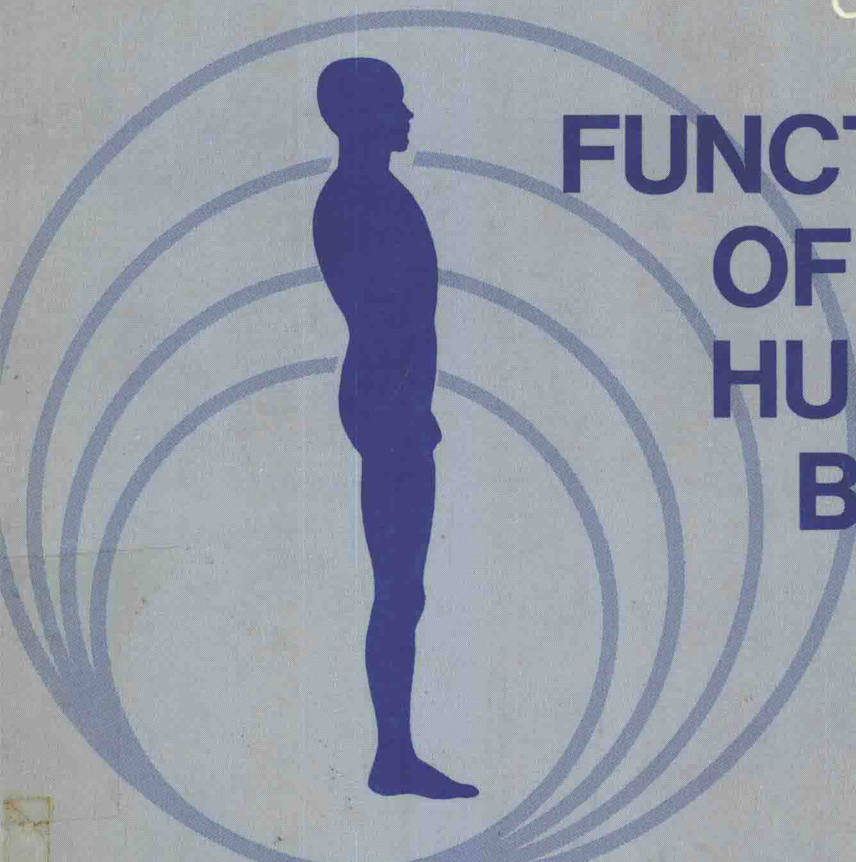
Armstrong

A Laboratory Manual for Guyton's



FUNCTION OF THE HUMAN BODY

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A Laboratory Manual for Guyton's

FUNCTION OF THE HUMAN BODY

THIRD EDITION

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FOREWORD TO THE FIRST EDITION

Dr. Armstrong has for many years had a special fondness for the student laboratory and for developing student laboratory experiments that *work*. For this reason all of us interested in the teaching of physiology welcome this laboratory manual.

In his characteristic thoroughness, Dr. Armstrong has chosen experiments, both fundamental and applied, that illustrate basic principles in each of the most important fields of human physiology. These experiments will give the student not only much additional knowledge of physiology itself but also a "feel" for experimental methods used both on the human being and on experimental animals. A wisely selected portion of the experiments demonstrates basic physical and chemical principles that are the very foundation of physiology. These attributes, I believe, are those that make a useful laboratory manual.

I believe also that the introduction Dr. Armstrong has written for each experiment, which reviews the physiology applicable to the day's work, will help make the experiment a successful learning experience and will add greatly to the meaningfulness of the results.

Special characteristics of Dr. Armstrong's manual are, first, the clarity with which the experimental procedures are described and, second, its presentation of detailed data for the benefit of the instructor, giving him instructions for preparing the necessary experimental materials. It is my sincere desire and belief that this manual will prove to be of great help to both teachers and students alike wherever introductory courses in physiology are taught.

ARTHUR C. GUYTON, M.D.

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PREFACE

This manual has been revised to provide laboratory exercises for courses in human physiology using Guyton's *The Function of the Human Body,* fourth edition, for a textbook. Arrangement of material is in the same order as it appears in this textbook.

Each laboratory exercise is referred to a specific "Textbook Assignment," and a brief description is given of the particular "Physiological Principles" required as background for the experiment. Data sheets provide tables for recording experimental results, graphs for displaying the relationships between the parameters and the variables, and questions to guide conclusions and discussions.

Where practical, the student is the experimental model. Where this is impractical, the albino rat, the frog, the turtle, or the dog is used.

The experiments are designed to utilize uncomplicated laboratory equipment. The emphasis is placed upon the physiology under study. Experience has confirmed the impression that when an initial exposure to physiological principles is complicated by highly sophisticated arrays of instrumentation, neither the instrumentation nor the physiology is well understood.

Human physiology is a paramedical discipline; therefore, medical terminology with its unique descriptive specificity is used throughout the manual. Hence, to assure adequate communication a medical dictionary should be available for consultation.

I wish to acknowledge the contributions made by colleagues the world over who established the basic principles upon which the experiments in this manual are based. In particular, appreciation is expressed to the many contributors to the compilations of teaching experiments so graciously furnished to all teachers of physiology by The American Physiological Society.

I am grateful to Dr. Arthur C. Guyton for many contributions and suggestions relative to the preparation of this manual and the personnel of W. B. Saunders Company for their cooperation and editorial excellence.

GEORGE G. ARMSTRONG

Houston, Texas

CONTENTS

Section IV

THE BODY FLUIDS AND THE URINARY SYSTEM

Section V

RESPIRATION

Section VI

THE NERVOUS SYSTEM

Section VII

THE GASTROINTESTINAL AND METABOLIC SYSTEMS

SECTION I

INTRODUCTION: CELL PHYSIOLOGY

1-1 GROSS ANATOMY OF THE ORGAN SYSTEMS

Textbook Assignment:

Pages 3–11.

INTRODUCTION

Demonstration of many physiological functions of the human body necessitates procedures which obviously cannot be performed on the human being. Therefore, physiologists often utilize animals ranging from subhuman primates to the most elemental forms in both their research and teaching endeavors.

The following exercise will serve to acquaint the student with the albino rat as an experimental mammalian model, to demonstrate the gross anatomy of the major organ systems, and to afford practice in certain procedures involved in other experiments.

EXPERIMENTAL PROCEDURES

Treatment and Handling of Albino Rats. Laboratory rats have for many generations been accustomed to handling by people. Therefore, remember, if this is your first contact with the albino rat, you are the novice. Laboratory raised rats seldom bite unless they are mistreated and, furthermore, it is impossible for a rat to bite if he cannot open his mouth. Hold the rat with the thumb and the forefinger encircling the head to extend around and beneath the lower jaw. Be sure the thumb and forefinger are beneath the lower jaw and not the throat, since the rat has difficulty understanding why his cooperation should be expected while he is being choked.

The tail makes an unusually convenient handle, and using it to lift the rat does him no harm. Indeed, when the tail is held, the rat tries to pull away; and thus while immobilized and preoccupied he is easy prey for the thumb and forefinger of the other hand. It is inadvisable to carry rats by their tails because their amazing dexterity enables them to flip their bodies upward and climb up their own tails. One could well expect a rat in this position to be tempted to bite the hand which has so greatly affronted his dignity.

Pronation of the hand holding the head will gently cradle the rat's body within the palm of the hand. Do not grasp or squeeze the body of the rat with the remaining fingers since pressure of this type causes discomfort and may encourage the rat to struggle.

A few minutes spent in handling the rat, lifting him from one box to another or to the surface of a table, and accustoming both the rat and the student to the feel of each other, are most properly expended.

Visual comparison of the rat with a convenient member of your class will immediately reveal more obvious differences than similarities. The most marked difference is size. Weight, which is related to size, is a convenient parameter for establishing drug dosages and normalizing physiological processes between different species. A dietary spring balance which has a maximal range of 500 grams and an accuracy to 1 gram is adequate for weighing rats. A weighing box placed on the platform is convenient; however, the scale must either be adjusted for the weight of the box or this weight subtracted from the total.

Always measure and record the weight of an experimental animal. The sex and the age, if known, should also be included on the data sheets.

Anesthetization. Ether will usually be used as the anesthetic agent for rat experiments. The bottom of a large wide-mouth jar is covered by a pad of cotton which has been sprinkled liberally with ethyl ether, and a folded paper towel is placed over the cotton. The rat is placed in the jar and the top covered.

Ether is very flammable; and when it is being used, there should be no open flames or smoking permitted within the laboratory.

Observe the rat very closely and immediately remove him when he collapses. Anesthesia is continued by the open drop method. A 50 ml beaker will fit very loosely over the rat's head, and a 2 X 2 gauze pad moistened with ether in the bottom of the beaker will usually support a reasonable ether-air mixture. Anesthesia is maintained by occasionally moistening the gauze pad with a few drops of ether.

The level of anesthesia must be continuously evaluated in order that the animal neither becomes unanesthetized nor induced to such a deep plane of anesthesia that the physiological responses being studied are severely depressed. The responses to painful stimuli (the elimination of pain perception is the primary function of anesthesia in experimental animals) may be evaluated by pinching the ears or tail and observing for flinching or reflex withdrawal. Another good criterion for the level of anesthesia is the respiratory pattern (rate and depth of respiration). The respiratory pattern of an awake rat should be observed; then the changes in this pattern as anesthesia develops should be followed. When the respiratory movements become very slow and shallow, the depth of anesthesia is too great. When the respiratory movements become very rapid and deep, the depth of anesthesia is too light. If the depth of anesthesia is too great and the rat still has his head within the ether-saturated atmosphere of the beaker, the level of anesthesia will get deeper. Therefore, when anesthesia appears too deep, the beaker should be removed. Do not become so involved in other aspects of the experiment that the beaker is left off until the rat awakens.

Proper anesthetization requires constant and close supervision; hence, for each experiment the responsibility for anesthesia is delegated to a specific student. This assignment is to be rotated among the members of the group for different experiments. The student who is designated anesthetist for a particular experiment will be responsible for the level of anesthesia throughout the experiment, and for the duration of that experiment he shall not under any circumstances subdelegate this responsibility to another member of the group for any period of time.

Weigh a rat, and then observe the respiratory rate and depth. Anesthetize the rat with ether. Observe respiratory rate and depth as anesthesia develops and when the rat collapses in the jar. Apply the mask (beaker containing ether-soaked gauze pad) and alternately deepen and lighten anesthesia. Note the time required to deepen anesthesia until a slow, shallow respiratory pattern develops, and then note the time required for the level to lighten to a rapid, deep respiratory pattern. Allow the rat to stabilize at a light level of anesthesia, and maintain this level during the next section.

Injections. The administration of chemical substances and drugs is made by three routes in the rat: subcutaneously, intraperitoneally, and intravenously.

Subcutaneous injection. This procedure is best performed with the rat held in a semi-squatting position on a table. The fourth finger of the left hand encircles the head and extends beneath the lower jaw to keep the mouth closed and the head fixed. A fold of loose skin on the back of the rat is lifted upward by the thumb and forefinger of the left hand. A 1/2 inch, 26 gauge hypodermic needle on a 1 ml tuberculin syringe, which is held in the right hand with the needle pointed toward the head, is thrust quickly through the pinched-up skin into the subcutaneous space. Inject 0.1 ml/100 gm body weight of isotonic saline (0.9% solution of sodium chloride). Quickly withdraw the needle and massage the site to close the puncture hole.

Each member of the group should make several practice subcutaneous injections into the anesthetized rat, and then obtain an unanesthetized rat and make at least one successful subcutaneous injection.

Intraperitoneal injection. The rat is held with the head encircled by the thumb and forefinger of the left hand, the body cradled in the palm of the hand, and the remaining fingers lightly encircling the body for restraint. The lower portion of the body is lightly pressed against the left side of your body to confine the rat's lower legs and stretch the skin of the abdomen taut.

A short quick thrust with a 1 ml syringe carrying a 1/2 inch, 26 gauge hypodermic needle into the midline of the lower portion of the abdomen will penetrate into the peritoneal cavity. The needle will rarely penetrate the gut or other abdominal organs if the thrust is terminated when a definite *give* is felt. Inject intraperitoneally 0.1 ml/100 gm body weight of normal saline. Fluids injected intraperitoneally do not have to be at body temperature but should be warmed.

Each member of the group should again make practice injections into the anesthetized rat, then complete at least one successful intraperitoneal injection into an unanesthetized rat.

Intravenous injection. Intravenous injections are made directly into the veins. This method will not be utilized in any of the following rat experiments. However, the laboratory instructor may wish to demonstrate intravenous injections via one of the large veins in the rat's tail. Intravenously injected fluids should first be warmed to body temperature.

Gross Anatomy of the Organ Systems. The rat, just as man, possesses specialized organ systems. These divisions are in many ways arbitrary since proper total function requires integrated activity of all organ systems, and the interrelationships between them afford overlap of the same parts functioning in several systems. The following classification roughly parallels the order of study to be followed in the textbook: (1) muscular system, (2) reticuloendothelial system, (3) cardiovascular system, (4) urinary system, (5) respiratory system, (6) nervous system, (7) gastrointestinal system, and (8) endocrine and reproductive systems.

The experiment will not follow this sequence of presentation since the convenience of dissection and the desirability of observing certain organs while the animal is still alive dictate a different order of procedure. Furthermore, some organs receiving attention in this exercise are not easily observable in the rat while others, such as the thymus and spleen, which may be very apparent receive little, if any, attention. Here again, the ultimate intent is to use the rat to learn the physiology of the human being, and not to learn the anatomy of the rat.

After the dissection has been completed, the entire revealed anatomy of the rat should be reviewed and simple diagrammatic sketches of the organ systems made and compared with those of the human being in the textbook.

Dissection of the Rat. Anesthesia should be maintained from the preceding portion of this exercise. After the thoracic cavity has been opened, the lungs can no longer function; therefore, the anesthesia may be discontinued when the thoracic cavity is opened. The effect of the residual ether in the rat will be sufficient to maintain anesthesia at a suitable level until the rat is no longer capable of perceiving painful stimuli. The central nervous system rapidly ceases to function when anoxic, and death will soon follow.

Stretch the rat out in the supine position on the dissecting board. Make a midline incision through the skin on the ventral surface extending from the symphysis pubis to the very tip of the lower jaw. Gently retract the skin over the thorax, and insert the point of a heavy pair of scissors directly in the midline just at the lower end of the rib cage. Slide the point as closely as possible to the underside of the sternum or breast bone, and cut through the center of the sternum toward the head to open the thoracic cage. Place hook retractors over the cut edges of the sternum and retract. Blot any blood from within the thoracic cavity.

The lungs are collapsed and no longer fill the thoracic cavity. The animal will make a few attempts to respire. Observe any activity of the respiratory muscles, especially the diaphragm, the large sheet-like muscles which divide the thoracic cavity from the abdominal cavity.

Slip a short length of large bore rubber tubing over the nose and mouth of the rat; gently blow into it and observe the lungs. Remove the tubing and note the flow of air from the lungs. Observe the beating of the heart as the animal grows more anoxemic.

Extend the incision from the thoracic cavity to the tip of the lower jaw. Trace the trachea downward to the tracheal carina. Note the right and left bronchi and the subsequent division into the smaller bronchioles which supply each major segment of both lungs. While making this observation, avoid cutting through the large vessels coming from the heart. Visualize the structures by blunt dissection and gentle retraction of the tissues from side to side. Cut off a small portion of the lung. Drop it into a beaker of water, and observe whether it floats or sinks.

Trace the trachea from the carina to the larynx or voice box. Insert a pair of fine-pointed scissors into the upper portion of the trachea, and divide the trachea longitudinally through the larynx. Then, using a larger pair of scissors, extend the incision upward, and divide the lower jaw in half. Examine the larynx carefully. Identify the epiglottis and the vocal cords.

Note the aorta and the large branches coming off the arch. These arteries arising from the arch of the aorta should be identified on the data sheet sketch. Trace the aorta downward to the diaphragm.

Observe the jugular veins in the neck. Trace them downward, noting the innominate vein, the superior vena cava, and the right atrium. Trace the inferior vena cava downward to the diaphragm. The right phrenic nerve, which innervates the right leaf of the diaphragm, runs downward alongside the inferior vena cava.

Remove the heart, the lungs, and the major vessels from the carcass. Rinse these structures under running water, and lay them out on the dissecting board. Using fine scissors and thumb forceps, dissect the heart loose from the surrounding tissues. Open the aorta and left ventricle with the fine-pointed scissors. Observe the aortic valve and count the cusps. Locate the left atrioventricular valve and count the cusps. A magnifying lens will be most useful since the heart and its structures are quite small.

Open the left atrium by cutting through the atrioventricular valve and the wall of the atrium. Identify the pulmonary veins emptying into the atrium. Cut the lungs loose from the heart, and cut through the wall of the right ventricle exposing the pulmonary valve and pulmonary artery. Observe the number of cusps in the pulmonary valve. Identify and note cusps of the right atrioventricular valve. Then cut through this valve and the wall of the right atrium. Observe where the vena cava empties into the right atrium.

Deepen the midline incision between the abdominal muscles into the abdominal cavity. Connect the thoracic and abdominal cavities by dividing the diaphragm. Retract widely the edges of the entire opening.

Identify the tongue. Trace the oral pharynx to the epiglottis. Locate the esophagus and trace it downward to the stomach. Trace the small intestine, identifying the duodenum, the jejunum, and the ileum. Trace the colon to the anus.

Observe the liver. Identify the site where the bile duct empties into the small intestine.

Diffusely scattered about the mesentery of the small intestine is the pancreas with many paired ducts emptying into the upper portion of the small intestine. Identify as much of this organ as possible. The pancreas has both exocrine (digestive enzymes) and endocrine (insulin and glucagon) glandular functions.

If the intestinal organs are shifted to one side to expose the back wall of the abdominal cavity, the kidneys are visible. Follow the ureter from the pelvis of the kidney to the bladder. Closely associated with the ureter are the renal artery and the renal vein. The adrenal glands should be easily observed cephalad to the kidney.

Remove one kidney; divide it in half with a sharp razor blade, and observe the papillae protruding into the pelvis.

Remove one adrenal gland and divide it with a sharp razor blade. Observe the structure.

The type of reproductive organs will depend upon whether the rat is male or female. If male, the testes may be either in the abdomen or in the scrotum. Remove a testis; divide it in half and observe its structure.

If the rat is female, the ovaries may be found close to the kidneys. Remove one ovary and look for graafian follicles. A magnifying lens will aid this examination. Locate the short oviducts lying near the ovaries, and trace these to the bicornate (two-horned) uterus. Compare this organ with the human uterus.

If time permits, the laboratory instructor may remove the brain and demonstrate the cerebral structures.

Name ______________________ Group __________ Date __________

DATA SHEET

General Data on Experimental Animal

Weight __________ Sex __________ Age __________

ANESTHESIA

CONDITION OF RAT	RESPIRATORY RATE	RESPIRATORY DEPTH	TIME REQUIRED (MIN)
Unanesthetized			
Collapsed in jar			
Deep anesthesia			
Shallow anesthesia			

Which requires the longer period of time, to increase or to decrease the depth of anesthesia? ______________________ How much longer? __________ min.

When the ether mask was removed, did the anesthetic level immediately decrease? __________________ Why? __________________

____

INJECTIONS

CONDITION OF RAT	NUMBER OF SUCCESSFUL INJECTIONS SUBCUTANEOUS	INTRAPERITONEAL
Anesthetized		
Awake		