indicator are as follows:

pH 9.0 Blue	pH 6.5 Yellow
pH 8.0 Dark Green	pH 5.5 Orange
pH 7.0 Yellowish Green	pH 4.0 Red

Make a careful comparison. The color changes are subtle.

2. Demonstration: In vivo demonstration of the in vitro inactivation of cholinesterase

Anesthetize a dog with pentobarbital Na (30 mg/kg i.v.) and prepare for a Master-Slave demonstration experiment. Record arterial blood pressure (Appendix II). Since acetylcholine produces bronchiolar constriction prepare to record respiration by two methods. Place a tracheal cannula and T-tube arrangement as described in Appendix II. This recording represents primarily the ventilation gas exchange. A second measurement of respiratory rate records thoracic size changes. Stretch a rubber tubing around the thoracic cage. Connect the side arm of a T-tube to a second venous pressure transducer. This recording is made on channel 3. Place electrodes for recording lead 2 of the EKG. The tachygraph should be used in this experiment. Use a venous catheter placed in the femoral vein for all injections. Inject i.v. 3 ml of the contents of tube 1. Allowing time for recovery between injections, administer 3 ml aliquots from each of the tubes in order. These tubes were prepared 20 to 30 minutes prior to injection by the addition, in the order listed, of the indicated reagents.

Tube 1
- 4 ml saline
- 1 ml acetylcholine (50 μg/ml saline)

Tube 2
- 3 ml saline
- 1 ml serum (dog)
- 1 ml acetylcholine (50 μg/ml saline)

Tube 3
- 2 ml saline
- 1 ml serum (dog)
- 1 ml physostigmine (500 μg/ml saline)
- 1 ml acetylcholine (50 μg/ml saline)

Table 4
- 3 ml saline
- 1 ml serum (dog)
- 1 ml physostigmine (500 μg/ml saline)

Interpret the observed effects of the above injections.

3. Demonstration: Methacholine-atropine antagonism

Use the same demonstration set-up as described under 2 for this part of the experiment.

A. Inject methacholine 0.5 mg/kg, s.c. Observe the changes in the recordings. Repeat the dose if necessary.

B. When marked parasympathetic stimulation is evidenced, inject atropine 1 mg/kg, i.v. Note antagonism of the cholinergic effects.

Animals: Dog

Drugs:
- Acetylcholine Br - 50 mg/ml - (in $NaHCO_3$)
- Neostigmine Br - 0.025 mg/ml - (in $NaHCO_3$)
- Physostigmine salicylate - 0.025 mg/ml - (in $NaHCO_3$)
- $NaHCO_3$ - 0.0005 M -- 0.042 mg/ml
- Buffer at pH 6.5
- Buffer at pH 5.5
- Universal indicator solution (Fisher)
- Dog serum

Drugs: (Demonstration)
- Atropine - 10 mg/ml
- Methacholine - 10 mg/ml
- Acetylcholine Br - 50 mg/ml (in saline)
- Physostigmine salicylate - 500 mg/ml (in saline)
- Pentobarbital Na - 50 mg/ml
- Dog serum
- Saline - 0.9%

Equipment:
- Test tube racks
- Dog board
- Muzzle
- Newspapers
- Animal clippers
- Gauze sponges
- Tourniquet
- Pneumograph
- Cannula: arterial
- Instruments and syringes
- Master-Slave Setup
- Other: available in desks

Experiment 14

Autonomic Stimulation and Blockade

Students will work in groups of four or five. Half the class will follow procedure A and half will follow procedure B. EACH STUDENT WILL RECORD ONLY THE DATA OBTAINED BY HIS GROUP, BUT IN HIS DISCUSSION MUST CONTRAST HIS RESULTS WITH THOSE OBTAINED BY OTHER STUDENTS USING THE OTHER PROCEDURE.

Procedure A is designed to demonstrate (1) the action of sympathomimetic amines, and other drugs, that may markedly affect blood pressure and (2) the effect upon these actions of a sympathetic blocking agent. Procedure B is designed to illustrate quantitatively the effect of a parasympathetic blocking agent upon the dose-response relationship manifested by the action of graded doses of acetylcholine upon the blood pressure of the dog.

Anesthetize a dog with pentobarbital Na (30 mg/kg) i. v. Cannulate the trachea and prepare the animal for blood pressure and tachograph recording. Prepare one femoral vein for the i. v. administration of drugs. Refer to Appendices II and III for methods of preparing the dog and the use of the Grass polygraph. Follow the protocol for the procedure assigned.

A. Record mean blood pressure and heart rate. After making control observations, proceed with the following protocol allowing ample time for effects to be manifest and for recovery between injections.

1.	Norepinephrine	0.3 μg/kg (10 μg/ml solution)
2.	Norepinephrine	1.0 μg/kg (10 μg/ml solution)
3.	Norepinephrine	3.0 μg/kg (100 μg/ml solution)
4.	Norepinephrine	3.0 μg/kg (100 μg/ml solution)
5.	Norepinephrine	10.0 μg/kg (100 μg/ml solution)
6.	Epinephrine	3.0 μg/kg
7.	Phenoxybenzamine	5,000 μg/kg
8.	Norepinephrine	3.0 μg/kg (100 μg/ml solution)
9.	Angiotensin	0.3 μg/kg

Wait at least 30 minutes after phenoxybenzamine before the next drug administration.

10.	Norepinephrine	3.0 μg/kg (100 μg/ml solution)
11.	Norepinephrine	10.0 μg/kg (100 μg/ml solution)
12.	Norepinephrine	30.0 μg/kg (100 μg/ml solution)
13.	Epinephrine	3.0 μg/kg
14.	Angiotensin	0.3 μg/kg

Those students doing part A should tabulate all the results in their report and, in addition, should graph the dose-response curves of norepinephrine on blood pressure before and after phenoxybenzamine. Use the 3-cycle semi-log paper provided. Plot the dose of norepinephrine on the abscissa (log scale) and the increase in pressure above preinjection level, in mm Hg, on the

ordinate (linear scale). Discuss the results, including the type of antagonism demonstrated.

B. Record mean blood pressure and heart rate. Set the sensitivity of the blood pressure recording at 10. After making control observations, carry out the following procedures allowing ample time for effects to be manifest and for recovery between injections of acetylcholine.

1. Find the smallest effective depressor dose of acetylcholine (probably about 0.01 μg/kg). Beginning with this dose, give progressively larger doses by multiples of approximately 3 (e.g. 0.01, 0.03, 0.10, 0.30, 1.0, etc.) until a drop in the blood pressure of 40-50 mm Hg occurs.

   Acetylcholine will be available in three concentrations (1, 10, 100 μg/ml). Choose and use that which will provide the required dose in a convenient volume.

2. Give atropine, 10 μg/kg, and wait 5 minutes before proceeding.

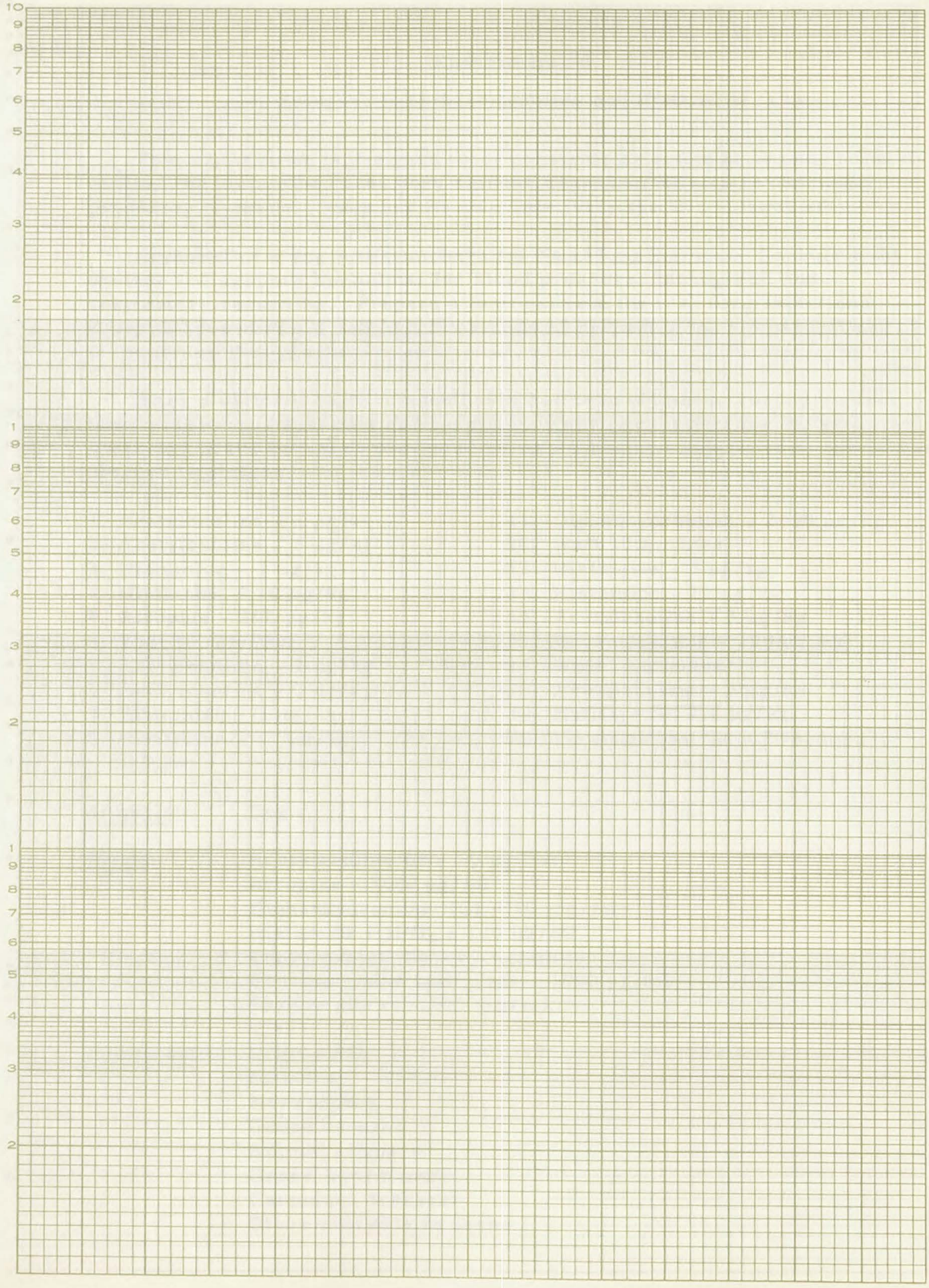
3. Repeat step 1, finding the minimum effective depressor dose and increasing the dose until a response is obtained that is comparable to that achieved at the conclusion of step 1.

4. Give atropine, 100 μg/kg, and wait 5 minutes before proceeding.

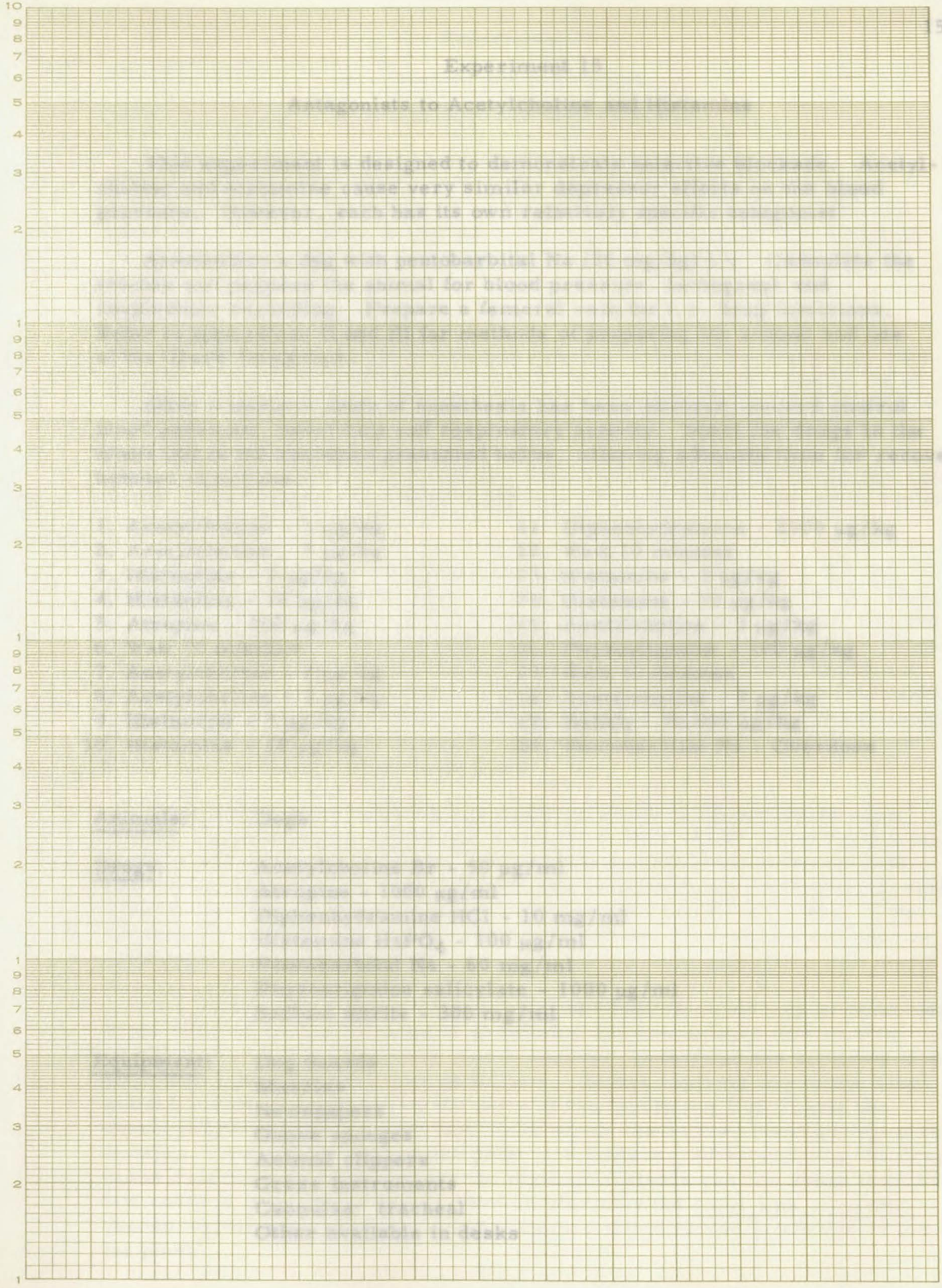
5. Repeat step 3.

Those students doing part B should tabulate all the results in their report and, in addition, should graph the three dose-response curves of acetylcholine on the blood pressure. Use the 4-cycle semi-log paper provided. Plot the dose of acetylcholine on the abscissa (log scale) and the decrease in pressure below preinjection level, in mm Hg, on the ordinate (linear scale). Discuss the results, including the type of antagonism demonstrated.

Animals:	Dogs	
Drugs:	Acetylcholine	- 100 μg/ml
		- 10 μg/ml
		- 1 μg/ml
	Atropine	- 250 μg/ml
	Norepinephrine	- 100 μg/ml
		- 10 μg/ml
	Epinephrine	- 100 μg/ml
	Pentobarbital Na	- 60 mg/ml
	Phenoxybenzamine	- 10,000 μg/ml
	Angiotensin	3 μg/ml
Equipment:	Dog boards	Animal clippers
	Muzzles	Grass instruments
	Newspapers	Cannulae-tracheal
	Gauze sponges	Artificial respiration hoses
		Other: available in desks



SEMI-LOGARITHMIC
3 CYCLES X 10 DIVISIONS PER INCH


10
9
8
7
6
5
4
3
2
1
4 CYCLES X 10 DIVISIONS PER INCH
SEMI-LOGARITHMIC

Experiment 15

Antagonists to Acetylcholine and Histamine

This experiment is designed to demonstrate selective blockade. Acetylcholine and histamine cause very similar depressor effects on the blood pressure. However, each has its own relatively specific antagonist.

Anesthetize a dog with pentobarbital Na (30 mg/kg) i.v. Cannulate the trachea and prepare the animal for blood pressure, tachograph and respiratory recording. Prepare a femoral vein for i.v. drug injections. Refer to appendices II and III for methods of preparing the animal and use of the Grass Polygraph.

After a uniform depth of anesthesia has been obtained, record control blood pressure, heart rate and respiratory activity. Inject the drugs in the doses and in the sequence presented below, allowing adequate time for recovery between injections.

1. Acetylcholine - 1 μg/kg
2. Acetylcholine - 3 μg/kg
3. Histamine - 3 μg/kg
4. Histamine - 10 μg/kg
5. Atropine - 100 μg/kg
6. Wait 10 minutes
7. Acetylcholine - 1 μg/kg
8. Acetylcholine - 3 μg/kg
9. Histamine - 3 μg/kg
10. Histamine - 10 μg/kg
11. Diphenhydramine - 2000 μg/kg
12. Wait 10 minutes
13. Histamine - 3 μg/kg
14. Histamine - 10 μg/kg
15. Acetylcholine - 3 μg/kg
16. Physostigmine - 100 μg/kg
17. Wait 10 minutes
18. Acetylcholine - 3 μg/kg
19. $NaNO_2$ - 30,000 μg/kg
20. Pentobarbital Na - Overdose

Animals: Dogs

Drugs:
- Acetylcholine Br - 50 μg/ml
- Atropine - 1000 μg/ml
- Diphenhydramine HCl - 10 mg/ml
- Histamine H_3PO_4 - 100 μg/ml
- Pentobarbital Na - 60 mg/ml
- Physostigmine salicylate - 1000 μg/ml
- Sodium nitrite - 300 mg/ml

Equipment:
- Dog boards
- Muzzles
- Newspapers
- Gauze sponges
- Animal clippers
- Grass Instruments
- Cannulae: tracheal
- Other available in desks

Experiment 16

Miotics and Mydriatics

(Bring Your Ophthalmoscope)

If you have a history of previous idiosyncrasy or intolerance to any of the drugs you are to take, consult the instructor who will rearrange your experiments. Students with a history of glaucoma must not take any mydriatic. When a drug is applied to the eye, be sure to prevent systemic absorption by occluding the lacrimal duct by finger pressure at the inner canthus.

Method (groups of 2 students)

Each student will serve as an experimental subject. Students will work in pairs in order to facilitate the application of the drugs and the making of the observations. All observations should be made under similar conditions before and after the use of the drugs. For example, those students who normally wear spectacles or contact lenses should wear them. If they are worn during the control observations, they must also be worn during the experimental observations. Use one eye for control observations, the other eye for experimental observations.

Pupillary Diameter. Estimate the normal size of the pupils with the Cogan entoptic pupillometer (Am. J. Ophthalmology; 24; 1431, 1941). This is a strip of metal containing a series of paired holes, the intervals between the holes progressively increasing by 0.5 mm. Hold the pupillometer to your eye and look at a lighted wall through any pair of holes. Cover the eye not being tested. Use the same area of the wall for all subsequent measurements. Lower or raise the pupillometer until you find that pair of holes which produces luminous discs which are tangential to one another. The distance between these holes is the diameter of the pupil.

Near Point. This is the nearest point at which an object can be seen clearly. The near point increases with age; normal values range from 7 cm in childhood to 40 cm in old age. Determine the near point in the control and experimental eyes before and after applying the drugs. Bring some reading material as close to the eyes as is consistent with clarity and measure the distance between the eye and the reading material. Record this distance in centimeters as the near point.

Far Point. This is the farthest point at which an object can be seen clearly. In the normal eye the far point is at infinity; for convenience this is arbitrarily taken as any point more than 6 meters. Determine the far point in the control and experimental eyes as follows. Before applying any drugs stand at the 6 meter line marked on the floor of the laboratory and choose the smallest line of letters on the test chart which can be read comfortably at that distance. Record the control far point as 6 meters. After applying the drug to the experimental eye measure the furthest distance you can stand from the test chart in order to be able to read comfortably the same line of letters chosen for the control observations. Record this distance in meters as the far point.

Drugs

Each student will receive one of the following combinations of drugs. Make control observations in both eyes before applying any drug to the one eye.

Students at Even-Numbered Benches

1. Cyclopentolate - Pilocarpine: Instill 1 drop of 0.5% cyclopentolate HCl into the conjunctival sac and make observations every five minutes for a total of at least 30 minutes. When no further change in pupillary size occurs, instill into the same eye 1 drop of 1% pilocarpine HCl. Repeat observations. Record all your data on the data sheet on page 16/4. For the purpose of compiling the class data submit only the maximum effects observed.

2. Pilocarpine - Cyclopentolate: Instill 1 drop of 1% pilocarpine HCl into the conjunctival sac and make observations every five minutes for a total of at least 30 minutes. When no further change in the pupil occurs, instill into the same eye 1 drop of 0.5% cyclopentolate HCl. Repeat observations. Record all your data on the data sheet on page 16/4. For the purpose of compiling the class data submit only the maximum effects observed.

Students at Odd-Numbered Benches

3. Phenylephrine-Physostigmine: Place a drop of 10% phenylephrine into the conjunctival sac and make observations every five minutes for a total of at least 30 minutes. When no further change in the pupil occurs, follow with 1 drop of 0.1% physostigmine salicylate. Repeat observations. Record all your data on the data sheet on page 16/4. For the purpose of compiling the class data submit only the maximum effects observed.

4. Physostigmine-Phenylephrine: Place a drop of 0.1% physostigmine salicylate into the conjunctival sac and make observations every five minutes for a total of at least 30 minutes. When no further change in the pupil occurs, follow with 1 drop of 10% phenylephrine. Repeat observations. Record all your data on the data sheet on page 16/4. For the purpose of compiling the class data submit only the maximum effects observed.

At the end of the laboratory period those students wishing to counteract the residual effects of the longer acting drugs, cyclopentolate and physostigmine, should do so as follows:

For residual effects of cyclopentolate add 1 drop of 1% pilocarpine
For residual effects of physostigmine add 1 drop of 0.5% cyclopentolate

Glossary of Terms

Miotic: constricts the pupil (Parasympathomimetics)
Mydriatic: dilates the pupil (Sympathomimetics and parasympathetic blocking agents)
Cycloplegic: paralyses ciliary muscle and hence accomodation (Parasympathetic blocking agents)
Myopia: near-sightedness, distant object focuses in front of retina. Far and near points decreased.

Hyperopia: far-sightedness, distant object focuses behind retina. Near point increased.

Animals: None

Drugs:

Cyclopentolate hydrochloride	0.5%
Pilocarpine hydrochloride	1.0%
Phenylephrine hydrochloride	10%
Physostigmine salicylate	0.1%

Equipment:
Meter sticks
Cogan entoptic pupillometers
Eye test charts

Experiment 16

Data Sheet

Drug	Time	Pupil Diameter (in mm.)		Near Point (in cm.)		Far Point (in meters)	
		control eye	treated eye	control eye	treated eye	control eye	treated eye
NONE							

Experiment 17

Histamime and Antagonists

Histamine has a wide range of biological activity. The most striking effects are those on smooth muscle, particularly bronchiolar smooth muscle. An injection of histamine or its intrinsic release in allergic conditions promotes the constriction of bronchiolar smooth muscle, thus reducing the bronchiolar lumina. This increases the resistance to both the inflow and outflow of air. Clinically residual air is increased.

The guinea pig is remarkably sensitive to the effects of histamine on bronchiolar smooth muscle. When breathed in a nebulized solution histamine often precipitates a fatal asthmatic attack.

The isolated ileum of the guinea pig is also quite sensitive to histamine. In vivo the effect of histamine on intestinal smooth muscle is not as marked as on the bronchioles.

The arteriolar capillaries in the skin are also sensitive to histamine and dilate in its presence. Clinically histamine is probably involved in local skin reactions with fixed antibodies. The reaction to an antigen is the characteristic wheal and flare response. This may be simulated by the intracutaneous injection of histamine.

The following experiments are designed to show these three effects. In addition, the experiment attempts to show the ability of certain histamine antagonists to prevent, or at least alter, these effects of histamine both in vivo and in vitro.

1. Histamine and antihistaminics in man

Clean an area on the right forearm with 70% alcohol. Place one drop of sterile histamine diphosphate (1:1000) on the cleaned and sponged area. Within an area approximately 2 mm square under the drop of histamine, gently puncture the epidermis 4 times. Do not draw blood! Observe the responses as follows: time required to form wheal; diameter of wheal; maximum diameter of flare; and duration of response (wheal or flare whichever is longer). Record data in the appropriate spaces on the blackboard.

Each student will take orally one of the following drugs: chlorpheniramine, diphenhydramine, tripelennamine, or a placebo. After 60 minutes have elapsed, repeat the procedure as outlined above for the cutaneous administration of histamine. Repeat the observations and record the data.

2. Demonstration: Effect of histamine on the bronchioles in vivo

Obtain and weigh 2 guinea pigs. Observe and record their rate and pattern of respiration, heart rate, and general body movements. Inject guinea pig A with 12.5 mg of diphenhydramine per kg, intraperitoneally.

After 15-20 minutes place both guinea pigs, A and B, in the metabolism chamber.

Place 3-4 ml of histamine (5 mg/ml) in the drug chamber.

Attach a hose from the air outlet on the table to the gas inlet on the metabolism jar, and aerate jar to nebulize histamine for 5 minutes.

Remove both guinea pigs from jar and observe as previously. Note lapse of time to death or recovery from exposure to histamine.

Cause of death?

3. Effect of histamine and antagonists on the isolated guinea pig ileum in vitro (work in pairs)

Students at odd-numbered desks will perform this portion of the experiment exactly as outlined in the procedure described below.

Students at even-numbered desks will perform the experiment as described with the single exception of using tripelennamine HCl at item 9.

Make a ring around the muscle chamber with a wax pencil 2 cm below the rim. When filled to this point the volume approximates 30 ml. This provides a means of diluting all substances added in the same volume. Add Sollmann's solution to the mark. Arrange to record with either a kymograph or Grass Polygraph as described in experiment 20 (Stimulation and Depression of the Intestine).

The isolated guinea pig ileum is not rhythmically spontaneous, as is the rabbit ileum (see experiment 20). Erratic pendular motion does occur. The experiment is performed at room temperature. This nearly eliminates spontaneous activity.

Determine the effects of histamine and diphenhydramine (or tripelennamine) according to the following protocol. Add all drugs through the opening in the top of the muscle chamber.

1. Add 0.4 ml of histamine diphosphate (1:100,000). Record the effect for one-half minute.

2. Wash twice with fresh Sollmann's and allow 3 minutes to elapse before proceeding to number 3.

3. Add 0.4 ml 5-hydroxytryptamine creatinine sulfate. Record the effect for one-half minute.

4. Repeat wash as in number 2.

5. Obtain a second control contraction with 0.4 ml of 1:100,000 histamine diphosphate, recording for one minute. If the second control varies from the first by more than 25%, obtain a third control.

6. Repeat wash as in number 2.

7. Repeat number 5 above substituting 0.4 ml 5-hydroxytryptamine creatinine sulfate for histamine diphosphate.

8. Repeat wash as in number 2.

9. Add 0.4 ml of diphenhydramine HCl (or tripelennamine HCl according to one's seating assignment) (1:250,000) and wait ONE MINUTE!

10. Repeat number 1 adding 0.4 ml of histamine diphosphate (1:100,000). Record the effect for one-half minute.

11. After the muscle has returned to the resting level, repeat number 3 above adding 0.4 ml 5-hydroxytryptamine creatinine sulfate. Record the effect for one-half minute.

12. Wash twice with fresh Sollmann's; wait 5 minutes and wash once more. Allow 5 additional minutes to elapse.

13. Obtain a control contraction with 0.4 ml of 1:100,000 histamine diphosphate. Record the effect for one-half minute. Why?

14. Repeat wash number 12 above.

15. Repeat number 3 above adding 0.4 ml 5-hydroxytryptamine creatinine sulfate. Record the effect for one-half minute.

16. Repeat wash as in number 2.

17. Repeat number 13.

Animals: Guinea Pigs

Drugs: Sollmann's solution
Diphenhydramine - 1:250,000 (in Sollmann's solution)
Histamine - 1:100,000 (in Sollmann's solution)
Tripelennamine - 1:250,000 (in Sollmann's solution)
Histamine $diPO_4$ - 5 mg/ml for Demonstration
Diphenhydramine - 10 mg/ml from vial for Demonstration
5-Hydroxytryptamine Creatinine Sulfate - 1:100,000 (in Sollmann's solution)

Drugs for Part 1:
Histamine $diPO_4$ - 1:1000 ampules (sterile)
Chlorpheniramine - 4 mg
Diphenhydramine - 50 mg
Tripelennamine - 50 mg
Alcohol - 70%
Placebo

Equipment:

Sterilizer	Aeration tube and needle
Needles	Muscle chamber and support
Metabolism jar	Other: available in desks

Experiment 18

Effect of Cardiac Glycosides on the Heart In Situ

This experiment reveals that a great deal of valuable knowledge may be attained with a minimum of apparatus - that careful observation is a tool which, when kept sharp, can cut its way through many obscurities. The overall picture of therapeutic and toxic digitalis action presented by this simple experiment can hardly be surpassed.

1. Observation of Glycoside Effects

Select a large active frog and inject into the dorsal lymph sac 2 ml of 10% urethane solution. When the animal becomes anesthetized, pin down in the frog pan and expose the heart with a minimum loss of blood (by a v-shaped incision from below the ensiform process upward and laterally). Keep the heart moist with Ringer's solution. Students should make the required observations and record same.

Observe carefully the sequence of the cardiac cycle and the form and color of the chambers in systole and diastole. Make several counts of heart rate; and when constant, proceed to apply directly to the surface of the heart with a hypodermic syringe and needle tiny droplets of the specified glycoside solution.

Students at odd-numbered desks will perform the experiment using ouabain (0.025 mg/ml).

Students at even-numbered desks will perform the experiment using digitoxin (0.05 mg/ml equivalent to 0.5 U.S.P. unit/ml).

Apply one drop at a time, at 2-minute intervals, and observe the heart for the following effects:

a) Change in rate;
b) changes in redness or paleness of ventricle in diastole and in systole;
c) changes in A-V interval

Record the above observations in tabular form. Compare the observed changes with the therapeutic effects of digitalis in man.

Continue to add the glycoside preparation at 2-minute intervals until toxic effects supervene. These may take the form of partial heart block, complete block, and ultimately ventricular standstill. The heart usually stops in systole but standstill may occasionally be diastolic. In this case, if the heart is mechanically stimulated, the standstill will become systolic with the auricles greatly engorged. Determine whether the stopped heart is irritable (still responds to mechanical stimulation or to the intraventricular injection of 0.2 ml Ringer's solution).

2. Recording of glycoside effects

Using a second frog repeat the above experiments; but this time in addition to the observations made in part 1, make a polygraph or kymograph record of the effects of the drug upon the heart. Use a small surgical needle and pass a fine thread through the apex of the ventricle.

A. If a recording is to be made with the Grass Polygraph:
Fasten the other end of the thread to a force transducer. Adjust the heart so it is under a slight load and set the sensitivity of the preamplifier so a 1-3 cm record of the contraction of the heart is obtained. If the tension and sensitivity are carefully adjusted it should be possible to obtain a record of both auricular and ventricular contractions. Adjust the paper speed so these components of the contraction can be recognized. It is not necessary to record continuously. A one foot record each five minutes or when noticeable changes in rate, rhythm or force are observed will be sufficient.

B. If a recording is to be made with a kymograph:
Fasten the other end of the thread to a heart lever. Carefully balance the heart lever in order to record both auricular and ventricular contractions. The kymograph drum should move about 2 inches in 10 seconds. It is not necessary to record continuously; a record of 10-20 seconds for each 6 minutes will suffice. Record a 10-second interval time line.

Regardless of the recording system do not neglect the value of visual observations and compare these with the polygraph or kymograph tracing.

Animals: Frogs

Drugs:
Ouabain - 0.025 mg/ml
Digitoxin - 0.05 mg/ml equivalent to 0.5 USP unit/ml
Frog-Ringer's solution
Urethane - 10% in Frog-Ringer's solution

Equipment:
Frog pans
Grass Polygraphs
Kymographs
Heart levers
Other: available in desks

Experiment 19

Pharmacology of Somatic Motor Neurones

It is well established that transmission of nerve impulses from the peripheral nerve to the skeletal muscle cell requires the presence of a chemical mediator, acetylcholine (abbreviation = ACh), which acts by depolarizing the post-synaptic membrane and initiating a propagated muscle impulse. The enzyme, cholinesterase, rapidly splits the highly active ACh into the relatively inactive choline and acetic acid. Several important drugs exert their action at the neuro-effector junction. Physostigmine and neostigmine inhibit cholinesterase so that any ACh released at the motor end plate remains active for a longer period of time. If sufficient ACh persists at the neuro-muscular junction, the resulting excessive depolarization paralyzes the effector cell. The muscular relaxants, decamethonium and succinylcholine, similarly, act through their excessive depolarizing effect upon the motor end plate. On the other hand, curare, tubocurarine and certain other curare-like drugs raise the threshold of skeletal muscles to stimulation by ACh by impeding depolarization of the motor end plate region.

1. Stimulation Action of ACh: (Work in Group of 2)

Pith a frog and pin it with the ventral surface up in the dissecting tray. Remove the skin from the ventral surface of the body and dissect the rectus abdominis muscle of one side from the pelvic girdle to its attachment in the cartilage of the pectoral girdle. Fasten a thread to each end of the muscle and free the muscle. Cover the remaining muscle with cotton moistened with Ringer's solution and set the animal aside for possible later use.

WITHOUT DELAY, suspend the isolated muscle in the muscle chamber, add 35 ml of Ringer's solution at room temperature, and fasten the other end of the muscle to the Force-Displacement Transducer FT03 attached to the third channel of the Grass Polygraph (see Appendix III). Apply a resting tension of about 1 gram to the muscle. A setting of 1 on the SENSITIVITY MV/CM KNOB will generally be about right.

In order to obtain adequate mixing of drugs as they are added to the chamber, connect a hose from the air outlet on the table to muscle chamber by inserting a 25-gauge needle into the rubber outlet tube of the chamber. Adjust the air current so that a fine stream of bubbles gently agitates the solution.

Set the paper speed at 0.25 mm/sec. The paper should be moving only during periods when drugs are being tested. Start the paper moving and by means of a tuberculin syringe, add to the muscle bath, 0.1 ml of a 0.015% ACh solution. Record the contraction on the paper for exactly 2 cm. Stop the paper.

Drain the solution from the chamber, add fresh Ringer's solution, and allow the muscle to stand in the fresh Ringer's for 5 minutes.

Replace the wash solution with fresh Ringer's solution. Start paper moving, add 0.2 ml of the 0.015% ACh solution and proceed as above.

Following the procedure outlined above, continue recording the responses to 0.4, 0.8 and 1.6 ml of the 0.015% ACh solution, until the maximum contraction is reached.

Construct a table showing the final concentration of ACh present in each case and the height of contraction at the point 2 cm past point of drug addition.

2. Sensitization by Physostigmine:

Record two control contractions of the muscle by adding to the chamber the volume of 0.015% ACh solution estimated from Section 1 to produce about 50% of the maximum contraction. The two control contractions should be of approximately the same magnitude. Each of these control contractions should be obtained as in Section 1; but, of course, using the same volume of 0.015% ACh each time.

After obtaining the two control contractions of approximately equal magnitude, add to 34 ml of fresh Ringer's solution 1.0 ml of 0.035% physostigmine and salicylate solution. Let the muscle stand in this solution for 10-15 minutes.

Add the same volume of 0.015% ACh used to obtain the control contractions (above in this Section), and record the response of the muscle. Compare this contraction with the controls.

Drain the chamber and wash the muscle with 2 or 3 portions of fresh Ringer's, allowing 10 minutes to elapse before proceeding to Section 3 below.

3. Inhibition by Tubocurarine:

Record two or more equal control contractions by adding the selected volume of 0.015% ACh used in Section 2 above. Drain the chamber after the last contraction and wash with 35 ml of fresh Ringer's solution. Drain after 5 minutes.

To 35 ml of fresh Ringer's solution, add 0.1 ml of 0.05% d-tubocurarine chloride. After exactly 30 seconds, add the selected volume of 0.015% ACh used to obtain control contractions, and record for 2 cm as previously.

Drain the chamber and wash the muscle with fresh Ringer's solution.

To 35 ml of fresh Ringer's solution, add 0.3 ml of the 0.05% d-tubocurarine chloride solution, proceed as above and record the response to the selected volume of 0.015% ACh used to obtain the control contractions.

Continue testing the response to increasing aliquots (0.5, 0.7, 0.9 ml) of the 0.05% d-tubocurarine chloride solution until marked paralysis is evident.

Construct a table as in Section 1 showing the final concentrations of d-tubocurarine versus the height of contraction.

Animals: Frogs

Drugs: Frog Ringer's solution
Acetylcholine Br - 0.015% in Ringer's
(15 mg per 100 ml)
Physostigmine salicylate - 0.035% in Ringer's
(35 mg per 100 ml)
d-Tubocurarine Cl - 0.05% in Ringer's
(50 mg per 100 ml)

Equipment: Grass Polygraphs
Muscle chamber and support
Aeration tube and needle
Other: available in desks

Experiment 20
Stimulation and Depression of the Intestine

The use of segments of the isolated ileum of the rabbit has been employed classically in physiological and pharmacological research. When removed and placed in physiological solution in the isolated organ bath the ileum rhythmically shortens and lengthens in a pendular motion. This unique property of the rabbit's isolated ileum provides an excellent preparation to study the effects of drugs on the spontaneous activity of smooth muscle.

Drugs will either increase tone, amplitude or oscillations per minute or decrease the same. In general drugs in this experiment will act through one of the following mechanisms:

1. Stimulation of sympathetic (adrenotropic) receptors
2. Stimulation of parasympathetic (muscarinic) receptors
3. Stimulation of autonomic ganglia

1. General Instructions

Fill the muscle bath with fresh warm Sollmann's solution and maintain at 36°C (do not let the temperature go below 35° or above 40°C). Adjust the air supply so that a fine stream of bubbles rises through the bath. This serves the double function of oxygenating and stirring the solution in the bath.

The muscle warmer should now be connected to the rheostat. The rheostat in turn is plugged into the alternating current. CAUTION: Do not turn the rheostat above the setting, number 8. Excessive heat will destroy your preparation and may ruin the equipment.

In a petri dish place sufficient warm Sollmann's solution to just cover a segment of ileum. Obtain a segment of ileum approximately 2 cm in length from the laboratory instructor. Attach one end of the segment with a fine cotton thread to the metallic portion of the muscle warmer. Attach the other end with a long piece of thread to the Grass strain gauge. Set the sensitivity on 2. Adjust the tension on the segment by moving the strain gauge up or down. Watch the excursion of the writing pen on the Grass Polygraph and tighten the supports of the strain gauge when the resting tension falls on 1 gram.

Those groups using the kymograph use the procedure outlined for the strain gauge except they will attach the free end of the segment by means of a thread to the writing lever. Adjust tension in this system with small pieces of clay until rhythmic pendular activity is apparent. Use an appropriate slow speed. There are three basic reasons for failure of this system:

(1) Tension (Insufficient; Excessive)
(2) Temperature (Usually Excessive)
(3) Drugs (Excessive)

2. Drugs

Add the various drug solutions cautiously using the minimum dose to get an effect in each case. This means adding the solution 0.03 ml at a time from a syringe and needle and waiting 30 seconds between additions until an effect is obtained or until it is obvious that there will be none. Remember that there is frequently a time lag before the action of a drug occurs, especially when a drug first has to antagonize the effect of the previous drug. Follow the procedure in the order given, changing to fresh Sollmann's solution only when directed:

(a) Norepinephrine - 0.1%
(b) Wash carefully by changing to fresh warm Sollmann's solution. Allow the tissue to recover its spontaneous rhythm and then proceed.
(c) Phenylephrine - 1.0%
(d) Wash as in (b)
(e) Isoproterenol - 0.1%
(f) Wash as in (b)
(g) Barium - 5%
(h) Wash as in (b)
(i) Cyclopentamine - 10%
(j) Wash twice at minute intervals
(k) Nicotine - 0.1% (May produce inhibition although most will observe stimulation)
(l) Wash as in (b)
(m) Pilocarpine - 1.0%
(n) Wash as in (b)
(o) Atropine - 1%
(p) Pilocarpine - 1.0% (No more than in step m)
(q) Nicotine - 0.1 % (No more than in step k)

Animals and Tissue: 2 to 4 kg male rabbits are killed by a blow on the head. The ileum is clamped at the ileocecal junction and dissected 20 cm proximally, severed, divided into 2 cm segments and placed in Sollmann's solution at 36°C.

Drugs:
Atropine SO_4 - 1%
Barium Cl - 5%
Cyclopentamine HCl - 10%
*Isoproterenol HCl - 0.1%
Nicotine SO_4 - 0.1%
*Norepinephrine HCl - 0.1%
*Phenylephrine HCl - 1.0%
Pilocarpine HCl - 1.0%
Sollmann's solution

*Norepinephrine, isoproterenol and phenylephrine deteriorate rapidly in a neutral solution containing no reducing agent. Thus, they must be dissolved in distilled water to which ascorbic acid has been added, 100 μg/ml, as a preservative.

Equipment:

(See Appendices - RE: strain gauge)
Heated muscle chamber and support
Rheostat (Do not operate above setting 8 unless so directed)
Aeration tube and needle
Water baths and heaters
Other: available in desks

Experiment 21

Drug Effects on the Cardiovascular System

The purpose of this experiment is to demonstrate the responses elicited by several cardiovascular drugs. Anesthesia is obtained by s.c. administration of morphine, 10 mg/kg, followed in 30 minutes by i.v. barbital sodium, 100 mg/kg. The trachea is cannulated and the animal is being ventilated by means of a Harvard respirator. The femoral artery and vein are cannulated. The catheter is passed into the thoracic aorta for measurements of aortic pressure. The venous catheter is passed up the vena cava to a point near the right atrium for venous pressure measurements. The venous pressure is recorded on Channel 2. The femoral vein on the opposite side is cannulated for injection of drugs. The chest is opened in the fourth interspace and a strain gauge arch is attached to the surface of the right ventricle. The strip of muscle between the two points of attachment of the strain gauge is stretched by approximately 20%. The procedure fixes the initial length of the muscle segment. The force of contraction of the myocardium is proportional to the amplitude of the recording. The EKG is recorded on Channel 4. Lead II is used.

All drug injections are made i.v. and flushed in with approximately 5 ml of saline.

Sequence of Drugs:

1. Nitroglycerin - 50 μg/kg
2. Norepinephrine infusion - 0.5 μg/kg/min.
3. Repeat nitroglycerin - 50 μg/kg injection during the period of the norepinephrine infusion
4. Methoxamine - 100 μg/kg
5. Isoproterenol - 0.5 μg/kg
6. Pentobarbital - 5 mg/kg. Repeat injections of 5 mg/kg until the contractility is markedly depressed.
7. Digitalis (twice the lethal dose given in divided doses over a 30-minute period)

Animals: Dog

Drugs:
Morphine Sulfate - 10 mg/ml
Barbital Sodium - 100 mg/ml
Norepinephrine Bitartrate - 5 μg/ml
Nitroglycerin - 1/100 gr tabs
Methoxamine Hydrochloride - 1 mg/ml
Isoproterenol Hydrochloride - 5 μg/ml
Digoxin - .25 mg/ml

Equipment: Master-Slave Unit and Accessories

Experiment 22

Drugs Influencing Urinary Output

The activity of the kidney depends mainly on three factors: (1) the amount of available water in the blood and tissues; (2) an ample amount of blood flowing through the kidneys in a given time; and (3) the rate of reabsorption in the kidney tubules.

Regarding the first factor, the normal water content of the blood is maintained by colloids (proteins). When the water content of the plasma increases, the excess water is excreted to maintain the normal ratio of colloid to water. The second factor, ample renal flow, is essential for adequate glomerular filtration, the first step in the process of excretion. Sufficient glomerular filtration requires both adequate blood pressure and an abundant flow. Pressure is essential to overcome the colloidal osmotic pressure of the plasma, which resists the passage of water out of the capillaries into the capsular lumen. Two forces resist the passage of fluid from the glomerular capillaries into the capsular lumen: (a) an osmotic pressure of about 30 mm Hg and (b) the "back-pressure" exerted by the fluid within the lumen of the nephron, about 5 mm Hg. Blood must enter the kidney at a pressure greater than the sum of these two forces. Hence, urinary secretion usually stops completely when the arterial pressure reaches 35-40 mm Hg. On the other hand, even with a high blood pressure, the urinary output may be low, unless the amount of glomerular blood flow is adequately maintained. Thus the amount of blood is as important as the pressure of blood. Lastly, the third factor, tubular reabsorption is very important because the urine volume is inversely related to the tubular reabsorption rate: a high rate yields a low urine volume and vice versa. In this experiment, hemodynamic factors will not be measured, but the student should bear in mind possible blood pressure changes which should be considered in the interpretation of the results.

In this experiment, the effects of representative diuretic agents on the urine output of unanesthetized rats will be determined. It is important to remember that we will be observing only the net effect on urine volume. Ideally, in evaluating the effectiveness of diuretics, total electrolyte excretion should be determined since this is the primary event in removing edema fluid from the body. If time permits electrolytes will be determined, and the details of the procedure(s) will be described at the laboratory.

Work in groups of 4; each student will be given one rat which has been fasted for 12 hours. Each group will be assigned a metabolism cage and, by assignment, will carry out one section of the experiment. Data sheets will be distributed. Record all data on the sheet and turn in this information at the end of the laboratory period.

A. Control:

1. To each rat administer slowly by stomach tube 50 ml/kg of tepid 0.9% NaCl solution.

2. Immediately after this inject s.c. 0.5 ml of 0.9% NaCl per rat. Record the injection time and place the rats in a metabolism cage arranged to collect urine without fecal contamination.

3. Collect the urine in a 10 ml graduate cylinder and measure the output every 30 minutes.

4. Using the urine volumes obtained above, calculate the percent of the volume of the administered saline load that was excreted during each collection period. In addition, calculate the cumulative percentage of the saline load that was excreted through each collection period by adding the combined volumes of all previous collection periods to the volume of that period.

B. Effects of Acetazoleamide:

1. Proceed as under A, 1-4, but administer 500 mg/kg of acetazoleamide orally. This is to be given with the tepid saline load, 50 ml/kg.

C. Effects of Hydrochlorothiazide:

1. Proceed as under A, 1-4, but administer 500 mg/kg of hydrochlorothiazide orally. This is to be given with the tepid saline, 50 ml/kg.

D. Excretion of Water Load:

1. Proceed as under A, 1-4, except omit the 0.9% NaCl load, and administer 50 ml/kg of tepid tap water.

E. Effects of Vasopressin:

1. Proceed as under D, but substitute 0.1 unit/kg of vasopressin s.c., instead of the 0.9% NaCl s.c. injection.

F. Effects of Mercaptomerin:

1. Proceed as under A, 1-4, but administer 10 mg/kg of mercaptomerin solution (calculated as Hg) i.m. instead of the saline s.c. Each ml of mercaptomerin solution contains approximately 40 mg of mercury. The onset of increased volume excretion is slow with the mercury diuretics. Initially, a decrease in urine volume output may be seen in normal rats. An increase in electrolytes is usually seen within the first hour. In the patient with edema increased volume output is seen within one hour.

Animals: Rats (fasted overnight)

Equipment:
Metabolism cages
10 ml graduates
Oral dosing needles
Other: available in desks

Drugs:
Sodium Chloride - 0.9%
Acetazoleamide - 500 mg vials
Vasopressin - 20 units/ml vials
Hydrochlorothiazide - 500 mg vials
Mercaptomerin - USP - 40 mg mercury per ml

Experiment 23

Absorption of Sulfadiazine

The activity of a drug is conditioned to a great extent by factors determining its ability to reach the specific site of action, the concentration it achieves at that site, and the length of time an effective concentration is maintained. The effective concentration of a drug may differ from its actual concentration either because it is bound to another substance such as the plasma proteins, or because its structure is modified by the body in such a manner as to produce an inactive compound, such as an acetylated sulfonamide. (The dog does not acetylate sulfonamide). The actual concentration is also influenced by the route of administration and the chemical properties of the drug. This experiment is designed to show the relationship between the dose, route of administration, time, and blood concentration of sulfonamides.

The sulfadiazine will be administered as follows:

100 mg Na salt/kg -- i.v.;
200 mg acid form/kg -- p.o.; and
400 mg acid form/kg -- p.o.

The p.o. dosages (by staff) will be via stomach tube prior to anesthetization with pentobarbital Na (30 mg/kg).

The i.v. dosage (by students) will be given after anesthetization with pentobarbital Na (30 mg/kg).

N.B. Refer to steps 5 and 6 concerning the necessity of a blood sample BEFORE administration of i.v. sulfonamide. (listed below)

Blood samples will be obtained via a short plastic catheter anchored in the femoral artery. From dogs dosed intravenously, take blood samples at 30, 60, 90 and, if possible, 120 minutes following administration of the drug. From dogs dosed orally in advance of the laboratory period, obtain at least three samples at 30-minute intervals. Results from these latter animals should be recorded in terms of the interval after an announced time of dosage.

Procedure for determination of free sulfonamide in blood:

This procedure is based on the Bratton and Marshall method for measuring sulfonamides (and compounds containing N on an aromatic ring) in biological materials (tissues and fluids). It is a standard analysis employed both in research and in the clinic. Reliable results with this very sensitive analytical procedure require care and precision of technique.

1. Collect about 2 ml of blood from the plastic catheter into a test tube containing several mg of sodium oxalate (specially provided).

2. Pipette 1.0 ml of the blood into an Erlenmeyer flask and add with shaking 14 ml of 0.05% saponin to cause rapid hemolysis and to dilute the blood.

Calculations: By using the sulfadiazine standard, all results will be in terms of free sulfadiazine. Report results as mg% of sulfadiazine in the original sample of whole blood and tabulate them on the blackboard.

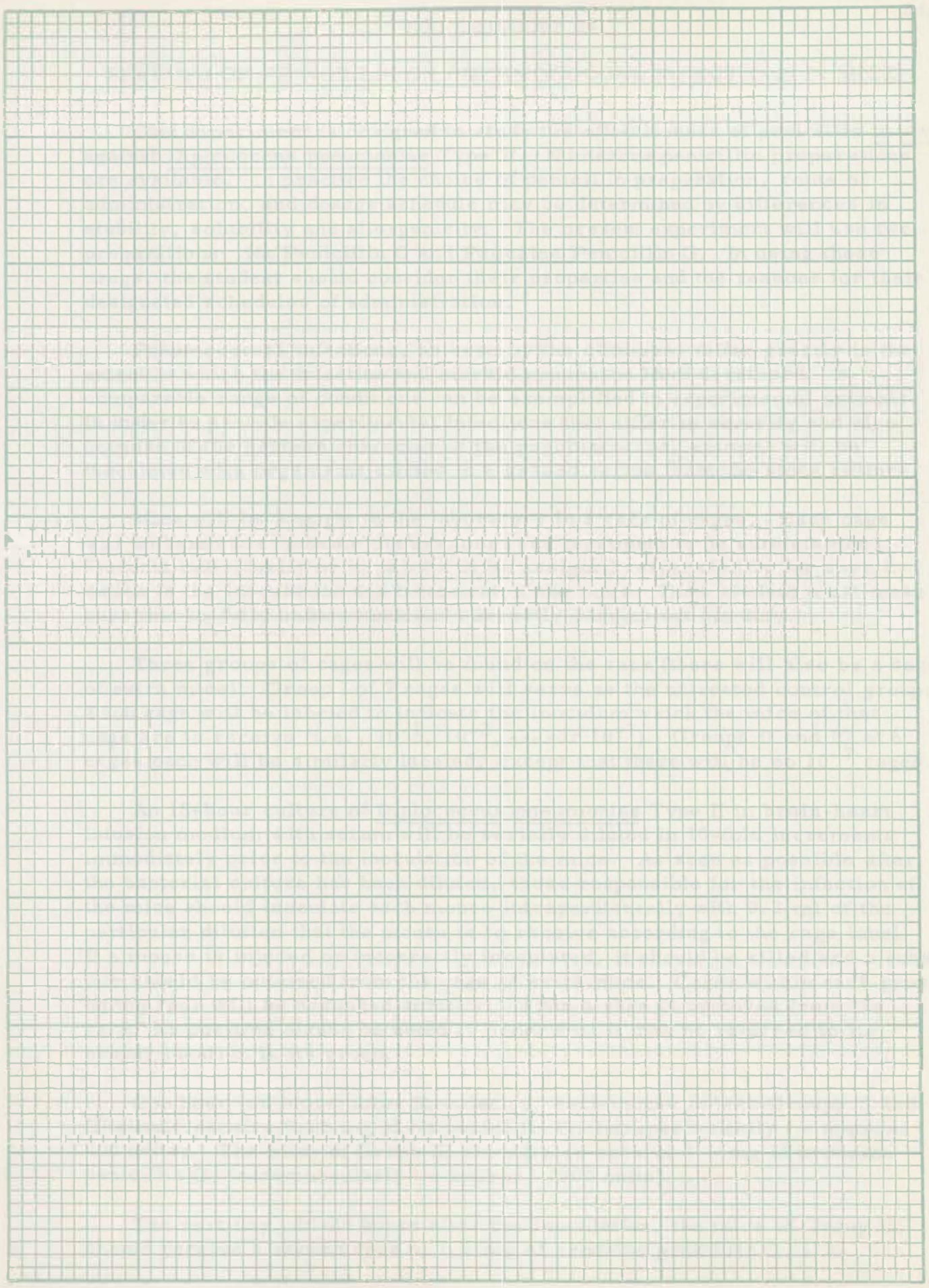
$$\text{Conc. of unknown} = \text{reading of unknown} \times \frac{\text{conc. of standard}}{\text{reading of standard}}$$

Reports: The class results will be duplicated and distributed to each student. Draw conclusions and construct pertinent graphs from the average data of the entire class.

Animals: Dogs (fasted overnight)

Drugs:
Ammonium sulfamate - 0.5%
N-(1-naphthyl)-ethylenediamine diHCl - 0.1%
Pentobarbital Na - 50 mg/ml
Saponin - 0.05%
Sodium nitrite - 0.1%
Sodium oxalate - 3%
Sodium sulfadiazine (standard) 0.05 mg/ml (5 mg%)
Sodium sulfadiazine - 50 mg/ml (for i.v. injection)
Sulfadiazine - 100 mg/ml
Trichloracetic acid - 15%
Trichloracetic acid - 2.9%

Equipment:
Colorimeter tubes
Erlenmeyer flasks, 50 ml
Coleman Junior Spectrophotometer
Test tubes (15 x 125 mm - oxalated)
Test tube racks
Filter paper
Burettes
Kimwipes
Parafilm patches
Polyethylene cannulae
Other: available in desks



"THE CHAMPION LINE" NO. 642
CROSS SECTION - 10 SQUARES TO INCH

EXPERIMENT 24

EFFECTS OF PENICILLIN ON PNEUMOCOCCAL INFECTIONS IN MICE

This experiment is designed to study the effectiveness of penicillin in vivo against Diplococcus pneumoniae Type II. Numerous penicillin preparations are available. In practice the choice of penicillin preparation, route of administration, and dosage schedule may be determined by differences in stability, solubility and allergenicity. The present experiment will use two preparations: Na Penicillin G and Procaine Penicillin G. In using these drugs, the student should consider their properties and the reasons for their designated schedules of administration.

Pneumococcal infections, as seen by the clinician, differ greatly in the extent to which the infection and morbid processes have developed. A comparable range of conditions can be exemplified in experimental animals. In order to provide experimental infections that differ in severity and also because of the protean character of the pneumococcus, animals will be inoculated with organisms diluted in a graded series from the stock culture.

Mice will be brought to the laboratory in a rack of cages. Each cage will be labeled to indicate the inoculum and treatment to be administered. Students individually or in pairs will be assigned responsibility for specific duties in the inoculation and treatment of the mice. Each student must make sure that he fully understands and correctly carries out his duty.

Three groups of cages will be found on the rack (there will also be a cage of extra mice). Group I of 7 cages will serve as the untreated control group. Each cage will be labeled to indicate the inoculum which the mice of that cage are to receive (i.e., 10^{-1}, 10^{-2}, ----or 10^{-7}). Groups II and III of 6 cages each will be similarly labeled, except that there will be no 10^{-7} cages.

An 18-hour culture of Diplococcus pneumoniae Type II in brain-heart infusion medium with serum will be serially diluted in 10-fold increments immediately prior to the inoculation of the mice. As soon as possible after the bacterial culture has been diluted, those responsible for the infection of the mice should inoculate each mouse intraperitoneally with 0.2 ml of the particular dilution that the mouse is to receive according to the cage label. If a mouse is injured or incorrectly inoculated, it should be killed and a substitute should be obtained from the cage of extra mice. Cages within each group on the rack will be arranged serially. When a cage is removed from the rack, its position should be noted in order that it may be returned to its proper location in its group.

One hour (or more) after inoculation, each mouse in both Groups II and III should receive 0.25 mg of Na penicillin G, intraperitoneally (= approx. 400 units). The mice of Group III should also receive 1.6 mg of procaine penicillin G, intramuscularly (= approx. 1600 units).

The next day, 20-24 hours after the inoculation, the mice of Group III should receive a second dose of 1.6 mg of procaine penicillin G, i.m.

All syringes and needles used for either inoculation or dosage should be boiled both before and after use.

During the course of the experiment, all animals will be checked at least twice daily. Deaths will be recorded and dead animals will be removed from the cages. The experiment will be terminated 72 hours (or more) after the institution of treatment. These duties will be carried out by a staff member. Students should, nevertheless, check daily on the progress of the experiment by looking at both the animals and the posted data sheet. A summary of the data will be distributed.

Based upon the percentage mortality data, at the end of the experiment, bar graphs should be prepared in sets of three (Groups I, II, III) for each dilution of the inoculum at which the data show differences between the three experimental groups.

Animals: Mice

Bacteria: Diplococcus pneumoniae Type II

Culture medium: Brain-heart infusion medium with serum

Drugs:
Na Penicillin 1 mg/ml (aqueous solution)
Procaine Penicillin G - 8 mg/ml (aqueous suspension)

Equipment:
Mouse cages
Cage rack
Covered jar for dead mice
Sterile serological pipettes (1 and 10 ml)
Sterile cotton plugged vials for diluted culture
Sterile serum vial stoppers
Sterile tuberculin syringes
Sterile hypodermic needles (20 ga.)
Sterilizer
Syringes and needles: available in desks

"THE CHAMPION LINE" NO. 642

CROSS SECTION - 10 SQUARES TO INCH

EXPERIMENT 25

BLOOD

Since the intravenous route is so frequently used in drug administration, an understanding of the characteristics of blood is essential. Certain drugs possess the ability to interfere with the coagulation of blood, others can shorten the clotting time. The former are referred to as "anticoagulants," and the latter as "thromboplastic" substances. The essential factors in blood clotting are summarized briefly as follows:

Thromboplastic + Prothrombin + Calcium ions ---> Thrombin
Thrombin + Fibrinogen ---> Fibrin (insoluble)

The prothrombin and fibrinogen are produced by the liver and are found in the plasma. Thromboplastin is produced by most tissues. It is the threads of fibrin together with blood cells which form the clot.

1. Effect of heparin on clotting time (groups of 4 students)

This section should be started first and sections 2 and 3 may be done in between samples and observations on the rabbit. The clotting time will be determined by Wright's capillary tube method.

Note the weight of a rabbit. Obtain blood samples from a marginal ear vein as described in Appendix VI. By capillary attraction, fill with blood 1 or 2 capillary tubes of about 10 cm. in length and 1 mm. in diameter. Maintain pressure over the puncture in the vein until the hemorrhage stops. At 30-second intervals, break off about 1 cm. of the tube until a fine thread of fibrin appears between the broken ends of the tube. The formation of a fine thread of fibrin represents the clotting time. With the later samples break off the ends of capillary tubing at 5 or 10 minute intervals. Care should be taken to keep the tubes at approximately the same temperature throughout the observations (do not hold in hand, and do not place under desk lamp).

After the first sample (control) has been taken, inject into the rabbit intravenously 3.0 mg per kg of heparin sodium. Determine the clotting time 10, 30, 60, and 120 minutes after administration of heparin. Fill 3 or 4 tubes with the 10 minute and 30 minute samples because they may take 2 hours or more to clot. If, after heparinization, the punctured vein bleeds persistently, apply a piece of filter paper to the ear and hold this in place with a slightly sprung paper clip. (Do not apply an artery clip or a hemostat to the rabbit's ear).

After the 10 minute post-heparinization sample, designated student groups should inject their rabbits intravenously with 3 mg/kg of protamine sulfate and determine clotting time at 30, 60 and 120 minutes after the administration of heparin.

Tabulate your results on the blackboard or on data sheets provided, recording clotting times in minutes for animals with and animals without protamine. In your report, present graphically the mean clotting times for the two

groups of animals and discuss the significance and clinical implications of these results.

2. Mechanism of clotting (groups of 4 students)

To 4 clean test tubes add the following materials as indicated:

Tube 1. 0.5 ml dog plasma* + 1.0 ml 0.9% NaCl
Tube 2. 0.5 ml dog plasma* + 0.5 ml 0.9% NaCl + 0.5 ml thromboplastin
Tube 3. 0.5 ml dog plasma* + 0.5 ml 0.9% NaCl + 0.5 ml 2% $CaCl_2$
Tube 4. 0.5 ml dog plasma* + 0.5 ml 2% $CaCl_2$ + 0.5 ml thromboplastin

*Citrated plasma

Use appropriate amounts of hot and cold tap water to provide a beaker of water at 37°C. Stopper the tubes, shake and immerse them in water at 37°C. Completely invert all tubes at intervals of 10 seconds and note the time of appearance of clotting. Interpret the facts about clotting revealed by these 4 tubes.

3. Hemolysis (groups of 4 students)

Hemolysis is a result of erythrocyte disintegration or of a greatly increased permeability of the blood cells which permits hemoglobin and salts to appear in the plasma or serum. The solution of the pigment hemoglobin causes the plasma to become red in color. Hemolysis can be caused by several agents, such as those which act on the cell surface causing precipitation, dissolution of lipoids, change in surface tension, etc., which may alter the permeability of the cells. The entrance of fluid into the cell (imbition) also causes hemolysis. This can occur by osmosis when the cell is placed in a solution of a weaker salt content than serum.

Carefully clean and dry 8 test tubes and measure 5 ml of one of the following into each tube:

a) 0.9% sodium chloride
b) 0.9% sodium chloride containing 0.1% saponin
c) 0.9% sodium chloride containing 0.1% saponin to which you have added 2 drops of cholesterol solution (in ether). Heat at 40°C for one hour.
d) 0.9% sodium chloride saturated with ether
e) 0.9% sodium chloride containing 2% urea
f) 2% urea
g) 5.5% glucose
h) distilled water

Add 2 drops of dog blood to each tube and mix well. Immediately, and after 30 minutes, note in which tubes hemolysis has taken place. Ability to see the type on this page clearly through the tube may be taken as the criterion of hemolysis. Explain the results.

Animals: Rabbits
Dog (blood donor)

Drugs:
- Calcium chloride - 2%
- Cholesterol - 1% in ether
- Dog blood (citrated)*
- Dog plasma (citrated)*
- Glucose - 5.5%
- Saponin - 0.1% in 0.9% NaCl
- Heparin sodium - 10 mg/ml
- Protamine sulfate - 10 mg/ml
- Sodium citrate - 5%
- Sodium chloride - 0.9%
- Sodium chloride - 0.9% saturated with ether
- Thromboplastin (comm. prep.)
- Urea - 2% (freshly prepared)
- Urea - 2% in 0.9% NaCl (freshly prepared)

Equipment:
- Scales for rabbits
- Razor blades
- Capillary tubing
- Gauze sponges
- Filter paper
- Paper clips
- Test tubes (small
- Small stoppers
- Test tube racks
- Thermometers
- Other: available in desks

*Blood, in a predetermined amount, should be drawn into a calibrated vessel containing sufficient anticoagulant solution to provide 5 mg of sodium citrate/ml of blood.

Experiment 26

Effect of Epinephrine and Insulin on Blood Glucose Level

Although hormones are physiological materials required for the normal functioning of the organism, as drugs they must be treated with respect and administered with caution. Many hormones, such as thyroid and the steroids, are slow in onset; others such as epinephrine and insulin are quick acting and may be observed experimentally in the course of a student laboratory period.

Students will work in groups of 4 and each group will be designated to carry out with a rabbit one of the 4 drug administrations described below. Take blood samples from the marginal vein of the ear as described in Appendix VI. Use a clean and dry 0.2 ml TC pipette and fill the pipette by both capillarity and gravity flow. A blood sample must be taken before the injection (i.e., at 0 time), and at 30, 60, and 120 minutes after the injection. The procedure for determining the blood glucose in each of these samples is outlined at the end of the exercise. Note that the first sample must be taken and the injection given early in the laboratory period in order that time will permit analysis of the 120-minute sample. Tabulate all the blood glucose values on the blackboard or on the data sheets provided.

1. Effect of epinephrine (groups of 4 students)

Inject the rabbit subcutaneously with epinephrine (0.1 mg/kg). In addition to the blood glucose observations, note any changes in the appearance or activity of the animal.

2. Effect of insulin in normal rabbits (groups of 4 students)

Insulin will be administered in two different dosages. Certain groups (2a) will inject their rabbits subcutaneously with 0.25 units of insulin per kg. Other groups (2b) will inject their rabbits subcutaneously with 1.5 units of insulin per kg. In addition to the blood glucose observations, note any changes in the appearance or activity of the animal. If the animal begins to convulse, inject 5 ml of 15% glucose solution intravenously. Whether convulsions are observed or not, inject all rabbits which have been given insulin with 15 ml of 15% glucose subcutaneously at the end of the laboratory period (inject in at least 3 locations).

3. Effect of insulin in diabetic rabbits (groups of 4 students)

Rabbits that have been given 150 mg of alloxan per kg 48 hours prior to the laboratory period will be provided. Some will be diabetic and some will be essentially normal. Inject subcutaneously 0.25 units of insulin per kg. These rabbits must be injected subcutaneously with 15 ml of 15% glucose at the end of the period as in section 2.

Procedure for the Determination of Blood Glucose

The method used in this laboratory is that of Nelson (J. Biol. Chem. 153, 375, 1944).

1. To 3.0 ml of water in a 13 x 100 mm test tube add 0.2 ml of blood from a TC (to contain) pipette rinsing back and forth three times. Mix and add 0.4 ml 0.3 N barium hydroxide. Mix and after the mixture has turned brown (2-3 minutes), add 0.4 ml 5% zinc sulfate. Mix and centrifuge. Remove a 1.0 ml aliquot of the supernatant into a 25 ml graduated Folin-Wu blood sugar tube.

2. Set up in blood sugar tubes 0, 40, and 100 μg of the glucose standard (0, 0.2 and 0.5 ml of glucose standard, 200 μg/ml) and dilute to a total of 1.0 ml with water.

3. To the 1.0 ml aliquot of protein-free filtrate or standard solution, add 1 ml of Copper Reagent (blue). Mix and heat for 20 minutes in boiling water bath. Cool tubes in a pan of cold water. Add 1 ml of Arsenomolybdate Reagent (yellow). Mix to complete evolution of CO_2. Dilute to the 25 ml mark with water and mix by inversion using Parafilm to cap end of tube. Read in Coleman Junior Spectrophotometer at 520 mμ using the per cent transmission scale. (See Appendix V for operation of instrument). The color is very stable so that the tubes may be read at any convenient time after color development.

4. Plot the standard curve on single cycle semilogarithmic paper provided and interpolate unknown readings. These readings represent the μg contained in 0.05 ml of original blood and must therefore be multiplied by a factor of 2 to convert the reading from μg/0.05 ml to mg/100 ml, the units normally used for expressing blood sugar.

5. If a tube gives a reading of 20% transmission or less, dilute its contents five-fold with water and read it again in the Spectrophotometer. Note the dilution factor so that you may multiply your final result accordingly.

Animals: Rabbits (1/4 pretreated with alloxan*)

Drugs:
Alloxan monohydrate - 5%
Epinephrine HCl - 0.01%
Glucose - 15%
Insulin Injection - 4 units/ml
0.4 units/ml

*Alloxan, 150 mg/kg, injected intravenously over a 10-minute period, 48 hours prior to day of experiment, of animals treated, should: a) kill, 1/6; b) render severely diabetic, 2/3; and c) leave "normal," 1/6. (Pincus, Hurwitz and Scott, 1954. P.S.E.B.M. 86: 553)

Reagents:

Glucose Standard, 200 μg/ml (Burrell J1070)

Copper Reagent: prepare day of use by mixing 250 ml Alkaline copper tartrate solution (Burrell J 115); 10 ml 15% copper sulfate (Burrell J780)

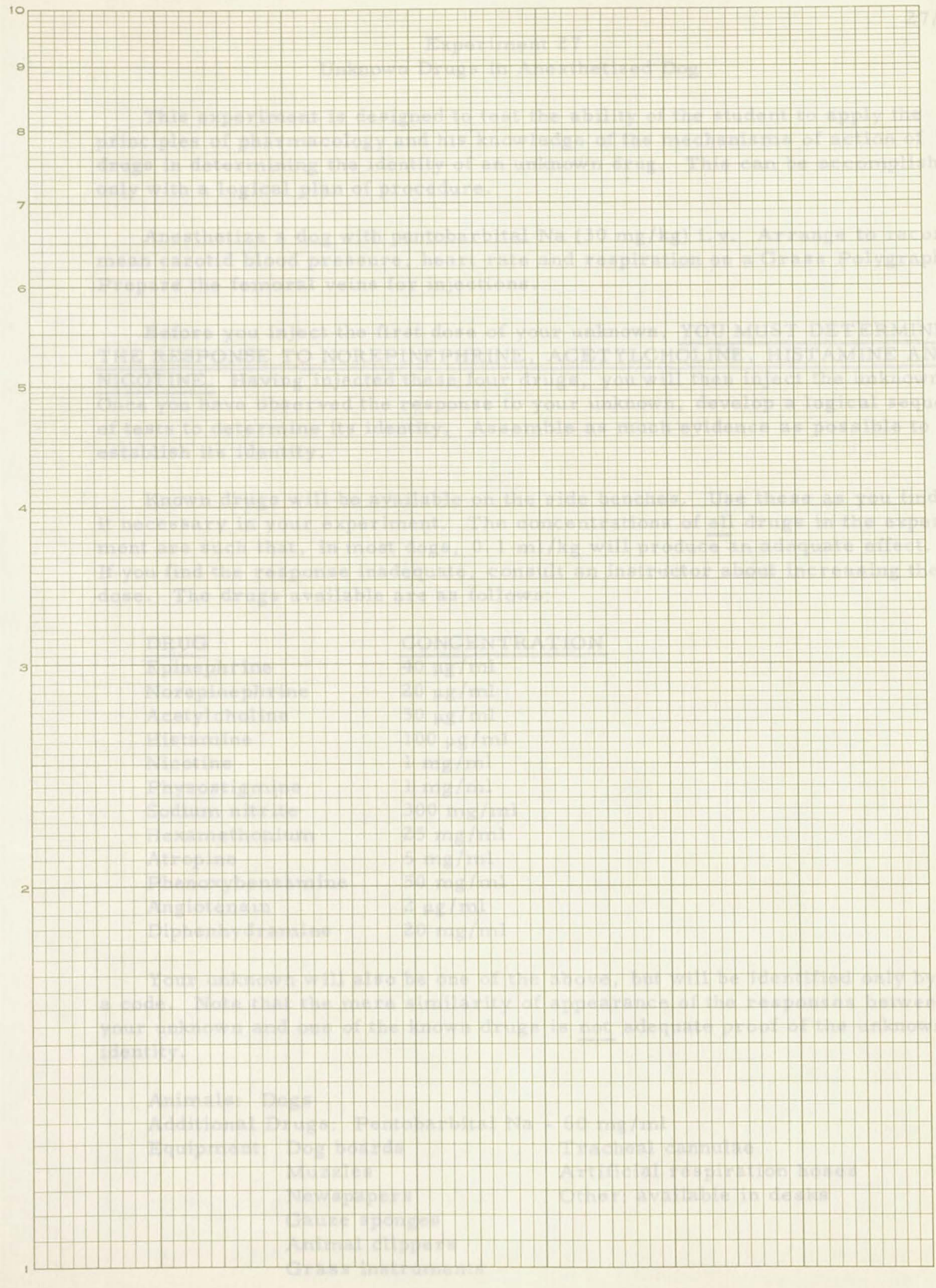
Arsenomolybdate Reagent: (Burrell J1350)

Zinc Sulfate 5% (Burrell J2715)

Barium hydroxide 0.3 N (Burrell J300)

Equipment:

Folin-Wu blood sugar tubes
Water baths and tube holders
Coleman Junior Spectrophotometer
Parafilm
Pipettes, 0.2 ml TC; 0.2 ml Mohr; 0.5 ml Ostwald-Folin;
1.0 ml volumetric; 5 ml serological
Test tubes 13 x 100 mm
Hot plates
Cuvettes
Kimwipes



Experiment 27
Unknown Drugs in Anesthetized Dog

This experiment is designed to test the ability of the student to apply the principles of pharmacology and his knowledge of the mechanisms of action of drugs in determining the identity of an unknown drug. This can be accomplished only with a logical plan of procedure.

Anesthetize a dog with pentobarbital Na (30 mg/kg) i.v. Arrange to record mean carotid blood pressure, heart rate and respiration on a Grass Polygraph. Prepare the femoral veins for injections.

Before you inject the first dose of your unknown, YOU MUST DETERMINE THE RESPONSE TO NOREPINEPHRINE, ACETYLCHOLINE, HISTAMINE AND NICOTINE. Having injected these four drugs, you will then inject the unknown. Once you have observed the response to your unknown, develop a logical sequence of tests to determine its identity. Assemble as much evidence as possible to establish its identity.

Known drugs will be available on the side benches. Use these as you find it necessary in your experiment. The concentrations of all drugs in the experiment are such that, in most dogs, 0.1 ml/kg will produce an adequate effect. If you find the response inadequate, consult an instructor about increasing the dose. The drugs available are as follows:

DRUG	CONCENTRATION
Epinephrine	40 μg/ml
Norepinephrine	20 μg/ml
Acetylcholine	30 μg/ml
Histamine	100 μg/ml
Nicotine	1 mg/ml
Physostigmine	1 mg/ml
Sodium nitrite	300 mg/ml
Hexamethonium	25 mg/ml
Atropine	5 mg/ml
Phenoxybenzamine	50 mg/ml
Angiotensin	2 μg/ml
Diphenhydramine	20 mg/ml

Your unknown will also be one of the above, but will be identified only by a code. Note that the mere similarity of appearance of the responses between your unknown and one of the known drugs is not adequate proof of the unknown's identity.

Animals: Dogs
Additional Drugs: Pentobarbital Na - 60 mg/ml
Equipment:
- Dog boards
- Muzzles
- Newspapers
- Gauze sponges
- Animal clippers
- Grass instruments
- Tracheal cannulae
- Artificial respiration hoses
- Other: available in desks

APPENDIX I

Useful Equivalents:

1 Kilogram (kg) = 1000 Grams (g) = approximately 2.2 Pounds Avdp.

1 Gram (g) = 1000 Milligrams (mg)

1 Milligram (mg) = 1000 Micrograms (μg, γ)

1 Microgram (μg) = 1×10^{-6} Grams

1 Nanogram (ng) = 1×10^{-9} Grams

1 Picogram (pg) = 1×10^{-12} Grams

Table of Weight - Volume Equivalents

Per cent	Ratio	Grams per Liter	mg per ml	mg%	μg per ml
10.	1:10	100.	100.	10,000.	100,000.
1.	1:100	10.	10.	1,000.	10,000.
0.1	1:1000	1.	1.	100.	1,000.
0.01	1:10,000	0.1	0.1	10.	100.
0.001	1:100,000	0.01	0.01	1.	10.
0.0001	1:1,000,000	0.001	0.001	0.1	1.

Solutions:

Solutions as provided generally will be weight-volume (w/v) solutions. Concentrations will be indicated in one of three ways:

Mass per volume (e.g., mg/ml)
percentage (e.g., 5% = 5 g/100 ml)
ratio (e.g., 1:1000 = 1 g/l = 1 mg/ml)

Calculation of Dosage:

The effects of drugs are determined both by the properties of the drug and the dose administered. Because laboratory animals differ greatly in weight, dosages will almost always be indicated in terms of mass per body weight. Care must therefore be exercised in determining the animal's weight in terms of the units specified in the dosage (e.g., conversion of pounds or grams to kilograms) and in calculating the volume of drug solution to be administered. These calculations involve only simple algebraic principles.

Generalized equations follow:

1. $\text{body weight} \times \frac{\text{dose}}{\text{body weight}} \div \frac{\text{mass of drug}}{\text{unit volume of solution}} = \text{ml of drug soln. to be administered.}$

Substituting:

2. $\frac{g}{1000} \times \frac{mg}{kg} \div \frac{mg}{ml}$ = ml of drug solution to be administered.

Example: Given a rat weighing 257 grams and a solution of pentobarbital Na (50 mg/ml); anesthetize animal by administering drug intraperitoneally in dosage of 35 mg/kg.

$$\frac{257}{1000} \times \frac{35}{1} \div \frac{50}{1} = \frac{.257 \times 35}{50} = 0.18 \text{ ml}$$

Solving:

3. $\frac{kg \times mg \times ml}{kg \times mg}$ = ml or $\frac{\cancel{g} \times \cancel{b} \times c}{\cancel{g} \times \cancel{b}} = c$

Dilution:

In certain cases it is necessary to dilute a stock solution to a more workable (less concentrated) solution. In such operations it is important to keep in mind the amount or volume of drug being diluted and the final volume to which it is diluted. Errors can be avoided if students remember that the volume being diluted is to be part of the final volume.

APPENDIX II

Preparation of Animals and Equipment for Mammalian Experiments

While it is possible to inject or infuse intravenously or even determine arterial blood pressure without exposing the vessels, the skill necessary to carry out these procedures, particularly when they must be repeated frequently, preclude their routine employment. Generally, therefore, vessels will be exposed and cannulae inserted both to facilitate the performance of the experiment and also to provide an opportunity to practice surgery on living animals. Although the experiments are acute (i.e., the animals are not permitted to survive) care should be exercised in order to avoid unnecessary trauma and hemorrhage.

I. Anesthetization and other preliminaries:

1. The animal should be kept on the floor until anesthetized. Muzzle the animal.
2. Remove hair from region of saphenous vein on lateral surface of lower part of hind limb. Clippers will be available but students must remember that these must be shared with other groups.
3. Restrain animal on its side.
4. Occlude vein by grasping the leg firmly above site of injection.
5. Pass a sharp 20 gauge needle through skin and into vein. Puncture of vein usually forces a small amount of blood into syringe. Withdraw a small volume of blood to make certain that needle is in vein and that system is patent.
6. Discontinue occlusion of vein and inject anesthetic. The injection should not be too rapid but should be given over a period of 15-30 seconds.
7. Upon completion of injection, remove needle from vein and maintain pressure on region of puncture to prevent development of a hematoma.
8. Remove muzzle.
9. Fasten dog to animal board in supine position.
10. Remove hair from area of operation.

*If great difficulty is experienced in intravenous administration of anesthetic, consult an instructor.

II. Cannulation of trachea:

Tracheal cannulation is usually advisable in order to insure a patent airway and to facilitate administration of gases or artificial respiration.

1. With a sharp scalpel make a midline incision through skin on the ventral aspect of neck. This incision should be 6 to 8 cm long and should be carried only through skin. Next, by blunt dissection widely separate pretracheal muscles in midline and expose trachea.

2. Make a T shaped incision in trachea, insert cannula of appropriate size and tie securely in place.

III. Cannulation of blood vessels:

The vessels most frequently used are the femoral veins and carotid arteries. As a general principle, if cannulation is for purpose of administering drugs insert cannula with flow; if cannulation is for purpose of recording pressure - insert cannula against flow.

A. Femoral vein:

1. The femoral vein and artery closely parallel one another in the femoral triangle and the artery may be located by palpation. As you proceed, repeatedly orient yourself by palpating artery.

2. With a sharp scalpel make an incision through skin overlying femoral artery (an incision 5 cm in length should be adequate). In practice, it is well to decide where cut is to be made and then, with fingers, draw the skin to one side before cutting. This procedure reduces the chance of cutting a major blood vessel. Blood from small hemorrhages may be removed by blotting with a gauze compress. "Skin bleeders" are not usually troublesome, but if persistent these may be controlled by means of a hemostat.

3. Use a blunt probe or hemostat to dissect through fascia overlying and surrounding vessels. It is better to work down to vessels by dissecting between muscles rather than by going through them. As you approach vessels care should be exercised that vessels or their branches are not nicked or severed because these will form persistent "bleeders" which must be controlled by a hemostat (later it may be desirable to ligate severed branches).

4. Locate femoral vein (typically mediad of artery), carefully expose vein and pass two threads under vein. Free one to two cm of vein from surrounding connective tissue.

5. A length of polyethylene tubing is supplied. This should be connected to the burette supplied in your desk setup. The system is filled with anticoagulant solution.

6. First retract more anterior thread and then retract more posterior thread. This procedure will trap a small volume of blood between threads.

7. With sharp scissors cut a small nick in vein between the two threads. Do not sever vein.

8. Ligate vein by tying more posterior thread.

9. Insert the polyethylene tubing into the vein, pass it forward 4 or 5 cm; tie securely in place with the anterior thread. The tie should be made approximately 1 cm from the opening in the vein. Make sure the cannula is open (not clotted or kinked).

10. Occasional flushing with 1 to 2 ml of anticoagulant solution may be necessary to prevent clotting.

*In cases where no large volume is to be administered cannulation may not be necessary. Injections through a 25 gauge needle may be made directly into vein. This procedure is facilitated by initially retracting a thread which has been passed under vein; needle is then inserted, ligature relaxed, injection made and needle withdrawn.

B. Carotid artery:

1. After cannulation of trachea (see II, above) locate carotid artery, by palpation, deep down on either side of trachea.

2. Free about 3 cm of carotid artery from connective tissue sheath and vagus nerve which passes along this sheath.

3. Pass 2 threads under artery. With one thread tie a ligature at distal end of freed artery segment. Place an artery clip (bulldog clamp) at proximal end of freed segment. Keep second thread between "bulldog" and ligature.

4. Defer actual cannulation until recording equipment and transducer are set up (see Appendix III). A length of polyethylene tubing is permanently attached to the transducer. A second length of tubing will just fit inside the tubing attached to the transducer. This system should be filled with anticoagulant solution (if bubbles are in the system, call your instructor).

5. Make an incision into the lumen of the artery to second thread. Insert polyethylene tubing of appropriate size. After the tubing has been advanced to the "bulldog" clamp place a light tie around the vessel between the "bulldog" and the opening in the vessel. Remove the "bulldog" and pass the tubing approximately 4 to 5 cm into the vessel. Tie securely in place. Make sure that the tie does not occlude the tubing. Check the recording. If the recording appears damped call your instructor.

IV. Exposure of vagus nerve:

The vagus nerves usually are lateral to, but closely associated with, the common carotid arteries.

1. Locate common carotid artery by palpation and following procedure described above, carefully expose artery and seek vagus nerve. If a carotid artery is to be cannulated, it might be well to expose vagus on opposite side.

2. Pass a thread under vagus nerve to facilitate finding nerve when it is needed.

V. Artificial respiration hose:

Respiratory failure is an emergency you will encounter either by accident or design and you should be prepared to deal with the situation effectively; first, by cannulation of the trachea (see above) and, second, by use of an artificial respiration hose which should be available at all times on a standby basis. This piece of equipment consists of a length of tubing with an open T interposed nearer one end than the other.

1. Connect the long end of the tube to the airline and open the valve to permit a gentle flow.

2. Connect the short end of the tube to the tracheal cannula.

3. Apply thumb to open end of T to inflate lungs. Avoid over inflation.

4. Remove thumb to permit exhalation.

5. Keep operation rhythmic.

VI. Pneumograph:

A large plastic T-tube fitted with a long and a short rubber tubing is supplied in your desk setup. Attach the T-tube to the tracheal cannula by means of the short tubing. The longer tubing should be fitted to the venous transducer. The Grass polygraph should be calibrated so that a deflection of approximately 1 cm occurs with each respiration (see Appendix III).

APPENDIX III

Grass Instrument

General Directions

1. Remove protective tips from pens and place them in the box provided.
2. On model 5D turn on the switch located just above the inkwells. This switch is absent in all other models in the laboratory.
3. Turn on D. C. Driver Amplifier switches.
4. Turn on all pen switches for those channels you will be using except number 4 (nearest operator). These switches are marked "oscillographs" on model 5D.
5. Set chart speed at .25 mm/sec.
6. Turn Driver Control Switch to the far right. On model 5D this switch is at bottom right hand corner of front panel and is marked CONSOLE AC POWER. On all other models, it is below the pen switches and unmarked.
7. Allow 10 minutes for warm up.
8. DO NOT TOUCH ANY KNOBS OR DIALS MARKED WITH RED DOTS.

RECORDING BLOOD PRESSURE

1. Be certain that the transducer marked 0-75 cm is attached to top channel.
2. The transducer will be supplied with polyethylene tubing attached to the top outlet of the chamber covering the pressure sensitive head. The side outlet of chamber will be capped and this cap should not be removed. The chamber and tubing will be filled with a permanent solution. Any shortage of fluid in the tubing should be replaced from the open end with anticoagulant solution. This fluid replacement should be accomplished by use of a hypodermic needle with an attached length of fine polyethylene tubing (see instructor). Care should be exercised to avoid undue pressure on

the confined fluid. DO NOT CLAMP, CUT OR SHARPLY BEND THE POLYETHYLENE TUBING SUPPLIED WITH THE TRANSDUCER. Join transducer tube with polyethylene tubing (supplied in drawer) of size suitable for use as a cannula. By the method employed above fill cannula with anticoagulant solution.

3. Set the SENSITIVITY MV/CM knob of the top preamplifier at 10 or 20. The instructor will advise which to use. (at a setting of 10: one cm = 50 mm Hg; at setting of 20: one cm = 100 mm Hg) The chart baseline (0 mm Hg) is the line 2 cm below center.
4. Set the 1.2 AMP HI FREQ. knob of the top D.C. Driver Amplifier at .1 to record mean blood pressure or 60 to record systolic and diastolic pressure.
5. Cannulate a carotid artery with polyethylene tubing.

RECORDING RESPIRATION

1. Be certain that the transducer marked 0-5 cm is attached to the second channel from the top.
2. Connect the top outlet of the transducer to the tracheal cannula with a piece of rubber tubing as described in Appendix II.
3. Set the SENSITIVITY MV/CM knob at whatever position provides the most suitable excursion of the pen.

RECORDING MUSCULAR CONTRACTION

1. Be certain that the Force-Displacement Transducer FT03 is attached to third channel from top.
2. Set the SENSITIVITY MV/CM knob at the sensitivity appropriate for the particular muscle. The table on the side of the polygraph gives you

the conversion factor for each sensitivity setting to allow conversion of cm of pen deflection into grams of force.

3. Tie a thread from the muscle to the small movable arm of the transducer. The thread should be perpendicular to the plane of the transducer.
4. Raise the transducer until there is a slight tension on the thread.

RECORDING EKG OR HEART RATE

A. Preparations for Recording

1. Attach electrode box to bottom channel.
2. Connect electrode wires (stored in desk drawer) to electrode box and insert needles subcutaneously as directed in Appendix II.
3. Set LEAD SELECTOR knob at II.

B. For Recording EKG

1. Set OUTPUT knob at EKG.
2. Set chart speed at 10.
3. Turn on pen switch #4. Never turn on unless, the electrodes are connected to the animal.
4. Adjust EKG SENSITIVITY to give suitable pen deflection.
5. If EKG recording goes off chart at any time, press TRACE RESTORER. Heart rate can be counted from the EKG, but a direct measurement of rate can be obtained by using the tachograph instead of the EKG.
6. Set 60 CYCLE FILTER at out unless otherwise instructed.

C. For Tachograph Record

1. Set 60 CYCLE FILTER at out.
2. Set OUTPUT knob at EKG.
3. Set TACHOGRAPH SENSITIVITY and BASE LINE controls at position required for range 100-300 beats/min (see table below preamplifier).

4. Turn TACHOGRAPH TRIGGER control as far as it will go counter-clockwise.
5. Set chart speed at 10.
6. Set OUTPUT knob at TACH.
7. Slowly turn TRIGGER control clockwise until pen deflects once for each heart beat, then advance TRIGGER control about 30° past this minimum setting. Turning the TRIGGER control too far or not turning it far enough is the most frequent cause of erratic recording.
8. Select chart speed such that successive pen deflections are superimposed. One may now switch back and forth between EKG and TACHOGRAPH merely by changing OUTPUT knob and picking appropriate chart speeds.
9. Measuring heart rate from the tachograph. The tachograph records only on the middle 40 mm of the chart. The rate is determined by the line at which the downward movement of the pen stops. The bottom line of this 40 mm range represents the minimum heart rate which can be measured (100 beats/min.). To measure the heart rate, determine the number of mm above this baseline at which the downward movement of the pen stops. Locate this value on the abscissa of the graph (next page) and find the corresponding heart rate on the ordinate. For rates below 100, switch to EKG and count the rate, making use of the known speed at which the chart paper is moving.

SIGNAL MARKER

The center pen makes a time signal every second. The instant at which a drug is injected may be indicated on the record by pressing the red signal button.

TACHOGRAPH CONVERSION GRAPH FOR 100 - 300 BEATS PER MINUTE RANGE

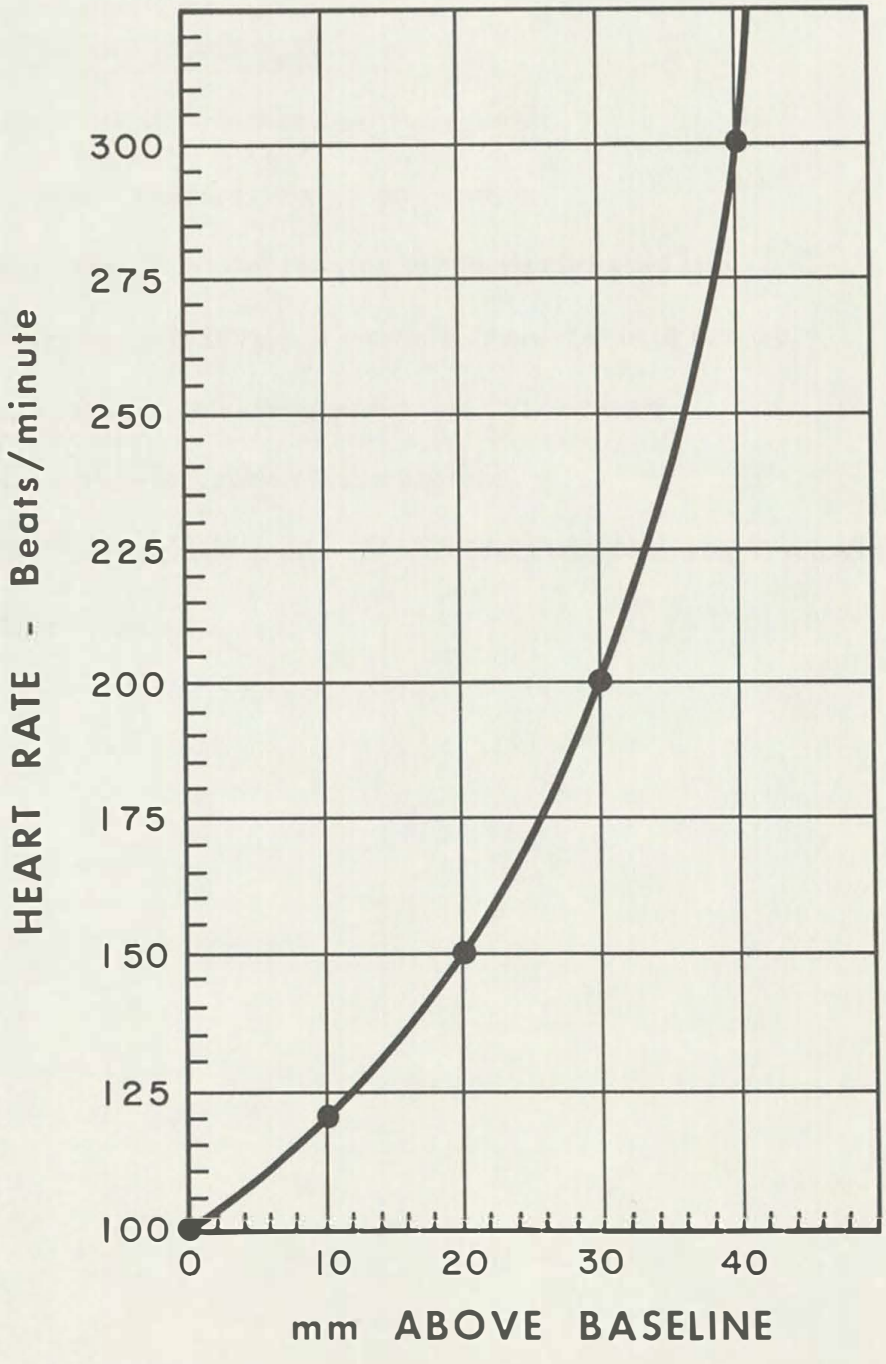


CHART SPEED

Always use the slowest chart speed compatible with the type of recording involved in the experiment. During intervals of the experiment in which recording is not necessary turn off the chart drive to conserve paper.

AT END OF EXPERIMENT

1. Turn off all switches on Polygraph.
2. Replace protective tips on pens.
3. Remove paper by tearing along perforated line.
4. Remove polyethylene cannula from carotid artery.
5. Disconnect pneumograph from transducer.
6. Remove electrodes from animal.
7. Disconnect electrodes from electrode box and place electrodes in desk drawer.

APPENDIX IV

INDUCTORIUM

The inductorium is a device that has been widely used to produce electrical currents of different intensities, particularly for the stimulation of nerves. The instrument consists of two coils: the primary coil is fixed in position; the secondary coil is movable on parallel rods. Current is induced in the secondary coil as current either begins or ceases to flow through the primary coil. The primary coil has three terminals which may be connected, in one of two combinations, with a source of direct current.

Make and Break Current:

When the direct current source is connected with the left hand and middle terminals of the primary coil a switch should be included in the circuit. With this arrangement, current is induced in the secondary coil when the switch is closed and again when the circuit is broken. A set-up of this sort is used to deliver the so-called "make and break" electrical stimulus. If "make and break" stimulation is to be provided at the option of the investigator the switch should be used. In this case care should be exercised that current does not flow through the primary coil in amount or for a time sufficient to heat the coil. In some instances it is desirable to provide a "make and break" stimulus at regular intervals. This may be accomplished by eliminating the switch and connecting the instrument with the laboratory's signal circuit (the staff will arrange for the proper operation of this power supply).

Tetanizing Current:

When the direct current source is connected with the right and left hand terminals of the primary coil a built-in interruptor makes and breaks the circuit at a relatively rapid rate (when properly adjusted) and this action induces in the secondary coil the so-called "tetanizing current." With this setup a switch should be included in the circuit between the primary coil and the power source and, again, care should be taken to avoid overheating of the coils.

A silver electrode stimulator may be connected with either the terminals of the secondary coil or terminals at the ends of the parallel rods on which the secondary coil is mounted. The intensity of the induced current is determined by the position of the secondary coil in relation to the primary coil. In practice, the inductorium is adjusted to deliver an effective stimulus by testing a potentially responsive system first, with the secondary coil at its maximum distance from the primary and then, repeating tests as the secondary coil (in a position parallel with the supporting rods) is slid toward or even over the primary coil. When a satisfactory position has been located for the secondary coil, it should be fastened in this position by the set screws available for this purpose. The blade switch, between the parallel rods, may be used to interrupt or prevent a stimulus without otherwise altering the setup.

APPENDIX V

OPERATION AND USE OF THE COLEMAN JUNIOR SPECTROPHOTOMETER

The Coleman Junior Spectrophotometer, Model 6A, is a simple instrument to operate and thus is very satisfactory for many routine methods of colorimetric analysis.

Readings can be made in either optical density or per cent transmission. The former is to be preferred for most situations because the readings give a straight line when plotted against the concentration of solute in the sample. However, the per cent transmission scale (lower) is easier to read; and by plotting the readings on the logarithmic scale of semilogarithmic graph paper, a straight line is obtained.

Operating directions:

1. Turn the instrument "on" by means of the toggle switch at the back of the instrument, and allow it to warm up for about 10 minutes before any measurements are made.

2. Verify the wave-length setting by checking the dial in the center of the instrument. It can be changed by rotating the selector knob below the dial.

3. Verify the galvanometer zero adjustment by inserting the cuvette adapter in the cuvette well, turned 90° from the keyway. This blocks the passage of light to the photocell. If, under these conditions, the galvanometer index (the dark line in the circle of light) is not exactly on zero per cent transmission, the instrument is out of adjustment and must be correctly adjusted by an instructor. Before continuing reinsert the cuvette adapter in its normal position with the lug in the keyway.

4. Insert in the cuvette well, at the lower right corner of the instrument, the cuvette or testtube containing the Blank solution.

5. By means of the COARSE and FINE knobs, adjust the galvanometer index to the 100 per cent transmission point.

6. After removing the Blank tube, insert the Standard tube and read the per cent transmission as indicated by the galvanometer index.

7. Similarly read the unknown samples, checking the Blank reading each time.

8. After reading the last sample, turn the instrument "off" by means of the toggle switch.

APPENDIX VI

Method for Obtaining Blood Samples from the Marginal Ear Vein of the Rabbit

Spread petrolatum over the upper surface of the ear. This makes the marginal ear vein readily visible along the posterior edge of the ear and also lowers the surface tension of the hair so the blood will pool instead of spreading when the vein is opened. This also minimizes clotting. Warm the ear under the table lamp and rub it gently until the vessels are fully dilated. With a sharp razor blade or scalpel, cut into the marginal ear vein at right angles to the course of the vessel. This should be a sharp cut, not over 2 mm in length so the edges of the cut will readily come together to stop the bleeding after the sample is obtained. Capillary tubes for clotting times and 0.2 ml pipettes can be filled by capillarity directly from large drops of blood formed on the petrolatum-coated surface of the ear. Larger samples should be collected in small polyethylene containers to which an anticoagulant has been added. The vessels will usually constrict and stop the bleeding as soon as the heat is removed from the ear. A thick coat of petrolatum over the wound will help stop bleeding and protect the area until time for the next sample. In rare instances, it will be necessary to place gauze or filter paper over the wound and apply gentle pressure with the thumb and forefinger for a minute or two. When ready for additional samples, again warm the ear; wipe away the petrolatum and open the clean wound by gently bending the ear. With good technique, it is possible to obtain 10 to 20 samples over a period of several hours from the single wound. If you need help in doing this procedure, consult the instructor. Do not traumatize the entire ear! !

APPENDIX VII
Prescription Writing

Prescriptions should be written following the example below:

You are to write a prescription for Phenoxymethyl Penicillin, USP, for a susceptible staphylococci infection in an adult; give a one week supply.

West Virginia University Medical Center

Practice Prescription

John Q. Public — 5/11/64
3152 Medical Center

℞ Phenoxymethyl Penicillin U.S.P. 125 mg.
Disp. #28
Sig.: One ac and hs

Label ☑ yes ☐ no

Non Rep. ✓ Refill X_____

Authorization is given for dispensing by generic name unless checked here. ☐

John M. Doe M. D.
Reg. No._____

1. Check block yes if prescription is to be labeled with contents and check block no if it is not.

2. Narcotic prescriptions cannot be refilled by law so you need not indicate this.

3. All narcotic prescriptions require your narcotic registration number placed in the provided space. Non-narcotic prescriptions do not require this number.

4. The patient's address and physician's address must also appear on narcotic prescriptions.

5. If the prescription is to be refilled indicate how many times in the lower left hand corner.

6. All prescriptions must be written and signed in ink.

APPENDIX 5B

MEDICAL PHARMACOLOGY SCHEDULES

FROM 2004 and 2018-19

Note increase in teaching faculty participation from 2004 to 2018-19

PCOL 761 Course Schedule, 2004
(Teaching faculty highlighted)

Date	Time	Lecture Title	Study Guide	Lecturer
3/24 (M)	8:00	Introduction to Course: Basic Definitions Routes of Drug Administration	1-4	Smith
	9:00	Membrane Transport Ionization and Protein Binding ALE #1: Drug Distribution	5-13 14-17	Smith
	10:00	Metabolism of Drugs	18-23	Stitzel
	11:00	Excretion of Drugs	18-23	Stitzel
3/25 (T)	8:00	Pharmacokinetics of Single Dose Administration	24-34	Craig
	9:00	Pharmacokinetics of Multiple Dose Administration	24-34	Craig
3/26 (W)	8:00	Receptors & Dose-Response Relationships	35-43	Fedan
	9:00	Mechanism of Drug Antagonism	44-48	Fedan
Date	Time	Lecture Title	Study Guide	Lecturer
3/26 (W) (cont.)	10:00	Drug Information Resources for Physicians: PubMed, Clinical Pharmacol 2000, & Drug Buddy	49-57	Abate Woodfork Smith
	11:00	Drug Information Resources (continued) Introduction to ALE #2: Medical Informatics and Pharmacology	49-57 58-63	Abate Woodfork Smith
3/27 (Th)	8:00	Introduction to the Autonomic Nervous System	64-68	Fedan
	9:00	Pharmacology of Noradrenergic Transmission	69-76	Fedan
	10:00	Herbal Medicine	77-86	Juckett
	11:00	Herbal Medicine Introduction to ALE #3: Case Based Exercises	77-86	Juckett
3/28 (F)	8:00	TBA		
	9:00	Pharmacology of Adrenoceptors	87-93	Fedan
	10:00	Adrenergic agonists	94-100	Fedan
	11:00	Pharmacology in an Aging Population	101-103	DeLaGarza
3/31 (M)	8:00	Alpha antagonists	103a-f	Fedan
	9:00	Beta Antagonists	103g-m	Fedan
	10:00	Prescription Writing and Drug Regulation	104-108	Graves
	11:00	Therapeutic Management of the Pediatric Patient		Ferrari
4/1 (T)	8:00	Cholinergic Nervous System I	109-121	Wonderlin

	9:00	Cholinergic Nervous System II	109-121	Wonderlin
4/2 (W)	8:00	ALE #2: Medical Informatics and Pharmacology		Small Group
	9:00	ALE #2 (continued)		Small Group
	10:00	Renal Pharmacology	122-129	Stitzel
	11:00	Renal Pharmacology	122-129	Stitzel
4/3 (Th)	8:00	Calcium Entry Antagonists	130-137	Wonderlin
	9:00	Antianginal Drugs	138-145	Fedan
	10:00	Exercise in computer simulation of hemodynamic action of autonomic drugs I		Rosen
	11:00	Exercise in computer simulation of hemodynamic action of autonomic drugs II		Rosen
4/4 (F)	8:00	ALE #3: Case Based Exercises in Herbal Medicine		Small Group
	9:00	ALE #3 (continued) Introduction to ALE #4: Drug Toxicity		Small Group
	10:00	Anticoagulant Drugs	149-155	Fedan
	11:00	Renin-Angiotensin System	156-163	Wonderlin
4/7 (M)	8:00-12:00	**EXAM 1**		
Date	Time	Lecture Title	Study Guide	Lecturer
4/8 (T)	8:00	Pharmacologic Management of Congestive Heart Failure	164-172	Finkel
	9:00	Pharmacologic Management of Congestive Heart Failure	164-172	Finkel
	4:00-6:00	Post Exam Review (Hostler Auditorium)		
4/9 (W)	8:00	ALE #4: Drug Toxicity		Small Group
	9:00	ALE #4 (continued) Introduction to ALE #5: Hypertension		Small Group
	10:00	Antihypertensive Drugs I	173-176	Wonderlin
	11:00	Antihypertensive Drugs II	173-176	Wonderlin
4/10 (Th)	8:00	Pharmacological Control of Plasma Lipids	177-187	Reasor
	9:00	Local Anesthetics	188-193	Smith
	10:00	Antiarrhythmic Drugs I	194-208	Wonderlin
	11:00	Antiarrhythmic Drugs II	194-208	Wonderlin
4/11 (F)	8:00	ALE #5: Hypertension		Small Group
	9:00	ALE #5 (continued)		Small Group
	10:00	Antiarrhythmic Drugs III	194-208	Wonderlin
	11:00	Diuretics in Intensive Care Medicine		Schiebel
4/14 (M)	8:00	Pharmacological Therapy of Arrhythmias	209-212	Schmidt
	9:00	Issues Effecting Drug Compliance/Hypertension		Arbogast

	10:00	Histamine	213-219	Smith
	11:00	TBA		
4/15 (T)	8:00	Introduction to Antiinflammatory Drugs: Steroids; Anti-rheumatoid Drugs and Therapy of Gout	220-228	Van Dyke
	9:00	Non-steroidal Antiinflammatory Drugs and Acetaminophen	229-236	Smith
4/16 (W)	8:00	PAF and prostaglandin/leukotiene antagonists Immunomodulatory Effect of Drugs I	237-248	Van Dyke
	9:00	Immunomodulatory Effect of Drugs II	249-263	Van Dyke
	10:00	Drug-Induced Allergies		Wilson
	11:00	TBA		
4/17 (Th)	8:00	Treatment of Rheumatoid Arthritis		DiBartolomeo
	9:00	Drug Use in Ophthalmology	264-269	Charlton
	10:00	Introduction and Pharmacology of the Central Nervous System	270-275	Craig
	11:00	TBA		
4/18 (F)		***Friday before Easter***		

Date	Time	Lecture Title	Study Guide	Lecturer
4/21 (M)	8:00	Sedative Hypnotics I	276-278	Craig
	9:00	Sedative Hypnotics II	279-283	Craig
	10:00	TBA		
	11:00	TBA		
4/22 (T)	8:00-12:00	**EXAM 2**		
4/23 (W)	8:00	Pharmacology of Myasthenia Gravis		Gutmann
	9:00	General Anesthesia I	284-296	Smith
	10:00	General Anesthesia II	284-296	Smith
	11:00	Neuromuscular Blocking Drugs	297-304	Johnstone
	4:00-6:00	Post Exam Review (Hostler Auditorium)		
4/24 (Th)	8:00	Pharmacological Management of Pain I	305-311	Smith
	9:00	Pharmacological Management of Pain II (includes NSAID, and chronic opioid use)	305-311	Smith
	10:00	Pharmacology of Asthma	312-319	Fedan
	11:00	Asthma (clinical case studies)		Hogan
4/25(F)	8:30-10:00	Neurodegenerative diseases Introduction to ALE # 6: Treatment of Parkinsonism	320-331	Schreurs
	10:00	Narcotic Analgesia I	332-342	Smith
	11:00	Narcotic Analgesia II Introduction to ALE #7: Geriatric Medicine	332-342	Smith
4/28 (M)	8:00	Antidepressants I	343-354	Woodfork
	9:00	Antidepressants II	343-354	Woodfork
	10:00	Anticonvulsants	355-360	Craig
	11:00	Epilepsy		Khan
4/29 (T)	8:00	Antipsychotics	361-368	Woodfork
	9:00	Anxiolytics	369-378	Woodfork
4/30(W)	8:00	ALE #6: Treatment of Parkinsonism		Small Group
	9:00	ALE #6 (continued) Introduction to ALE #7: Geriatric Medicine		Small Group
	10:00	Attention-Deficit Hyperactivity Disorder	379-386	Woodfork
	11:00	Therapy of Psychiatric Illness		Selby

Date	Time	Lecture Title	Study Guide	Lecturer
5/1 (Th)	8:00	Drugs of Abuse	387-391	Craig
	9:00	Case Studies of Chronic Pain Management		Vaglienti
	10:00	Drug Impaired Physician		Barbaccia
	11:00	Serotonin includes migraine therapy		Graber
5/2 (F)	8:00	ALE#7: Geriatric Medicine		Small Group
	9:00	ALE #7 (continued) Introduction to ALE #8: Cancer Chemotherapy		Small Group
	10:00	Pharmacology of G.I. Tract	392-405	Connors
	11:00	Pharmacology of G.I. Tract	392-405	Connors
5/5 (M)	8:00-12:00	**EXAM 3**		
5/6 (T)	8:00	Cancer Chemotherapy I	406-417	Strobl
	9:00	Cancer Chemotherapy II	406-417	Strobl
	4:00-6:00	Exam Review (Room 2116 HSN)		
5/7 (W)	8:00-10:00	Review Session		Faculty
	10:00	Cancer Chemotherapy III	406-417	Strobl
	11:00	Pharmacogenomics	418-424	Strobl
5/8 (Th)	8:00	Developmental Toxicology	425-431	Reasor
	9:00	Metals Toxicity	432-438	Reasor
	10:00	Insulin and Hypoglycemic Agents	439-448	Mawhinney
	11:00	Adrenocortical Steroids	449-454	Mawhinney
5/9 (F)	8:00	ALE #8: Cancer Chemotherapy		Small Group
	9:00	ALE #8 (continued)		Small Group
	10:00	Estrogen and Progestins I	455-468	Mawhinney
	11:00	Estrogen and Progestins II	455-468	Mawhinney
5/12 (M)	8:00	Androgens	469-471	Mawhinney
	9:00	Thyroid and Antithyroid Therapy	472-481	Connors
	10:00	TBA		
	11:00	Mechanisms of Antidotal Therapy	482-484	Reasor
5/13 (T)	8:00	Solvent Toxicity	485-489	Davis
	9:00	Pesticide Toxicity	490-492	Davis
5/14 (W)	8:00-12:00	**EXAM 4**		
5/15 (Th)	8:00-12:00	**Study day**		
	4:00-6:00	Post Exam Review (Room 2116 HSN)		
5/16 (F)	8:00-12:00	**SHELF BOARD in Pharmacology**		

Exam Coverage				
Exam	**Exam Date**	**Classes From**	**Classes To**	**# of Lectures**
Exam 1	4/7/03	3/24/03	4/3/03	25
Exam 2	4/22/03	4/4/03	4/17/03	23
Exam 3	5/5/03	4/21/03	5/1/03	25
Exam 4	5/14/03	5/2/03	5/13/03	19
Shelf Board	5/16/03	-	-	-
Exam questions are generally taken from the material presented in clinical correlations and regular lectures.				

PCOL801, 2018-1019 (Teaching faculty highlighted in yellow)

Block	Date		Title	Presenter
1	13-Aug	Mon 9:00	Introduction to Pharmacology	Karen Woodfork
1	14-Aug	Tues 10:00	Absorption and Distribution, Ionization and Protein Binding 1	Karen Woodfork
1	14-Aug	Tues 11:00	Absorption and Distribution, Ionization and Protein Binding 2	Karen Woodfork
1	15-Aug	Wed 8:00	Receptors and Dose-Response Relationships	Mary Davis
1	15-Aug	Wed 9:00	Mechanisms of Drug Antagonism	Mary Davis
1	16-Aug	Thurs 8:00	Metabolism of Drugs 1	Leah Hammer
1	16-Aug	Thurs 9:00	Metabolism of Drugs 2	Leah Hammer
1	16-Aug	Thurs 11:00	Problem-Solving Activity: Drug Ionization and Distribution	Karen Woodfork
1	17-Aug	Fri 10:00	Excretion of Drugs	Leah Hammer
1	17-Aug	Fri 11:00	Adverse Drug Reactions	Leah Hammer
1	22-Aug	Wed 10:00	Pharmacokinetics 1	Mary Davis
1	22-Aug	Wed 11:00	Pharmacokinetics 2	Mary Davis
1	23-Aug	Thurs 10:00	Problem Solving Activity: Pharmacokinetics 1	Karen Woodfork
1	23-Aug	Thurs 11:00	Problem Solving Activity: Pharmacokinetics 2	Karen Woodfork
1	24-Aug	Fri 10:00	Prescription Writing and Drug Regulation	Charles Ponte
1	27-Aug	Mon 8:00	Introduction to Clinical Pharmacogenomics	William Petros
1	27-Aug	Mon 9:00	Drug Development and Good Clinical Practice	Julie Lockman
1	29-Aug	Wed 9:00	Self-Directed Learning: DIY Exam	Karen Woodfork
2	7-Sep	Fri 10:00	Antibiotics 1	Julie Lockman
2	7-Sep	Fri 11:00	Antibiotics 2	Julie Lockman
2	10-Sep	Mon 10:00	Antibiotics 3	Julie Lockman
2	10-Sep	Mon 11:00	Antibiotics 4	Julie Lockman
2	11-Sep	Tues 10:00	Antibiotics 5	Julie Lockman
2	11-Sep	Tues 11:00	Antibiotics 6	Douglas Slain
2	12-Sep	Wed 10:00	Immunosuppressants 1	Karen Woodfork
2	12-Sep	Wed 11:00	Immunosuppressants 2/Immunostimulants	Karen Woodfork
2	13-Sep	Thurs 11:00	Inflammation and Glucocorticoids - CAMTASIA ONLY	David Siderovski
2	18-Sep	Tues 10:00	Eicosanoid Drugs In-Class Session	Siderovski/Woodfork

2	17-Sep	Mon	9:00	Antihistamines - CAMTASIA ONLY	Karen Woodfork
2	17-Sep	Mon	10:00	NSAIDs & Eicosanoid Drugs 1 - CAMTASIA ONLY	David Siderovski
2	17-Sep	Mon	11:00	NSAIDs & Eicosanoid Drugs 2 - CAMTASIA ONLY	Siderovski/Woodfork
2	18-Sep	Tues	11:00	Drug-Induced Allergy	Paul Siegel
2	19-Sep	Wed	9:00	Self-Directed Learning: DIY Exam	Karen Woodfork
3	24-Sep	Mon	11:00	Cancer Chemotherapy 1	Jun Liu
3	26-Sep	Wed	9:00	Cancer Chemotherapy 2	Jun Liu
3	26-Sep	Wed	10:00	Cancer Chemotherapy 3	Jun Liu
3	28-Sep	Fri	9:00	Cancer Chemotherapy 4	Jun Liu
3	8-Oct	Mon	10:00	Antivirals	Douglas Slain
3	8-Oct	Mon	11:00	Antifungals	Douglas Slain
4	15-Oct	Mon	10:00	Intro to Autonomic Pharmacology	Leah Hammer
4	15-Oct	Mon	11:00	Noradrenergic Transmission and Adrenoceptors	Leah Hammer
4	16-Oct	Tues	10:00	Adrenoceptor Agonists	Karen Woodfork
4	17-Oct	Wed	8:00	Drug Adherence	Greg Doyle
4	17-Oct	Wed	9:00	Alpha-Adrenoceptor Antagonists	Karen Woodfork
4	17-Oct	Wed	10:00	Beta-Adrenoceptor Antagonists	Karen Woodfork
4	18-Oct	Thurs	10:00	Cholinergic Pharmacology 1	Julie Lockman
4	18-Oct	Thurs	11:00	Cholinergic Pharmacology 2	Julie Lockman
4	19-Oct	Fri	8:00	Clinical Management of Skin Disorders 1	Roxann Powers
4	19-Oct	Fri	9:00	Clinical Management of Skin Disorders 2	Roxann Powers
4	19-Oct	Fri	10:00	Diuretics 1	TBA
4	19-Oct	Fri	11:00	Diuretics 2	TBA
4	22-Oct	Mon	8:00	Renin-Angiotensin System	TBA
4	22-Oct	Mon	9:00	Calcium Entry Antagonists	TBA
4	22-Oct	Mon	10:00	Problem Solving: Imaginary Human Exercise	Karen Woodfork
4	23-Oct	Tues	10:00	Antihypertensive Drugs 1	Karen Woodfork
4	23-Oct	Tues	11:00	Antihypertensive Drugs 2	Karen Woodfork
4	24-Oct	Wed	8:00	Treatment of Congestive Heart Failure	Karen Woodfork
4	24-Oct	Wed	9:00	Treatment of Myocardial Ischemia	Karen Woodfork
4	25-Oct	Thurs	10:00	Antiarrhythmic Drugs 1	Karen Woodfork

4	25-Oct	Thurs	11:00	Antiarrhythmic Drugs 2	Karen Woodfork
4	26-Oct	Fri	8:00	Clinical Management of Hypertension	James Mills
4	26-Oct	Fri	9:00	Clinical Management of Congestive Heart Failure	James Mills
4	26-Oct	Fri	10:00	Clinical Management of Arrhythmias	Stanley Schmidt
4	26-Oct	Fri	11:00	Lipid-lowering Drugs	David Siderovski
4	29-Oct	Mon	11:00	Problem Solving: Cardiovascular Disease	Karen Woodfork
5	8-Nov	Thurs	11:00	Mucoactive Drugs - CAMTASIA ONLY	David Siderovski
5	9-Nov	Fri	9:00	Pulmonary Drugs - in-class session	David Siderovski
5	9-Nov	Fri	10:00	Pharmacological Therapy of Asthma - CAMTASIA ONLY - watch before in-cl	David Siderovski
5	27-Nov	Tues	8:00	CAMTASIA: Antimycobacterial Drugs (25 min)	Karen Woodfork
5	27-Nov	Tues	10:00	Intro to CNS	Julie Lockman
5	27-Nov	Tues	11:00	Anxiolytics	David Siderovski
5	28-Nov	Wed	8:30	Sedative Hypnotics	TBA
6	3-Dec	Mon	8:00	Drugs of Abuse 1	Han Ting Zhang
6	3-Dec	Mon	9:00	Drugs of Abuse 2	Han Ting Zhang
6	3-Dec	Mon	10:00	Self-Directed Learning: Journal Club (2 hour)	
6	3-Dec	Mon	11:00	Self-Directed Learning: Journal Club (2 hour)	
6	4-Dec	Tues	8:00	Antidepressants 1 - CAMTASIA ONLY	David Siderovski
6	4-Dec	Tues	9:00	Antidepressants 2, Bipolar Disorder - CAMTASIA ONLY	David Siderovski
6	4-Dec	Tues	10:00	Alcohols	Han Ting Zhang
6	5-Dec	Wed	9:00	Antipsychotics - CAMTASIA ONLY	David Siderovski
6	6-Dec	Thurs	10:00	Psychotherapeutic Drugs In-Class Session	David Siderovski
6	6-Dec	Thurs	11:00	Clinical Management of Psychiatric Disorders	Joseph Selby
6	10-Dec	Mon	11:00	Drug Therapy of Anemia	Knox Van Dyke
6	17-Dec	Mon	11:00	Antimalarial Drugs	Karen Woodfork
6	18-Dec	Tues	10:00	Anticoagulants 1	Richard Johnston
6	18-Dec	Tues	11:00	Anticoagulants 2	Richard Johnston
7	11-Jan	Fri	10:00	Antiparasitic drugs, GI parasites	Karen Woodfork
7	11-Jan	Fri	11:00	Antiemetic and prokinetic drugs	Leah Hammer
7	15-Jan	Tues	11:00	CAMTASIA: Drugs for Viral Hepatits	TBA

7	17-Jan	Thurs	11:00	Treatment of Inflammatory Bowel Disease	Syed Mustafa
7	18-Jan	Fri	9:00	Treatment of peptic ulcer disorders	Syed Mustafa
7	18-Jan	Fri	10:00	Laxatives and cathartics	Syed Mustafa
7	22-Jan	Tues	11:00	CAMTASIA: Drugs for Erectile Dysfunction	Karen Woodfork
8	31-Jan	Thurs	10:00	Pituitary and Hypothalamic Diseases	John Connors
8	31-Jan	Thurs	11:00	Thyroid and Antithyroid Therapy	John Connors
8	8-Feb	Fri	10:00	Video: Diabetes Drugs 1	Julie Lockman
8	8-Feb	Fri	11:00	Video: Diabetes Drugs 2	Julie Lockman
8	4-Feb	Mon	8:00	Video: Female Reproductive Drugs 1	Karen Woodfork
8	4-Feb	Mon	9:00	Video: Female Reproductive Drugs 2	Karen Woodfork
8	5-Feb	Tues	9:00	Video: Male Reproductive Drugs	David Siderovski
8	11-Feb	Mon	9:00	A&E: Reproductive Drugs Case-Based Questions	Woodfork/Siderovski
8	11-Feb	Mon	10:00	Pre-diabetes	Rosemarie Lorenzetti
8	13-Feb	Wed	8:00	A&E: Diabetes Therapeutics Case-Based Questions	Charles Ponte
9	19-Feb	Tues	9:00	Drugs Affecting Bone Mineral Metabolism	John Connors
9	21-Feb	Thurs	10:00	Drugs for Gout	Karen Woodfork
9	21-Feb	Thurs	11:00	Disease-Modifying Anti-Rheumatic Drugs	Karen Woodfork
9	1-Mar	Fri	9:00	Supplements 1	Karen Woodfork
9	1-Mar	Fri	10:00	Supplements 2	Karen Woodfork
9	27-Feb	Wed	8:00	Intro to Toxicology	Karen Woodfork
9	27-Feb	Wed	9:00	Metals Toxicity	Karen Woodfork
9	28-Feb	Thurs	9:00	Developmental/Reproductive Toxicity; Solvent Toxicity	Karen Woodfork
9	28-Feb	Thurs	10:00	Pesticides and Cholinesterase Inhibitors	Mary Davis
9	28-Feb	Thurs	11:00	Mechanism of Antidotal Therapy	Syed Mustafa
9	1-Mar	Fri	8:00	Drugs Used in Ophthalmology	Brian McMillan
10	18-Mar	Mon	10:00	VIDEO: Opioid Analgesia 1	Leah Hammer
10	18-Mar	Mon	11:00	VIDEO: Opioid Analgesia 2	Leah Hammer
10	21-Mar	Thurs	10:00	General Anesthesia 1	David Siderovski
10	21-Mar	Thurs	11:00	General Anesthesia 2	David Siderovski
10	27-Mar	Wed	9:00	Clinical Management of Epilepsy	John Magruder

10	27-Mar	Wed	10:00	A&E: Pain Management Cases 1	Richard Vaglienti
10	27-Mar	Wed	11:00	A&E: Pain Management Cases2	Richard Vaglienti
10	28-Mar	Thurs	8:00	Neurodegenerative Diseases 1	Bernard Schreurs
10	28-Mar	Thurs	9:00	Neurodegenerative Diseases 2	Bernard Schreurs
10	28-Mar	Thurs	10:00	Migraines	Leah Hammer
10	28-Mar	Thurs	11:00	Skeletal Muscle Relaxants	Leah Hammer
10	29-Mar	Fri	9:00	Anticonvulsants	David Siderovski
10	29-Mar	Fri	10:00	Medical Marijuana	Karen Woodfork
10	29-Mar	Fri	11:00	Local Anesthesia	Karen Woodfork
10	1-Apr	Mon	9:00	A&E: Clinical Management of Anesthesia	Manuel Vallejo
10	1-Apr	Mon	10:00	Drug Impaired Physician	Robert Johnstone
10	3-Apr	Wed	8:00	A&E: Parkinson's Computer Simulation	Karen Woodfork
10	3-Apr	Wed	9:00	A&E: Parkinson's Computer Simulation	Karen Woodfork
11	11-Apr	Thurs	10:00	Antiretroviral Drugs	Douglas Slain
11	12-Apr	Fri	10:00	Vitamins	Knox Van Dyke
11	12-Apr	Fri	11:00	VIDEO: Translational Research in Pharmacology	Julie Lockman
11	18-Apr	Thurs	9:00	Pediatric Medicine	Norman Ferrari
11	22-Apr	Mon	11:00	A&E: Understanding Antimicrobial Problems	Julie Lockman
11	23-Apr	Tues	9:00	Geriatric Pharmacology	Treah Haggerty
	5-Dec	Wed	8:00	Drugs for ADHD - CAMTASIA ONLY	David Siderovski
	17-Dec	Mon	10:00	Antiparasitic Drugs - Blood	Karen Woodfork
	30-Apr	Tues		NBME Exam: Pharmacology	

APPENDIX 5C
PHARMACOLOGY TEACHING IN THE TRANSITION YEAR, 2020-2021

PHARMACOLOGY TEACHING, FALL 2020

PCOL 801 (Old curriculum)

Month	Wk	Subject	Presenters
Aug	2	Pharmacology principles	Hammer 2*, Woodfork 4*
Aug	3	Pharmacology principles	Hammer 1, Woodfork 4, Petros 1
Aug	4	Drug development, Exam 1	Woodfork 2
Sep	1	Antibiotics	Lockman 5, Woodfork 1, Slain 3
Sep	2	Immunosuppressants, Exam 2	Lockman 1, Woodfork 3
Sep	3	Cancer chemotherapy	Liu 5, Woodfork 3
Sep	4	Adrenergic & cholenergic drugs	Hammer 1, Woodfork 5, Memon 2
Oct	1	Review, office hours, Exam 3	Hammer 2, Woodfork 4
Oct	2	Diuretics	Memon 2
Oct	3	Renal & CV pharmacology	Woodfork 4, Memon 2
Oct	4	Arrythmias, review, Exam 4	Hammer 1, Woodfork 1
Nov	2	Pulmonary & CNS pharmacology	Hammer 1, Woodfork 1, Lockman 1, Memon 1
Nov	3	CNS pharmacology, Exam 5	Hammer 1, Memon 1
		THANKSGIVING BREAK	
Dec	1	Drugs of abuse, journal club	Zhang 3
Dec	2	Anticoagulants, antivirals, Exam 6	Johnston 2, Woodfork 2, Memon 1
		CHRISTMAS BREAK	

*Number of contact hours for each presenter in this week

PHARMACOLOGY TEACHING, SPRING 2021

PCOL801 (Old)

Month	Wk	Subject	Presenters
Jan	1	GI PCOL	
	2	GI PCOL	Hammer 1, Woodfork 6
Jan	3	Review, Exam 7	Hammer 1, Woodfork 2
Jan	4	Endocrine PCOL	Hammer 2, Woodfork 2
Feb	1	Endocrine PCOL	Hammer 2, Woodfork 4
Feb	2	Review, Exam 8	
Feb	3	Bone PCOL, gout	Hammer 1, Woodfork 2
Feb	4	Toxicology	Hammer 2, Woodfork 4
Mar	1	Review, Exam 9	
Mar	2	Opioids, anticonvulsants	Hammer 3, Schreurs 1
Mar	3	Anesthsia/manage pain	Hammer 2, Woodfork 4
Mar	4	Eye, review, Exam 10	Hammer 2, Woodfork 4
Mar	5		
April	1	Miscellaneous	Lockman 1, Woodfork 3
April	2	Reviews	Lockman 1, Woodfork 1,
April	3	Exam 11	
April	4	NBME Exam	

PCOL 812 (New)

Subject	Presenters
Foundations, cancer	Hammer 4, Woodfork 8
Foundations, Exam	Woodfork 4
Antibiotics	Lockman 5, Woodfork 1
Immunosuppressants	Woodfork 3, Slain 1
NSAIDS, acteaminophen	Lockman 1, Woodfork 2
Miscellaneous	Woodfork 4, Petros 1
Antivirals, antifungals	Woodfork 1, Slain 4
Exam	
Autonomic PCOL	Hammer 3
Skeletal muscle PCOL	Hammer 1, Schreurs 1
Review and Exam	
Opioids	Hammer 3, Schreurs 1
Anesthetics	Woodfork 3
CNS PCOL	Hammer 9, Woodfork 2

APPENDIX 5D

CURRENT MEDICAL PHARMACOLOGY SCHEDULES

PCOL812 (Spring MS1) and PCOL820 (MS2)

PCOL812

Schedule of MS1 Curriculum

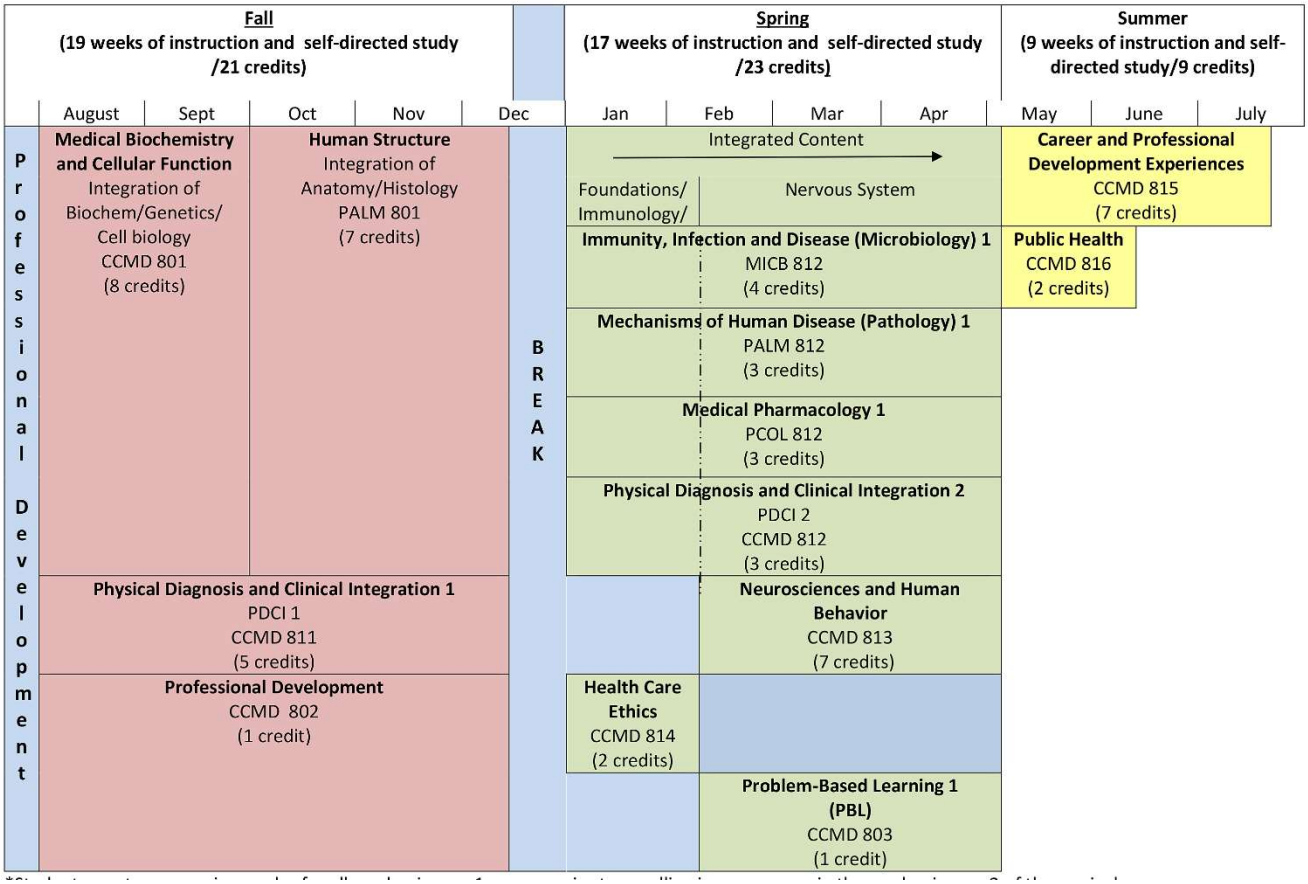
Summary of Classes in Spring Semester

Lectures in PCOL812

Schedule of MS1 Curriculum

Pre-Clerkship Phase: Academic Year 1 of the Curriculum

45 Weeks of instruction, experiential and self-directed study (53 credits Hours)



Summary of Classes in MS1 Spring Semester

MS1 CURRICULUM, SPRING SEMSTER

Block	Month	Wk	Subject	Classes
8	Dec	3	Orientation to Spring classes	PATH 1, PDCI 1
			CHRISTMAS BREAK	
8	Jan	1	Foundation/immunology	PCOL 2, MICRO 29, PATH 2, PDCI 6
8	Jan	2	Immunology, PCOL principles & cancer drugs	PCOL 11, MICRO 11, PATH 2, PDCI 6
9	Jan	3	Toxicology, pharmacokinetics, review & exams	PCOL 5, MICRO 6, PATH 2, PDCI 8, Ethics 3
9	Jan	4	Bacteriology, antibiotics	PCOL 4, MICRO 13, PATH 4, PDCI 5
9		5	Immunology, antibiotics	PCOL 7, MICRO 14, PATH 1, PDCI 7
10	Feb	1	Anti-inflammatory drugs, review & exams	PCOL 6, MICRO 6, PATH 1, PDCI 8
10	Feb	2	Antihistamins; Intro to neuroscience	PCOL 1, Neuro 15, PDCI 5
10	Feb	3	Antivirals; neuro lab, myelin	PCOL 5, Neuro 9, PATH 3, PDCI 5
11	Feb	4	Neuro lab, Exams	PCOL 1, Neuro 6, PATH 2, PDCI 7
11	March	1	Peripheral nervous system; Autonomic PCOL	PCOL 3, Neuro 19, PATH 2, PDCI 6
11	March	2	Special senses; neuropharmacology	PCOL 2, Neuro 13, PATH 2, PDCI 6
12	March	3	Central nervous sytem; Exams	PCOL 2, Neuro 11, PATH 2, PDCI 4
12	March	4	CNS disorders; short spring break	Neuro 19, PATH 2, PDCI 7
12	Apr	1	Hypothalamic & cortical functions	Neuro 12, MICRO 2, PATH 1, PDCI 6
13	Apr	2	Group presentations; opioids & CNS PCOL	PCOL 4, Neuro 21, PATH 3 , PDCI 3
13	Apr	3	Anesthetics & pain managements; exams	PCOL 5, Neuro 4, MICRO 2, PATH 1
13	Apr	4	Psychoactive drugs; Reviews	PCOL 13, PCDI 2
14	May	1	PCOL exam	
14	May	2	Neuro exams	

MS1 **PHARMACOLOGY (PCOL812)**

Block		Date	Day	Time	Title	Presenter
FOUNDATIONS						
General	8	1/4/2023	Wednesday	8:00	Absorption and Distribution, Ionization and Protein Binding 1	Karen Woodfork
princples	8	1/5/2023	Thursday	11:00	Absorption and Distribution, Ionization and Protein Binding 2	Karen Woodfork
	8					
	8	1/9/2023	Monday	11:00	**Problem Solving: Drug Ionization and Distribution	Karen Woodfork
	8	1/10/2023	Tuesday	9:00	Receptors & Dose-Response Relationships 1	Karen Woodfork
	8	1/10/2023	Tuesday	9:00	Receptors & Dose-Response Relationships 2/Drug Metabolism 1	Leah Hammer
	8	1/10/2023	Tuesday	10:00	Metabolism of Drugs 2	Leah Hammer
	8	1/11/2023	Wednesday	8:00	Excretion of Drugs	Leah Hammer
	8	1/11/2023	Wednesday	9:00	Adverse Drug Reactions	Leah Hammer
	8	1/12/2023	Thursday	8:00	Cancer Chemotherapy 1	Karen Woodfork
Chemo-	8	1/12/2023	Thursday	9:00	Cancer Chemotherapy 2	Karen Woodfork
therapy	8	1/12/2023	Thursday	10:00	Cancer Chemotherapy 3	Karen Woodfork
	8	1/12/2023	Thursday	11:00	Cancer Chemotherapy 4	Karen Woodfork
	8	1/13/2023	Friday	10:00	**Active Review: Cancer Chemotherapy	Karen Woodfork
	9	1/17/2023	Tuesday	9:00	**Problem Solving: Dose-Response Graphs, Pharmacokinetics	Karen Woodfork
Pharmaco-	9	1/18/2023	Wednesday	8:00	Principles of Toxicology	Karen Woodfork
kinetics	9	1/18/2023	Wednesday	9:00	Pharmacokinetics 1	Karen Woodfork
	9	1/18/2023	Wednesday	9:00	Pharmacokinetics 2	Karen Woodfork
	9	1/20/2023	Friday	8:15	PCOL Exam Block 1 (Foundations 1)	
Anti-	9	1/24/2023	Tuesday	10:00	Antibiotics 1	Julie Lockman
microbials	9	1/24/2023	Tuesday	11:00	Antibiotics 2	Julie Lockman
	9	1/25/2023	Wednesday	11:00	Antibiotics 3	Julie Lockman
	9	1/27/2023	Friday	11:00	Drug Development and Human Subject Research	Karen Woodfork
	9	1/30/2023	Monday	8:00	Antibiotics 4	Julie Lockman
	9	1/30/2023	Monday	9:00	Antibiotics 5	Julie Lockman
	9	1/30/2023	Monday	10:00	Antibiotics 6	Douglas Slain

Immuno-	9	1/30/2023	Monday	11:00	Inflammation and Glucocorticoids	Karen Woodfork
pharmacology	9	2/1/2023	Wednesday	10:00	Immunosuppressants 1	Karen Woodfork
		2/1/2023	Wednesday	11:00	Immunosuppressants 2/Immunomodulators	Karen Woodfork
	9	2/2/2023	Thursday	10:00	Drug-Induced Allergy	
	10	2/6/2023	Monday	10:00	Eicosanoid Drugs	Karen Woodfork
	10	2/7/2023	Tuesday	9:00	**Clinical Correlation: Prescription Writing and Drug Adherence	Charles D. Ponte
	10	2/7/2023	Tuesday	10:00	**Active Review: Immune System Drugs	Karen Woodfork
	10	2/7/2023	Tuesday	11:00	**Active Review: Antibiotics	Julie Lockman
	10	2/6/2023	Monday	11:00	NSAIDS and Acetaminophen	Karen Woodfork
	10	2/15/2023	Wednesday	10:00	Antihistamines	Karen Woodfork
	10	2/16/2023	Thursday	10:00	Local Anesthetics	Karen Woodfork
	10	2/16/2023	Thursday	11:00	Clinical Pharmacogenomics	William Petros
Antivirals	10	2/21/2023	Tuesday	8:00	Antivirals	Douglas Slain
	10	2/21/2023	Tuesday	9:00	Antiretroviral Drugs	Douglas Slain
	10	2/22/2023	Wednesday	10:00	Antifungals	Douglas Slain
	10	2/23/2023	Thursday	10:00	Antivirals, Antiretrovirals, Antifungals Mini-Review	Douglas Slain
	10	2/24/2023	Friday	10:25	**Active Review: Antihistamines, Local Anesthetics	Karen Woodfork
	11	3/3/2023	Friday	8:30	PCOL Exam Block 3 (Neuro 1)	
NEUROBIOLOGY						
Peripheral NS	11	3/8/2023	Wednesday	11:00	Intro to Autonomic Pharmacology	Leah Hammer
	11	3/9/2023	Thursday	10:00	Receptors and Signal Transduction 1	Leah Hammer
	11	3/9/2023	Thursday	11:00	Receptors and Signal Transduction 2	Leah Hammer
	11	3/16/2023	Thursday	8:00	Drugs for Ophthalmology	
	11	3/16/2023	Thursday	9:00	Neuromuscular Blockers and Skeletal Muscle Relaxants	Leah Hammer
	11	3/16/2023	Thursday	10:00	Drugs for Movement Disorders	Berny Schreurs
	12	3/22/2023	Wednesday	11:05	Block 4 Non-Mandatory Review Autonomics & Receptors	

	12	3/24/2023	Friday	8:30	PCOL Exam Block 4 (Neuro 2)	
Central NS	13	4/12/2023	Wednesday	8:00	Opioid Analgesics 1	Leah Hammer
		4/12/2023	Wednesday	8:30	Opioid Analgesics 2	Leah Hammer
	13	4/12/2023	Wednesday	9:00	PABS Instruction: 4/12	Walter Byrd
	13	4/13/2023	Thursday	8:00	Drugs for Dementia	Berny Schreurs
	13	4/13/2023	Thursday	9:00	Migraines	Leah Hammer
	13	4/18/2023	Tuesday	8:00	General Anesthesia 1	Karen Woodfork
	13	4/18/2023	Tuesday	9:00	General Anesthesia 2	Karen Woodfork
	13	4/19/2023	Wednesday	8:00	**Clinical Management of Anesthesia	Manuel Vallejo
	13	4/19/2023	Wednesday	9:00	**Active Review Session: Opioids, Anesthesia	Karen Woodfork
	13	4/19/2023	Wednesday	10:00	**Pain Management Cases	
Psycho-	13	4/24/2023	Monday	8:00	Antiseizure Drugs	Aman Dabir
therpeutics	13	4/24/2023	Monday	9:00	**Clinical Correlation: Management of Seizure Disorders	Aman Dabir
	13	4/24/2023	Monday	8:00	Antiseizure Drugs	Aman Dabir
	13	4/24/2023	Monday	9:00	**Clinical Correlation: Management of Seizure Disorders	Aman Dabir
	13	4/25/2023	Tuesday	9:00	Medical Cannabis	Karen Woodfork
	13	4/25/2023	Tuesday	10:00	Anxiolytics and Sedative Hypnotics	Leah Hammer
	13	4/26/2023	Wednesday	8:00	Antipsychotics	Leah Hammer
	13	4/26/2023	Wednesday	9:00	Alcohols	Leah Hammer
	13	4/26/2023	Wednesday	10:00	Antidepressants 1	Leah Hammer
	13	4/26/2023	Wednesday	11:00	CNS Toxicitiy: Drug Toxicities and Environmental Exposures	Karen Woodfork
	13	4/27/2023	Thursday	10:00	Antidepressants 2 & Drugs for Bipolar Disorder	Leah Hammer
	13	4/27/2023	Thursday	10:00	Drugs for ADHD	Leah Hammer
	13	4/27/2023	Thursday	11:00	Drugs of Abuse 1	Leah Hammer
	13	4/28/2023	Friday	8:00	Drugs of Abuse 2	Leah Hammer
	13	4/28/2023	Friday	9:00	**Active Review Block 6	Leah Hammer
	14	5/2/2023	Tuesday	8:30	Block 6 Exam	

PCOL820

Schedule of MS2 Curriculum

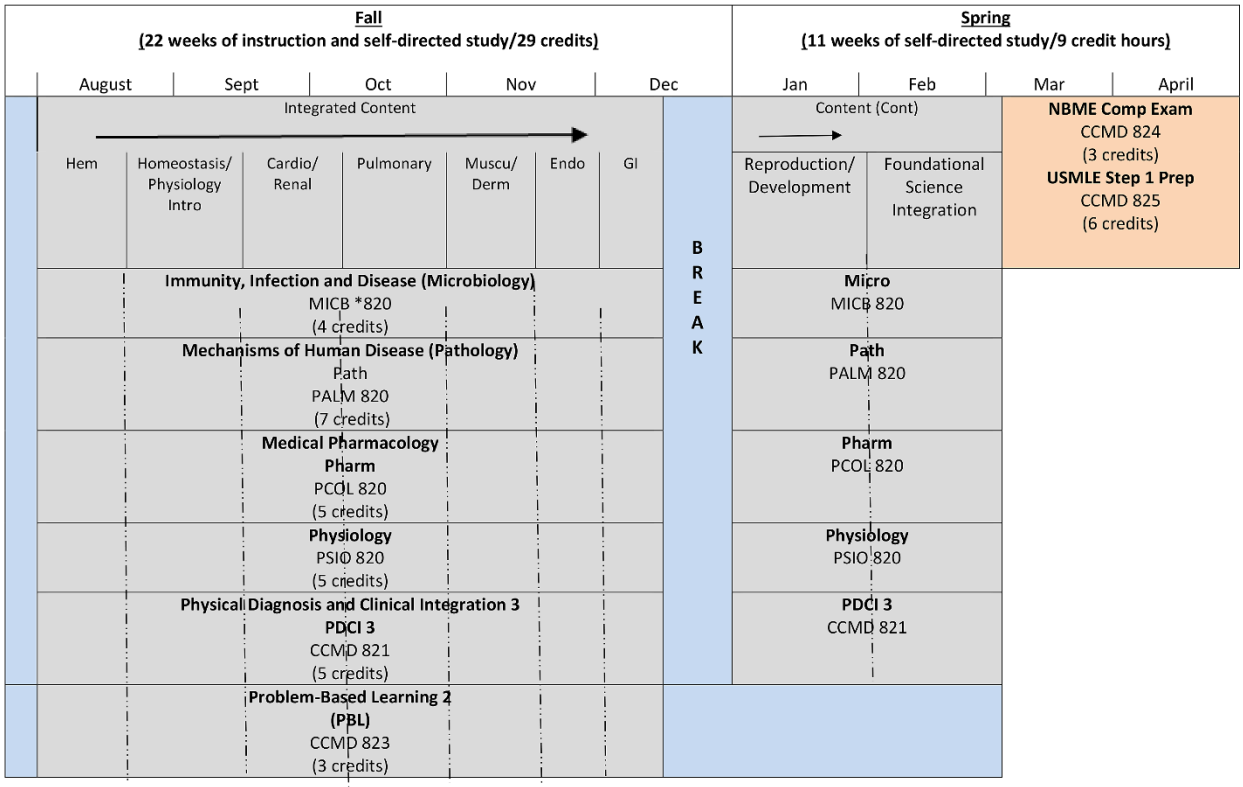
Summary of Topics in MS2 Curriculum

Lectures in PCOL820

OVERVIEW OF MS2 CURRICULUM

Pre-Clerkship Phase: Academic Year 2 of the Curriculum

33 weeks of instruction, experiential and self-directed study (38 credit hours)



TOPIC COVERAGE IN MS2 CURRICULUM

Block	Month	Week	Subject	Classes
1	Aug	2	Hematology	**PCOL 7***, PATH 8*, MICRO 3*, PDCI 4*
1	Aug	3	Hematology	**PCOL 4**, PATH 4, MICRO 5, PDCI 6
			Exams	
2	Aug	4	CV& Renal (CV)	PHYS 27
2	Aug	5	CV& Renal (renal)	PHYS 14, PATH 1, MICRO 7
			Exams	
3	Sept	1	CV & Renal (CV)	**PCOL 7**, PATH 3, PCDI 3
3	Sept	2	CV & Renal	**PCOL 9**, PATH 3, PDCI 7
3	Sept	3	CV & Renal	**PCOL 5**, PATH 2, MICRO 4
	Sept	4	CV & Renal	PATH 1, PDCI 7
			Exams	
4	Oct	1	Pulmonary	PHYS 14, PATH 1, MICRO 7
4	Oct	2	Pulmonary	**PCOL 3**, PATH 1, MICRO 9
4	Oct	3	Pulmonary	**PCOL 2**, PATH 1, MICRO 4, PDCI 7
			Exams	
5	Oct	4	Skeletal muscle & bone	PHYS 6, **PCOL 4**, PATH 6, MICRO 1, PDCI 2
5	Nov	1	Skeletal muscle & bone	**PCOL 1**, PATH 1, MICRO 4, PDCI 5
			Exams	
6	Nov	2	Endocrinology	PHYS 8, **PCOL 1**, PATH 3, PDCI 1
6	Nov	3	Endocrinology	**PCOL 5**, PDCI 5
			Exams	
	Nov	4	THANKSGIVING BREAK	
7	Nov	5	Gastrointestinal track	PHYS 13, PATH 2, MICRO 3, PDCI 1
7	Dec	1	Gastrointestinal track	**PCOL 8**, PATH 1, MICRO 7, PDCI 6
7	Dec	2	Gastrointestinal track	**PCOL 2**, PATH 2, MICRO 1, PDCI 11
			Exams	
			CHRISTMAS BREAK	
8	Jan	1	Reproduction & Development	PHYS 6, **PCOL 2**, PATH 3, MICRO 2, PDCI 3
			Physiology Review	PHYS 7
8	Jan	2	Reproduction & Development	**PCOL 5**, PATH 4, MICRO 1, PDCI 13
8	Jan	3	Reproduction & Development	PATH 1, MICRO 3, PDCI 3
			Exams	
	Jan	4	General reviews	PHYS 2, **PCOL 5**, PATH 1, MICRO 8, PDCI 9
9	Feb	1	Integration & review	PHYS 4, **PCOL 2**, PATH 1, MICRO 2, PDCI 14
9	Feb	2	Study days & exams	PHYS, **PCOL**
9	Feb	3	Study days & exams	PATH, MICRO
9	Feb	4	Study days & exams	PDCI

* Number of contact hours for each class in week

MS2 Pharmacology (PCOL820)

Block	Date	Day	Time	Title	Presenter
1	8/8/2022	Monday	8:00	MS2 Course Introductions	
1	8/11/2022	Thursday	8:00	Anticoagulant, Antiplatelet, and Fibrinolytic Drugs 1	Richard Johnston
1	8/11/2022	Thursday	9:00	Anticoagulant, Antiplatelet, and Fibrinolytic Drugs 2	Richard Johnston
1	8/11/2022	Thursday	10:00	Drug Therapy of Anemia	Roseane Santos
1	8/11/2022	Thursday	11:00	Hematological Toxicology	Karen Woodfork
2	8/15/2022	Monday	10:00	Antimalarial Drugs	Karen Woodfork
2	8/15/2022	Monday	11:00	Antiparasitic Drugs - Blood and Tissue Parasites	Karen Woodfork
2	8/17/2022	Wednesday	11:00	**Active Review: Drugs Affecting the Hematological System	Richard Johnston
2	8/19/2022	Friday	8:00	PCOL Exam 1	
3	9/6/2022	Tuesday	8:00	Adrenoceptor Agonists	Karen Woodfork
3	9/6/2022	Tuesday	9:00	Alpha Adrenoceptor Antagonists	Karen Woodfork
3	9/6/2022	Tuesday	10:00	Beta-Adrenoceptor Antagonists	Karen Woodfork
3	9/7/2022	Wednesday	0:00	Diuretics 1	Roseane Santos
3	9/7/2022	Wednesday	8:00	Cholinergic and Anticholinergic Drugs 1	Leah Hammer
3	9/7/2022	Wednesday	9:00	Cholinergic and Anticholinergic Drugs 2	Leah Hammer
3	9/7/2022	Wednesday	11:00	Diuretics 2	Roseane Santos
3	9/12/2022	Monday	10:00	**Imaginary Human Exercise	Karen Woodfork
3	9/13/2022	Tuesday	10:00	Renin-Angiotensin System Inhibitors	Roseane Santos
3	9/13/2022	Tuesday	11:00	Calcium Entry Antagonists	Roseane Santos
3	9/15/2022	Thursday	8:00	Antihypertensive Drugs 1	Roseane Santos
3	9/15/2022	Thursday	9:00	Antihypertensive Drugs 2	Roseane Santos
3	9/15/2022	Thursday	10:00	Antiarrhythmic Drugs 1	Karen Woodfork
3	9/15/2022	Thursday	11:00	Antiarrhythmic Drugs 2	Karen Woodfork
3	9/16/2022	Friday	10:00	Treatment of Congestive Heart Failure	Karen Woodfork
3	9/16/2022	Friday	11:00	Treatment of Myocardial Ischemia	Karen Woodfork

3	9/19/2022	Monday	8:00	Drugs for Dyslipidemias	Leah Hammer
3	9/19/2022	Monday	12:23	**Clinical Management of Hypertension	
3	9/20/2022	Tuesday	10:00	**Clinical Management of Acute Heart Failure	George Sokos
3	9/21/2022	Wednesday	11:00	Cardiovascular & Renal Toxicology	Karen Woodfork
3	9/23/2022	Friday	10:00	**Problem Solving: Cardiovascular Disease	Karen Woodfork
3	9/23/2022	Friday	11:00	**Problem Solving: Cardiovascular Disease	Karen Woodfork
3	9/30/2022	Friday	8:00	PCOL Exam 3	
4	10/13/2022	Thursday	8:00	Drugs for Asthma and COPD	Richard Johnston
4	10/13/2022	Thursday	9:00	Mucolytic Drugs	Richard Johnston
4	10/13/2022	Thursday	10:00	Antimycobacterial Drugs	Karen Woodfork
5	10/17/2022	Monday	11:00	Respiratory Toxicology	Karen Woodfork
5	10/18/2022	Tuesday	10:00	**Problem Solving: Pulmonary Drugs	Richard Johnston
5	10/21/2022	Friday	8:00	PCOL Block 4 Exam	
5	10/26/2022	Wednesday	10:00	Clinical Management of Skin Disorders 1	Roxann Powers
5	10/28/2022	Friday	8:00	**Clinical Management of Skin Disorders 2	Roxann Powers
5	10/28/2022	Friday	10:00	Drugs Affecting Bone Mineral Metabolism	Karen Woodfork
5	10/28/2022	Friday	11:00	Disease-Modifying Anti-Rheumatic Drugs	Karen Woodfork
5	10/31/2022	Monday	10:00	Drugs for Gout	Karen Woodfork
5	11/4/2022	Friday	8:00	PCOL Exam 5	
6	11/11/2022	Friday	10:00	Thyroid and Antithyroid Therapy	Leah Hammer
6	11/14/2022	Monday	8:00	Antidiabetic Drugs 1	Roseane Santos
6	11/14/2022	Monday	9:00	Antidiabetic Drugs 2	Roseane Santos
6	11/14/2022	Monday	9:59	**Clinical Correlation: Pre-Diabetes (lecture via Zoom)	
6	11/15/2022	Tuesday	10:00	Drugs for Hypothalamus & Pituitary	Karen Woodfork
6	11/16/2022	Wednesday	10:00	**Clinical Correlation: Diabetes Therapeutics Cases	Charles D. Ponte
6	11/18/2022	Friday	8:00	PCOL Exam 6	

7	12/7/2022	Wednesday	8:00	Antiemetic Drugs	Leah Hammer
7	12/7/2022	Wednesday	9:00	Treatment of Inflammatory Bowel Disease	Karen Woodfork
7	12/7/2022	Wednesday	10:00	Treatment of Peptic Ulcer Disorders	Leah Hammer
7	12/7/2022	Wednesday	11:00	Laxatives and Cathartics	Leah Hammer
7	12/8/2022	Thursday	9:00	Vitamins & Supplements 1	Karen Woodfork
7	12/8/2022	Thursday	10:00	Vitamins & Supplements 2	Karen Woodfork
7	12/8/2022	Thursday	11:00	Antiparasitic Drugs (GI parasites)	Karen Woodfork
7	12/9/2022	Friday	8:00	GI Toxicology	Karen Woodfork
7	12/12/2022	Monday	9:00	Drugs for Viral Hepatitis	Karen Woodfork
7	12/13/2022	Tuesday	11:00	**Pharmacology Active Review: GI Drugs	Leah Hammer
7	12/16/2022	Friday	8:00	PCOL Exam 7	
7	1/5/2023	Thursday	10:00	Estrogens, Progestins, and Related Drugs	Roseane Santos
7	1/5/2023	Thursday	11:00	Androgens & Related Drugs, Drugs for Erectile Dysfunction	Roseane Santos
8	1/10/2023	Tuesday	11:00	Developmental/Reproductive Toxicity	Karen Woodfork
8	1/10/2023	Tuesday	11:30	Gender-Affirming Hormonal Therapy	Kacie M. Kidd
8	1/12/2023	Thursday	10:00	Pediatric Medicine	Hilary E. Morley
8	1/12/2023	Thursday	11:00	Geriatric Medicine	Treah Haggerty
8	1/13/2023	Friday	10:00	**Active Review: Reproductive Drugs	Roseane Santos
8	1/20/2023	Friday	8:00	PCOL Exam 8	
8	1/25/2023	Wednesday	10:00	Mechanism of Antidotal Therapy	Karen Woodfork
8	1/25/2023	Wednesday	11:00	Translational Research in Pharmacology	Julie Lockman
8	1/26/2023	Thursday	8:00	**Active Review: Pharmacokinetics	Karen Woodfork
8	1/26/2023	Thursday	9:00	**Active Review: Pharmacodynamics	Karen Woodfork
8	1/26/2023	Thursday	10:00	**Active Review: Autonomic Drugs	Karen Woodfork
9	1/30/2023	Monday	8:00	**Active Review: CNS Drugs	Leah Hammer
9	1/31/2023	Tuesday	11:00	**Active Review: Antibiotics	Julie Lockman
9	2/3/2023	Friday	10:00	**Active Review: Cardiovascular Drugs	Karen Woodfork

9	2/10/2023	Friday	8:00	PCOL Exam 9
9	2/17/2023	Friday	8:00	NBME Subject Exam - Pharmacology