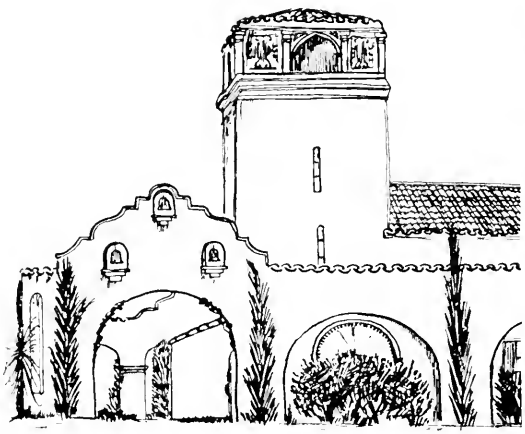
Presented in honor of

Louis C. Chandler, D.O.



COLLEGE OF OSTEOPATHIC PHYSICIANS
AND SURGEONS • LOS ANGELES, CALIFORNIA

FUNCTIONAL DIAGNOSIS

FUNCTIONAL DIAGNOSIS

BY

MAX KAHN, M.A. PH.D., M.D.

DIRECTOR OF THE DEPARTMENT OF LABORATORIES, AND CHIEF OF CLINIC OF DISEASES OF METABOLISM, BETH ISRAEL HOSPITAL; ASSOCIATE IN BIOLOGICAL CHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK

IN COLLABORATION WITH

MORRIS HIRSCH KAHN, M.D.

CHIEF OF CLINIC IN CARDIOVASCULAR DISEASES AND INTERNAL MEDICINE, MOUNT SINAI HOSPITAL DISPENSARY; ASSISTANT, CARDIOGRAPHIC LABORATORY, MOUNT SINAI HOSPITAL; CHIEF CARDIOGRAPHIST, BETH ISRAEL HOSPITAL, NEW YORK

AND

JACOB ROSENBLOOM, PH.D., M.D.

FORMERLY ASSISTANT PROFESSOR OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF PITTSBURGH; FORMERLY ASSOCIATE IN BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK

FOREWORD BY

WILLIAM J. GIES, M.S., SC.D., PH.D.

PROFESSOR OF BIOLOGICAL CHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK

NEW YORK

W. F. PRIOR COMPANY, INC.

1920

Printed in the United States of America

DEDICATED

WITH

AFFECTION AND GRATITUDE

TO

PROFESSOR WILLIAM J. GIES

FOREWORD

This volume is another milestone on the highway of progress in Medicine. It marks another advance in the march toward prevention and control of disease.

The necessity for the use of tests of function, in attaining accuracy and completeness in the diagnosis of many diseases, and in determining the degree or the extent of physiological disorder, is obvious. The reliability of many of the available tests of function has been firmly and convincingly established. The futility of some of the proposed methods for this purpose has likewise been clearly indicated. Separation of the wheat from the chaff is in progress in this field as in every other in Medicine. The promise of development in the scope and proficiency of the methodology of function-testing appears to be limited only by the amount and character of the clinical observation and laboratory research this general means of study and diagnosis will attract and receive.

An obstacle in the way of rapidity of extension in the use and evaluation of many of the reliable functional tests, and of the development and improvement of such methods, is the wide dispersion of their descriptions throughout the great mass of original medical literature, with the consequent mechanical difficulty imposed on many laboratory workers and practitioners of thus studying and understanding these methods. This book, one of the first of its kind, represents an earnest and successful attempt to assemble, and render easily accessible, full and accurate descriptions of all the published procedures of actual or presumed value in functional diagnosis, and to present these descriptions in a classified arrangement, with indications of the relative values of the tests in each group, that will render selection and use of methods convenient and satisfactory under all conditions involving their application.

Many of the most valuable functional tests now in current use, and in increasing employment, are biochemical in character and also biochemical in functional import. It is, accordingly, a very great pleasure for me to commend this excellent volume to the attention of medical men generally, for its author, Dr. Max Kahn, and one of his two collaborators, Dr. Jacob Rosenbloom, are not only successful practitioners of medicine, with extended clinical and laboratory experience in the use of function tests, but for several years were ardent and able investigators and officers of instruction in the Biochemical Laboratory at the College of Physicians and Surgeons, each receiving the Columbia degree of Ph.D. in Biochemistry in recognition of his original work in this field, and both carrying with them into their life-work the esteem, affection, and abiding good wishes of the writer.

My fellow students of some years ago have registered, in this notable

volume, the scientific ability, fidelity, and earnestness that characterized their efforts in the laboratory of the College of Physicians and Surgeons during their terms as students and teachers; and I am confident this work will be accorded a degree of professional acceptance, because of its worth and usefulness, that will give them the very deep gratification which is the highest possible reward for fruitful public service.

WILLIAM J. GIES

NEW YORK CITY
January, 1920.

CONTENTS

LIST OF ILLUSTRATIONS

KIDNEY FUNCTION TESTS

FUNCTIONAL DIAGNOSIS

INTRODUCTION

The attempts at the investigation of the functional capacity of various organs have yielded profitable results, and the literature of the past several decades is replete with suggestions for such examinations. The question whether there is such a phenomenon as a functional disease of an organ without some underlying structural pathology is a mooted one, and the preponderance of opinion seems to be that there can be no derangement in the functional activity without some inflammatory, neoplastic or other process as a causative factor. Nevertheless, the poor functioning of a certain tissue may be due to some pathology in a neighboring organ, rather than in the tissue itself; thus, for example, gastric derangement is recognized as a common manifestation in appendicitis, gall-bladder disease, and structural diseases of the colon. As Stockton says, "An unprejudiced view would seem to grant that a disordered nervous system may at times give rise to cardiospasm or pylorospasm, but the warning should be kept in mind that we should seek the cause in some marked irritation at or near the abnormal contraction."

The purpose of function testing is of double significance. If we assume that a derangement of activity of a certain gland is the result of some structural changes in that gland, the finding of such lessened function will give us a clue to the diagnosis. For instance, if we were to find that the functional activity of the pancreas is below par, we may assume (from the above hypothesis) that there is some pathological change in this gland. So also with the stomach, intestines, etc. On the other hand, granted that there is a diseased state in a special gland, for example, the liver or kidneys, what is the functional capacity of the organ? A patient who has chronic hepatitis or chronic nephritis, may still have enough functional compensation to carry him through many years of life. From the prognostic and therapeutic viewpoints, therefore, it is essential to know just exactly how much we can expect a certain organ to perform. It should be distinctly understood, however, that a function test does not make a diagnosis for the clinician as to the type of underlying pathology in the involved tissue. Thus, one cannot expect the biochemist to give a verdict of cancer of the stomach after having performed the Rehfuss fractional analysis of the gastric contents. The biochemist can state that at the time of the analysis the stomach of the patient showed an absence of free acid, etc., which may be due to carcinoma or to several other causes. The result of the test is simply an additional symptom for the clinician to take into considera-

tion. The best results can be obtained when the physician and the laboratory scientist work in collaboration, i. e., discuss the history of the case and the other symptoms present. It is remarkable how much information a test-tube examination can give us, but to expect it to give us *all* the information about an individual case is a rather premature hope with the present state of knowledge. It is a precarious undertaking to make a *pathological* diagnosis with one function test, in many instances.

It is the purpose in these chapters to place before the profession a review (which we have endeavored to make as complete as possible) of the tests that have been suggested for examining the functional activity of the various organs that have been studied. The work is complete in itself, in that all the chemical and biochemical methods used in the conduction of these tests have been described in detail. Many of the tests described have fallen into disrepute, and we have discussed them for tigation.

We wish to thank Prof. P. B. Hawk for his kind permission to quote from his excellent textbook several of the directions for the the sake of completeness. Much remains to be done in this field of invesanalysis of the blood and urine.

CHAPTER I

GASTRO-INTESTINAL FUNCTION TESTS

We shall first discuss the tests that determine the secretive functions of the stomach. It has been the custom to administer to the patient a certain test-diet and after allowing it to be digested in the stomach for some time, to remove the gastric contents and analyze it for the various constituents. We shall enumerate several of the test-diets suggested:

Test-Meals

Test-Breakfast of Ewald and Boas.

Diet: 35 grams wheat bread. 400 c.c. water or weak tea, without sugar.

Method: Give as breakfast on empty stomach. (In cases of dilatation do previous lavage.)

Remove one hour later, at time of maximum acidity.

Principle: Bland stimulant of gastric juice.

Test-Breakfast of Boas.

Diet: Oatmeal soup—500 c.c. (made of one-half ounce rolled oats in water).

Remove one hour later.

Principle: Does not introduce lactic acid which is present in all kinds of bread. Employed in cases of cancer of stomach where certain and quantitative tests for lactic acid are desired. The stomach is washed the night before.

Test-Breakfast of Dock.

Patient receives one shredded wheat biscuit and a glass of water. This breakfast contains no lactic acid, and in this has the advantage over Ewald's test-diet.

Oatmeal Test-Breakfast of Boas.

One tablespoonful of oatmeal is boiled in 800 c.c. water until the volume is reduced to 400 c.c. and is then given to the patient. This is free of lactic acid.

Dry Test-Breakfast of Boas.

Five Albert cakes are given to the patient to be masticated thoroughly, and swallowed without fluid of any sort.

Test-Dinner of Riegel.

Diet: Plate of soup 400 c.c. Beefsteak 150-200 grams. Mashed potatoes 150 grams.

Remove after four hours (time of maximum acidity).

Disadvantages: (1) Positive lactic acid reaction obtained from the sarcolactic acid, irrespective of HCl present. (2) Stomach tube may be occluded by undigested meat.

Test-Meal of Fischer.

This consists of an Ewald or Dock breakfast together with a quarter of a pound of finely chopped lean beef, broiled and slightly seasoned. It is removed after three hours (if the gastric secretory power is being examined), or after seven hours (if the gastric motility is investigated).

Test-Supper of Boas.

This consists of 100 grams white bread with a little butter, and cold meat, together with two cups of tea with sugar. Some distinguishing ingredient like raisins or spinach or currants is added. This meal is taken at bedtime, and the gastric contents removed in the morning before breakfast.

Determination of Peptic Activity.—Method of Mett as Modified by Nirenstein and Schiff.—*Principle.*—Nirenstein and Schiff recommend that the gastric juice be diluted so as to overcome the effect of the inhibiting substances. Capillary glass tubes are filled with egg-white and are allowed to lie in the diluted gastric juice in the incubator for a definite length of time. Depending upon the amount of pepsin present, the protein is digested at both ends of the tube. For clinical purposes this method is entirely satisfactory.

Method of Preparing Mett's Tubes.—Christiansen gives the following technic: The whites of several eggs are strained through gauze or cheesecloth, so that a homogeneous, clear, air-bubble free mixture is obtained. This is sucked up in cleaned and dried glass tubes of about 10 inches long and ½ mm. internal diameter. These tubes are then introduced into a large vessel of water which has been previously boiled and cooled to about 85° C. The tubes are allowed to lie in the 85° C. warm water until the water is cooled. In order to obtain tubes of uniform digestibility, when a subsequent batch is prepared, the tubes are heated at varying degrees, i.e., certain tubes are coagulated at 90° C., others at 89°, 88°,

etc., down to 80° C., and their digestibility is contrasted with the standard tube initially prepared at 85° C. The tubes are sealed with wax or paraffin, and may be kept indefinitely until used. They should be cut in lengths of about three-quarters of an inch when used.

Procedure.—Two tubes prepared as above are placed in an Erlenmeyer flask of about 25 c.c. capacity, in which has been placed 1 c.c. of gastric juice (filtered) and 15 c.c. N/20 HCl (= 0.18 per cent. HCl). The flask is stoppered and allowed to stand in the incubator for 24 hours at 37° C. The amount of digestion is determined by means of a low power microscope and a millimeter scale. Normal human gastric juice digests from 2 to 4 mm. of protein on both ends. In cases of gastric malignancy, gastroptosis, pernicious anemia, achylia, etc., the digestion may be nil. In conditions of gastric ulceration, hyperacidity and reflex stomach irritation due to duodenal ulcer, cholelithiasis, etc., the digestion may be markedly increased.

Calculation.—The peptic digestion is expressed as the square of the number of millimeters digested (Schutz-Borissow). A gastric juice digesting three millimeters has an amount of pepsin equal to 9 times that of a gastric juice digesting only 1 millimeter of protein.*

Determination of Tryptic Activity.—It frequently happens that there is regurgitation of the duodenal contents into the stomach, so that while trypsin is not a gastric enzyme, but one of pancreatic origin, it may still be present in the stomach contents. Hawk and his collaborators have found trypsin very frequently in the contents of the normal human stomach.

Spencer's Method.—Spencer's method is as follows:

"(*a*) Prepare five reagent tubes, Nos. 1, 2, 3, 4, and 5; more if desired. To tubes 1 and 2 add 0.5 c.c. of gastric contents (filter if cloudy).

"(*b*) To tubes 2, 3, 4, and 5 add 0.5 c.c. of distilled water.

"(*c*) From tube 2 remove 0.5 c.c. of its mixed contents and add to tube 3. Mix thoroughly and add 0.5 c.c. from tube 3 to tube 4. Repeat for tube 5.

"We now have dilutions of gastric contents of 1, ½, ¼, ⅛ and 1/16.

"(*d*) To each tube add one drop of phenolphthalein solution (phenolphthalein 1 gram; alcohol (95 per cent.) 100 c.c.); then add drop by drop a 2 per cent. sodium bicarbonate solution until a light pink color is produced.

"(*e*) To tubes 1, 2, 3 and 4 add 0.5 c.c. of casein solution. Tube 5 must receive 1 c.c. of casein solution, since it contains 1 c.c. of the diluted gastric contents. For the casein solution, dissolve 0.4 gram of casein in 40 c.c. of N/10 NaOH. Add 130 c.c. of distilled water, then 30 c.c. N/10 HCl. This leaves the solution alkaline to the extent of 10 c.c. of N/10 NaOH, minus about 3 c.c. neutralized by the casein.

"(*f*) Incubate for five hours at 40° C.

"(*g*) Precipitate the undigested casein by dropwise addition of a solution of the following composition: glacial acetic acid 1 c.c., alcohol (95

* For other methods of determining peptic activity, consult P. B. Hawk, "Practical Physiological Chemistry," 1916, p. 167.

per cent.) 50 c.c., distilled water 50 c.c. The tubes in which digestion has been complete remain clear; others become turbid.

"(*h*) The tryptic values are expressed in terms of dilution. Thus, complete digestion in tube 3 (a dilution of 1/4) shows four times the tryptic power of undiluted gastric juice; taking 1 as a standard, its tryptic value is 4."

Detection of Rennin.—Riegel's Method.—To 10 c.c. of fresh milk are added 5 c.c. of neutralized gastric juice, and placed in the incubator for 15 minutes. If rennin is present, distinct coagulation will be observed.

Detection of Organic Acid.—In cases of marked stasis of the stomach contents, as in gastric carcinoma, gastroptosis, etc., the food undergoes a process of fermentation, with the resulting liberation of organic acids such as acetic, butyric, lactic, oleic, etc. These are especially present in the marked diminution of free hydrochloric acid. To test for these acids, the following methods may be used.

1. Detection of Lactic Acid.—(*a*) *Uffelmann's Reaction.*—To 5 c.c. of a 1 per cent. solution of phenol add a few drops of a 5 per cent. solution of ferric chlorid until an amethyst-blue color develops. To this add a few c.c. of the filtered gastric juice. A positive reaction is indicated if a canary-yellow color develops. It responds to the presence of 0.01 per cent. or more of lactic acid.

(*b*) *Kelling's Test.*—To a test-tube full of water, add a few drops of a 10 per cent. solution of ferric chlorid. Divide the contents of the tube into two portions. To one portion add a few drops of filtered gastric juice. If a distinct canary-yellow color (ferric lactate) is produced, lactic acid is present.

(*c*) *Strauss' Test.*—It is best to extract 5 c.c. of the gastric contents with ether in a separatory funnel (thus separating out the lactic acid) and then test the ethereal extract according to the method of Kelling.

(*d*) *Hopkins' Test.*—To several c.c. of concentrated sulphuric acid in a test-tube, add one drop of a concentrated solution of copper sulphate, and a few drops of filtered gastric juice. Heat it in a beaker of boiling water for two minutes. The tube is now cooled under running water, and to it are added a few drops of a dilute alcoholic solution of thiophene, C_5H_5S, and the tube is replaced in the beaker. If lactic acid is present, a cherry-red color develops quite rapidly. For clinical purposes this test is very cumbersome and more expensive to carry out as well as consuming more time, without being much more accurate, than the Uffelmann test.

Certain foods, such as milk, bread and meat, contain a certain amount of lactic acid. In performing analyses of the gastric contents this is to be kept in mind. The advantages of the various test-meals, as well as their disadvantages, have been discussed (see page 3).

2. Detection of Butyric Acid.—Under physiological conditions, butyric acid may be present in the gastric contents if the individual has partaken of much milk or butter. In infants, it is frequently present in the gastric contents. The vomitus of an infant will often have the char-

acteristic rancid butter odor. In cases of carcinoma of the stomach with marked obstruction, there may be present much butyric acid in the stomach contents.

The characteristic, disagreeable odor of butyric acid is the best test for its qualitative detection. It is best, however, to shake out the liquid under examination with ether, wash the ether with a little water and then add a few crystals of calcium chlorid. The butyric acid will separate out as oily globules, easily detected by the odor.

To the ethereal extract add a few drops of concentrated sulphuric acid and ethyl alcohol, and warm slightly. An odor of pineapples will develop if butyric acid is present.

3. Detection of Acetic Acid.—Due to the action of fungi, acetic acid may be produced in the stomach. It is sometimes mistaken for lactic acid. The gastric contents are extracted with ether, and the ethereal residue reëxtracted with water. This watery extract is neutralized with sodium carbonate. Add a few drops of dilute ferric chlorid solution, and a red color will appear if acetic acid is present.

Topfer's Method of Gastric Analysis.—This method is much less elaborate than many others, but is sufficiently accurate for ordinary clinical purposes. The method embraces the volumetric determination of (1) total acidity, (2) free acidity (organic and inorganic), and (3) free hydrochloric acid, and the subsequent calculation of (4) combined acidity and (5) acidity due to organic acids and acid salts, from the data thus obtained.

General Procedure.—Feed the Ewald test-meal as directed previously. At the end of one hour remove the entire stomach contents and analyze as directed below. This method of procedure is less accurate than the fractional method. Measure the volume of the gastric contents, strain it through cheese-cloth and introduce 10 c.c. of the strained material into each of three small beakers or porcelain dishes. Label the vessels A, B and C respectively and proceed with the analysis according to the directions given below. The volume of fluid present in the stomach one hour after an Ewald meal varies under normal conditions between 50 and 100 c.c. In cases of hypersecretion or defective motility 200-300 c.c. may be found. Very excessive volumes, e.g., 500-3000 c.c., are indicative of dilatation of the stomach and suggest pyloric stenosis, either benign or malignant.

1. Total Acidity.—Add 3 drops of a 1 per cent. alcoholic solution of phenolphthalein to the contents of vessel A and titrate with N/10 sodium hydroxid solution until a faint pink color is produced and persists for almost two minutes. Take the burette reading and calculate the total acidity.

Calculation.—The total acidity may be expressed in the following ways:

(*a*) The number of cubic centimeters of N/10 sodium hydroxid solution necessary to neutralize 100 c.c. of gastric juice.

(*b*) The weight (in grams) of sodium hydroxid necessary to neutralize 100 c.c. of gastric juice.

(*c*) The weight (in grams) of hydrochloric acid which the total acidity of 100 c.c. of gastric juice represents, i.e., percentage of hydrochloric acid.

The forms of expression most frequently employed are *a* and *c*, preference being given to the former, particularly in clinical work.

In making the calculation note the number of cubic centimeters of N/10 sodium hydroxid required to neutralize 10 c.c. of the gastric juice and multiply it by 10 to obtain the number of cubic centimeters necessary to neutralize 100 c.c. of the fluid. If it is desired to express the acidity of 100 c.c. of gastric juice in terms of hydrochloric acid by weight, multiply the value just obtained by 0.00365.

2. Free Acidity (*Organic and Inorganic*).—Add 3 drops of sodium alizarin sulphonate solution to the contents of vessel B and titrate with N/10 sodium hydroxid solution until a violet color is produced. In this titration the red color, which appears after the tinge of yellow due to the addition of the indicator has disappeared, must be entirely replaced by a distinct violet color. Take the burette reading and calculate the free acidity due to organic and inorganic acids.

Calculation.—Since the indicator used reacts to both organic and inorganic acids, the number of cubic centimeters of N/10 sodium hydroxid used indicates the free acidity of 10 c.c. of gastric juice.

3. Free Hydrochloric Acid.—Add 4 drops of dimethylaminoazobenzene (Töpfer's reagent) solution to the contents of the vessel C and titrate with N/10 sodium hydroxid solution until the initial red color is replaced by orange yellow. Take the burette reading and calculate the free acidity.

Calculation.—The indicator used reacts only to free hydrochloric acid, hence the number of cubic centimeters of N/10 sodium hydroxid used indicates the volume necessary to neutralize the free hydrochloric acid of 10 c.c. of gastric juice. To determine the data for 100 c.c. of gastric juice proceed according to the directions given under Total Acidity.

4. Combined Acidity.—This value may be obtained by subtracting the number of cubic centimeters of N/10 sodium hydroxid used in neutralizing the contents of vessel B from the number of cubic centimeters of N/10 sodium hydroxid used in neutralizing A. The data for 100 c.c. of gastric juice may be calculated according to directions given under Total Acidity.

5. Acidity Due to Organic Acids and Acid Salts.—This volume may be conveniently calculated by subtracting the number of cubic centimeters of N/10 sodium hydroxid used in neutralizing the contents of vessel C from the number of c.c. of N/10 sodium hydroxid solution used in neutralizing the contents of vessel B. The remainder indicates the number of c.c. of N/10 sodium hydroxid solution necessary to neutralize the acidity due to organic acids and acid salts present in 10 c.c. of gastric juice. The data for 100 c.c. of gastric juice may be calculated according to directions given under Total Acidity.

According to Seidl, the gastric contents obtained after a test-break-

fast possess, as a rule, a higher degree of acidity than the filtrate. The higher acidity degree is caused by a mechanical combination of the acid with the starchy material. Gastric mucus has the property of binding much alkali. For this reason acidimetry in the presence of considerable admixture of mucus with the gastric contents should be performed with the filtrate only.

On the other hand, Weinberg states that titration of the unfiltered gastric juice gives more reliable results than titration of the filtered specimen. If possible, titration of both filtered and unfiltered gastric contents should be made in order to obtain data concerning the distribution of the acid contents (Hawk).

Glutzinski's Method for Gastric Acidity.—Smithies states that in gastric cancer or in cases where a peptic ulcer is suspected of being carcinomatous, the Glutzinski method is particularly instructive. The method of procedure is as follows: The contents of the fasting stomach are siphoned out in the morning and the findings recorded as to amount, color, odor, relics of food, blood, etc., litmus reaction, free acid with Congo paper and free acid with phloroglucin vanillin; lactic acid with Strauss or Uffelmann tests, and occult blood with the guaiac, turpentine, or benzidin tests. The amount of free acid and total acid is determined by titration. The microscopical findings are also recorded. Then the stomach is washed out clean with tepid water and Ewald-Boas test-breakfast is given. Forty-five minutes later the stomach contents are siphoned out again and the stomach thoroughly rinsed out anew. The test-dinner is then given: about 100 grams of chopped roast veal or boiled beef; 150 grams potato cooked with 20 grams fat and no fluid. The stomach contents are siphoned out anew after 2 hours. All this is done on one day. The findings of larger amounts of hydrochloric acid after the test-dinner speak for ulcer; smaller proportions for cancer. The insufficiency of the stomach mucosa is revealed by the lack of acid after the test-dinner, even when some acid is found after the test-breakfast. Fonis regards such findings as absolutely conclusive in doubtful cases. He tabulates the findings in 26 ulcer cases and comments on the value of the information thus derived. In four cases the findings proved dubious and the course of the cases showed that the ulcer at the time must have been just starting malignant degeneration. The procedure, says Smithies, is one of much promise, and should be tried in doubtful cases as a matter of routine. Negative information should never, however, postpone laparotomy where the clinical history is suggestive.

Rusca does not believe in the reliability of the Glutzinski test. According to him there are too many factors that play an important part in determining the amount of free hydrochloric acid. Kuttner also could not substantiate Glutzinski's claims. Nevertheless, Nicolayessen, of Christiania, reported before the International Congress of Surgeons, New York, 1914, that he and his collaborators have obtained good results with this method.

According to Zoeppritz, who employed Glutzinski's method in 462

cases, so-called relative secretory insufficiency occurs comparatively often in the presence of gastric carcinoma, but may also be observed in other affections of the stomach and even when the stomach is healthy. The method is useless in the differential diagnosis between stenotic and non-stenotic carcinoma and between carcinoma and ulcer, and in the early diagnosis of malignant disease (Zoeppritz).

Volume of Gastric Secretion.—In order to determine whether the patient is suffering from hypersecretion, it is necessary to determine the quantity of gastric contents before and after a test-meal. If the volume is the same as the volume taken in, with the test-meal, it is indicative of motor insufficiency. In normal subjects, it is possible to withdraw only a portion of the ingested volume taken in the test-meal, the rest having passed into the duodenum.

It has been observed by London and Polowzowa, and by London and Sagelmann, that all foodstuffs do not leave the stomach with the same rapidity. They demonstrated that carbohydrates leave more quickly than proteins, and proteins leave more quickly than fats. This so-called "selective capacity" of the stomach for certain foodstuffs has been disputed by Scheunert and by Grinnerer, but the investigations of Cannon have demonstrated that there is such a "selective capacity." Cannon explains the reason why different foodstuffs leave the stomach with unequal rapidity, as due to the chemoreflex action of the hydrochloric acid upon the pyloric sphincter. The proteins combine with hydrochloric acid and hence its action upon the sphincter becomes weaker, while this is not the case with the carbohydrates. According to him, if the carbohydrates are moistened with alkali they leave the stomach more slowly than usual and the acid proteins, on the contrary, leave the stomach earlier than other proteins.

It has been found by Boldyreff that after administration of fat to a patient, there is a marked regurgitation of bile, duodenal and pancreatic secretions into the stomach, so that gastric digestion in these cases is essentially brought about by pancreatic juice. The experiments of Babkin, Boldyreff and others have demonstrated that the acid concentration of freshly secreted gastric juice of man is similar to that of the dog, that is, 0.4 to 0.5 per cent., and that this initial high acid content is reduced by means of the regurgitated alkaline fluids of the duodenum. This claim was corroborated by the experiments of Migai and Zaitzeff in Russia, and by Hawk and his collaborators in America.

Method of Mathieu-Remond.—The patient receives a definite volume of water, which is thoroughly mixed with the gastric contents by means of massage, only blowing air into the stomach through a tube. The acidity of the gastric contents is determined before and after the addition of the water. From the amount of dilution which the residue undergoes is determined the volume of the gastric contents.

Let x = volume of the residuum (before the intake of the water).

q = volume of water added.

v = volume of gastric contents removed before the administration of the water.

a = acidity of the gastric contents removed before dilution.

a′ = acidity of diluted residuum.

$$\therefore ax + a'q = a'x,$$

$$\therefore x = \frac{a'q}{a - a'},$$

$$\therefore x = v + \frac{a'q}{a - a'}.$$

Method of Strauss.—A portion of the gastric contents is removed, and the volume and specific gravity are estimated. A measured volume of water is then introduced into the stomach, and is allowed to mix intimately with the gastric contents. The entire gastric contents are now removed and the specific gravity of the diluted gastric contents is determined.

x = volume of gastric residuum before the water intake.
S = specific gravity of undiluted contents.
S′ = specific gravity of diluted contents.
V = volume of diluted contents removed.
a = volume of water taken in.

$$\therefore x = \frac{V S + (a - V) S' - a}{S - S'}.$$

Method of Elsner.—The Mathieu-Remond formula having been determined, the stomach is thoroughly washed out until the gastric washings come out clear. These washings are collected in graduated cylinders and allowed to stand 24 hours. The sediment is now read off. Normally this volume should be between 30 and 100 c.c. A volume above 100 c.c. speaks for motor insufficiency. Prym has absolutely denied the value of these tests, but Mathieu energetically opposes the views of Prym.

Method of Hayem and Winter.—This method determines the change in concentration of the gastric juice by estimating the quantity of dissolved solids in one cubic centimeter of the juice. A measured volume of juice is evaporated to dryness in a weighed vessel, and the dry residue thus determined. Winter suggests that the patient receive the Ewald test-breakfast together with 10 grams sugar. At the beginning of digestion the concentration is 0.10; after 1 hour with the Ewald meal it is 0.06. When the stomach is nearly empty the concentration is 0.01. In this way it is possible to determine at which period of the digestion the specimen was removed.

Rehfuss Method of Fractional Analysis of Gastric Secretion.—As Rehfuss, to whom we are greatly indebted for the following data, says, in the literature the idea of making a fractional examination of gastric digestion is by no means new. Perhaps to Hayem belongs the credit of studying most carefully the secretory output in various conditions, but his method was crude and his curves show the irregular intervals necessitated by such an examination. Practically all examinations which were conducted on the gastric secretion in the human subject used the method by which the subject received a definite test-meal which was emptied in ten minutes on one day, on the following day in twenty minutes, on the third day after thirty minutes, and so on, until the entire cycle of gastric digestion was completed. Many authors have investigated the gastric secretion at various periods. Ewald and Boas, then Reichmann, von Jaksch and Kornemann, in 1885, published a most comprehensive series of observations on the digestion of milk in a healthy young man of 20. After habituating his subject to the passage of the tube, he commenced his series, removing the milk on successive days after increasing time intervals.

Von Jaksch records a series of tables indicating the effect of milk, tea, carbohydrates and meat in children. These were administered and removed at various intervals by means of the sound. His observations are of interest and he shows that the position of the high point differs according to the diet ingested. He makes the statement, however, that after the administration of weak tea and saccharin, free HCl is detected in 78 per cent. of the cases in three-quarters of an hour, but after feeding milk or meat, he could detect its presence in the first quarter of an hour.

Schule's observations are quite complete, but unfortunately restricted to four individuals, although they were repeated, and different articles of diet were investigated. After tea and toast, for instance, while the majority of high points were encountered at sixty minutes, several were found at forty-five and seventy-five minutes, respectively.

Kornemann gives fifty minutes as the point at which the free HCl reaches its height after the administration of a test-breakfast. With 35 grams of roll and 250 grams of tea, he finds the stomach empty in two and one-half hours.

Ehrenreich, perhaps, published the first satisfactory results of a complete examination of the gastric cycle and utilized for that purpose a Nelaton catheter. He showed that examinations at quarter-hour intervals demonstrated that the pepsin concentration rapidly reached its maximum (after twenty to thirty minutes) and then had a tendency to remain constant, while the acid concentration reached its maximum only after seventy minutes. Gregerson criticizes the method, in as much as apparently a great deal of saliva and mucus is formed by Ehrenreich's method. Some twenty-two cases were enumerated by Ehrenreich, insufficient to draw any interpretation of the interesting findings which he recorded (Rehfuss).

Gregerson's cases, while few in number, are interesting and instruc-

tive, but the method, as were other methods enumerated above, is entirely impractical.

In 1914, Rehfuss described his method of studying gastric secretion. He devised a tube by which it is possible to follow the entire cycle of gastric digestion with practically no discomfort and by which it is possible at any given moment to draw off any quantity desired of the juice to perform the necessary chemical examinations. The following is the procedure as described by Professor Hawk, in whose laboratory Rehfuss carried out his investigations:

Removal of Residuum.—If the so-called "empty" stomach is examined in the morning before any food or drink has been taken, it will be found to contain considerable material. This is termed residuum. Before a test-meal is introduced into the stomach, this organ should be emptied. If this is not done we cannot consider the samples withdrawn after the test-meal is eaten as representing the secretory activity

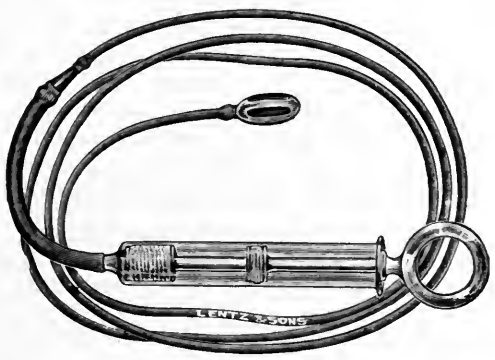


Fig. 1.—Rehfuss Stomach Tube.

of the gastric cells under the influence of the stimulation of the test-meal. It has been generally recognized, clinically, that a residuum above 50 c.c. is pathological. Such a volume has been considered as indicative of hypersecretion, and this in turn in many cases indicates an organic lesion. The observations indicating that a residuum of over 10 c.c. was pathological, were made upon residuums removed by means of the old type of stomach tube, which does not completely empty the stomach. When the residuum is completely removed by means of the Rehfuss tube it has been demonstrated that the normal residuum is practically always over 20 c.c. and that the average is about 50 c.c. The normal residuum has been found to possess all the qualities of a physiologically active gastric juice with an average total acidity of 30 and an average free acidity of 18.5. The residuum is often colored by bile. This is particularly true if the fluid has a relatively high acidity. Trypsin is also generally present. These findings indicate regurgitation. A residuum of large volume possessing a total acidity value of 70 or over may indicate ulcer.

The Test-Meal.—Before making an analysis of the stomach con-

tents it is customary to introduce something into the stomach which will stimulate the gastric cells. The response to this stimulation is then measured clinically by the determination of total acidity, free acidity and pepsin in the stomach contents. Many forms of test-meal have been used.

The test-meal most widely employed is the Ewald test-meal. This consists of 2 pieces (35 grams) of toast and 8 ounces (250 c.c.) of water. Inasmuch as it was demonstrated by Rehfuss that water gave a similar gastric stimulation to that produced by the Ewald meal, he suggested that a simple water-meal might be substituted for the Ewald meal. This water-meal also has the added advantage of enabling one to determine the presence of food rests and to test more accurately for lactic acid, blood and bile.

The Retention-Meal.—In order to obtain more information regarding gastric motility than is furnished by the ordinary test-meal described above, the patient may be fed a so-called retention-meal. This meal is fed in place of the regular evening meal and contains substances readily detected. In the morning before breakfast (7-8 A. M.) remove the stomach contents (residuum) by aspiration and examine for food rests. The normal stomach should give no evidences of food retention. A satisfactory retention-meal consists of 4 ounces each of boiled string beans and rice. Diets containing prunes, raspberry marmalade, lycopodium powder, etc., have also been employed. In many instances an ordinary mixed diet will serve the purpose (Hawk).

Removal of Samples for Analysis.—At intervals of exactly 15 minutes from the time the test-meal is eaten until the stomach is empty 5-6 c.c. samples of gastric contents are withdrawn from the stomach by means of aspiration.

In removal of samples from the stomach, it is essential that very little traction be employed. To empty the stomach completely, aspiration is practiced in four positions: (*a*) on the back; (*b*) on the stomach; (*c*) on right side; (*d*) on left side. This results in complete evacuation of the stomach. Three tests may be employed to determine whether the stomach is empty: (1) No more material can be aspirated in any position; (2) injection of air and auscultation over the stomach with a stethoscope reveals a sticky râle and not a series of gurgling râles such as are heard when there is material in the stomach; (3) lavage or irrigation through the tube which shows the absence of all food in the stomach.

Plotting of Curves.—The results of these examinations are plotted, the abscissa being the time, in minutes, at which the gastric contents were removed and the ordinate being the number of c.c. decinormal sodium hydroxid solution necessary to titrate the free acidity and the total acidity of the gastric contents.

The normal curves that may be obtained are of three types, according to Rehfuss and his co-workers: (1) The *"isosecretory" type* shows a steady rise, high point, in terms of tenth normal sodium hydroxid, 40 for free acid and 60 for total acid, usually sustained for from half

an hour to an hour, and then a gradual decline with total disappearance of the food residues in from two to two and a half hours (Fig. 2).

(2) "The *hypersecretory type* shows a rapid response to stimuli, often a marked change in the acidity even of the five-minute samples, rapid increase in acidity, high point from 70 to 100 or over, either sustained or abrupt, and a slow decline or none at all in the usual time. The food left the stomach in normal time from two to two and one-half hours, but even after the passage of all food material, there was often encountered an outpouring of pure gastric juice for half an hour, one

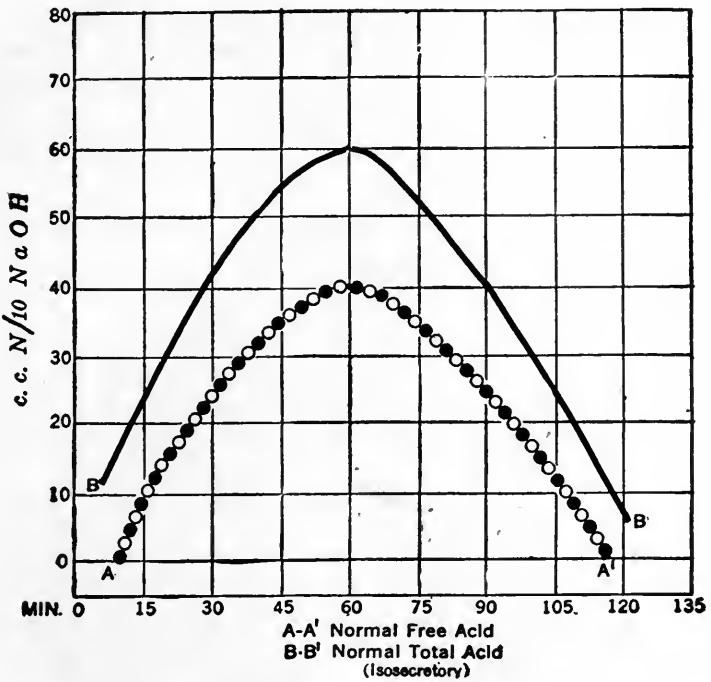


FIG. 2.—ISOSECRETORY CURVE.

hour or even several hours. This finding, which was obtained in many cases, is so pronounced and distinct that they call it *continued digestive secretion* in contradistinction to hypersecretion because it occurs in normal symptomless persons. This type they call the *hypersecretory type* because of the general tendency of the acidity to assume exaggerated proportions" (Rehfuss, Bergeim and Hawk).

(3) The *hyposecretory type* shows a slower ascent than the isosecretory curve, a slower response to stimuli, and a high point from 40 to 50. This type is rarely met with.

CONCLUSIONS OF USUAL TEST-MEAL ANALYSIS.—It is in the change of function due to gastric disease that this curve is of such great diagnostic aid. From this curve we can obtain information which a single analysis after one hour following an Ewald test-meal could never yield.

The author cannot do better than quote the conclusions of Rehfuss as to the limitations of the usual test-meal analysis:

1. It is impossible to interpret the figures obtained by the examination of the test-meal removed in one hour by the usual technic.

2. The one-hour period represents but one phase in the constantly changing cycle of gastric digestion. While it is true that in a certain proportion of normal cases the high point is to be found at the one-hour interval, this is by no means always the case, and pathologically every deviation from this type may be encountered.

3. It is impossible to judge what has preceded or what will follow this point, data absolutely necessary to a complete understanding of the case.

4. Delayed digestion, many forms of hyperacidity, hypersecretion, symptoms of early catarrh, occult bleeding, are in many cases entirely overlooked by the customary examination.

5. So-called normal figures at the one-hour point cannot be interpreted in the light of a simple isolated phase examination. They may mean (*a*) a perfectly normal curve; (*b*) they may be followed by a marked hyperacidity, hypersecretion, and motility disturbances at a later period; (*c*) they may be only one point in a continued high acidity and hypersecretion such as is encountered in obstructive cases; (*d*) they may show a form of larval hyperacidity.

6. Hyperacid figures may be part of an abrupt rise and equally rapid fall, or they may be part of a sustained persistent hyperacidity accompanied by marked hypersecretion and evidences of beginning or pronounced motor disturbances, factors impossible to demonstrate by the ordinary examination.

7. Subacid figures may be part of a general subacid curve, or they may mean a simple delay in digestion with its complete evolution at a later period. Finally, by no means rare, subacid figures at the one-hour point may be followed by hyperacid figures at a later stage in digestion.

8. The ordinary method can give us evidence of nothing but the crudest anomalies in motor function. The fractional method enables us to determine precisely the end point of gastric digestion.

9. In the studies of the complete gastric cycle, every form of secretory and motor disturbance has been found. A symptom like the actual motor secretory disturbance by no means respects the hour period and may be found depending on the nature of the case at any point in the gastric cycle.

In a paper on "The Impossibility of Interpreting the Findings Obtained by the Customary Examination of the Test-Meal," Rehfuss makes clear the following points:

1. This point records but one phase in the ever changing cycle of gastric digestion.

2. A large number of observations have shown that this point is by no means the high point or the representative one.

3. Many changes may precede or follow this point.

4. Many forms of hyperacidity, hypersecretion, occult bleeding, are entirely overlooked in this way.

5. Delay in digestion may mean that figures at the one-hour point may be doubled at the second hour.

6. No idea of evacuation or the motor function of the stomach can be obtained by this method.

7. Entirely erroneous conclusions may be drawn.

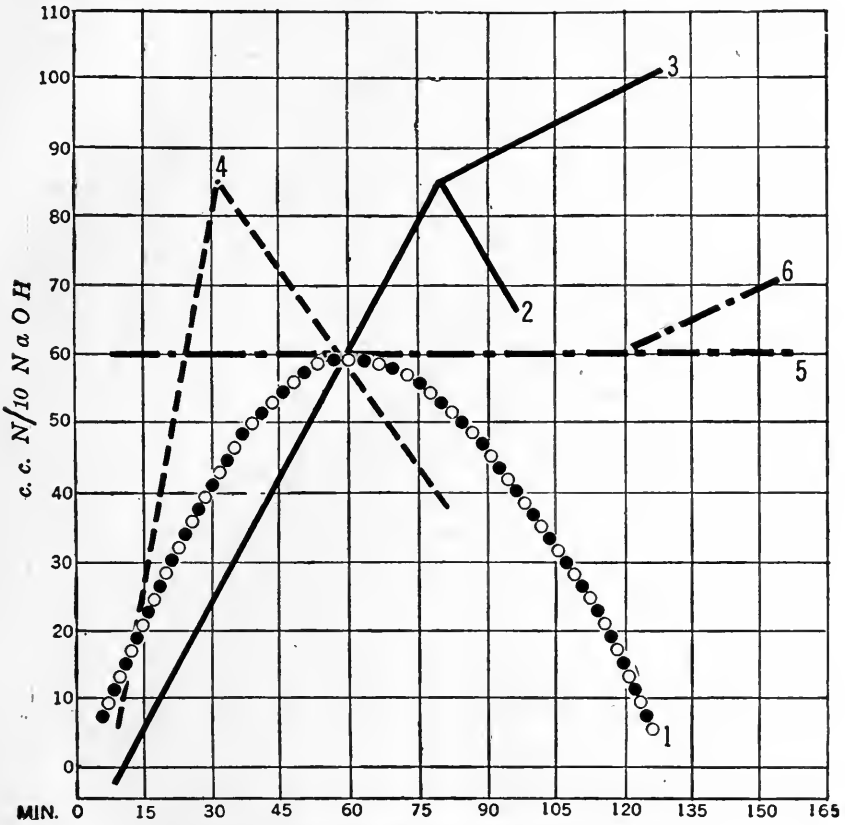


FIG. 3.—FALLACIES OF THE EWALD TEST-MEAL.

The possibilities of the gastric curve with a normal acidity at the one-hour period: (1) Normal curve; (2) hyperacidity; (3) persistent hyperacidity; (4) larval hyperacidity; (5) continued hypersecretion; (6) prolonged digestion.

CONCLUSIONS FROM REHFUSS FRACTIONAL METHOD.—The curves that may be obtained by the Rehfuss fractional method in gastric ulcer, duodenal ulcer and in gastric cancer are rather typical.

In cases of *gastric ulcer* the ascent of the curve is rapid, and may reach its maximum before the hour or a little after. The high point in the free acidity may be between 60 and 70 and the total acidity between 100 and 110. There is then a gradual or sudden decline as the stomach empties itself. Blood may, of course, be found in the fractions removed. Two typical analyses of the secretion in cases of gastric ulceration, confirmed by operation, are given in Table 1.

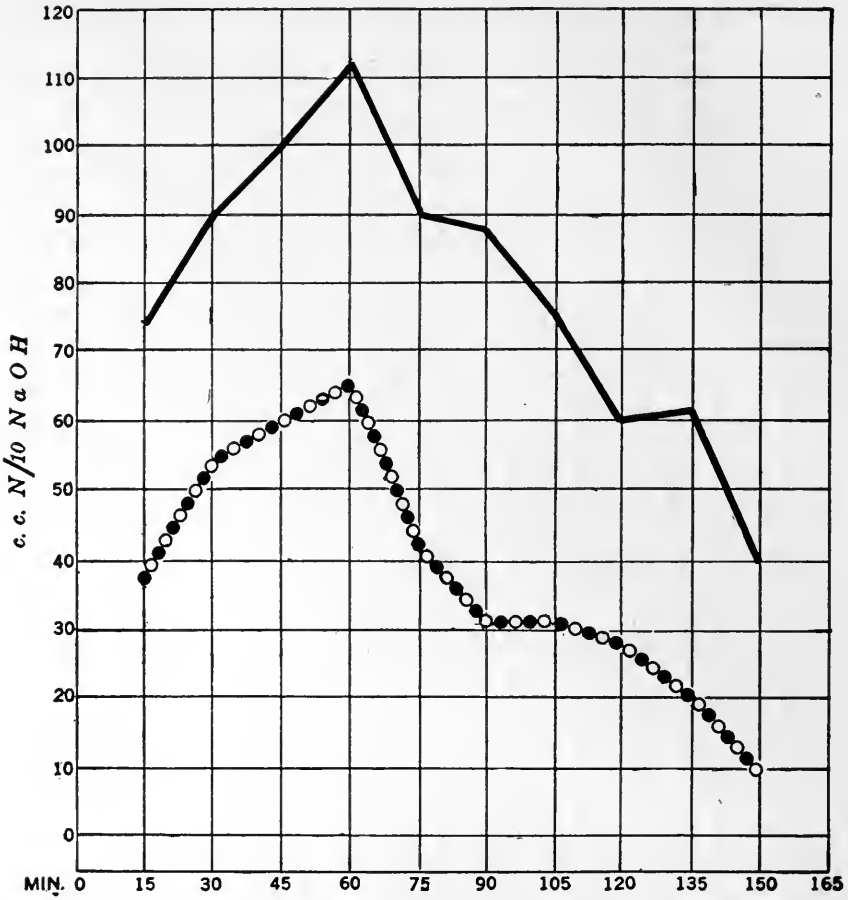


FIG. 4.—CURVES IN A CASE OF GASTRIC ULCER. (Table 1, Miss W.)
Upper curve: total acid; lower curve: free acid.

TABLE 1. CURVES IN CASES OF GASTRIC ULCER.

Time	Miss W.		Mr. M.	
	Free Acid	Total Acid	Free Acid	Total Acid
15	38	74	28	46
30	54	90	50	60
45	60	100	52	80
60	64	112	54	84
75	42	90	64	90
90	32	88	65	95
105	32	75	40	70
120	28	60	24	44
135	20	62	20	36
150	10	40	empty	empty

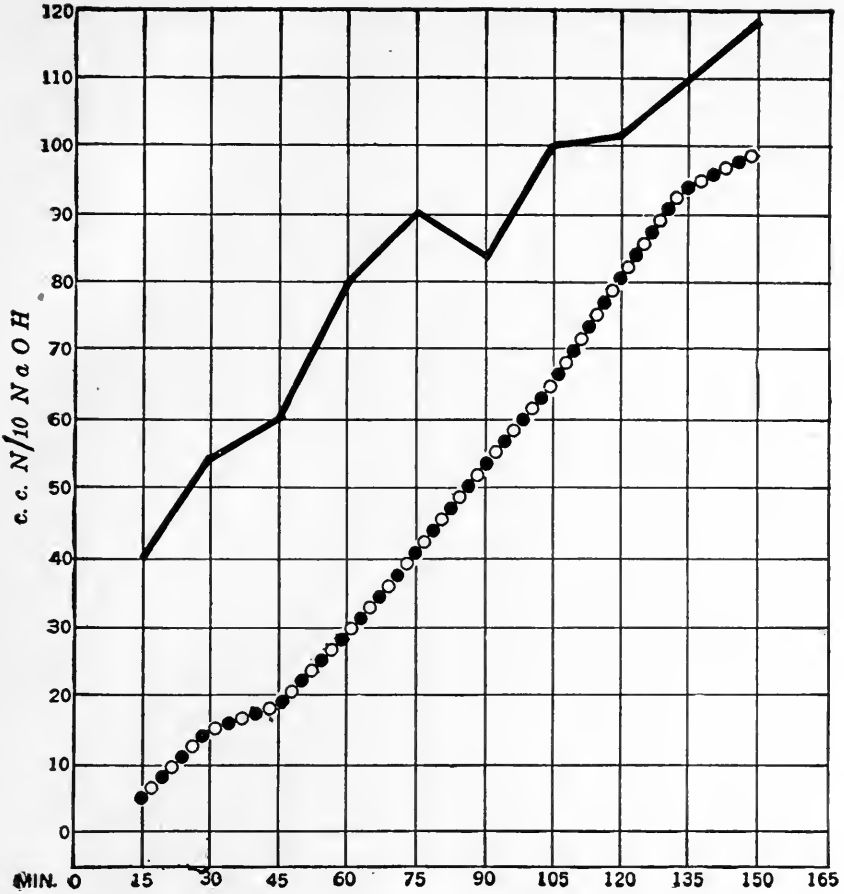


FIG. 5.—CURVES IN A CASE OF DUODENAL ULCER. (Table 2, Mrs. B.)

TABLE 2. CURVES IN CASES OF DUODENAL ULCER.

Time	Mrs. B.		Mrs. F.	
	Free Acid	Total Acid	Free Acid	Total Acid
15	6	40	0	20
30	14	54	12	32
45	18	60	32	46
60	28	80	38	56
75	40	90	42	68
90	54	84	48	82
105	66	100	56	80
120	82	102	80	98
135	96	110	96	112
150	98	118	98	116

In *duodenal ulcers* the ascent of the curve is gradual. The height of the curve seems to be reached when the stomach is emptying itself, and the reflex irritation of the food passing over the diseased duodenum, stimulates the secretion of the gastric juice. Table 2 shows the result of the analyses by the fractional method of two cases of duodenal ulcer, confirmed by operation.

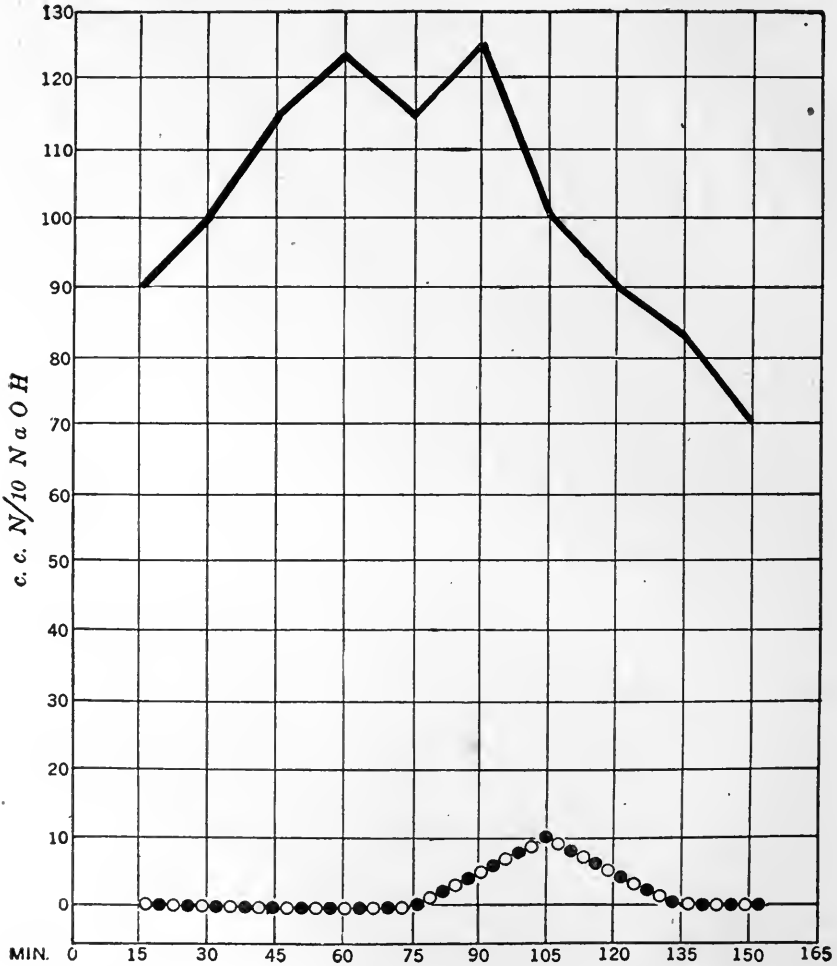


FIG. 6.—CURVES IN A CASE OF PYLORIC CANCER. (Table 3, Mrs. C.)

It will be seen from these analyses how inefficient the ordinary one-hour examination would have been. The report would have come—"Total acid 56 and free acid 38" (Mrs. F.), and the conclusion would have been reached that this was a case of hypo-acidity, and one would begin to suspect stasis, malignancy, etc. In reality, as one sees from the fractional method, this is a case of hyperacidity, pointing toward a duodenal ulcer.

In *gastric carcinoma* with obstruction, the curve usually present is, in the author's experience, the following. (Table 3, Fig. 6.) The free acid is either entirely absent, or rises to a point between 10 and 15 after one hour. The total acidity may, on the other hand, be normal or even above normal. The following analysis of gastric carcinoma, with obstruction, confirmed by operation, is rather typical. Blood and lactic acid were very heavy.

Table 3. Curves in Pyloric Cancer. Mrs. C. (Fig. 6).

Time	Free Acid	Total Acid
15	0	90
30	0	102
45	0	115
60	0	126
75	0	120
90	5	130
105	10	100
120	5	90
135	0	84
150	0	70

In the author's opinion, analyses of the gastric secretion by the Rehfuss fractional method yield results of great significance and of distinct aid in the diagnosis of diseases of the stomach and duodenum.

Reflex irritation due to gall-stones, appendicitis, etc., may influence the gastric curve markedly and give results stimulating ulcer. This must always be borne in mind.

In carcinoma of the fundus of the stomach (with no obstruction at the pylorus), both the total and free acid is markedly subnormal (Fig. 7).

The Gastric Residuum.—Harmar and Dodd have shown, by means of the roentgen rays, that the complete removal of the gastric residuum with the aid of the usual stomach-tube, is by no means certain. This finding has been further confirmed by Rehfuss, Bergeim and Hawk, who have succeeded in removing, from the normal fasting stomach, quantities of residuum greatly in excess of that obtainable by the old method. Loeper, Zweig, Kemp, Wolff, Strauss, Riegal, and Soupalt * assert that the quantity of residuum in the normal fasting stomach should not exceed 20 c.c., and that there should be no microscopic food residues. Rosin and Schreiber * declare that *the quantity of residuum* is not of much importance, as a pathological factor. In thirteen samples of normal residues obtained by Rehfuss, Bergeim and Hawk, none were found to be so small in volume as the 20 c.c. suggested by Loeper and others, while five were very near the 60 c.c. limit suggested by Rosin and Schreiber, and four were greatly in excess of this figure. Oettinger says that when the amount of residuum exceeds 150 c.c., food débris or retention is almost constantly observed, and that a diagnosis of stenosis can

* Cited by Loeper.

be made unhesitatingly. However, Rehfuss, Bergeim and Hawk had one case of 180 c.c. of residuum, and another of 120 c.c.; yet in neither were any microscopic food residues found. This absence of microscopic food residues was true of all the cases examined, although in a few cases fat globules and vegetable fibers were found microscopically.

In regard to the *cause* of the residuum, it has been suggested by Loeper that there is an increased molecular concentration of the residuum, and that this favors pylorospasm and a consequent retention of material. The presence of bile in a number of the residua of Rehfuss, Bergeim and Hawk, and the absence of pylorospasm, would not tend to favor this theory. On the contrary, if the closure of the pylorus is

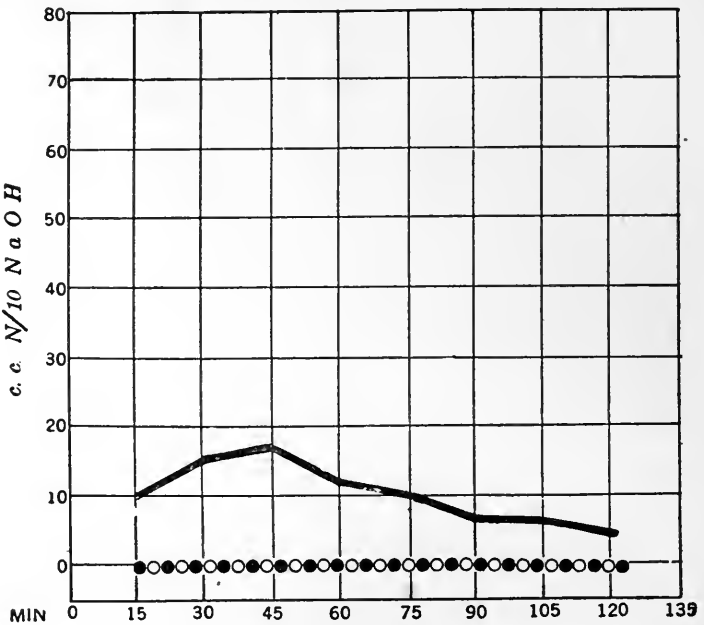


FIG. 7.—CURVES IN A CASE OF CARDIAC CANCER.

due to an acid reflex on the mucous membrane of the duodenum, it is more probable that, in the absence of acid, the pylorus is in a relaxed condition. Sokolow showed that saliva, bile and pancreatic juice were all capable of causing gastric secretion. Grobbel maintains that saliva is always expelled from the stomach at the close of the meal.

Oettinger suggested that although the bile reaction of the residuum might be negative, still there would be, in some cases, a distinct yellowish, or greenish-yellow color. This fact has been further substantiated, by the work of Rehfuss, Bergeim and Hawk, and by the present contribution. Sartory showed the presence of a yeast in the gastric residuum, to which the color was due. He designated it as *Cryptococcus salmoneus*. Fowler, Rehfuss and Hawk have shown, however, that not in all cases is the color due to yeast, as many times a strong bile reac-

tion was demonstrable. Oettinger suggests that, as the acidity of the residuum becomes stronger, it becomes the richer in yeasts, and the poorer in microbes.

Loeper has suggested the use of the volumes of residua in relation to the diagnosis of ulcer. However, as has been shown by Rehfuss, Bergeim and Hawk, this is open to great error, and its value is very questionable. Recently Meunier found that the freezing-point lowering (cryoscopic index) of the stomach contents was 0.35° C. He suggested the use of this fact in the administration of medicaments. He demonstrated that when medicaments were given at the concentration having the same freezing point lowering, they passed through the stomach with maximum rapidity. He concludes that if medicines are given in solutions capable of lowering the freezing-point 0.35° C., the least gastric disturbance will result.

Fowler, Rehfuss and Hawk draw the following conclusions from their study of gastric residua:

1. They have been able to confirm the finding of their previous investigation, that "the accepted limit of the normal residuum of the empty stomach as 20 c.c. is false." The average volume of the residuum, in over 100 normal cases, in the present investigation, was 52 c.c. This finding throws considerable question on the value of an increased residuum in the diagnosis of ulcer.

2. The residuum found by them in every case had all the qualities of a physiologically active secretion. They believe that the gastric glands are never inactive, and cite experiments to demonstrate that, even in the absence of normal stimuli (food, psychic, etc.), these secretions appear.

3. There is a definite relationship between the character of the residuum, and the character of the gastric secretory response to a stimulus, as a test-meal, for instance.

4. From the standpoint of osmotic pressure, there is a constant tendency toward the formation of a secretion in the stomach.

5. Both colorless and bile-colored residua may be found, and the two may occur in the same individual.

6. The colored residuums appear more frequently in higher activities, and vice versa, a fact which is explained by the greater frequency of regurgitation in the former condition. This regurgitation has for its purpose the partial neutralization of the high acid stomach contents.

7. Total and free acidity vary directly with each other. It is pointed out that free acidity was rarely encountered until the total acidity exceeded 10. The averages in this series of studies were 29.9, total acidity, and 18.5, free acidity. The value of acid figures over 70, with an increased quantity of residuum, is emphasized as of diagnostic value.

8. There is a definite relationship between the quantity of pepsin, and the total acidity for low acid values. As high acid values are approached, this relationship disappears.

9. Trypsin was found almost constantly in the residuum. It is

shown to be inversely proportional to the free acidity. This is explained by the more complete closure of the pylorus in high acid conditions, in conjunction with the destructive action of acid on trypsin.

10. The average cryoscopic index of —0.470, as compared to an index of the blood equivalent to —0.560, gives evidence of a tendency for osmosis of material to take place from the blood into the lumen of the stomach.

11. High acidities are shown, by a comparison of the cryoscopic index and specific gravity data, to be accompanied by a throwing out of solution of certain molecules, the character of which is unknown at the present time.

12. The residuum is one of the lightest fluids of the body, having an average specific gravity of 1.0056. This point is not without significance.

Fowler and Zentmire obtained the following data:

Eighty-one samples of residua were collected from sixty different subjects; twenty-one of the subjects submitted to the passage of the tube twice.

Averages on the compilation of the normal gastric residuum:

1. The average quantity removed in eighty-one cases was 49.44 c.c., the largest quantity being 135 c.c., and the smallest 12 c.c.

2. In sixty-nine cases noted, the residuum was in twenty-two cases (32 per cent.) colorless, and in forty-seven cases (68.1 per cent.) yellow or green.

3. The total acidity recorded in eighty-one instances averaged 30.3 in terms of tenth-normal sodium hydroxid, with the highest figure at 86.0 and the lowest at 4.0.

4. The free acid (seventy-nine cases) averaged 15.6, with figures ranging from 74.5 to 0.

5. The pepsin concentration as measured by the Mette method was determined in seventy-nine cases, and gave an average of (3.3), highest (6.7), and lowest (0.0).

6. Trypsin was tested for in sixty-three cases, with an average of 5.2 units; ten cases gave 16 units, and fifteen, no reaction. Thus in 23.8 per cent. of the cases there was no evidence of trypsin.

7. Microscopical examination revealed on no occasion gross food residues. On several occasions vegetable débris was found, but never meat fibers. Leukocytes, in considerable number, were found almost constantly, and occasionally swallowed material from the throat with bacteria.

The gastric curve produced by three cases of pernicious anemia (Kahn and Barsky) strikingly resembles the curve of carcinoma of the stomach. The free acid was absent and the total acid varied between 15 and 45. No blood was present; a trace of lactic acid was found in one case; pepsin was absent. The gastric mucosa appears to be altogether non-functionating, due to the complete atrophy that is doubtless present of the mucous membrane. The patients were complaining of gastric distress, belching and anorexia, and they stated that they felt better

when a solution of hydrochloric acid and pepsin was administered to them.

Method for the Simultaneous Fractional Analyses of Gastric and Duodenal Contents (Kahn).—It is possible to study simultaneously the duodenal and gastric secretions by the following method: An Einhorn tube is passed into the duodenum of the patient, using the technic of Einhorn. Next morning a Rehfuss tube is inserted into the stomach of the patient. The patient is then given an Ewald test-meal, and the gastric and duodenal contents removed simultaneously at varying intervals of time. The extractions are usually made every fifteen minutes for a period of two and a half or three hours. The gastric contents are analyzed for the acid secretions and the enzymes. The duodenal contents are analyzed for the various enzymes. The results are charted in the form of a curve.

A gastro-duodenal tube has been devised which obviates the necessity of passing two tubes. This tube is composed of two compartments—one ending ten inches above the duodenal opening. The tube bifurcates at its free end, and the openings are distinctly labeled G and D to indicate the opening leading to the stomach and to the duodenum.

Gastro-albumorrhea Test.—1. Salomon Test.—On the assumption that malignant disease of the stomach is accompanied by degeneration processes which liberate débris and protein matter into the stomach cavity, Salomon recommended a test, which he thought was diagnostic of cancer of the stomach. This theoretical assumption is in accordance with our knowledge of cancer in general, and the results reported by various observers would seem to bear out the theory. It must be remarked, however, that such diseases as gastric ulcer would also cause a gastro-albumorrhea, as has been demonstrated by several opponents of Salomon's Test.

Salomon's method for testing the stomach contents for the albumin fraction is as follows: The stomach is first carefully washed on the evening before testing, after a preliminary non-albuminous fluid diet has been administered for twenty-four hours. On the next morning the stomach is thoroughly washed with normal saline solution (400 c.c.), the same fluid being repeatedly used and then tested for the quantity of nitrogen by the Kjeldahl method, and for the quantity of albumin by the Esbach method.

Salomon found the nitrogen content in non-carcinomatous cases to be between 0 and 16 milligrams per 100 c.c. of fluid. His study of six cases of cancer of the stomach revealed between 10 and 70 milligrams of nitrogen per c.c., and the albumin content was between 0.06 and 0.5 parts per thousand. According to Salomon, a case is extremely suspicious of carcinoma if the nitrogen content is more than 20 milligrams per 100 c.c. of the fluid, or if the Esbach test gives a distinct precipitate.

Siegel concurred with Salomon's opinion, concluding from his own results, that a figure over 25 milligrams of nitrogen per 100 c.c. is suspicious of gastric cancer, Orlowski, Schittenhelm and Lowes, Zirkelbach,

Witte, and Schupfer, are convinced that the Salomon test is of value. Gerster regards this test as useful in cancer of the lesser curvature without stenosis, unless the cancer has formed on an old ulcer, in which case the little hydrochloric acid present would digest the albumin present. Zirkelbach, however, is of the opinion that the minimum nitrogen content suggestive of cancer is 30 milligrams nitrogen per 100 c.c. of the washing fluid. Berent and Guttmann, Romano, Minkowski, and Yague have reported very unfavorable results with this test.

2. Wolff-Junghans Method.—This method is well described by Smithies, and we shall quote from him: In the normal aspirated test-meal there are demonstrable relatively large quantities of soluble albumin, by means of precipitating reagents. This soluble albumin appears only through the agency of the gastric enzymes. This fact is proved by testing for soluble albumin, a similar test-meal which had been chymified, but not swallowed. In such event, only minute quantities of dissolved albumin are present.

Acting on these observed facts, Wolff and Junghans fed similar meals to sets of individuals, revealing malignant and benign achylias. Their work appeared to show that in the malignant achylias aspirated test-meals were rich in soluble albumin, while in benign achylias very little of the albumin could be demonstrated.

Three suppositions have been advanced to explain this increased volume of dissolved albumin in the malignant achylias. It has been suggested that the excess of albumin is due (*a*) to interference with albuminous absorption; (*b*) to a "cancer milk" rich in albumin, which exudes from malignant growths, and (*c*) to a specific, peptid-splitting ferment from the neoplasm, capable of carrying protein digestion as far as the completely soluble stage.

Clinically, the reaction was shown to be positive in 18 of a series of 20 gastric cancers, and negative in 14 of a series of 15 cases of simple achylia in Ewald's service. Recently Ralph reported positive tests in all of 7 cases where cancer was present in the stomach, or secondarily involved that viscus. In 8 cases of benign achylia the test proved negative. Ralph states that gastric contents contaminated with blood beyond a dilution of 1 to 3000 may give the reaction and cautions against positive interpretation in instances where there is high combined acid present. In such event, peptone is usually present. He claims that cancer of the cardia is not so likely to give a positive reaction as is cancer in other parts of the stomach.

(*a*) *Technic of Smithies.*—Smithies used the following technic:

Preparation for Test.—The day previous to the examination of his gastric extract the patient was given one ounce of castor oil at 4 P. M. This was followed at 6 P. M. by a motor test-meal consisting of mixed food. At 7 P. M. twenty raw, seedless raisins were given. Twelve hours later (7 A. M. the following morning) the patient was fed 60 grams of second-day bread and 200 c.c. of water. This secretory test-meal was removed from 50 to 60 minutes after administering. The specimen secured was thoroughly mixed, filtered through double, hydrochloric

acid washed papers, and tested for dissolved albumin within an hour of its being obtained from the stomach. On account of the fact that only 52.2 per cent. of cases of gastric cancer yield gastric extract, revealing the absence of free hydrochloric acid, and that in 15.7 per cent. of cases free hydrochloric acid ranged between 20 and 50 per cent., he deemed it advisable to apply the test for soluble albumin not only to achylias but also to gastric extracts where the free hydrochloric acid was below 20 per cent. In a few instances of suspected malignant ulcer he has performed the test upon gastric extracts with higher free hydrochloric acid content. In such he has been fully alive to the possibilities of error, but for the purpose of gaining information and for comparison he has deemed it wise to make the test.

Mode of Procedure.—Six absolutely clean test-tubes are required for each test. Those of the narrow type and of 20 c.c. capacity answer very well. The tubes are numbered serially from 1 to 6. They receive respectively 1 c.c., 0.5 c.c., 0.25 c.c., 0.1 c.c., 0.05 c.c., and 0.025 c.c. of the filtered gastric extract. These amounts are readily measured by means of a 1 c.c. pipet, graduated into 1/100 c.c.; the volume in each test-tube is next consecutively brought up to 10 c.c. volume with distilled water. This gives from the tubes 1 to 6 dilutions of gastric juice varying respectively from 1 to 10 to 1 to 400 (viz., 1 to 10, 1 to 20, 1 to 40, 1 to 100, 1 to 200, and 1 to 400). These figures he has termed "units" of precipitable albumin. The tubes are then inverted several times to insure complete mixture of their contents. One c.c. of the reagent to precipitate the albumin in solution is then carefully layered upon the contents of each tube. The precipitating reagent suggested by Wolff has proved satisfactory.

It has the following formula:

Phosphotungstic acid	3 grams
Hydrochloric acid (concentrated).....	10 c.c.
Alcohol (96 per cent.).....	200 c.c.
Water	2000 c.c.

Mix and keep in a glass or rubber-stoppered flask in a cool place.

The results are interpreted in the following manner:

If the white ring of precipitated albumin appears in tubes 1, 2 and 3 (namely, units of albumin from 10 up to 50), and no further manifestations are present in the remaining three tubes, the test is called negative. If tubes 1, 2, 3 and 4 exhibit rings (units of albumin from 10 to 100) the reaction is considered suspicious. The presence of white rings in tubes 1, 2, 3, 4, 5 and above (units of albumin ranging from 10 to 200 to 400) is considered a positive test.

Results.—The gross results of Smithies' work were as follows: of 747 gastric extracts of the class described above, 318 (42.6 per cent.) gave 200 to 400 units of precipitable albumin; 112 (15.7 per cent.) exhibited 100 units, and 317 (42.4 per cent.) showed less than 100 units.

In this grouping 71.5 per cent. of the gastric extracts were from cases showing some degree of gastric retention.

Smithies summarizes his experience with this test in the following words:

1. When carefully performed and interpreted, the Wolff-Junghans test for demonstration of dissolved albumin in gastric extracts was positive or suspicious in 80 per cent. of the series of gastric cancer. In this series it was a more constant finding in gastric extracts than were the absence of free hydrochloric acid, the presence of lactic acid, and a positive glycyltryptophan test. It was rather more constant than the demonstration of occult blood and of gastric motor efficiency. It was not so consistent in its manifestation as the demonstration of organisms of the Oppler-group or the increase in the formal index.

2. In extragastric malignancy, gastric syphilis and nephritis, the Wolff-Junghans test seems inconstant.

3. In the differentiation between malignant and non-malignant achylias, the Wolff-Junghans test, when interpreted in connection with other clinical and laboratory data, is of considerable value. Positive reactions are rarely obtained in the achylias of primary anemia, simple achylia gastrica, and simple achlorhydrias when such are unassociated with gastric motor inefficiency.

4. Simple gastric and duodenal ulcers, especially when accompanied by pyloric stenosis or gastric atony, may give confusing responses to the Wolff-Junghans' test.

5. The presence of blood in gastric extracts may be a factor in the production of certain positive tests.

The gastro-albumorrhea test was applied in cases of pernicious anemia by Kahn and Barsky. The total nitrogen in 100 c.c. of gastric contents was 4.1 mg. and 3.7 mg. No albumin was present as determined by the phosphotungstic acid precipitation method. It is to be assumed that there is no discharge of protein from the gastric mucosa, although there no doubt is a chronic inflammatory process, in quantities sufficient to be tested.

(*b*) *Method of Rehfuss and Clarke.*—By means of the fractional method, Rehfuss and Clarke have studied the protein content of the gastric juice in every phase of digestion. The regular Wolff technic was used, the specimens being removed every 15 minutes for analysis.

One point is worthy of especial mention. The patient is warned to be particularly careful about swallowing oral and bronchial secretions. An estimation of the protein content of saliva in six normal individuals showed that it gave a reaction in five cases at 1:80 and in one case for some unknown reason at 1:160. These figures can be greatly surpassed in the sputum encountered in certain pulmonary cases, and it is an everyday observation that these individuals swallow much of their sputum.

Clarke and Rehfuss state that the protein on various occasions found in the gastric juice must come from a variety of sources. Briefly enumerated, it may come from food ingested, saliva or bronchial secre-

tions swallowed, bleeding owing to an ulcerative lesion, regurgitation of intestinal contents, and finally in cases in which there is a gastric lesion, from specific protein products derived from the lesion itself.

That the pure gastric juice derived from normal individuals contains but little protein, must be evident from the fact that on various occasions the juice obtained from normal individuals after the administration of a water-meal (a test-meal consisting of 200 c.c. of water which is aspirated at intervals) never showed a protein content exceeding 1:40. This is significant inasmuch as it indicates that the pure normal juice has a very low protein content, any increase of which must be of pathologic importance. But the customary Wolff test is performed by administering the ordinary Ewald test-meal, and testing at the one-hour interval. Concerning this method, we have some data from Wolff, Einstein and Rolph; the results, however, of testing merely one phase of the digestive curve is obviously wrong. It merely registers a single point in the whole mechanism of gastric digestion and gives no information regarding the elaboration of the protein, a point not without importance.

Clarke and Rehfuss, therefore, made studies (1) to determine if possible the actual content of the test-meal and its fluctuation during digestion, and (2) the modification of this curve in normal and pathologic cases. These latter are recorded merely in the hope that a possible interpretation may be obtained for some of the curious phenomena recorded.

They found that tea alone gave no reaction even at a dilution of 1:10, and that a regular Ewald meal consisting of 8 ounces of tea and two pieces of toast gave a reaction at 1:20, immediately after thorough maceration. After standing for fifteen hours in the incubator at 38° C. a reaction of 1:40 was obtained, showing that there was practically no autodigestion. A solution of pepsin without hydrochloric acid gave no protein action whatever.

In interpreting any protein curve, the question arises as to the quantity and source of the protein. Clarke and Rehfuss have pointed out that, normally, in the absence of any extrinsic factor, the quantity of dissolved protein runs more or less parallel to the duration of time in the stomach and the acidity. Any marked deviation must therefore be accounted for. On the basis of this finding rests the value of the test for diagnostic purposes. If the protein curve simply follows the acid curve, it merely represents the action of the gastric juice on the bread ingested, and may be taken as an index of gastric function. This is rarely given in the acid curve. But abnormal or pathologic possibilities exaggerate the curve, out of all proportion to the acid curve, either in the very beginning, during, or at the termination of the curve. Therefore, if bread alone can only give us a definite amount of protein within a definite time, an exaggerated quantity or the presence of high figures must mean that the protein comes from other sources than bread. In the present work, the separation and recognition of the different forms of protein has not seemed practical for routine work. The method of interpretation has been based on a comparison of the acid and protein

curves. It is therefore perfectly evident that a certain protein concentration in a given time can be produced from a standard meal. What are the extrinsic sources of protein? These may be (1) blood; (2) the presence of pus either intragastric in origin or swallowed; (3) the end-products of protein digestion still in the stomach through atony or obstruction; therefore stasis, lack of motor tone or actual obstruction may unduly increase the protein concentration, all of which are removed by emptying the stomach before administering the meal; (4) a possible exudation of lymph or serum from ulcer; (5) the exudation from a malignant lesion.

Clarke and Rehfuss draw the following conclusions from their work:

1. The gastric juice in health shows definitely a protein content of very low degree.

2. This content is increased in disease by the addition of an exudation of protein material from inflammatory, ulcerous, or carcinomatous mucous membranes, or by the addition of partially digested and retained food residues, or the swallowing of protein material such as certain forms of sputum.

3. Bread and tea alone, following the composition of the Ewald meal, will show in the absence of any pathologic factor, a definite amount of protein, corresponding to the curve or the digestive power of the juice secreted. A mixture of maceration of bread in tea will show a protein content on 1:20-1:40; if the mixture is acted on by an artificial gastric juice *in vitro* the protein content of the juice rises steadily within the next two hours and may reach 1:320 in seventy-five minutes. In other words, there is a transformation and liberation of soluble protein which may be demonstrated by the Wolff technic.

4. The pathologic significance arises when a curve shows any marked deviation from this recognized standard, that is to say, when there is an undue concentration of protein out of all proportion to that normally found at that particular phase in digestion. If, therefore, a marked increase in protein does not conform in a general way to the acid curve it can be definitely stated that the protein is coming from other sources than the proteins of the bread.

5. An analysis of the protein would seem to demonstrate that normally it is of the nature of a proteose, but in inflammatory or ulcerative conditions it is probably serum protein removed to a large extent by saturation with ammonium sulphate.

6. Attention is called to the interesting curves found in ulcer, many of which showed traces of blood, several of which can be explained on the basis of protein retention, some of which must be explained on the basis of hypothetic exudation.

7. In the differentiation of achylia carcinoma, we pointed out that the test was of value in direct proportion as the case approached a true achylia and the added factors (extrinsic) such as swallowed pus, bleeding and protein residues could be ruled out. They likewise pointed out that the one-hour point was insufficient for examination and that the characteristic for carcinoma in these cases is a divergence of the protein

curve out of all proportion to the acid curve. Infected catarrh, hemorrhagic erosions, achlorhydria, hemorrhagica gastrica, may give high findings, but they do not have the tendency to give a steadily increasing protein content.

8. They believe that a study of the protein curve may yield information of the greatest value, provided that all the precautions have been observed.

(*c*) *Investigations of Hess.*—Hess, who studied the gastric secretion of very young infants (several hours old), came to the following conclusions from his work:

New-born infants regularly secrete a considerable amount of hydrochloric acid before they are given any food. Among fifty-two infants varying in age from one-half hour to eighteen hours, only one did not have hydrochloric acid in the stomach; in all but one instance free acid was obtained.

The hydrochloric acid varies greatly in amount. Exceptionally it was found almost lacking on repeated tests (congenital hypochlorhydria of hyposecretion), or very profuse (congenital hyperchlorhydria or hypersecretion).

In almost all cases acid was obtained throughout prolonged tests, in spite of the fact that food was not given to stimulate secretion. In one instance 17 c.c. of highly acid juice was aspirated in one hour and fifty minutes. Rennin, pepsin and lipase were also obtained in the (unfed) new-born.

Prevailing physiologic views cannot account for the gastric secretion immediately after birth. It is not the result of mechanical stimulation by means of the catheter, as the juice was obtained immediately on the introduction of the tube, without an intervening latent period. It may be prenatal in origin. Nor is it clear what stimulates the continued secretion which was obtained for hours. Experiments showed that the saliva is not the exciting agent; the effect of sucking could not be determined. Comparative tests of the same infants at birth and later, during the first week of life, showed that the stimulus to gastric secretion may be greater in the new-born infant which has not been fed.

This chlorhydria of the new-born is not usually associated with increased tonicity of the pyloric sphincter, as the duodenal catheter can readily be passed through the pylorus. Even when 0.4 per cent. hydrochloric acid is instilled into the gastric cavity the catheter can be readily passed into the duodenum. However, the high acidity may at times be related to the pylorospasm or to duodenal ulcer met with in infancy.

Although gastric secretion is so marked in the new-born, duodenal and pancreatic secretion is very scanty. Nor can this secretion be readily stimulated by allowing hydrochloric acid to enter the duodenum. Evidently the mechanism of pancreatic secretion is not as easily activated in the new-born as in later infancy.

Hess used a soft rubber catheter for the removal of the gastric contents.

Gastric Emptying Power.—Smithies, in his excellent book on "Can-

cer of the Stomach," describes in detail the physiologic method for estimating the gastric emptying power. We shall quote from him:

1. Physiologic Method of Smithies.—Its object is to prove whether food can pass into or out of the stomach. The contra-indications are few: recent severe hemorrhage; clinical evidence of perforation; coma; extreme asthenia; severe cardiorenal disturbances or mental upsets.

The patient's stomach should be washed free from whatever material it contains. Following the lavage (provided the clinical history of marked stenoses at the orifices has not been obtained), 2 ounces of castor oil are administered through the stomach-tube or per oram. Three hours later the patient is allowed to eat a moderate-sized meal of mixed food, in the manner that he ordinarily follows. This meal contains among other ingredients, at least 50 grams of cold meat, 2 leaves of head lettuce and 20 raw raisins. Instructions are sometimes needed to insure the patient's swallowing the skins of the raisins. It is important that they should be eaten. Beverages are allowed, preferably water, milk or weak tea. This meal has the advantages of being readily available, palatable, and of sufficient bulk. The last consideration is of essential value in any motor meal. It is impossible to establish evidence of the anatomic condition of the stomach orifices by motor meals of the baby pap type. An interval of 8-12 hours is permitted to elapse before a stomach-tube is passed and attempts at the recovery of remains of the motor meal are made. Experience has taught us that food remnants present constantly in a stomach after 8 hours, generally indicate some mechanical hindrance to their free exit from the viscus. It is not uncommon in healthy individuals to demonstrate the presence of food in the stomach after 4 to 6 hours, i.e., the common time limit for estimation of gastric emptying power. In many instances of pyloric spasm, associated with peptic ulcer, disease of the gall-bladder, the appendix, etc., intermittent (and sometimes marked) 6 to 8 hours' residues may be removed from the stomach. In pronounced atony, rather more than 4 per cent. of cases exhibit food retained longer than 6 hours.

Some degree of 12-hour retention was demonstrated in 483 cases (69 per cent.) in Smithies' series of 701 instances where test-meal data are available. Frequently the amount varied considerably upon repeated examinations, but there were but 2 per cent. of cases, in which the neoplasm was located at the antrum or distal to it, where some trace of food retained for from 8 to 12 hours could not be persistently demonstrated (Smithies).

Smithies gives a table, which we shall reproduce here, showing the clinical inter-relationship existing between important test-meal findings and the presence of abdominal tumor. (*See* Table 4.)

2. Salol Test of Gastric Motility (Ewald and Sievers).—Phenylsalicylate (salol) decomposes into phenol and salicylic acid when in alkaline solution. It is understood, therefore, that in the gastric passage it neither decomposes nor is it absorbed. In the intestines, however, salol is split up, and absorbed, and the urine shows the presence of salicyluric acid.

TABLE 4. RELATION BETWEEN TEST-MEAL FINDINGS AND GASTRIC TUMOR.

	Total	Sarcinæ	Yeasts	Oppler-Boas	Lactic Acid	No Free HCl	Food Remnants	Tumor	Occult Blood
Sarcinæ	71	0	36	65	20	31	42	56	48
Yeasts	150	36	0	116	38	76	116	117	104
Oppler-Boas	221	65	116	0	50	132	178	186	172
Lactic Acid	85	20	38	50	0	44	57	64	76
No Free HCl	180	31	76	132	44	0	130	128	120
Food Remnants	194	42	116	178	57	130	0	146	142
Tumor	208	46	117	186	64	128	146	0	147
Occult Blood	202	48	104	172	76	120	142	147	0

The method of performing this test is as follows: The patient is given one gram of salol immediately after dinner or breakfast. The urine is collected, and tested every fifteen minutes for salicyluric acid by the addition of ferric chlorid, which imparts a violet color to the urine in the presence of the aryl derivative. Normally, the salicyluric acid appears in the urine in from 30 to 75 minutes. A delay of more than 75 minutes means motor insufficiency. If for 24 hours the delay persists, it is presumptive evidence of pyloric stenosis. In cases of gastroptosis or gastrectasis, the salol may still be present in the urine after 30 hours, although normally, the salicylates are totally excreted within 24 hours.

Sometimes the salol may decompose in the stomach due to the presence of alkaline mucus, or of regurgitated pancreatic juice (Brunner). On the other hand it sometimes happens that owing to acid fermentation in the intestines, the salol fails to decompose after it has passed the stomach.

Huber has recommended that the time that it takes for the salicyluric acid to disappear from the urine be measured. When the motility of the stomach is normal, the salicyluric reaction disappears by the twenty-fourth to the twenty-seventh hour. Accordingly, the patient, emptying the bladder at the end of twenty-seven hours, is asked to pass urine every three hours thereafter until the salicyluric acid reaction, if still present, disappears. The delay of the end of the reaction is said to be directly proportionate to the slowing of the motility of the stomach.

3. LEUBE'S TEST OF MOTOR POWER OF STOMACH.—The patient is given a Riegel test-meal. This is removed six hours later, and the stomach is washed with a liter of water. If only slight traces of food are present, the motor power of the stomach is normal. This method is, perhaps, the most convenient for practical purposes.

4. IODOFORM TEST FOR GASTRIC MOTILITY.—Fleischer administers to the patient 0.1 gram iodoform, with, or just after, the main meal. The urine is then examined for iodin. With normal gastric motility, iodin appears in the urine, in from 60 to 90 minutes.

5. WINTERNITZ TEST.—The author of this test recommends the use of iodipin instead of salol. This substance is not affected by the gastric

contents, but is acted upon in the intestine by the pancreatic secretion, with the liberation of iodin, which may be tested for in the saliva by the addition of a little starch paste.

6. Sahli's Desmoid Reaction.—This is a method for testing gastric function without using the stomach-tube. The underlying principle of the test is the fact that raw catgut may be digested in gastric juice but is entirely indigestible in pancreatic juice. The test is made as follows: A methylene-blue pill is introduced into a small rubber bag and the mouth of the bag subsequently tied with catgut. The small bag is then ingested immediately after the midday meal and the urine examined, 5, 7, 9, and 18-20 hours later for methylene blue. If methylene blue is present in appreciable quantity, it will impart to the urine a greenish-blue color. If not present in sufficient amount to impart this color, the urine should be boiled with one-fifth its volume of glacial acetic acid, whereupon a greenish-blue color results if the chromogen of methylene blue is present. This contingency seldom arises, however, inasmuch as in most cases of uncolored urine it will be found that the rubber bag has passed through the stomach unopened. If the methylene blue is found in the urine inside of 18-20 hours a satisfactory gastric function is indicated.

7. Günzburg's Method.—Webster describes this method as follows: "A tablet of 0.2 gram of potassium iodid is placed in a piece of the thinnest possible, strongly vulcanized rubber tubing, measuring about 2.5 cm. in length. The ends of the tubing are folded and the package tied with three threads of fibrin which have been hardened in alcohol. The package is now tested by placing it in warm water for several hours and examining the water for potassium iodid. The patient swallows one of these packages three-quarters of an hour after an Ewald meal, the saliva being tested for potassium iodid at intervals of 15 minutes. In the presence of free hydrochloric acid in normal amounts, the threads of fibrin are dissolved and the potassium iodid is absorbed, giving a reaction in the saliva in from one to one and three-quarter hours. In cases of hypochlorhydria the reaction is delayed, a delay of 6 hours indicating a practical absence of free hydrochloric acid. This test very frequently gives reliable results, but the threads of fibrin soon become brittle and break on swallowing the package, so that a reaction for potassium iodid under these conditions would have no value."

Motility of Intestine.—1. Carmine Test.—In order to determine the motility of the intestine, certain substances which color the feces are given at a definite time, and the evacuations are watched to ascertain the interval necessary for these substances to appear in the feces. Carmine colors the feces red. The patient is given the Schmidt diet (see page 37), together with a capsule containing 0.5 gram carmine. As Basch remarks, in the carmine test, we have a simple, harmless, reliable and convenient means for the demarcation of stools and the estimation of gastro-intestinal motility and patency for the detection of fistulous communications of the alimentary canal with exterior or with other hollow organs, for the location of the distal end of a duodenal

tube, and to aid in the differentiation between esophageal diverticulum and dilatation (Aaron).

Hymanson studied the carmine test in infants and children. He states that a complete passage of carmine, when given by mouth, of less than fourteen hours is abnormal. In children with intestinal catarrh, the passage time varies from 9 to 12 hours, and in hyperemic and ulcerated forms of catarrh from 3 to 8 hours. The main point deduced is that the more severe the condition, the higher up the lesion. These quick passages have a certain diagnostic value. If carmine given by mouth is in the stools within a definite interval, there might be good reason to suspect the existence of pyloric obstruction. Carmine is given to children in doses of ½ to 2 grains.

2. Charcoal Test.—Instead of carmine, charcoal may be administered with similar results.

3. Einhorn's Bead Test.—Einhorn describes a method of estimating the motor functions of the digestive apparatus. It consists in having the patient swallow a number of small porcelain or glass beads. The time that elapses between the taking of the beads and their reappearance, indicates the length of their sojourn in the digestive apparatus. Solid foodstuffs are attached to glass or porcelain beads by being drawn through the opening in the bead and tied on with a silk thread; obviously on passing through the stomach and bowel, the substance attached to the bead, if entirely digestible, will have disappeared, whereas indigestible substances will be found in the feces attached to the bead. It is possible in this way to ascertain the digestibility of many food substances in normal as well as in pathological conditions.

Six test-substances are usually attached to the beads: (1) catgut; (2) fish bone; (3) meat; (4) potato; (5) mutton fat; (6) thymus gland. Physiologically, the first two substances (catgut and fish bone) are usually digested in the stomach, and the remaining four (meat, potato, mutton fat, thymus) in the intestine.

All the beads usually appear in the stool under normal conditions in one or two days, either all empty, or with a trace of fat, thymus or fish bone left. Deviations from this rule point to pathologic conditions.

With regard to the functions of the digestive apparatus, the following conclusions may be drawn: In case all the beads reappear in a much shorter time than 24 hours, there is an accelerated motility; if they reappear after forty-eight hours, a retarded motility exists. The digestive function is good if all the beads are empty, or if there are but traces of fat, thymus or fish bone left. A reappearance of catgut or meat, potato, much fat or much thymus, always indicates a poor digestive function for the food substance in question. If all these test substances reappear in the stool, an absolutely poor digestive function exists.

Preparation of Food Beads.—(1) Catgut: Take raw catgut No. 00, draw it through the bead and tie the ends together.

(2) Fish Bone: As the ordinary fish bone breaks when tied in a knot, it is best to use the long bones from a pickled herring. The bones are washed in water first, then rubbed off with a cloth and kept in water in a bottle. When wanted they are taken out of the water, drawn through the bead, and tied in the same manner as the catgut.

(3) Meat: The muscle-fibers of raw beef are cut lengthwise in the direction of the fibers and in pieces 5 to 6 cm. long, and 1 cm. thick. These are preserved in a bottle of alcohol. Take a piece of meat from the alcohol bottle, tear off lengthwise a muscle fiber, 2 to 3 cm. long and 1 mm. thick; draw the same through the bead and allow the ends to overlap; next tie the ends fast together over the bead with a silk thread.

(4) Thymus: Raw sweetbread from the calf is cut in cubes and preserved in alcohol. For use, lay a small piece, about 2 cubic millimeters, within a small square of gauze, fold the four ends of the gauze together and tie with thread, so that the small piece of thymus is enclosed as in a purse; then fasten the gauze purse to a bead.

(5) Mutton Fat: Beads with large opening, 1.5 to 2 mm. in diameter, are placed in hot rendered mutton fat and after a minute are taken out with a forceps and placed in a vessel of cold water. This congeals the fat. Then they are laid on a clean piece of filter paper, and allowed to remain until thoroughly dried; the beads can thus be kept as long as desired, and are ready when wanted for use.

(6) Potato: Cook a piece of potato with the peel on, in boiling water for 2 minutes. Take out of the water and cool. Cut a small piece of potato with peel, 1 cm. long, 0.5 cm. wide, and 1.5 mm. thick, and attach it to a bead. Two or more substances may be attached to one bead, for instance, catgut and fish bone, meat and thymus. The test beads can all be kept on hand with the exception of the potato, which must always be freshly prepared. Meat and thymus beads are best kept in alcohol. Catgut, fish bone, and fat beads are simply preserved dry. The bead when prepared should be tied with a string, and the string placed in a gelatin capsule and so administered, best shortly after a meal. The bead test gives a very fair idea of how long the food remains in the intestinal tract, which the test-diet does not. The bead test is designed to show the digestibility of protein, fat, and carbohydrates and the motility of the gastro-intestinal tract.

4. Schmidt Test-Diet.—Intestinal sufficiency may be tested by the following method of Schmidt, as described by Aaron:

The Test-Diet and Its Administration.—The demands to be made upon a suitable diet are many. It should be made up in such a way as to be equally acceptable to healthy, and to intestinally diseased individuals. It should be almost, but not entirely, free from indigestible matter, so that the irritation normally supplied by the intestinal contents may not be entirely absent. Furthermore, it should satisfy the minimum caloric requirements in physical rest; it should contain a suitable proportion of protein, fat and carbohydrates; it should be easily

procurable and easy to prepare. In Schmidt's original test-diet, importance was attached to the exact measurement of all the articles of nutrition contained.

It was as follows:

Morning: Milk 0.5 liter, or if milk is not well tolerated, cocoa 0.5 liter, prepared with 20 grams of powdered cocoa, 10 grams of sugar, 400 grams of water, and 100 grams of milk, together with 50 grams of biscuits.

Forenoon: One-half liter of oatmeal gruel (made from oatmeal 40 grams, butter 10 grams, milk 200 grams, water 300 grams, 1 egg, and a little salt; strained).

Midday: Chopped beef (125 grams gross weight) slightly roasted with 20 grams of butter, care being taken that the inside remains rare. Mashed potatoes 250 grams (potatoes 190 grams, milk 100 grams, butter 10 grams and a little salt).

Afternoon: Like the morning diet.

Evening: Like the forenoon diet.

This diet contains: milk 1.5 liters, 2 eggs, 100 grams of biscuits, oatmeal 80 grams, butter 50 grams, beef 125 grams, potatoes 190 grams, having the following composition:

	Protein	Fat	Carbohydrates
Milk, 1.5 liters	45.0	53.2	67.6
Two eggs	11.3	10.9	0.5
Biscuits, 100 grams	8.55	0.98	75.1
Oatmeal, 80 grams	1.76	1.2	8.2
Butter, 50 grams	0.37	42.2	
Beef, 125 grams	26.0	1.96	
Potatoes, 190 grams	3.95	0.28	39.9
	97.03	110.72	191.3

Calculating the protein at 4 calories, the fat at 9, the carbohydrates at 4, this test-diet would yield 2131.8 gross calories. According to Lohrisch, the direct combustion of this test-diet in the calorimeter yields 2146.3 calories, which corresponds closely enough. Also, according to Lohrisch, the cellulose content of one day's test-diet amounts to 0.8916 grams. However, in the course of years, it has been found that, for practical purposes, the precise quantitative determination of various nutritive ingredients is not at all necessary. It should simply be borne in mind, that the test should be composed of milk in not too restricted quantities (½ to 1½ liters); white bread or crackers, about 100 grams; potato purée, 100 to 250 grams; chopped beef, 120 grams. But many additions or omissions may be resorted to, to suit the taste and requirements of the patient. The accurately measured diet is now used only for exact clinical examination and quantitative analyses. For practical purposes Schmidt now lays down the following changed and amplified form of his test-diet:

Morning: Milk one-half liter, or tea or cocoa with much milk if acceptable; 1 roll and a soft boiled egg.

Breakfast: Oatmeal gruel, strained, one plate, with a little salt or sugar, if desired; farinaceous soup or porridge may be substituted.

Midday: Lean beef, well chopped and slightly roasted (inside rare), with potato purée, finely strained, the quantity not to be too small.

Afternoon: Like the morning diet but without the egg.

Evening: Milk ½ liter, or a plate of soup (as for breakfast), 1 roll with butter, and 1 or 2 soft boiled eggs or scrambled eggs. A little wine is also permitted, also the addition of weak coffee, or tea, bouillon and chopped cold roast veal.

This is an absolutely bland and non-irritating diet, which as far as possible meets the requirements and the personal taste of the patient, with no difficulty whatever in the way of procuring or preparing the same, as it provides only for the simplest and always obtainable articles of nutrition.

In spite of many objections this test-diet has met with general approval. The objections were principally to the effect that the diet list was not the only possible one—which, of course, is correct. It is quite possible to compose a different test-diet which would meet the demands laid down. But the value of the Schmidt formula lies in the fact that upon it as a basis, and through the labors of Schmidt, Strasburger, and their co-workers, our entire system of modern coprology and functional intestinal diagnosis has been constructed. All the numerous analyses and stool examinations which have furnished material for establishing systematic intestinal diagnosis have been made with this test-diet. If at the present time we are able to speak of "normal feces," we are indebted for this achievement to the application of just this test-diet. As soon, moreover, as we make quantitative or qualitative changes in the material points of this test-diet, we rob the fecal examination of its firm and assured foundation and destroy the object of comparison—the normal feces—of which we must always avail ourselves in judging pathologic conditions.

As a rule, this test-diet is well tolerated. Milk, possibly, might occasionally give rise to diarrhea. In such cases the milk is boiled together with cocoa or replaced by the latter article entirely.

For purposes of examination, the test-diet is taken for two or three succeeding days or, at *all events, for a sufficient time to make sure of the stool being derived from it.*

Examination of the Test-diet Stool.—The test-diet stool is collected in a chamber, transferred to a glass or tin vessel specially reserved for this purpose, and sent to the physician. If the feces are hard or thick a wooden spatula may be used to transfer them from the chamber; fluid feces may be poured into the receptacle for transportation.

Having thus obtained the test-stool, the next step is its examination. This should be made microscopically and chemically.

(*a*) *Macroscopical Examination.*—The feces should always be examined as soon as possible after defecation. They should first be inspected

and examined as to color, consistency, odor, gross admixtures of mucus, blood, pus and helminths.

The next step in the macroscopical examination is the trituration of the feces. This is done in the following manner: The entire quantity is thoroughly mixed with a wooden spatula, so that it becomes a homogeneous mass and it is certain that a sample taken from it represents the mixture. Of these stirred feces a small portion, the size of a walnut, is carefully triturated in a mortar, with gradual addition of water until the mass is of the consistency of soup. The trituration should be done so carefully that no coherent non-triturated particles will be visible to the eye. These feces, triturated to the finest possible consistency, are poured out and spread upon a black plate where it will be possible to observe with the greatest accuracy and distinctness whether any parts of the test-diet, and which, have been evacuated in a macroscopically visible form—i.e., have not been assimilated and digested. The macroscopical examination, therefore, includes a search for connective tissue of the meat particles of muscle, potato remnants, fat and cellulose residue. Furthermore, in this examination it will be possible to recognize constituents which do not originate from the test-diet, but from the intestine itself, as, for instance, the important matter of mucus, small pus flakes, and large crystals of ammonia-magnesium phosphate.

(*b*) *Microscopical Examination.*—The microscopical examination serves to supplement the macroscopical, and requires three different procedures:

1. Inspection of a small particle of the untriturated feces, spread in a thin layer under the cover-glass: examination as to the presence of muscle particles, fat in its various forms, potato cells, cellulose remnants, cocoa remnants, mucus, pus, and parasitic eggs.

2. A small particle of feces is thoroughly triturated on a slide with a few drops of a 30 per cent. acetic acid solution, by means of a needle; heated for a moment over a flame to the boiling point, and inspected under the cover-glass. By this process all the fat remnants are temporarily melted, the entire quantity of fat being shown in the warm preparation in the shape of liquid globules of fatty acid, spread over the entire surface. As the preparation cools, the drops coagulate into intransparent masses of fatty acid. From this preparation it is possible to approximately estimate the fat content of the feces.

3. A small particle of the feces is carefully triturated on the slide with a droplet of a strong compound solution of iodin (iodin 1, potassium iodid 2, distilled water 50), and inspected under a strong light under the cover-glass.

By this preparation any remnant of starch, either enveloped in cellulose or free, which stains blue with iodin will be recognized. At the same time it is possible to observe any blue-stain iodin fungi and yellow-stained yeast cells.

(*c*) *Chemical Examination.*—(i) Reaction Test.—The simplest method of testing the reaction consists in bringing a strip of red and blue litmus paper, soaked in water, into contact with the feces, and

noting the change of color on the outer side of the paper. Schmidt recommends azolitmin paper, prepared from pure litmus coloring substance.

(ii) Schmidt's Sublimate Test.—The sublimate test serves to discover whether the feces contain the normal fecal pigment, hydrobilirubin or pathologically unchanged biliary pigment (bilirubin). The test is based upon the fact that hydrobilirubin stains intensely tile-red owing to the formation of mercurial chlorid of hydrobilirubin, while bilirubin with sublimate stains green owing to the oxidation of the bilirubin, changing it to biliverdin. For this purpose it is necessary to triturate in a mortar a walnut-sized piece of feces to a thin consistency, adding a generous portion of concentrated aqueous sublimate solution (corrosive sublimate 28, sodium chlorid 25, distilled water 500), which is thoroughly mixed with the feces. The tile-red stain of hydrobilirubin will then rapidly occur with fresh feces. Feces which have been standing for some time produce a reddish-brown to a dirty-brown color. The mixture should be allowed to stand for 24 hours, when any unchanged biliary pigment that may be present will be found to be stained green. In that case, either the entire quantity of feces is stained green or only a few macroscopic or microscopic particles may be visible.

(iii) Schmidt's Incubator Test.—The incubator test is instituted to show whether the feces incline to carbohydrate fermentation or to protein putrefaction, or whether they are negative. For this purpose Strasburger's fermentation tube is employed. This fermentation tube is connected with a bottom vessel, into which 5 grams of formed feces are put with a wooden spatula and well stirred with water. If the stool is hard a smaller quantity is taken, a larger one if it is liquid. The bottom vessel is closed with a one-bale rubber stopper, through which runs a glass tube connecting it with the fermentation tube. Another glass tube connects the fermentation tube with a vessel filled with tap water. The latter vessel has a two-hole rubber stopper, through one perforation of which the glass tube just referred to leads. Through the other a V-shaped glass tube enters, one end of which dips down into the tap water while the other enters the one-hole rubber stopper of a third vessel, stopping short as it enters the top. The apparatus is kept in the incubator for twenty-four hours at a temperature of 37° C. Should gas develop, it will enter from the bottom vessel into the fermentation tube, displacing the water in the middle vessel, and thence into the empty vessel. Carbohydrate fermentation is assumed to exist if after twenty-four hours the outer tube is about half filled with water, if the reaction of the feces has become distinctly acid, if the feces in the bottom vessel when it is opened have an odor of butyric acid and their color has turned light yellow. Albuminous putrefaction has taken place if the reaction of the feces has become strongly alkaline. There is a distinct odor of putrefaction, the feces have assumed a dark color and there is but slight development of gas. Baurmeister has modified the fermentation tube so that it is easily manipulated and more durable. The modification consists of three ordinary wide-mouthed bottles connected with glass tubing through perforated rubber stoppers. The ground feces are placed in the first bottle, the second bottle is filled

TABLE 5.—ANALYSIS OF FECES AFTER SCHMIDT-STRASSBURGER TEST-DIET. (*Pernicious Anemia.*)

Case	Day	Weight Dry Feces (Grams)	Total Nitrogen		Fat	
			Grams	Per cent. Intake	Grams	Per cent. Intake
1	1	157.5	2.343	14.2	4.51	4.3
	2	162.4	2.544	15.4	6.05	5.5
	3	175.5	1.897	11.5	5.72	5.2
	Av.	165.1	2.261	13.7	5.42	5.0
2	1	98.3	1.669	9.7	6.71	6.1
	2	75.5	1.926	11.2	4.29	3.9
	3	108.2	1.857	10.8	5.28	4.8
	Av.	94.0	1.817	10.5	5.42	4.9
3	1	117.0	1.144	7.2	4.62	4.2
	2	110.5	1.033	6.5	3.96	3.6
	3	86.5	1.319	8.3	4.84	4.4
	Av.	104.6	1.132	7.3	4.47	4.06

with water, and the third bottle remains empty. In the presence of fermentation or putrefaction the generated gas forces the water with some of the feces into the third bottle. When the test-diet feces are normal the second and third bottles remain the same as when first placed in the incubator. In the presence of fermentation, some of the yellow feces are forced into the two other bottles. The feces are usually darker in the presence of putrefaction.

(iv) Examination for Dissolved Protein.—For this purpose the following procedure is instituted: The feces (daily quantity) are well triturated, water being added slowly, and further diluted with water until a rather liquid consistency (about 500 c.c.) is obtained. This fluid is allowed to stand for a few hours and is then filtered through a double filter. The turbid filtrate is passed for clarification through a silicated filter, after which the filtrate is usually clear. If it is desired to test the clear filtrate for dissolved protein (albumin, albumose), it will be necessary to first remove the nucleoprotein which is present in every fecal extract. This is effected by the careful addition, in droplets, of a 30 per cent. acetic acid solution to the liquid contained in the test-tube. The precipitated nucleoproteins cause a turbidity of the previously clear filtrate which must now be passed through a double layer of filters. If the resulting filtrate is limpid and free from nucleoproteins, a few more drops of a 3 to 5 per cent. solution of acetic acid should be added in order to make doubly sure that all of the nucleoproteins have been precipitated, after which the usual albumin test —boiling with acetic acid—the ring test with nitric acid or the ferro-cyanid-of-potassium test should be instituted. Should the filtrate, turbid from the precipitated nucleoproteins, remain so after the second filtration, it should once more be filtered through a silicated filter, which will clarify it and make it ready for examination for albumin. Quanti-

tatively, the protein in the nucleoprotein-free fecal extract can be determined by Esbach's reagent, or Tsuchiya's 1 per cent. solution of alcoholic phosphotungstic acid (phosphotungstic acid 1, hydrochloric acid 5.96 per cent., alcohol 100).

The analyses of the feces in three cases of pernicious anemia are given in Table 5 (Kahn and Barsky).

In this series of cases of pernicious anemia the bulk of feces was very much increased, from 75.5 grams to 175.5 grams. It will be seen that the average daily excretion of dry feces in the three cases was 165.1, 114, and 104.6 grams, an increase of from 100 to 200 per cent. as compared with the normal figures of Schmidt. The nitrogen elimination was also much increased in the first and second cases, being an evidence of some deficiency of protein absorption. The average excretion of nitrogen in the feces in the three cases was 13.7, 10.5 and 7.3 per cent. respectively. The fecal fat does not seem to vary much from the normal, the average figures for the three cases being 5.0, 4.9 and 4.06 per cent.

Study of Intestinal Putrefaction (*Ethereal Sulphate Estimation*).—Perhaps as index of *intestinal putrefaction* attention may be paid to the urinary sulphur partition. It is known that in cases of intestinal stasis, etc., where the flora of the intestines are abnormal, there is an increased production of aryl compounds which are conjugated in the liver with sulphuric and glycuronic acids, and are excreted in the urine.

There are normally present in the urine a certain number of organic substances, demonstrated to be conjugation products of aromatic substances with sulphuric acid. These are called ethereal sulphates.

The presence of these aromatic sulphates has been known for a long time. Berzelius had demonstrated that the sulphates in the urine are in a form other than, and in addition to, the inorganic sulphates. In 1848, Städeler demonstrated the presence of phenol and phenolic substances in the urine. It was in 1876 that Baumann, by his brilliant researches, proved that there were present in the urine a number of phenol substances in conjugation with sulphuric acid, and he demonstrated the presence of phenolsulphuric acid and cresolsulphuric acid.

The prolific number of articles that have appeared on the ethereal sulphates of the urine, since the time of Baumann, shows the interest which this subject has aroused, and the attempts which have been made to make practical application of the knowledge of this subject in the field of medicine in general, and of auto-intoxication in particular.

According to Martin, "blue substances" were known to be present in the urine by Hippocrates and Galen. Sabatier (1837), Wastel (1840) and Béchillon (1840) reported respectively cases of a blue substance in the urine. Prout (1840) erroneously identified this substance as Prussian blue. Similar cases were reported by Heller, Martin, Scherer, Virchow, Hill Cassal, von Sicherer, Gubler and others.*

Schunck, in 1857, found that upon treatment of urine with mineral acids or upon air exposure, a blue substance is formed, which he con-

* We are indebted to Gustav Baar's "Die Indicanurie," Berlin and Wien, 1912, for the historical summary on Indican.

sidered identical with the indican that occurs in such plants as *Indigofera tinctoria, Isatris tinctoria,* etc. Two years later Carter proved that this substance is present in the urine of normal and diseased subjects, and that it also occurs in blood.

Bayer showed that indol was the mother-substance of indican. Brieger found that intestinal putrefaction produces the same substances as are produced by pancreatic digestion. Kühne, and later Nenaki, proved that indol is a product of pancreatic digestion of proteins. Hoppe-Seyler demonstrated that fibrin decomposes to indol without the action of bacteria. Radziewsky showed that indol was of normal and constant occurrence in the feces. Jaffe stated that the fecal indican, when absorbed, is the cause of the urinary indican.

That indican is produced by decomposition of protein was confirmed by Yasnopolsky. It was demonstrated by Baumann, Nencki, Brieger and Thieman that indican was indoxyl sulphate. Indican formation is due to the same factors that cause, in general, the excretion of ethereal sulphates in the urine.

Nuttal and Thierfelder stated that the only cause of indol formation was intestinal putrefaction.

It was suggested by Hoppe-Seyler that indol and other ethereal sulphates were derived in part from the tissue proteins, and he therefore considered two types of indicanuria—"metabolic" and intestinal. Salkowski was inclined, at that time, to concur with the view of Hoppe-Seyler. Folin, also, believes in the metabolic formation of ethereal sulphates.

Many investigators, however, are opposed to this view, and have advanced many proofs to show that there was no "metabolic" indicanuria.

Tuczek demonstrated that in insane individuals who refrained from food, no indican was present in the urine. In such cases, indican reappeared in the urine after the ingestion of some protein. Rabbas found the same to be the case in a patient who was starving. In fever, where we expect high endogenous catabolism, Jaffé found no increase in indican.

Salkowski stated that while he could find no indican in the urine of starving human beings, still he could demonstrate traces of indican in the urine of starving dogs. But Herter explained this indicanuria in starving dogs as due to the decomposition of the slight quantities of intestinal secretions in the animal's alimentary canal.

A number of authors have attempted to influence the endogenous metabolism and thus observe the formation of "metabolic" ethereal sulphates. The work of Lewin with phlorizin seemed to show such a possibility. Harnack injected oxalate solutions into animals and found an increased excretion of indican in the urine. Blumenthal and Rosenfeld obtained similar results. Kisch also concluded that oxaluria is an evidence of metabolic derangement.

At the instigation of M. Jaffé, H. Scholz repeated the work of Harnack and of Lewin and could not confirm their results. Mayer also contradicted Lewin's findings.

In the professional faster, Müller failed to find indican in the urine after the third day of the fast. Sherwin and Hawk found indican in the urine of their dog, who was fasted once for 117 days, and the second time 105 days. They summarize their findings thus:

"The course of intestinal putrefaction, as measured by the urinary indican excretion, was followed in two experiments upon the fasting dog. The initial fast was one of 117 days in length, and the indican output was continuous and fairly high throughout. The 'repeated' fast was 105 days in length, during which the indican values were much lower than during the initial fast. There was an absolute absence of indican in all urine passed during the last 48 days of the 'repeated' fast, i.e., after the 57th fasting day. The finding of diminished intestinal putrefaction as a result of 'repeated' fasting is in line with other observations from our laboratory which have shown that 'repeated' fasting is accompanied by greater resistance; a less rapid loss in body weight; less pronounced protein catabolism; a general physical and mental improvement."

Besides the gastro-intestinal tract, indican and ethereal sulphates may be increased in the urine due to putrefaction going on in abscesses, empyema, tuberculosis with cavity formation, fetid bronchitis, gangrene, retention of a dead fetus, etc. These types of indicanuria were called "septic" indicanuria by MacKee and "extra-intestinal" indicanuria by De Santos Saxe.

Hartmann found increased excretion of indican in pus formation anywhere in the body. Wells found that pyothorax increased the indican excretion. Similar results were obtained by Keilmann (in abscess of the knee and furunculosis), by Porter (in abscess formation), and by Herter (in intestinal putrefaction). The latter author attempted to demonstrate that the formation of indican was due to the activities of the *Bacillus coli* within the intestinal tract.

Combe claims that "leaving out organic suppurations, the 'sulpho-ethers' are solely derived from the microbic intestinal putrefaction produced at the expense of the proteids, the nucleo-albumins, the pancreatic and intestinal juices, the bile and the intestinal mucus." He also thinks that the quantity of the so-called "sulpho-ethers" is proportional to the degree and intensity of the putrefaction taking place in the intestine.

Stern, however, believes that the excretion of ethereal sulphates in the urine is not proportional to the intensity of the intestinal putrefaction, but is proportional to the power of absorption, which varies greatly according to the individual.

The influence of the kind and method of nutrition on the excretion of the ethereal sulphates was studied by many authors.

In 1884, Smirnow (Petrograd) studied the effect of consuming the daily food at frequent intervals upon the assimilation and metabolism of nitrogen, and upon the intestinal putrefactive bacteria. The term fractional nutrition has been applied to such a division of the daily ration. The subjects were eight healthy young men. The experiments were of twelve days' duration and each was divided into 2 equal periods.

The same quantities of bread, milk, butter and meat were consumed daily. Weak tea with a little sugar was taken as a beverage. As an index of bacterial action, the ratio of preformed sulphuric acid to ethereal sulphuric acid in the urine was determined. The preformed sulphuric acid was determined by the Salkowski method and the ethereal sulphates by Baumann's method. The ratio of one to the other was not affected by fractional nutrition.

Strauss and Philippsohn found that the administration of lactose caused a very marked reduction in the excretion of ethereal sulphates.

Poehl, Biernacki, Hirschler and Winternitz obtained the same result by modifying the diet and giving only carbohydrates. Cohendy and also Leva found a lessened excretion of ethereal sulphates after the administration of lactic acid bacilli. Mester showed that partaking of high or tainted foods greatly increased the proportion of the urinary ethereal sulphates. Hoppe-Seyler found that a vegetarian diet diminished the ethereal sulphates of the urine, and Strauss observed a decrease of more than one-half by adding 100 grm. lactose to the diet. Biernacki, Matteoda, and Winternitz noted a decrease in the urinary ethereal sulphate with a milk diet; Poehl showed that sour milk diminished the amount of urinary ethereal sulphates.

Rovighi and Embden found a decreased excretion in the ethereal sulphates by the use of kefir, and Rothmann, Gottwald and Krauss and Hirschler found a decreased excretion with a farinaceous diet.

Investigations reported by Hawk indicated that the drinking of copious (1000 c.c.) or moderate (500 c.c.) volumes of water with meals decreased intestinal putrefaction as measured by the urinary indican output, and that copious water drinking caused a more pronounced lessening of the putrefactive processes than did moderate water drinking. Softened water was employed in the experiments in question.

Combe states that the normal excretion of ethereal sulphates in an adult is 0.1 to 0.15 grams per diem. This amount may be increased by a meat diet, hypochlorhydria, continued use of sodium bicarbonate, jaundice, and constipation (Baar). The amount of ethereal sulphates may be reduced by vegetarian diet, lactose, milk-diet, sour-milk, farinaceous diet, diarrhea, and the internal use of hydrochloric acid.

Metschnikoff and Tissier found that the use of certain milk bacilli (bacillus of Massol) decreased the ethereal sulphates in the urine. Similar results were obtained by Dunn (with buttermilk), Gaillard, Fournier (who used lactic acid ferment). Baar, however, could not confirm these findings.

Labbé and Vitry concluded that it was possible to establish a definite relationship between the variety of injested albumin and the ethereal sulphates. They found that:

1 gram of bread protein yielded 0.00355 grams ethereal sulphate.
1 gram of egg protein yielded 0.00289 grams ethereal sulphate.
1 gram of meat protein yielded 0.00236 grams ethereal sulphate.
1 gram of milk protein yielded 0.00200 grams ethereal sulphate.

Upon feeding dogs with fat, Nasse found an increase in the ethereal sulphates, but as Magrangeas points out, Nasse does not say whether the fat was pure or contained some protein. Backmann found that the feeding of butter and cream did not influence the excretion of the ethereal sulphates.

Labbé and Vitry found no effect on the ethereal sulphates of the feeding of fats.

Hoppe-Seyler and Krause found that the feeding of carbohydrates reduced the output of ethereal sulphates in the urine. Eisenstadt obtained similar results.

Adrian found that fractional feeding did not influence the excretion of ethereal sulphates in the urine.

Schumann found that a dry diet increased the excretion of ethereal sulphates in the urine.

Wereschagin experimented on ten healthy young men to see the effect of the feeding of glucose on intestinal decomposition of protein. He found that there was a marked reduction of the ethereal sulphates in the urine, and an increase of the neutral sulphur.

Barteschevitch found that during constipation there was a marked increase in the urinary ethereal sulphates. He found

During diarrhea—ethereal sulphates..... 0.269 grams
During constipation—ethereal sulphates..... 0.497 grams

Combe obtained similar results. Hoppe-Seyler found that in simple constipation there was no increase in the urinary ethereal sulphates. Pfungen, on the other hand, found an increased excretion of ethereal sulphates during intestinal stagnation. Magrangeas concludes: "Constipation does not seem to have any direct influence whatever upon the elimination of sulpho-ethers."

Bunge and many others have found the ethereal sulphates of the urine increased four times with a meat diet. Stadelmann showed that the prolonged administration of sodium bicarbonate increased the urinary ethereal sulphates, and Wasbutzki noted that in all gastric conditions with hypo-acidity there was a considerable increase in the ethereal sulphates in the urine.

Biernacki, Stadelmann, Kast, Jaweil, Schmitz and others found that the administration of hydrochloric acid lessened the quantity of ethereal sulphates in the urine. I. Strauss contradicted these results. Von Noorden stated that hyperacidity and subacidity of the stomach had no effect on the output of ethereal sulphates.

In diseases of the gastro-intestinal tract, like typhoid fever and cholera, Bouchard reported increased amounts of ethereal sulphates in the urine.

Simon stated that simple constipation was seldom accompanied by indicanuria. Jones, Haughton and others obtained similar results.

Von Moraczewski found that feeding of thyroid tissue caused an increase in the elimination of indican. Fever has similar effects.

De Lacrousville remarked that as long as the kidneys functionated

normally and excreted indican and ethereal sulphates, there was no danger of auto-intoxication, which was due to excess of indican in the blood.

Haughton, Williams, MacKee, Darenberg, Perroy and others have found increased blood-pressure and albuminuria as accompanying factors of indicanuria.

Baar found the following positive indican reaction in men and women:

Men (975)		Women (1117)	
Indican Positive	Indican Negative	Indican Positive	Indican Negative
1930	1759	2224	2187

No tests have been devised as yet to determine the functional capacity of the various portions of the small intestine and colon.

BIBLIOGRAPHY

Determination of Peptic Activity

Hawk, P. B. Practical physiological chemistry, 1916, p. 167.

Töpfer's Method of Gastric Analysis

Seidl. Arch. f. Verdauungskr., 1916, xxi, No. 2.
Weinberg. *Ibid.*, 1916, xxi, No. 4.
Zoeppritz. Mitt. a. d. Grenz. d. Med. u. Chir., 1916, xxix, 25.

Volume of Gastric Secretion

Brugsch and Schittenhelm. Lehrbuch der klinischen Untersuchungsmethoden, 1911, p. 330.
Elsner. Lehrbuch der Magenkrankheiten, 1909, p. 172.
Hayem. Evolution pathologique de la digestion stomacale, 1907.
Mathieu. Arch. f. Verdauungskr., 1896, i, 348.
Prym. Arch. f. klin. Med., 1907, cx, 321.
Remond. Ergebn. d. inn. Med. u. Kinderh., 1910, v.

The Gastric Residuum

Best. Jour. Am. Med. Assn., 1916, lii, 803.
Ehrenbeich. Ztschr. f. klin. Med., 1912, 231.
Ewald and Boas. Arch. f. path. Anat., 1885, ci, 325.
Fowler, Rehfuss, and Hawk. Jour. Am. Med. Assn., 1915, lxv, 1021.
Fowler and Zentmire. *Ibid.*, 1917, lxviii, 167.
Gregerson. Arch. f. Verdauungskr., 1913, xix, 263.
Grobbel. Ztschr. f. physiol. Chem., 1914, lxxxix, 1.
Harmer, T. W., and Dodd, W. L. Sources of error in the use of the stomach tube for diagnosis. Arch. Int. Med., November, 1913, 488.
Hayem. Maladies de la tube digestive. Brouardel and Gilbert, 1905.
Kemp. Diseases of the stomach, intestines and pancreas, 1912, p. 133.
Kornemann. Arch. f. Verdauungskr., 1912, xviii, 369.
Loeper. Leçons de pathologie digestive. Ser. 2, 1912, 17, 19.
Meunier. Bull. d. sc. pharmacol, xxi, 389.

Oettinger. Arch. de mal. de l'appar. digest., 1910, 563.
Rehfuss. Am. Jour. Med. Sc., 1915, cl, 72.
——— *Ibid.*, 1914, cxlviii, 848.
——— New York Med. Jour., 1916, 1115.
Rehfuss, Bergein and Hawk. Jour. Am. Med. Assn., 1914, lxiii, 11.
Rehfuss, M. E., Bergeim, Olaf, and Hawk, P. B. Gastro-intestinal studies, 1. The question of the residuum found in the empty stomach. *Ibid.*, July 4, 1914, 11.
——— *Ibid.*, lxiii, 909.
Reichmann. Ztschr. f. klin. Med., 1885, xxiv, 565.
Sartory. Compt. rend Soc. de biol., 1906, lx, 619.
Schule. Ztschr. f. klin. Med., 1895, xxxvii, 461.
Sokolow. Dissertation, St. Petersburg, 1904 (Babkin: Die Aeussere Sekretion der Verdauungsdrusen, 1914, p. 150).
von Jaksch. Ztschr. f. klin. Med., 1890, xxix, 383.
Wolff. Taschenbuch der Magen- und Darmkrankheiten, p. 22.
Zweig. Magen- und Darmkrankheiten, p. 459.

Gastro-albumorrhea Test

Berent and Guttmann. Deutsch. med. Wchnschr., 1907, xxxiii, 154.
Clarke and Rehfuss. Biochem. Bull., 1915, iv, 211.
Goodman. Med. Bull., 1913, xxii, 70.
Kahn and Jacobowitz. Med. Record, Dec. 11, 1915, cxxxviii, 1006.
Orlowski. Ztschr. f. klin. Med., 1908, lxv.
Salomon. See Kahn, Arch. Diagn., July, 1914, 254; Oct., 1914, 356.
Schittenhelm and Loewes. Centralbl. f. Stoffwechsel- u. Verdauungskr., 1905, xviii, 409.
Schupfer. Deutsch. med. Wchnschr., 1907, xxxiii, 154.
Siegel. Berl. klin. Wchnschr., 1914, xxi, 229, 338.
Smithers. Jour. Am. Med. Assn., 1912, lviii, 1008.
Witte. Ztschr. f. klin. Med., 1908, lxv, 30.
Wolff and Junghans. Berl. klin. Wchnschr., 1912, May 27, 1912.
Yague. München. med. Wchnschr., 1907, liv, 850.
Zirkelbach. Arch. f. Verdauungskr., 1906, xii, 534.

Gastric Emptying Power

Brown. Am. Jour. Med. Sc., 1912, cxliv, 682.
Brunner. Deutsch. med. Wchnschr., 1889, xv, 128.
Ewald and Sievers. Therap. Monatsh., August, 1887, i, 287-291.
Hess. Am. Jour. Dis. Child., 1913, vi, 264.
Huber. Cor.-Bl. f. schweiz. Aerzte, 1890, xx, 65-74.
Smithies and Ochsner. Cancer of the stomach, 1916.
Walsham and Overend. Lancet, 1915, i, 650.

Motility of Intestine

Aaron. Diseases of the digestive organs, 1915.
Hymanson. Am. Jour. Dis. Child., February, 1916, x, 455-459.

Intestinal Putrefaction

Albertoni. Ann. di chem. e di farm., 1893, xviii, 396.
——— Jahr. f. Tierchem., 1893, 622.
Baar, G. Die Indicanurie. Berlin, 1912.
Baccharini. Riforma med., 1898, ii, 231; 1899, iii, 411.
Barteschevitsch. Ztschr. f. klin. Med., 1890, xvii, 56.

BAUMANN. Pflüg. Arch., 1876, xii, 69; 1877, xiii, 285.
——— Ztschr. f. physiol. Chem., 1879, iii, 156; 1892, xvi, 221.
BAUMAN AND HERTER. *Ibid.*, 1877, i, 247.
BENEDICT. Jour. Biol. Chem., 1909, vi, 363.
BIERNACKI. Arch. f. klin. Med., 1891, xlix, 87.
BLUMENTHAL. Engelmann's Arch. f. Anat. u. Physiol., 1901, 275; 1902, 374.
BLUMENTHAL AND ROSENFELD. Char. Ann., 1902, xxvii, 46.
BRIEGER. Ztschr. f. physiol. Chem., 1871, ii, 241.
——— Ztschr. f. klin. Med., 1881, iii, 461, 465.
BULIGINSKI. Hoppe-Seyler's Medizinische-Chemische Untersuchungen, 1886, p. 234.
BUNGE. Handbuch, p. 315.
CARLETTI. Arch. di farm. e terap., 1899, vii, 323.
COHENDY. Compt. rend. Soc. de biol., 1906, lviii, 602.
COMBE. Intestinal auto-intoxication. New York, 1910, p. 201.
DOMINICIS. Arch. der Med. Experiment, 1893, l, 496.
ECKERT. Dissertation. St. Petersburg, 1896.
ELLINGER. Ztschr. f. physiol. Chem., 1903, xxxix, 44.
EMBDEN. *Ibid.*, 1892, xvi, 30.
EMERSON. Clinical Diagnosis. 1913, p. 143.
FOLIN. Jour. Biol. Chem., 1905, i, 13.
——— Am. Jour. Physiol., 1905, xiii, 97.
GAVA. Ungar. Arch. f. Med., 1891, i, 288.
——— Jahr. der Tierchem., 1892, 309.
GILBERT AND WEIL. Compt. rend. Soc. d. biol., 1900, lii, 685.
GOTTWALD. Chem. Centralbl., 1889, i, 29.
GUELPE. Auto-intoxication et desintoxication. Paris, 1910.
——— Proc. Int. Cong. Med., 1914, 392.
HALDANE. Jour. Physiol., 1888, ix, 213.
HABERLIN. Deutsch. Arch. f. klin. Med., 1890, xlv, 339.
HARLEY. Cited by Mann. Physiology and pathology of urine, 1908, p. 16.
HENNIGE. Deutsch. Arch. f. klin. Med., 1879, xxiii, 271.
HERTER AND WAKEMAN. *See* von Noorden's Metabolism and practical medicine. 1907, Vol. III, p. 626.
HIRSCHLER. Ztschr. f. physiol. Chem., 1886, x, 35.
HOPPE-SEYLER. Pflüg. Arch., 1882, v, 470.
——— Ztschr. f. physiol. Chem., 1889, xii, No. 1, 31, 36.
——— Berl. klin. Wchnschr., 1892, 43.
——— Habilitationsschr. Kiel, 1887.
JAFFE. Deutsch. Arch. f. klin. Med., 1903, ii, 219.
KAST. Festchr. z. Eröffn. d. Krankenhauses. Hamburg, 1889.
KRAUSS. Ztschr. f. physiol. Chem., 1893, xviii, 167.
LEO. Die Krankheit der Verdauungsorgane, 1890, p. 316.
LEPINE. Rev. de méd., 1896, xvi, 594.
LEVA. Berl. klin. Wchnschr., 1908, xlv, 922.
LEWIN. Beitr. z. chem. Phys. u. Path., 1902, i, 472.
LEYDEN. Festschr., 1902, ii, 267.
MAGRANGEAS. Contribution a l'étude des sulfo-ethers urinaires. Thèse, Paris, 1908.
MATTEODA. Dissertation. Geneva, 1894.
——— Jahr. f. Tierchem., 1894, 537.
MAYER. Beitr. z. chem. Phys. u. Path., 1902, ii, 217.
MESTER. Ztschr. f. klin. Med., 1894, xxiv, 441.
MORACZEWSKI. Ztschr. f. klin. Med., 1903, li, 475.
——— Virchow's Arch. 1900, clix, 221; 1898, cli, 22.
MORAX. Ztschr. f. physiol. Chem., 1886, x, 318.
MUELLER. Ztschr. f. klin. Med., 1887, xii, 63.
——— Berl. klin. Wchnschr., 1887, 433.
——— Virchow's Arch., 1893, cxxxi, 128.

von Noorden. Ztschr. f. klin. Med., 1890, xvii, 529.
——— Nothnagel's Spec. Path. u. Therap., 1897, iii, No. 2.
——— Pathologie der Stoffwechsel, 1893, p. 405.
Nuttall and Thierfelder. Ztschr. f. physiol. Chem., 1896, xxii, 71.
Ortweiler. Mitt. aus dem med. klin. zu Würzburg, 1885, ii, 153.
Pfungen. Ztschr. f. klin. Med., 1892, xxi, 118.
Pœhl. Med. Wchnschr. Petersburg, 1897, 50.
——— Maly's Jahr. Tierchem., 1887, xvii, 277.
Reale. Nuova clin. terap. iii, No. 5.
Rethers. Dissertat. Berlin, 1891.
Rosenblum. Interstate Med. Jour., 1916, xxiii, 125.
Rothmann, Gottwald, and Krauss. Ztschr. f. physiol. Chem., 1889, xii, 16
——— Pflüg. Arch., 1882, xxix, 525.
Rovighi. Ztschr. f. physiol. Chem., 1892, xvi, 20, 30.
Salkowski. Ztschr. f. physiol. Chem., 1889, vii, 223; xii, 85.
Schmidt. Ztschr. f. klin. Med., 1898, xxxiv, 263; 1892, xvii, 401.
Schmitz. Ztschr. f. physiol. Chem., 1894, xix, 385, 401.
——— Berl. klin. Wchnschr., 1890, xxvii, 772.
Senator. Centralbl. f. d. med. Wissensch., 1877, xv, 337, 370, 388.
Stadelmann. Ueber den Einfluss d. Alkaline auf Stoffwechsel. Stuttgart, 1890.
——— Dissertation. Stuttgart, 1890.
Stadmueller and Rosenbloom. Arch. Int. Med., 1913, xii, 276.
Städler. Ann. Chem. u. Pharm., 1851, lxxvii, 17.
Steiff. Ztschr. f. klin. Med., 1889, xvi, 331.
von Stejskal and Erben. *Ibid.*, 1900, xl, 165; 1900, xxxix, 151.
Stern. Dissertation. Breslau, 1892.
Stokvis. Nederl. Tijdschr. v. Geneesk., 1889, ii, 409.
Strauss. Ztschr. f. klin. Med., 1894, xxiv, 441.
Strauss and Philippsohn. *Ibid.*, 1900, xl, 396.
Taylor. Zentralbl. f. Inn. Med., 1897, xviii, 873.
Töpfer and Freund. Wien. klin. Wchnschr., 1899, xii, 1299.
Vannini. Policlinico, 1900, vii, 6.
——— Virchow's Arch., 1904, clxxvi, 375.
Wasbutzki. Arch. f. Exper. Path. u. Pharm., 1889, xxvi, 133, 135.
Winternitz. Ztschr. f. physiol. Chem., 1892, xvi, No. 17, 474.
Wereschagin. Dissertation. Petrograd, 1895.
Wohlgemuth. Berl. klin. Wchnschr., 1906, xliii, 508.
Velden. Virchow's Arch., 1872, lxx, 343.
Ziemke. Jahr. f. Tierchem., 1893, 270.

CHAPTER II

PANCREATIC FUNCTION TESTS

Sladden classifies the tests of pancreatic sufficiency under two main headings, with a number of subdivisions, thus:

I. Tests of external secretion, dependent upon abnormalities in the ferments of the pancreatic juice.
   1. Oil test-breakfast.
   2. Duodenal intubation.
   3. Sahli's glutoid capsule test.
   4. Tests of nucleoprotein digestion (Schmidt, Kashiwado, Franzig).
   5. Azotorrhea.
   6. Creatorrhea.
   7. Tests for tryptic power of feces (Müller and Schlecht, Gross).
   8. Steatorrhea.
   9. Analysis of fat in feces.
   10. Tests for lipolytic power of feces.
   11. Von Ehrmann's palmin test-meal.
   12. Test for diastatic power of duodenal contents.
   13. Test for diastatic power of feces.
   14. Estimation of lecithin in feces.
   15. Effect of administration of pancreatic preparations.

II. Tests dependent upon other functions of the pancreas.
   1. The simpler characteristics of the urine.
   2. Ferments in the urine—diastase.
   3. Pentose derivatives in the urine—Cammidge.
   4. Glycosuria—actual or potential.
   5. Loewi's adrenalin mydriasis test.

While this classification is quite a suitable, if cumbrous one, we shall discuss the tests of pancreatic function under the following headings:

1. Analysis of duodenal contents.
2. Analysis of gastric contents after various test-meals.
3. Analysis of feces.
4. Analysis of urine.

Analysis of Duodenal Contents.—Einhorn has devised a tube whereby it is quite feasible to obtain the duodenal contents for analysis. Usually no difficulty is encountered in the insertion of the Einhorn duodenal tube (Fig. 8). The patient is given a cup of tea with sugar to drink on a fasting stomach. Forty-five minutes later the duodenal pump is inserted, and after the tube has reached the duodenum, the contents are pumped out and analyzed. The insertion of the tube is accomplished in the following manner: The capsule of the duodenal pump, as well as the lower part of the duodenal pump, is moistened with warm water and put into the pharynx of the patient. Then the latter drinks some water and the instrument thus soon passes into the stomach. To be certain that the capsule has not become stuck in the esophagus,

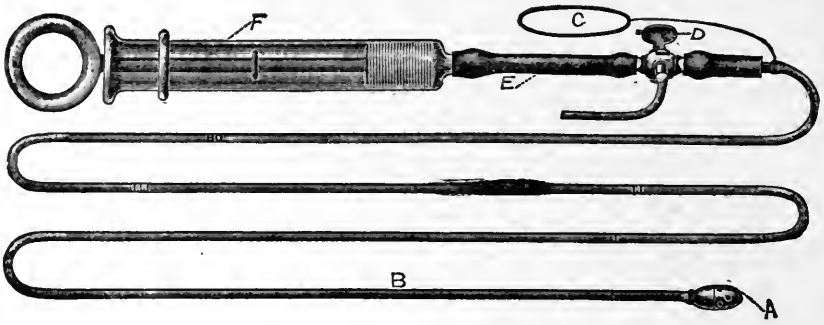


FIG. 8.—THE DUODENAL PUMP.

A, metal capsule, lower half provided with numerous holes, the upper half communicating with tube B. I, II, III, marks showing distance from capsule: I = 40, II = 56, III = 70 cm. C, rubber band with silk attached to end of tubing, which can be placed over the ear of patient. D, three-way stopcock. E, collapsible connecting tube. F, aspirating syringe. (Einhorn.)

it is well to have the patient shake his abdomen and to aspirate a syringeful of chyme. This can easily be identified as gastric contents. Now a syringeful of water and then one of air are passed through the instrument. The rubber tube is then clamped off and left alone for about one hour. The patient is told not to close his mouth too tightly, so that the tube is not retarded in its wanderings. The patient must also avoid intentional swallowing of the tube. Through the peristalsis of the stomach the capsule is pushed on farther, and usually passes through the pylorus into the duodenum and later into the beginning of the small intestine. It is best that the patient read some light literature in order to divert his attention. After one hour the distance that the capsule has progressed is examined. If the capsule is in the duodenum, a clear, golden-yellow, or watery liquid of alkaline reaction and somewhat viscid consistency is generally obtained. If, however, the capsule is in the stomach, an acid liquid resembling the one first obtained is removed.

It is sometimes necessary to administer a dose of atropin to relax the pylorus and permit the duodenal tube to pass through the sphincter.

The contents withdrawn are assumed to be duodenal contents if: (1)

a roentgenogram shows the tube *in situ* in the duodenum; or (2) if upon slowly withdrawing the tube while aspirating, a distinct difference is noted between the contents obtained at the point marked 80 cm. and the contents withdrawn after the metallic capsule is felt suddenly to enter the larger cavity of the stomach (56 cm.). When the capsule lies in the duodenum, one obtains in the course of five minutes 10 to 40 c.c. of golden-yellow fluid. This material enters the aspirating syringe drop by drop, or rhythmically, every 20 to 30 seconds, with a rapid gush of 1 to 2 c.c. of material. This latter phenomenon is probably due to a peristaltic acceleration of the secretions entering the duodenum at the moment and to the periodic expulsion of gastric juice (Crohn).

The contents of the duodenum are analyzed for amylase, lipase, and alkali protease (trypsin). The following methods are used:

Amylase.—In every one of several test-tubes is placed one c.c. of the fluid to be tested. Increasing amounts, from 0.5 to 6.0 c.c. of 1 per cent. starch solution (Kahlbaum's soluble starch) are added to the successive test-tubes, and then water to bring the volume up to 10 c.c. Incubation proceeds at 40° C. for one hour. The material is then tested by adding Lugol's solution drop by drop until the excess of iodin is apparent. The last tube in the series which fails to react for starch is the tube from which the reading is taken. The number of c.c. of starch solution in this test-tube multiplied by the dilution (three) gives the factor accepted as representing the amylolytic activity of 1 c.c. of duodenal contents.

Lipase.—To 10 c.c. of distilled water are added 1 c.c. of duodenal juice, 1 c.c. of ethyl butyrate, 1 c.c. of toluol, and a drop of 1 per cent. alcoholic solution of phenolphthalein, the whole is then made exactly neutral with N/10 NaOH and the total amount of fluid brought up to 25 c.c. After stoppering, the flask is shaken forcefully for about fifteen seconds and again brought to the exact neutral point. A control test is always prepared, the duodenal juice of the control being boiled actively for five minutes before addition to the flask. After incubation for 24 hours at 40° C. the two flasks are titrated for free acid in the test-flask. The result multiplied by three (the dilution of the duodenal juice) denotes the lipolytic strength of the test-material.

Protease (*Trypsin*).—To test for alkali-protease, Mett tubes, coagulated egg-albumen cubes, Fermi gelatin tubes, and casein are utilized. The Gross-Fuld casein method is carried out as follows:

This method is based upon the principle that faintly alkaline solutions of casein are precipitated upon the addition of dilute (1 per cent.) acetic acid, whereas its digestion products are not so precipitated. The method follows: Prepare a series of tubes each containing 10 c.c. of a 0.1 per cent. solution of pure, fat-free casein which has been heated to a temperature of 40° C. Add to the contents of the series of tubes increasing amounts of trypsin solution under examination, and place them at 40° C. for fifteen minutes. At the end of this time remove the tubes and acidify the contents of each with a few drops of dilute acetic acid. The tubes in which the casein is completely digested will

remain clear when acidified, while those tubes which contain undigested casein will become more or less turbid under these conditions. Select the first tube in the series which exhibits no turbidity upon acidification, thus indicating complete digestion of the casein, and calculate the tryptic activity of the duodenal contents.

The unit of tryptic activity is an expression of the power of 1 c.c. of the duodenal contents exerted for a period of fifteen minutes on 10 c.c. of a 0.1 per cent. casein solution.

From his study of the pancreatic function as measured by examination of the duodenal contents, Crohn came to the following conclusions:

The quantitative examination of the duodenal ferments is the most rational and accurate method of studying the external secretion of the pancreas. Diminution of such enzyme activity of the pancreas is a reliable sign of organic disease of the gland. Occasionally, though rarely, a diminution of ferments occurs as a symptom of advanced organic disease elsewhere in the body. Roughly the diminution of ferments is directly proportional to the extent of organic destruction which has taken place. The absorption of fat and nitrogen from the intestine is independent of the condition of the external secretion of the pancreas, or even of its presence. Absorption may be poor with an intact gland, or good with a gland of which only a fragment survives the disease. The functional activity of the gland, not its organic condition, determines the degree of absorption; this is probably controlled by an internal secretion, or *hormone*. Duodenal ferment tests give the index of the organic condition of the pancreas. Absorption tests give the index of the functional activity of the gland.

Frank succeeded in obtaining duodenal contents in 60 per cent. of the persons he examined. The cases were chosen at random and suffered no pancreatic disease. Examination of the duodenal contents in all these instances showed active alkali protease. The other ferments were not investigated. The inability of Frank to obtain the duodenal material desired in 40 per cent. of his assays is probably due to too short a time being allowed for the metallic capsule to enter the duodenum. This was obviated in Crohn's series of cases by passing the pump in the evening and allowing the entire night to elapse before aspirating the desired material. Even then more than one attempt is sometimes required before success is attained. The procedure is a mild one and only rarely objectionable to the patient.

Einhorn reports his study of the pancreatic secretion by means of the duodenal pump. In order to ascertain the condition of the duodenal contents in health, several apparently perfectly healthy persons were examined with regard to the state of their pancreatic secretion. They took the duodenal tube either at night before retiring or between 4 and 5 A. M. with a glassful of water. They then slept, rose at 8 A. M. and called at his office in the fasting condition at 9 A. M., when the duodenal contents were obtained. The amount of ferments in normal individuals fluctuated as follows: Amylopsin 4 to 8 mm., steapsin 2 to 5 mm., trypsin 0.5 to 5 mm. The alkalinity as determined by titration with

N/10 HCl (using methyl orange as an indicator) varied between 15 and 40. The rennet ferment was present in all. In looking over the results obtained by these examinations there exists a noticeable independence among the three different ferments with regard to the quantity in the same individual—one ferment may be present in large amounts, while the other two may be present in small amounts or may be altogether absent. The quantity of one ferment does not justify assuming that an equivalent quantity of another ferment is also present. It is thus necessary to test for each of the other two ferments separately.

Landau and Reasnicki also strongly advised that the duodenal contents be analyzed for the three enzymes. The results that they obtained were very favorable.

According to Chase and Myers, whose results in general corroborated those of Einhorn and his co-workers, the acidity of the gastric juice appears to be without influence upon the activity of the enzymes present in the duodenal juice. In a case of carcinoma of the gall-ducts and pylorus with biliary obstruction, there was an entire absence of bile from the duodenal juice. In a case of chronic pancreatitis, the amylolytic and proteolytic activity was entirely negative, while the lipolytic activity was comparatively weak. The absence of pancreatic enzymes from the duodenal juice would appear to be positive evidence of either pancreatitis or non-patency of the pancreatic ducts, while the lack of bile would appear to afford similar evidence of the occlusion of the common bile-duct.

White used the duodenal tube in 90 cases: 56 for diagnosis and 34 for treatment. The results that he obtained were very encouraging.

In our experience the examination of the duodenal contents for pancreatic enzymes has yielded very valuable information. The test-fluid can be easily obtained, and the analytical methods are extremely simple, so that clinicians should devote more attention to this source of information regarding pancreatic sufficiency and the condition of the duodenal contents.

It must be remembered, however, that the ptyalin of the saliva may yield results simulating pancreatic amylase, that the pepsin of the stomach and the erepsin of the small intestines may hide the absence of trypsin, and that gastric lipase may be present and disguise the absence of pancreatic steapsin. If proper precautions are taken, however, these difficulties may be overcome.

The accompanying table taken from Crohn's work will show the results he obtained upon analyses of the duodenal contents and stools in a series of cases (Table 1).

Of six cases of pancreatic disease studied by Einhorn, four showed absence of one or two ferments in the duodenal contents. In two cases of tumor of the pancreas the ferments were present. Cloudy turbid bile indicates gall-bladder or duct disease (Einhorn).

Sladden has the following criticism for this method of pancreatic study: "From the standpoint of the practical clinician, the method is open to serious objections; patients do not appreciate spending the

TABLE 1.—ANALYSIS OF

		Amount	Reaction	Bile
				Group A
Case I	Cholelithiasis	7 c. c.	Acid	—
Case II	Cholelithiasis	15 c. c.	Acid	—
Case III*	Cholelithiasis	35 c. c.	Acid	—
Case IV*	Cholelithiasis	10 c. c.	Neutral	—
Case V	Cholelithiasis (before operation)	27 c. c.	Acid	—
				Group B
Case VI	Acute pancreatitis	10 c. c.	Acid	—
				Group C
Case VII	Obstructive jaundice, new growth	12 c. c.	Neutral	0
Case VIII*	Obstructive jaundice, stricture	10 c. c.	Acid	0
Case IX*	Obstructive jaundice (*a*)	10 c. c.	Acid	0
	Duodenal sarcoma (*b*)	5 c. c.	Acid	—
				Group D
Case X	Hypertrophic cirrhosis of liver	25 c. c.	Neutral	—
Case XI	Hypertrophic cirrhosis of liver	50 c. c.	Acid	—
				Group E
Case XII	Duodenal ulcer	20 c. c.	Acid	—
Case XIII	Gastric neurosis	.....	Alkaline	—
Case XIV	Gastric neurosis	8 c. c.	Acid	—
Case XV	Duodenal ulcer	15 c. c.	Acid	—
Case XVI	Duodenal ulcer, hour-glass	3.5 c. c.	Alkaline	—
Case XVII*	Carcinoma of stomach	7 c. c.	Acid	—
Case XVIII	Achylia gastrica simplex	4 c. c.	Acid	—
				Group F
Case XIX	Diabetes (juvenile)	25 c. c.	Acid	—
Case XX	Diabetes	9 c. c.	Acid	—
Case XXI	Diabetes (syphilis)	15 c. c.	Acid	—
Case XXII	Diabetes	3.5 c. c.	Acid	—
Case XXIII	Diabetes, Milk diet	.....	Acid	+
	Fuller diet	25 c. c.	Acid	—
Case XXIV	Diabetes, Chronic pancreatitis (?)	3 c. c.	Alkaline	—
				Group G
Case XXV*	Retroperitoneal Hodgkin's disease	40 c. c.	Acid	—
Case XXVI*	Abdominal sarcoma	12 c. c.	Neutral	—
				Group H
Case XXVII	Chronic colitis	.....	.....	+

* Diagnosis confirmed by operation or autopsy (Crohn).

night, or even a few hours with a tube down the esophagus, nor does the risk of injury to the stomach or the duodenum, where ulceration already exists, seem entirely remote." In our experience, however, patients do not object very strenuously to this method, and we have had no accidents that would point to any danger arising from the intubation.

A method has been devised for the simultaneous fractional analysis of the gastric and duodenal contents. (*See* p. 25.) We have found the height of pancreatic activity to be two hours after the Ewald test-meal in normal individuals. With an ulceration of the duodenum, the

DUODENAL CONTENTS (CROHN).

Duodenal Contents					Stool				
Amylase	Lipase	Casein	Fermi	Mett	Cubes	Amylase	Lipase	Casein	Fermi
10 c. c.	0	1 to 5800	—	—	—	—	—	—	—
12 c. c.	6.3	1 to 12000	—	0	—	—	—	—	—
30 c. c.	10.0	1 to 1000	3	—	—	0	0.1	1 to 700	—
24 c. c.	3.6	1 to 18000	10	2	+	10	0.25	1 to 50000	2
0	0	1 to 5000	10	—	—	—	—	—	—
0	1.8	0	—	—	—	0	0.2	0	—
4	2.4	1 to 4000	3	0.5	+	0	0.3	0	0
30	0	1 to 4000	(3) (5)	—	—	—	—	—	—
0	0.5	0	—	0	—	0	0	0	—
4	3.4	1 to 600	3	0	—	2	0	1 to 200	0
27	—	1 to 18000	7	0	+	8	1.5	1 to 3000	0
15	1.0	1 to 4000	1	0	—	—	—	—	—
20	—	1 to 6000	—	—	—	++	1.5	1 to 5000	—
+	—	++	—	—	—	0	0	1 to 5000	—
0	0	+	—	+	—	(1.0)	—	—	—
+++	0	++	—	—	—	(5.0)	—	—	—
—	2.4	1 to 1400	10	—	—	—	—	—	—
9	5.4	0	—	0	—	—	—	—	—
3	8.4	1 to 4000	3	0.5	Rennet Neg.	6.0	0.1	1 to 30000	—
6	4.5	+++	—	2	—	—	—	—	—
9	12.6	1 to 4000	—	0	—	—	—	—	—
30	2.7	1 to 6000	(3) (7)	0	+	1	0	1 to 700	—
1.5	6.0	1 to 4000	2.5	—	+	8	0.4	0	0
(6)	0	1 to 34	(1.5)	—	—	—	—	—	—
(6)	(0.6)	1 to 4000	(15)	1	+	—	—	—	—
1.5	1.2	1 to 500	(5) (11)	1	+	2.5	3	1 to 125	—
0	5.6	1 to 4000	8	1	++	—	—	—	—
4	3.3	1 to 500	4	1.5	—	10	0.15	1 to 1700	0
0	0.6	1 to 12000	—	—	—	—	—	—	—

maximum activity of the enzymes may be reached after three hours. In cases of reflex irritation due to appendicitis, gall-stones, etc., the curve of pancreatic digestion seems to be influenced to this extent—that there is a secondary rise in the amount of pancreatic digestion, after a fall which may last three-quarters of an hour.

Analysis of Regurgitated Duodenal Contents by Examination of the Stomach Contents.—Spencer, Meyer, Rehfuss and Hawk reported a study of the duodenal regurgitation in the normal human stomach. They employed fractional removal of the gastric contents by means of the Rehfuss tube. The experiments were all carried out on normal in-

dividuals whose last meal was that of the previous evening. The presence of bile and trypsin was used in determining whether regurgitation of duodenal contents had occurred. The authors found that trypsin is almost constantly demonstrable in the fasting and digesting contents of the normal human subject. The authors incline to the view that the human pylorus is controlled from the duodenum, acid fluid keeping the pylorus closed until the fluid in the duodenum is neutralized.

Landau and Reasnicki found that the detection of trypsin in the stomach contents is more readily accomplished in the presence of a low degree of acidity. For clinical purposes only positive results are of value. If trypsin cannot be demonstrated in the stomach contents it does not by any means indicate that the external secretion of the pancreas is pathologically altered. In such cases direct intubation of the duodenum becomes necessary. The examination of the gastric contents for diastase in order to diagnose pancreatic disease or to prove a regurgitation of the duodenal contents is of no clinical value whatever (Landau and Reasnicki).

1. Ehrmann's Test.—Based upon the fact that neutral fat, free from fatty acid, is split up by the lipase from the pancreas, the resulting acid forming green salts with copper, Ehrmann devised a color reaction which will enable one to determine the functional activity of the pancreas. Commercial palmin has proved to be the most suitable neutral fat. Other fats, such as butter and oil, cannot be used. Emulsified fat in the form of milk or cream is also unsuitable, since it may be hydrolyzed by ferments other than pancreatic, although the butyric acid odor noted after the removal of these foods from the stomach is due chiefly to the action of pancreatic enzyme. The patient takes the following test-breakfast on the fasting stomach: About 30 grams of ordinary rice starch are dissolved in about one-fourth of a liter of water, and warmed somewhat. In this solution about 57 grams of palmin are stirred; the palmin is dissolved by heating. The mixture is drunk from a glass, and is allowed to remain in the stomach from two to two and one-half hours, when it is removed by means of a stomach-tube. In testing the gastric contents two solutions are required: Solution I, petroleum ether 90, benzol 10; Solution II, cupric acetate 3, distilled water to 100. A portion of the fatty gastric contents is shaken vigorously with an equal quantity of Solution I. The ether layer, after separation, is poured into a second test-tube, and is then shaken with an equal volume of Solution II. The ether which again separates is stained more or less intensely emerald-green, depending upon the concentration of fatty acids in the gastric contents. With no hydrolysis of the palmin, the ether remains water-clear. If the gastric contents are very acid, the reaction may be weak or may fail even with normal pancreatic secretion. In such a case it is advisable to repeat the test-breakfast, adding to it a teaspoonful of sodium bicarbonate.

2. Boldyreff-Volhard Oil Test-breakfast.—Pavloff and Boldyreff had found that the administration of oil on an empty stomach causes

a regurgitation of the duodenal contents through the pylorus. About 200 or 250 c.c. of olive oil or cream are administered on a fasting stomach, and in from thirty to forty-five minutes the stomach contents, which have been rendered less acid by administration of milk of magnesia (as suggested by Lewinski), are removed and the tryptic activity ascertained.

Frank regards the test as valuable to prove complete achylia pancreatica, or if a series of tests be made on one patient, to gain useful information as to the tryptic function of the pancreas. In von Ehrmann's case (1910) of chronic pancreatitis the oil test-breakfast was successful, and Mahlenburg, too, favored this method and rarely failed to find trypsin unless there was complete absence of pancreatic juice. A full account of this subject was given by Michailow in 1912, who collected 450 cases, in nearly 400 of which trypsin was found in the gastric contents after the test-meal. His analyses of the cases where trypsin was absent are not altogether convincing as evidence for the value of the test for diagnosis.

The possibility of failure to regurgitate is always present. Michailow insists that it is important for the patient not to retch, no easy thing to secure after swallowing about five ounces of olive oil neat (Sladden).

Analysis of Feces.—1. Analysis of Feces for Pancreatic Enzymes.—*Trypsin.*—The fact that the feces normally contain traces of a proteolytic ferment was shown by Leo, Baginsky, Schmidt and others, while Hemmeter proved that it was trypsin, and not pepsin, since it digests fibrin in an alkaline or neutral, not an acid, medium. The experiments of Frank and Schittenhelm with fecal extracts passed through a porcelain filter have shown that the proteolytic action of the feces is not dependent upon the presence of bacteria. The earlier experiments were carried out with fibrin, or Mett's tubes filled with white of egg or blood-serum, and it was not until Müller showed that drops of the fluid feces obtained by the administration of a purgative, such as calomel, or an emulsion of formed stool with glycerin, placed on a serum plate containing dextrose broth (Löffler), and incubated at 50° to 60° C., gave, under normal conditions, pits due to the digestion of the solid serum, that the examination of the stools for trypsin as a diagnostic measure began to attract much attention. If the pancreas is functionating normally, evidences of digestive changes in the serum plate should be obvious in about half an hour. If no change has taken place in twenty-four hours, it may be concluded that there is pancreatic insufficiency. This method has, however, inherent difficulties which militate against its general use, and the test devised by Gross, or one of its modifications, is now more frequently employed.

While some observers have failed to find trypsin in meconium by these methods, others state that it is usually present. There can be no doubt, however, that it quickly makes its appearance, and may usually be detected within a short time after birth. In normal persons the tryptic activity of the feces is uninfluenced by the diet or by a diminution in the acidity of the gastric juice by the administration of large doses of sodium bicarbonate (Schlecht). It is increased in diarrhea

and in conditions which stimulate peristalsis, thus hindering the absorption and destruction of the ferment. Constipation, on the other hand, diminishes the quantity of trypsin in the stools.

Schlecht states that he obtained only a feeble reaction in several cases of carcinoma of the stomach in which there was no mechanical obstruction of the pancreatic ducts, and explains this result by suggesting that a diminished activity of the pancreas was produced by the gastric disease or by the associated cachexia. In a case of poisoning by corrosive sublimate, with markedly bloody stools, no proteolytic action could be obtained with the feces owing to the antiferment present in the blood-serum. In the experience of most observers, a negative result is most constantly obtained in cases of cancer of the head of the pancreas, and it is therefore an exceedingly useful test in the diagnosis of that disease. Cirrhosis of the pancreas and obstruction of the duct by gallstones, etc., interfere more or less with the digestion of proteins by extracts of the feces, but rarely give rise to such very striking results as are seen in cases of growth in the head of the pancreas.

Crohn thus discusses the identity of the proteolytic ferments of the duodenal contents and of the stool:

"To return to a consideration of the alkali-protease found in duodenal content, one must consider that we are dealing with two ferments, trypsin and erepsin. Erepsin originates from two sources, the duodenal mucosa (Cohnheim) and from the pancreas (Bayliss and Starling). Schäffer and Terroine, experimenting with the excretion of an artificial pancreatic fistula in the dog, showed that in fluid in which trypsinogen was present but not activated by enterokinase, an ereptic ferment with peptone-splitting properties was still present. Of the test for alkali-protease, neither the Mett tubes nor the coagulated egg-albumen cubes are attacked by erepsin; nor are the Fermi gelatin tubes digested by erepsin. To establish this latter point, three fresh extracts of duodenal mucous membranes containing active erepsin (one cat, one dog, and one human intestine) were prepared after the method of Cohnheim. None of these extracts liquefied gelatin even after three days.

"These same extracts in their most concentrated form were tested for the casein-digesting power of the intestinal mucosa. That the digestive power of these intestinal extracts is only a very slight fraction of the same power of the pancreatic secretion is seen by a comparison of the results obtained. Thus cat mucosa extract in dilution of 1 to 15, dog mucosa extract 1 to 140, human mucosa extract 1 to 10 digested 10 c.c. of 0.1 per cent. casein solution; normal human duodenal contents containing pancreatic secretion digests the same amount of casein in dilution up to 1 to 10,000. It seems, therefore, fair to deduce that the amount of erepsin present both in the mucous membrane of the duodenum and in the pancreatic secretion could not account for the active proteolysis of casein as found in duodenal contents. Hence, we seem justified in assuming that the pancreatic trypsin is the active factor here and erepsin, while unquestionably present, yet of little moment in the tests, as carried out.

"A similar process of reasoning seems justified in discussing the results of the stool examinations; for, if the concentrated extract of normal duodenal mucosa digests casein in dilution of only 1 to 10, how can we explain the proteolysis of casein in dilutions of the stool up to 1 to 10,000 or 1 to 20,000, as frequently found, except on the hypothesis that it is the much more powerful pancreatic trypsin that is appearing in the stool."

Frank and Schittenhelm, by means of complicated polypeptic splitting experiments, seem to demonstrate that the protease present in the stool is erepsin, rather than trypsin. It is difficult to harmonize their findings with such simple facts as the above. The occasional finding of a ferment in the stool which liquefies gelatin, would tend to confirm the impression that this ferment derives its origin from the pancreas. That bacteria do not stimulate the results of the human ferments seems established by the fact that a case in which the pancreatic ducts have been proved to be closed gave complete negative results in both duodenal and stool analyses.

Trypsin is demonstrated in the stools by the following methods:

(*a*) *The Serum-plate Method.*—Müller and Schlect found that trypsin would act upon the surface of a serum agar plate, producing small depressions. They demonstrated by this method the regular occurrence of trypsin in normal feces. The plates were kept at a temperature of 50° or 60° C., so that bacterial action was prevented. In several cases of primary and secondary disease of the pancreas trypsin was absent from the feces or greatly diminished. A number of investigators have found this method of value. It yielded positive results in 5 out of 6 cases of pancreatic disease examined by Hirschberg.

(*b*) *The Casein Method.*—Casein in alkaline solution is precipitated by acidifying with dilute acetic acid. When the casein is digested by trypsin the addition of acetic acid produces no clouding of the solution. This is the basis of a method introduced by Gross for detecting the presence of trypsin. More than 200 stools were examined by him, and in all cases in which disease of the pancreas could be excluded a protein-splitting ferment was present in the feces. Brugsch and Masuda have concluded from their investigations that the strong splitting of casein produced by fecal extracts cannot be attributed to erepsin. Spooner and Pratt, in a recent case of cancer of the pancreas, found that the power of the feces to digest casein was entirely lost. In a case of fatty diarrhea, probably due to pancreatic hypochylia, the amount of trypsin in the feces was greatly reduced. In this case the cell-nuclei were not digested in the Schmidt's beef cubes, and after administration of Sahli's glutoid capsules no reaction was obtained in the urine even at the end of twenty-four hours.

Gray and Pickman studied pancreatic ferments in cases of pulmonary tuberculosis. Trypsin and amylopsin were determined in the stools in a series of nearly one hundred cases of tuberculosis and it was found that the pancreatic secretion was seriously reduced by the toxins of tuberculosis. Rest, either in bed or by means of pneumothorax, reduced

the formation of toxins and permitted the pancreatic ferments to return toward normal. A persistently low trypsin index was found to be of bad prognostic significance, but low amylopsin readings were less unfavorable. The interpretation of the index must always take into consideration anorexia, overeating and diarrhea.

Zuccola states that an examination of ferments in the feces is a useful aid in the diagnosis of pancreatic efficiency when taken in conjunction with other methods. It is not absolute in its indications, nor does it yield results which can be regarded as quantitative. As with so many other tests of pancreatic activity, a positive outcome pointing to absence of trypsin has more value than a negative result, especially in view of possible interference from other proteolytic enzymes (Sladden).

Amylase (Diastase).—The presence of diastase is shown by the digestive action that it has upon starch, using a solution of iodin as the indicator.

The following is *Robert and Strassburger's method,* as modified by Goiffon and Tallarico: A 1-per cent. solution of starch is mixed with an equal part of 10-per cent. solution of the feces in thymol water, neutralized, filtered. The filtrate is placed in the incubator at 37° C., and at regular intervals a drop is brought in contact with a drop of iodin solution. When it ceases to give a blue color, the digestion of the starch is considered to be complete. The stool should be fresh, and there should not be the slightest admixture of urine. It is often enough merely to mix the stool and the starch solution in a test-tube, heat in a water-bath, and apply the iodin test. If an abundance of amylase is present the starch will be digested in about five minutes.

Wohlgemuth has adopted the following quantitative method for determining the diastase in the stools. The fresh feces are well mixed, and five grams are thoroughly ground in a mortar with 20 c.c. of 1 per cent. solution of sodium chlorid, added a small quantity at a time. The emulsion is then left for half an hour at the room temperature, and frequently stirred meanwhile. It is now divided equally into two portions of 10 c.c. each, and is transferred to graduated centrifuge tubes, which are centrifugalized until all the solid material is collected at the bottom and stands at the same height in both tubes. The quantities of sediment and supernatant fluid are noted. Nine test-tubes are now taken. Into the first three are placed 1.0 c.c., 0.5 c.c., 0.25 c.c. of undiluted extract; into the next three, 1.0 c.c., 0.5 c.c., 0.25 c.c. of an eight-fold dilution of the original extract, made with 1 per cent. sodium chlorid; and into the last three, 1.0 c.c., 0.5 c.c., 0.25 c.c. of a sixty-four-fold dilution, so that each tube contains half the fecal extract of the preceding:

1st tube ... 1.0	6th tube ... 0.0312
2nd tube ... 1.5	7th tube ... 0.0156
3rd tube 1.25	8th tube ... 0.0078
4th tube 0.125	9th tube ... 0.0039
5th tube 0.0625	

To each tube 5 c.c. of a 1 per cent. solution of starch are then added. The tubes are now plugged with wool, or closed with corks, and placed in the incubator at 38° C. for twenty-four hours. At the end of that time, they are filled, within a fingerbreadth of the brim, with cold distilled water, one drop of a decinormal iodin solution is added to each, and the lowest dilution giving a blue reaction looked for. It is then assumed that the tube next lowest in order contains sufficient diastase to convert all the added starch, and from this the quantity of 1 per cent. starch solution fermented by 1 c.c. of the fecal extract can be calculated. Knowing the proportion of solid residue liquid extract in the 5 grams of feces, the quantity of ferment corresponding to 1 c.c. of this residue can be determined, and from this the diastatic power of the total daily mass of feces can be determined. According to Wohlgemuth and Wynhausen, the average diastatic value of the feces lies between 470 and 500. To obtain satisfactory results, the feces must be homogeneous and alkaline in reaction, as diastase does not act in an acid medium. It is advisable to place the patient on a simple mixed diet, calculated to stimulate the functions of the pancreas to normal activity, for a couple of days before the feces are collected for examination.

Quantitative Determination of Fecal Amylase.—Hawk's Method.—Weigh accurately about 2 grams of fresh feces into a mortar, add 8 c.c. of a phosphate-chlorid solution (0.1 mol dihydrogen sodium phosphate and 0.2 mol disodium hydrogen phosphate per liter of 1 per cent. sodium chlorid), 2 c.c. at a time, rubbing the feces mixture to a homogeneous consistency after each addition of the extraction medium. Permit the mixture to stand at room temperature for a half-hour with frequent stirring. We now have a neutral fecal suspension. Transfer this suspension to a 15 c.c. graduated centrifuge tube, being sure to wash the mortar and pestle carefully with the phosphate-chlorid solution and add all washings to the suspension in the centrifuge tube. The suspension is now made up to the 15 c.c. mark with the phosphate-chlorid solution and centrifugated for a 15-minute period, or longer if necessary, to secure satisfactory sedimentation. At this point, read and record the height of the sediment column. Remove the supernatant liquid by means of a bent pipet, transfer it to a 50 c.c. volumetric flask and dilute it to the 50 c.c. mark with the phosphate-chlorid solution. Mix the fecal extract thoroughly by shaking and determine its amylolytic activity. For this purpose a series of six graduated tubes is prepared, containing volumes of the extract ranging from 2.5 c.c. to 0.078 c.c. Each of the intermediate tubes in this series will thus contain one-half as much fluid as the preceding tube. Now make the contents of each tube 2.5 c.c. by means of the phosphate-chlorid solution in order to secure a uniform electrolyte concentration. Introduce 5 c.c. of a 1 per cent. soluble starch solution and three drops of toluol into each tube, thoroughly mix the contents by shaking, close the tubes by means of stoppers and place them in an incubator at 38° C. for 24 hours. At the end of this time remove the tubes, fill each to within half an inch of the top with ice-water, add 1 drop of tenth-normal iodin solution, thoroughly mix the

contents and examine the tubes carefully with the aid of a strong light. Select the last tube in the series which shows entire absence of blue color, thus indicating that the starch has been completely transformed into dextrin and sugar, and calculate the amylolytic activity on the basis of this dilution. In case of indecision between two tubes, add an extra drop of the iodin solution and observe them again.

The amylolytic value, Df, of a given stool, may be expressed in terms of 1 c.c. of the sediment obtained by centrifugation as above described. For example, if it is found that 0.31 c.c. of the phosphate-chlorid extract of the stool acting at 38° C. for 24 hours completely transformed the starch in 5 c.c. of a 1 per cent. starch solution, then we would have the following proportion:

$$0.31 \text{ c.c. extract} : 5 \text{ c.c. starch} :: 1 \text{ c.c. extract} : x$$

The value of x in this case is 16.1, which means that 1 c.c. of the fecal extract possesses the power of completely digesting 16.1 c.c. of a 1 per cent. starch solution in 24 hours at 38° C.

Inasmuch as stools vary so greatly as to water content, it is essential to an accurate comparison of stools that such comparison be made on the basis of the solid matter. Supposing, for example, that in the above determination we had 6.2 c.c. of sediment. Since the supernatant fluid was removed and made up to 50 c.c. before testing its amylolytic value, it is evident that 1 c.c. of this sediment is equivalent to 8.1 c.c. of extract. Therefore, in order to derive the amylolytic value of 1 c.c. of sediment, we must multiply the value (16.1) as obtained above for the extract, by 8.1. This yields 130.4 and enables us to express the activity as follows:

$$\mathrm{Df}\,{}^{38^\circ}_{24^h} = 130.4$$

The above method of calculation is that suggested by Wohlgemuth. In case time and facilities permit of the determination of the moisture content of the feces, it is much more accurate and satisfactory to place the amylolytic values of the stools on a "gram of dry matter" basis. The amylolytic values of the stools are expressed as the number of c.c. of 1 per cent. starch solution which the amylase content of 1 gram of dry feces is capable of digesting.

Amylase was first demonstrated in the feces of infants by Wegscheider. Later von Jaksch, Maro, Allaira, and others showed that the feces of children constantly contain it. It is found during the first week of life in abundance, and Pottevin proved that it is constantly present in meconium. The quantity appears to diminish somewhat in later life, but, according to Strassburger, it never entirely disappears. It has been suggested that the diastatic action of fecal extracts on starch might be due to the contained bacteria, but the experiments of Kerley, Mason and Craig have proved that an extract freed from bacteria by filtration through a Berkefeld filter has an unchanged action on starch. The

amount present in the stools appears to vary within very wide limits normally, perhaps as a result of changes in the diet. Diarrhea increases the quantity, and constipation generally diminishes it. In diseases of the pancreas interfering with the flow of pancreatic juice into the intestine, the digestive action of an extract of the feces for starch is diminished, or may be altogether abolished; thus in many cases of cancer of the head of the pancreas Cammidge has obtained an unchanged blue reaction with iodin after twelve or even twenty-four hours' incubation; but with growths of the gall-bladder and common duct that did not obstruct the pancreatic duct, starch digestion has not been interfered with.

From Brown's study on the diastase content of feces in normal and in certain pathologic conditions the following conclusions are drawn: The stool, if a rigorously exact method is carried out as to food, purgative employed, preservation of specimen, estimation of ferment, etc., furnishes a diastase content within definite limits. The effect of waiting too long after the stool has been obtained before making the examination, and the influence of variations in temperature in the place in which it is kept and of different laxatives and different foods, are so great as to render results, obtained by methods in which insistence on such a rigorous technic has not been made, of much less value.

Extensive carcinoma of the pancreas showed no diastase in the tube of lowest dilution in Brown's method, and this absence of ferment should prove of great help in the diagnosis of this condition. In chronic pancreatitis diastase was present in the stool, but in markedly diminished amounts. In achylia gastrica the diastase content of the stool was practically normal in all the cases examined. This, in the first place, suggests that in the absence of hydrochloric acid some other method of pancreas activation is called into play, and, in the second place, that the diarrhea met with in certain of these cases of achylia gastrica—the so-called gastrogenous diarrhea—is not of pancreatic origin.

Gerganoff points out the possibility of error in the quantitative determination of enzymes in the feces through the admixture of blood. Blood, whether it be from the stomach or intestines, may lead to considerable increase of the fecal ferments (Gerganoff studied diastase particularly). Especially intestinal hemorrhages, when large, produce a decided increase. But gastric hemorrhages, when hydrochloric acid is lacking, may lead to similar results. When free hydrochloric acid is present in the stomach it may be concluded with reserve that a bloody stool rich in diastase is not due to gastric hemorrhage, but to bleeding from the duodenum or lower portions of the intestine. This point may prove useful in the diagnosis of a gastric ulcer or in its exclusion.

As has been previously stated, Crohn considers the quantitative analyses of the feces and duodenal contents for enzymes to be the most logical and fruitful method for the diagnosis of pancreatic insufficiency. There are, of course, other factors which influence the absorption of food besides the pancreatic ferments. But, generally speaking, a lessening of the pancreatic enzymes is directly indicative of some organic pathological

process in the gland. According to Crohn, the absorption and assimilation of foods may run an independent curve, altogether neglectful of the diminution of the pancreatic enzyme output, and he states that absorption may be poor with an intact gland or good with a gland of which only a fragment survives the disease. While the author cannot entirely subscribe to this opinion, it can be stated that the examination of the various secretions and excretions for pancreatic enzymes yields excellent diagnostic results.

Lipase in the stools has little significance so far as pancreatic disease is concerned. In 1875, Pfeiffer showed that the feces contain a fat-splitting ferment. It is not derived from the pancreas, but appears to come from the intestinal mucous membrane, although a bacterial origin cannot be altogether excluded. Hecht has proved that the stools of infants contain a ferment which has the power of splitting the fats contained in the yolk of eggs by the *Voldard-Stade method:*

The yolks of three eggs are emulsified with 100 c.c. of water. Ten c.c. of this are mixed with the specimen to be tested, and the mixture is placed in the incubator for two or three hours. It is then well shaken with 75 c.c. of ether and left to stand, the separation of the ether being promoted by the addition of a few c.c. of neutral alcohol. With a pipet, 50 c.c. of the ether are removed and mixed with 75 c.c. of neutral alcohol and titrated with decinormal soda. The mixture is then placed in a flask, 10 c.c. of normal soda solution are added, the flask is well corked, and left at the room temperature for twenty-four hours. Ten c.c. of normal hydrochloric acid are now added, and the mixture is again titrated with decinormal soda. In both titrations phenolphthalein is used as the indicator. The result of the first titration gives the fatty acids, and the second the soaps that have been formed. From these the amount of fat that has undergone saponification can be reckoned.

Another method is to incubate the admixture of fluid to be tested with ethyl butyrate for a few hours. If the mixture has been previously rendered neutral, the presence of butyric acid can be recognized by its action on neutral litmus, or it may be titrated with decinormal soda and phenolphthalein.

According to Hemmeter, the fat-splitting ferment contained in an extract of feces does not act upon olive oil. Hecht could not find any parallelism between the quantity of lipase in the stools and the amount of neutral fat, fatty acids, and soaps in the feces.

2. Chemical Analysis of the Feces Following the Schmidt-Strassburger Diet.—The diet consists of "1.5 liters milk, 100 grams zwieback, 2 eggs, 50 grams butter, 125 grams beef, 190 grams potatoes, and gruel of 80 grams oatmeal. It contains about 102 grams albumin, 111 grams fat, 191 grams carbohydrates, or a total of 2234 calories."

In the morning: 0.5 liter milk (or if milk does not agree, 0.5 liter cocoa prepared from 20 grams cocoa powder, 10 grams sugar, 400 grams water and 100 grams milk) and 50 grams zwieback.

In the forenoon: 0.5 liter oatmeal gruel (made from 40 grams oatmeal, 10 grams butter, 200 c.c. milk, 300 c.c. water, 1 egg; strained).

At noon: 125 grams chopped beef (raw weight), broiled rare with 20 grams of butter, so that the interior will still remain raw, and 250 grams potato broth (made of 190 grams mashed potatoes, 100 c.c. milk and 10 grams butter).

In the afternoon: As in the morning.

In the evening: As in the forenoon.

In the recognization of severe pancreatic disease there is no single symptom of greater significance than bulkiness of the stools. This is a diagnostic sign to which Osler, Musser, and others have called attention. Much information can often be gained from the weight of dried stools, and this can be ascertained even when facilities are not available for exact chemical analyses. All that is necessary in addition to scales for weighing is a water bath and a ventilating hood. With pancreatic juice absent from the intestine, not only are the stools voluminous, but the dried residue is much in excess of the normal.

In a series of six healthy individuals placed on the test-diet for three days, Schmidt found the average weight of the dried feces to be 54.3 grams. The maximum was 62 grams and the minimum 45 grams. Pratt found in a case of obstructive jaundice associated with malignant disease of the pancreas that the weight of the dried feces was 419 grams in one metabolism period of three days, and 355 grams in another. In a patient with chronic fatty diarrhea and glycosuria without icterus the feces weighed 438 grams.

The increase in weight of the feces which results from shutting off the pancreatic juice from the intestine was well shown in our animal experiments. In a preliminary absorption test with the dog in normal condition the weight of the dried food in a period of three days was 624 grams, and the weight of the feces 140.4 grams. In a metabolism experiment of the same duration begun five days after separating the pancreas from the duodenum, the weight of the dried food was reduced to 416 grams and that of the feces increased to 302.7 grams.

In none of the cases studied by Schmidt was such a marked increase in weight of the feces observed as in Pratt's 2 cases of pancreatic disease. The average weight of the feces in 5 cases of "fermentative dyspepsia" reported by him was 127.4 grams; the average in "gastrogenous diarrhea" with achylia was 98.9 grams. His highest figures were in obstruction of the common bile-duct, where the average weight was 175.6 grams, and the maximum 215.4 grams. There are no observations on cases of obstruction of the pancreatic ducts given by Schmidt. It seems to Pratt that the possibility of shutting off the pancreatic secretion by an obstruction in the lower part of the common bile duct should be recognized. This may be the explanation of the heavy weight of the feces in 2 of his cases.

In a number of cases of pancreatic disease, metabolism studies have shown a great interference with the absorption of fat of nitrogen. Morrison and Pratt made a metabolism experiment on a patient presenting the typical symptoms of total obstruction of the pancreatic ducts. There was no jaundice. It was found that 58.9 per cent. of the fat of the

food was excreted in the feces. The percentage of nitrogen unabsorbed was 50.9 per cent. Normally not over 5 or 10 per cent. of the fat or nitrogen of the food is lost in the feces.

In a metabolism experiment on a patient with cancer of the pancreas and obstructive jaundice, Spooner and Pratt found that 79.9 per cent. of the fat of the food was excreted in the feces and 34.8 per cent. of the nitrogen.

Harley has reported a case of probable obstruction of the pancreatic ducts without jaundice, in which there was a fat loss of 73.1 per cent. In a case of cirrhosis and atrophy of the pancreas, combined with cirrhosis of the liver, Weintraub found a fat loss of 25.2 per cent.; Deucher, in cancer of the pancreas, found fat losses of 82.9 per cent. and 52.6 per cent.; Brugsch and König, in a case of abscess of the pancreas, 59.7 per cent. (absorption experiment of only one day's duration). Blässner and Siegel, in a case of atrophy of the pancreas due to a calculus, found a fat loss of 56.1 per cent.; Gigon, in a case of pancreatic calculi with obstruction of the ducts, a maximum fat loss of 47.4 per cent. and a minimum of 13.5 per cent.; Ehrmann, in atrophy of the pancreas, 50.2 per cent.; Tileston, in 5 cases of cancer of the pancreas with icterus, fat losses of 75.6 per cent., 68 per cent., 52.6 per cent., 45.6 per cent., 49.1 per cent.

In Harley's case there was a nitrogen loss of 40 per cent.; Weintraub found a nitrogen loss of 60.6 per cent.; Deucher, 29.6 per cent. in one case and 19 per cent. in the other. Glaessner and Siegel 41.5 per cent.; Gigon, 24.7 per cent.; and Ehrmann, 42.8 per cent.; Tileston in three cases, 19.8 per cent.; 14.5 per cent.; and 21.1 per cent. Brugsch found an average fat loss of 45 per cent. in three cases of icterus, but the nitrogen loss averaged only 11 per cent. If 50 per cent. or more of the fat and 25 per cent. of the nitrogen of the food are recovered from the feces the conclusion is warranted that pancreatic insufficiency exists.

While the method of chemical analysis of the feces is more difficult than the other tests, it yields results which are of greater value. Of course these examinations can only be carried out in a well-equipped laboratory, and better still in a hospital laboratory.

3. Azotorrhea.—Azotorrhea as a test for pancreatic insufficiency is well discussed by Sladden.

The term azotorrhea is used to denote an excessive excretion of nitrogen compounds in the feces, the inference being made that such compounds are present owing either to failure of digestion of proteins or to failure of absorption of products of digestion.

The determination of the nitrogen content of the feces is a process only to be undertaken by a skilled chemical pathologist, and as a clinical test, it is ruled out in ordinary conditions of practice. Moreover, to place such determinations upon a sound basis, a complete metabolism experiment has to be made, with estimations of nitrogen intake and output.

As with all other investigations of the proteolytic activity of pancreatic juice, the disturbing effect of other ferments, pepsin, erepsin, and

proteolytic agents of bacterial origin, complicates and obscures the issue; while, on the other hand, disease of the absorbing areas of the intestine, tuberculosis ulceration, or degeneration and atrophy of the mucous membrane may lead to deficient absorption of nitrogen products which have not suffered from lack of pancreatic juice to break them down. This being so, one can hardly agree with Barbour that azotorrhea is a cardinal sign of pancreatic disease except with the proviso that the mechanism of absorption in the small intestine is not deranged.

A review of work done on this subject shows quite unmistakably that azotorrhea is a common feature of pancreatic disease Deaver, Tilestone, Pratt, von Ehrmann, O. Gross, Barbour and Brugsch have all communicated cases in point, whereas there appear to be few records of completely normal utilization of nitrogen in the presence of definite pancreatic disease. Delfino has published a case of pancreatic cyst where this was so.

A very interesting feature in most of the cases recorded is the tendency of extracts of pancreatic gland to improve nitrogen absorption. Von Ehrmann's patient (1910), with chronic pancreatitis and a loss of nitrogen equal to 43 per cent. of the intake, when given pancreatin lost only 17 per cent. of the nitrogen in the diet, although this represented an increased intake. In an interesting and valuable series of tests on two cases, one with chronic pancreatitis without icterus, the other with icterus, the result of syphilitic cirrhosis, the same author found the nitrogen loss in the pancreatic case was 34 per cent. of the intake, that of the icteric case, 12 per cent. Pratt's account of seven cases with pancreatic disease showed a great increase of nitrogen loss, from the normal 5 per cent. or 10 per cent. to 30 per cent. or 40 per cent., and animal experiments confirm this. He quoted Tilestone for cases of icterus with carcinoma of the pancreas where loss of both fat and nitrogen was increased; that icterus alone, however, has but little influence on nitrogen absorption is shown by Ehrmann's case above mentioned and by Brugsch's experience that in uncomplicated icterus the nitrogen loss may be 11 per cent., but with a pancreatic lesion added it will rise to 33 per cent.

Brugsch makes an interesting suggestion that in cases where acidosis is present, as an advanced diabetes, this may reduce the alkalinity of the duodenal and intestinal contents and so impair ferment activity and lead perhaps to an augmentation of fat and nitrogen excretion. There does not appear to be any experimental work in support of this theory, but against it can be adduced the experiments of Long and Muhlmann, who showed that *in vitro* trypsin can withstand an acidity of more than 0.3 per cent. HCl for half an hour at 40° C. If pepsin be present also a distinct retardation of tryptic activity is caused. Their experiments also showed that commercial preparations of pancreatic gland rapidly deteriorate below the pharmacopeial standards. In two cases reported by Gross doses of twenty tablets daily of pankreon (rhenania) produced no effect on nitrogen absorption, but by doubling the dose a marked benefit was obtained. With the use of raw pig's pancreas, 75 grams per day, an even greater improvement occurred. Cammidge's experience with

new gland as compared with dry preparations is similar. Mosenthal, in a case of glycosuria with creatorrhea, steatorrhea and absence of trypsin in the stools, gave pankreon without effect, but on substituting 150 grams of raw sheep's pancreas he observed very great improvement in fat and protein digestion. The improvement was so great in the case of fat that acidosis tended to increase.

Although other and simpler tests render observations on nitrogen loss in the stools a rather unsatisfactory method for diagnosis, nevertheless it is probably true that pancreatic insufficiency is the most usual cause of azotorrhea, and if metabolism experiments be undertaken with observations on the influence of pancreatic preparations on nitrogen absorption, much valuable information can be gained both for diagnosis and the direction of treatment. Tilestone (1912) regards the determination of nitrogen loss quite as valuable for diagnosis as fat determinations, and in the absence of intestinal disease impairing absorption he regards a nitrogen loss of more than 30 per cent as being strongly suggestive of pancreatic disease.

4. Steatorrhea.—Müller observed that the feces contained a larger percentage of unsplit fat, that is, of neutral fat, in his cases of pancreatic disease than in other conditions studied. Zoja asserted that a low percentage of soaps was of diagnostic value. Fitz, in 1903, collected 11 cases from the literature in which an analysis of the fecal fat had been made; while in health, from 20 to 30 per cent. is in the form of neutral fat, in 9 out of the 11 cases of disease of the pancreas the percentage of neutral fat was increased.

Pratt has made analyses of the fecal fat in 7 cases of undoubted pancreatic disease. The fat extractions were made according to Rosenfeld's method. In the determination of the neutral fat, fatty acids, and soaps, Müller's procedure was followed.

In all but one of these cases steatorrhea occurred. The percentage of fat ranged from 28.8 to 72.8, while normally the feces contained less than 25 per cent. In 4 of the 7 cases there was an increase in the neutral fat, but not sufficiently great to be of distinct diagnostic value. A very low percentage of soaps was found in 3 of the 7 cases.

Cammidge gives the tables quoted below of fat analysis of the feces in pancreatic and non-pancreatic disease. (Tables 2-9.)

Cammidge states: "Considering first the relation of the 'unsoaped' fat (neutral fat and free fatty acids) to combined fatty acids, it will be seen that the effect of interference with the functions of the pancreas has been as a rule to increase the proportion of unsoaped fats, while obstruction of the bile flow and intestinal catarrhs have tended to raise the percentage of combined fatty acids. The influence of disease of the pancreas in this direction is well seen in both series of cirrhosis of the gland, for in the eight cases comprised in the first series and in 90 per cent. of those in the second series, the unsoaped fats were in excess. Again in the case where pancreatic cyst was present, it will be seen that, although the total fat was not high (20.5 per cent.), nearly three-quarters of this (15 per cent.) was in the unsoaped condition, and only about a

TABLE 2.—ANALYSIS OF FECES.

Series A. Group I. Pancreatic Disease—No Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Combined Fatty Acids Soap (Per Cent.)	"Unsoaped" Fat	
					More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)
1. Stones in common bile-duct.	25	36.5 (71.3–16.0)	19.5 (51.0–4.5)	17.0 (37.9–0.3)	56	44
2. Stones in gall-bladder.	11	27.1 (44.0–16.2)	14.7 (22.4–4.3)	12.4 (21.2–2.6)	54	46
3. Intestinal catarrh.	83	28.8 (79.9–6.5)	12.5 (45.7–1.0)	16.3 (38.2–1.3)	30	70
4. Ulcer (duodenum).	15	30.5 (48.0–7.2)	14.7 (26.4–1.0)	15.8 (28.2–6.2)	46	54
5. Ulcer (stomach).	3	27.6 (47.2–7.5)	16.6 (20.2–5.4)	11.0 (27.2–4.1)	33	67
6. Sprue.	8	57.0 (76.1–39.8)	31.0 (42.4–7.4)	26.0 (34.0–16.6)	50	50
7. Pernicious anemia.	2	48.1 (50.4–45.8)	19.0 (9.4–8.6)	39.1 (41.8–36.5)	—	100
8. Tuberculous enteritis.	1	60.5	40.2	20.3	100	—
9. Cirrhosis of liver and pancreas.	9	39.0 (68.0–12.6)	22.9 (36.2–3.4)	16.1 (34.0–4.5)	89	11
10. Cirrhosis of pancreas.	8	36.2 (61.2–15.3)	22.6 (44.1–11.7)	13.6 (27.0–3.5)	100	—
11. Arteriosclerosis.	8	27.1 (53.8–15.1)	14.9 (50.8–0.5)	12.2 (34.1–3.0)	50	50
12. Heart disease.	4	19.1 (25.7–11.7)	10.7 (16.7–6.1)	8.4 (15.7–4.1)	75	25
13. Chronic pancreatitis.	47	28.3 (72.2–18.0)	16.3 (52.0–2.2)	12 (29.3–0.5)	49	51
14. Cyst of pancreas.	1	20.5	15.0	5.5	100	—
15. Pancreatic infantilism.	1	57.4	48.5	8.9	100	—

TABLE 3.—ANALYSIS OF FECES.

Series A. Group II. Jaundice—No Pancreatic Disease. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Combined Fatty Acids Soap (Per Cent.)	"Unsoaped" Fat	
					More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)
1. Stones in common bile-duct.	16	54.8 (81.3–25.7)	21.7 (46.3–10.0)	33.1 (54.1–8.2)	25	75
2. Growth of bile-duct.	5	74.6 (90.4–65.0)	30.7 (37.1–30.0)	44.0 (57.5–32.0)	—	100
3. Simple stricture of common bile-duct.	1	79.9	27.8	52.1	—	100
4. Catarrhal jaundice.	2	31.9 (32.9–31.0)	11.3 (11.6–11.0)	20.6 (21.3–20.0)	—	100
5. Growth of gall-bladder.	2	25.6 (27.3–23.9)	12.4 (13.5–11.3)	13.2 (14.8–12.6)	—	100

quarter (5.5 per cent.) in the form of combined fatty acids. On the other hand, in a case of simple stricture of the common bile-duct, I found 52.1 per cent. of the dry weight of the feces consisted of soaps, and only 27.8 per cent. of neutral fats and free fatty acids. Sixteen cases of simple obstruction of the common bile-duct by gall-stones, with no associated pancreatitis, showed an average of 21.7 per cent. of unsoaped fat to 31.1 per cent. of combined fatty acids. The latter were in excess of the former in all but four cases, and in these the jaundice was not marked and the difference was slight.

"Simple uncomplicated cases of pancreatic disease or jaundice are, however, comparatively rare. We have most frequently to consider the effects of the two together, or to take into account a coexistent intestinal condition with one or the other. In cancer of the pancreas, where as a rule there is complete blocking of the common bile-duct and serious interference with the functions of the pancreas, since the growth is most commonly situated in the head of the gland, the proportions of soaps and unsoaped fats vary very considerably. In my first series of 38 cases there was an excess of unsoaped fat in 57 per cent., but in the second series of 16 cases such an excess was only found in 13 per cent. This difference is, I think, to be explained by the fact that a small quantity of bile was finding its way into the intestine in a large number of the cases of the second group than of the first, and in many of them there was evidence of abnormal putrefactive changes in the intestinal contents, so that more of the fat was being split through the agency of bacteria and a larger proportion was being turned into soap. That more efficient fat absorption was as a rule taking place in these cases is sug-

TABLE 4.—ANALYSIS OF FECES.

Series A. Group III. Pancreatic Disease with Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Combined Fatty Acids Soap (Per Cent.)	"Unsoaped" Fat	
					More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)
1. Stones in common bile-duct.	51	56.6 (87.2–15.8)	30.2 (59.8–5.5)	26.4 (55.8–2.3)	57	43
2. Growth of common bile-duct.	2	49.5 (72.7–26.2)	29.6 (33.2–25.9)	19.9 (39.5–0.3)	50	50
3. Growth of ampulla of Vater.	1	65.7	35.1	30.6	100	—
4. Catarrhal jaundice.	24	45.0 (62.9–9.2)	27.0 (69.7–3.7)	18.0 (42.7–1.8)	58	42
5. Growth of pancreas secondary to gall-bladder.	4	55.4 (66.3–39.6)	38.2 (52.7–30.1)	17.2 (30.0–7.8)	100	—
6. Growth of pancreas secondary to stomach.	8	34.0 (63.7–19.2)	12.3 (31.6–5.6)	21.7 (41.1–4.3)	37	63
7. Growth of pancreas secondary to intestine.	6	26.2 (39.8–12.7)	10.5 (13.3–6.6)	15.7 (27.6–6.1)	33	67
8. Secondary growth of pancreas, primary elsewhere.	4	33.3 (39.6–12.7)	20.7 (23.1–14.9)	12.6 (18.1–7.0)	75	25
9. Cancer of pancreas.	38	71.3 (93.3–22.3)	41.0 (69.0–7.0)	30.3 (63.8–3.6)	57	43

gested by the lower average percentage of total fat. Chronic pancreatitis associated with the presence of gall-stones in the common bile-duct, and jaundice, gave an excess of unsoaped fat in 57 per cent. of the cases in the first series and 45 per cent. in the second. In both, very wide variations were met with, depending apparently upon the extent to which the bile flow was interfered with and the amount of damage to the pancreas."

Cammidge examined the feces from seven cases of growth of the common bile-duct. In five there was no evidence of involvement of the pancreas, and in these the combined fatty acids were considerably in excess of the unsoaped fats. Of the two in which the pancreas was involved in the growth, one showed an excess of combined fatty acids and the other of unsoaped fats, but as the patient with the low percentage of unsoaped fat (.3 per cent.) was on an exclusively milk diet, the results

TABLE 5.—ANALYSIS OF FECES.

Series A. Group IV. No Pancreatic Disease—No Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Combined Fatty Acids Soap (Per Cent.)	"Unsoaped" Fat	
					More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)
1. Stones in common bile-duct.	9	33.2 (62.3–21.2)	15.3 (34.0–3.5)	17.9 (38.8–3.8)	44	56
2. Stones, gall-bladder or cystic duct.	23	32.1 (79.9–11.6)	15.4 (27.8–4.4)	16.7 (52.1–0.5)	47	53
3. Intestinal catarrh.	18	25.3 (39.5–13.8)	8.1 (13.3–2.0)	17.2 (34.2–9.8)	5	95
4. Ulcer (duodenum).	6	26.4 (36.8–14.0)	7.8 (11.3–2.9)	18.6 (27.2–8.8)	—	100
5. Ulcer (stomach).	13	20.6 (27.9–16.8)	8.1 (12.2–4.2)	12.5 (15.7–8.9)	—	100
6. Sprue.	5	48.8 (57.7–34.5)	25.5 (26.7–11.7)	23.3 (32.0–19.0)	40	60
7. Pernicious anemia.	1	23.1	12.7	10.4	100	—
8. Appendicitis.	3	20.5 (22.1–18.6)	6.5 (7.0–5.6)	14.0 (15.3–11.0)	—	100
9. Chronic colitis.	30	20.9 (42.1–8.6)	5.6 (12.7–0.5)	15.3 (33.4–6.2)	—	100
10. Tuberculous enteritis.	3	39.8 (61.3–25.1)	14.9 (23.5–4.6)	24.9 (30.7–22.1)	—	100
11. Dilated stomach.	4	26.4 (28.5–20.4)	4.5 (6.4–2.1)	21.9 (23.6–12.4)	—	100
12. Growth of stomach.	15	25.1 (30.4–11.1)	10.0 (14.5–6.1)	15.1 (18.3–2.7)	33	67
13. Growth of intestine.	19	30.4 (39.8–22.1)	11.5 (17.1–8.0)	18.9 (27.6–7.5)	5	95
14. Growth of other organs.	10	25.4 (31.4–22.7)	10.8 (15.9–6.3)	14.6 (18.7–4.8)	40	60
15. Cirrhosis of liver.	5	20.2 (26.6–10.5)	10.6 (13.1–5.3)	9.6 (13.2–5.2)	80	10
16. Infantile acholia.	1	39.2	14.1	25.2	—	100
17. Congenital family steatorrhea.	1	83.1	37.2	45.9	—	100
18. Normal.	25	20.6 (25.0–9.1)	10.2 (15.6–6.1)	10.4 (10.2–4.3)	48	52

TABLE 6.—ANALYSIS OF FECES.

Series B. Group I. Pancreatic Disease—No Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Neutral Fat (Per Cent.)	Free Fatty Acids (Per Cent.)	Combined Fatty Acids (Per Cent.)	"Unsoaped" Fat		Average "Unsoaped" to Soaped Fat	Average Neutral Fat to Free Fatty Acid
							More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)		
1. Cirrhosis of pancreas.....	20	26.5 (76.0–10.0)	16.3 (51.4–4.0)	5.1 (22.0–1.9)	11.2 (21.8–1.7)	10.2 (26.0–2.0)	90	10	1.6:1.0	1:2.0
2. Catarrhal pancreatitis.....	25	23.5 (54.5–7.9)	9.5 (31.3–3.0)	2.1 (5.0–1.2)	7.4 (28.4–0.9)	14.0 (23.1–9.7)	72	28	1.0:1.5	1:3.5
3. Pancreatic infantilism.....	1	23.3	6.0	1.1	4.9	17.3	—	100	1.0:2 9	1:4.4

TABLE 7.—ANALYSIS OF FECES.

Group 2. Pancreatic Disease—Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Neutral Fat (Per Cent.)	Free Fatty Acids (Per Cent.)	Combined Fatty Acids (Per Cent.)	"Unsoaped" Fat		Average "Unsoaped" to Soaped Fat	Average Neutral Fat to Free Fatty Acid
							More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)		
1. Stones in common bile-duct	36	57.1 (78.0–42.4)	26.0 (44.4–20.0)	4.3 (7.2–2.4)	22.0 (42.0–12.4)	31.0 (45.0–22.4)	25	75	1.0:1.2	1:5.1
2. Cancer of pancreas.....	16	64.6 (89.6–45.4)	25.1 (41.2–13.0)	3.1 (7.7–1.1)	22.0 (49.1–11.0)	39.5 (50.7–11.8)	13	87	1.0:1.5	1:7.1

TABLE 8.—ANALYSIS OF FECES.

Group 3. Intestinal Disease—No Jaundice. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Neutral Fat (Per Cent.)	Free Fatty Acids (Per Cent.)	Combined Fatty Acids (Per Cent.)	"Unsoaped" Fat		Average "Unsoaped" to Soaped Fat	Average Neutral Fat to Free Fatty Acid
							More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)		
1. Chronic intestinal catarrh..	34	29.0 (40.8–11.1)	9.8 (14.0–2.1)	0.9 (1.2–0.2)	8.9 (12.8–1.9)	19.2 (27.9–9.0)	—	100	1.0:2.0	1:9.9
2. Acute intestinal catarrh...	1	12.8	5.0	2.8	2.2	7.8	—	100	1.0:1.5	1:1.3
3. Chronic colitis.....	18	19.6 (45.0–5.3)	6.2 (14.0–1.6)	0.5 (2.0–0.2)	5.7 (15.6–1.4)	13.4 (41.0–3.7)	—	100	1.0:2.3	1:8.0

TABLE 9.—ANALYSIS OF FECES.

Group 4. Normal. (Cammidge.)

	No. of Cases	Total Fat (Per Cent.)	"Unsoaped" Fat (Per Cent.)	Neutral Fat (Per Cent.)	Free Fatty Acids (Per Cent.)	Combined Fatty Acids (Per Cent.)	"Unsoaped" Fat		Average "Unsoaped" to Soaped Fat	Average Neutral Fat to Free Fatty Acid
							More than Soaps (Per Cent.)	Less than Soaps (Per Cent.)		
Normal.....	10	21.0 (25.2–8.5)	10.0 (15.0–5.2)	1.0 (2.0–0.8)	9.0 (13.0–8.0)	11.0 (16.0–4.8)	50	60	1.0:1.0	1:9.0

are not strictly comparable with those given by the other cases who are taking a mixed diet.

The influence of intestinal affections on the relation between the soaps and unsoaped fats of the stool is well illustrated by the cases diagnosed as "intestinal catarrh" or "enteritis," in which there was neither jaundice nor pancreatitis to complicate the issue, for in 95 per cent. of these in the first series and 100 per cent. of these in the second, the combined fatty acids were present in excess. Similar results were also obtained with the feces of patients suffering from duodenal ulcer, gastric ulcer, and appendicitis. As all these conditions are associated with a pathological state of the intestinal mucous membrane and an abnormal intestinal flora, which may affect the pancreas either directly through the ducts or indirectly by way of the lymphatics, one sometimes meets with cases in which there is an excess of unsoaped fats. This is most common in connection with ulcers of the duodenum. There was evidence of such a secondary pancreatitis in fifteen out of the twenty-one cases that Cammidge included in the tables, and in about half of these there was a relative excess of unsoaped fats. A similar complication was found in three out of the sixteen cases of gastric ulcer, but only one of these showed an excess of neutral fats and free fatty acids in relation to combined fatty acids. Even when well-marked pancreatic insufficiency is present, and there are other indications that the digestive functions of the gland are interfered with, the excess of unsoaped fat that might be expected is often replaced by an abnormally high percentage of soaps, owing to the fat-splitting action of the intestinal bacteria. Thus, out of 83 cases of pancreatitis associated with an intestinal catarrh, the soaps were in excess in 70 per cent., and in only 30 per cent. were the unsoaped fats and combined fatty acids equal, or the former in excess.

It is obvious that when there is interference with the functions of the pancreas from cirrhosis consequent on inflammatory changes, and also an abnormal activity of the fat-splitting bacteria of the intestine, the relation between the soaps and the unsoaped fats will vary with the relative intensity of the two, and no hard and fast rule can be laid down; each case must be judged on its merits and on the indications to be obtained by other methods of examination. Of these, the presence of indicanuria, an excess of inorganic ash in the feces, and the results of the pancreatic insufficiency tests are the most important. The varying relations found in my cases of duodenal ulcer and gastric ulcer are examples of this, and the same explanation probably holds good for the cases of sprue and pernicious anemia that Cammidge examined. Stones in the common bile-duct and gall-bladder have frequently been found not to be associated with a disturbance in the relations between the unsoaped fats and combined fatty acids as might theoretically be expected, especially when there has been no obstruction to the bile flow. Cammidge is inclined to think that this may be due to the abnormal activity of fat-splitting bacteria, for in such cases there is generally evidence of cholangeitis, and this, as well as the gall-stone formation, is probably consequent upon the invasion of the biliary tract by intestinal

organisms which have ascended beyond their normal habitat to the level of the common bile-duct.

The following methods are used for analyzing fecal fat (Cammidge): "A weighed quantity of the feces, which have been dried to a constant weight, if necessary with the addition of alcohol but without any added sulphuric acid, is extracted with ether in a Soxhlet apparatus continuously for forty-eight hours. After the completion of the extraction the ether is evaporated off, the flask containing the ether extract is heated in a steam oven for an hour, cooled and weighed. This extract (A) gives the weight of the neutral fats and free fatty acids contained in the weight of feces taken. If the extraction thimble is allowed to stand in the air until the smell of ether has disappeared, and is then dried and weighed, this weight, plus the weight of the extract and less the weight of the thimble, will give the weight of the quantity of dry feces used for the extraction, and may therefore be used as a check on this, or may replace the original weighing process if desired. The residue from the thimble is now transferred to a porcelain dish and thoroughly ground up with a dilute watery or alcoholic solution of hydrochloric acid. The mixture is tested to see that it is strongly acid and then evaporated to dryness on a boiling water-bath. The perfectly dry residue is now transferred to the thimble from which it came, and extracted in a Soxhlet apparatus with ether continuously for twenty-four hours. The ether is then distilled off, the flask is heated in a steam oven, cooled, and weighed as before. This ether extract (B) contains the fatty acids present as soaps. The volatile fatty acids in the first extract (A) may now be estimated by washing with hot water, filtering, washing the residue on the filter, first with hot water and then with ether, adding the ether to the residue in the flask, drying, and weighing the flask after the ether has been evaporated off. The non-volatile fatty acids are then determined by dissolving the residue in the flask in a considerable excess of absolute alcohol, or a mixture of alcohol and ether, and titrating with a decinormal solution of alcoholic potash, using phenolphthalein as the indicator. Since 1 c.c. of decinormal potash corresponds to .0284 gram of stearic acid, the weight of the fatty acids present can be calculated in terms of stearic acid by multiplying the number of cubic centimeters of decinormal soda used by .0284. The difference between this result and the weight of the ether extract previously determined will give the weight of the neutral fat and any lipoids that may be present. The amount of the latter is so small, that for practical purposes it may be neglected and the value obtained, taken as being that of the neutral fat. If it is considered necessary to estimate the cholesterin separately, the solution with which the titration was carried out is evaporated to dryness and warmed with alcoholic potash. The amount of lecithin is then estimated from the phosphoric acid content of the ash. The weight of the saponified fatty acids in the second extract (B) can be checked by dissolving the dried residue in alcohol and titrating with decinormal alcoholic potash, and then expressing the result in terms of stearic acid in the way employed for the estimation of the non-volatile fatty acids

in the extract (A). As a rule, it will be found that the figures obtained by weighing and titration agree fairly closely, but those given by the latter method are to be preferred.

"The neutral fats and fatty acids in the ether extract (A) may also be estimated by Hoppe-Seyler's method. The extract is dissolved in ether, and shaken with an excess of dilute sodium carbonate solution. The mixture is then placed in a separating funnel and left to stand for a few hours. The watery solution is separated and well shaken with ether, which is again separated. The two ether extracts are now combined, evaporated to dryness, cooled, and weighed. The result gives the weight of the neutral fat (and lipoids), while the difference between this and the original weight of the dry ether extract (A) gives the weight of the free fatty acids."

For routine analyses, Cammidge employs the following method:

About 0.5 gram of the dried feces is weighed out and introduced into the lower bulb of a Schmidt-Werner milk tube as described for the rapid estimation of the total fat. Ten c.c. of distilled water are then added, and the tube is heated for a quarter of an hour in a boiling water-bath, occasionally rotated to mix its contents well. After being cooled, the tube is filled up to the 50 c.c. mark with ether, and the consequent steps of the process carried out as for the estimation of the total fat; in fact, the two experiments are usually carried out side by side, the tubes being labeled A and B respectively. The ether extract of the A tube, the contents of which have been heated with dilute hydrochloric acid, gives the total fat; the ether extract of the B tube, in which water only was used, gives the sum of the neutral fats and free fatty acids, and for convenience of reference Cammidge generally speaks of the latter as the "unsaponified," or better, "unsoaped," fats, while the difference between the two gives the combined fatty acids or soaps. To determine the relation between the neutral fats and free fatty acids, the ether extract from the B tube is dissolved in an excess of ether (about 20 c.c.), a few drops of an alcoholic solution of phenolphthalein are added, and it is then titrated with decinormal alcoholic soda. The number of c.c. used to neutralize the solution multiplied by 0.0284 grams, gives the weight of free fatty acids contained in the quantity of dry feces originally used, and the difference between this and the weight of the ether extract gives the quantity of neutral fat (and lipoids).

The whole process can be carried out in the space of an hour or so after the feces have been dried down, and if only sufficient of the mixed moist stool to yield a couple of grams of dry material are employed, the whole of the estimations may be made from beginning to end in six to eight hours, most of the time being occupied by the drying of the feces to a constant weight and waiting for the ether extracts to clear.

Considerable variations occur in the percentage of the fatty constituents of the feces, even in health, but as a rule a normal adult, taking an ordinary mixed diet, gives figures which lie between the following extremes:

Total fat	15-25	per cent. of the dry weight
"Unsoaped" fat	10-15	" " " " " "
Neutral fat	1-2	" " " " " "
Free fatty acids	9-13	" " " " " "
Combined fatty acids	10-15	" " " " " "

By means of the nephelometric principle, which has been used by Bloor for the determination of the fat in blood and milk, Laws and Bloor have found it possible to make an accurate determination of the fat in the stools in about one hour. The method consists essentially in extracting directly with acidified alcohol and ether, filtering the extract, then precipitating the fat in a watery solution and comparing the cloudy suspension so obtained with that of a similarly prepared standard solution.

5. Creatorrhea.—As Sladden says, creatorrhea is a natural corollary of azotorrhea. This phenomenon has been known for many years. It is very simple to recognize, since in a film of normal feces examined under a microscope it is rare to find more than one or two muscle-fibers in a field, and most often they are entirely absent.

According to Kleineberger the finding of creatorrhea is about the best evidence for pancreatic insufficiency; Wertheimer concurs, as does Von Ehrmann, who stipulates, however, that the patient's meat ration for a few days previously should not exceed two ounces daily. He compared the effect of giving pancreatin upon azotorrhea and creatorrhea, and came to the conclusion that the effect was quantitative, not qualitative, i.e., there was still impaired digestion of muscle-fiber, although the digestion of nitrogenous food as a whole was so improved that the nitrogen loss became normal. In a later paper (1913) von Ehrmann still maintains this view, which has also been confirmed by Oscar Gross. How this apparently selective action of pancreatic extract comes to pass is not at all clear; Allen, arguing from a case of carcinoma of the duodenum with complete occlusion of the pancreatic duct, in which no creatorrhea could be found, suggested the possibility of an internal secretion of the pancreas taking an essential part in the digestion of muscle-fibers. In Barbour's case and in the experience of Sladden the administration of pancreatic extract may clear up a creatorrhea, and in any case it appears to be a policy of rather doubtful utility to call in a fresh internal secretion to account for an observation otherwise "unexplained."

The creatorrhea test is not free from fallacies—diarrhea or even rather abnormal peristalsis may lead to appearance of muscle-fibers in the feces, and Allen states that a condition of achylia gastrica may produce a similar result. If these possibilities are excluded a finding of creatorrhea has great positive significance. A negative observation has not, however, the same value for the exclusion of pancreatic disease, although, as Pratt remarks, in cases where pancreatic insufficiency has been definitely excluded well-marked creatorrhea has not been observed. Glaessner drew attention to the possible action of proteolytic enzymes of

intestinal or bacterial origin, to render a negative observation of less value for diagnosis.

6. Sahli's Glutoid Capsule Test.—In 1897, Sahli recommended the following test for pancreatic sufficiency: Gelatine capsules are made and hardened in formalin. The capsule is filled with iodoform, methylene blue or salicylic acid. This capsule is not soluble in gastric juice, but is acted upon in the presence of pancreatic juice with the liberation of its contents. In a normal subject the contents should be found in the urine or saliva within four hours. When iodoform is used the saliva will react with starch to give a blue color, showing the presence of iodin. When salicylic acid is used, the urine will give a port wine or red color with a solution of ferric chlorid if the salicylates are excreted in the saliva. If methylene blue is used, the urine will be blue or green in color.

Mention may be made here of Müller and Schlecht's adaptation of Sahli's glutoid capsule test to the detection of the tryptic ferment in the feces. A gelatin capsule hardened in an alcoholic solution or formalin is filled with powdered wood charcoal and floated on 10 or 15 c.c. of a liquid stool, which must not have been filtered, in a wide test-tube, so that it does not touch the walls, and incubated at 37° C. If the feces contain a normal proportion of trypsin, the capsule should dissolve in about half an hour, and its contents will stain the fluid in the tube black. If no trypsin is present, the capsule will remain unaltered for a day or so before it finally dissolves.

This test has the same drawbacks as Sahli's, and particularly the difficulty of properly adjusting the hardness of the gelatin and preventing accidental opening of the capsule. It has now been replaced by the more satisfactory casein tests.

Wallenfang found the reaction delayed in four out of eight healthy individuals.

According to Pratt, this test is unreliable since one case with definite disease of the pancreas gave a normal reaction, whereas another case with normal pancreas gave a very much delayed reaction.

Ferreira introduced a modification, giving encapsulated salicin, but as the nature and origin of the ferment which splits salicin is not known, the method merely adds a further objection to those urged against the simpler test of Sahli.

7. Schmidt Cell-Nuclei Test.—The observation made by Schmidt that cell-nuclei are digested only by the pancreatic secretion is the physiological basis of the test. The method has been assailed on theoretical as well as on practical grounds. Brugsch and his pupil Hesse have been the chief opponents of the method, but they have failed to prove that it is untrustworthy, and Schmidt's assertion that the gastric juice does not act upon cell-nuclei seems to be true. Strauch, working in Abderhalden's laboratory, showed that pure pancreatic juice completely dissolved the cell-nuclei in six to eight hours; pure intestinal juice (erepsin) and pure gastric juice, on the contrary, did not digest them.

Pratt found in a dog with complete pancreatic achylia, produced by separating the pancreas from the duodenum, that the cell-nuclei were not digested. The gastric juice was normal, and there was no disease of the intestine. Thus clear evidence was presented by this experiment that the normal secretions of stomach and intestine were unable to digest the cell-nuclei.

Raw beef containing a fair amount of fibrous tissue is cut into cubes measuring about 0.5 cm. in size. These are hardened in alcohol, and placed in small bags made of silk gauze. They should be immersed in water for several hours before using. The bags are recovered from the stools and the meat cubes examined for the presence of nuclei after paraffin sections have been prepared. The test is positive if the nuclei are preserved and take the stain, negative if the nuclei are digested. Einhorn has used thymus gland instead of beef. This modification appears to be a distinct improvement.

Recently Kashiwado, a pupil of Schmidt, has simplified the nucleus test. By gastric digestion the nuclei of the thymus gland are isolated. The nuclei are then stained with hematoxylin, and a powder prepared, composed of equal parts of lycopodium and nuclei. Two capsules, each containing 0.25 gram of the mixture, are administered after dinner or supper. If the nuclei are not digested they are easily recognized in the stools.

Pratt has not observed a definite case of pancreatic insufficiency in which the nucleus test was negative. The value of the test is impaired by the fact that the meat cubes must remain in the intestine not less than six nor more than thirty hours. If the period is too short the nuclei are undigested by normal pancreatic juice. Putrefaction may cause the disappearance of the nuclei if the meat cubes remain more than a day in the intestine. In one of his cases the silk bags were recovered in the feces five days after they were taken into the stomach.

The preservation of the nuclei, with the test properly carried out, does not necessarily imply complete absence of the pancreatic secretion. In a case of fatty diarrhea with achylia gastrica the nuclei were not digested, but Spooner and Pratt demonstrated trypsin in the stomach by Volhard's method. If with the nuclei test one can recognize cases in which there is a deficient secretion of pancreatic juice, the value of the method will not be diminished but enhanced. As already stated, there are few cases of pancreatic disease in which total absence of pancreatic secretion occurs, and these are readily diagnosticated.

In 1906 Schmidt described cases in which he maintained that there was functional pancreatic achylia.

Pratt also concurs with the view that there are cases of functional pancreatic hypochylia.

According to Sladden, it seems probable that if diarrhea can be excluded a positive outcome of the test may be of help, but a negative result is hardly to be trusted; a positive result, moreover, is only likely to be obtained when the pancreas is practically completely out of action.

Fronzig modified the Schmidt test by using the nucleated red cells

of the blood of a goose as a source for his nucleoprotein, mixing the defibrinated blood with barium sulphate, which acts as an indicator in the feces which have to be searched for undigested red corpuscles. It is noteworthy that Wohlgemuth, von Westemijk, and Glaessner and Popper have all denied the presence of a nucleolytic ferment in the pancreatic juice, but Fronzig's work seems to contravene this denial (Sladden).

The Schmidt nuclear test has not lacked critics who have attributed some digestive power or nuclear tissue to intestinal and even to gastric juice, not to mention bacterial agencies. At the Johns Hopkins Hospital it has been possible to apply a crucial test, so to speak, to the problem involved by making observations on a patient known to have a complete absence of pancreatic secretion in the intestinal tract. Atchley added thymus gland, one of the tissues richest in nucleoprotein, to the food of the patient, and estimated the urinary output of uric acid. Inasmuch as the metabolism of the purins derived from the digestion of nucleoproteins leads to a prompt increase in the production of uric acid which is readily demonstrated in a healthy subject, this factor can be used to ascertain whether the nuclear tissue has been digested and the purins have been made available in the gastro-intestinal tract. That this conversion actually occurred in the absence of both bile and pancreatic juice in Atchley's patient was attested by the marked rise in uric acid excretion following the ingestion of 150 grams of thymus as a supplement to a purin-free diet. The exogenous output of uric acid exceeded the endogenous level by more than half a gram. This demonstration of the digestion of thymus nuclei with the production of uric acid independently of the pancreatic secretion, together with a negative result of the Schmidt test in the same case and in many others reported, "definitely points out the worthlessness of the Schmidt nuclear test for pancreatic function."

Analysis of Urine.—1. Diastase (Amylase) in the Urine.—Wohlgemuth has shown that laceration of the dog's pancreas gives rise to a rapid and marked increase in the quantity of diastase in both blood and urine. The method he employed required twenty-four hours for its completion, which is a great disadvantage in the study of human cases, and he has, therefore, so modified it that the result may be obtained in one-half hour. Using this method with normal human sera (150 cases), Wohlgemuth and Noguchi found the normal value to be 8 to 16; the highest normal value found was 32. Thus, if a lesion of the pancreas is suspected in a patient who has received a severe blow on the abdomen, a value of 64 greatly strengthens the supposition.

Marino reports a quantitative study of urinary diastase in various diseases. He used the method of Wohlgemuth. The author finds (1) that the excretion of diastase in the urine is greatly lessened in nephritis and in diabetes mellitus. (2) In pancreatic disease the urinary diastase is increased in quantity. This, the author believes, is a very important sign of pancreatic disease. (3) As a functional test of the kidney, the quantitative estimation of diastase is valuable. (4) In pernicious anemia

and in secondary anemia, the diastase of the urine is markedly decreased. The diminution seems to be greater in pernicious than in secondary anemia, though the number of cases studied was too small to formulate a rule.

In making determinations of the diastatic ferments in the urine, according to Wohlgemuth's method, Neumann calls attention to the fact that reliable estimations should be based upon the twenty-four-hour output. The total diastatic ferment amount, *per diem,* varies much with the same individual and appears to be influenced by psychic factors rather than by changes in the diet. Generally the diastatic power of the blood-serum is less than that of the urine. This is found to be definitely decreased in diabetes mellitus, the amount of reduction being of some prognostic value. It is also diminished in pernicious anemia, Basedow's disease and in some forms of nephritis. The notable increase in pancreatic disease is of real diagnostic worth. There is a slight increase in urinary diastase and in some febrile conditions. Investigations carried out in a number of other diseases showed no great deviation from the normal.

The work of Wohlgemuth has been confirmed by Corbett, Yvon, Noguchi, and others. Hirschberger found a large amount of diastase in the urine of two cases of pancreatitis, and Wynhausen in two cases of cancer of the pancreas.

In our experience, the determination of amylase in the urine by the method of Wohlgemuth throws very much light on the condition of the pancreas. In obstruction of the duct of Wirsung either by cancer, gallstone, enlarged glands, etc., the amylase of the urine is much increased in output. In organic disease of the pancreas, a similar state of affairs exists.

Amylolytic ferment is present in definite quantity, 6.6 to 8 units, according to Stocks in the blood-serum and urine of all healthy individuals, and has also been found in all the body fluids examined. The level is practically constant in the blood-serum. The level in the urine is subject to diurnal variations due chiefly to the digestive functions. The ferment is of pancreatic origin and is absorbed directly by the blood. No proof of the action of antiamylase has been found. Disease of the kidneys causing any diminished permeability of these organs reduces the amount of ferment in the urine and consequently raises the amount in the blood. Any disturbance in the ratio D (blood) : D and M (urine) indicates renal insufficiency in all such cases. Severe passive congestion also raises the amount of amylase in the blood. With these exceptions any increase of the ferment in the blood-serum denotes pancreatic mischief. The values have been found raised by Stocks in all cases of pancreatic disease, the increase depending on the degree of obstruction in any part of the gland or its ducts, and on the acuteness of the condition. The highest values were found in a case of acute pancreatitis. The estimation of the amylolytic capacity of the blood-serum and the urine is a most delicate test of the efficiency of the pancreas, and consequently is a most delicate and reliable test for disease of the

pancreas. A simplified modification of Wohlgemuth's method was used by Stocks in his work.

According to Schleicher the value of the various methods of testing the external secretion of the pancreas is still *sub judice*. In order to determine the reliability of the various tests, the author has employed the most popular methods in 22 instances in which the external pancreatic secretion seemed to be abnormal. The methods of Gross and Müller for the detection of trypsin and the diastase test of Wohlgemuth are reliable; the nuclein test of Schmidt, however, is less trustworthy. A definite opinion concerning the methods of Winternitz and Ehrmann cannot as yet be given. The oil-breakfast of Boldyreff-Volhard furnishes reliable results. On the other hand, the test of Schlecht and the glutoid capsules of Sahli gives less reliable results. The methods of Gross and Wohlgemuth may be recommended for acute cases; here the qualitative and quantitative demonstration of trypsin and diastase in the feces as well as in the urine must be made. Both tests are very reliable; they alone will evince the degree of functional pancreatic activity. Other tests may be employed to corroborate the findings obtained with the Gross and Wohlgemuth methods.

Quantitative Determination of Amylolytic Activity.—Wohlgemuth's Method (as described by Hawk):

Arrange a series of test-tubes with diminishing quantities of urine, introduce into each tube 5 c.c. of 1 per cent. solution of soluble starch and place each tube at once in a bath of ice-water. When all the tubes have been prepared in this way and placed in the ice-water bath, they are transferred to a water-bath or incubator and kept at 38° C. for from 30 minutes to an hour. At the end of this digestion period the tubes are again removed to the bath of ice-water in order that the action of the enzyme may be stopped.

Dilute the contents of each tube to within about ½ inch of the top, with water, add one drop of a N/10 solution of iodin and shake the tube and contents thoroughly. A series of colors ranging from dark blue through bluish-violet and reddish-yellow to yellow, will be formed. The dark blue color shows the presence of unchanged starch, the bluish-violet indicates a mixture of starch and erythrodextrin, whereas the reddish-yellow signifies that erythrodextrin and maltose are present and the yellow solution denotes the complete transformation of starch into maltose. Examine the tubes carefully before a white background and select the last tube in the series which shows the entire absence of all blue color, thus indicating that the starch has been completely transformed into dextrins and sugar. In case of indecision between two tubes, add an extra drop of the iodin solution, and observe them again, after shaking.

Calculation.—The amylolytic activity of a given solution is expressed in terms of the activity of 1 c.c. of such a solution. For example, if it is found that 0.02 c.c. of an amylolytic solution, acting at 38° C., completely transforms the starch in 5 c.c. of a 1 per cent. starch solution

in 30 minutes, the amylolytic activity of such a solution would be expressed as follows:

$$D = 250$$

This indicates that 1 c.c. of the solution under examination possesses the power of completely digesting 250 c.c. of 1 per cent. starch solution in 30 minutes at 38° C.

2. Cammidge Reaction.—The reaction of Cammidge, about which so much has lately been written, does not seem to be so successful in the hands of others as in those of the author. Cammidge says: "My experience with the improved method has been most satisfactory, for in every case where pancreatitis has been found to be present, the urine has given more or less marked reaction, corresponding to the extent of the lesions. Normal urines have given no reaction, and control cases . . . where there was no pancreatic lesion, have also proved negative."

In careful studies recently reported by Wilson, Kenney, Whipple, and others, little value is accorded the test. Wilson, reporting on 504 tests from Mayo's clinic, says: "The end results, judged by Mr. Cammidge's own criteria, must be considered as a means of diagnosticating disease of the pancreas, as both valueless and misleading. There is no apparent clinical relationship between disease of the pancreas and any of our various types of end reaction."

Kinney, reporting from Deaver's service in the German Hospital, Philadelphia, says: "Very little dependence can be put upon a negative reaction, and a positive reaction can only be considered of value as a confirmatory examination."

Cammidge examined 1500 samples of urine derived from 1475 cases, with special reference to this reaction. Of 17 cases of acute and subacute pancreatitis, 13 gave a positive pancreatic reaction. Six of these at operation showed an acute inflammation. In 3 the pancreatitis had been the sequel to an attack of mumps. In 859 cases of chronic pancreatitis the reaction was obtained 364 times; 40 times in 60 cases of stone in the common duct; 18 times in 7 cases of stone in the gall-bladder; 4 times in 10 cases of growth in the common duct; 103 times in 194 cases of intestinal catarrh; 42 times in 53 cases of catarrhal jaundice; 27 times in 50 cases of duodenal ulcer; 5 times in 47 cases of gastric ulcer; 8 times in 13 cases of sprue; 4 times in 6 cases of pernicious anemia; 2 times in 11 cases of tuberculosis; 6 times in 8 cases of typhoid fever; and 14 times in 21 cases of cirrhosis of the liver and pancreas. All of these conditions Cammidge considered as causes of chronic pancreatitis. In 4 cases of pancreatic calculi the reaction was obtained 3 times: twice in 4 cases of pancreatic cyst and once in a case of pancreatic infantilism. In 73 cases of cancer of the pancreas 24 positive reactions were obtained. In a miscellaneous group, 461 of the 467 cases gave a negative pancreatic reaction. In the last group are specimens of urine from 50 presumably healthy individuals, none of which gave a positive pancreatic reaction. Cammidge believed that his experience in these five years confirmed the claim that he made—that the

reaction is clinically useful in its improved form. He did not think that it was pathognomonic or that taken alone it would enable one to form a correct opinion in every instance. But he did think that when the results of the examination of the urine were considered in conjunction with the clinical symptoms and an analysis of the feces, a trustworthy diagnosis might be arrived at in nearly every case of pancreatic disease. As to the nature of the reaction, it is probably due to the destruction of some substance with a glyconucleoprotein content which yields a pentose on hydrolysis, and since the percentage of pentose in the dry weight of the pancreas is nearly five times as great as in any other organ of the body, and is more easily combined and more readily set free than the corresponding sugar in other tissues, the reaction is obtained with much greater frequency and more constantly in lesions involving active degenerative changes in that organ than in others. A strongly positive reaction cannot be accepted as a conclusive indication of pancreatic infection. Cirrhosis of the pancreas will not give rise to the reaction unless there is also some active inflammation going on at the same time. So, too, in cancer of the pancreas, there is usually no reaction, for no destruction of gland substance takes place unless secondary inflammation sets in.

To perform the Cammidge test, the following *procedure* may be followed: A twenty-four-hour specimen of urine is collected and preserved with thymol or toluene, or chloroform. It is filtered, and the filtrate tested for albumin and for glucose. If albumin is present, the urine is boiled and acidified and the precipitated protein is filtered off, the test of Cammidge being performed on the filtrate. If glucose is present, the urine is fermented for 12 to 24 hours, in order to get rid of the glucose, filtered and tested. If both substances are present, a combination of the above processes should be resorted to.

After the urine is made protein- and glucose-free, it is treated as follows: 20 c.c. of the clear, filtered urine are placed in a small flask, covered with an inverted funnel to act as a condenser, and 1 c.c. of strong hydrochloric acid is added. It is now boiled for 10 minutes on a sand bath. The boiling should not be too vigorous, and the flame should be turned low for the greater part of the time.

The flask is now cooled in cold running water, and brought up to 20 c.c. with distilled water. Four grams of lead carbonate are now slowly added, and shaken, at first gently and then more thoroughly. The shaking should be repeated at intervals until no more carbon dioxid is evolved. The contents of the flask are now filtered through a moistened filter paper.

Four grams of powdered basic lead acetate are added. The mixture is thoroughly shaken for some minutes, and allowed to stand. It is filtered through a moistened filter paper.

To the clear, colorless filtrate 2 grams of powdered sodium sulphate are added and well shaken for several minutes; it is slowly brought up to the boiling point on a sand bath, shaking from time to time. The ex-

cess of lead is now removed, and it is important that the heating and shaking be done carefully.

The mixture is cooled and filtered. Ten c.c. of filtrate are measured and 8 c.c. of water added. To this are added 8 grams of phenylhydrazin hydrochlorid, 2 grams of sodium acetate and 1 c.c. of 50 per cent. acetic acid. The mixture is boiled in a flask with a funnel condenser on a sand bath for 10 minutes. It must not boil too vigorously. It is filtered hot through a moistened filter paper, and brought up to 15 c.c. with hot distilled water. It should be allowed to stand for 4 or 5 hours at room temperature or in the ice-box.

If the test is positive, typical osazone crystals are produced which are more circular and tuft-like than the glucosazone crystals. The crystals are soluble in 33 per cent. sulphuric acid.

Loewi's Adrenalin Test.—Loewi, in 1908, found in a series of animal experiments relative to diabetes and pancreatic function that adrenalin chlorid in a strength of 1-1000, when instilled into the conjunctival sac of dogs and cats normally caused mydriasis. In animals, however, from which the pancreas had been removed, thus setting up a complete pancreatic insufficiency, mydriasis occurred constantly, in from 24 to 65 hours after operation. In another series of dogs in which the pancreatic juice was diverted through an external fistula no mydriasis occurred, even after many months. From this Loewi concluded that mydriasis was the result of the failure of the internal secretion of the gland. Of the basis of further experiments in dogs in which diabetes had been established, he concluded that the internal secretion had at least two independent functions, the glycogenic function and the adrenalin function. His argument in explanation of the phenomenon of mydriasis is that the pancreas furnishes through its internal secretion a chemical substance which is a depressor, or inhibitor of the sympathetic nervous system. So, when adrenalin, which is a sympathetic excitant, is instilled into the normal eye, the sympathetic nerves supplying the ciliary muscle are stimulated, but not strongly enough to overcome the pancreatic inhibition. If by reason of disease this inhibitory control of the pancreas is vitiated or lost, adrenalin is unobstructed or less obstructed in its action and causes mydriasis.

Whether this hypothesis of Loewi is the true explanation of the phenomenon or not, is open to question. In view of the present uncertainty regarding the antagonism of pancreas and adrenals, and indeed the influence of all glands of internal secretion upon one another, one perhaps should be slow to accept it. At least, however, no more rational theory than Loewi's has come to our notice. Loewi found that normally the human eye did not react to adrenalin. He tried the reaction in 48 clinical cases embracing such conditions as carcinoma, nephritis, tuberculosis, pneumonia, rheumatic fever, and diabetes. In 36 there was a dilatation of the pupil averaging one millimeter. His series included 18 cases of diabetes, of which 10 had mydriasis, and 3 cases of Graves' disease, all of which reacted positively. This latter result Loewi explained on the ground of a hyperactivity of the sympathetic system

in hyperthyroidism, which is in excess of the pancreatic depressor influence. One writer, Cords, has since accounted for mydriasis in the presence of exophthalmos on the basis of a corneal inflammation, leading to a more rapid absorption of the drug.

Since Loewi published his paper in 1908 there have been comparatively few reports in the literature of experience with the test. It is looked upon with favor by several English clinicians, notably Garrod, Humphrey and Sladden. Zak, in Germany, found it undependable as a pancreatic guide; he obtained positive results in many cases with lesions of the stomach, intestine and peritoneum.

Sladden, however, at St. Bartholomew's Hospital, in a series of 51 tests found 11 positive cases. Of these 11, in 5 pancreatic disease was demonstrated, in 4 it was probable, in 1 there was exophthalmic goiter, and in the last one only it was excluded at operation. On the basis of this experience Sladden writes that "the adrenalin reaction is associated with pancreatic disease, and with lesions closely associated with the pancreas either anatomically or physiologically so frequently as to render the phenomenon worthy of serious attention as a guide to diagnosis.

Decker, working in the author's laboratory, found that of 500 cases, which covered a wide range of injury and disease, there were 18 positive reactions, only 2 of which were known to have pancreatic lesions: (1) a case of carcinoma of the pancreas; (2) chronic pancreatitis associated with gall-stones. On the other hand, in cases known definitely to be pancreatic, there were seven negative reactions distributed as follows: carcinoma, 5; cyst, 1; lymphangitis, 1.

It might be of interest to note on account of the etiological relationship which is thought to exist between gall-bladder and pancreatic disease that in 15 gall-bladder cases there were 3 positive reactions. In only one of these was the pancreas involved.

From this record it is apparent (1) that the reaction is not pathognomonic of pancreatic disease; (2) that it is absent in cases which by other methods are proved to have pancreatic lesions. Whether or not in 16 of the 18 positive cases there was an associated pancreatic lesion, it is impossible to tell without a doubt. At least, judging either by clinical history, physical examination, tests of the urine or stools, or intra-abdominal examination at the time of the operation, there was no definite evidence of pancreatic disturbance.

The technic of the test is as follows: Three drops of adrenalin chlorid solution 1-1000 are instilled into one conjunctival sac, the other eye being used as control, and followed in five minutes by three more drops, the point being to fill the conjunctival sac. Aside from a slight smarting for a few seconds, the patient suffers no discomfort, and no subsequent harmful effects. Dilatation of the pupil should occur within an hour if the test is positive. The amount of enlargement varies from less than a millimeter to complete pupillary dilatation and seldom occurs in less than a 15-minute period. The length of time taken for action seems to be dependent on the facility with which the adrenalin

is absorbed rather than the amount used. Care must be taken to avoid impaired and inflamed eyes.

Carbohydrate Tests.—ALIMENTARY GLYCOSURIA.—The patient is given 100 grams of glucose, dissolved in tea or water, two hours after breakfast, and the urine is examined for sugar every two hours until six hours have passed, and also at the end of twenty-four hours.

In cases where the pancreas is suspected of being the seat of disease, a positive result with this test will accentuate the suspicion, but should not be taken as a pathognomonic sign of pancreatic involvement.

BIBLIOGRAPHY

General References

ALBU. Beitrag zur Diagnostik der inneren und chirurgischen Pancreaserkrankungen. 1911.
CAMMIDGE. Glycosuria and allied disorders. 1913.
——— The Feces of children and adults. 1915.
FRANK. Arch. f. Verdauungskr., 1912, xviii, 367.
GLAESSNER. Ergebn. d. inn. Med. u. Kinderh., 1910, vi, 29.
KÖRTE. Die chirurgische Krankheiten des Pancreas. 1898.
OPIE. Diseases of the pancreas. 1903.
OSER. Nothnagel's "Practice." 1903.
ROBSON AND CAMMIDGE. Surgery of the pancreas. 1913.
SCHMIDT AND STRASSBURGER. Die Faeces des Menschen, 1911.
SLADDEN. Quart. Jour. Med., 1914, vii, 455.

Analysis of Duodenal Contents

VON BARTH-WEHRENALT. Internat. Beitr. z. Path. u. Therap. d. Ernährungsstör., 1910, i, 530.
CROHN. Am. Jour. Med. Sc., 1913, cxlv, 393.
CHASE AND MYERS. Arch. of Int. Med., 1913, xii, 528.
EINHORN. Berl. klin. Wchnschr., 1910, xlvii, 522.
EINHORN AND ROSENBLOOM. Arch. of Int. Med., 1910, vi, 666.
FRANK. Arch. f. Verdauungskr., 1912, xviii, 367.
GROSS. Deutsch. Arch. f. klin. Med., 1912, cviii, 106.
HESS. Arch. Int. Med., 1912, x, 37.
JUNGHANS. Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs., 1911, 49.
KAHN. Surg., Gynec. and Obst., 1917, xxiv, 333.
KLEINBERGER. Berl. klin. Wchnschr., 1909, xlvi, 2329.

Analysis of Regurgitated Duodenal Contents by Examination of Stomach Contents

BOLDYREFF. Arch. f. d. ges. Physiol., 1908, cxxi, 13.
——— Russk. Vratch, 1904, iv, 39.
v. EHRMANN. Berl. klin. Wchnschr., 1912, xlix, 1363.
——— Ztschr. f. klin. Med., 1910, lxix, 319.
LANDAU AND REASNICKI. *Ibid.*, 1916, lxxx, Nos. 3, 4, 307-350.
LONG AND MUHLEMANN. Arch. Int. Med., 1914, xiii, 314.
MAHLENBERG. Ztschr. f. d. ges. Physiol. u. Path. d. Stoffwechs, 1909, iv, 643, 689.
MICHAILOW. Arch. f. Verdauungskr., 1912, xviii, 273.
SPENCER, MEYER, REHFUSS AND HAWK. Am. Jour. Physiol., 1916, xxxix, 459.
VOLHARD. München. med. Wchnschr., 1907, liv, 403.

Analysis of Feces for Pancreatic Enzymes

BALINT AND MOLNAR. Berl. klin. Wchnschr., 1910, xlvii, 1620.
GROSS. Deutsch. Arch. f. klin. Med., 1912, cviii, 106.
HAWK. Arch. Int. Med., 1911, viii, 382, 552.
HIRSCHBERG. Deutsch. med. Wchnschr., 1910, li, 1992.
HUAYAMA. Ztschr. f. exper. Path. u. Therap., 1911, viii, 624.
LIFSCHUTZ. Arch. f. Verdauungskr., 1913, xix, 562.
MATKO. *Ibid.*, 1913, xix, 662.
MULLER AND SCHLECHT. Med. Klin., 1909, v, 573, 618.
OPPENHEIMER. Die Fermente, 1911.
ROTKY. München. med. Wchnschr., 1913, lx, 2158.
SCHLECHT AND WIENS. Zentralbl. f. inn. Med., 1909, 12.
STANIEK. Med. Klin., 1910, vi, 1023.
WEIZBERG. Arch. f. Verdauungskr., 1911, xvii, 533.
WOHLGEMUTH. Biochem. Ztschr., 1912, xxxix, 302, 324.
WYNHAUSEN. Berl. klin. Wchnschr., 1909, xlvi, 1406.
ZUCCOLA. Riforma med., 1913, xxix, 393.

Azotorrhea as Test for Pancreatic Insufficiency

BARBOUR. Arch. Int. Med., 1911, viii, 5, 662.
BRUGSCH. Deutsch. med. Wchnschr., 1909, 2307.
——— Ztschr. f. klin. Med., 1906, lviii, 518.
CAMMIDGE. Practitioner, 1914, xcii, 621.
DEAVER. Jour. Am. Med. Assn., 1911, lvi, 1079.
DELFINO. Deutsch. Ztschr. f. Chir., 1913, cxxi, 280.
v. EHRMANN. Berl. klin. Wchnschr., 1912, xlix, 1363.
GROSS. Ergebn. d. Wissensch. Med., 1911.
MOSENTHAL. Arch. Int. Med., 1912, ix, 339.
PRATT. Am. Jour. Med. Sc., 1912, cxliii, 313.
TILESTONE. Arch. Int. Med., 1912, ix, 526.

Steatorrhea as Test for Pancreatic Insufficiency

BITTORF. Deutsch. med. Wchnschr., 1912, xxxviii, 1034.
BRIGHT. Tr. Med.-Chir., Edinburgh, 1833, xviii, 1.
BRUGSCH. Deutsch. med. Wchnschr., 1909, 2307.
COHN AND PEISER. *Ibid.*, 1912, ii, 60.
v. EHRMANN. Berl. klin. Wchnschr., 1912, xlix, 1363.
v. EHRMANN AND KRUSPE. Ztschr. f. klin. Med., 1913, lxxviii, 122.
FALTA. Ztschr. f. klin. Med., 1910, lxxi, 17.
GANOD AND HURTLEY. Quart. Jour. Med., 1912, vi, 242.
JANSEN. Arch. di Pharmak., Specim., 1913, xiii, 15.
KEUTHE. Berl. klin. Wchnschr., 1909, xlvi, 47.
MULLER. Ztschr. f. klin. Med., 1887, xii, 45.
PRATT. Am. Jour. Med. Sc., 1913, cxliii, 313.
SALOMON. Deutsch. Klin. xii, 533.
TAUBER. Arch. f. Verdauungskr., 1912, xviii, 627.
WERTHEIMER. Ztschr. f. klin. Med., 1912, lxxvi, 57.
ZOJA. Morgagni, 1898, xl, 617; 1899, xli, 1.

Creatorrhea as a Test for Pancreatic Insufficiency

FERREIRA. Cited by Frank, Arch. f. Verdauungskr., 1912, xviii.
PRATT. Am. Jour. Med. Sc., 1912, cxliii, 313.
SAHLI. Deutsch. med. Wchnschr., 1897, xxiii, 7.
——— Deutsch. Arch. f. klin. Med., 1898, lxi, 445.

Schmidt Cell-Nuclei Test

ATCHLEY. Arch. Int. Med., 1915, xiv, 654.
BARBOUR. Arch. Int. Med., 1911, viii, 5, 662.
FRONZIG. Ztschr. f. klin. Med., 1913, lxxvii, 40.
GLAESSNER AND POPPER. Deutsch. Arch. f. klin. Med., 1909, xciv, 46.
KASHIWADO. *Ibid.*, 1911, civ, 584.
SCHMIDT. *Ibid.*, 1906, lxxxvii, 456.
——— München. med. Wchnschr., 1909, lvi, 1803.
VAN WESTENRIJK. Ztsch. f. exper. Path. u. Therap., 1910, 8.
WOHLGEMUTH. Berl. klin. Wchnschr., 1910, xlvii, 92.

Analysis of Urine, Diastase Test

COHNHEIM. Arch. f. path. Anat. u. Physiol., 1863, xxviii, 241.
COLE. Jour. Phys., 1904, xxx, 202.
CORBETT. Quart. Jour. Med., 1913, vi, 351.
GEYELIN. Arch. Int. Med., 1914, xiii, 96.
STOCKS. Quart. Jour. Med., 1916, ix, 180.
WALLIS. St. Bartholomew's Hosp. Rep., 1914, xlix.
WOHLGEMUTH AND NOGUCHI. Berl. klin. Wchnschr., 1886, xxiii, 34.

Analysis of Urine, Cammidge Reaction

CAMMIDGE. Lancet, 1904, 1, 782.
CARO AND WORNER. Berl. klin. Wchnschr., 1909, xlvi, 359.
DEAVER. Jour. Am. Med. Assn., 1911, lvi, 1079.
EICHLER AND SCHIROKAUER. Berl. klin. Wchnschr., 1909, xlvi, 352.
EVANS. Jour. of Physiol., 1912, xliv, 220.
GLAESSNER AND SIEGEL. *Ibid.*, 1904, xli, 440.
HAGEN. Beitr. z. klin. Chir., 1909, lxi, 3.
KEHR. München. med. Wchnschr., 1909, xlvi, 1062.
KORTE. Ann. Surg., 1912, iv, 23.
LANGER. Wien. klin. Wchnschr., 1913, xxvi, 9.
LIBERTINI. Riv. crit. di Clin. med., 1912, xi, 161.
MAASS. Med. Klin., 1909, v, 177.
MAYESINA. Mitt. a. d. Grenzgeb. d. Med. u. Chir., xxv, 403.
PILCHER. Ann. Surg., 1910, li, 88.
ROTH. Ztschr. f. klin. Med., 1909, lxvii, 222.
SLADDEN. St. Bartholomew's Hosp. Rep., 1914, xlix, 19.

Loewi's Adrenalin Test

CORDS. Die Adrenalinmydriasis. Wiesbaden, 1911.
DECKER. Bull. Allegheny County Med. Soc., 1916; Boston Med. and Surg. Jour., 1917, clxxvi, 867.
GLEY. Internat. Cong. Med., 1913, Section on Medicine, J., 44.
LOEWI. Arch. f. exper. Path. u. Pharmak., 1908, lix, 83.
PEMBERTON AND SWEET. Arch. Int. Med., 1912, x, 169.

CHAPTER III

LIVER FUNCTION TESTS

In order to study the functional activity of an organ, it has been customary to apply certain specific tests to the individual functions of that organ. Thus if an organ has several functions, tests are applied to one of these functions, and conclusions are drawn therefrom as to the capability of the organ to perform all of its offices. This has been especially the case in the investigation of the condition of the liver.

The liver has a multiplicity of duties to perform in the body, all of which are of essential importance. It is probable that each individual cell of the hepatic structure takes part in all of the liver functions; it is also possible that different portions of the liver lobule, and different conglomerations of the liver lobules may have specific functions. In the former case it is most likely that a reduction in the ability of the liver to perform one function will be accompanied by a proportional reduction in all the liver functions; in the latter case, one or more functions of the liver may be disturbed without affecting the other hepatic functions.

In order to appreciate the various methods for determining the liver functions, it is best to enumerate the different functions of the liver:

1. Secretion of bile.
2. Relation to carbohydrate metabolism.
   (*a*) Glycogen formation.
3. Relation to nitrogen metabolism.
   (*a*) Formation of the urea.
4. Detoxification function.
   (*a*) Formation of the conjugate sulphates and glycuronates.
   (*b*) Withholding of toxins and poisons.
5. The decomposition of the erythrocytes.
6. The formation of fibrinogen.
7. The formation of antithrombin.

The methods for the study of the liver functions are several. These tests can be classified in the following way:

1. A study of the carbohydrate tolerance of the liver; this will include the tests of general carbohydrate metabolism, tests of tolerance for special carbohydrates; for example, Bauer's galactose test, Strauss's levulose test, etc.
2. A study of the nitrogen excretion in the urine, including the urea, amino, and ammonia nitrogen fractions.
3. The urobilinogen excretion in the urine.
4. Analysis of the fibrinogen of the blood, which was found to disappear from the blood after liver extirpation.
5. A study of lipase and fibrinolytic ferments of the blood.
6. The phenoltetrachlorphthalein test.
7. The liver conjugation function of toxic substances.

Carbohydrate Tolerance Tests.—Strauss in 1898-1900 attempted to explain the reason for the conflicting results obtained by the French and German schools as to the value of the carbohydrate tolerance studies in liver disease. Roger, Achard and Castaigne, Baylac, and Bierens de Haan reported that valuable information may be obtained by a study of the carbohydrate metabolism in cases of hepatic disease. On the other hand, Quincke, Frerichs, von Noorden, Kraus and Ludwig, Bloch, and Müller obtained results which led them to conclude that there is no appreciable change in the sugar tolerance in such patients. According to Strauss, the contradictory results are due to the methods used by the various observers, to the difference in the carbohydrate used, and to the quantity of carbohydrate administered.

In 1873, Legg reported that obstruction of the bile duct prevents the appearance of glycosuria after Claude Bernard's *piqure*. From the results of Wittich, Kulz and Frerichs, Cohnheim, Dastre and Arthus, and Hergenhahn, it would seem that stagnation of bile is followed by a reduction or disappearance of the glycogen from the liver. As Weintraud points out, it does not follow from these experiments that the liver does not still form glycogen, but only that it has lost the power of *storing* glycogen. If this reasoning is correct, and is applicable to man, it is probable that, under conditions which require the collaboration of the liver for the preliminary storing of large amounts of carbohydrates, the icteric patient would show more easily than the healthy an alimentary glycosuria after a rich glucose intake (Weintraud).

On an ordinary diet, Kulz and Frerichs failed to find sugar in the urine of such patients. Frerichs administered 100 to 200 grams of glucose to 19 cases of hepatic disease, and only in two patients (phosphorus poisoning) did he find traces of glucose in the urine. In the other seventeen cases, no glucose was present. Roger recorded positive results in two cases of catarrhal jaundice and in one of gall-stones. Both von Noorden and Strauss never observed glycosuria in jaundiced individuals following the administration of 150 grams of glucose on a fasting stomach.

Lépine, in repeating Colrat's investigations (in French literature the testing of alimentary glycosuria is generally called *epreuve de Colrat*), supposed that not only disturbances of the portal circulation, but also any alteration of the liver cells, may be the cause of a glycosuria, owing to an insufficient retention of sugar in the liver. He, however, obtained positive results only in cases of atrophic cirrhosis, and not in carcinoma of the liver or fatty liver.

The results which others obtained after him may be best observed in the following table:

Table 1.—Results of Carbohydrate Tolerance Tests.

	Date	Atrophic Cirrhosis		Hypertrophic Cirrhosis	
		Positive	Negative	Positive	Negative
Lépine	1876	3	—	—	—
Couturier	1876	2	—	—	—
Robineaud	1878	2	—	—	3
Valmont	1879	2	4	—	2
Hardy	1879	—	1	—	—
Vulpian-Reymond	1879	—	—	—	1
Roger	1887	5	2	1	2
Moscatelli	1889	—	1	—	—
Bouchard	1890	2	—	—	—
Schapiro	1891	1	—	1	—
Kraus and Ludwig	1891	3	4	—	4
Bloch	1892	—	3	—	1
Surmount	1892	2	1	—	3
Colasanti	1895	—	7	—	—
Bierens de Haan	1895	2	1	11	7
Achard-Castaigne	1898	2	—	—	—
Strauss	1898	1	10	—	6
Naunyn	1900	—	8	—	—
Bruining (dextrose)	1902	2	11	—	—
" (saccharose)	1902	13	—	—	—

According to this table, negative results preponderate in the more recent investigations, in which the authors were not convinced of the presence of sugar by the mere positive results of the reduction tests.

1. Saccharose Test of Colrat and of Lépine.—First, 150-200 grams of saccharose (cane or beet-sugar) are administered to the subject on an empty stomach. The urine is collected and each specimen is examined by the Benedict solution. If glucose is present in the urine, the test is considered positive.

Barton states that a "weighty theoretical objection is the fact that cane-sugar must be converted into glucose in the alimentary tract before it can be utilized by the organism, and the power of the intestinal juices to produce this conversion is in each case an unknown quantity, and, therefore, a source of error."

2. The Levulose Test.—In 1899, Sachs recorded that upon extirpation of the liver of frogs, there was a lessened tolerance for levulose,

while the assimilability of other carbohydrates, such as glucose, galactose and arabinose was normal. Based upon these experiments, Strauss introduced the *levulose test for hepatic insufficiency.*

Method.—The patient is fasted over night. Then 100 grams of levulose are administered, dissolved in a glass of water; the urine voided for five to six hours after this test-meal is collected and analyzed for levulose by the Seliwanoff reagent.* It sometimes happens that patients feel nauseated after the partaking of this quantity of levulose and they may vomit.

Strauss concluded from seventy-seven experiments that about 80 per cent. of all patients with liver disease react positively to this levulose test. He found that only 4.5 per cent. of all hepatic cases show an alimentary glycosuria, thus demonstrating the superiority of levulose over dextrose as a means of testing liver function.

Normally the tolerance for levulose is not less than the tolerance for glucose. This is true of human beings (Voit, von Noorden), of rabbits (Blumenthal) and of dogs (Schlesinger), so that the meaning of alimentary levulosuria in hepatic disease is rather difficult of explanation. From his collective review of literature, Strauss, in 1913, reported that positive results with his levulose tests are obtained in the following conditions:

Table 2. Results with Levulose Test.

Normal individuals	15	per cent.
Congested liver	17	" "
Hepatic cirrhosis	83	" "
Syphilitic jaundice	75	" "
Obstructive jaundice	62.5	" "
Hepatic tumors	38	" "

Rowntree, Marshall and Chesney obtained only two positive results with this test in fourteen cases of cirrhosis of the liver. They have tabulated the results of Strauss, Ferranini, Landsberg, Chajes, von Halasz, Hohlweg, von Frey, Churchman, Falk and Saxl and Bruining.

The table on the following page taken from Rowntree, Hurwitz and Bloomfield shows the attitude of various workers concerning the value of the levulose test.

According to this table, it appears that the test is far from satisfactory, and only relative significance can be attributed to the findings both from the diagnostic and prognostic viewpoints.

3. Galactose Test.—Bauer, in 1906, recommended that galactose be used as a test for liver function. Strauss advises that 30 grams of galactose be administered in the morning on a fasting stomach, and the urine collected for the next five to six hours. The urine is tested for

* To 5 c.c. of Seliwanoff's reagent (prepared by dissolving 0.05 gram of resorcinol in 100 c.c. of dilute, 1:2, hydrochloric acid) in a test-tube add a few drops of the urine and heat the mixture to boiling. A positive reaction is indicated by the production of a red color and the separation of a red-brown precipitate. The latter may be dissolved in alcohol to which it will impart a striking red color.

TABLE 3.—RESULTS WITH LEVULOSE TEST.

Author	Normal Livers		Abnormal Livers		Remarks
	Cases	Positive	Cases	Positive	
Strauss.....	58	6	25	23	Considers test of value.
Ferranini.....	—	—	16	15	Test preferable to glucose test, which showed only 10 positive.
Landsberg.....	7	4	21	9	Test is of no importance.
Chajes.....	21	2	—	—	Thinks that positive findings are rare in normal cases.
von Halasz.....	20	1	23	8	The 8 positive findings were in cirrhosis. Test of value.
Hohlweg.....	—	—	30	9	Ten cases were chronic congestion.
von Frey.....	—	—	26	14	Considering only those positive with more than 0.1 gram sugar in urine.
Churchman.....	38	9	12	10	Test not conclusive.
Falk and Saxl....	—	—	351	259	Collected from literature.
Bruining.....	—	—	30	27	Considers test of value.

galactose by Fehling's reagent or Benedict's reagent, and more specifically by Tollen's reaction.*

Bauer found that it was of special value in cases of catarrhal jaundice, and this has been confirmed by Bondi and König, Reiss and Jehn, and Hirose.

Roubitschek studied the galactose tolerance of dogs during phosphorus poisoning. He found that galactosuria occurred with the acute degenerative changes produced in the liver cells. He believes it is only

* To equal volumes of urine and hydrochloric acid (sp. gr. 1.09) add a little phloroglucinol, and test the mixture in a boiling water bath. Galactose, pentrose and glycuronic acid will be indicated by the appearance of a red color. Galactose may be differentiated from the two latter substances in that its solutions exhibit no absorption bands upon spectroscopical examination, and that upon oxidation it yields uric acid.

the acute conditions injuring the liver parenchyma that interfere with the power of the organ to synthesize galactose to glycogen.

In chronic conditions, cirrhosis, passive congestion, etc., regeneration of liver cells may compensate functionally to a sufficient degree to prevent the occurrence of galactosuria.

Falk and Saxl, von Frey, and Hirose have observed that this test is not constant in diseases of the liver other than catarrhal jaundice.

Worner and Reiss have compared the levulose and galactose tests for liver insufficiency. They declare that when 40 grams of galactose are given for the test, an elimination of 3 grams and more must be regarded as positively pathologic. Levulose given for this purpose must be in the dose of 100 grams, and more than 0.7 grams must be eliminated before we can speak of a pathologic output. Alimentary levulosuria testifies in a general way to some injury of the liver. Pathologic alimentary galactosuria, on the other hand, testifies to the existence of certain definite liver affections and thus permits differential diagnosis of catarrhal jaundice, phosphorus poisoning and fatty liver. Circumscribed liver affections, gall-stones and cancer do not lower the tolerance for galactose unless infection is present.

Strauss, from a comparative study of the levulose and galactose tests, found that the former gave a positive finding more than twice as frequently as did the latter. In the fourteen cases of hepatic cirrhosis, two were found positive with the galactose test (Rowntree, Marshall and Chesney).

Bloomfield and Hurwitz concluded from their studies of *lactose tolerance* in chloroform and phosphorus poisoned dogs, that little significance can be attached to carbohydrate tolerance tests (by means of any sugar) as an index of liver function.

Maliwa tabulates the findings in twelve cases of liver disease, in ten of orthostatic albuminuria, and in twenty-one other patients, after ingestion of 40 grams galactose, fasting. The data demonstrate that intolerance for galactose is by no means an unequivocal symptom of insufficiency of the liver. The kidneys often have something to do with it. When the latter is the case, the galactose elimination begins abruptly and in large amounts, dropping off equally abruptly. With insufficiency of the liver, the curve of elimination is generally more protracted.

Friedmann and Strouse have drawn the following conclusions from their work: "We feel justified in stating that whereas tests for carbohydrate tolerance, when grouped as we have grouped our results (*see* Table 4), may indicate in a general way functional disability of an organ under suspicion, the tests can never be interpreted in an absolutely specific manner. The possibility of implication of other organs must always be borne in mind. Functional inefficiency of one gland, sufficient to lower the threshold of carbohydrate metabolism, may be entirely compensated for by functional hyperactivity of a correlated gland. Even in apparently clean-cut individual cases the tests

TABLE 4.—GROUP-REACTIONS TO CARBOHYDRATE TESTS.

Group	Levulose		Dextrose		Total
	Number	Per Cent. Positive	Number	Per Cent. Positive	
Liver:					
Cirrhosis	6	12.5	10	70.0	16
Cholecystitis pancreatitis	6	66.0	2	100.0	8
Cancer liver and pancreas	2	100.0	2	100.0	4
Catarrhal jaundice	4	50.0	4	50.0	8
Thyroid:					
Simple goiter	5	0.0	3	0.0	8
Hyperthyroidism	18	50.0	11	63.5	29
Hypothyroidism	3	0.0	—	—	3
Hypophysis	7	43.0	3	67.0	10
Pluriglandular	7	58.0	5	20.0	12
Neurasthenia	33	12.0	14	28.0	47
Miscellaneous	30	10.0	17	35.0	47

do not follow the course one might logically expect, and, therefore, in doubtful cases their value must remain very limited."

Urinary Nitrogen Partition.—In the liver certain stages in the intermediary metabolism of protein are completed. The amino-acids are broken down to ammonia, which is transformed to urea. As Weintraud says: "There remains to be investigated whether, in individual diseases of the liver, the urine yields any information regarding marked disturbances of these relations; likewise whether, instead of the end product of protein decomposition—namely, of urea—intermediary products appear which, like ammonia and the amino-acids, may be regarded as precursors of urea, and, finally, whether a lessened activity of the liver may be made entirely responsible for this insufficient conversion of nitrogenous substances."

In obstructive jaundice the urinary nitrogen partition has been usually found to be normal. Such reports were made by Mörner and Sjoquist, von Noorden and Friedrichsen, von Jaksch, Landau, Halpern, Simnitzki and Rodoslowow.

In cirrhosis of the liver, however, the nitrogen partition has been found to be abnormal. The ammonia values in cases of cirrhosis are remarkably high. Hallervorden and others obtained high values in comparison with the total nitrogen decomposition. For example, Mörner and Sjoquist in one case found the total daily nitrogen to be 20.748 grams, the total twenty-four hours' ammonia to be 2.4 grams, the ammonia nitrogen being 9.5 per cent. of the total nitrogen, and the urea nitrogen 84.6 per cent. In five cases Fawitzki calculated the ammonia nitrogen, on the basis of 100 parts of total nitrogen, as follows: 17.5, 10.7, 7.6, 10.0, 9.8 parts. Gumlich found 5.7 grams total nitrogen and 0.86 gram ammonia, the ammonia making up 12.3 per cent., and the urea 70 per cent. of the total nitrogen. In von Noorden's cases the

ammonia nitrogen reached 8.5 to 12.6 per cent. of the total nitrogen, the normal value being 3 to 5 per cent. One of Schubert's cases yielded ammonia nitrogen constantly over 10 per cent. of that of total nitrogen (in one day up to 18.17 per cent.). Such high values are, however, far from being usual. Indeed, in the cases of Stadelmann and Weintraud there were many which showed absolutely no increase in the ammonia output.

The relative figures found for urea are all below those of the normal value—90 per cent. of the total nitrogen (Landau). In Fawitski's six cases the average urea nitrogen values of long series of observations were found to be 78.9, 88.4, 87.1, 89.2, 88.8, 89.0 per cent. of the total nitrogen; in Munzer's cases, 82.2 and 86.7 per cent.; in Mörner and Sjoquist's, 84.6, 73.2, and 84.9 per cent. In von Noorden's observations, however, the values were 77 to 79 per cent., and in Gumlich's cases only 70 to 77.6 per cent. Urea given per os to such patients is promptly excreted (Setti and De Stefanio).

In 1907, Glaessner showed that in most instances of liver disease, an unusually high excretion of amino-acid nitrogen occurred and that the ratio of amino-acid nitrogen to total non-protein nitrogen was increased. On the other hand, Levene and Van Slyke and Yoshida each reported two cases of liver cirrhosis in which a normal percentage of amino-acid nitrogen existed.

Falk and Saxl compared several tests to determine disturbance of liver function in cases of hepatic disease. For purposes of study they have divided affections of the liver into four groups: Group I includes tumors of the liver, like cancer, sarcoma, ecchinococcus, amyloid liver, leukemia, and chronic passive congestion. The parenchyma is affected secondarily. In Group II they place all infections and intoxications (typhoid, pneumonia, tuberculosis, chloroform, alcohol, phosphorus, for example). Group III contains those conditions in which the liver may be pathologically affected by the escape of bile from its normal passages (icterus from gall-stones, from complete closure of the common duct, and from catarrhal conditions). In Group IV are placed the atrophic and hypertrophic cirrhosis of the liver. As functional tests, Falk and Saxl employed only those of supposedly known value, i.e., levulose, urobilin, and the nitrogenous bodies—amino-acids, polypeptids, ammonia. Each of these tests was applied to their cases. The analysis of their results and of those reported in literature shows that a marked disturbance of liver function is disclosed, particularly in cirrhosis of the liver. Nitrogen ratios, urobilin excretion, and tolerance for levulose all reveal abnormalities. Such constancy of findings is seen in no other hepatic disease. In Falk and Saxl's cases these disturbances of function appeared early in the course of the disease. Often it was possible, by finding urobilinuria, levulosuria, and especially abnormal nitrogenous ratios, to arrive at the correct diagnosis at a stage of the affection when only vague gastric symptoms were present. In the remaining three groups the findings were less useful in a diagnostic way.

Von Moraczewski and Herzfeld studied the excretion of certain

urinary constituents in hepatic disease. Examination of healthy persons as well as of 4 cases of cirrhosis of the liver, 2 cases of catarrhal jaundice, one of diabetes, one of pernicious anemia, one of leukemic tumor of the liver and spleen, one of acid intoxication and 2 of chronic renal disease showed that, in general, there ensues a certain form of excretion in hepatic diseases which resembles the excretory conditions while fasting. There is a high degree of uric acid, ammonia and acetone excretion. Large amounts of volatile fatty acids and indican are always found in the urine. The acetone increase is especially noticeable when on a milk regimen. The increase of certain urinary constituents runs parallel with a decrease of others. Nitrogen, for instance, is markedly decreased. In pernicious anemia and leukemia, uric acid is augmented; this, however, is not the case with ammonia, acetone and indican. In diabetes, acetone and ammonia are increased; uric acid and the volatile fatty acids are not increased. In acid intoxication the volatile fatty acids, ammonia and acetone, are increased, while the excretion of uric acid remains about normal. In nephritis nothing of import was noted in the excretion of the urinary constituents. Hence, in diseases of the liver, all aforementioned substances were increased, while in affections of the blood but a few of these substances were excreted in larger amounts.

In eclampsia the liver is, of course, markedly involved. In such conditions, Ewing and Wolf found a high ammonia excretion and a low urea output. Edgar reports similar observations. Van Slyke has recently pointed out that this is, perhaps, the only constant factor in eclampsia.

The total nitrogen, urea nitrogen, ammonia nitrogen and amino-nitrogen of the urine are determined in the following ways: A 24-hour specimen of urine is collected, preserved with thymol, and analyzed.

1. Total Nitrogen.—*Kjeldahl Method* (Hawk).—*Principle.*—The principle of this method is the conversion of the various nitrogenous bodies of the urine into ammonium sulphate by boiling with concentrated sulphuric acid, the subsequent decomposition of the ammonium sulphate by means of a fixed alkali (NaOH) and the collection of the liberated ammonia in an acid of known strength. Finally, this partly neutralized acid solution is titrated with an alkali of known strength and the nitrogen content of the urine under examination computed.

Procedure.—Place 5 c.c. of urine in a 500 c.c. long-necked Jena-glass Kjeldahl flask, add 20 c.c. of concentrated sulphuric acid and about 0.2 gram of copper sulphate and boil the mixture for some time after it is colorless (about one hour). If a suitable hood or fume chamber is not available the sulphuric acid vapors may be carried away by suction. Connect the outlet tube of a 2-3 liter wash bottle filled with caustic soda solution with a suction pump. Connect the inlet tube with a Folin fume absorption tube. If such a tube is not at hand a small funnel may be attached. Place the absorption tube loosely over the

mouth of the digestion flask and pass a constant current of air through the apparatus.

Allow the flask to cool and dilute the contents with about 200 c.c. of ammonia-free water. Add a little more of a concentrated solution of NaOH than is necessary to neutralize the sulphuric acid and introduce into the flask a little coarse pumice stone or a few pieces of granulated zinc, to prevent bumping, and a small piece of paraffin to lessen the tendency to froth. By means of a safety-tube connect the flask with a condenser so arranged that the delivery-tube passes into a vessel containing a known volume (the volume used depending upon the nitrogen content of the urine) of N/10 sulphuric acid, using care that the end of the delivery-tube reaches beneath the surface of the fluid. Mix the contents of the distillation flask very thoroughly by shaking and distill the mixture until its volume has diminished about one-half. Titrate the partly neutralized N/10 sulphuric acid solution by means of N/10 sodium hydroxid, using congo red as an indicator, and calculate the content of nitrogen of the urine examined.

Calculation.—Subtract the number of cubic centimeters of N/10 sodium hydroxid used in the titration from the number of c.c. of N/10 sulphuric acid taken. The remainder is equivalent to the number of c.c. of N/10 sulphuric acid, neutralized by the ammonia of the urine. One c.c. of N/10 sulphuric acid is equivalent to 0.0014 gram of nitrogen. Therefore, if y represents the volume of urine used in the determination, and y the number of cubic centimeters of N/10 sulphuric acid neutralized by the ammonia of the urine, we have the following proportion:

$$y : 100 :: y \times 0.0014 : x \text{ (percentage of nitrogen in the urine examined).}$$

2. Urea Nitrogen.—(*a*) *Marshall's Urease Method.—Procedure.*—Two 5 c.c. portions of the urine are measured into flasks of 200-300 c.c. capacity and diluted with distilled water to about 100-125 c.c. One c.c. of a 10 per cent. solution of urease is added to one flask, a few drops of toluene to each and the solution allowed to remain, well-stoppered, at room temperature over night (or five hours). The fluid in each flask is titrated to a distinct pink color with N/10 hydrochloric acid using methyl orange as an indicator. A few cubic centimeters of the enzyme solution used should also be titrated to determine the amount of N/10 hydrochloric acid required to neutralize 1 c.c.

Calculation.—The amount of hydrochloric acid required for the contents of the flask containing the urine and enzyme solution, less the amount used for 5 c.c. of urine alone and that previously determined for 1 c.c. of enzyme solution, corresponds to the urea originally present in the sample of urine. Since 1 c.c. of N/10 HCL is equivalent to 3 mg. of urea, the number of c.c. required, multiplied by 0.6, gives the value of urea expressed in grams per liter of urine.

(*b*) *Benedict's Method.*—The urea is decomposed by heating with a mixture of potassium bisulphate and zinc sulphate. The fact that the hydrolyzing agent is a salt and that the digestion takes place in

the practical absence of water seems to insure less decomposition of substances other than urea. The ammonia formed is distilled off and determined in the usual manner.

Procedure.—Five c.c. of urine are introduced into a rather wide Jena-glass test-tube, about 3 grams of potassium bisulphate and 1-2 grams of zinc sulphate added, a small quantity of powdered pumice and a bit of paraffin are introduced and the mixture boiled almost to dryness either over a free flame or by immersion in a sulphuric acid bath at about 130° F. The tubes are then weighed (a screw clamp is convenient) and immersed for three-fourths of their length in a bath of sulphuric acid at a temperature of 162°-165° F. (not lower) for one hour.

The contents of the tube are then washed into an 800 c.c. Kjeldahl distillation flask, diluted to about 400 c.c. with water, made alkaline by the addition of 15-20 c.c. of 10 per cent. KOH (or 25 c.c. 15 per cent. Na_2CO_3) and distilled as usual in the Kjeldahl method. The value obtained must be corrected for ammonia by a separate determination of the latter.

3. Ammonia Nitrogen.—*Folin's Method.—Principle.*—The ammonia of the urine is set free by the addition of an alkali and this ammonia is then carried over by an air current into a flask containing a measured amount of standard acid. The excess acid is then titrated. The necessity for distillation is avoided.

Procedure.—Place 25 c.c. of urine in an aërometer cylinder, 30-40 cm. in height, add about 1 gram of dry sodium carbonate and introduce some crude petroleum to prevent foaming. Insert into the neck of the cylinder a rubber stopper provided with two perforations, into each of which passes a glass tube, one of which reaches below the surface of the liquid. The shorter tube (10 cm. in length) is connected with a calcium chlorid tube filled with cotton, and this tube is in turn joined to a glass tube extending to the bottom of a 500 c.c. wide-mouthed flask which is intended to absorb the ammonia, and for this purpose should contain 20 c.c. of N/10 sulphuric acid, 200 c.c. of ammonia-free distilled water and a few drops of an indicator (alizarin red or Congo red). To insure the complete absorption of the ammonia the absorption flask is provided with a Folin improved absorption tube, which is very effective in causing the air passing from the cylinder to come into intimate contact with the acid in the absorption flask. In order to exclude any error due to the presence of ammonia in the air, a similar absorption apparatus to the one just described is attached to the other side of the aërometer cylinder, thus insuring the passage of ammonia-free air into the cylinder. With an ordinary filter-pump and good water-pressure the last trace of ammonia should be removed from the cylinder in about one and one-half hours. The number of c.c. of the N/10 sulphuric acid neutralized by the ammonia of the urine may be determined by direct titration with N/10 sodium hydroxid.

Calculation.—Subtract the number of c.c. of N/10 sodium hydroxid used in the titration from the number of c.c. of N/10 sulphuric acid

taken. The remainder is the number of c.c. of N/10 sulphuric acid neutralized by the NH_3 of the urine. One c.c. of N/10 sulphuric acid is equivalent to 0.0017 gram of NH_3. Therefore, if y represents the volume of urine used in the determination and y' the number of c.c. of N/10 sulphuric acid neutralized by the NH_3 of the urine, we have the following proportion:

$$y : 100 :: y' \times 0.0017 : x \text{ (percentage of } NH_3 \text{ in the urine examined).}$$

4. Amino-acid Nitrogen.—*Henriques-Sörensen Method.—Procedure.*—The determination of the amino-acids is carried out as follows: The solution to be analyzed, if carbonates, phosphates and ammonia are absent, is made neutral to litmus (paper) and the solution titrated with formaldehyd as below. In case carbonates, phosphates or ammonia are present a preliminary treatment is necessary which will vary according to the quantity of ammonia present.

(1) *For Small Amounts of Ammonia.*—Applicable to most urines. Fifty c.c. of the material under examination is pipetted into a 100 c.c. measuring flask and 1 c.c. phenolphthalein solution and 2 grams of solid barium chlorid are added; the whole is shaken, to saturate the solution with barium chlorid; saturated barium hydroxid solution is added until the red color of the phenolphthalein develops and then an excess of 5 c.c. is added. The flask is filled to the graduation mark with water, shaken and permitted to stand for 15 minutes after which it is filtered through a dry filter. Eighty c.c. of the clear red filtrate (which corresponds to 40 c.c. of the liquid under examination) are placed in a 100 c.c. measuring flask, neutralized to litmus and diluted to 100 c.c. with freshly boiled water. Equal portions of this solution, 40 c.c. (equivalent to 16 c.c. of the original solution), may be taken for analysis, one for the formal titration and the other for the determination of ammonia nitrogen.

(2) *For Large Amounts of Ammonia.*—After the treatment with phenolphthalein, barium chlorid and barium hydroxid, and after the solution has been diluted to 100 c.c. as in (1) above, the ammonia is distilled off, *in vacuo.*

In case the solution is deeply colored, as in protein digests, it may be necessary to decolorize before the titration is attempted.

Final Titration.—For the final titration a volume of from 20-40 c.c. which contains approximately 0.025 gram of nitrogen is the most desirable. A control solution is run composed of an equal volume of boiled distilled water and 20 c.c. of the formaldehyd mixture. This control solution is colored so that its tint matches that of the solution to be titrated.

To this control is added about half the volume of N/5 alkali which will be used in the titration of the solution under investigation and it is then titrated with N/5 acid to a faint red (first stage).

An additional drop of N/5 alkali is added, which imparts a distinct red to the solution (second stage).

The solution to be analyzed is now titrated to the color produced

TABLE 5.—COMPARATIVE STUDY OF LIVER FUNCTION TESTS (Rowntree, Marshall and Chesney).

	Total Number of Tests	Total Number of Positive Tests	Cirrhosis of Liver		Myocardial Insufficiency		Carcinoma		Syphilitic Hepatitis		Pernicious Anemia		Polyserositis		Miscellaneous	
No. of cases tested			14		12		3		2		2		2		10	
Name of Test				+		+		+		+		+		+		+
Phthalein in feces	42	16	*14*	6	*9*	1	*3*	2	*2*	1	*2*	2	*2*	0	*9*	4
Phthalein in urine	34	21	*11*	7	*10*	5	*3*	2	*2*	1	*1*	0	*2*	2	*5*	4
Fibrinogen	36	8	*14*	6	*11*	1	*1*	0	*2*	0	—	—	*2*	0	*1*	1
Fibrinolytic ferment	40	7	*14*	7	*12*	0	*1*	0	*2*	0	—	—	*2*	0	*9*	0
Lipase	39	5	*14*	2	*11*	1	*1*	0	*2*	0	—	—	*2*	0	*9*	0
Galactose	29	4	*11*	2	*7*	0	*1*	0	*2*	1	*1*	0	*2*	0	*4*	2
Levulose	18	2	*10*	2	*3*	0	*0*	0	—	—	*1*	0	*1*	0	*3*	0
Blood:																
Urea N, per cent	37	9	*14*	5	*12*	1	*1*	0	*2*	1	—	—	*2*	2	*6*	0
NH_2N	35	25	*14*	10	*9*	7	*1*	1	*2*	1	—	—	*2*	1	*6*	4
Urine:																
Urea N, per cent:	41	11	*14*	3	*11*	3	*2*	0	*2*	2	*2*	1	*2*	0	*8*	2
NH_3N, per cent	41	28	*14*	10	*11*	7	*2*	0	*2*	2	*2*	2	*2*	2	*8*	5
NH_2N, per cent	22	12	*7*	5	*6*	2	*1*	0	*1*	2	*1*	0	*1*	1	*5*	2

Explanation: The figures in Italics represent the number of times tests were made and the other figures indicate the number of positive results in the various types of diseases.

in the second stage of the control. The formaldehyd mixture is now added: 10 c.c. for each 20 c.c. of the solution, and the mixture again titrated to the second stage with N/5 alkali.

Two drops of the N/5 alkali are now added to the control solution, which assumes a deep red color (third stage). Fifth normal alkali is added to the solution under examination until it assumes a color corresponding to the third stage of the control. This completes the titration.

Calculation.—The calculations are similar to those which pertain to any acidimetry procedure. Each c.c. of an N/5 alkali or acid solution is equivalent to 0.0028 gram of nitrogen. An example will illustrate the procedure: 40 c.c. of solution (16 c.c. of urine) required 5.10 c.c. N/5 NaOH; control, 0.10 c.c. N/5 NaOH; total required for amino-acids 5.00 c.c. equivalent to 0.0014 gram of nitrogen. Ammonia nitrogen in 16 c.c. of urine, 0.007 gram N. Then 0.014 — 0.007 = 0.007 gram amino-acid nitrogen in 16 c.c. of urine.

Rowntree, Marshall and Chesney made a comparative study of a number of liver function tests in 45 cases. The results obtained may best be represented by Table 5, taken from their published report.

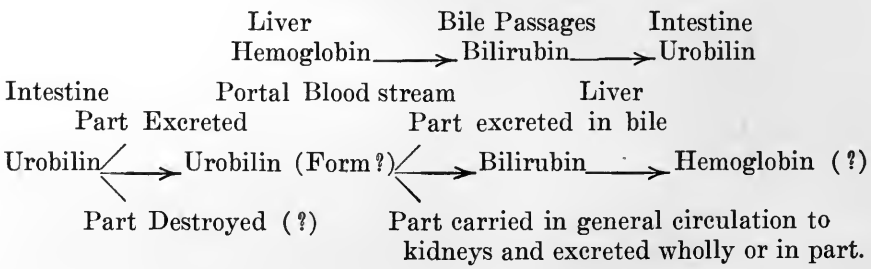
Urobilinogen Excretion.—Significance of Urobilin Excretion.—Wilbur and Addis draw the following conclusions as to the significance of urobilin excretion.

1. The intestinal formation of urobilin from the decomposition of bile present within the bowel is the usual mode of origin. In complete closure of the common duct due to carcinoma, for instance, there is usually absence of urobilin from the urine, bile and stools.

2. There is evidence that the diseased liver may originate urobilin either directly as a product of its cells, or indirectly from decomposition of bilirubin within the bile passages. Thus, patients in whom no bile is reaching the intestine may nevertheless show urobilin in large amounts in the bile, and to some extent even in the urine and stools if the liver itself is diseased or functionally deficient. The urobilin in the urine and stools of such cases must be due to excretion from the blood of urobilin absorbed from the liver.

The usual sequence in hemoglobin metabolism may be represented by the accompanying scheme (Wilbur and Addis).

Schematic Representation of Usual Sequence in Hemoglobin Metabolism



On this hypothesis, bilirubin is broken down to urobilin in the intestine and the absorbed urobilin is synthesized in the liver to bilirubin and perhaps by a reverse action again to hemoglobin.

Under abnormal conditions in the liver, formation of bilirubin from the absorbed urobilin from the intestine may fail, and further, there may be a reduction of bilirubin to urobilin in the liver itself, a reversal of the normal process.

Under these circumstances, the accumulated urobilin in the liver will be absorbed by the blood stream and excreted in the urine.

3. An increased quantity of urobilin in the stools indicates increased blood destruction.

In such cases, urobilin may appear in excessive amount in the urine apart from obvious disease of the liver, because, the quantity of urobilin carried to the liver from the intestine being so much increased, the fraction carried past the liver into the general circulation and so to the kidneys is sufficient to lead to an obvious urobilinuria.

4. There is a close association between increase of urobilin in the urine and disturbance of liver function. But even if increased blood destruction is excluded by urobilin estimations in the stools, it is not at present possible to regard the amount of urobilin in the urine as an approximate guide to the functional efficiency of the liver. The occasional occurrence of large amounts of urobilin in the urine of patients in whom there is no reason to suspect damage to the liver, the great and irregular fluctuations of urobilin excretion in cases of liver disease from hour to hour and from day to day, and the want of knowledge as to the fate of urobilin in the blood and tissues make it necessary to recognize that there may be other factors besides the condition of the liver and the amount of blood destruction which influence the excretion of urobilin in the urine.

Value of Urobilin Test.—The conclusions of Wilbur and Addis, so far as the clinical value of the test is concerned, are as follows:

1. *Hepatic Cirrhosis.*—Urobilinuria is of marked value as evidence of a definite pathological change in the enlarged liver of alcoholic persons and occurs almost constantly in the hypertrophic stage of hepatic cirrhosis. In our cases, we did not find any increase of urobilin in the stools. While in advanced cases, part of the urobilin in the urine may be derived from the formation of urobilin from bilirubin in the liver, it is probable that as a rule it is due to failure on the part of the liver to synthesize to bilirubin, the urobilin brought to it from the intestine.

2. *Hepatic Stasis.*—Estimations of urobilin in the urine are of value in judging the amount of damage done to the liver parenchyma by chronic passive congestion. Hence, a marked increase of it is of ominous prognostic significance in cardiac decompensation.

3. *Jaundice.*—Urobilinuria is absent or insignificant in cases of obstructive jaundice. Its intermittent occurrence points to an incomplete obstruction with concomitant damage to the liver. It is present in the icterus of cases of increased destruction of the red blood-cells. Careful

studies for urobilin should be made in suspected cases of chloroform poisoning, acute yellow atrophy and other similar conditions in which the liver is apt to be damaged.

4. *Malaria.*—The great increase of urobilin in the stools and the urobilinuria which occurs in severe cases of malaria is of diagnostic importance in obscure febrile conditions. The persistence of urobilinuria after malarial attacks, when there is no fever and no increase of urobilin in the stools, can be taken as evidence of some complication such as hepatic cirrhosis or abscess.

5. *Anemias.*—By means of urobilin estimations in the stools and urine, those forms of anemia associated with an increased blood destruction may be differentiated. It is probably in this field that the most valuable clinical results will be obtained. The contrast between the very large total urobilin elimination in the author's cases of pernicious anemia as compared with the small amount found in secondary anemias following hemorrhage or carcinoma was very striking.

6. *Pneumonia.*—A slight increase of urobilin with additional amounts at the time of resolution does not indicate severe damage of the liver parenchyma in this disease. It is probably due to a clogging of the liver with the products of resolution and absorbed hemoglobin from the affected lung. A study of the curve of this elimination may be of value in certain pneumonias. Allowances must always be made for other factors, such as constipation, diarrhea and the inability of the kidney to eliminate urobilin readily in the presence of a complicating severe nephritis. The early appearance of large quantities of urobilin in the urine in pneumonia, particularly in the presence of jaundice, makes the prognosis grave. The occurrence of urobilin in the serum of pneumonia patients is of the gravest prognostic significance. It is recognized apparently by our present methods only in the presence of a degree of cyanosis markedly unfavorable to the life of the individual.

7. *Carcinoma.*—Except in those cases in which there is practically a complete disappearance of urobilin from the excretions, due to an obstruction of the common duct from carcinoma, the author has not found its estimation in carcinomatous cases to be of evident clinical value.

8. *Infections.*—In infectious processes causing parenchymatous changes in the liver or accompanied by hemolysis, urobilin estimations are of definite value in estimating both of these factors in any given case. There is apparently an approximation between the urobilin excretion in the urine and feces and the amount of blood destruction. In such infectious processes as amebic colitis, the appearance and constant presence of urobilin may be of marked value in indicating the presence of inflammatory liver conditions, particularly abscess.

9. *Scarlet Fever, Measles.*—The majority of measles cases present urobilinuria in excess, but, as might be expected, the amount is less and the duration shorter than in scarlet fever with its more definite damage to the hepatic cells.

10. *Decompensation.*—As indicated previously, marked urobilinuria

is indicative of pathological change in the liver in the stasis of decompensation. This circulatory stasis calls for the use of increasing amounts of hemoglobin within the body and is associated with a greater production and elimination of bilirubin leading to an inability on the part of the overburdened and congested liver to modify the abnormal amounts of urobilin reaching it. A return of compensation is often indicated by the disappearance of a qualitative urobilinogen or urobilin test. Decompensations in markedly anemic persons are not so apt to be accompanied by increased amounts of urobilin until very profound stasis occurs.

11. *Nephritis.*—Severe nephritis of various types, even in the presence of increased production of urobilin, may prevent definite urobilinuria. Large amounts of urobilin in the urine may be taken as indirect evidence of a certain degree of efficiency in the renal epithelium.

Quantitative Methods for Urobilin Estimation (Wilbur and Addis).—1. *In Urine.*—Following is a summary of only the most essential points of the methods which are in use:

Almost all the work done has been with the urine. The methods may be divided roughly into those requiring a separation of the urobilin from the urine by means of precipitation with ammonium sulphate; secondly, those depending on the spectroscopic absorption bands of urobilin; and thirdly, those based on the property of fluorescence, and lastly colorimetric methods depending on the violet color produced by copper sulphate in urobilin solutions.

(*a*) *Methods Involving Salting Out of Urobilin by Means of Ammonium Sulphate.*—Hoppe-Seyler in 1891 estimated urobilin in urine gravimetrically. The acidified urine was saturated with ammonium sulphate, and the precipitate extracted with chloroform and alcohol. The solution was evaporated, the residue dissolved in ether, filtered and evaporated, and the residue dissolved in alcohol, evaporated and weighed.

Viglezio, in the same year, recommended a method which depended on the salting out of the urobilin with ammonium sulphate and its solution in alcohol. The amount was gauged by noting how much of this solution was required to produce a certain grade of fluorescence or the appearance of the spectroscopic absorption bands, when it was added to a solution of zinc chlorid.

Studenski added copper sulphate to the urine, precipitated with ammonium sulphate, and dissolved the copper compound of urobilin in chloroform. This color was compared with that produced by copper sulphate in a solution of urobilin of known strength.

Ladage recognized the necessity of taking the urobilinogen into account and recommended that iodin be added to convert it to urobilin. The urobilin was then precipitated by saturation of the urine with ammonium sulphate, and the acid chloroform solution diluted until the spectroscopic absorption became invisible. In Fr. Müller and Huppert's method a mixture of barium chlorid and barium hydrate is added to the urine, and the precipitate is filtered off and washed with

hot water. The excess of barium in the filtrate is removed by sodium sulphate, and the filtrate after neutralization with sulphuric acid is saturated with ammonium sulphate. The precipitated urobilin is filtered off, allowed to dry in the air and extracted three times after acidification with a warm mixture of alcohol and ether. This solution is then evaporated to a convenient bulk and the amount of urobilin estimated spectrophotometrically.

Charnas, in 1909, gave as an alternative method to the one based on the spectroscopic characteristics of urobilin, a gravimetric method in which after all urobilinogen in an ether extract of the urine has been converted into urobilin by exposure to sunlight, the urobilin is separated by water from the ether, and after filtration precipitated by ammonium sulphate, dried, dissolved in alcohol, the alcohol evaporated in vacuo and the residue weighed.

(*b*) *Spectroscopic Methods.*—Gerhardt (1889) and Beck (1895) made estimations by means of the spectrophotometer using Vierordt's tables of the grade of light extinction in different regions of the spectroscope in normal urine and solutions of urobilin.

Saillet (1897) extracted the freshly passed urine with acetic ether. In another specimen the urobilinogen was converted into urobilin by exposure to light and then extracted. The two extracts were added together and diluted until the spectroscopic band disappeared.

Conner and Roper (1918) made approximate measurements of the amount of urobilin in urine by noting the number of dilutions required to obliterate the urobilin band in the urine. They recommend the addition of a few drops of Lugol's solution to the urine in order to convert the urobilinogen into urobilin.

Auche (1909) found that a solution of urobilin of 1:200,000, prepared by a method he describes, is sufficient to make the five absorption bands of potassium permanganate all of equal intensity, although without it some are lighter than others. He, therefore, superimposes the urine, which in some cases has to be extracted with a thymol-chloroform solution, over a solution of potassium permanganate and dilutes the urine or its extract until all the bands are of equal intensity.

Simpson (1910) acidifies the urine with sulphuric acid and exposes it to light for some time in order to convert urobilinogen to urobilin. It is then diluted until the urobilin band disappears. This method gives fairly accurate results when a dilution of fifteen or more volumes is necessary. With smaller amounts the reading is apt to be obscured by other pigments.

Hausmann (1913) adds copper sulphate to the urine and dilutes until the spectrum becomes invisible.

Henocque, Hayem, Gautretet, Deniques, Hildebrandt, Riva and Zoja have used one modification or another of these spectroscopic methods.

Charnas (1909) makes the urine alkaline, allows it to ferment at 37° C. (98.6° F.) for from twenty-four to forty-eight hours, acidifies with tartaric acid and extracts with ether and petroleum benzin. To a portion of the ether extract an ethereal solution of paradimethylamino-

benzaldehyd is added and two to three drops of absolute alcohol saturated with hydrochloric acid gas. After dilution, if necessary, with alcohol, a spectrophotometric reading is made.

(*c*) *Methods Depending on the Property of Fluorescence.*—Grimm (1893), Fischler (1906), Grigant and Monod (1909), and Descomps (1909) estimated the amount of urobilin by the intensity of the fluorescence present in the zinc salt filtrate of the urine. Various devices are adopted to aid the conversion of urobilinogen into urobilin.

(*d*) *Colorimetric Methods.*—Bogomaloff (1892) used a colorimetric method depending on the depth of the red-violet color produced in a chloroform extract of urine containing urobilin by the addition of copper sulphate.

Braunstein (1903) used a modification of this method.

Flatow and Brunell (1913) estimate the amount of urobilinogen in fresh urine from the depth of the red color given on the addition of Ehrlich's reagent. As a standard they use a phenolphthalein solution to which a few particles of metallic sodium have been added.

Brugsch and Retzlaff (1912) modify Charnas' method by extracting the alkaline urine first with ligroin. They make the final reading with Plesch's colorimeter, using a solution of Bordeaux red as the standard.

2. *The Stools.*—Quantitative estimations of urobilin in the stools have been carried out by comparatively few investigators. Gerhardt at first used a somewhat complicated process of extraction and purification, but later, except in special cases, simply extracted with acid alcohol and read spectrophotometrically. Auche extracts with alcohol, removes other pigments by shaking with ligroin and thereafter makes the same spectroscopic determination as he employs in urine extracts.

Simpson (1910) mixes the day's feces with water and adds enough dilute sulphuric acid to make the mixture distinctly acid. For one or two days full exposure to light is allowed to convert the chromogen into urobilin. Filtration is then carried out and the residue repeatedly extracted until the filtrate becomes colorless. Direct examination of the filtrates is made and the amount determined from the degree of dilution required to obliterate the spectroscopic band.

Brugsch and Retzlaff (1912) attempt to convert all the urobilin into urobilinogen by allowing the stools to stand for some time before extracting with ligroin, to remove indol derivatives, and then with acetic ether After the addition of paradimethylamino-benzaldehyd they make a colorimetric determination, as in their method with urine.

A. Edelmann describes a modification of Schmidt's urobilin test which is applicable to both urine and feces and which is much quicker than the original test. Instead of a saturated aqueous solution of bichlorid of mercury, he employs a saturated alcoholic solution; the latter contains about twice as much bichlorid as the aqueous solution. The technic for applying the test to the *urine* is as follows: Two reagents are necessary: (1) a concentrated alcoholic solution of bichlorid of mercury; (2) a 10 per cent. alcoholic solution of zinc chlorid, and

amyl alcohol. About 10 c.c. of urine in a test-tube are treated with half the volume of concentrated alcoholic sublimate solution, mixed, and then shaken with amyl alcohol (which is best accomplished by pouring the contents down the wall of a second test-tube several times). To the clear amyl alcohol layer which quickly separates above, several c.c. of the alcoholic zinc chlorid solution are added; or the amyl alcohol may be poured into another tube and treated with zinc chlorid. With large amounts of urobilin the amyl alcohol is saturated with the pigment and shows a beautiful rose-red color (only with pathological amounts of urobilin) and the addition of zinc chlorid produces an intense green fluorescence. With small amounts of urobilin, if the fluorescence is not visible with diffuse light, the light may be focused on the tube with a convex lens, or the light from a small electric flash may be employed. By this means traces of urobilin may be detected. In applying this test to the *stools*, several grams of feces are rubbed in a mortar with a very small amount of water. Then an excess of reagent I is added, and rubbed a minute longer and filtered into a clean test-tube. The addition of a few c.c. of solution II to the filtrate, which is red in the presence of urobilin, causes a green fluorescence.

3. *In Serum.*—No quantitative work has been done with serum. The methods employed for the detection of urobilin in serum were reviewed by Conner and Roper. We shall refer, therefore, only to those which have appeared since their publication.

Morel and Monod (1908) add alcohol to the serum and heat to boiling for half an hour. The filtrate is concentrated and a drop of Obermeyer's reagent and an alcoholic solution of zinc acetate and acetic acid are added. After twenty-four hours the filtrate is examined for fluorescence.

Auche (1909) adds a solution of iodin in potassium iodid to the serum and afterward some zinc cyanate in ammonia and filters. The filtrate is examined spectroscopically.

Fromholdt and Nersessoff (1912) precipitate the oxalated plasma with alcohol, filter, concentrate, acidify, and shake with amyl alcohol, and then with alkaline water. The water is then made acid and shaken with amyl alcohol and examined spectroscopically.

Von Jaksch (1892) considered the presence of urobilinogen in the urine as indicative of hepatic disease. Neubauer demonstrated that Ehrlich's paradimethylamino-benzaldehyd test given by certain urines is really a test of urobilinogen.* The work of Munzer, Munzer and Bloch, Fischler, Bauer, Falk and Saxl and others indicates that urobilinogen occurs in the urine in many forms of liver disease.

* The solution is prepared in the following way:

Paradimethylamino-benzaldehyd	2 grams
Hydrochloric acid—concentrated	50 c.c.
Water—to	100 c.c.

A pink or red color results with a positive urine upon addition of a few drops of the solution to 5 c.c. of the urine.

In six cases of diseases of the liver, Wilbur and Addis found the following dilution values of urobilin in urine and stools:

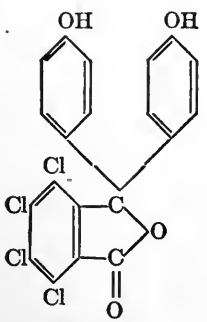
TABLE 6.—DISEASES OF THE LIVER WITHOUT MUCH, IF ANY, INTERFERENCE WITH THE ENTRANCE OF BILE INTO THE INTESTINES.

No.	Length of Period of Observation (Days)	Diagnosis	Average Daily Urine	Urobilin Stools
1	53	Portal cirrhosis	572	3,647
2	10	Portal cirrhosis	241	3,088
3	14	Cirrhosis (syphilitic)	456	3,451.
4	30	Bronzed diabetes	5,800	7,400
5	4	Hanot's cirrhosis	2,034	3,360
6	15	Abscess of the liver	1,713	4,047

Methylene Blue Test.—This test is, in the author's opinion, of absolutely no value. It will be described here only for the sake of completeness. In 1899, Chauffard and Castaigne advised the following test to determine the functional sufficiency of the liver: One c.c. of a 5 per cent. solution is injected subcutaneously or, as Roche recommended, 0.002 gram of the dye is taken by mouth in a capsule in the morning on a fasting stomach. The urine should become colored in one-half hour, becoming deepest in color from three to four hours after the injection. If the elimination is delayed, etc., or occurs at irregular intervals, the test is considered positive. Of course, kidney disease will interfere with this test.

Phenoltetrachlorphthalein Test.—In 1909, Abel and Rowntree conducted pharmacological experiments on animals with phenoltetrachlorphthalein, which was synthesized by Professor Orndorff, of Cornell University.

Its formula is:



Phenoltetrachlorphthalein

They found that this substance, when injected intravenously, was excreted in the bile. At the suggestion of Rowntree, Whipple, Mason, and Peightal studied the excretion of this substance in the bile when the

liver was subjected to artificial lesions. These authors found that in dogs which had been poisoned by phosphorus, for example, there was interference with the excretion of the phthalein. It was then that Rowntree, Marshall, and Chesney applied the tests clinically and obtained rather encouraging results.

The phenoltetrachlorphthalein test is applied in the following manner:

The dye is to be prepared for use each time. One gram of the substance is placed in a 200 c.c. Erlenmeyer flask, with two c.c. of 2/N sodium hydroxid solution and 18 c.c. of freshly distilled water. This is boiled for twenty minutes under a reflux condenser. The solution is filtered into a 100 c.c. flask, and is ready for use. This gives approximately a 5 per cent. solution, which is almost isotonic with blood. The solution is of an intense purplish-red color; it will not keep for more than a few days. Arbitrarily 8 c.c. of this solution, approximately 400 mg. of the phthalein has been selected. This amount is sufficient to give a most intense purplish-red color to 20 liters of water. Its administration in health is never followed by the appearance of the dye in the urine, and this amount insures in health an intense color in the final preparation of the feces, which is used for the quantitative determination. The dye is administered intravenously by gravity with antiseptic and aseptic precaution and with the usual intravenous technic. The funnel and system are filled with freshly distilled water, and after the flow is well established the phthalein solution is added. From 50 to 100 c.c. of water are used and the phthalein solution is washed in with freshly distilled water until the fluid entering the vein is colorless. Ten to fifteen minutes are required for its administration. Physiological salt solution may be preferable to distilled water for use in this injection.

Active purgation is instituted prior to the administration of the dye, and throughout the time of observation, usually by means of compound cathartic pills. The stools are collected for forty-eight hours, the urine for twenty-four hours. In the event that little or no feces are obtained, enemata are used, but unless a normal amount of dye is recovered the test must be discarded, since low findings under this condition could not be accepted.

The total forty-eight-hour feces are placed in a two-liter bottle and diluted with water to one or 1.5 liter, depending on their amount. This is placed in a shaking machine for from five to twenty minutes. Without allowing time for sedimentation, one-tenth of the total is placed in a one-liter flask and to this is added 5 c.c. of 40 per cent. sodium hydroxid, which causes the mixture to take on a very red color. Dilution is made with water to 1 liter. A stopper is inserted and the mixture thoroughly shaken; 100 c.c. of this preparation is placed in a 200 c.c. flask, 5 c.c. of lead acetate added, resulting in a decolorization of the mixture through the throwing out of a heavy lead precipitate which carries down the pigments, leaving a clear colorless supernatant fluid. Then 5 c.c. of 40 per cent. sodium hydroxid are added; this again elicits the red phthalein color, but does not redissolve the other lead pigment

combination. In certain instances 5 c.c. of sodium hydroxid at this point are not sufficient to elicit the maximum intensity of red, and more should be added until maximum intensity is reached, but not sufficient to free the other pigments from their insoluble lead combinations. The contents of the flask are made up to 200 c.c., shaken, and a small part filtered off, or the solution is allowed to stand for five minutes, when in many cases a clear red, supernatant fluid ready for estimation can be decanted. This solution is compared in a Rowntree and Geraghty modification of the Autenreith and Königsberger colorimeter with 20 mg. to a liter solution of the disodium salt of tetrachlorphenolphthalein (e.g., 0.4 c.c. of original solution to one liter, plus sufficient sodium hydroxid to insure maximum color). With these dilutions the amount of dye present is indicated directly in percentages.

When the amount recovered is below normal, it is advisable to add 2 to 3 c.c. more alkali to the 200 c.c. preparation, and redetermine, thus insuring that the maximum color has been elicited. The addition of large quantities of alkalis is undesirable, since it sets free the other pigments, rendering the solution yellowish-red instead of purplish-red. Not more than ten minutes are required to carry out this test after the feces are removed from the shaker. Where difficulty is experienced on account of the quality of the color, the following procedure may prove of some value in certain instances: After the addition of about 10 c.c. of 40 per cent. sodium hydroxid, the feces are made up with water to 1 liter. To one-tenth of this is added 5 c.c. sodium hydroxid and water up to 1 liter. Of this 100 c.c. are placed in a 200 c.c. flask and to it are added 5 to 10 c.c. or more of calcium chlorid mixture until the best quality of color is elicited. Dilution is made to 200 c.c., the mixture is allowed to stand from one-half to twenty-four hours, and a small amount of the supernatant fluid is filtered off and read against the standard.

Rowntree and his collaborators are quite enthusiastic as to the value of this test. Their results may be presented in the following table:

Table 7.—Results of the Phthalein Test.

	Examined	Less than 30 Per Cent.	Urine Examined	Positive
Cirrhosis	18	9	11	7
Congestion	32	11	10	6
Carcinoma of liver	6	6	3	2
Carcinoma	5	3	3	2
Luetic liver	5	2	2	1
Severe anemia	9	7	1	0
Miscellaneous	5	2	2	2
Total	80	40	32	20
Normals	33	2	22	3
Total	113	42	54	23

They state that it is evident that a phthalein output of less than 30 per cent. and the appearance of phthalein in the urine is infrequent in health and frequent in anatomically diseased livers. A normal phthalein output does not indicate the absence of a diseased liver, but that demonstrable functional changes do not accompany the anatomical changes. In the eleven cases coming to autopsy, pathological findings contraverting the functional findings have not been encountered. Absence of marked diffuse pathological changes has been noted in several instances in which the functional studies showed a good function where clinically severe liver injury was suspected. The autopsy findings serve to increase our confidence in the test. In one instance a microscopic study of the liver suggested greater injury than was shown by the phthalein.

Kahn and Johnston, however, could not confirm these results. According to them the test is not as easy to carry out as the description indicates. It is rather difficult, and in many cases almost impossible to impress the nurse with the importance of collecting the entire quantity of feces. The duty is rather a disagreeable one and complaints are likely to arise. The chemical analysis is also a disagreeable procedure and in a number of instances almost discouraging. In these cases it is almost impossible to obtain a color which can be compared with the standard. In general, this test is not easy; it requires some experience and it needs a well-equipped laboratory.

Kahn and Johnston show (in Table 8, on the opposite page) the percentages of dye recovered in the various cases. Certain cases which clinically were typical cases of liver involvement gave rather high figures for the phthalein excretion in the urine, whereas in other cases in which the diagnosis pointed to non-hepatic involvement, there was frequently observed a very low phthalein output. McLester and Frazier also concluded that this test had no clinical value.

Fibrinogen Test.—Following the observations of Doyon and Kareff and Nolf that the fibrinogen disappeared from the blood after liver extirpation, and of Doyon and his associates that the content was decreased in chloroform poisoning, and of Corin and Ansiaux and of Jacoby that it was decreased in phosphorus poisoning, Whipple and his co-workers developed a quantitative method which they have applied in studies of the fibrinogen content of the blood in health and disease, in clinical and experimental conditions. Their findings in acute and chronic hepatic injury make it reasonably certain that the liver is very active in the formation of fibrinogen and is the most important factor in maintaining a constant fibrinogen balance.

To determine fibrinogen, the heat coagulation method as devised by Whipple is used. It is as follows: Clear plasma, obtained from the oxalated blood, was measured into a centrifuge tube and heated in a water-bath at 58 to 60° C., for twenty-five minutes. The precipitate was collected by centrifugalization, washed thoroughly with cold and hot water and alcohol, with repeated centrifuging, washed into a weighed Gooch crucible with alcohol, washed with alcohol and ether, and dried

TABLE 8.—RESULTS OF THE PHTHALEIN TEST.

No.	Name	Diagnosis	Output of Phthalein Per Cent.	Remarks
1	B.	Fracture	14	
2	B.	Gastritis	28	
3	B.	Fracture	31	
4	S.	Mitral insufficiency	19	
5	H.	Jaundice	5	
6	B.	Fracture	12	
7	S.	Congestion of liver	32	
8	B.	Congestion of liver	34	
9	D.	Cholelithiasis	28	
10	S.	Chronic gonorrhea	25	
11	H.	Amputation	22	
12	R.	Hernia	27	
13	K.	Renal tuberculosis	32	
14	K.	Burns	24	
15	D.	Tuberculosis	21	
16	S.	Malaria	34	
17	D.	Cholelithiasis	20	Jaundice
18	F.	Cholecystitis	21	
19	R.	Fracture	14	
20	R.	No diagnosis	23	
21	D.	Liver congestion	25	
22	H. V.	Atrophic cirrhosis	25	
23	W. J.	Atrophic cirrhosis	17	
24	E. P.	Atrophic cirrhosis	21	
25	H. C.	Syphilis of liver	24	
26	C. L.	Abscess of liver	19	
27	T. M.	Cholecystitis	25	Jaundice
28	P. A.	Cholecystitis	30	
29	R. S.	Gall-stones	18	Jaundice
30	H. O.	Gall-stones	27	Jaundice
31	W. K.	Gall-stones	26	
32	L. Y.	Cancer of liver	17	
33	H. P.	Chronic gastro-enteritis	32	
34	G. L.	Mucocolitis	35	

to constant weight at 110° to 115° C. This method has been carefully studied by Whipple and has been shown to give constant results and has been controlled by the plasma-serum coagulum method. However, where the fibrinogen content is very low, the method may give slightly too low figures.

Whipple, in a series of thirty observations of the fibrinogen content of normal dogs, found variations from 200 to 867 mg. per 100 c.c. Individual fluctuation was very large even in a short space of time. However, in human cases no such wide variation was noted. In a series of approximately normal cases the fibrinogen varied between 385 to 618 mg. per 100 c.c. Rowntree and his co-workers placed the lower limit of normal at 350 mg.

In 14 cases of hepatic cirrhosis studied by Rowntree, Marshall and Chesney with this method, 6 gave positive results. According to them negative findings have no value.

The Total Non-protein Nitrogen, Urea Nitrogen and Amino-acid Nitrogen of the Blood.—Rowntree, Marshall and Chesney were the first to utilize these analyses in the study of liver function. They make the following statements:

"The fact that the liver is generally considered as being the organ mainly concerned with the formation of urea, and the metabolism of amino-acids, led us to make these determinations in this connection. In a series of sixteen strictly normal individuals Folin and Denis found the total non-protein nitrogen 22 to 26 mg., and urea nitrogen 11 to 13 mg. per 100 grams of whole blood. As soon, however, as one goes from the strictly normal, wide variations occur. Out of 63 cases of syphilis only 13 showed normal values, the variations being 20 to 45 mg. of total non-protein nitrogen and 10 to 26 mg. of urea nitrogen. Our experience was similar to this, and in papers on kidney function we have not regarded values below 50 mg. for total non-protein nitrogen and 25 mg. of urea nitrogen per 100 c.c. serum as of any diagnostic or prognostic import. Determinations of the amino-acid content of the blood either in health or disease are practically lacking. Van Slyke and Meyer found in dogs the amino-acid nitrogen of the whole blood between 3.1 to 5.4 mg. per 100 c.c."

In considering what one should regard as normal in this connection, it seems fairer to work with hospital or dispensary patients who give no evidence of kidney or liver disturbance. Rowntree, Marshall and Chesney have made these three blood determinations on a series of such cases, the results being tabulated below.

Table 9.

	Mg. per 100 c. c. Serum			Of Total Non-protein Nitrogen	
Clinical Diagnosis	Total Non-protein Nitrogen	Urea N	NH_2N	Urea N (Per Cent.)	NH_2N (Per Cent.)
Chronic urethritis, verumontanitis, prostatitis.	32	22	2.8	70	8.2
Chronic urethritis.....	24	12	2.5	50	9.6
Chronic urethritis.....	28	13	2.8	46	9.3
Bubo.....	22	10	2.6	45	10.8
Pyelitis.....	38	16	2.1	50	6.1
Normal.....	31	16	2.6	52	8.4
Normal.....	19	9	2.0	47	10.5
Normal.....	22	9	1.7	41	7.8
Normal.....	25	11	2.0	44	8.0

They have, therefore, considered anything below 40 per cent. for the urea N as abnormal, and above 3.0 mg. per 100 c.c. the upper limit of normal for amino-N. Of course the series of normals is small, and great variations may be found to occur in health.

The methods for the determination of total non-protein nitrogen, urea nitrogen and amino-acid nitrogen in the blood are as follows (the directions are quoted from Hawk's "Practical Physiological Chemistry"):

1. Non-protein Nitrogen.—(*a*) *Colorimetric Method of Folin and Denis.—Principle.*—This method, which is simple and convenient, depends upon the removal of the proteins from a sample of blood by precipitation with methyl alcohol, and the estimation of nitrogen in the methyl alcohol solution (after the removal of the proteins) by means of oxidation and Nesslerization. The details of the procedure are carried out in the following manner:

Method of Drawing Blood.—Attach, by means of a short piece of pure gum-tubing, a hypodermic needle about 1 mm. in diameter and 25 mm. in length (previously sterilized and paraffined) to the tip of a 2 or 5 c.c. pipet. Introduce into the upper end of the pipet (which must be perfectly clean and dry) a small pinch of powdered potassium oxalate, and allow it to run down into the tip and the needle. Attach a piece of rubber tubing to the upper end of the pipet, and to this a mouthpiece consisting of a short, tapering glass tube. Place a pinchcock over the rubber tube near the top of the pipet. To draw the blood, insert the needle into the vein or artery and regulate the flow by means of the pinchcock and suction. The exact quantity of blood desired is thus obtained without any waste or clotting.

Method of Isolating Non-protein Nitrogen Constituents.—Methyl alcohol and zinc chlorid are employed as precipitants for the protein materials of the blood, and the determination of the non-protein nitrogen is then carried out upon a portion of the methyl alcohol extract. The procedure is as follows: Transfer the blood, as soon as drawn, to a measuring flask which is half filled with pure methyl alcohol (it must be acetone free). Fill to the mark with methyl alcohol and shake thoroughly. (If 1 c.c. of blood is taken, 25 c.c. flasks are used for the precipitation, while for 5 c.c. of blood 50 c.c. flasks are used.) Allow the flask to stand for at least two hours and at the end of that time, or later, filter the contents through dry filter paper. Add 2-3 drops of a saturated alcoholic solution of zinc chlorid to the filtrate and filter again through a dry filter paper after a few minutes. The zinc chlorid brings down an appreciable precipitate and the last traces of coloring matter, so that the second filtrate obtained is perfectly colorless and clear. This filtrate is used for the determination of non-protein nitrogen.

Trichloracetic Acid Modification.—Greenwald has suggested the use of trichloracetic acid as the precipitant for the proteins of the blood, as being more satisfactory than the methyl alcohol and zinc chlorid. The objection to the methyl alcohol is that some of the amino-acids (creatin, asparagin, and tyrosin) are insoluble in it and hence precipitated along with the proteins. These acids are not removed by the trichloracetic acid. Certain nitrogenous lipoid substances are precipitated by the trichloracetic acid and not by the methyl alcohol. Greenwald suggests that these substances, even though non-protein in character, should not be included with the non-protein nitrogen of the amino-acids and urea.

Procedure.—Dilute the blood to ten times its original volume with a 2.5 per cent. trichloracetic acid solution. Let stand 30 minutes and then filter. Shake the filtrate with about four grams of kaolin per 100 c.c. and filter again. An aliquot of this final filtrate is taken, digested with sulphuric acid and nitrogen determined in the usual way.

Determination of Total Non-protein Nitrogen.—Transfer 5 c.c. of the alcoholic filtrate to a large Jena test-tube of the same kind as is used in urine analysis. Add 1 drop of concentrated sulphuric acid, 1 drop of kerosene, and a small pebble or glass bead to prevent bumping. Immerse the test-tube in a beaker of boiling water for five or ten minutes to drive off the methyl alcohol. When the alcohol is removed add 1 c.c. of concentrated sulphuric acid, 1 gram of potassium sulphate and 1 drop of copper sulphate solution. Boil, cool, dilute and aërate the solution as described in the determination of total nitrogen in urine with the exception that the ammonia is collected in a large test-tube instead of in a 100 c.c. flask. Nesslerize the solution, using 7 to 8 c.c. of diluted Nessler reagent (dilution 1:5), dilute to 25 or 50 c.c. according to the amount of color, and compare with a standard solution containing 1 mg. of ammonia nitrogen, nesslerized and diluted to 100 c.c. and the colorimeter prism set at 20 mm.

Calculations.—If 5 c.c. of blood are diluted to 50 c.c. and 10 c.c. of the alcoholic extract (equivalent to 1 c.c. of blood) are used for the determination, the amount of non-protein nitrogen (as milligrams per 100 c.c. of the blood) can be obtained by use of the formula $\frac{20}{R} \times D$, in which R stands for the reading of the unknown and D represents the volume to which its ammonia has been diluted. If the equivalent of 0.4 c.c. of blood has been taken for the determination the formula $\frac{50}{R} \times D$ is used, and if the equivalent of 0.5 c.c. of blood has been taken the formula becomes $\frac{40}{R} \times D$.

2. Urea Nitrogen.—(*a*) *The Urease Method.*—Van Slyke and Cullen's modification of Marshall's Method.

Procedure.—Run 3 c.c. of fresh blood (carefully measured with an accurate pipet) into a 100 c.c. test-tube containing 1 c.c. of a 3 per cent. solution of potassium citrate (to prevent clotting). Add 1.5 c.c. of the urease solution and 2 or 3 drops of caprylic alcohol (to prevent foaming). After ten minutes add 15 c.c. of a saturated solution of potassium carbonate, and drive off the ammonia by aspiration into another tube containing 15 c.c. of hundredth-normal hydrochloric or sulphuric acid. Titrate the excess of acid with hundredth-normal sodium hydroxid or potassium hydroxid, using methyl red or alizarin as indicator.

Calculations.—Each c.c. of acid neutralized indicates 0.01 gram of urea per 100 c.c. of blood, or 0.0467 gram of urea nitrogen per 100 c.c. of blood. In case the blood should be one of the rare samples containing over 0.15 per cent. of urea, all the acid will be neutralized, and it will

be necessary to repeat the determinations, using in the determination only 1 c.c. of blood. Fresh blood contains so little ammonia that it may be disregarded.

3. Amino-acid Nitrogen.—(*a*) *Method of Van Slyke and Meyer.*—*Principle.*—The protein of the blood is removed by precipitation with alcohol and the amino-acid nitrogen determined in the filtrate by the nitrous acid method.

Procedure.—From 30 to 50 c.c. of freshly drawn blood are mixed with 9 or 10 volumes of 95 per cent. alcohol to precipitate the proteins. The volume of the alcohol-blood mixture must be known, but in case it is not convenient to use a graduated cylinder for the mixture, its volume can be taken as the sum of the volumes of the alcohol and blood without essentially affecting the results. The alcohol and blood are thoroughly mixed, the vessel containing them is closed and 24 hours are allowed for precipitation of the proteins to become complete. The solution is filtered through a dry folded filter into a measuring cylinder without washing the precipitate. The volume of filtrate is noted and is taken for analysis as an aliquot part of the total blood-alcohol mixture. The filtrate is then concentrated to a volume of 3-5 c.c. and used for determination of amino-nitrogen by the Van Slyke nitrous acid method. The use of a few drops of caprylic alcohol to prevent foaming is advisable.

(*b*) *Method of Constantino.*—This is based on the formol titration procedure. One hundred c.c. of blood or serum are mixed with a measured (500 c.c.) volume of 2 per cent. mercuric chlorid solution containing 0.8 per cent. hydrochloric acid. The mixture is shaken vigorously in a stoppered flask and allowed to stand a few hours. Centrifugate for 10 minutes; pour the supernatant liquid through a dry filter into a graduated cylinder. An aliquot of the filtrate is taken, the mercury is removed with hydrogen sulphid and the latter by a current of air. The liquid is exactly neutralized and concentrated on the water-bath, or better, at 50° C. in a vacuum, MgO added, and the mixture distilled in a vacuum at 45° C. to get rid of ammonia. The volume should now be about 30 c.c. A little solid barium chlorid and barium hydroxid are added, and 1.5 c.c. of 0.5 per cent. solution of phenolphthalein. Filter. Neutralize accurately to sensitive litmus paper. Add neutral formalin solution and titrate with N/5 NaOH.

Lipase of the Blood.—In 1913, Whipple, Mason and Peightal, utilizing Loevenhardt's technic, demonstrated that the lipolytic activity of the blood varied from normal in certain diseases of the liver. Severe experimental injury to the liver resulting from chloroform, phosphorus and hydrazin always produced increase in plasma lipase to from two to eight times the normal. Clinically Whipple found an increased lipolytic activity in several cases of eclampsia, invariably so in those showing hemorrhagic portal vein necrosis, in pneumonia, peritonitis, leukemia and in the early stages and sometimes in the late stages of cirrhosis. The lipolytic activity of the blood in pernicious vomiting, uremia, jaundice, and obstructive jaundice, was normal. He therefore concludes

that high lipase values will be found in practically all cases of eclampsia, liver injury with necrosis due to poisons, intoxications or infections, acute yellow atrophy, cholangeitis, and abscess of liver with considerable destruction of liver tissue.

Lipase Determination.—This determination can be made either on oxalated plasma or serum, there being little difference in the results, except that the serum lipase is slightly higher owing to the dilution of the plasma by the oxalate. All our estimations have been made on plasma lipase with the exception of two or three cases, where serum was utilized. Loevenhardt's method of determining the lipolytic activity was utilized. The technic is as follows: Four tubes are prepared, each containing 1 c.c. of plasma and to each is added 4 c.c. of distilled water and 0.3 c.c. of toluol. To two of the tubes is added 0.26 c.c. of ethyl butyrate, the other two serving as controls. The tubes are stoppered and shaken and incubated at 38° C. for eighteen to twenty-four hours. They are now titrated to neutrality with N/10 acid and N/10 alkali, using azolitmin as indicator. The controls show the titrable blood alkalinity to be about 0.1 c.c. N/10 acid. The ethyl butyrate tubes give the amount of acid production above the neutral point and the sum represents the total acid production or lipolytic activity.

The serum of patients suffering from bacterial diseases, Sagal claims, shows a slight increase in enzyme activity, while the serum obtained from sufferers of diseases of non-bacterial origin show a slight depression. Bile-stained serum and the serum from hepatic cirrhosis show marked decrease in enzyme activity. Lipase activity gradually decreases with increasing age. Variations of lipase activity in diseased conditions, as shown by the hydrolysis of ethyl butyrate, are so small as to be devoid of diagnostic or prognostic value. (Sagal.)

Rowntree, Marshall and Chesney draw the following conclusions from their experiments on the experimental efficiency of the various liver function tests:

1. Outspoken changes in liver function can be demonstrated in most cases of advanced liver cirrhosis, in markedly congested livers associated with myocardial insufficiency, in carcinoma of liver, in luetic livers, and in conditions of cachexia with marked anemia.

2. Functional changes have been most marked in cirrhosis, in neoplasm of liver and in cachectic conditions with severe grades of anemia. The functional changes in chronic passive congestion have not been frequent or pronounced.

3. Harmony in the findings of the tests is present in some cases, i.e., most of the tests indicating a decreased function or indicating a normal function, but in other instances, the function in an individual case appears normal by some tests and diminished by others and absolutely no parallelism exists between the findings of the various tests in the latter instance, i.e., with one test indicating decrease in function it is impossible to predict what the other tests will show.

4. From this small series of cases it is impossible to reach definite conclusions concerning the absolute and relative value and limitations

of these various tests, but the following impressions are the outcome of our limited experience:

(*a*) Under clinical conditions a phthalein output under 30 per cent. or the appearance of phthalein in the urine is of unquestionable significance. When in accord, i.e., both positive or both negative, the evidence is of more value than single or discordant findings. Positive value is not claimed for negative findings. A marked decrease in phthalein means a decided injury to liver function. Autopsies in 11 cases have increased our belief in the value of this test.

(*b*) Low fibrinogen values are frequently but inconstantly encountered in cirrhosis, which confirms the results reported by Whipple. Marked positive findings may carry prognostic significance, although they may not appear until shortly before death. Negative findings have no value.

(*c*) The determination of the lipolytic activity of the blood-plasma furnishes very little or no information of prognostic or diagnostic significance in these types of clinical cases. In only two or three instances have the clinical findings been comparable with our findings or those of Whipple in chloroform- or phosphorus-poisoning. High values probably carry prognostic significance.

(*d*) Dr. Goodpasture's fibrinolytic ferment studies on this series of cases show that this ferment is present only in cirrhosis and hence, when present, is of definite diagnostic importance.

(*e*) Bauer's galactose test is applicable without discomfort to the patient, but yields no information of consequence.

(*f*) Strauss's levulose test was attended by technical difficulties —nausea and vomiting frequently following its employment—and yielded information of no consequence in the limited number of cases in which it was successfully carried out.

(*g*) Blood N partition: cumulative phenomena have not been encountered in this series except with coexistent renal disease.

The urea N percentage of total has been 40 per cent. or less in several instances, and especially low in cases of advanced cirrhosis.

The amino-nitrogen has been high in a considerable proportion of the clinical cases. In phosphorus-poisoning the amino-nitrogen increase was always present and was associated with increase in the urea N and total non-protein nitrogen. In chloroform-poisoning the absolute and relative values of the various forms of nitrogen did not vary from normal.

(*h*) Urinary nitrogen partition: No instance of absolute normal urinary nitrogen partition has been encountered. However, the low level of protein metabolism so often present, together with the non-exclusion of acidosis, renders the interpretation of the N distribution somewhat difficult. Practically all the cirrhosis cases show definite N partition changes.

The ammonia nitrogen and amino-nitrogen were definitely increased in most of the cases studied and particularly in cirrhosis.

Concerning the relative merits of these tests it appears that the

phthalein, fibrinogen, the blood and urine nitrogen partitions are of decided value in determining the presence and to a less degree the extent of functional involvement, while the demonstration of the presence of a fibrinolytic ferment is of decided diagnostic importance. The determination of sugar tolerance and of the lipolytic activity of the blood apparently afford information of much less value.

Ghedini's Ferment Test.—In 1913, Ghedini proposed a test which is based upon the presence in the blood-serum of a ferment which he claims arises in the liver-cells, and which is capable of converting glycogen into maltose, isomaltose, and glucose. In short, as Rowntree, Hurwitz and Bloomfield point out this must be a test for two blood ferments, diastase and maltase. Diastase varies tremendously in normal sera, consequently, *a priori,* this in itself would vitiate the test. Sufficient evidence has not been presented to establish the liver as the sole source of the ferment or ferments involved.

The Fibrinolytic Ferment of the Blood.—In 1914, Goodpasture reported that in some cases of cirrhosis of the liver, there is present a ferment in the blood stream that dissolves fibrin, that is to say, will dissolve a blood-clot. This ferment, according to him, is specific for fibrin, does not attack fibrinogen, is destroyed by heating at 56°C. for one-half hour and is easily recognized.

The technic of the test is as follows: Blood is drawn aseptically from a vein of the forearm by means of a needle and syringe, and allowed to stand in a sterile glass tube, whereupon the clot forms as normally. If this clot be allowed to stand at 37°C. for several hours, and if the ferments be present, at the end of 6 to 8 hours it will be found, on examination, that the clot has entirely dissolved.

It would seem that this ferment, when present, has definite diagnostic significance, since it was present in 7 cases of cirrhosis of the liver and was not found in any other condition in a series of 45 cases studied by Rowntree, Marshall and Chesney.

The results obtained by the authors do not justify any credence in the reliability of this test.

Sulphoconjugation Test (Kahn).—The cause and the location of the formation of the ethereal sulphates and of indican have been studied by a number of investigators.

Since Städeler found phenol in cow's and horse's urine, Landolt, Lieben, Hoppe-Seyler, Buliginsky and Munk found traces of it in normal human urine, and Salkowski observed that in ileus and other obstructive intestinal disease, the excretion of phenol in the urine is much increased.

This formation of phenol and phenolic substances, cresol, indol, skatol, etc., has been ascribed to the action of the intestinal bacterial flora. Such organisms as the *Bacillus coli communis,* which is a normal inhabitant of the intestinal canal, are harmless under normal circumstances. In conditions of injury to the intestinal mucosa, these organisms become virulent (Fermi and Salto). Other organisms, like the *Bacillus putrificus, Bacillus aërogenes capsulatus,* which are obligatory

anaërobes, thrive in the colon where there is no oxygen (Herter), and break up protein in the carbocyclic, toxic substances.

It was demonstrated by Baumann that these split products are very toxic, but that, when they are united with sulphuric acid, they lose their poisonous effect.

Baumann found that phenol sulphate is a normal urinary constituent and that the administration of phenol increases the phenol sulphate in the urine.

Baumann and Herter reported that not only phenol, but also other substances were excreted in the urine as ethereal sulphates. They also observed that phenol unites not only with sulphuric acid but with other radicals. This was confirmed by Schmiedeberg, who found that phenol unites with glycuronic acid.

Upon poisoning dogs with phenol, he found that the liver became rich in phenol sulphates. For example, in 100 parts of liver he found 19 times as much tribrom phenol as in 100 parts of blood. This phenomenon seemed to prove that the liver is the seat of conjugation of the phenolic and indolic radicals with sulphuric acid.

Lang determined the quantity of ethereal sulphates in the urine of geese before and after extirpation of the liver. His figures are rather small, and should not be taken conclusively, but he was led to believe that the synthesis of the ethereal sulphates was not exclusively performed in the liver.

In experiments performed *in vitro,* Koch also demonstrated, so it appeared to him, that the liver was not the only seat of sulphoconjugation. He took liver, kidney, pancreas, thymus, muscle, and minced each organ respectively, and added phenol and disodium sulphate. He kept these mixtures at body temperature or else at 8° to 12° C. He reported that all the tissues, save the thymus, took part in the synthesis. He obtained similar results with orth-, meta-, and paradi-oxyphenol.

Landi repeated the experiments of Koch, using only the liver tissue. But, as he says, due to the fact that decomposition sets in so very soon, he could not confirm Koch's findings. In order to throw more light on the subject, he made perfusion experiments with the liver, and he came to the final conclusion that the seat of conjugation of the phenolic and sulphuric radicals was not the liver but the intestines.

The results of Landi are directly negated by the findings of Embden and Glaessner. They performed perfusion experiments on the organs of dogs, using the liver, muscle, kidneys, lungs and small intestines. From their investigations they conclude that the liver is the most important organ for the formation of the ethereal sulphates. Smaller quantities of ethereal sulphates are produced in the lungs and the kidneys, but the muscle tissue and the small intestine play a very insignificant rôle in the formation of the ethereal sulphates.

Reale, from his observations, was of the firm opinion that the liver was the seat of the synthesis of the ethereal sulphates.

Finizio confirmed Reale from his clinical findings. In normal individuals and in a case of echinococcus hepatic cyst, he found that the

administration of thymol caused an increased excretion of ethereal sulphates in the urine. When, however, he administered thymol to a patient suffering from hepatic cirrhosis, he found no increase of the ethereal sulphates in the urine.

In normal conditions of the alimentary tract, Strauss and Philipsohn found no phenol in the urine, and they concluded that, under normal conditions, the phenol and other radicals were conjugated with sulphuric acid. According to these authors, the liver is the seat of the synthesis of the ethereal sulphates.

Herter and Wakeman took 7 grams of liver, kidney, muscle, brain and blood, respectively, which were minced, and treated each tissue with 10 c.c. of a weak phenol solution, and allowed to stand for two to three hours. The mixtures were then distilled, and they found that there was a loss in the phenol distilled. The liver retained most of the phenol, then came in order the kidneys, muscle, brain.

In conditions of jaundice, Biernacki found four times the amount of ethereal sulphates normally present. Darenberg and Perroy found an increased excretion of indol and skatol in jaundiced individuals. Labbe and Vitry obtained similar results. Magrageas obtained varying quantities of ethereal sulphates in icteric patients.

Amann found that in the healthy subject there is a direct proportion between the quantities of ethereal sulphates and the total nitrogen in the urine. The coefficient of Amann may be thus expressed:

$$\frac{\text{Eth. S.} \times 100}{\text{N in Urine}}$$

The value of this coefficient varies between 1.4 and 1.5. This was confirmed by Guerbet and Rouen. Slightly smaller coefficients were obtained by Magrageas.

The question has been discussed by Eiger and Hopadze whether the aromatic compounds formed in the system are diminished in amount and destroyed under normal conditions of hepatic activity, and whether, in cases of disturbance of the function of the liver, these compounds are obviously increased and placed at the disposal of the liver for conjugation with sulphuric acid. The subject is more important in its relation to cases of disease of the hepatic parenchyma than in simple biliary stasis. The ethereal sulphuric acids are most frequently, both absolutely and relatively, increased in atrophic cirrhosis of the liver, and most markedly in tumors of the liver.

In normal urine 14 to 25 per cent. of the total sulphur is present as the so-called neutral sulphur. The easily oxidizable portion of this must arise from the sulphocyanate of the saliva, and from other partly unknown substances, while the remainder is regarded—in part, at least—as a derivative of the taurin of the bile (Lépine). This latter bears, in the nomenclature of the French physiologists, the name "biliary sulphur of the urine."

Lépine found that in incipient cases of obstructive jaundice in ani-

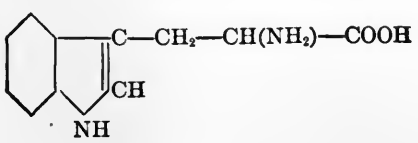
mals and in man, the biliary sulphur was absolutely and relatively increased as regards the oxidized sulphur (up to 30 to 43 per cent. of the total sulphur). After a few days of the biliary obstruction, the sulphur became approximately normal, and after long continuance of the disturbance showed a decrease.

F. Müller, who studied a case of jaundice from gall-stones of somewhat long standing, found on three days the values of the neutral sulphur to be 22.9, 15.7, 10.7 per cent. of the total sulphur. Later in the same case, but with different diet the values were 19.2 and 17.4 per cent. In a case of carcinoma of the stomach and liver, accompanied by jaundice, the findings were 29.0, 21.1 and 16.1 per cent. These figures confirm Lépine's idea that the neutral sulphur diminishes the longer the jaundice continues.

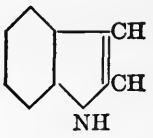
On the other hand, a marked decrease, and even a lowering of the normal values, should be expected in chronic obstructive jaundice, provided the assumption is correct that in cases of disturbed outflow of bile into the intestines the production of biliary acids is markedly reduced by the interruption of the circulation of bile acids. Since this is not observed, the relation of the hardly oxidizable sulphur to taurocholic acid must be reinvestigated before an opinion on the formation of bile acids can be based on the excretion of neutral sulphur. Hence it does not follow that Schmidt should assume that the production of bile acids, even in long-continued jaundice, suffers no reduction, because he but rarely found high values for the neutral sulphur in his case of jaundice. According to Benedict, a portion of the non-oxidized sulphur compounds, which may be excreted in increased amounts as a result of toxic action on the protein constituents of the body, are to be regarded as intermediary bodies which resist the further oxidation to sulphuric acid. Corresponding to their presence in the bile (Bial) conjugated glycuronic acids are regularly observed in the urine in cases of biliary obstruction (Van Leersum) (von Noorden, "Metabolism and Practical Medicine").

The toxic aromatic radicals produced by decomposition of protein are conjugated in the liver with sulphuric or glycuronic acid, and are then excreted in the urine. If we should take indol as an example, the following process would take place.

Tryptophan, or beta-indol-alpha-aminoproprionic acid, is one of the products of decomposition and putrefaction of proteins. It is the mother substance of indol and skatol, etc. Upon the breaking down of tryptophan, indol, which is very toxic, is produced.

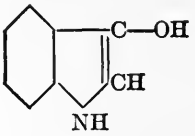


Tryptophan



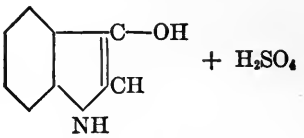
Indol

Indol is oxidized in the intestines to indoxyl.

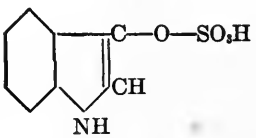


Indoxyl

If indol or indoxyl enters the general circulation marked toxinemia results with its concomitant symptoms. The protective mechanism of the body against this toxinemia is to conjugate the indoxyl with sulphuric acid in the liver, producing a substance which is almost non-toxic—indican.

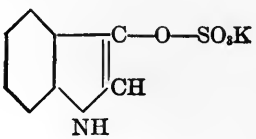


Indoxyl



Indoxyl sulphuric acid

In the presence of potassium salts:



Indican

Similar results are obtained with any of the aromatic radicals, as phenol, cresol, tyrosin, skatol, etc.

It is well known that the total sulphur in the urine may be partitioned into three distinct fractions:

(*a*) The inorganic sulphates.
(*b*) The ethereal sulphates.
(*c*) The neutral sulphur.

It has been definitely established that, normally, the inorganic sulphates form about 70 per cent. of the total sulphur and the remaining 30 per cent. are divided almost equally between the ethereal sulphates and the neutral sulphur.

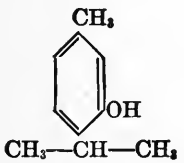
The ethereal sulphates are the conjugated aromatic sulphonic acids. It is this fraction that is of special interest to us now.

It is, of course, impossible to rely upon the excretion of ethereal sulphates as a symptom of hepatic function. The proteins which are ingested daily give rise to their quota of aromatic radicals which influence the quantity of the conjugated sulphates. The condition of the intestinal flora plays a rôle in the formation of aromatic radicals; thus it is known that in intestinal putrefaction there is a marked increase in the conjugated sulphates excreted.

The author, therefore, adopted the following technic for the determination of liver function by means of the ethereal sulphate output:

The patient received a dose of castor oil to clean out his bowels. He was then kept on a known diet for two days, during which time the urine was collected, preserved, and analyzed for total sulphur and ethereal sulphates. On the third day the patient received a capsule containing one-half gram of thymol. The urine was collected for the next two days, preserved, and analyzed for total sulphur and ethereal sulphates.

Thymol is iso-propyl-meta-cresol:



Iso-propyl-meta-cresol

If all the thymol were absorbed and if all the thymol were conjugated with sulphuric acid and none with glycuronic acid, the 0.5 gram of thymol would be excreted as 0.7666 grams of thymol sulphuric acid. This would cause a marked increase in the percentage of ethereal sulphates. If the liver were not functionating properly, the thymol would not be conjugated, and the percentage of ethereal sulphates would be only slightly different from what it had been on the first two days. One objection to the study of the function of any organ as an index of disease of that organ, is, that it is perhaps possible for the healthy part of the diseased organ to compensate and assume the work of the whole gland. In such a condition, of course, the functional output of the organ may be normal, and would be no index of the pathological anatomy of the

organ. Under these circumstances only marked destructive changes would leave their impress on the functional activity of the organ.

TABLE 10.—ETHEREAL SULPHATE ELIMINATION BEFORE AND AFTER THYMOL ADMINISTRATION. (Kahn).

Case No.	Diagnosis	Total Sulphur (Grams)		Ethereal Sulphate Sulphur (Grams)		Ethereal Sulphate Sulphur Per Cent. of Total Sulphur	
		Before Thymol	After Thymol	Before Thymol	After Thymol	Before Thymol	After Thymol
1	Normal.....	2.0375	2.1295	0.2893	0.5646	14.2	26.8
2	Gastritis.....	1.9428	1.7427	0.1457	0.3380	7.5	19.4
3	Fracture.....	2.7467	2.5527	0.3131	0.6024	11.4	23.6
4	Congestion of liver..	0.9852	1.0734	0.1753	0.7069	17.8	28.6
5	Congestion of liver..	1.7345	1.6982	0.2480	0.3610	14.3	21.2
6	Gall-stones.....	2.7628	2.8075	0.7597	1.0303	27.5	36.7
7	Gall-stones.....	3.0042	2.6826	0.3965	0.8474	13.2	29.4
8	Cholecystitis.....	2.7807	2.6437	0.4866	0.7428	17.5	28.1
9	Atrophic cirrhosis...	2.2328	2.3029	0.2791	0.3400	12.5	15.2
10	Tumor of liver.....	1.9492	1.8757	0.1637	0.3676	8.4	19.6
11	Cancer of liver.....	2.7526	2.6278	0.6083	0.6648	22.1	25.3
12	Syphilis of liver....	2.8104	2.9075	0.3990	0.5437	14.2	18.7

In the author's experience, however, disturbances in the structure of the liver go hand in hand with disturbances of function, especially as is indicated by sulphuric acid conjugation of the aromatic radicals. He found that in cirrhosis of the liver, the conjugation of thymol with sulphuric acid does not take place to as marked an extent as in the normal state.

It will be observed that in the non-hepatic diseases, and in the non-destructive diseases of the liver, a marked increase in the excretion of ethereal sulphates was observed on the day after the thymol administration. In diseases of the liver, like atrophic cirrhosis, cancer of the liver, or syphilis of the liver, this organ has lost its power to conjugate the thymol with sulphuric acid. Case Number 10 was a benign tumor of the liver, and it seems no destructive changes went on in the hepatic tissue.

In cases of pernicious anemia, Kahn and Barsky found a deficiency in the detoxicating function of the liver (Table 11).

The following methods may be used for the determination of total and ethereal sulphates (Hawk):

BENEDICT'S TOTAL SULPHUR METHOD.—*Principle.*—The urine is evaporated and ignited with a solution of copper nitrate and potassium chlorate. Organic matter is thus destroyed and all unoxidized sulphur is oxidized to the sulphate form and can be readily precipitated with barium chlorid in the usual manner. The method is very convenient and accurate.

TABLE 11.—SULPHOCONJUGATION TEST OF HEPATIC FUNCTION IN PERNICIOUS ANEMIA. (Kahn and Barsky.)

Case	Total Sulphur (Grams)		Ethereal Sulphate Sulphur (Grams)		Ethereal Sulphate Sulphur, Per Cent. of Total Sulphur	
	Before[1]	After	Before	After	Before	After
X[2]	2.0375	2.1295	0.2893	0.5646	14.2	26.8
1	1.536	1.6205	0.3993	0.5055	26.0	31.2
2	1.139	1.0974	0.3075	0.3237	27.0	29.5
3	1.208	1.1522	0.1691	0.2154	14.0	18.7

[1] Before the administration of thymol and after.
[2] This was a normal individual.

Procedure.—Ten c.c. of urine are measured into a small (7-8 cm.) porcelain evaporating dish and 5 c.c. of Benedict's sulphur reagent added. The contents of the dish are evaporated over a free flame regulated to keep the solution just below the boiling-point, so that there can be no loss through spattering. When dryness is reached the flame is raised slightly until the entire residue has blackened. The flame is then turned up in two stages to the full heat of the Bunsen burner and the contents of the dish thus heated to redness for ten minutes after the black residue (which first fuses) has become dry. This heating is to decompose the last traces of nitrate (and chlorate). The flame is then removed and the dish allowed to cool more or less completely. Ten to 20 c.c. of dilute (1:4) hydrochloric acid is then added to the residue in the dish, which is then warmed gently until the contents have completely dissolved and a perfectly clear, sparkling solution is obtained. This dissolving of the residue requires scarcely two minutes. With the aid of a stirring rod the solution is washed into a small Erlenmeyer flask, diluted with cold, distilled water to 100-150 c.c., 10 c.c. of 10 per cent. barium chlorid solution are added drop by drop, and the solution is allowed to stand for about an hour. It is then shaken up and filtered as usual through a weighed Gooch crucible. Controls should be run on the oxidizing mixture.

Benedict's solution consists of:

Crystallized copper nitrate (sulphur-free).... 200 grams
Sodium or potassium chlorate ..... 50 grams
Water to .....1000 c.c.

FOLIN'S ETHEREAL SULPHATES METHOD.—*Principle.*—The inorganic sulphates are removed with barium chlorid and the conjugated sulphates then determined after hydrolysis.

Procedure.—Place 125 c.c. of urine in an Erlenmeyer flask of suitable size, dilute with 75 c.c. of water and acidify the mixture with 30 c.c. of dilute hydrochloric acid (1 volume of concentrated HCl to 4 volumes of water). To the cold solution add 20 c.c. of a 5 per cent. solution

of barium chlorid, drop by drop. Allow the mixture to stand about one hour, then filter it through a dry filter paper. Collect 125 c.c. of the filtrate and boil it gently for at least one-half hour. Cool the solution, filter off the precipitate of $BaSO_4$, wash, dry, and ignite.

The Glycuronic Conjugation Test.—Similar to the sulphoconjugation, there is also a glycuronic conjugation in the liver. Roger has made this a basis for his study of liver function, and Gautier, Chiray and others have confirmed his results.

Gautier found that in 100 healthy persons examined, the glycuronic acid content was pronounced, and a dose of camphor was followed by little if any increase in the glycuronuria on an average diet. The fasting healthy subject showed a slight increase after the camphor test. In cases of heart or kidney disease with insufficiency of the liver there was always a notable transient elimination of glycuronic acid after ingestion of the test dose of camphor. In fifteen diabetics the camphor test always proved negative. In advanced cirrhosis the liver is unable to respond to the camphor test. Roger believes that the glycuronic acid is manufactured by the liver to combine with certain toxic bodies in the organism and thus eliminate them. This assumption is confirmed by a recent case of attempted suicide with a preparation of phenol. The urine was black and showed the highest proportion of glycuronic acid Gautier has ever encountered. Then followed the phase in which there was no glycuronuria, after which normal conditions were gradually restored. With cirrhosis of the liver, the total absence of glycuronuria is a sign of a speedily fatal outcome. In tests of alimentary glycosuria, the glycuronuria was not modified even by ingestion of 150 grams sugar, confirming the view that the glycuronic acid is produced only when needed to take care of toxic substances and get them eliminated. The Grimbert and Bernier test for glycuronic acid is reliable if the reagents are pure.

The Hippuric Acid Conjugation Test.—Morse, working along similar lines, has recommended the administration of benzoic acid to patients, and the analysis of the urine for hippuric acid, the result of the synthesis of the benzoic acid with glycocoll. Since the kidney also plays a rôle in the synthesis of this substance, the value of this test for hepatic function is doubtful.

BIBLIOGRAPHY

Carbohydrate Tolerance Tests

BARTON. Manual of vital function-testing methods and their interpretation. Boston, 1916.
BAUER. Wien. med. Wchnschr., 1906, lvi, 2537.
——— Deutsch. med. Wchnschr., 1908, xxxiv, 1505.
BAYLAC AND ARNAUD. Congres. franç. de méd., Toulouse, 1902.
BERNARD. Diabetes, 1878, p. 160.
BLOOMFIELD AND HURWITZ. Johns Hopkins Hosp. Bull., 1913, xxiv, 375, 380.
BONCHARD. Malade par relentissement de la nutrition, 1890, p. 159.
BONDI AND KÖNIG. Wien. med. Wchnschr., 1910, lx, 2617.

BRANDT. Arch. f. exper. Path. u. Pharmak., 1913, lxxii, 457.
BRUINING. Berl. klin. Wchnschr., 1902, xxxix, 587.
CHAJES. Deutsch. Med. Wchnschr., 1904, xxx, 699.
CHURCHMAN. Johns Hopkins Hosp. Bull., 1912, xxiii, 10.
COLRAT. Lyon méd., 1875, xviii, 553.
CRISAFI. Zentralbl. f. d. ges. Med., 1903, i, 1.
FALK AND SAXL. Ztschr. f. klin. Med., 1911, lxxiii, 325.
FERRANINI. Centralbl. f. inn. Med., 1902, xxiii, 921.
VON FREY. Ztschr. f. klin. Med., 1911, lxxii, 383.
FRIEDMANN AND STROUSE. Arch. Int. Med., 1914, xiv, 531.
GOODMAN. Jour. Am. Med. Assn., 1909, liii, 2050.
VON HALASZ. Wien. klin. Wchnschr., 1903, xxi, 44.
HIROSE. Deutsch. med. Wchnschr., 1912, xxxviii, 2, 1414.
KING. Intern. Beitr. z. Path. u. Therap. d. Ernährungsstör, etc., Berlin.
LANDSBERG. Deutsch. med. Wchnschr., 1903, xxix, 563.
LEGG. St. Bartholomew's Hosp. Rep., 1873, ix, 175.
LEPINE. Semaine méd., 1901, iv, 3.
QUINCKE. Berl. klin. Wchnschr., 1876, 529.
RASPIDE. Thése de Toulouse, 1903.
RIESS AND JEHN. Deutsch. Arch. f. klin. Med., 1912, cviii, 187.
ROGER. Revue de Méd., 1896, xvi, 935.
ROUBITSCHEK. Deutsch. Arch. f. klin. Med., 1912, cviii, 225.
ROWNTREE, HURWITZ AND BLOOMFIELD. Johns Hopkins Hosp. Bull., 1913, xxiv, 327.
SACHS. Ztschr. f. klin. Med., 1899, xxxviii, 87.
SAMBERGER. Arch. f. Dermat. u. Syph., 1903, lxvii, 89.
SCHEDE. Jahrest. f. Kinderh., 1915, N. F., lxxxii, 45.
SCHIWKAUER. Ztschr. f. klin. Med., 1913, lxxviii, 462.
STEINHAUS. Arch. f. klin. Med., 1902, lxxiv, 537.
STRAUSS. Berl. klin. Wchnschr., 1898, xxxv, 398.
——— Deutsch. med. Wchnschr., 1901, xvii, 756.
WAGNER. Ztschr. f. klin. Med., 1914, lxxx, 174.
WEINTRAUD. Von Noorden's Metabolism and practical medicine, 1907, Vol. II, p. 282.
WORNER AND REISS. Deutsch. med. Wchnschr., 1915, xl, 895.

Urinary Nitrogen Partition

ASCOLI. Deutsch. Arch. f. klin. Med., 1901, lxxi, 387.
BIERENS DE HAAN. Dissertation. Leiden, 1897.
BREITMANN. Zentralbl. f. inn. Med., 1913, xxxiv, 857.
BRILLE AND GARBAN. Gaz. d. hôp., 1914, lxxxvii, 405.
CALABRESE. Zentralbl. f. inn. Med., 1898, 55.
FALK AND HESKY. Ztschr. f. klin. Med., 1910, lxxi, 261.
FALK AND SAXL. *Ibid.*, 1911, lxxiii, 325.
FAWITZKY. Arch f. klin. Med., 1889, xlv, 429.
GLAESSNER. Ztschr. f. exp. Path. u. Therap., 1907, iv, 336.
GUMLICH. Ztschr. physiol. Chem., 1882, xvii, 10.
HALLERVORDEN. Exper. Arch., 1880, xii, 274.
HAWK. Practical physiological chemistry, 1916, p. 483.
JANSEN. Jour. Biol. Chem., 1915, xxi, 557.
LABBE AND BITH. Bull. et mém. Soc. méd. d. hôp., 1913, xxix, 510.
LAMB, HOLKER AND SCOTT. Med. Chron., 1915, lxi, 125.
LANDAU. Deutsch. Arch. f. klin. Med., 1904, lxxix, 417.
LEVENE AND VAN SLYKE. Jour. Biol. Chem., 1912, xii, 301.
MACADAM. Jour. Path. and Bacteriol., 1913, xviii, 281.
MELLANBY. Internat. Cong. Med., 1913, Sect. 3, (a), Chem. Path., pt. 2, 91.
V. MORACZEWSKI AND HERZFELD. Ztschr. f. klin. Med., 1916, lxxxii, 27.

Munzer. Deutsch. Arch. f. klin. Med., 1893, xxxiii, 164.
Riecke. Dissertation. Würzburg, 1886.
Rowntree, Marshall and Chesney. Tr. Assn. Am. Phys., 1914, xxix, 586.
Stadelmann. Deutsch. Arch. f. klin. Med., 1893, xxxiii, 526.
Yoshida. Biochem. Ztschr., 1909, xxiii, 239.

Urobilinogen Excretion

Bauer. Zentralbl. f. inn. Med., 1905, xxvi, 833.
Eustis. New Orleans, Med. and Surg. Jour., 1912, lxv, 415.
Fischler. München. med. Wchnschr., 1908, lv, 1421.
von Jaksch. Klin. Diagnostik, 1892, 348.
Munzer. Med. Klin., 1913, ix, 586.
Munzer and Bloch. Arch. f. Verdauungskr., 1911, xvii, 260.
Newbauer. Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol., in München., 1903.
von Noorden. Proc. XVII Cong. Med., VI, Part 2, 55.
Wilbur and Addis. Arch. Int. Med., 1913, xiii, 235.

Methylene Blue Test

Babaliantz. Thése. Geneva, 1912.
Castaigne. Jour. de Phys. et de Path., May, 1899, i, 627.
Chauffard. Presse méd., April 23, 1898, i, 129.

Phenoltetrachorphthalein Test

Abel and Rowntree. Jour. Pharmacol. and Exper. Therap., 1909. i, 233.
Kahn and Johnston. New York Med. Jour., 1915, cii, 848.
Krumbhar. *Ibid.*, 1914, c, 719.
McLester and Frazier. Jour. Am. Med. Assn., 1915, lx, 383.
Orndorff and Black. Am. Chem. Jour., 1901, xli, 349.
Rowntree, Hurwitz and Bloomfield. Johns Hopkins Hosp Bull., 1913, xxiv, 327.
Sicson. Arch. Int. Medicine, 1914, xiv, 804.
Whipple, Peightal and Clark. Johns Hopkins Hosp. Bull., 1913, xxiv, 343.

Fibrinogen Test

Corin and Ansiaux. Maly's Jahresb. ü. d. Fortschr. d. Thierchem., 1894, xxiv, 642.
Doyon. Compt. rend. Soc. de biol., 1905, lviii, 30.
Doyon and Kareff. *Ibid.*, 1904, lvi, 612.
Doyon, Morel and Billet. *Ibid.*, 1905, lviii, 108.
Goodpasture. Am. Jour. Physiol., 1914, xxxiii, 70.
Nolf. Arch. internat. de Physiol., 1905, iii, 1.
Whipple. Am. Jour. Physiol., 1914, xxxiii, 56.
——— Arch. Int. Med., 1912, ix, 365.
Whipple and Hurwitz. Jour. Exper. Med., 1911, xiii, 136.
Whipple, Mason and Peightal. Johns Hopkins Hosp. Bull., 1913, xxiv, 207.

The Total Non-Protein Nitrogen, Urea Nitrogen and Amino-Acid Nitrogen of the Blood

Hawk, P. B. Practical physiological chemistry.
Rowntree, Marshall and Chesney. Tr. Assn. Am. Phys., 1914, xxix, 586.

Lipase of the Blood

LOEVENHARDT. Am. Jour. Physiol., 1902, vi, 331.
SAGAL. Jour. Med. Research, 1916, xxxiv, 160.
WHIPPLE. Am. Jour. Physiol., 1914, xxxiii, 50.
WHIPPLE, MASON AND PEIGHTAL. Johns Hopkins Hosp. Bull., 1913, xxiv, 207.
WHIPPLE, PEIGHTAL AND CLARK. *Ibid.*, 1913, xxiv, 343.

Ghedini's Ferment Test

GHEDINI. Gazz. degli Osped., 1913, xxxiv, 41.

The Fibrinolytic Ferment of the Blood

GOODPASTURE. Johns Hopkins Hosp. Bull., 1914, xxv, 33, 330.

Sulphoconjugation Test

AMANN. Rev. med. de la Suisse Rom., 1896, xvi, 339-349; 657-661.
BIERNACKI. Arch. f. klin. Med., 1891, **xlix, 87.**
BUBGINSKY AND MUNK. Med. Chem. Untersuch., 1866, 234.
FINIZIO. Pediatria, Napoli, 1899, vii, 275.
FOSTER AND KAHN. Jour. Lab. and Clin. Med., 1916, ii, 146.
HERTER. Brit. Med. Jour., 1897, ii, 1847.
HOPPE-SEYLER. Ztschr. f. physiol. Chem., 1889, xiii, 31.
KAHN, MAX. Am. Jour. Med. Sc., 1918, clv, 668.
KAHN, M., AND BARSKY, J. Arch. Int. Med., 1919, xxiii, 334.
LEPINE. Rev. de méd., 1881, xxvii, 911.
MAGRAGEAS. Thése de Paris, 1910.
MULLER. Berl. klin. Wchnschr., 1876, xiii, 529.

CHAPTER IV

TESTS OF THE FUNCTIONAL CAPACITY OF THE CIRCULATION

"The heart, consequently, is the beginning of life; the sun of the microcosm, even as the sun in his turn might well be designated the heart of the world; for it is the heart by whose virtue and pulse the blood is moved, perfected, made apt to nourish, and is preserved from corruption and coagulation; it is the household divinity which, discharging its function, nourishes, cherishes, quickens the whole body, and is indeed the foundation of life, the source of all action."—William Harvey, "An Anatomical Disquisition on the Motion of the Heart and Blood in Animals." London, 1628 (Chapter VIII).

The short contractile tube which propels forward the blood contained in the perivisceral cavity of an ascidian, is neither structurally nor functionally much entangled with the other organs of the animal. But on passing upward through higher types of life, this simple tube is replaced by closed arterial and venous systems ramifying minutely in every corner of every organ. We find that the circulatory apparatus, while it has become structurally interwoven with the whole body, has become unable properly to fulfill its office without the help of offices that are quite separated from its own, and unless all of its parts act coordinately, each adequately fulfilling its own distinct and special function.

The efficiency of the cardiac motive power, the vasomotor system, the nervous system and the condition of the other organs of the body all affect the adequacy of the circulation. These factors are variable and, especially the first three, are closely related to each other.

Given the limits of what we are to consider as normal, all variations from the normal must be taken as indicating disturbance of function. *No single test alone is, or need be, made the criterion for an absolute functional diagnosis or prognosis.* Estimation of the cardiac efficiency by one of these tests would only partly disclose the functional state of the circulation. However, by combined study of the functional

organization by means of clinical observation and the use of various technical methods, a fair estimate may be obtained of the state of efficiency of the circulation.

The Cardiac Motive Force.—That the heart shall continue to act normally, its substance must be continuously fed with an abundant supply of arterial blood. Given this fuel, the heart possesses motor powers complete within itself.

It carries in its structure a system of specialized cells which generate automatically the impulses causing its contractions. One group of cells, the so-called sino-auricular node of Keith and Flack,[1] is placed between the sulcus terminalis and the junction of the superior vena cava with the right auricle. This is the normal pace-maker of the heart.[2] Another group of cells, the atrioventricular node of Tawara,[3] is situated in the posterior wall of the right auricle near the interauricular septum. This group assumes the function of pace-maker for the ventricle if the sinus node ceases for some reason to dominate the heart's contractions.[4]

It should be noted that there are a number of other places where an independent contraction may arise. That is, if any part of the heart muscle is rendered more excitable than the nodal structure, the contraction would proceed from that part.[5]

The function of the heart represented by the specialized cells is called the chronotropic function, the function of impulse-formation or of rhythm in the heart's contraction. Their probable mode of action is by conversion of chemical into kinetic force. This function is so specially delegated that any abnormalities of it may be directly attributed to alteration of sensitiveness, disease or destruction of the cells controlling it.

From the sinus region the cardiac impulse normally travels from the auricles to the ventricles along a bundle of Purkinje fibers. This, the bundle of His, divides into two branches which pass to the right and left ventricles and arborize just beneath the endocardium to supply the papillary muscles and the walls of the ventricles. Saigo found that these fibers are much richer in glycogen granules than the ordinary muscle-fibers.[6] For any variation of the function of conductivity (dromotropic function) this specialized system of fibers is therefore responsible.

The property of cardiac muscle to receive stimuli is termed its excitability or bathmotropic function.

The fundamental power of contraction of the heart muscle (inotropic function) differs from the characteristic contractility of skeletal muscle. The nerve distribution in skeletal muscles regulates their contractility. With the heart muscle a stimulus which is just strong enough to cause a contraction will cause the strongest contraction which that heart can make under the given conditions,[7] due to the arborization of the conducting tissue. After the contraction the heart loses for the time being its ability again to respond to a stimulus. This is called the refractory period.

Finally, the heart muscle possesses the property of tonicity which controls the degree of its relaxation.[8] The recognition of the state of tone of the heart musculature is very necessary to understand the failing heart properly.

Perfect functioning of the heart would imply a state in which all the qualities of the cardiac structure are normal and coördinate. In no organ is exemplified to a higher degree the fact that the mutual dependence of functions is proportionate to their specialization. *If any of the qualities are deranged, to that extent will the normal function of the whole organ be disturbed. The recognition of such derangements of function and of their significance forms a part of the study of the efficiency of the circulation.*

The complementary action of the vasomotor system is indispensable in maintaining adequate circulation. The size of the field of distribution of the blood is under vascular control. The degree to which the heart could compensate for extreme loss of vasomotor control would indicate, perhaps more than any other observation, how satisfactory mechanically the organ is.

The Reserve Force of the Heart.—"While all the functions when exercised use all the force they possess, they nevertheless manifest a quality whereby they can respond, under certain circumstances, with a greater activity. Thus the rate may be suddenly increased, and at the same time the stimulus passed from the auricle to the ventricle with increased rapidity, and the contraction be executed quicker. These changes are to a great extent under the control of the nervous system."[9] It is this power of responding to effort that gives us the clue to the real state of the heart.

Under normal conditions an increased demand for blood by an organ may be fully supplied by vasomotor regulation. If the demand becomes general, with a deficiency of oxygen in the circulation throughout the body, as occurs during strenuous muscular exercise, adequate circulation cannot be maintained through vasomotor action alone; the heart must partake in this compensatory process.

During its normal activity the heart possesses an available reserve force by means of which the amount of circulation may be increased. In a state of vasomotor incompetence, therefore, the task of transporting the oxygen and of increasing the velocity of the circulation devolves upon the cardiac motive force.

The reserve force may evince itself in two ways, i.e., either by an increase in rate or by an increase in the systolic output with each beat.

Different individuals react differently in this respect.[10] *In untrained hearts, as well as in the chronic infections and cachectic diseases and in states of nervous or psychic stimulation, the heart usually reacts by an increase in rate. In athletes, on the other hand, or in cases in which exercise has been practiced, the heart usually reacts by increased diastolic intake and systolic output to make up for the circulatory needs.* In the latter case, the heart dilates, increasing its capacity, so that with each

contraction there ensues the expulsion of a larger quantity of blood into the systemic circulation.

In the first instance, the increased rate, if excessive, may partly defeat its purpose. Complete and prolonged systolic contraction usually takes place at the expense of the diastolic period. The tachycardia may become so marked that the period of diastole will be shortened to such a degree that the filling of the ventricles will be very deficient. A small systolic output will naturally occur. The *x*-ray in such an instance would show the size of the heart during diastole to be less than normal.

In the second instance also the demand upon the heart may exceed its capacity. Normally a small amount of residual blood remains in the heart even after complete contraction. If the diastolic intake is abundant and the heart muscle is too weak to expel the entire content with each systole, an increasing amount of residual blood remains in the left ventricle after each heart-beat. The *x*-ray in this instance would show a heart dilated above normal during its diastolic phase.[11]

Upon this fact are based some of the methods of testing the heart's functional capacity. If either of the modes of reaction of the heart to muscular exertion fails to enhance and to maintain the circulation effectively, heart-failure results. The venous pressure increases, the tissues become laden with carbon dioxid and their supply of oxygen from the arterial current becomes inadequate. On the other hand, if the strain is not continued, after a sufficient period of rest, the heart regains its former volume and later its former tonicity and once more may acquire its original strength.

Excessive dilatation of the heart during or after exercise indicates a lowering of tonicity and the onset of overstrain. It represents a pathological condition in which the heart has overstepped its limits. The condition usually recedes and leaves no traces unless the heart is again overstrained while still in a dilated condition.

Compensation and Decompensation.—From the clinical point of view, as long as the heart is able to maintain an adequate amount of blood-flow throughout the circulation, the latter may be said to be compensated. Good cardiac compensation implies that the heart is able not only to do justice to a normal or an increased demand during rest, but also to accommodate itself to extraordinary effort.

Decompensation, or heart-failure, as Mackenzie puts it, is really the outcome of the impairment of the functions of the heart muscle itself. That is, heart-failure is a physiological effect and is frequently not at all relevant to the anatomical changes that have taken place in the heart-valves as result of disease. In like manner, the symptoms of heart-failure are not the outcome of the lesion affecting the heart-valves, but rather they are the expression of impaired function of the heart muscle and furnish evidence of the insufficiency of the reserve force.

"The functional efficiency of the heart muscle depends upon the amount of reserve force, and it is by estimating the amount of reserve force that we recognize the presence and degree of heart-failure, and the bearing of any sign or symptom on the heart's efficiency, which is the

essential matter in the study of all forms of affection of the heart."[12]

Aim of the Functional Diagnosis of the Heart.—Changes in the flow of the blood throughout the body may be occasioned by nervous influences, vascular dilatation and contraction, and by alteration of the heart's action. The first two factors are very variable.

It is the aim of functional diagnosis to estimate the functional integrity of the heart as a pump, to learn if the heart may submit to the usual demands of active life and if it can undergo an anticipated amount of strain, such as is entailed by work, anesthesia, child-birth, febrile toxemia, etc.

Functional diagnosis should also serve as a clinical index of the increase or diminution of the heart's efficiency as the result of treatment or muscular work.

In a comprehensive study of circulatory efficiency, therefore, it should be our aim to ascertain which of the various portions of the cardiovascular mechanism are intact and which are deranged, to what extent the derangement of structure and function affects the circulation of the blood, and to what degree it affects the normal activity and longevity of the individual.

The complementary action of the vasomotor system is very important in maintaining adequate circulation. This factor is different in different individuals and its variations are perhaps the greatest source of error in determining the efficiency of the circulation.

Most of the tests of heart function are based upon circulatory changes produced by muscular exertion. *The clinical symptoms and physical signs may indicate the direction, but probably not the exact level of cardiac efficiency.* Changes of pulse-rate and blood-pressure reveal important criteria in the study of heart function. The technical methods, the sphygmograph and electrocardiograph and the roentgen ray have proven themselves valuable aids.

A more or less complete analysis of heart function, therefore, should comprise the following studies:

I. Significant physical signs and symptoms.
   1. Irregularities of the heart.
   2. Variations in rate—bradycardia and tachycardia.
   3. Changed quality of the pulse—pulsus alternans, etc.
   4. Auscultatory signs.
   5. The evidence in other organs of impaired heart function.

II. Blood-pressure studies.
   1. Cardiac efficiency factor of Tiegerstedt.
   2. The second tone phase test of Tornai.
   3. Cardiac strength—cardiac weakness ratio.

III. Cardiovascular response to a general demand for increased circulation.
   1. Methods depending upon variations in pulse-rate:
      (*a*) The effect of change of posture upon the pulse-rate.

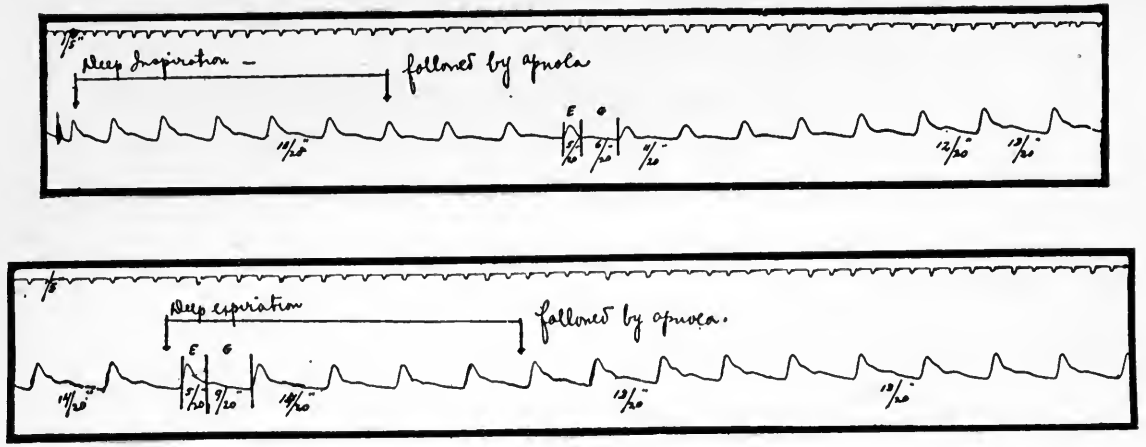


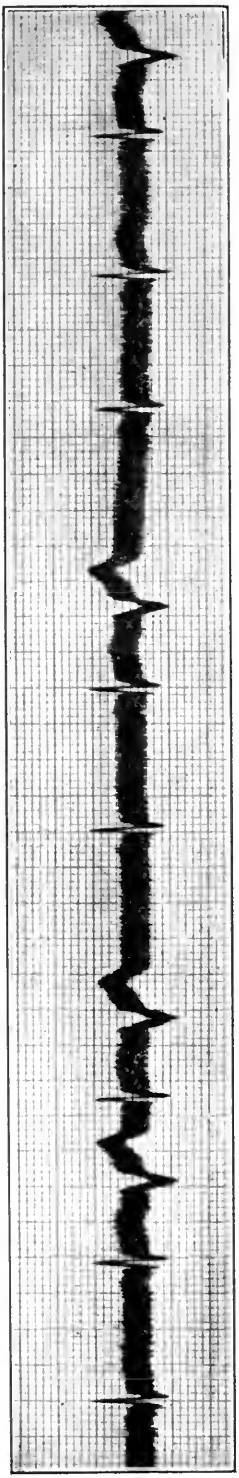
FIG. 9.—PULSUS IRREGULARIS RESPIRATORIUS.

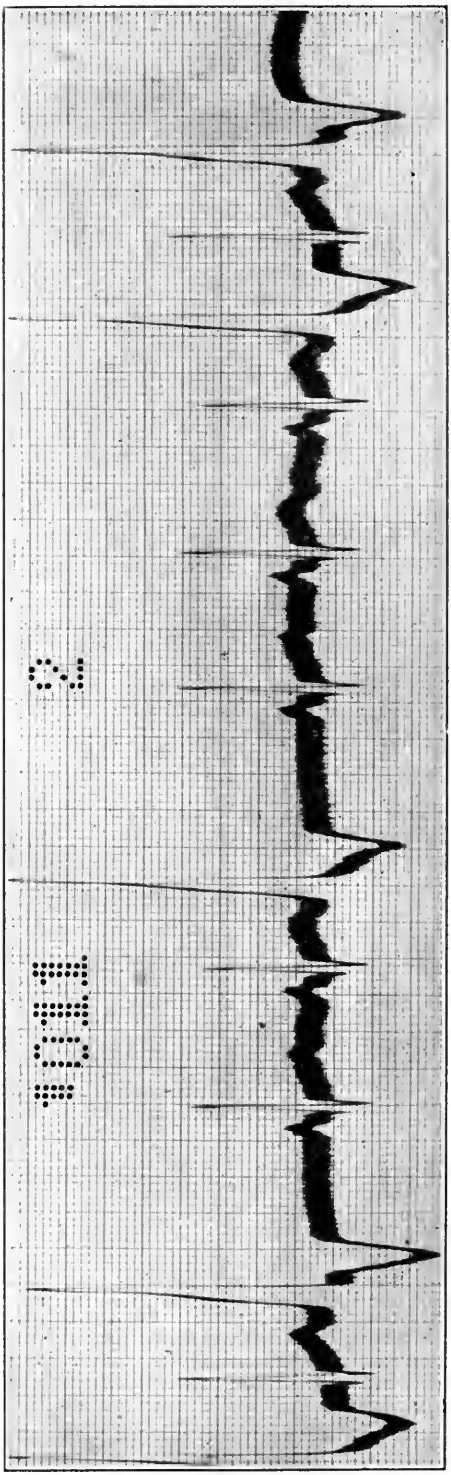
Respiratory variations in the frequency of the pulse are usually due to chronotropic influences (altered stimulus production), probably of vagus origin. Inspiration increases the pulse-rate and expiration decreases it. In sinus irregularities, it is the variation in the length of the diastolic period that is the chief characteristic.

Positive chronotropic influences are often accompanied by those affecting the other functions, the altered rate being associated up to a certain point with variations in contractility and conductivity.

In the tracing the irregularity is seen to be due to variations in the length of the diastole of the heart (period G), the systolic period (E) remaining constant. A deep breath increased the pulse-rate from 92 to 109 per minute. Expiration slowed the pulse to 87.

The tracing also shows the reduction of arterial blood-pressure produced by inspiration.





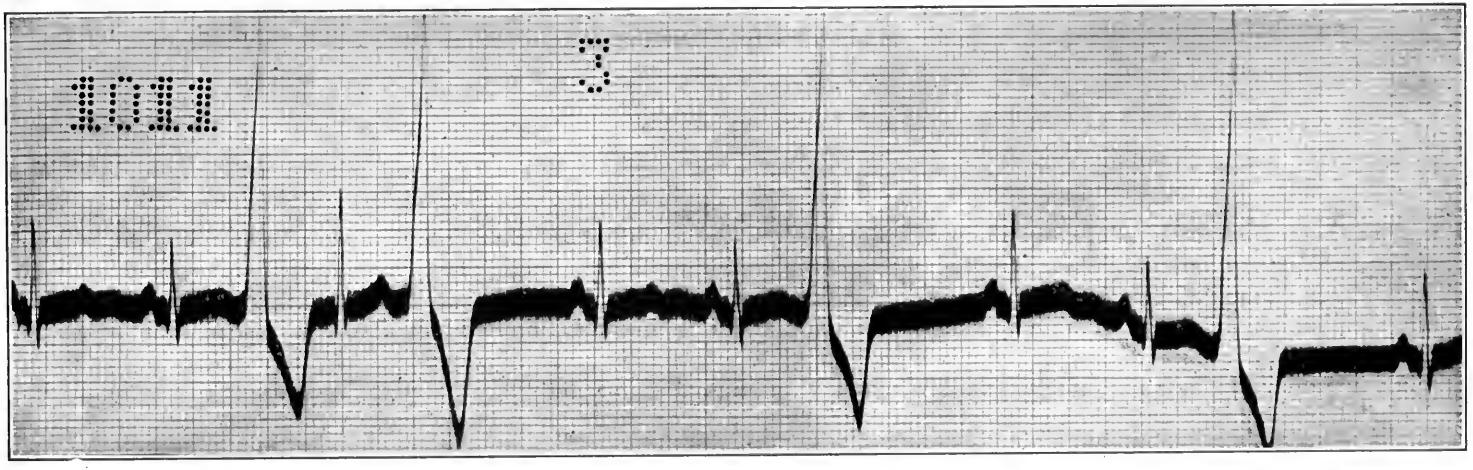


FIG. 10.—ELECTROCARDIOGRAM SHOWING PREMATURE VENTRICULAR CONTRACTIONS.

(*b*) Mendelsohn test.
(*c*) Herz's test.

2. Methods depending upon variations in blood-pressure:
   (*a*) The effect of change of posture upon the blood-pressure.
   (*b*) Effect of exercise upon the blood-pressure.
   (*c*) Stair-climbing test of Selig.
   (*d*) Herz's gymnastic resistance test.
   (*e*) Katzenstein's test—compression of femorals.
   (*f*) Epinephrin test.
   (*g*) Graupner's exercise test.
   (*h*) Barringer's test—"delayed rise" of pressure.

IV. Tests of efficiency of the right side of the heart.
   1. The venous pressure.
   2. Breathing tests.

V. The polygraph.
   1. Interpretation of sphygmographic tracings.
   2. Significant pulse and apex tracings in myocardial degeneration.

VI. The electrocardiograph.
   1. Interpretation of the electrocardiogram.
   2. Significant electrocardiogram in myocardial degeneration.

VII. The roentgen ray and orthocardiograph.
   1. Variations in the size of the heart during and after work.
   2. The reflex effects of external irritation on the size of the heart.

VIII. Metabolic changes in impaired heart function.
   1. Vital capacity of the lungs in relation to heart function.
   2. The elimination of salt as an index of heart function.

IX. Functional tests in congenital and abnormal hearts, in cardiac displacements, and in special diseases.

I. The Significant Physical Signs and Symptoms of Impaired Function.—1. IRREGULARITIES OF THE HEART.—Respiratory arrhythmia and the "youthful irregularity" of Mackenzie do not indicate any disturbance of heart function. In fact, sinus arrhythmia in the young individual indicates normal heart muscle [13] (Fig. 9).

All other forms of arrhythmia are not to be overlooked as they indicate impairment of the motor mechanism of the heart. *When we find irregular action we may assume that a waste of the heart's energy occurs.* After a premature ventricular contraction, there occurs a long pause during which the ventricle is overfilled with blood. The following contraction is necessarily exaggerated in order to expel the increased content. When extra-systoles are frequent, as in pulsus bigeminus, or in pulsus trigeminus, abnormally large quantities of blood are mobilized

with each normal systole. This may eventually lead to fatigue of the ventricular muscle (Fig. 10).

With the pulsus irregularis perpetuus, of auricular fibrillation, a great number of the heart's contractions are incomplete and have little effect upon or value in the circulation. The heart is overstimulated with many futile and unavailing contractions. The waste of the heart's energy is at a maximum and exhaustion of its musculature follows.

2. Variations in Rate.—*Tachycardia.*—As before explained, this may impair the circulation. The beats, following each other in rapid succession, do not permit of proper filling of the ventricle. To maintain the circulation, a slower rate would suffice. The heart, acting rapidly, is therefore working out of proportion to the demand put upon it, and will easily become fatigued. During tachycardia the heart is smaller in its diastolic phase than in normal diastole. With fatigue of the left heart, dilatation takes place, systolic contractions become incomplete and each diastole finds the heart wider than before. In this way a state of tachycardia, apparently harmless, without any recognizable organic basis, can become clinically extremely significant.

Bradycardia.—Disturbances of conduction resulting in prolonged diastole cause an increased intraventricular tension. If there exists complete heart-block, the function of the heart is usually better than in cases of partial or changing heart-block. In complete heart-block, the ventricular musculature adapts itself to the increased amount of blood forced into it by the successive auricular contractions. It dilates and hypertrophies and after every so many auricular contractions propels a larger volume of blood into the systemic vessels. If, however, the ventricle responds from time to time to the auricular stimulus—and it must respond with its complete contraction—there occurs a waste of its energy if it contracts after a short diastole, or it lacks power to completely empty itself if the diastole has been prolonged. Temporary stasis then occurs because the blood is not propelled into the aorta; and signs of congestion of the visceral organs, especially of the brain, develop—such as dizziness, unconsciousness and even convulsions, as in Stokes-Adams syndrome (Fig. 11).

3. Changed Quality of Pulse.—Alternation of the pulse indicates a state of myocardial exhaustion. Pulsus alternans is an irregularity due to a variation in the force of the beats of the heart, a strong beat alternating with a weak one. The rhythm, however, is perfectly regular. The real cause of this form of pulse wave has escaped detection. "The disturbance is dependent upon some unexplained anomaly of the ventricular systoles, whereby at each alternate systole of the left ventricle a greater or smaller quantity of blood is thrown into the systemic arteries."[14] It occurs in cases of tachycardia, where it does not seem to have any very serious import. It also occurs where the heart-rate lies within normal limits, and at such times it is a sign of much clinical value.

In the vast majority of cases it is associated with degenerative changes in the heart muscle, and indicates some degree of heart exhaustion. It

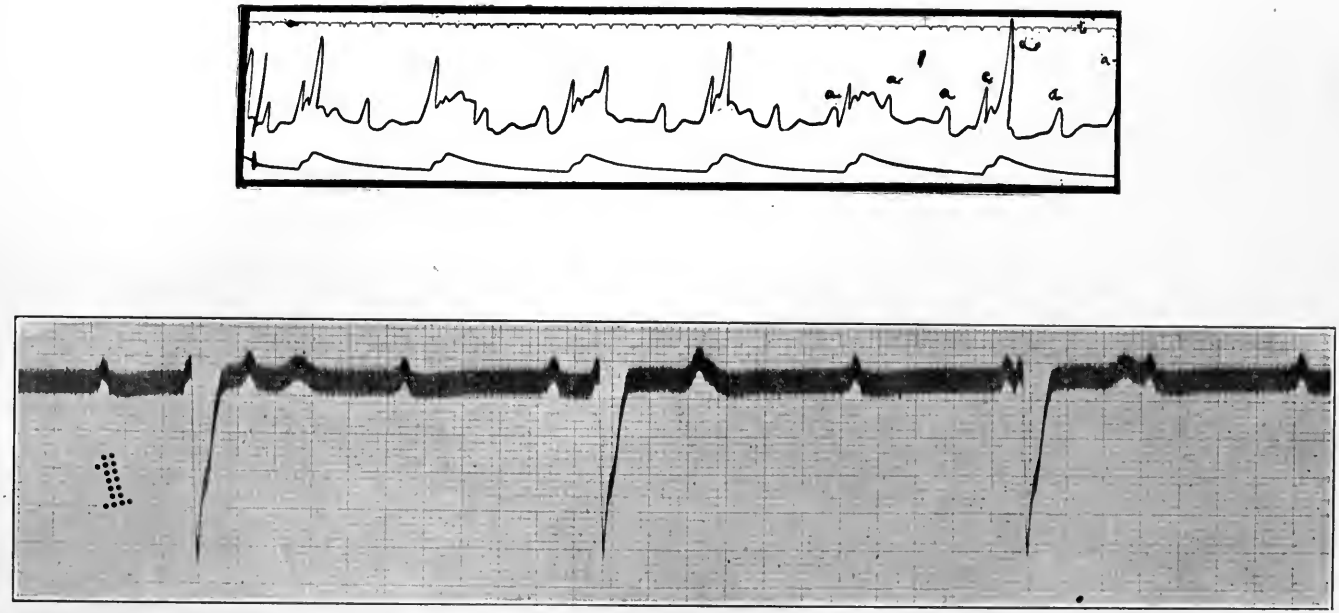


FIG. 11.—POLYGRAPHIC AND ELECTROCARDIOGRAPHIC TRACINGS FROM A CASE OF COMPLETE HEART-BLOCK.

is, therefore, of very grave significance. The alternation itself causes no symptoms, except those referable to the myocardial changes responsible for the pulse anomaly. It may first be discovered upon measuring the systolic blood-pressure in the arm (Fig. 12).

4. Auscultatory Signs of Heart Function.—Auscultation will yield much valuable information as to the sufficiency of the heart. If there is an insufficiency of the power of the left ventricle through diminution of its contractility, or elasticity, the aortic second sound will show a relative reduction in intensity following brief exercise, owing to a smaller systolic output of blood. If under such circumstances the right ventricle remains sufficient, there will be a simultaneous accentuation of the second pulmonic sound. Cases which present this combination of signs are likely to end favorably.

Insufficiency of the right ventricle is also shown by a relative diminution in the intensity of the pulmonic second sound following exercise, and when this is combined with evidence of sufficient power of the left ventricle, the prognosis is less favorable.

If the accentuation of the aortic first sound is greater than that of the second, it is an indication of the hyperexcitability of the vasomotor center which leads to vascular dilatation during work to relieve the strain on the heart. If the vasomotor disturbance is considerable and the aortic sound much reduced, this may result in an insufficient peripheral circulation.[15]

A special form of stethoscope has been devised for the purpose of estimating the comparative intensity of the various heart-sounds.[16] The value of this stethoscope (Bock) depends upon the fact that the normal ratio of intensities of the first sound at the apex to the second sound at the base is approximately 2:1. According to some authors who have used the instrument, this ratio is frequently altered when the myocardium is affected, and may become 1:1.[17]

It is important to differentiate *functional* from *organic* heart murmurs. Functional murmurs show decided changes of intensity, and, in contrast to organic murmurs, rather become weaker than more pronounced by energetic cardiac action, and may disappear entirely if the heart is under stimulation, particularly by digitalis.[18]

5. Symptoms in Other Organs.—When the heart is forced to exercise for a period at the full extent of its power, heart-failure sets in. This produces an insufficiency of circulation in the various organs. The functional impairment of the latter may produce symptoms indicating the debilitated power of the heart.

The symptoms of heart-failure, therefore, are very variable, depending upon which organs in different individuals are most affected by the deficient blood supply.

Dyspnea is one of the earliest symptoms of functional disturbance of the heart. The respiratory center is extremely sensitive to changes of the oxygen and CO_2 content of the blood. Therefore it was maintained that dyspnea results from insufficient oxygenation of the medullary center. This, however, has been contradicted by Kraus, who showed

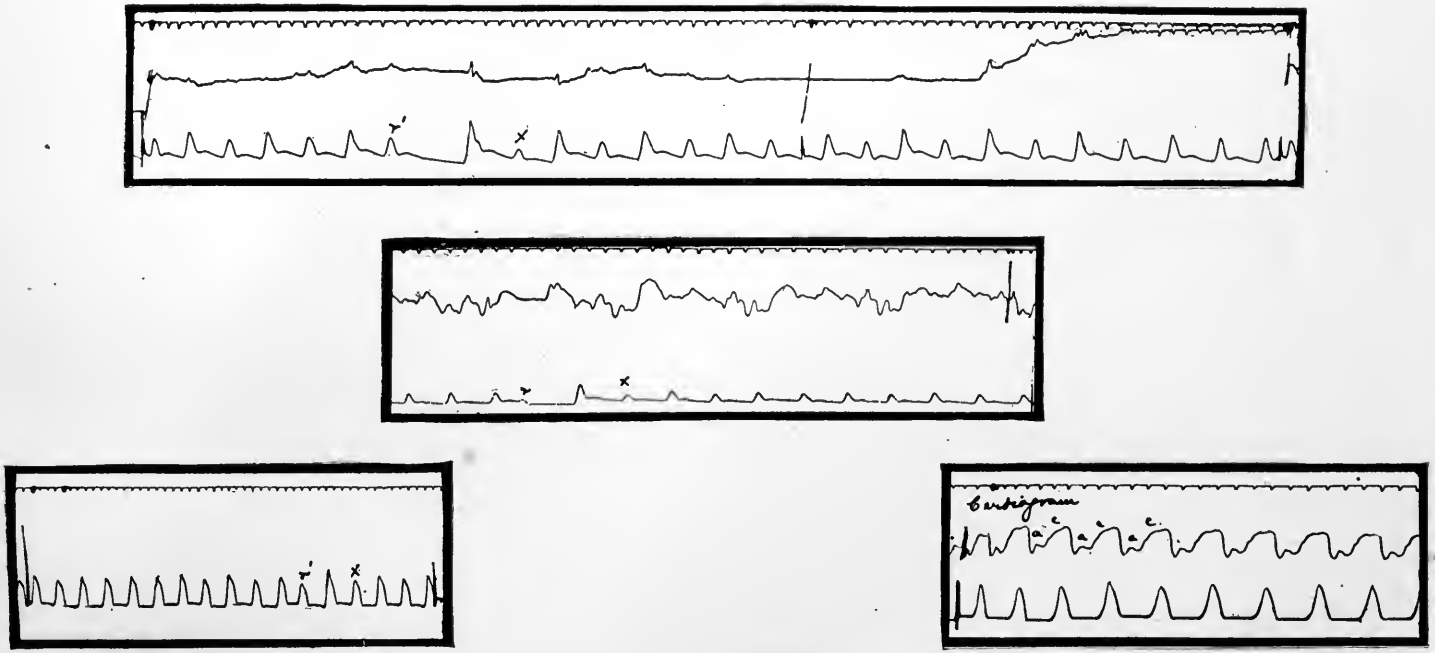


FIG. 12.—PULSUS ALTERNANS.

In the first and second tracings, the alternating character of the pulse is more marked after the long pause following the extrasystole (r'). The second beat (x) following the pause appears at the normal interval, but is greatly reduced in size.

The two lower tracings are from a case of syphilitic myocardial degeneration. Note the marked a wave in the cardiogram.

that the amount of oxygen taken up by the blood and of CO_2 given off per minute is practically unchanged in cardiac failure. That is, the rapid breathing induces a greater amount of air to enter the lungs per minute without altering the amount of oxygen taken up by the blood.[19]

It is, therefore, probable that stasis in the pulmonary capillaries stimulates the vagus endings in the same way as does CO_2. The elasticity of the lung is probably diminished and its volume is increased.[20]

This explanation of the dyspnea in heart-failure finds support in the clinical experience that severe dyspnea, panting, is an early sign of mitral lesions, while in aortic disease it is a sign of severe decompensation. Welch demonstrated that in conditions in which the force of the left heart is impaired without impairment of the right, dyspnea and the other respiratory disturbances occurred.[21] Failure of the right ventricle substitutes a state of broken systemic compensation and the dyspnea may become much relieved and give place to cyanosis, enlargement of the liver and edema.[22]

Associated with dyspnea, perhaps the earliest sign of heart-failure will be found in the appearance of fine *crepitations at the bases of the lungs.* In patients with mitral lesions particularly, the bases of the lungs will be found to show signs of edema in the early stages of breakdown.

Stasis Phenomena.—Stasis phenomena in heart disease, such as edema, ascites, hydrothorax, are evidence of failure of compensation of the right side of the heart. When the force of the heart no longer maintains the arterial pressure at the height necessary for efficient circulation in the tissues, we get the symptoms in the remote organs, with the development of dropsy, ascites and enlarged liver. The disappearance of dropsy is often a definite sign of the restoration of the heart's tone.

Angina Pectoris.—Mackenzie has advanced the view, most significant from the standpoint of functional diagnosis, that "angina pectoris is but the expression of an exhausted muscle." The exhaustion may arise from any cause that overtaxes the heart. The gravity of the case does not depend upon the violence of the symptoms. Its importance must be estimated by an examination of the conditions that have induced the muscular exhaustion. The presence of other phenomena, such as cardiac asthma, pulsus alternans, or Cheyne-Stokes' breathing, is an additional evidence of such advanced exhaustion and renders the outlook very grave.

Allbutt rejects this theory and believes that the angina is a pain located in the ascending or suprasigmoid portion of the ascending aorta, and that this pain is due to tension or stretching of the nerve endings in the adventitia. He believes that the sudden death in angina is due to vagus inhibition as a result of the pain.[23]

II. Blood-pressure Studies—The Absolute Work Done by the Heart Muscle.—1. SPHYGMOMANOMETRY.—The estimation of the arterial pressure is of great importance in the examination of the circulatory system. Not alone is this necessary for the proper interpretation of the various tests later to be described, but it is only from a careful

analysis of the factors entering into its production that the absolute and relative amount of work done by the heart can be ascertained.

With each systole, the left ventricle expels its content of blood into the arterial system. The larger arteries are thus distended to a slight degree. The elastic coats of the arterial walls cause them to recoil and to compress the column of blood within them. This serves to keep the pressure at a certain height in order to maintain a continuous flow of blood through the capillaries. The pressure within the arteries is spoken of as the blood-pressure or arterial pressure.

The factors thus concerned in the production and maintenance of the blood-pressure are: (1) the volume output of the left ventricle; (2) the rate of the heart-beat; (3) the elastic recoil of the arteries, and (4) the peripheral resistance of the capillaries. The viscosity of the blood and the total amount of blood in the body are lesser factors, influencing the peripheral resistance.[24]

2. Methods of Measuring the Blood-Pressure.—*Digital examination* of the pulse reveals a number of important data. The palpation of the arterial wall, the resistance it offers to the compressing finger, the size of each beat, the manner of its rise, the duration of the pulse-wave, and the diastolic pressure after the wave has subsided, are indicators of value, some of which the finger alone can detect. This method, however, offers no mathematical accuracy in interpretation and must therefore be supplemented by the instrumental methods which have come to play a most important rôle in the study of the circulation. However, we must not deprive ourselves of those pulse impressions which the finger alone will reveal to us and some of which cannot be acquired in any other way.

Many *instruments* have been devised to measure the arterial pressure. Some are manometric and other methods are graphic. Most of the instruments, however, depend upon compression of the brachial artery by an air-bag placed around the arm. The bag is inflated to a pressure above the maximal pressure in the artery when the radial pulse is found to disappear. The air in the bag is then allowed to escape and the pressure noted at which the radial pulse-beats return. The pressure in the bag is measured by a mercurial or spring manometer to which it is attached by rubber tubing. It is necessary to use a wide cuff, at least 12 cm., as it has been shown that the possibility of error with a narrow cuff may be as much as 40 per cent. of the reading.[25]

The deduction is that the amount of pressure required to just obliterate the pulse is exactly equivalent to the intra-arterial blood-pressure. One important source of error must not be overlooked. It was formerly assumed that a very small amount of pressure would suffice to completely compress the normal artery.[26] This was evidently fallacious, as it has been definitely shown that it may require a considerable amount of pressure, even up to 30 mm. of mercury, to overcome the resistance of the arterial wall alone in certain cases in which the artery is thick, contracted or sclerosed.[27]

The instruments used are in the main mechanical variations of the

Riva-Rocci type.[28] They all consist of an inflatable air-bag which surrounds the arm. *Hill's* instrument consists of a small bag which is compressed over the radial artery.[29] The bag is connected by a tube to a mercury, compressed air or spring manometer. The bag is inflated through another tube and the pressure read off directly from a scale on the manometer.

Inasmuch as the graphic methods of blood-pressure estimation are not for general clinical adaptation, mention of them will suffice.

The *Erlanger sphygmomanometer* consists of a blood-pressure instrument as above described.[30] The bag, however, is indirectly connected with a Marey tambour and the pressure changes are recorded by the movement of the tambour upon smoked paper on a small drum.

Uskoff's sphygmotonograph consists of a Jacquet sphygmograph combined with a sphygmomanometer.[31] Together with the pulse-wave, the absolute blood-pressure is recorded automatically by means of a float upon the mercury manometer.

The *sphygmobolometer* (Sahli) aims to record the pulsations in the cuff by a float on top of the mercury column.[32]

The *plethysmograph* was the first useful apparatus devised for estimating the blood-pressure in man.[33] The hand or forearm is placed in a glass container filled with water and possessing an accurately fitting rubber cuff to make it air-tight. The inside of the vessel leads by a rubber tubing to a recording pen and drum. It shows the volume changes in the arm caused by variations in blood-pressure.

3. Auscultatory Method of Estimating Blood-Pressure.—Korotkow in 1905 first advocated the use of the auscultatory method of estimating blood-pressure.[34] The usual cuff and manometer are used. A stethoscope is applied 2-4 cm. below the cuff over the brachial artery. The blood-current is shut off and when the mercury is permitted to fall gradually, a clear tone is heard corresponding to the point of maximum systolic pressure. A sequence of sounds then follows as the pressure is gradually reduced in the cuff, until all sound disappears from the brachial artery; sometimes a low, dull tone may persist. This point corresponds to the minimum or diastolic blood-pressure.

Numerous studies have been made to discover the significance of changes in maximal and minimal arterial pressures and careful analyses of the cycle of sounds in the artery below the point of compression have suggested certain tests of myocardial function.

We need not enter into the theoretical considerations regarding the formation and interpretation of the sounds. The factors involved are: (1) the impulse given to the blood-current by the heart's contraction;[34] (2) the suddenness of the distension of the vessel;[35] (3) the amplitude of the blood-wave;[36] (4) the diameter of the vessel,[37] and (5) the resonating character of the cuff.[38]

There are described five phases during the lowering of the external pressure from above the obliteration point.

1. A clear, sharp sound—the index of systolic pressure.
2. A murmur of variable duration replacing the above.

3. A clear, usually loud and snappy sound replacing the murmur.

4. A transformation (usually sudden, at other times more gradual) of the clear sound into a dull one, the index of diastolic pressure.

5. The disappearance of all sound.

The maximal blood-pressure in the arteries occurs during ventricular contraction. The diastolic is the minimal arterial pressure and represents the ebb to which the arterial pressure falls while the aortic valves remain closed. Von Recklinghausen found that in taking the reading by the mercury manometer the large oscillatory waves which occur in the mercury column begin and end abruptly and these points he considers the points of maximal and minimal pressure.[39]

The amplitude or range of arterial pressure between systole and diastole is the result of the volume output into the circulation of the heart and is called the pulse-pressure. It indicates the extent to which the heart overcomes the peripheral resistance. It represents that excess over diastolic pressure which opens the aortic valves and distends the arterial walls.

We shall concern ourselves with the significance of the blood-pressure as an index of the functional capacity of the heart.

The normal systolic blood-pressure of adults up to forty years varies from 115 to 125. It is less in younger individuals and females, and usually increases with age, so that a pressure of 140 to 150 may be considered normal in people over fifty years. The normal diastolic pressure ranges from 30 to 50 mm. mercury below the systolic pressure.

Gibson has suggested that certain arithmetical relations normally exist between the three divisions of the blood-pressure:[40]

$$\text{Diastolic}:\ \text{Systolic} = 2:3$$

and

$$\text{Pulse Pressure}:\ \text{Systolic Pressure} = 1:3$$

Based upon the ratio of these factors to each other, certain methods have been suggested for the estimation of the efficiency of the heart considered as a pump.

4. Tests for the Estimation of the Efficiency of the Heart.—(*a*) *Cardiac Efficiency Factor of Tigerstedt.*—Tigerstedt[41] utilizes the formula of Poiseulle[42] which is used for fluids circulating in horizontal tubes. Reasoning from that formula, he suggested the following:

The pulse-pressure or volume output of the heart multiplied by the pulse-rate represents the velocity of the circulation. The systolic pressure multiplied by the pulse-rate represents the total amount of work done in the circulation. The quotient of the velocity of circulation divided by the total work in the circulation gives the efficiency of the heart as a pump. Thus:

$$\frac{\text{P. P.} \times \text{Pulse-rate} = \text{Vel.}}{\text{Syst. P.} \times \text{Pulse-rate} = \text{Work}}$$

$$\frac{\text{P. P.}}{\text{S. P.}} = \text{Efficiency of heart as a pump.}$$

This is called the blood-pressure coefficient. In the normal individual this coefficient is 25 to 35 per cent. A cardiac efficiency factor below 20 per cent. or over 40 per cent. would indicate myocardial inefficiency. To illustrate, a systolic pressure of 130 mm., while within normal limits, may in truth represent the total power of a failing heart if accompanied by a diastolic pressure of 110 and a pulse-pressure of 20 mm.—as the formula would show:

$$\frac{\text{P. P.}}{\text{S. P.}} = \frac{20}{130} = 15 \text{ per cent.}$$

The same conditions may be present with a high systolic and a high diastolic pressure. Great increase of the P. P. is pathological and may be more than the normal heart can stand. The myocardium may, to a limited extent, hypertrophy, to be enabled to bear the overload of volume output. If the overload becomes excessive and the heart begins to fail, the pulse-pressure would fall at the expense of the maximal pressure. Thus in compensated and advanced arteriosclerosis and chronic interstitial nephritis a systolic pressure of 180 to 200 mm. accompanied by a diastolic pressure of 110 to 120 mm. is frequently seen, and in such cases when the heart begins to fail the systolic pressure falls, while the diastolic pressure, owing to the rigid arteries and the increased pulse-rate occasioned by the dilating heart, may remain relatively high, and as a result the pulse-pressure falls until the head of pressure in the arteries is no longer sufficient to maintain the circulation.

Stone has suggested the determination of a cardiac load and overload factor based on auscultatory blood-pressure determinations.[43] He considers that the pulse-pressure measures the energy of the heart in systole in excess of the diastolic pressure. For clinical purposes, it represents the load of the heart. Under normal conditions it is approximately 50 per cent. of the diastolic pressure. The myocardial load, according to Stone, may therefore be expressed by the fraction

$$\frac{\text{Pulse Pressure}}{\text{Diastolic Pressure}} \text{ or } \frac{\text{P. P.}}{\text{D. P.}}$$

Since the diastolic pressure measures the peripheral resistance, it is a better index of hypertension than the systolic pressure. A sustained diastolic pressure of 100 to 110 signifies hypertension. The diastolic is less influenced by physiologic factors than the systolic pressure.

(*b*) *Tornai's Test: Auscultatory Phase-relations in Functional Diagnosis of the Heart.*—Korotkow recognized three phases in the arterial tones produced by compression of an artery by a Riva-Rocci cuff, a maximum pressure tone, a minimum pressure tone and a murmur mixed with these occurring at a variable point between these two. Later authors recognized (Ettinger,[37] Fischer [44]) a fourth dull tone below the highest diastolic point.

Tornai discerns as many as six phases and he bases his functional

diagnosis upon certain variations in the relationship of these tones to one another.[45]

Technic of the Test.—Tornai finds a narrow cuff (modified by Sahli) most convenient for auscultatory estimations. The minimal or diastolic blood-pressure is first determined. The cuff is then inflated to ascertain the maximal pressure. Gradually the air is permitted to escape from the cuff and the pressure is noted at which the arterial murmur appears. The pressure is further lowered and the point noted at which the murmur disappears. Note is kept of whether the murmur is loud or not and whether it is pure or mixed with the pulse-sound.

The patient then executes a moderate amount of work—such as sitting up and lying down several times, or raising his feet in the air a number of times. It is important to eliminate as much as possible any excitement or undue strain. For each of several minutes after cessation of the muscular exercise the blood-pressure readings are taken with especial reference to the time, duration, character and intensity of the arterial murmur.

These measurements indicate the duration of the altered heart action and how long it takes the heart to return to normal.

Standards.—In healthy people there is normally a period in the blood-pressure reading during which an arterial murmur is audible. This murmur increases in duration after moderate exercise, above the maximal point. Within 3-5 minutes after work, however, the normal heart returns to its normal phase-relations.

After fatiguing work, 8-10 minutes may elapse before a return to normal. During this period after fatiguing exercise the maximal and minimal pressure as well as the position of the murmur are lower than before.

When we find, therefore, that after exercise the arterial murmur begins higher and nearer the maximal pressure and that the maximal and minimal pressures have not fallen, we may conclude that the heart in that case possesses a sufficient reserve force (for the amount of work executed).

In heart disease diminished reserve force will be indicated by a shortening of the murmur phase and its depression after exertion.

In cases of marked hypertrophy of the left ventricle Tornai found that a normal reaction to exercise may take place because of the increased output with each beat, although the functional capacity of the heart may be affected by its valvular trouble.

He tabulated the findings in a number of cases before and during treatment. In one case of Addison's disease phase-variations in the blood-pressure were restored approximately to normal during a systematic course of adrenalin treatment. In a second similar case no benefit was derived from the treatment and the abnormal auscultatory findings persisted.

(*c*) *Cardiac Strength-Cardiac Weakness Factor as Measured by Auscultatory Phases.*—Goodman and Howell have measured the five different phases of the cycle in figures of pressure on the manometer.[46] With

the normal pressure, systolic 130 mm. and diastolic 88 mm., the phases are fairly distinct and bear a certain relation to the extremes of pressure.

The first phase, or tone phase, serves principally as an index of the height of systolic pressure. The second or murmur phase seems to be especially dependent upon cardiac effectiveness. It is in this phase alone that the individual sounds possess a distinct element of duration. Many authors have agreed that the second phase or murmur phase of the auscultatory blood-pressure observation indicates cardiac strength. Tornai was among the first of these, and he has developed the test of myocardial efficiency based upon it (described above).

The third phase, or second tone phase, according to Goodman and Howell, depends not alone on cardiac efficiency, but also on the character of the vessel wall. The more sclerotic the vessel and the greater the cardiac hypertrophy, the more favorable are the conditions for the production of a clear tone.

The fourth phase indicates the point of diastolic pressure. Goodman and Howell found the following percentages normal:

1st phase	31.1
2nd phase	44.4
3rd phase	11.1
4th phase	13.3

They considered that the cardiac strength as indicated by the sum of the second and third phases bears normally a ratio of 5 to 4 to the sum of the first and fourth phases.

It is, however, not always practically applicable, because the second sound is sometimes absent normally and difficult to determine and also because a short second phase is very frequently made up by a long third phase.

III. Cardiovascular Response to a General Demand for Increased Circulation.—1. Methods Depending upon Variations of the Pulse-Rate.—(*a*) *The Effect of Change of Posture upon Pulse-Rate.*—As early as 1833, Donnell showed that the pulse-rate is normally slower in the recumbent than in the erect or semi-erect positions.[47] Schapiro made the observation that the normal difference disappears when the heart is seriously weakened.[48] Hogerstedt and Graupner noted the return of the pulse-difference, after the effect of digitalis upon the heart was established.[118] In one hundred cases recently tested, it was found that the slowing of 7 to 15 beats per minute, which recumbency normally produces, is diminished or altogether lost in cases of incompetent valvular disease or when the heart is seriously weakened by any cause.[49] Geigel found that a variation of pulse-rate above 30 between lying and standing or an inversion of the normal relationship between lying and standing indicates a weakened heart function.[50]

Psychic factors may considerably disturb the correctness of the esti-

mate in any particular case. The normal increase from reclining to standing posture should never be more than twenty beats per minute.

In a group of 124 soldiers with clinically normal hearts studied by the author, the pulse-rate rose an average of 16 beats on change from the seated to the erect posture. The variations from the average were comparatively slight; the maximum rise was 28 in a few cases, and the minimum in one case was 0; the most frequent figures were 12, 18 and 24. Whenever a variation of 30 or over occurred in this series of normal cases, subsequent tests proved that this was not constant and probably due to adventitious influence.[51]

Prével considers the mechanism causing the acceleration of the pulse on changing from the reclining to the upright position an abdomino-cardiac reflex. The contents of the abdomen slide down, inducing an acceleration of the pulse as the gastric ramifications of the pneumogastric are stretched.[52]

(*b*) *Mendelsohn's Test: The Effect of Exercise upon the Pulse-Rate.*—The variations of the pulse-rate following exercise were the first to be studied in their functional significance. Mendelsohn and Graupner estimated the length of time it takes for a normal heart to return to its previous rate after a measured amount of work.[53] The time it takes for any given heart to return to normal after a definite amount of exercise was then suggested as a measure of its functional capacity. The longer the time it takes to return to the normal rate, the less efficient the heart is considered.

The technic of the test is very simple. The pulse-rate is noted after a period of rest in the standing and the recumbent postures. The patient then performs a measured amount of work, either on a Gaertner ergostat or in any other way. Cabot and Bruce use stair-climbing and calculate the number of foot-pounds of work by multiplying the height climbed by the weight of the individual in pounds. The pulse is counted at rest after the work stops and the time noted until its return to normal.

Mendelsohn finds that after 100 to 200 kilograms of work the pulse in healthy hearts returns immediately to normal. After 200 to 500 kilograms, the pulse returns to normal in 2-3 minutes. Sometimes it falls slightly below normal, but returns to normal within 2-3 minutes. Above 500 kilograms of work the normal heart may increase in rate for a short period even after the cessation of work, or it may fall below normal to return to its original rate after a shorter or longer period.

Mendelsohn asserts it as a principle that, the greater the amount of work done with prompt return to the normal rate, the greater is the functional capacity of the heart. He emphasizes the point that the amount of work should be considered as of relative value only. Absolute amounts of work cannot be laid down as the normal for any person because the capacity for work varies with the weight, muscular development and general makeup of the individual.

In cases of heart disease, often after 25-50 kilograms of work, the heightened rate will maintain even after a prolonged period of rest.

(*c*) *Stair-Climbing Test of Selig.*—Selig experimented with 100 per-

sons in various states of health before and after exercise. The exercise consisted in stair-climbing and, in several cases, a game of football. Immediately after the exercise the change in outline of the heart was noted by percussion, and the pulse and blood-pressure taken.[54]

Selig found that in healthy individuals the pulse rose an average of 20 beats per minute, the highest rate in his series being 153. Various authors give slightly different figures of the highest pulse-rate after work which is compatible with normal cardiac efficiency—thus, Staehlin, 156[55]; Tantweiler, 170[56]; Grünbaum and Amson, 173[57]; and Goodall, 180.[58]

In most of the 46 cases of various general diseases tested the pulse-rate increased slightly more than normally and the blood-pressure fell after the work. The results showed, however, considerable difference between the various types of cases.

In the 43 cases which showed heart disease, the pulse increased an average of 19 beats per minute.

This staircase test is perhaps the most commonly employed on account of its simplicity and the fact that it can be performed without any special apparatus. In doing this test, we find almost always complete corroboration of the patient's statements regarding his cardiac capacity. Stair-climbing or merely walking is an experiment every cardiac patient necessarily performs upon himself in the usual course of his life and the symptoms of fatigue come perhaps concomitantly with the physical signs we discover.

The "hopping test," or bending the knees or the trunk, are modifications of the Selig test. These are used not only for the study of heart function, but frequently as a routine method to elicit a latent valvular lesion. These tests have the defect that the actual amount of work performed by the individual cannot be computed.

Lian found the following modification of the exercise test of value. It is based upon counting the pulse after an exactly measured physical exercise.[117] The test consists in having the subject, after the pulse-rate at rest in the standing position has been carefully ascertained, execute running steps on one spot, with the legs bending to a right angle with the thighs, at the precise rate of two steps a second. At the end of one whole minute the subject stops and remains at rest, standing, while the pulse-rate is counted for fifteen seconds in every minute until it returns to normal or nearly so. The heart is considered functionally adequate when this takes place before three minutes have elapsed after the exercise. A rise in the rate to over 120 a minute from the normal resting rate of 70 to 80 is held to show slight cardiac weakness. When the rate rises as high as 152 and returns to normal only after four or five minutes, functional deficiency is distinct.

Acceleration of the heart at the beginning of voluntary exercise is chiefly due to the decrease in tone of the cardio-inhibitory center. This is based upon evidence provided by Gasser and Meek.[59] After the removal of the accelerators and subsequent section of the vagi, marked acceleration of the heart may still be produced by a short period of exercise. Acceleration may, however, be brought about through the accel-

erators if necessary. These are a factor of safety and in exercise their action is superimposed on that of the vagi only in times of great need. Aside from this, their chief function is maintaining the level of the resting pulse. Snyder [60] regards the respiratory change of heart-rate also as a depression of the vagus center, an automatic effect from the inspiratory center.

(*d*) *The Hopping Test.*—The "hopping test" used in a study of the author is a modification of the stair-climbing test commonly employed, and when standardized yields equal service with the latter. In performing the prescribed exercise, the author considered that the body weight is raised, on an average, three inches or one-quarter of a foot. The toes are raised from the ground perhaps one inch and the knee is raised about two inches. Taking the average weight as 150 pounds, the amount of work performed in 100 hoppings would be about 150 × 3/12 (feet) × 100, or 3750 foot-pounds. When the individual's weight is known, as it was in all of our cases, the approximate of work performed was easily computed. The weight of the individual was considered the only variant, as the height of hopping was controlled as much as it could be to about the average.

In the group of 124 normal cases, the pulse-rate rose an average of 25 beats above that of the standing posture as counted immediately after the exercise, or of 41 beats above that of the sitting position. During the two minutes following the exercise, it fell an average of 29 beats with the patient seated. There were no extreme variations in these figures, so that the results here obtained, as shown in Fig. 13, may be taken as the normal pulse variations from the sitting to the standing postures, and also following the prescribed form and degree of exercise.

Observations were made by the author upon a series of 32 cases of simple tachycardia. They all presented moderate tachycardia, above 100, and in most instances 120 beats a minute, more or less persistent, but without discernible cause and with otherwise normal hearts.

The increase of pulse-rate in simple tachycardia, as a result of exercise, over the rate in the erect posture, was much less than normal. The average increase after exercise was only 11 beats a minute; the variations above and below this were slight.

In two cases of sinus bradycardia with persistent pulse of 60 and less, the results of comparative tests revealed what may be interpreted as vagotonic influence upon the pace-maker. Thus the pulse rose 25 beats in changing from sitting to standing posture, but exercise did not increase it at all, the effect being more complete than in simple tachycardia. The pulse then fell even lower than before. The systolic and diastolic pressure changes were quite normal.

In 12 cases of fully compensated mitral regurgitation the blood-pressure reactions were normal. The changes of pulse-rate were almost exactly like those in our group of cases of simple tachycardia. That is, change of posture from sitting to standing increased the rate more than normally; exercise, however, effected the same change as appeared so characteristically in the cases of simple tachycardia, and was

even more marked, i.e., there was only a slight increase of pulse-rate, an average of 6 beats per minute.

(*e*) *Herz's Test* (*"Selbsthemmungsprobe"* [61]).—The technic of this test is as follows: The pulse-rate is taken with the patient in the recumbent posture. The patient is then instructed to alternately flex and extend one forearm. In doing this, however, the patient is instructed to contract both the protagonist and antagonist muscles at the same time throughout the exercise, thus in each case resisting his own movements. The movements are slow and strenuous. They can be controlled by the examiner if he guides the elbow and wrist in their motions.

Grünbaum and Amson have previously shown that normally, with this exercise, a very slight rise of pulse-rate may occur, or the rate may remain unchanged. In cases of muscular heart affection, Herz found that the pulse is inevitably slowed by this "Selbsthemmungsprobe." According to the author, hypertrophy and dilatation of the heart both give the same "S" reaction. The findings of Herz are apparently empirical. He offers no theoretical explanation for his findings. There does not appear to be any physiological explanation for this reaction, and psychic effect may be sufficient to cause a change of rate. Hirschfelder believes that the influence of the vagus in certain cases is a factor in producing Herz's results.[62]

Cabot and Bruce found that normally Herz's test was relative, but that in some of their cases of myocarditis the pulse slowed 5 to 20 beats. They, however, did not follow Herz's technic in performing the test. They made it a rule that extension shall consume a full minute's time and flexion also a minute, a condition which would inevitably introduce the psychic and nervous effects. They also restricted muscular contraction, which Herz especially emphasizes. The results of their studies with those of Herz are therefore not exactly comparable.

2. Methods Depending upon Variations in Blood-Pressure.—(*a*) *Effect of Change of Position upon the Blood-Pressure.*—The auscultatory method for blood-pressure examination should properly be applied with the patient's arm at the side of the chest, as the normal reading varies considerably with the arm in different positions. This holds for both the seated and the standing postures and even in the recumbent position, and was carefully adhered to in all the author's observations.

Change of posture causes a vasomotor effect that manifests itself by a change of systolic and diastolic pressure and of pulse-pressure. This is the more marked the greater and the quicker the change of posture is executed. Crampton found that the systolic pressure is increased normally on change from the recumbent to the standing position, while in conditions of lowered vasomotor tone it remains the same or is decreased.[63]

From the seated to the standing position the change is distinct and normally quite characteristic. In our group of normal cases the systolic pressure fell an average of 2 mm. of mercury on change to the standing position. In the large majority of cases the change ranged between

a rise of 10 mm. or less and a fall of 10 mm. or less. In one case a rise of 26 and in two cases a fall of 24 mm. of mercury were the extremes.

The diastolic pressure rose an average of 7 mm. from the sitting to the standing posture. It rose in almost every case and in a few cases as high as 20 mm. of mercury; in a few cases it fell 10 mm.; in only one case it fell 27 mm. The pulse-pressure fell an average of 9 mm. of mercury on change from the sitting to the standing position.

(*b*) *Effect of Exercise upon the Blood-Pressure.*—Arno Lehndorff showed experimentally that stimulation of the splanchnics produces their contraction with increase of blood-pressure to a varying degree. This is also the normal effect of muscular work. If the heart's action becomes insufficient during exercise, the blood-pressure falls.[64]

The "hopping exercise," as previously described, was used in the author's study. The systolic pressure in the group of 124 normal cases, measured 30 seconds after the completion of the exercise, with the patient seated, had risen an average of 21 mm. in the group of normal cases on change from the previous sitting position (Fig. 13). The largest number showed a rise of between 6 and 30 mm., the highest rise being 60 mm. in one case. In three cases only was there a fall of systolic pressure of 4, 5 and 9 mm. of mercury respectively.

The diastolic pressure, 30 seconds after exercise, had fallen an average of 4 mm. on change from the previous sitting posture. A slight fall of diastolic pressure was the usual finding, although a rise of 10 mm. or less occurred in a number of cases. In one case the diastolic pressure rose 25 mm. The pulse-pressure increased considerably with the exercise.

The systolic and diastolic pressures and the pulse-pressure gradually returned to almost the normal within about two minutes following the cessation of the exercise. Table 1 (page 196), entitled "Functional Capacity of the Circulation in Neurocirculatory Asthenia," presents the form in which our data were collected.

(*c*) *Stair-climbing Test of Selig.*—In the healthy cases tested by Selig, the blood-pressure rose an average of 8 mm. after work, and the outlines of the heart and the position of the apex beat remained unchanged. In his cases with heart disease the blood-pressure rose an average of 10 mm. of mercury. However, in some of these cases with valvular defects, the climbing of as high as 2000 steps often did not fatigue, whereas in the cases of myocardial weakness, even a very few steps were at times fatiguing, and these showed a dilated heart after light work.

(*d*) *Herz's Gymnastic Resistance Test.*—Herz suggested another test in which the change of blood-pressure after resisted movements is supposed to indicate the functional reaction of the heart.[65]

The patient is seated and executes movements of flexion and extension of the forearm or abduction and adduction of the thighs, or separation and approximation of the extended lower limbs. These motions are controlled by resistance exerted by the examiner.

The blood-pressure is taken while at rest and then after each of the

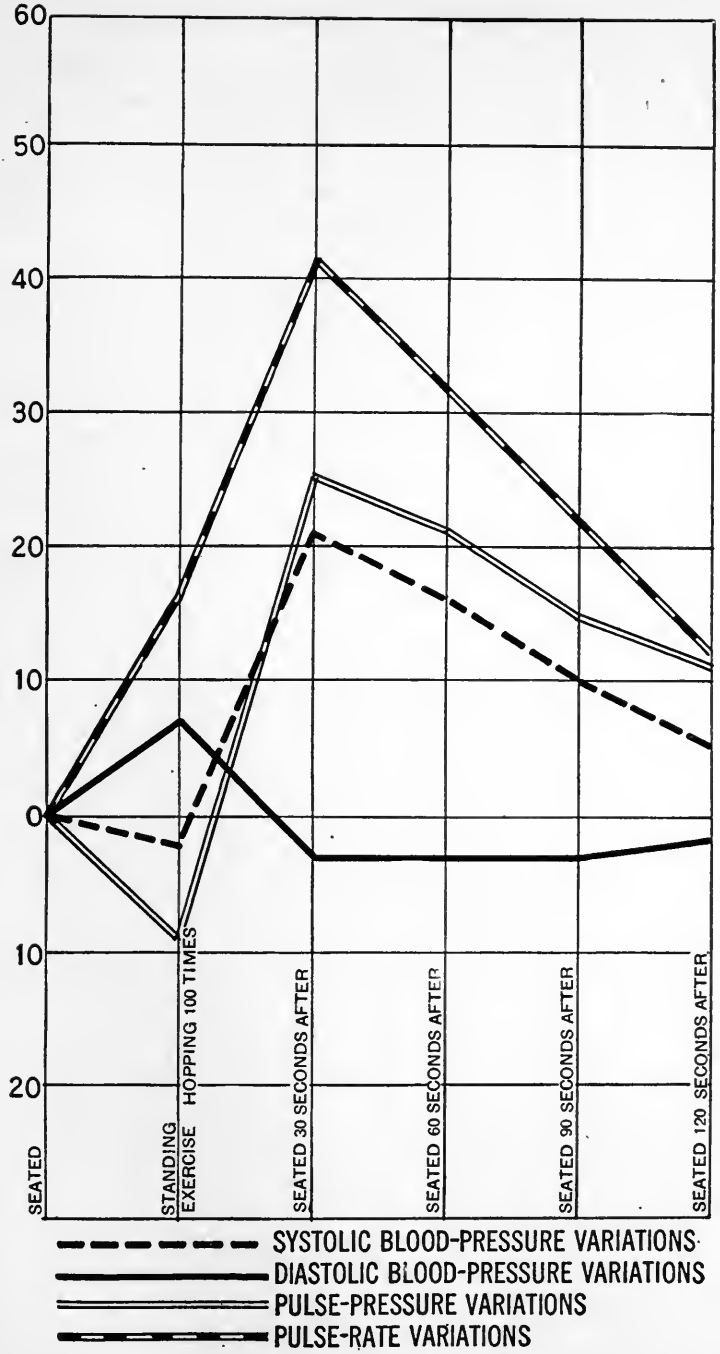


FIG. 13.—ANALYSIS OF STUDIES MADE UPON 124 INDIVIDUALS WITH CLINICALLY NORMAL HEARTS.

The complete series of observations were made by the author in practically all the cases.

various forms of resisted movements. The normal change of blood-pressure as a result of the exercise is not more than 10-15 mm. of mercury.

In cases of decompensated circulation the difference in blood-pressure may reach 20-30 mm. This test is crude and indefinite. The limits of the normal variation are not exact and the vasomotor influence is so great proportionately, that the test is not serviceable to indicate a deficiency of cardiac motive force.

(*e*) *Katzenstein Test.*—Marey and Weber have found that the blood-pressure increases after ligation of a large artery, such as the femoral or iliac, or after compression of the abdominal aorta in laboratory experiments.[66] If we increase the peripheral resistance by suddenly diminishing the vascular field, a marked rise in blood-pressure occurs. The heart normally does not increase in rate but there occurs an increase in the ventricular output, the strain falling directly upon the left ventricle—corresponding to the second form of heart reaction before described (*see* page 138).

Experimentally, hypertrophy of the ventricle takes place. In weak animals, however, the heart does not stand the increased strain and dilates, the animal dying of heart-failure.

This principle is used by Katzenstein as a test of the heart function.[67] The patient lies quietly in the recumbent position. The pulse-rate and blood-pressure are carefully taken. Both iliac arteries are then compressed digitally by an assistant for 2-2½ minutes, although Katzenstein advises compression even up to 5 minutes. Compression may be made by an Esmarch bandage, or, according to Merelli, by an inflatable rubber cuff.[68]

Katzenstein deducts the following standards from his clinical studies and animal experimentation:

1. In normal compensated hearts, after compression of the iliac arteries, the blood-pressure rises 5 to 15 mm. of mercury and the pulse-rate falls.

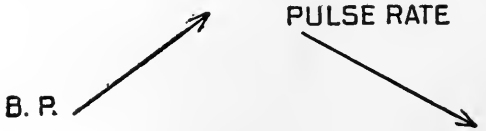


Fig. 14.

2. In hypertrophied compensating hearts, the blood-pressure rises 15-40 mm. mercury, and the pulse-rate falls or may remain unchanged.

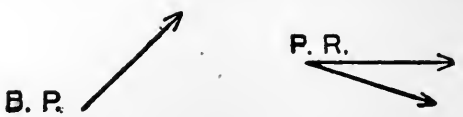


Fig. 15.

3. In beginning decompensation the blood-pressure remains unchanged while the pulse-rate increases.

FIG. 16.

4. In cases of cardiac decompensation the blood-pressure falls while the pulse-rate increases considerably.

FIG. 17.

Pain is sometimes produced by the digital pressure required to obliterate the femoral or iliac pulse. The psychic influences here are particularly disturbing and the pain may of itself cause variations of pulse-rate and blood-pressure.

Levy has corroborated the findings,[69] but several clinicians could not carry out the test extensively because of pain caused by digital pressure.[49]

Recently Elliott reported observations on the effect of partial and complete occlusion of blood-vessels on the general blood-pressure in man.[70] He found that annular compression of the thighs causes a rise in blood-pressure, larger and more immediate the higher the point of compression. This is due to the increase in arterial resistance produced by diminishing the available capacity of the arterial system. The heart-rate is somewhat slowed. Decompression of the thighs causes a fall in blood-pressure, more immediate and more steep than the previous rise. The heart rate is then somewhat accelerated.

There was no evidence that compression of an arm, as in making blood determinations on man with the aid of a sphygmomanometer cuff, produces any effect on general blood-pressure.

These results have been further substantiated in a case of femoral arteriovenous aneurysm. Closure of the communication between the femoral artery and vein produced a tremendous rise of blood-pressure and a slowing up of the pulse-rate. Opening it, of course, had the opposite effect. The slowing of the heart-rate may be induced directly by the increased blood-pressure.

Norris made investigations with the Katzenstein test and found in general confirmatory results.[71] In many cases, other factors were necessary to form a fuller opinion of the heart's efficiency. He felt that the test was not absolute, but that it was of some value as a corroborative observation.

It is of interest that Katzenstein used the test to determine the risk and prognosis of anesthesia and surgical procedures on his cases. He found that an abnormal reaction to the test should interdict general anesthesia or major operations.

(*f*) *Epinephrin Functional Test of the Heart.*—Epinephrin induces vasoconstriction, which raises the blood-pressure, thus throwing extra work on the heart. The sound heart responds with energy, and roentgenoscopy shows slight if any change in the outline of the heart an hour later. A weak heart dilates at once and the dilatation persists for some time. This indicates weakness of the myocardium, either of the muscle itself or its innervation, simple exhaustion or a valvular lesion. The dilatation is most pronounced with mitral disease. With aortic insufficiency, the aorta may become dilated under the epinephrin test while the apex region shows no change.

The test dose of epinephrin is 1 mg., and the subject has to keep absolutely still, against the table used in the test. The roentgenoscopy is repeated three times at half-hour intervals; the findings at one hour are the criterion. The normal heart shows a very slight shrinking of the outline after an hour and a half, testifying to the secondary constricting action on the musculature of the heart.

Loeper[72] and his co-workers studied 100 cases before and after administration of epinephrin. The response may vary with the amount of epinephrin already circulating in the blood, also with the degree of arteriosclerosis that may be present, and with other conditions. The response may thus vary widely in different positions and in the same person at various times. But combining the test with careful investigation of the pulse and blood-pressure, before and after, provides an important means of estimating conditions in the cardiovascular apparatus with reactions similar to those in the Katzenstein test.

Meek and Eyster[73] observed that the intravenous injection of epinephrin normally causes a decrease in heart-rate. Its action is twofold, accelerating the heart by direct stimulation and inhibiting it reflexly through the vagus, but the net result of this balanced mechanism is normally a decrease in pulse-rate.

(*g*) *Graupner's Test.*—Graupner's Test[74] for estimating the functional capacity of the heart depends upon the principle that the reaction of the weakened heart to a measured amount of work differs from the reaction of the normal heart. In this test a definite amount of work is executed by a group of muscles measured by a Zuntz ergometer, and blood-pressure estimations are made before, during and after the work.

Graupner reached the following conclusions:

(1) A moderate amount of work, in normal hearts, will cause a rise of blood-pressure after the work. This either promptly returns to normal or remains constant at the higher level for a period, but does not fluctuate and gradually returns to normal.

(2) The greater the amount of work done, the higher the rise of blood-pressure, and the quicker the return to normal, the more efficient is the myocardium.

(3) A sinking of the blood-pressure after muscular exertion, declining from the start, or even a very slight rise after work of about 10 mm. mercury, which falls again almost immediately to below the original point is evidence of incapacity of that heart for that amount of work. This indicates physiological or functional insufficiency.

(4) If the blood-pressure remains high for a period after the work and then suddenly falls, it is evidence of overstrain or fatigue.

(5) If the blood-pressure after work is lower than normal and then slowly returns to normal but does not rise above normal, a primary myocardial weakness exists. This reaction is characteristic of myocardial insufficiency.

Graupner's test depends upon the fact that the ventricle reacts to muscular work which at first increases the blood-pressure. If the ventricle proves unequal to the task of maintaining the pressure, there occurs a compensatory increase in pulse-rate but a fall in blood-pressure.

(*h*) *Barringer's Test of the Functional Capacity of the Circulation.*—Arno Lehndorff showed experimentally in 1908 that stimulation of the splanchnics produces their contraction with increase of blood-pressure to a varying degree. If the heart's action becomes insufficient, however, the blood-pressure falls. With the recovery of the heart's contraction, the pressure rises again.[64]

Recently Barringer described a test of heart function, using Graupner's method of making frequent readings of the pulse-rate and systolic blood-pressure after a measured amount of work, and clinically obtaining Lehndorff's experimental results in insufficiency of the heart.[75]

The *theoretical considerations* upon which his test depends, as Barringer presents them, are as follows:

Muscular work increases the CO_2 content of the blood. This stimulates the nervous centers controlling the suprarenal glands. An increase in the adrenalin content of the blood is thereby produced, which causes a constriction of the vessels in the splanchnic area and a resulting rise in blood-pressure. The quickened heart-rate accompanying muscular work causes an increase in the quantity of blood discharged by the heart per minute and this also contributes to the rise in blood-pressure.

The systolic pressure during work, therefore, mounts rapidly and the left ventricle finds it more and more difficult to expel its contents against this increasing resistance. At a certain height of aortic pressure the ventricle probably does not empty itself completely and a steadily increasing volume of blood remains in the heart after each systole. In other words, an insufficiency exists. At this moment the roentgen ray would possibly show a heart decidedly increased in size. If the work stops, the CO_2 content of the blood falls, the activity of the suprarenal gland decreases and the splanchnic vessels relax. The blood-pressure, therefore, begins to fall. But the heart now works more efficiently against the lowered aortic pressure and expels a larger quantity of the increased residual blood at each stroke until it finally

empties itself completely with each systole. The increased quantity of blood which the recovering heart thus throws into the aorta more than compensates for the lowered pressure. The pressure, therefore, again rises briefly, and this delayed rise of blood-pressure after the cessation of muscular work is the significant point in Barringer's test.

When the work stops, if the heart is much dilated, it probably requires a short time to reach its maximum efficiency to expel its increased content and there results a slowly mounting blood-pressure.

Technic.—Graduated work is furnished by movements of flexion, extension and swinging with iron dumb-bells weighing from 3 to 20 pounds each. These afford a practical means of furnishing graduated work for testing the heart functioning capacity. Computation of the amount of work performed through dumb-bell exercises is made according to the following example: If a 5-pound dumb-bell is extended upward from the shoulder through 2 feet to arm-length, 10 foot-pounds of work is performed with each extension. With a 10-pound dumb-bell 20 foot-pounds, or twice as much work, would be performed in the same space of time.

The clinical experiments reported by Barringer indicate that in blood-pressure reactions to graduated work we possess a valid test of the heart's functional capacity as far as it can be measured at the present time. If the systolic blood-pressure reaches its greatest height not immediately after work, but from 30 to 120 seconds later, or if the pressure immediately after work is lower than the original level, that work, whatever its amount, according to Barringer, has overtaxed the heart's functional capacity and may be taken as an accurate measure of the heart's efficiency.

Discussion.—A possible error in making the readings lies in the fact that the auscultatory systolic blood-pressure is greater in expiration than in inspiration. In cases of asthma the writer found it to be as much as 12 mm. mercury higher during expiration than inspiration.

Another difficulty, which applies, however, to some of the other methods as well, lies in the determination of delayed rises in cases of auricular fibrillation. Here the blood-pressure variations represent the varied amounts of diastolic filling of the left ventricle.[76]

This, like the other tests based upon blood-pressure findings, omits a very important factor which in life has an effect upon the cardiac efficiency. That is the psychic and nervous influences which are accountable for a great deal of what we call heart-strain. In actual life, the situations are not so simple as the lifting of a dumb-bell under a physician's direction. The complex-processes of life call for cardiac reaction to visual and auditory stimuli and other sensations not measurable in terms of physical work. For example, while crossing a road, the sudden approach of a vehicle or a cry will startle the patient. One cannot estimate in foot-pounds of work the effect the excitement has upon the heart, and until we can translate the indefinite effects into numerical data, the problem of the heart's functional capacity with the exactions of ordinary living will be only incompletely solved.

Barringer found that the delayed rise in systolic blood-pressure was obtained after large amounts of work which varied normally according to the subject's physique and condition of muscular training.

In a recent study of Barringer's method in a military hospital in London, Rapport asserts that Barringer's method of ascertaining the shape of the blood-pressure curve excludes the variations which occur in the fractions of a minute following accomplished exercise. He states that the variations of pulse-rate and blood-pressure may be an exact expression of real events, but do not indicate the capacity of the heart to do work.[77]

Mann studied the circulatory reactions of ten patients convalescing from acute infectious disease by means of Barringer's method. He found that during the progress of convalescence there was a progressive increase in the amount of work necessary to produce a "delayed summit." This was synchronous with the clinical and subjective improvement, thus corroborating the value of the test.[78]

The writer's findings with this test are reported under the heading, Tests of the Functional Capacity of the Circulation in Special Diseases (p. 191).

IV. Test of Efficiency of the Right Heart.—1. Methods of Determining Venous Pressure.—In the clinical study of venous blood-pressure, the veins of the hand and forearm lend themselves most easily for observation. Venous pressure serves as an index of the accumulation of the blood in the systemic circulation and indirectly therefore of right-sided heart-failure.

As early as 1897 von Frey suggested a method of measuring the venous blood-pressure.[79] He describes a small bracelet with a lever placed over the vein and pressure exerted on the lever. The venous pressure is considered equal to the amount of pressure required to retard or stop the venous return. This method appears to be inaccurate.

In making a venous blood-pressure estimation, it is necessary, as has been recognized by all the workers in this field, to have the vein or veins under observation at the level of the heart. If this is impossible it is then necessary to correct the reading for the force of gravity thus brought into play. Levels above the heart decrease and those below the heart correspondingly increase the reading. The venous blood-pressure, for example, in the forearm or the hand hanging at the side, is greater than it would be when the forearm or hand is placed at the level of the heart. The costal angle, as suggested by von Recklinghausen, has been the point chosen as marking the heart level.[80] This landmark is especially appropriate as it localizes the right heart which is such an important factor in the maintenance of venous blood-pressure.

Gärtner determined the venous pressure by considering it equal to the height above the angle of Ludwig at which the veins of the hand could be seen to collapse.[81] Normally, in the standing or sitting posture the veins collapse when raised to the level of the third, fourth or fifth rib. In cases of right heart-failure with overdistension of the right auricle, the arm has to be raised to the level of the clavicle or even higher.

The author calls this the gravity method because it depends for its explanation on gravimetric principles. It is therefore serviceable in extreme cases where the right heart is overfilled. According to Schott, if the arm is raised to an angle of 60 degrees, the normal rise in venous pressure does not exceed 3 cm. Any reading above this denotes cardiac insufficiency.[82]

Von Basch made a practical advance on this method, using air-pressure, and making it possible to take the readings in cms. of water through a glass cylinder placed over the veins.[83] The error here was considerable, the variant being the amount of pressure with which the cylinder was applied.

Von Recklinghausen [84] and later Eyster and Hooker [85] used direct compression of the veins by air to determine the intravenous pressure. Von Recklinghausen substituted a rubber chamber with three openings. One of these was placed over the vein, another was covered by a glass through which the vein could be viewed, and through the third air could be introduced and the pressure measured on a water manometer. The point at which the vein is seen to disappear indicates the intravenous pressure.

Moritz and Tabora introduce a cannula into a vein at the elbow and connect it with a capillary manometer containing sterile normal saline solution.[86] The solution is allowed to run into the vein to a level below which it will not fall. This level above the plane of the cannula is taken as the reading of the intravenous pressure. In other words, the pressure which is just insufficient to cause a further passage of fluid from the manometer into the vein is read; as the fluids within the vein and the manometer are in equilibrium at this point, this reading is, of course, the venous pressure.

The normal venous pressure determined by the method of von Recklinghausen is 5-10 cm. H_2O (4 mm. Hg.). These figures agree well with the direct manometric determination of Moritz and Tabora. Changes in pressure in the systemic veins, which show how well the right ventricle is pumping, often afford an excellent index of the break in systemic compensation, rising from the normal pressure of 5-10 cm. H_2O to a height of 20-25 cm. It usually rises as the patient's condition becomes worse and falls as improvement sets in. In cases of heart disease the pressure may rise to 27 cm. of water.

Howell described a newer method for clinical use to determine the venous blood-pressure.[87] He applies the cuff used in making ordinary blood-pressure readings to the arm and another similar cuff patterned to fit the tapering forearm, made of light rubber tissue with a light inelastic covering. Each of the two cuffs is connected with a separate water manometer. A slight pressure, say of 3 cm., is introduced into the lower cuff that it may the more accurately fit the forearm without affecting the intravenous pressure. The upper cuff is then inflated, causing constriction of the arm and, therefore, swelling the veins in the forearm. This increase is registered in the manometer attached to the

lower cuff. The pressure required in the upper cuff to cause the first rise in the manometer of the lower cuff is the venous blood-pressure.

The average venous pressure in normal cases, looked on from a cardiovascular standpoint, is 7.6 cm. of water. Hooker has recorded observations on the venous blood-pressure in normal individuals at different ages and found a progressive increase with age, from about 10 cm. of water in the second decade, to 26 cm. between the ages of 75 and 85 years.[88]

2. Breathing Tests (*The Valsalva Experiment—The Russian Test—"Holding the Breath" Test*).—In carrying out any exercise involving muscular strain, the individual involuntarily closes his glottis and executes an attempt at forced expiration.

The result of this is a tremendous increase in intrathoracic pressure which hinders the outflow of blood from the right ventricle as well as the inflow into the right auricle.

The result of these two factors is dilatation of the right ventricle and stasis in the systemic veins. This is shown by cyanosis of the face and distension of the veins that accompany all such exercises, even in trained athletes.

The high pressure within the lungs stimulates the sensory endings of the vagus which in turn reflexly stimulate the motor nucleus of the vagus and the vasomotor center in the medulla and cause both slowing of the pulse and rise of blood-pressure.

Forced expiration with closed glottis is a test applicable clinically. The patient takes a deep breath and then follows a period of apnea for as long a time as possible. This results in an increase in CO_2 and decrease in O_2 in the venous blood and in a rise in the venous pressure, often to as high as 20 mm. mercury.

Voluntary apnea, with a normal right heart, should be endurable a certain length of time, and lessened time of endurance would indicate right-sided cardiac insufficiency. Dyspnea resulting from toxic causes, as in the infectious diseases, or from mechanical causes, as in pleural effusion or ascites, would naturally invalidate this test.

According to Cooper, in normal individuals the breath may be held in inspiration from 40 to 70 seconds; in expiration from 20 to 35 seconds $\frac{40\text{-}70}{20\text{-}35}$. In patients with cardiac insufficiency this ratio is preserved, though the periods are shortened, i.e., $\frac{25}{15}$. In bronchial asthma the breath can be held longer in expiration than in inspiration and the ratio is reversed $\frac{15\text{-}25}{25\text{-}35}$. It seems probable that this ratio will prove of great value in differentiating between attacks of bronchial asthma and asthma due to acute failure of the left ventricle.[89]

V. The Polygraph.—A fuller and deeper knowledge of the mechanism of the circulation was promised by the development of the polygraphic method. The polygraph simultaneously records tracings of the

arterial and venous pulsations in the neck as well as the movements of the apex of the heart, and of the pulsating liver.

Many instruments have been devised for recording the movement of the heart and the pulsations of the arteries and veins, but for practical clinical purposes the three most serviceable are a modification of the Dudgeon sphygmograph, Von Jacquet's sphygmograph and the ink-polygraph of Mackenzie. The latter is the most commonly used apparatus. It consists of two tambours (B) with pen levers (F). The tambours are connected by rubber tubing to two cups, one covering the radial artery (C) and the other to be placed over the jugular bulb, apex of the heart or any other point from which pulsations are to be traced (E). The two pens come in contact with a moving roll of paper (D)

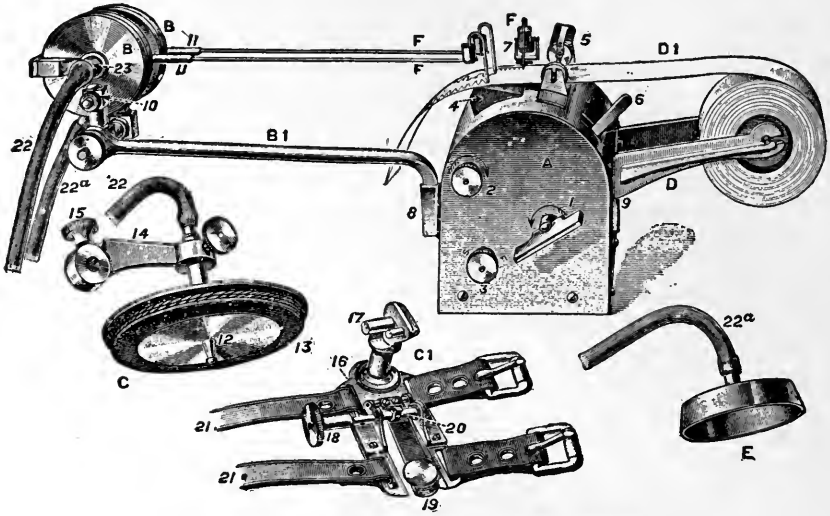


FIG. 18.—THE INK-POLYGRAPH OF MACKENZIE.

and record their movements upon it. At the same time a small pen (F-7) is attached to a clock-work mechanism in the apparatus and serves to mark time on the paper in fifths of a second. The illustration may be elucidative of the details of the apparatus. (*See* Fig. 18.)

1. INTERPRETATION OF THE SPHYGMOGRAPHIC TRACINGS.—The sphygmogram represents the variations in pressure occurring within the vessel wall, magnified in some degree by the mechanism of the sphygmograph. It records the rate and rhythm of the heart, and yields information concerning the heart-beat. The sphygmogram is of great assistance in analyzing the tracings of the pulsations obtained from the veins in the neck. From it we can determine the period during which the aortic and pulmonary valves are open—the sphygmic period (E in Fig. 9). This begins with the upstroke of the arterial tracing and ends at the dicrotic notch.

The study of the jugular pulse reveals information concerning the movements in the heart, especially the effects of the systole and diastole

of the right auricle and of the systole and diastole of the right ventricle. The jugular bulb is part of a venous cistern (*see* Fig. 19) and consequently, tracings obtained from this bulb represent the fluctuations in the pressure of the blood contained within the cistern. The cistern is formed by the superior vena cava, the innominate, iliac, hepatic

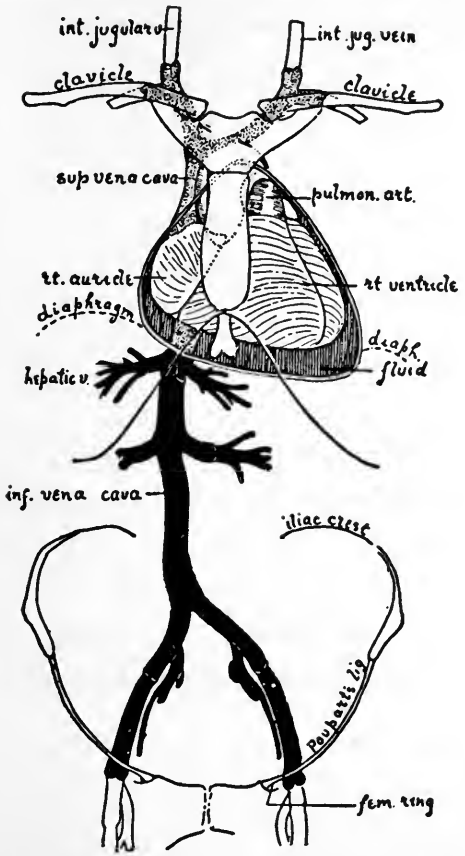


FIG. 19.—DIAGRAM OF THE VENOUS CISTERN FROM WHICH THE HEART IS FILLED, AS SEEN IN A MAN AGED FIFTY-FIVE, IN WHOM THE PERICARDIUM WAS DISTENDED AND THE HEART COMPRESSED BY A LARGE EFFUSION OF FLUID.

The abdominal or infradiaphragmatic part of the cistern is indicated in black; the thoracic or supradiaphragmatic is stippled. The heart is compressed upward and backward against its attachments. (Keith.)

and renal veins; it is shut off from the venous system of the lower extremities by strong valves situated in the common femoral veins, from 10-25 cm. below Poupart's ligament, from the venous system of the upper extremities by equally strong valves in the subclavian vein, from the venous system of the head and neck by the valves above the jugular bulb.

A combined tracing of the movements in the carotid artery and

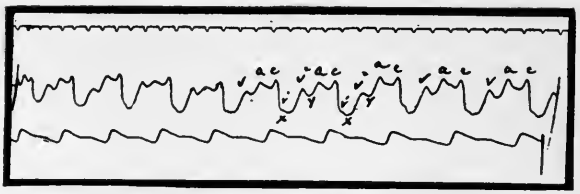


FIG. 20.—NORMAL CERVICAL TRACING, WITH *a-c-v* SEQUENCE.
Simultaneous radial tracing.

jugular vein would, therefore, reveal the variations of pressure (1) within the auricle, (2) within the ventricle and (3) within the aorta.

The normal cervical tracing presents a series of waves in the fol-

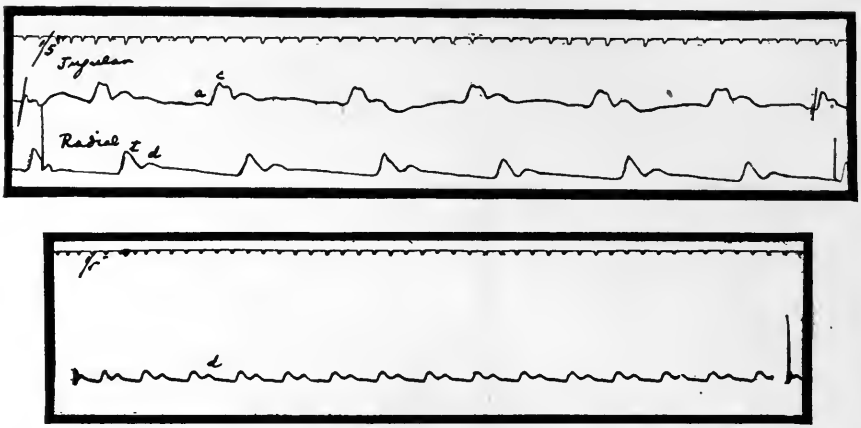


FIG. 21.—DICROTIC PULSE.
The dicrotic wave is so great as to resemble a premature pulse-beat.

lowing sequence (*see* Fig. 20). First, the wave "*a*" is due to auricular systole. This is followed by the wave "*c*," due to ventricular systole and distension of the carotid artery. After this a wave "*v*" takes place, which is the result of the filling of the auricle and the increased pres-

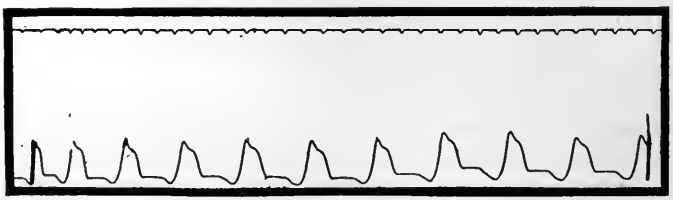


FIG. 22.—PULSUS CELER. (Water-hammer pulse.)
From a case of aortic regurgitation. In very low arterial pressure, the dicrotic notch occurs at a very low level in the tracing. Its height is a more certain guide to the arterial pressure than the character of the systolic wave.

sure in the jugular vein. The negative phase "*x*" which follows the "*c*" wave represents the diastole of the auricle as it is receiving blood from the veins. A negative phase ("*y*") follows later when the tricuspid valves open. The wave "*v*" is increased by all factors which tend towards the more rapid filling of the right auricle; it is immaterial whether the blood comes from the vein or from the right ventricle.

From the functional point, therefore, we may take it that the earlier "*v*" begins in the phase "*x*," the greater is the engorgement of the auricle.

The *a*—*c* interval is one-fifth of a second and is almost constant in normally acting hearts. It represents the interval between the beginning of auricular systole and the opening of the aortic valves, or ventricular systole.

By means of the polygraphic tracings all those irregularities of the heart with variations in the rate and quality of the pulse, discussed earlier in the chapter, are ideally revealed. In this way is the polygraphic method of invaluable service in the estimation of the heart's functional state.[90]

The figures are briefly discussed in the respective notes and will serve to illustrate the value of polygraphic tracings in the study of heart function (Figs. 21-24).

2. The Effect of Cough upon the Curve of the Sphygmogram and Its Functional Significance.—It is evident that the changes of blood-pressure are reflected in the curve of the arterial tracing. The level of the arterial curve is also affected by other influences, such as muscular contractions, while the tracing is being taken, and the vagus influences of respiration. Besides these, the mechanical conditions under which the tracings are made will alter their shape. It has been shown that compression of an artery will cause the pulse below to become more prominent up to a certain degree and then the wave will fall to a lower level.

A consideration which must be discussed before entering upon the discussion of the effects of cough upon the pulse tracings is the effect of voluntary muscular contractions of the forearm and the changes of the radial pulse produced by them. A tremor of the hand or of the muscles of the forearm would give the picture of a tremogram superimposed upon the curve of the radial wave. Sudden coarse muscular contraction would produce an elevation anywhere in the course of the figure, but would not otherwise affect the general shape of the pulse waves preceding or following.

In a case of degeneration of the heart muscle in a syphilitic patient, who also had a right bundle-branch lesion, the writer noted a particular effect of coughing upon the shape of the radial pulse wave. It was constant in his case and an analysis of the tracing shows that the change of curve bore a relation to the blood-pressure effects induced by the strain of coughing. The cough exaggerated the graphic evidence of alternation and altogether affected the curve more than in normal cases. The susceptibility of the radial curve to marked variations after

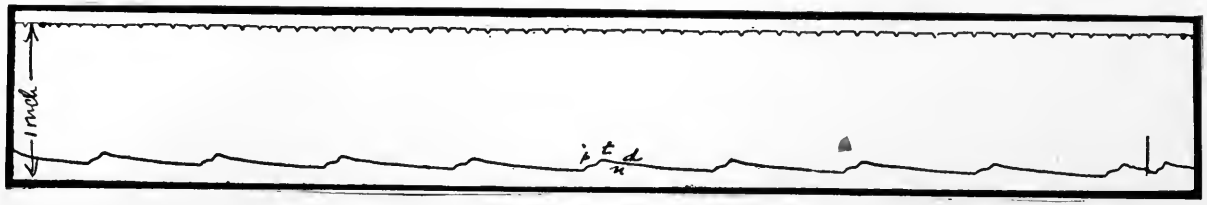


FIG. 23.—ANACROTIC PULSE IN HYPERTENSION.

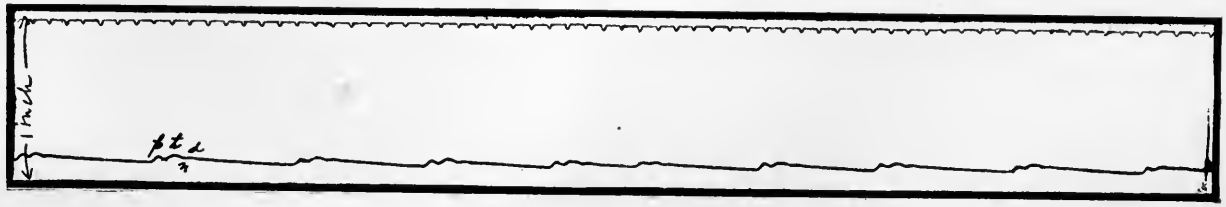


FIG. 24.—PULSUS BISFERIENS.

From a case of aortic stenosis with absolute irregularity of the pulse. It has been suggested that the ventricle contracts in two stages in certain cases of aortic stenosis, and that this is shown by the fact that the tidal wave is sometimes found split in two. In true pulsus bisferiens, the first of the two waves is always lower than the other.

coughing is an indication of marked myocardial degeneration. In the case cited, the ideal condition existed for the test, inasmuch as the loss of tonicity of the musculature of the left ventricle was extreme, associated with complete destructive lesion of the right branch of the bundle (Fig. 25).

3. The Cardiographic Evidence of Loss of Tonicity of the Heart Muscle.—The impulse of the apex of the heart produces a graphic curve which varies slightly in shape in different individuals. It is most frequently a trapezoid, presenting an elevation to a varying length and then a slight fall to a plateau, which turns abruptly to the base line. (*See* Fig. 26.) Von Martius identifies various points of the curve with events in the heart-cycle: (1) The beginning of the presphygmic period, in which all the valves in the heart are closed. This is the point at which a shock is felt on palpation, caused by the sudden hardening of the ventricular muscle. (2) The systolic expulsion of the blood, beginning with the opening of the semilunar valves. This corre-

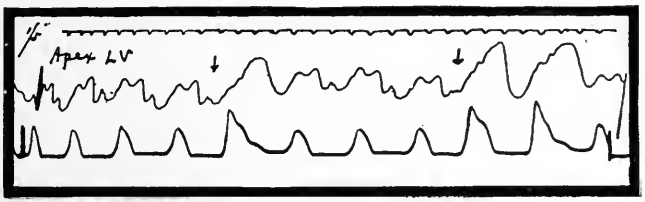


Fig. 25.—Effect of Coughing upon the Radial Tracing in a Case of Marked Myocardial Degeneration.

sponds to the thrust due to the pressure of the apex of the left ventricle against the chest wall. (3) The closure of the semilunar valves. The triangular and pointed apex curves are not quite as common as the trapezoidal form, but the shape of the curve will necessarily vary with hypertrophy of one or the other ventricle, with the type of endocardial lesion, with the shape of the chest, and the state of emphysema of the lungs.[91]

Up to the present time, but one important change in the cardiogram has been known to have pathological significance; that is, the change from a positive to a negative cardiogram. In these cases, instead of the cardiographic curve rising above the abscissa, it presents a fall, corresponding to a drawing in at the time of the apex-impulse. Negative curves of this character obtained over the lowest and outermost portion of the apex-impulse indicate hypertrophy of the right ventricle.

Similar negative curves may be obtained from the right side of the apex-beat and are usual when the apex impulse is due directly to the right ventricle, due to its hypertrophy, i.e., when the lowermost and outermost portion of the apex-beat is caused by the systole of the right ventricle.

Occasionally a slight elevation precedes the apex-impulse and is attributable to the auricular contraction, forcing blood into the ventricle.

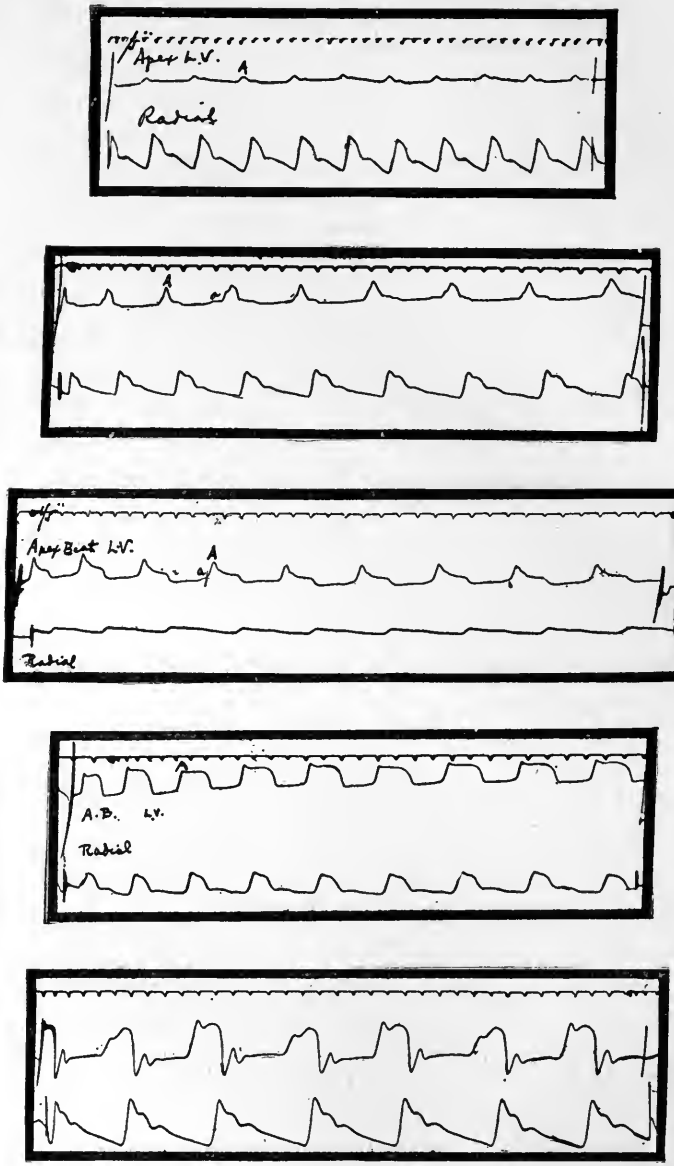


FIG. 26.—CARDIOGRAPHIC CURVES, REPRESENTING THE DIFFERENT FORMS OF THE APEX BEAT.

Showing apex beat (A), radial wave and wave due to auricular systole (*a*).

This elevation is normally slight, rising gradually to a point from which the sudden impulse due to the apex-beat springs. In the negative cardiogram the auricular wave may be present as a slight elevation terminating in the descending wave of the cardiogram.

Normally the wave caused by the auricles discharging their contents into the ventricles may be absent or may present itself only as a slight elevation just preceding the systole of the ventricle. The resistance to the auricular current offered by the wall of the ventricle is analogous to that offered by contracted arteries to the systole of the heart.[92]

There are two important factors which would have the effect of increasing the size of the wave due to auricular systole: (1) Relative hypertrophy of the auricles with increase in the force of the impulse and (2) loss of tonicity of the ventricles with dilatation and thinning of their wall.

(1) Relative hypertrophy of the auricles, especially of the left side, is induced by obstruction to the flow of blood into the ventricles, most typically and markedly in stenosis of the mitral valve.

With reference to the wave "*a*" of the cardiogram in cases of mitral stenosis it shows itself as a thrill or series of waves at the beginning of systole, and not as a distinct single impulse. Besides, the clinical features of the condition are very apparent, and are not to be mistaken for the condition in which there occurs also a characteristic cardiogram.

(2) This condition is the loss of tonicity of the ventricular muscle. This influences the prominence of the "*a*" wave in the curve of the cardiogram. Depression of tonicity of the ventricular muscle is usually manifested by dilatation of the heart with thinning of the walls of the ventricles. The heart lies frequently markedly dilated against the chest wall. Its impulse is diffuse, feeble, and electrocardiographically it may present evidence of degenerative changes in the myocardium. It is in such cases that one obtains the alternating form of pulse, so significant of myocardial disease.

Together with this evidence and the effect of cough upon the form of the radial curve (above described), the other significant and characteristic feature is the presence of a prominent and distinct "*a*" wave in the cardiogram. The characteristics of the "*a*" wave are typical of low tonicity of the ventricular muscle. The rise of the "*a*" wave is abrupt, the curve of filling is steeper than normal. The impulse due to auricular contraction rises to a maximum and falls when the ventricle is partially filled, indicating that it was the low tonicity of the wall that permitted the transmission of the wave through the chest wall.

This is well illustrated in Fig. 27, in which the typical cardiogram is associated with pulsus alternans, and in which case the electrocardiogram showed marked widening of the Q-R-S interval, indicating intraventricular or arborization block.

It is, however, important to realize that cardiograms vary greatly, and many of them are difficult to analyze. Much has yet to be done

before a decided opinion can be given as to the exact meaning of some of these records. In cases of myodegeneration, however, the apex-beat may prove of some service, as it would present a prominent "*a*" wave without evidence of mitral stenosis.

VI. The Electrocardiograph and Its Value in Functional Diagnosis.—Every muscular action in the body is accompanied by the generation and conduction of an electric current. The human body may therefore be regarded as a battery with the two arms and the two legs as its natural and convenient poles. When the body is at rest, the predominating electrical current is that associated with the contraction of the heart muscle.

If now, with the body at rest, the two hands, or one hand and one foot, are connected by wires into a circuit, with a very sensitive gal-

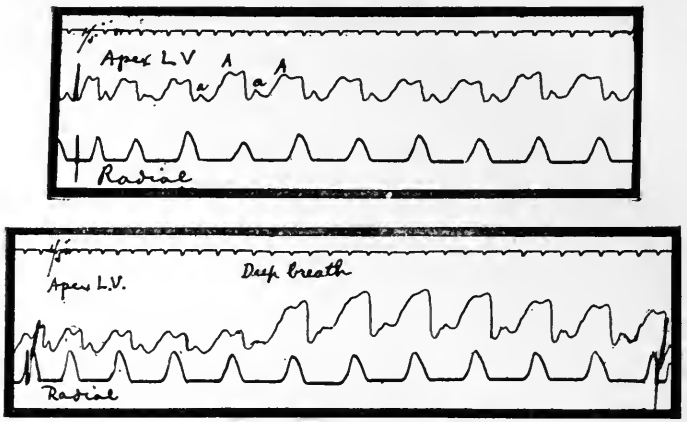


FIG. 27.—TYPICAL CARDIOGRAM IN CASE OF MARKED MYOCARDIAL AND SUBENDOCARDIAL DISEASE OF LUETIC ORIGIN.

This patient showed bundle-branch block, widening of the Q-R-S interval and pulsus alternans. Note prominent "a" wave in the cardiogram.

vanometer between them, each time that the heart-beats the needle of the galvanometer will be deflected to one side or the other, depending upon the direction of the current; and the extent of its deflection will be proportionate to the amount of current and the difference in potential between the two poles. Not only will the galvanometer needle be deflected each time that the heart-beats, but it will be deflected with the auricular contraction first and then with the ventricular contraction separately. A graphic record of the deflections can be obtained by photographing the movements of the needle on a moving film.

Einthoven of Leyden invented a galvanometer which is exceedingly sensitive to very minute electrical currents or differences in potential.[93] It consists of a fine quartz or platinum thread suspended vertically in an electromagnetic field. Its ends are connected by wires to the poles of the body to be examined. When the current from the body passes through the thread, it is attracted to one or the other side of the mag-

netic field. This thread is magnified by a powerful lens and is illumined by a powerful arc-lamp. Its shadow is focused on a camera in which it is photographed on a moving film.

By this method it is possible to study very small currents. The electric variations accompanying the cardiac action have an intensity of from 1 to 3 millivolts. The current is led off to the string in three directions—Lead I contains the right hand and the left hand in the circuit, Lead II the right hand and left foot, and Lead III carries the current from the left hand and left foot. The current from the body

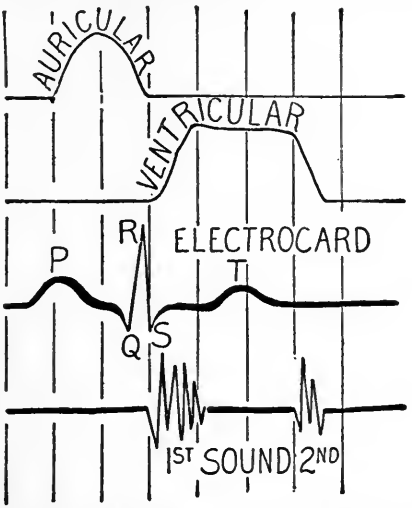


FIG. 28.—DIAGRAM SHOWING THE TIME RELATIONS BETWEEN THE WAVES OF THE ELECTROCARDIOGRAM, THE HEART SOUNDS AND THE CONTRACTIONS OF AURICLES AND VENTRICLES. (Barker, Hirschfelder and Bond, in Journal of the American Medical Association.)

Vertical divisions indicate tenths of a second.

enters the string at its upper end. An electrocardiogram is obtained from each of the three leads in cases studied.

1. INTERPRETATION OF THE ELECTROCARDIOGRAM.—The normal electrocardiogram consists of a series of waves, the result of auricular and ventricular activity. (*See* Fig. 28.) As can be seen, the first wave is small and is due to the activity of the auricle—designated by Einthoven the P wave. This is followed by a very small depression of the curve below the base line, and is called the Q depression. Then comes a series of waves due to the action of the ventricles: a large wave R which rises suddenly and falls to below the base line (the S depression), and finally, a second smaller wave T which occurs during the middle of systole.

Observations made by Lewis and Gilder indicate that certain clinical valvular conditions cause more or less typical changes in the form of the waves of the electrocardiogram.[94]

In mitral stenosis the average curve in Lead I shows a diminution of R and increase of S; in Lead III it shows a conspicuous increase of R

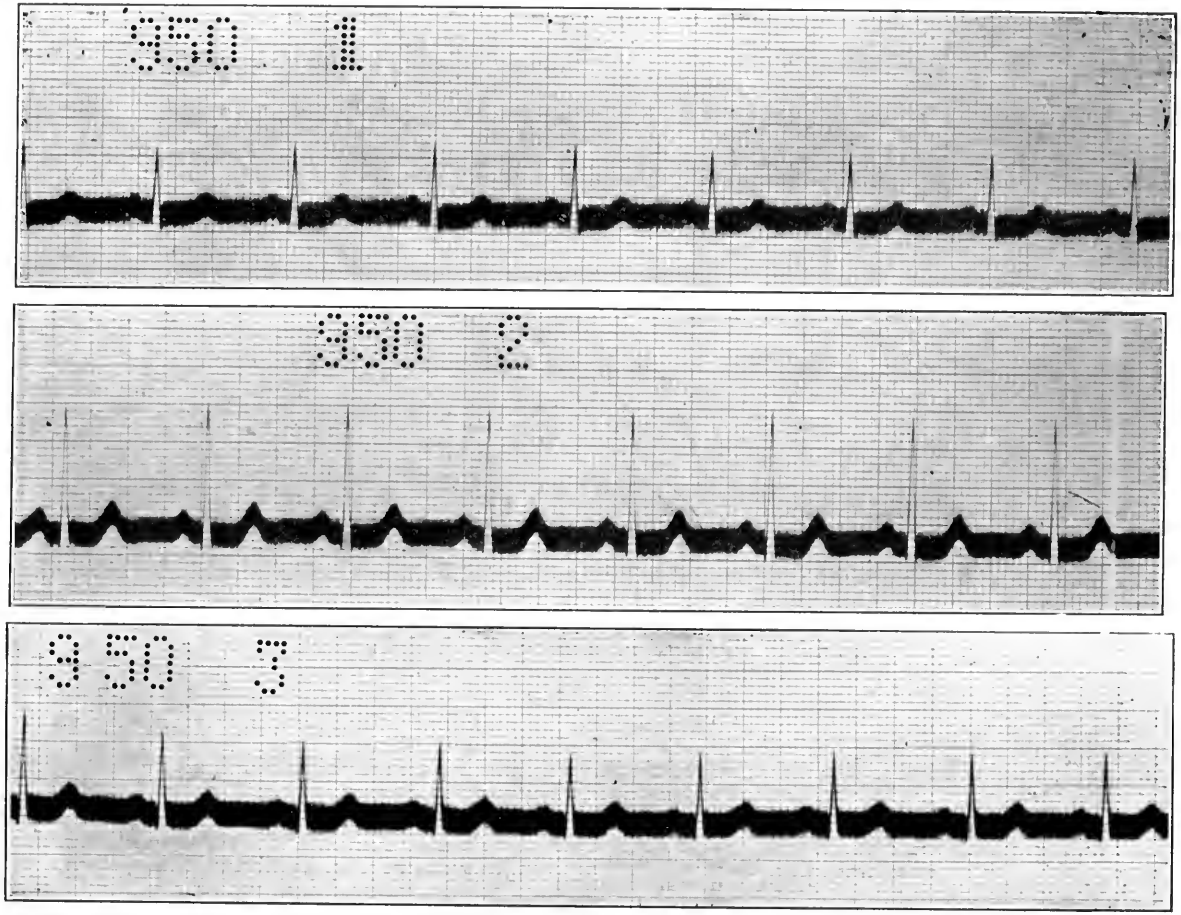


FIG. 29.—NORMAL ELECTROCARDIOGRAM; P WAVE, Q-R-S INTERVAL AND T WAVES NORMAL IN ALL THREE LEADS.

and a decrease of S, as compared to the normal. This is the sign of right ventricular preponderance, as shown by Einthoven. The right side of the heart may be so predominatingly hypertrophied as to cause an actual inversion of R below the base line in Lead I.

In aortic cases, on the contrary, the picture is reversed. In Lead I, R is increased and S diminished, while in Lead III, R is diminished and S is increased. In more marked hypertrophy, R in Lead III is inverted below the base line. These changes constitute the sign of left ventricular preponderance.[95]

Defects of conduction are clearly shown by the electrocardiogram and a reference to the accompanying figures will show the electrocardiographic evidence obtained in a wide variety of cases (Figs. 29-32).

2. Electrocardiographic Changes Associated with Myocardial Involvement.—What is probably the most important advance in the interpretation of the electrocardiograph with particular reference to the estimation of myocardial involvement and prognosis has recently been made by Oppenheimer and Rothschild and reported by them from the Cardiographic Laboratory of Mount Sinai Hospital.[96] The author will quote freely from their publications on the subject.

"The normal electrocardiogram is to be considered the result of the passage of an impulse at a normal velocity through the usual channels, that is, node of Tawara, main stem, bundle branches and arborizations, which consist of the so-called Purkinje fibers. The latter form a network covering practically the entire endocardial surface of the ventricles. The velocity of the impulse through Purkinje fibers is at least ten times faster than its rate through ordinary ventricular musculature. The impulse reaches the ventricle normally through the Purkinje fibers, stimulating the ventricular walls practically as a whole.

"It is conceivable that the passage of this impulse may be hindered at any point in the conducting system.

"A lesion only partly involving either bundle branch, or an extensive lesion of the arborizations of a branch such as occurs in human pathology, would cause a delay in the transmission of the excitation wave to the area supplied by the damaged conduction fibers. Such a lesion, if sufficiently extensive, should give observable changes in the electrocardiogram, and it is probable that these changes will be distinguishable from the changes that are produced by most other cardiac abnormalities.

"By intraventricular block we mean any delay in conduction below the main stem of the bundle of His. Intraventricular block includes: (1) bundle-branch block, and (2) arborization or Purkinje block, by which we mean interference with the conduction beyond the two chief branches of the bundle of His.

"This disturbance of conduction may be permanent or temporary. The permanent changes we consider indicative of a definite pathologic lesion, generally myodegeneration (Figs. 33-34).

"The criteria in the electrocardiograms that we have used are in general as follows:

"1. Abnormal prolongation of the time interval of the Q-R-S group

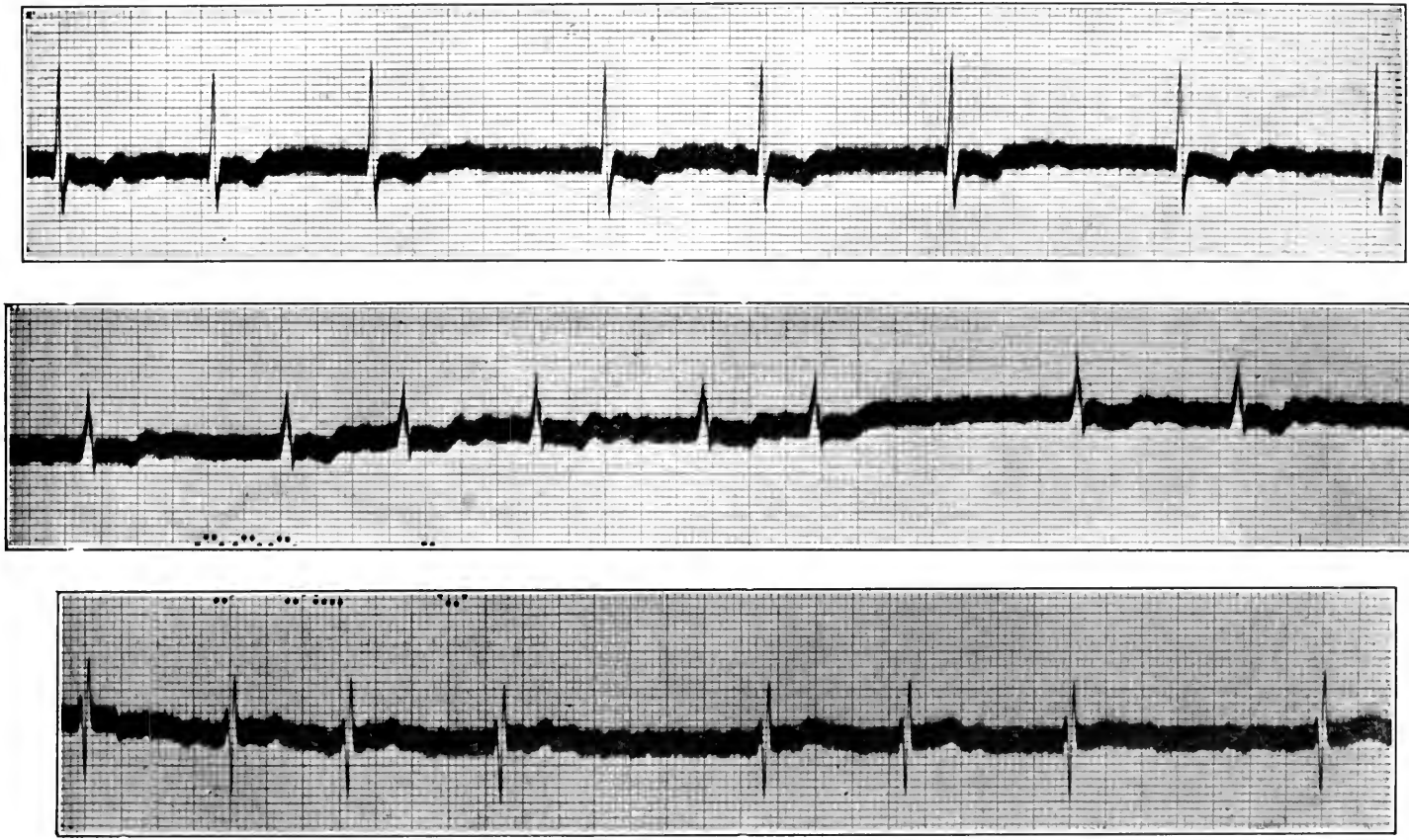


FIG. 30.—ELECTROCARDIOGRAM FROM A CASE OF AURICULAR FIBRILLATION.

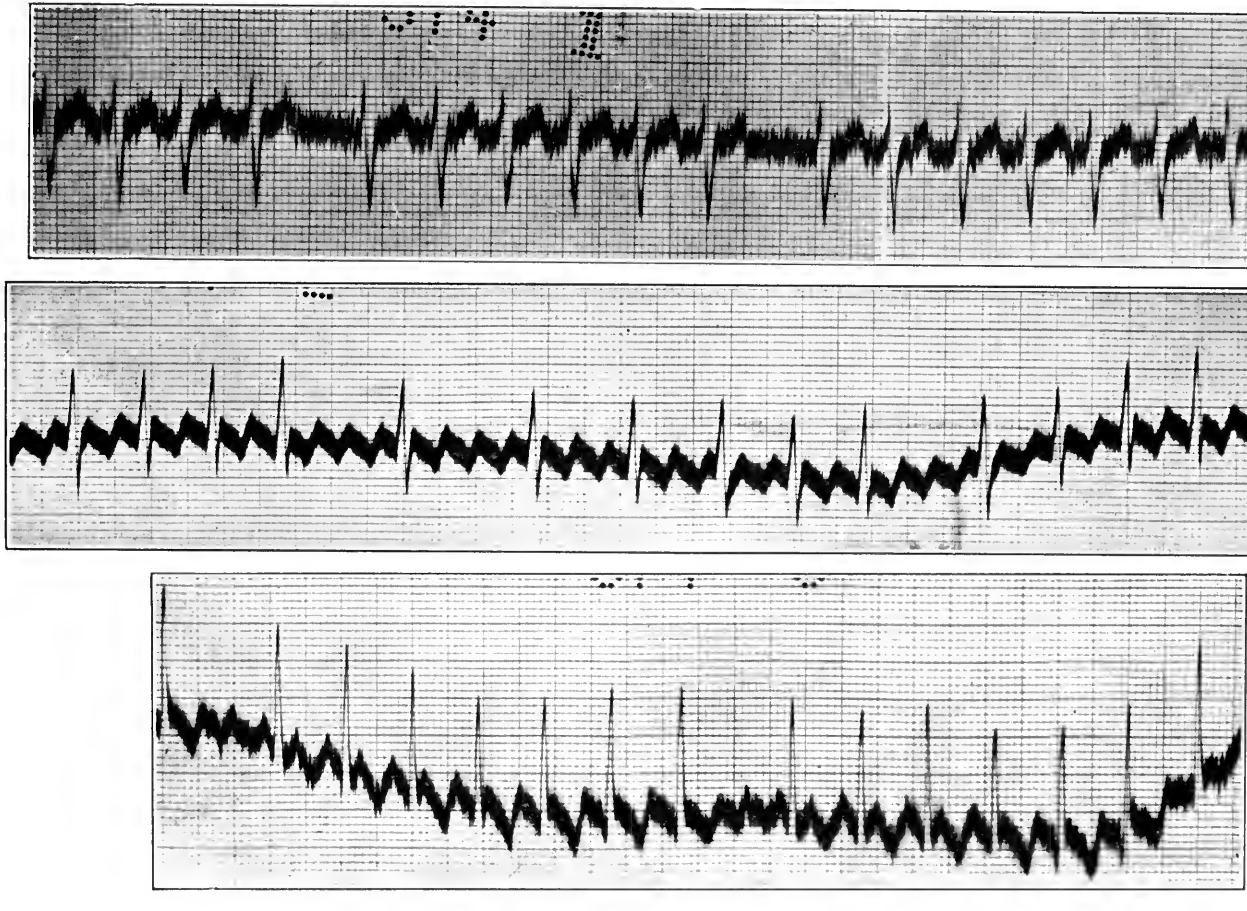


FIG. 31.—ELECTROCARDIOGRAM FROM A CASE OF AURICULAR FLUTTER.

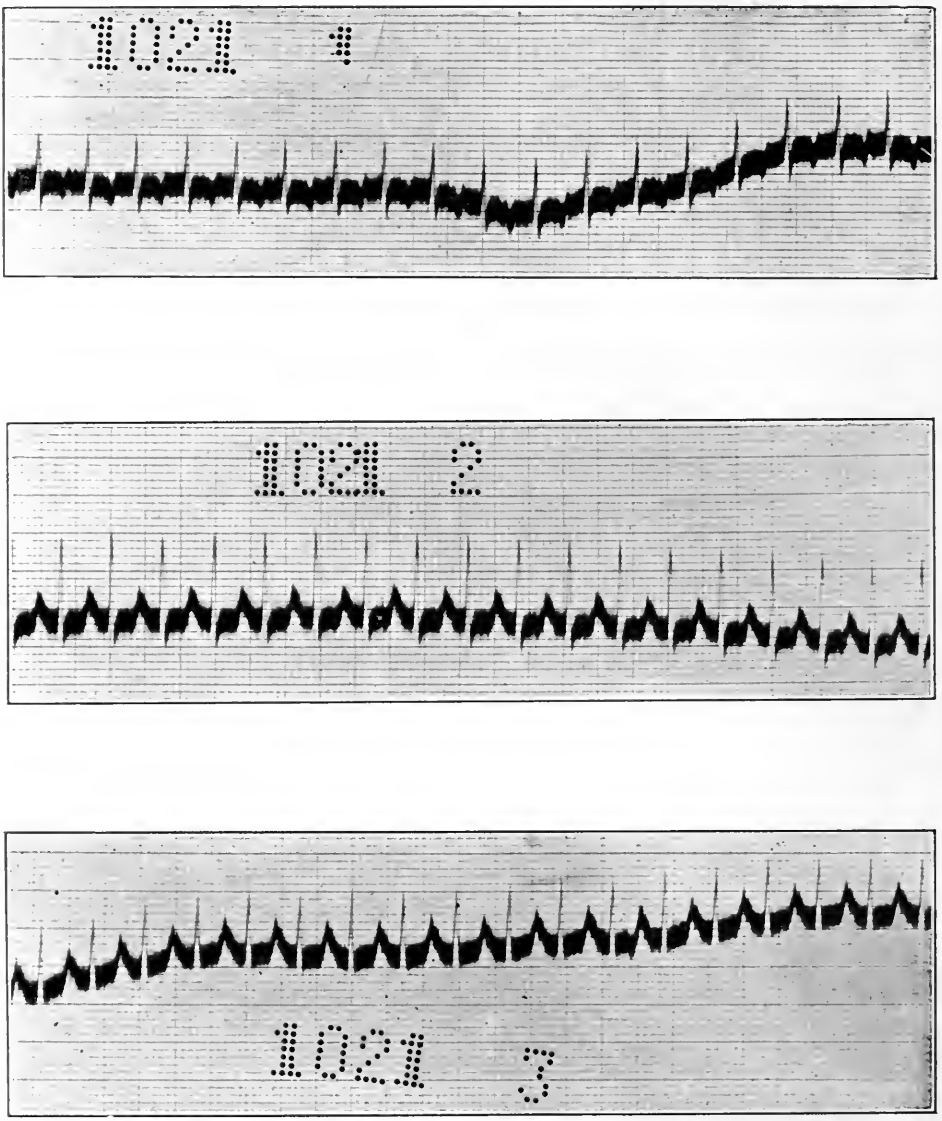


FIG. 32.—ELECTROCARDIOGRAM FROM A CASE OF PAROXYSMAL TACHYCARDIA OF AURICULAR ORIGIN.

beyond the normal limit of 0.1 second. This prolongation is most manifest in a widening of the R wave, so that its foot points are abnormally separated. The R wave no longer has its slender, tall, spine-like appearance, but is broader and sometimes blunter than normally.

"2. Notching of the R wave. This notching may appear on the ascending or descending limb, on both limbs, or at the peak. It may be multiple, and its degree and location may vary slightly from beat to beat. In arrhythmias, the shorter the preceding interventricular interval, the more pronounced is the evidence of disturbed intraventricular conduction.

"3. Low voltage as expressed by a low amplitude of the waves in all three leads. This change is not uniformly present, but when it occurs it helps to differentiate this type from the electrocardiograms typical of bundle-branch block.

"4. Absence of the typical diphasic curves with huge T waves found in experimental bundle-branch block.

"*Prognosis.*—Special emphasis should be laid on the serious prognosis in patients showing electrocardiograms indicative of intraventricular block. Of the 58 patients studied whose fate is known, the mortality has been 48 per cent. within two years.

"On physical examination two signs have been especially noted: (1) a muffled, poor or practically absent first heart-sound, and (2) gallop rhythm. One is often struck by the fact that the heart is hypertrophied, but that the first sound, instead of being booming, has a poor or muffled quality."

VII. The Roentgen Ray and Orthodiagraph.—1. Variations in the Size of the Heart during and after Work.—The position and size of the heart can to a great degree be ascertained by the usual methods of physical diagnosis.

The *x*-ray, however, is more complete in its revelations of the outlines of the heart and blood-vessels. It pierces the edges of emphysematous lungs, which often cover the heart borders and can reveal the lower border of the heart when hidden or displaced by a distended stomach. The large vessels in the mediastinum are shown distinctly responding to the cardiac impulse. From the functional standpoint, the roentgen ray and orthodiagraph show the variations in size of the heart during and after work, and serve as excellent controls in many of the functional tests above described.

The normal cardiac shadow thrown upon the fluoroscopic screen resembles the area of relative dullness on percussion, except that it also extends upward behind the manubrium. In the second left interspace the shadow of the pulmonary artery is seen and in the second right that of the aorta.

This shadow is larger than the heart because of the divergence of the rays from the tube to the screen. To obviate this when measuring out the heart, F. Moritz devised an instrument known as the orthodiagraph. By means of this the exact contour of the heart can be marked off, both on the body and on a paper held in a frame behind

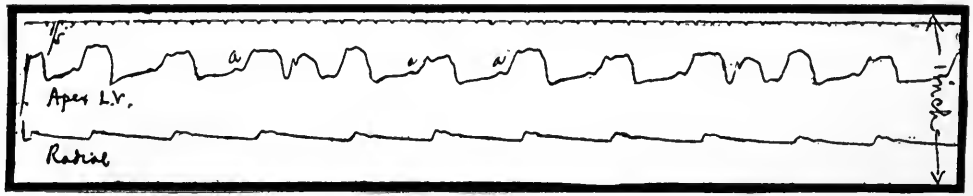
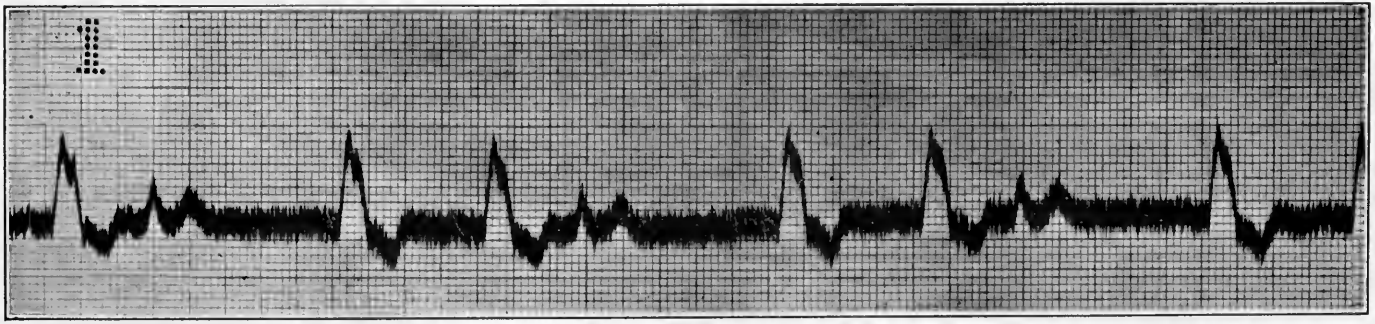


FIG. 33.—CARDIOGRAM IN CASE OF INTRAVENTRICULAR OR ARBORIZATION BLOCK.
Note the prominent "*a*" wave, indicating loss of tonicity of the ventricular musculature.



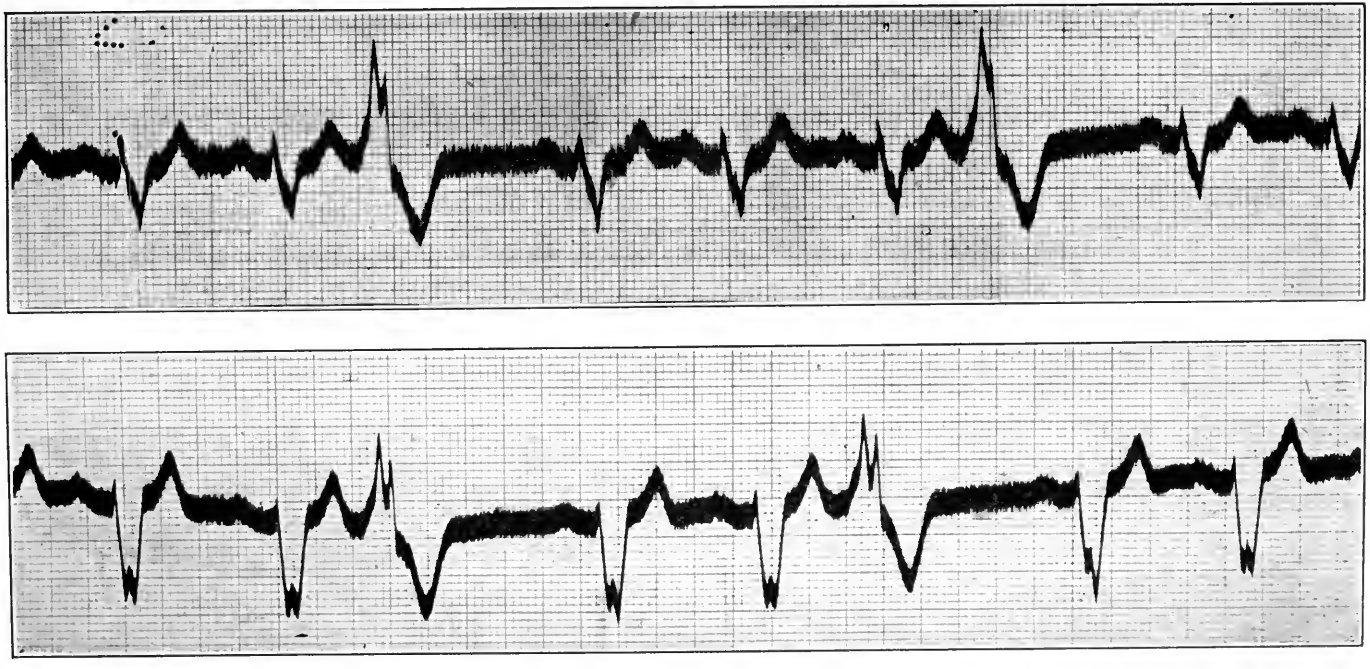


FIG. 34.—MARKED WIDENING OF Q-R-S INTERVAL IN A CASE OF INTRAVENTRICULAR OR ARBORIZATION BLOCK.

the patient. This furnishes a means of determining the size of the heart, or of any organ, with accuracy.[97]

Sudden variations in the size of the heart are dependent upon or associated with variations in the volume of blood contained in the heart. The heart walls remain practically unchanged during short periods, affected only by the slight changes in coronary circulation. It is not necessary to consider the hypertrophy of the heart which develops only during long periods of time.

Nicolai and Zuntz studied the changes in the size of the heart during work by means of the roentgen ray. The work was done on a stationary ergometer. That is, the *x*-ray apparatus was applied while the patient was walking on an inclined plane. The plates were so exposed as to obtain the shadow during diastole and during systole, and the plates obtained before, during and after work were compared.[98]

The authors show that, during work, the heart increases in its diastolic size, but is normal in its systolic size.

The increased diastole, during work, they attribute to the muscular and respiratory activity incident to work. The heart is normal in its systolic size because, during work, it empties itself almost completely with each beat. Such a reaction is normal, and the diastolic increase in size alone is not evidence of insufficiency of the heart.

Only then when the residue in the heart chambers after systole is greater than normal, does an insufficiency exist. This is, therefore, an important mode of measuring the insufficiency of the heart for a measured amount of work. The heart is orthodiagraphed in normal systole. A measured amount of work is executed and *x*-ray measurements taken during diastole and systole respectively. If a greater quantity of blood than normal remains in the heart after systole, insufficiency may be said to exist. The normal heart expels only about one half its contents during systole. The normal reaction after work is a fall in both the diastolic and systolic diameters of the heart. This is attributed to the sudden decrease in the quantity of blood pumped into the heart as a result of the stopping of the muscular activity.

The same fall in size of the heart can be observed in any person after closure of the glottis followed by forced expiration. This serves to shut off the blood from entering the heart, and naturally the latter becomes smaller with each systole, emptying itself of a small amount with each beat.

Nicolai and Zuntz estimate the relative volumes of blood in the heart by taking the radius (half of the horizontal diameter of the heart) and multiplying it by πr,[2] considering the heart spherical.

2. The Myocardial Retraction Reflex.—Attention was directed in 1898 to a heart-reflex by means of *x*-rays and it has since been suggested as of value in determining myocardial tonicity.[99]

The reflex under consideration consists of a contraction of the myocardium of varying duration, which results when the skin of the precordial region is irritated. The cutaneous irritant may vary from a spray of ether to massage with a towel or by a series of percussion blows.

The most effective site for inducing the heart-reflex is the spinous process of the seventh cervical vertebra.

Myocardial contraction ensues in the sagittal and transverse diameters of the organ. Percussion of the muscles causes a retraction of the right ventricle only (myopathic heart reflex).

The reflex in question is easily observed with the *x*-rays, although accurate percussion will show that, after outlining the heart, a notable retraction may be demonstrated after cutaneous irritation. The contraction of the myocardium implicates both ventricles of the heart after cutaneous irritation, although as a rule it is more evident in the left than in the right ventricle. It may also be noted that the myocardial recession persists even after the source of cutaneous irritation is removed.[100]

Minerbi[101] called attention to the prompt retraction of the sound heart when the precordial region is tapped. This tapping with twenty strokes of the hammer and plexiometer is followed by retraction of the auricle, atrium or ventricle according as one or the other is nearest the line of strokes. The heart outline will then be found 1,2 or even more centimeters inside of the previously drawn outline. The retraction is evidently due to the direct or autonomic excitability of the muscle tissue.

In very large dilatations and in advanced myocardial degeneration, the heart does not respond to precordial excitation and is not favorably influenced by balneological treatment, or the latter may even be contraindicated on account of the probable development of cyanosis.[102] If one can demonstrate the heart reflex even in high grades of cardiac insufficiency, the prognosis is more favorable.

3. Myocardial Dilatation Reflex.—A counter reflex exists of dilatation of the heart muscle as a result of peripheral irritation.[103] This may be evoked by percussing the spinous processes of the ninth, tenth, eleventh and twelfth dorsal vertebræ. This reflex has not been studied with regard to its evidence in cases of myocardial insufficiency.

Breccia[104] called attention to the rapid enlargement of the heart in certain persons when the upper abdomen is briskly and repeatedly tapped. The heart returns to its former outline in a few minutes. This reaction indicates that the heart is weakened too much to respond quite normally to the extra demands made upon it by the mechanical stimulation thus induced in the abdominal aorta. It professes thus to be a rapid sign that the heart is below par. In 100 cases of mitral defect, Breccia found the reaction negative in the perfectly compensated cases and positive in the decompensated and weak.

VIII. Metabolic Changes in Impaired Heart Function.—1. Vital Capacity of the Lungs as a Test of Myocardial Function.—It has long been known that in patients with heart disease the vital capacity of the lungs, that is, the volume of the greatest possible expiration after the deepest inspiration, is decreased below normal.[105] Determinations of the vital capacity in cases of cardiac disease are often of practical value as they give quantitative information as to the tendency to dyspnea,

and thus, indirectly, as to the clinical condition and the reserve power of the patient.

Peabody and his co-workers have made extensive graphic and clinical studies of the respiration by means of the following method:[106]

"Briefly it consists in having the subject breathe through valves which separate the expired from the inspired air. The expired air passes through a closed circuit and is then rebreathed, so that the inspired air contains a progressively rising percentage of carbon dioxid and a falling percentage of oxygen. Samples of the inspired air are then taken every two minutes and analyzed for carbon dioxid. A calibrated volumetric recording spirometer is connected with the closed circuit and the volume of each respiration is registered on the drum of a kymograph. With the aid of an electric-timer, the minute-volume of air breathed can be thus easily determined."

"From the studies of Peabody and his collaborators it is evident that there exists a remarkably close relationship between the clinical condition of cardiac compensation and the vital capacity of the lungs. Thus, in general, patients with a vital capacity of 90 per cent. or more of the normal standard adopted for their sex and height have little or no abnormal tendency to dyspnea. Patients with a vital capacity of from 70 to 90 per cent. of the normal become short of breath on unusual exertion and must lead a restricted life, although many of them can do light work. Patients with a vital capacity of from 40 to 70 per cent. of the normal are much more limited in their activities. They become dyspneic on moderate or slight exertion, are rarely able to work and frequently suffer from cardiac decompensation. Those with a vital capacity of less than 40 per cent. of the normal are decompensated patients, usually confined to bed, and the mortality in this group is high. There is, moreover, a close correspondence in the individual case between changes in the vital capacity and variations in the tendency to dyspnea. In stages of decompensation the vital capacity falls, and with recovery the vital capacity rises."

2. The Elimination of Salt as an Index of Heart Function.—Koranyi's method is based upon the principle that the concentration of the urinary ingredients depends upon the velocity of the circulation.[107] That is, with reduced circulation, the NaCl output will be reduced, and with acceleration it will be increased. Therefore the molecular concentration of NaCl in the urine as measured by the freezing point may be regarded as a test of circulatory efficiency; i.e., $\frac{\Delta}{\text{NaCl}}$ is increased in slowing circulation and lessened by rapid circulation.

Koranyi examined 24 hour urinary collections. Loeb and Knecht modified the method (more accurately) as follows[108]: The patient takes his evening meal at 6 P. M. He voids at 12 midnight and 6 A. M. He then gets 100 grams milk and after that urinates every 1½ hours. These urinary portions are each examined for $\frac{\Delta}{\text{NaCl}}$. These specimens of urine are not affected by previous ingesta. The effect of exercise can

then be studied as has been done by Loeb, and more recently by von Ritook.[109]

The latter compared the Koranyi test with the von Recklinghausen-Strassburger test[110] in the same cases. He examined 41 cardiac cases suffering from failing compensation.

The urine of 1½ hourly fractions was first examined and at the same time the pulse-rate (F), the minimal blood-pressure (D) and systolic pressure (S) were taken.

$S - D =$ amplitude (A), from which is derived $\frac{A}{S} = Q$ (Strassburger's Quotient), and A × pulse-rate is calculated.

Then the patient was given some work on the ergostat, the pulse and blood-pressure again taken and after one hour the urine again studied for $\frac{\Delta}{NaCl}$.

The authors frequently found contradictory results. They found that the amplitude estimation as a test of beat-volume, and that the A × pulse-rate as an index of rapidity of circulation are not accurate. This was also found by Klemperer.[111]

IX. Tests of the Functional Capacity of the Circulation in Special Diseases.—The author made studies upon soldiers between the ages of twenty and thirty years recruited into the Army in Kentucky. The observations were all carefully recorded and were compiled as a mass of figures. An analysis has discovered certain characteristic results.

Studies were made upon the pulse-rate, systolic and diastolic blood-pressure in the seated posture and repeated with the patient standing and again after exercise. The blood-pressure was measured by the auscultatory method, using a vertical mercury manometer and a wide cuff. Hopping one hundred times on one foot was the exercise used, that being the one prescribed for the examination of the soldier. In most cases a series of observations was made and repeated and the average figure calculated.[51]

Paroxysmal Tachycardia.—Lewis pointed out a distinguishing feature between paroxysmal and simple tachycardia.[112] He showed that effort has no effect on the pulse-rate during an attack of paroxysmal tachycardia while it increases the rate in other tachycardias. Lewis' assertion was not meant for the intervals between paroxysms. During these intervals, the pulse-rate increases a little more than in the normal cases both after change of posture and after exercise. Effort may induce a paroxysm, while on the other hand, it is used by some patients to stop an attack.

The value of testing the functional capacity of the circulation in cases of paroxysmal tachycardia should be emphasized as shown by the author in a previous study of the subject. Low arterial pressure is a constant finding in all these cases, though most marked during the attacks. The reason for this lies in the deficient filling of the ventricles during the very short diastolic periods. The period of ventricular systole is never more than 0.2 of a second. If the rate is over 200 per

minute, the period during which filling of the ventricles can take place is very short. As a result venous stasis takes place and the arterial pressure falls.

From a study of the average pulse and blood-pressure changes in the author's series of five cases, no characteristic findings can be attributed to this condition, following the postural and exercise tests that were used in this study.[113]

Thyrotoxic Tachycardia and Exophthalmic Goiter.—Nine cases of exophthalmic goiter were grouped together to ascertain the relationship of hyperthyroidism to the functional capacity of the circulation. The findings unequivocally support the belief that hyperthyroidism lessens markedly the cardiac and circulatory efficiency. This is evidenced by almost all of the tests used. First, the average increase of pulse-rate on changing from the seated to the standing posture was more than normal. Much more exaggerated than this, however, was the sudden tachycardia following exercise. In this series the pulse rose an average of 65 beats above what it was previously in the seated posture. The instability of pulse-rate in these cases is associated with an instability of blood-pressure. The fluctuation of diastolic pressure is quite marked. It rises with change of posture almost twice as much as it does normally and falls three times as much as normally following exercise. The diastolic pressure then remains low during the time of the test. (Fig. 35.)

The systolic pressure showed a delayed rise following exercise in a few cases, and gives the average of a sustained rise in the curve. This delayed rise or sustained level of blood-pressure signifies deficient circulatory efficiency.[114] The pulse-pressure likewise showed a delayed increase, which is perhaps of equal significance. There were only slight variations from the average in the results of individual cases; the curves constructed from readings in separate cases resemble the combined curve shown in Fig. 35.

Functional tests therefore confirm the attitude toward these cases that they are unfit subjects for strenuous work or prolonged exercise and strain.

Neurocirculatory Asthenia.—Neurocirculatory asthenia, or the "effort syndrome" of symptoms, is met with not alone in military life, but also under stress of civic and social relations. The disorder depends upon a vasomotor instability occurring in highly strung nervous individuals, and presents the following symptoms: breathlessness, precordial pain, a feeling of exhaustion, giddiness and fainting, palpitation, headache, lassitude, irritability, tachycardia, apical systolic thrill, high systolic blood-pressure and nervous instability. All these symptoms are exaggerated by effort and excitement.

In passing, it may not be out of place to point out a differential point between the thrill in this malady and that in organic mitral stenosis. The thrill in this disease, as also in some cases of thyrotoxic heart, is due to the diffusion of the apex beat as it strikes over several rib spaces with a forcible or jerky movement. This systolic shock gives to the palpating hand the impression of a presystolic thrill. The thrill is

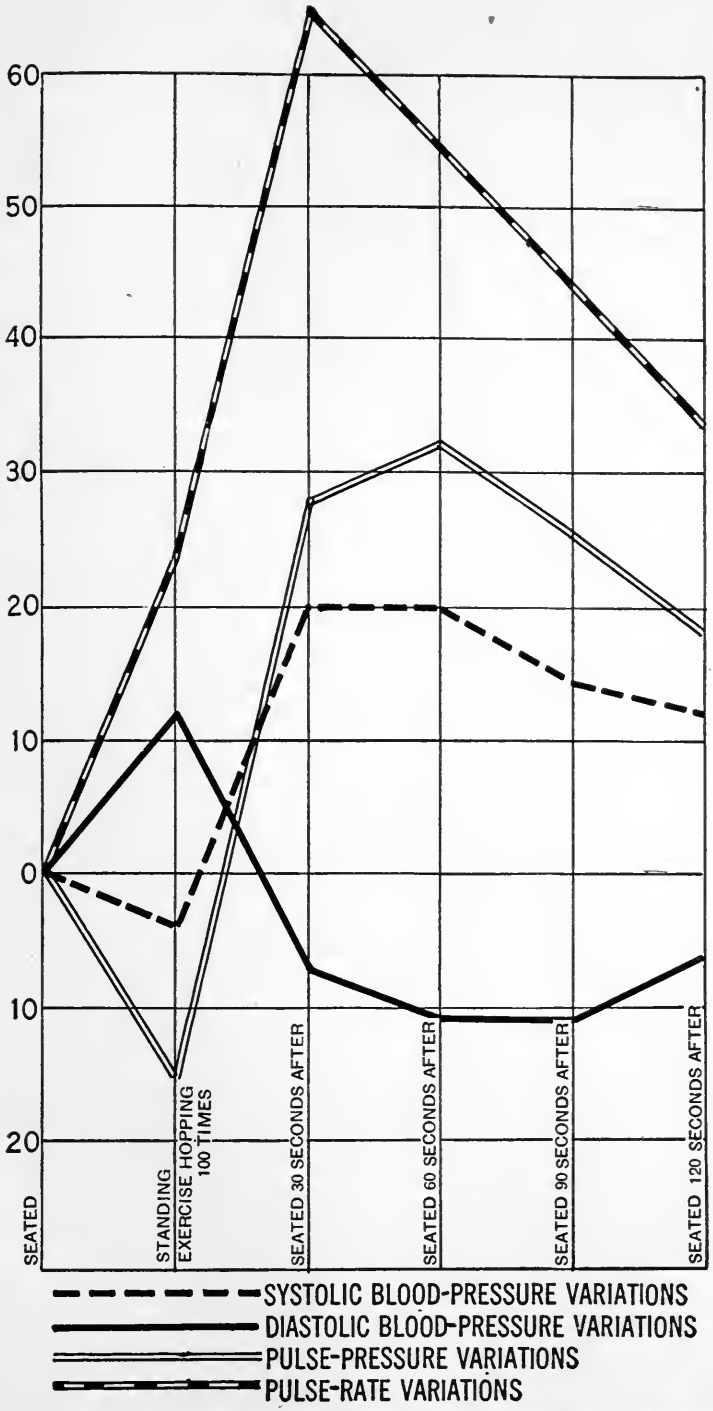


FIG. 35.—FUNCTIONAL TESTS IN THYROTOXIC TACHYCARDIA AND EXOPHTHALMIC GOITER.

obtained characteristically when the chest-piece of the stethoscope covers a whole interspace, being applied over two ribs.

If now the stethoscope is placed obliquely upon the chest wall, so that its edge lies within an interspace and its mouth inclines toward either the upper or the lower rib, the thrill disappears. This is characteristic and almost always to be noted when there is a thrill in these cases. In mitral stenosis, however, the distinct presystolic wave persists against any adventitious attempts to obliterate it. The cardiogram in mitral stenosis is also different from that of this condition.[51]

The one important feature that dominates the cardiac signs of this malady is the hypertonicity of the heart muscle. This can be recognized on palpation and auscultation, and is exaggerated by exercise. The hypertonic condition and action of the heart muscle depends upon the hypertensive nervous state that is responsible for most of the other symptoms. Tests for increased tonicity of the heart muscle have not as yet been developed and we must therefore rely upon our clinical acumen and experience to recognize and estimate it.

McNee and Dunn,[115] and more recently Brooks,[116] found the thyroid gland enlarged in many of these cases and consider the condition thyrotoxic. We believe that the clinical picture is neurogenic and psychogenic primarily, rather than induced by endocrinal disturbance. Neurocirculatory asthenia presents a tremogram different from that obtained in hyperthyroidism. The thyrotoxic tremor is fine and at a fairly regular rate of about eight to the second. The tremogram of neurocirculatory asthenia is rather coarse and irregular and the irregularity increases in rate and amplitude upon effort.

The curve that we have constructed from studies made upon 28 cases of neurocirculatory disorder presenting the classical symptoms, shows: first, increase of pulse-rate on change of posture and following exercise greater than normal; secondly, fall after exercise more gradual than normal; thirdly, increase of systolic blood-pressure and of pulse-pressure more than in the normal cases immediately after exercise (Fig. 36).

It must be emphasized, however, that the hypertonic state of the heart muscle as a feature of this disease is of equal importance with the exaggeration of the symptoms and signs by effort or excitement.

With regard to the pulse-rate, what is probably more characteristic than its exaggeration after exercise and its more gradual return than normally, is the instability of rate due to psychic factors.

Table 1 shows characteristic figures from our protocols of cases of neurocirculatory asthenia. Each double column is from a separate case.

It is apparent from the curve and from the table that patients with the effort syndrome may have normal cardiac reserve power. There is no delayed rise of the systolic blood-pressure after the exercise. This accords with the findings in three cases examined by Robey and Boas.[117]

Tests were made in cases of neurocirculatory asthenia of the effect of change from the lying to the seated position and from the lying to the standing position. The changes depend upon splanchnic effects.

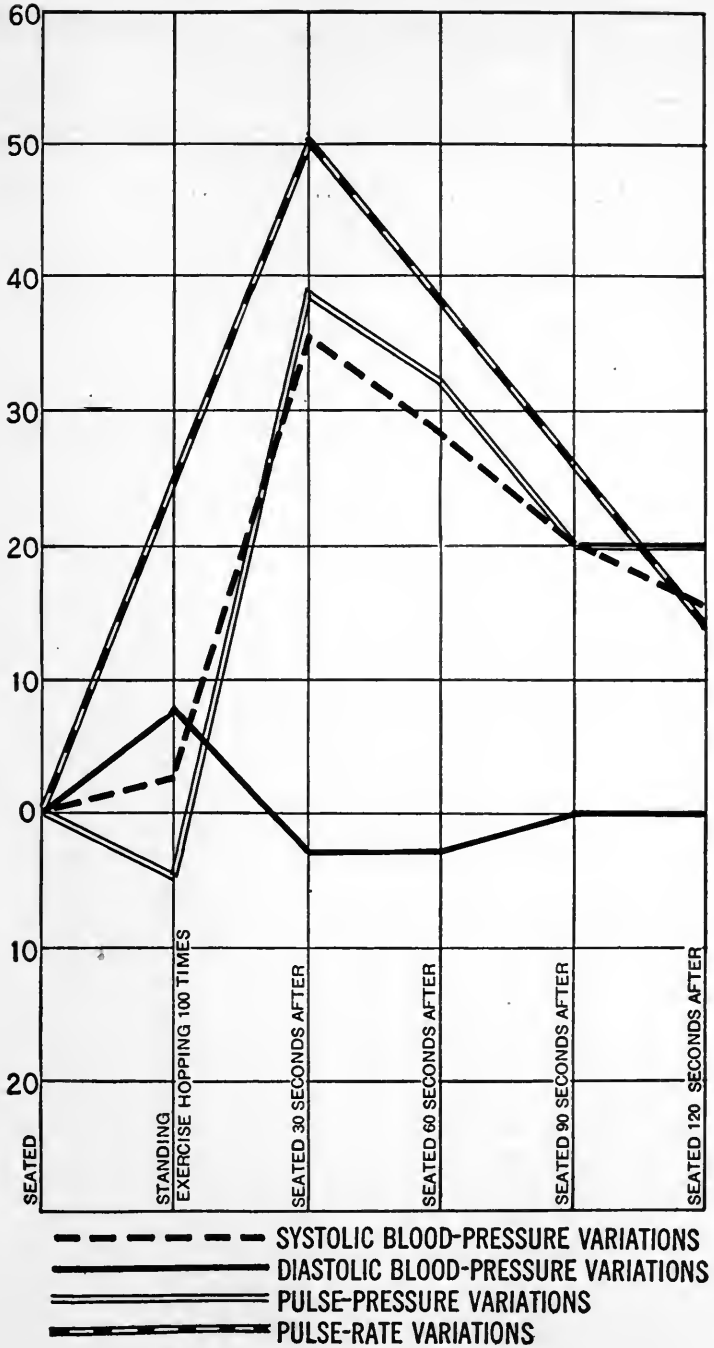


FIG. 36.—NEUROCIRCULATORY ASTHENIA.

TABLE 1.—FUNCTIONAL CAPACITY OF THE CIRCULATION IN NEUROCIRCULATORY ASTHENIA.

	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.
Pulse-rate, seated.....	84	..	96	..	90	..	114	..	96	..
Pulse-rate, standing....	132	..	132	..	132	..	132	..	108	..
Blood-pressure, seated..	115	75	130	64	108	68	120	70	122	70
Blood-pressure, standing	145	90	138	84	120	86	115	75	128	84
Exercise performed.....	Hopping One Hundred Times on One Foot									
Foot-pounds of work....	3425		4250		4685		3375		3225	
Pulse immediately after.	156	..	144	..	156	..	192	..	120	..
Blood-pressure 30 seconds after.....	145	80	144	76	154	72	160	70	156	90
Blood-pressure 60 seconds after.....	140	80	144	76	144	68	160	65	136	80
Blood-pressure 90 seconds after.....	120	85	142	72	138	66	150	65	140	84
Blood-pressure 120 seconds after.....	135	80	140	68	134	64	134	70	124	76
Pulse-rate 2 minutes after.....	120	..	78	..	132	..	132	..	108	..

Sudden change from the recumbent to the standing position causes a slight fall of blood-pressure. But after about 15 seconds both the systolic and diastolic pressure rises as in the normal cases.

NEPHRITIC HYPERTENSION.—The author studied 11 cases of hypertension of nephritic origin to note what general effect hypertension alone may have upon the functional capacity of the circulation. The lowest diastolic pressure in the series was 90 mm. of mercury. The pulse-rate was augmented more than normally by change from the seated to the erect posture. The diastolic pressure rose on standing and fell after exercise, giving a curve very much like the normal (Fig. 37). The systolic pressure was uninfluenced by change of posture from seating to standing. Exercise increased the systolic pressure almost twice as much as normally, and what is more important, the Barringer test, i. e., the systolic pressure taken 30, 60 and 90 seconds after exercise, indicates, if not a delayed rise, an increased pressure maintained longer than normally. The pulse-pressure does show a distinct delayed rise. That is, cases of nephritic hypertension, as concluded from the evidence, have a diminished functional capacity of the circulation. Because of the distress after exertion, one is prompted to caution in these cases and no more strenuous exercises were used for the observations.

CONGENITAL LESIONS AND CARDIAC DISPLACEMENT.—These tests were not done extensively enough to warrant detailed analysis. The findings may be briefly summarized.

In one case of congenital dextrocardia with transposition of the

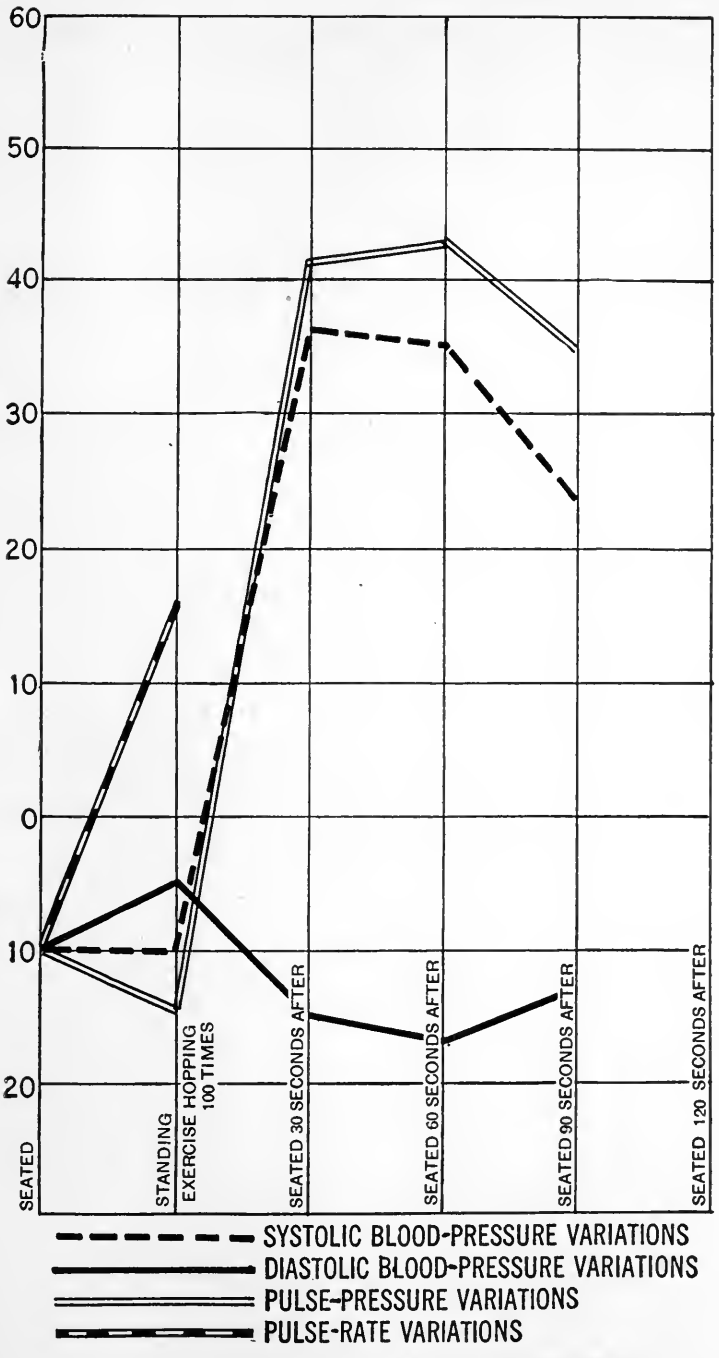


FIG. 37.—FUNCTIONAL TESTS IN NEPHRITIC HYPERTENSION.

viscera, findings were obtained quite different from the normal. The main feature was a marked fall, 14 mm. of mercury, in the diastolic pressure on change of position from sitting to standing and its return to 5 mm. above the previous reading after exercise.

The same feature singularly characterized a case of patent ductus arteriosus. A fall of diastolic pressure of 30 mm. occurred in this case and persisted after exercise. It is hoped that the effect of congenital heart lesions upon functional efficiency will be given further study.

In two cases of markedly displaced heart, very peculiar findings were obtained, chiefly an instability of the pulse-rate variations following exercise. The findings are not sufficiently convincing or numerous to report in detail.

REFERENCES

General Introduction

1. Keith and Flack. Jour. Anat. and Physiol., 1907, xli, 172.
2. Lewis. Heart, 1910-11, ii, 23.
   Lewis, Oppenheimer and Oppenheimer. *Ibid.*, 147.
   Wybau. Arch. internat. de physiol., 1910, x, 78.
3. Tawara. Zentralbl. f. Physiol., 1905, xix, 70.
4. Engelmann. Arch. f. Anat. u. Physiol., 1903, 505.
   Lohmann. *Ibid.*, 1904, 431.
   Meek and Eyster. Am. Jour. Physiol., 1914, xxxiv, No. 4, 368.
5. Mackenzie. Diseases of the heart. London, 1914, Chap. VIII, p. 33.
6. Lewis. Lectures on the heart. New York, 1915, Chap. I, p. 31.
   Saigo. Verh. d. Deutsch. Path. Ges., Jena, 1908, xii, 165.
7. Bowditch. Ber. d. Math. phys. Cl. d. Königl. Sächs. Gesellsch. d. Wissensch., Leipzic, 1871, xxiii, 652.
8. Mackenzie. Brit. Med. Jour., London, 1905, ii, 1689.
9. ——— Diseases of the heart, London, Chap. VII, p. 31.
10. Hoffmann. Funktionelle Diagnostik und Therapie der Herzkrankheiten, Wiesbaden, 1911, Chap. V, 240.
11. Nicolai and Zuntz. Berl. klin. Wchnschr., 1914, li, 821.
12. Mackenzie. Diseases of the heart. London, Chap. VII, p. 12.

I. *The Significant Physical Signs and Symptoms of Impaired Heart Function*

13. Mackenzie. Diseases of the heart. London, 1914, Chap. XXVI, p. 187.
14. Lewis. Clinical disorders of the heart-beat. New York, 1916, Chap. VIII, p. 103.
15. Rehfisch. Berl. klin. Wchnschr., 1915, lii, 1225.
16. Bock. München. med. Wchnschr., 1908, lv, 551.
17. Leyton. Proc. Roy. Soc. Med., 1916, ix, 49.
    Ramsay. Brit. Med. Jour., London, 1916, ii, 521.
18. von Leube. Special medical diagnosis. Philadelphia, 1907.
19. Kraus. Deutsch. med. Wchnschr., 1905, xxxi, 1.
20. Basch, S. Klinische und experimentelle Untersuchungen aus dem Laboratorium von S. Basch. Berlin, 1892, Vol. II, p. 198.
21. Welch. Virchow's Arch. f. path. Anat., Berlin, 1878, lxxii, 375.
22. King, T. W. Guy's Hosp. Rep., London, 1837, ii, 104.
23. Allbutt. Diseases of the arteries and angina pectoris. London, 1918.

II. *Blood-pressure Studies*

24. Determann. Ztschr. f. klin. Med., Berlin, 1906, lix, 283.
25. von Recklinghausen. Arch. f. exper. Path. and Pharmak., 1906, lv, 375.
26. Schmidt. Arch. f. Physiol., Leipzig, 1909, 323.
    Scholtyssek. *Ibid.*, 331.
27. Janeway and Park. Arch. Int. Med., 1910, vi, 586.
    MacWilliam and Kesson. Heart, 1913, iv, 297.
    Russel. Brit. Med. Jour., 1912, i, 659.
    ——— Arterial hypertonus, sclerosis and blood-pressure. Philadelphia, 1908.
28. Riva-Rocci. Gaz. Med. di Qurino, 1896, Nos. 50-51.
29. Hill, L. Heart, 1909, i, 73.
    Hill and Barnard. Brit. Med. Jour., London, 1897, ii, 904.
30. Erlanger. Johns Hopkins Hosp. Bull., 1904, xii, 56.
31. Uskoff. Ztschr. f. klin. Med., 1908, lxvi, 90.
32. Sahli. Deutsch. med. Wchnschr., 1907, xxxiii, 628.
33. Müller, O. Arch. f. Anat. u. Physiol., 1904, 203.
34. Korotkow. Mitt. d. k. Milit.-Mediz. Akad. z. St. Petersburg, 1905, xi, 365.
35. Ehret. München. med. Wchnschr., 1909, lvi, 959.
36. Etinger. Wien. klin. Wchnschr., 1907, xx, 992.
37. Kryloff. Ber. d. Kaiserl. Militärärztlichen Akad., 1906, xiii, No. 2.
38. Gittings. Arch. Int. Med., 1910, vi, 196.
39. von Recklinghausen. Arch. f. exper. Path. u. Pharmakol., 1906, lvi, 1.
40. Gibson. Proc. Roy. Soc., Edinburgh, 1907, xxviii, 343.
41. Tigerstedt. Ergebn. d. Physiol., 1907, vi, 265.
42. Poiseulle. Ann. d. chim. et phys., Paris, 1847, i, 21.
43. Stone. Jour. Am. Med. Assn., 1913, lxi, 1256.
44. Fischer. Ztschr. f. diätet. u. physik. Therap., Leipzig, 1908, xii, 389.
45. Tornai. *Ibid.*, 1910, xiii, 504.
    ——— Ztschr. f. klin. Med., 1910, lxx, 235.
46. Goodman and Howell. Am. Jour. Med. Sc., 1911, cxlii, 334.
    ——— Univ. Penn. Med. Bull., 1910, xxiii, 469.

III. *Cardiovascular Response to a General Demand for Increased Circulation*

1. Methods Depending upon Variations in Pulse-rate

47. Donnell. Cited by Barton, Manual of vital function, Boston, 1917.
48. Schapiro. Russk. Vratch, 1903, ii, Nos. 10-11.
49. Cabot and Bruce. Am. Jour. Med. Sc., 1907, cxxiv, 491.
50. Geigel. Deutsch. Arch. f. klin. Med., 1910, xcix, 31.
51. Kahn, Morris H. Am. Jour. Med. Sc., 1919, clvii, 634.
52. Prével. Paris méd., 1917, vii, 138.
53. Graupner. Deutsch. med. Wchnschr., 1906, No. 26, 1028.
54. Selig. Prag. med. Wchnschr., 1905, xxx, 418; 1905, xxxi, 432.
55. Staehlin. Deutsch. Arch. f. klin. Med., 1897, lix, 79.
56. Tantweiler. Quoted by Selig, in Deutsch. Arch. f. klin. Med., 1900, lxvii, 147.
57. Grünbaum and Amson. Wien. med. Presse, 1902, xliii, 2224.
58. Goodall. Brit. Med. Jour., 1916, ii, 519.
59. Gasser and Meek. Am. Jour. Physiol., 1914, xxxiv, No. 1, 48-71.
60. Snyder. *Ibid.*, 1915, xxxvii, No. 1.
61. Herz. Deutsch. med. Wchnschr., 1905, xxxi, 215.
62. Hirschfelder. Diseases of the heart and aorta. Philadelphia, 1918.

2. Methods Depending upon Variations in Blood-pressure

63. Crampton. New York Med. Jour., 1913, xcviii, 385.
64. Lehndorff. Arch. f. Anat. u. Physiol., 1908, 362.
65. Herz. Lehrbuch der Heilgymnastik, Wien, 1904.
66. Marey and Weber. Cited by Katzenstein, Ref. 67.
67. Katzenstein. Deutsch. med. Wchnschr., 1904, xxx, 807; 1915, xli, 457.
68. Merelli. Cited by Barton, Manual of vital function, Boston, 1917.
69. Levy. Zeit. f. klin. med., 1906, lx, 74.
70. Elliott. Am. Jour. Physiol., 1917, xlii, 290.
71. Norris. Internat. Clinics, 1907, i, 66.
72. Loeper, Wagner and Roquebert. Progrès méd., Paris, 1918, xxxiii, 83.
73. Meek and Eyster. Am. Jour. Physiol., 1915, xxxviii, No. 1, 62-66.
74. Graupner. Die Messung der Herzkraft. München, 1905.
75. Barringer. Arch. Int. Med., 1916, 363, 670.
——— *Ibid.*, 1917, xx, 829.
——— *Ibid.*, 1918, xxii, 363.
76. James and Hart. Am. Jour. Med. Sc., 1914, 147, 63.
77. Rapport. Arch. Int. Med., 1917, xix, 981.
Ghedini. Gaz. degli Osped. e delle Cliniche, Milan, 1918, xxxix, 385.
78. Mann, Hubert. Arch. Int. Med., 1918, xxi, 682.

IV. *Tests of Efficiency of the Right Heart*

79. Frey. Deutsch. Arch. f. klin. Med., 1902, lxxiii, 511.
80. von Recklinghausen. Arch. f. exper. Path. u. Pharmakol., Leipzig, 1901, xlvi, 78.
81. Gärtner. München. med. Wchnschr., 1904, lxxiv, 2038; 1903, l, No. 47, 2038.
82. Schott. Deutsch. med. Wchnschr., 1901, xxvii, 356.
——— New York Med. Jour., 1902, lxxv, 75.
——— Jour. Am. Med. Assn., 1907, xlviii, 1423.
83. Basch. Arch. d. sc. biol., 1904, Sup. xi, 117.
——— Wien. med. Presse, 1904, xlv, 962.
84. von Recklinghausen. Arch. f. exper. Path. u. Pharmak., 1908, lv, 463.
85. Eyster and Hooker. Johns Hopkins Hosp. Bull., 1908, xix, 274.
86. Moritz and Tabora. Deutsch. Arch. f. klin. Med., 1909-1910, xcviii, 475.
87. Howell. Arch. Int. Med., 1912, ix, 149.
88. Hooker, D. R. Am. Jour. Physiol., 1916, xl, 43.
89. Cooper, C. M. Jour. Am. Med. Assn., 1909, lii, 1182.

V. *The Polygraph*

90. Hay. Heart disease, graphic methods. London, 1909.
91. Martius. Deutsch. med. Wchnschr., 1888, xiv, No. 13, 241.
92. MacWilliams and Kesson. Heart, 1913, iv, 279.

VI. *The Electrocardiograph*

93. Einthoven. Arch. Internat. de Physiol., 1906, iv, 132.
——— Arch. f. d. ges. Physiol., 1908, cxxii, 517.
94. Lewis and Gilder. Phil. Tr. Roy. Soc., 1912, cciii, 351.
95. Lewis. Heart, 1913-1914, v, 367.
96. Oppenheimer and Rothschild. Proc. Soc. Exper. Biol. and Med., 1916, xiv, 57.
——— Jour. Am. Med. Assn., 1917, lxix, 429.

VII. *The Roentgen Ray and Orthodiagraph*

97. Moritz, F. München. med. Wchnschr., 1908, No. 13, iv, 671.
98. Nicolai and Zuntz. Berl. klin. Wchnschr., May 14, 1914, 821.
99. Abrams. New York Med. Jour., Oct. 29, 1910, xcii, 850.
100. Merklen and Hertz. Examen et séméiotique du coeur. Paris, 1910.
101. Minerbi. Riv. crit. di clin. med., Florence, 1917, xviii, 213.
102. Schott. Deutsch. med. Wchnschr., 1897, 495.
103. Meri. *See* Barton.
104. Breccia. Riforma med., Naples, 1917, xxxiii, 1133.

VIII. *Metabolic Changes in Impaired Heart Function*

105. Rubow. Deutsch. Arch. f. klin. med., 1908, xcii, 255.
106. Peabody. Arch. Int. Med., 1917, xx, 433.
107. Koranyi. Ztschr. f. klin. Med., 1897, xxxiii, 1.
108. Loeb and Knecht. Deutsch. Arch. f. klin. Med., 1905, lxxxiii, 462.
109. Ritoók. Deutsch. med. Wchnschr., 1909, xxxv, No. 34, 1471.
110. Strassburger. Deutsch. Arch. f. klin. Med., 1907, xci, 378.
111. Klemperer. *Ibid.*, 1907.

IX. *Tests for the Functional Capacity of the Circulation in Special Diseases*

112. Lewis, T. Clinical disorders of the heart-beat. New York, 1916, Chap. V, p. 66.
113. Kahn, Morris H. Med. Clin. North America, 1918, ii, 427.
114. Barringer, T. B. Arch. Int. Med., 1917, xx, 829; 1918, xxii, 363.
115. McNee and Dunn. Spec. Rep., 1917, Ser. No. 8, Med. Res. Comm., London.
116. Brooks, Harlow. Jour. Am. Med. Assn., 1918, lxx, 728.
——— Med. Clin. of North America, 1918, ii, 477.
117. Robey and Boas. Jour. Am. Med. Assn., 1918, lxxi, 525.
Lian, C. Ann. de méd., Paris, 1918, v, 345.
118. Hogerstedt and Graupner. Quoted by Herz in Deutsch. med. Wchnschr., xxxi, 215.

CHAPTER V

FUNCTIONAL TESTS OF THE ENDOCRINE GLANDS

Thyroid Gland

Tests for Hyperthyroidism.—1. Iodin Tests for Hyperthyroidism.—It has been a well-known fact that the administration of iodids over long periods to cases of goiter may produce symptoms of hyperthyroidism.[1] Fr. v. Mueller suggested the use of iodin as a means of disclosing hyperthyroidism, as those suffering from this disease often exhibit intolerance to iodin by showing emaciation and tachycardia, after its administration.

However, the administration of thyroid gland, iodin or iodid of potassium to show a latent hyperthyroidism is too dangerous a procedure to be used as a routine clinical test. This is especially true when signs of emaciation are present.[2]

2. Marinesco-Roseo Complement-Deviation Test for Hyperthyroidism.—Marinesco[3] and, later, Roseo[4] suggested that in states of hyperthyroidism there is sufficient thyroid substance (antigen) present in the blood-serum to give rise to the formation of antibodies in the patient's blood. They proposed to test for these antibodies by means of

an antigen prepared from thyroid gland removed at operation from a case of hyperthyroidism. In the experiments an aqueous extract of goiter, from a case of hyperthyroidism, was used as antigen, and the serum of the same patient was used for antibody and also the serum of four other cases of hyperthyroidism. In the case whose goiter furnished the antigen the fixation was complete; in the two other cases there was incomplete hemolysis; and in the fourth the hemolysis was complete, as in a normal control.

They have also studied this reaction, using ethereal and alcoholic extracts of the gland. They found that the aqueous, alcoholic and ethereal extracts acted in about the same way; at times the ethereal extract was more active. In most of the cases of hyperthyroidism there was found either a total absence of hemolysis, or an incomplete or partial hemolysis. On the other hand, the serum of cases of hyperthyroidism never fixed complement in the presence of normal thyroid tissue, with the exception that the serum of a syphilitic patient gave a partial hemolysis with ether and alcoholic extracts of a normal thyroid.

No definite opinion as to the value of this test can be formulated at present, as considerable work is necessary to determine its value in cases of latent hyperthyroidism.

3. Abderhalden's Serodiagnosis: Application of Specific Ferment Test to Diagnosis of Hyperthyroidism.—Lampe[5] and his coworkers have studied the possibilities of applying the Abderhalden specific ferment test to the diagnosis of thyroid states. They believe that the blood-serum of patients with hyperthyroidism contains ferments which are specific for thyroid tissue. They think that if, in cases of hyperthyroidism, there is an overproduction of normal thyroid secretion, a negative result with the Abderhalden test should be obtained, because in this case there is an introduction into the blood of a purely native protein, only in increased amounts, and therefore no production of ferments. If, on the other hand, we have in cases of hyperthyroidism the presence in the blood of a protein that is secreted by the thyroid —but of an altered composition, on account of the pathological changes in the gland—then this foreign protein would stimulate the production of a specific ferment and the Abderhalden reaction would be positive.

They studied the effect of the serum from cases of hyperthyroidism upon normal thyroid gland, exophthalmic goiter gland, cystic and parenchymatous gland, normal thymus, hyperthyroidism thymus, ovary, testicle, kidney, suprarenal, pancreas, etc. In all instances where the serum from cases of hyperthyroidism was allowed to act upon hyperthyroidism tissue, the tissue was digested. In a few cases the reaction was positive when the normal thyroid tissue was used. The reaction was also positive in four out of five of the cystic goiter cases and in the thymus and ovarian tissue cases. With the other tissues the reaction was negative.

On account of these results they believe that in cases of hyperthyroidism, so called, there is a true dysthyroidism, not a simple hyperthyroidism.

Two distinct methods of detecting the presence of these antibodies have been devised. The first, the optic method, is capable of very wide application to the diagnosis of different conditions and should prove extremely useful in solving many problems of great clinical importance. It requires, however, considerable skill and technical ability as well as a rather expensive apparatus. The second, the dialyzation method, is much simpler, both in technic and in necessary equipment. Both of these methods require the most assiduous attention to the various details given, if any dependence is to be placed upon the results of the tests.

1. *The Optic Method.*—The basis of this test is as follows: A solution of peptone in physiologic salt solution has a definite power of rotating the plane of polarized light. Likewise, the serum, both suspected and normal, has a similar action. The degree of rotation of either, however, remains permanent for some time at 37° C. If a solution of peptone and normal serum are mixed and the degree of rotation of this mixture determined, no appreciable change will be observed between the initial and final polarimetric readings. If, however, a solution of peptone, whose polarizing is known, is treated with a serum containing the specific ferments above mentioned, digestion of the peptone occurs with the formation of products showing rotatory powers sufficient to change the initial rotation of the mixture to quite an extent. These changes may be observed at different intervals and interpreted as described later.

Preparation of Thyroid Peptone.—The fresh thyroid is made blood-free by cutting it into small pieces and placing these under running water for about 15 minutes. The pieces should be dried between folds of filter paper and placed in about five times their weight of 70 per cent. sulphuric acid. The mixture is allowed to stand for three days at room temperature and the container shaken frequently. At the end of this time, the container is placed in ice water and the contents diluted with 10 volumes of distilled water, stirring constantly. The sulphuric acid is removed by adding approximately the calculated amount of finely powdered barium hydrate and the precipitation completed with a known solution of this salt, the mixture being stirred constantly. When the reaction of the mixture becomes neutral to litmus paper, the barium sulphate is filtered off. If the filtrate is turbid, refiltration is necessary, until a perfectly clear filtrate is obtained. The separation of the barium sulphate is much facilitated by the use of a large centrifuge, if such is at hand. The precipitate is then washed with a large amount of cold water and the filtrate and washings combined. The mixture should be tested for both barium and sulphuric acid. If either is present, it must be removed. This barium- and sulphuric-acid-free solution is then evaporated to dryness on the water-bath, under reduced pressure, at a temperature not exceeding 40° or 50° C. It is wise to test the evaporated material at several intervals for the presence of either barium or sulphuric acid, as these sometimes appear on concentrating the mixture. It is important that these be removed, as their presence will result in further hydrolysis of the peptone and, in consequence, will lessen the

value of the final product. A thick, yellow syrup or a foamy mass remains after this evaporation. The product may be used in this form, but it is preferable to purify it, if reliable results are to be invariable.

This yellowish residue is dissolved in methyl alcohol with the aid of heat, and the hot solution is poured into absolute ethyl alcohol. The peptone is thrown down as a yellow powder, which is soluble in water to a clear yellowish solution of weakly acid or amphoteric reaction. This powder is not hygroscopic. A further purification is still advisable. The yellow powder, above mentioned, is dissolved in water up to a 5 per cent. solution and a 10 per cent. solution of phosphotungstic acid added as long as a precipitate forms. This is then filtered and washed several times with water. Following this, the precipitate is rubbed up in a mortar with some water and twice its weight of barium hydrate. This is again filtered and the excess of barium removed from the filtrate, with sulphuric acid. The barium sulphate is filtered off and the filtrate evaporated to dryness under reduced pressure at 40 to 50 degrees, as outlined above. This product is snow white and is permanent.

It is absolutely essential for the successful application of the optic test that the peptone be as pure as possible. The same product is not always obtained by the above method, as the hydrolysis may proceed further than the peptone stage. Such products are unsuitable for the test. It is wise, therefore, to work as quickly and as carefully as possible with a large amount of thyroid substance, so that one may obtain an appreciable amount of peptone. If the product is found serviceable, it may be kept for years. A further point to be considered in the use of a prepared peptone is that the solution of this product must give absolutely no turbidity with the serum to be tested. Such a finding is not infrequent, owing to the probable presence of precipitins in the product. Such a peptone cannot be used.

A further important property of the prepared peptone must be its power of rotating the plane of polarized light. The degree must not be too small or the product will prove of little value. It will be seen, therefore, that the preparation of a serviceable and proper peptone is a matter of considerable difficulty and is essential to the successful performance of the test.

One may preserve the peptone, prepared as above, either in the solid state or in the form of a solution. The advantage of a solution is that one has on hand a large amount of material, which will give good comparative results, as the solution is permanent. Abderhalden formerly used solutions of 0.5 to 2.5 per cent. strength. This solution must be absolutely clear and colorless. If not clear and colorless, it may be filtered through thick paper or a Berkefeld. This clear solution may be preserved by overlaying its surface with toluol. When required for the test, the solution is withdrawn by a pipet dipping below the toluol. If care is taken to keep a layer of toluol over the solution, the stock material will be permanent for a long period. Should this solution become turbid at any time, the material should be thrown away and a

new stock solution prepared, as above. The optical activity of this stock solution must be tested before each test.

Obtaining the Serum.—The serum of the patient is obtained by withdrawing 10 to 15 c.c. of blood. The blood, as drawn, should be put directly into a sterilized centrifuge tube, so that all cellular elements may be completely separated. For a successful test the serum must not show signs of the presence of cells. A further precaution to be taken is that no sign of hemolysis must be present in the serum; for this reason, the cells should be separated rapidly. It is wise to make the test on the same day on which the blood is taken, although, if the precautions above mentioned are observed, a delay of 24 to 48 hours does not materially affect the activity of the serum.

Technic.—Having prepared the 10 per cent. solution of peptone and having proven that it answers all the requirements mentioned above, place 1 c.c. of this clear solution (withdrawn by a pipet) in a small, clean, sterile test-tube. Add 2 c.c. of the clear suspected serum and shake the tube several times. Examine the mixture carefully for any turbidity or precipitation. If any is observed, the test cannot be carried out. Add sufficient physiologic salt solution to the mixture to fill the 1 decimeter polarimetric tube. This mixture with salt solution is preferably made in this way, rather than by adding the salt solution after the peptone solution and serum have been placed in the polarizing tube. Any turbidity may be much more easily detected. Pour the above mixture into the 1 decimeter tube, whose mantle has been filled with water at 37° C.

Carefully determine the initial rotatory power of the mixture, checking the readings several times and controlling them by subsequent ones after 5 or 10 minutes. No change should be observed in these readings. Place the tube and its contents in the incubator at 37° C. and repeat the readings every hour for a few periods, and then continue every 6 to 8 hours. Do not extend the investigation over more than 48 hours. Record all readings and interpret them as given below.

Control tubes must be arranged as follows: (1) the peptone solution alone; (2) the suspected serum alone; (3) peptone solution plus normal serum; (4) peptone solution plus known positive serum; (5) peptone solution plus inactivated (heated to 60° C.) suspected serum. In all of these controls the same conditions must be maintained and the same length of polarizing tube must be used as in the test itself. If any turbidity occurs in any of the control mixtures or tubes, these must be disregarded in interpreting the test, as turbid solutions give variable results with the polarimeter.

It goes without saying that this test requires the very best equipment possible. The cheap polariscopes are absolutely useless, as they are not delicate enough to detect the fine variations given. The three-shadow instrument of Landolt-Lippich is especially to be recommended. The polarizing tubes used are, preferably, the 1 decimeter tubes which are furnished with a mantle which may be filled with water at any desired temperature. If different length tubes are used in any of the

tests, a correction must be made in order that comparative figures may be obtained.

In performing this test, even to a greater degree than when the instrument is used in other work, much depends upon the ability of the worker to detect slight variations in the degree of the rotatory powers of the mixtures under investigation. The method is easy to learn, but the special sensibility toward such changes cannot be taught. Abderhalden cautions any one who shows a working error of as much as 0.04° in his observations against attempting to interpret the test.

In reporting the result of this test, Abderhalden employs the following method:

Deviations within 0.04°	Negative
Deviations between 0.05° and 0.1°	Positive (+)
Deviations between 0.11° and 0.2°	Positive (++)
Deviations over 0.2°	Positive (+++)

2. *The Dialyzation Method.*—This method is much more simple than the optic method, as regards both technic and apparatus. It must not be thought, however, that any less care is necessary in carrying out the details of the test. In fact, erroneous results are, perhaps, more easily obtained by careless manipulation when this method is employed.

The basis of this method is the conversion of the colloidal non-dialyzable protein into dialyzable products, through the activity of the ferments above mentioned. These products are, then, detected by simple color reactions in the dialysate.

Preparation of Thyroid Protein.—As autolysis proceeds fairly rapidly in thyroid tissue, prepare the albumin from thyroids which should be as fresh as possible. Remove the external portions of the thyroid, and wipe away as much blood as possible. Cut the material into small pieces and wash for a short time in running water.* While this is being done, boil about 2 liters of water, to which are added 2 drops of glacial acetic acid. Throw the washed bits of thyroid into this boiling water and boil for 5 to 15 minutes. Pour the mixture upon a loose, quick-acting filter and boil the pieces again with a second portion of acidulated water for 5 to 15 minutes. Pour off this water and test with the triketohydrinden hydrate reaction (*see* page 210). If a positive reaction results, the thyroid tissue must be again boiled with acidulated water until a negative reaction occurs. The essential points in this process are rapid and complete coagulation of the protein and the removal of all soluble dialyzable material which may react with the reagent mentioned above.**

As soon as a negative result is obtained with the extractive water, pour the mixture into a wide-mouth flask, add some chloroform, and then overlay the fluid with toluol and stopper the flask; or else

* It is essential that all visible blood be removed, as its presence will introduce a large error.

** Care must be taken not to add an excess of acetic acid to the water, as this may interfere with the ninhydrin test and thus give rise to an appreciable error.

place the material in several smaller glass jars and overlay with toluol. This protein keeps almost indefinitely and may be removed from the containers as desired. It should be tested, from time to time, to show that it contains, in itself, nothing which may react with the reagents used in the later test.*

Obtaining the Serum.—The serum is obtained by venous puncture, the blood (about 10 c.c.) being drawn directly into a sterilized centrifuge tube. The cellular elements are separated as quickly as possible and the serum drawn off into a clean sterile tube. It is of especial importance in this test that the serum show no sign of hemolysis. As it has been shown that amino-acids are present in the blood during digestion and may, therefore, give a positive reaction with triketohydrinden hydrate, it is wise to take the blood in the morning before breakfast in all cases. If this is not done, a smaller amount of serum must be used in the later test to compensate for this possible error.

Selecting the Dialyzing Tubes.—It is evident that this part of the preparation for the test is of extreme importance. The dialyzing thimbles must be permeable for peptone but not for protein. Unless these conditions obtain, the test is valueless. Not all of the thimbles on the market are by any means suitable. Abderhalden advises the use of the diffusion shells No. 579 A., of Schleicher and Schull. Not all of these will answer the purpose.

It is necessary, therefore, that all the dialyzing thimbles used in the test should have been previously tested and known to fulfill the above requirements. As the thimbles are usually dry and hard when obtained, they should be soaked in cold water for a few hours, placed in boiling water for a few seconds and kept in water covered with toluol.

To test these thimbles for their permeability for protein, proceed as follows: Remove the thimble from the water and place in it 5 c.c. of serum or of a solution of egg albumen. Add a few drops of toluol to prevent bacterial action. Place 20 c.c. of distilled water in the dialyzing vessel and overlay this with toluol. This dialyzing vessel should be quite narrow, the distance between the wall and the thimble (when in place) being about ¼ cm. These vessels are kept plugged with cotton and are sterilized before use. Now suspend the thimble with its albuminous contents in the dialyzing tube in such a way that the fluid outside is as high or, preferably, a little higher than that within the thimble. Plug the vessel with cotton to prevent contamination and put the apparatus in the incubator at 37° C. for 18 to 24 hours. At the end of this time, test the dialysate (outside fluid) for protein by the biuret or triketohydrinden hydrate reactions given below. Those thimbles giving negative results are retained to be tested for their permeability to peptone. The shells permitting the passage of protein cannot be used in the test.

Select those thimbles showing impermeability to protein and wash

* Abderhalden recommends that this protein be tested with ninhydrin before being used in any test. This is a vital point.

them thoroughly in water. Place in them 5 c.c. of a 1-1,000 solution of Witte's or, preferably, peptone from silk (peptone La Roche) and add sufficient toluol to cover the solution. Dialyze as above against 20 c.c. of distilled water, placing the apparatus in the incubator for 18 hours at 37° C. Those thimbles which permit the passage of peptone, as shown by the triketohydrinden hydrate tests, are kept for use in these tests and the non-permeable ones are laid aside. The properly tested and selected thimbles are then preserved in water overlaid with toluol.

Technic.—Remove a few pieces of the coagulated protein from the container, wash in distilled water and dry between filter paper.* Break this up into very small bits or grind up in a mortar. Weigh out three portions of ½ gram each. Place ½ gram in each of three tested dialyzing thimbles in such a way that none of the material touches or remains upon the top or outside of the shells. Carefully wash off the outside of the thimble by means of a stream of distilled water or hold it under running water. This is done to remove any possible adhering protein, which would vitiate the test later made. Now add to Tube No. 1, 1 to 1.5 c.c. of clear hemoglobin-free serum to be tested. Withdraw this serum from its container by means of a sterile graduated pipet. Overlay the surface of the mixture in the thimble with toluol. Then place this thimble in a sterile dialyzing tube, as described above, containing 20 c.c. of distilled water, which should stand slightly higher than the fluid in the thimble. Overlay the external fluid with toluol and plug the dialyzing vessel with cotton to prevent contamination. Place the apparatus in the incubator at 37° C. for 18 hours and then test the dialysate for peptone, as outlined below.

Controls.—Charge Thimble No. 2 with ½ gram of thyroid protein and 1 c.c. of serum of a known positive control. Overlay with toluol and arrange as above.

Charge Thimble No. 3 with ½ gram thyroid protein and 1 c.c. of a known negative serum or with the inactivated (heated to 60° C.) serum used in the test.

A further control should be run, using 1 c.c. of the serum alone without the addition of protein, to prove that it does not contain any dialyzable substances which will give the later reactions.

The tests with all of these controls are carried out exactly as the test itself, every precaution being taken to prevent the introduction of errors. The tests for cleavage products of protein are made with one of the following tests, the latter being in some respects preferable.

(*a*) *The Biuret Test.*—This was the test formerly employed by Abderhalden and has some advantages, one being that it does not react with certain dialyzable products not infrequently present in the serum of normal subjects. It requires considerable care and skill in manipulation as well as in interpretation. Doubtful results are very frequent unless every precaution is taken.

Remove about 10 c.c. of the dialysate by means of a pipet, dipping

* Test the material before use with the triketohydrinden hydrate reaction. Absolutely no trace of a blue coloration should be obtained.

below the toluol. Place this in a test-tube and add 5 c.c. of a 33 per cent. sodium-hydrate solution. Mix by careful shaking and add very carefully, drop by drop, from a buret a very dilute (0.25 per cent.) solution of copper sulphate in such a way that a distinct contact ring is formed. If peptone is present, a violet-red to a pure red contact ring will be observed, sharply differentiated from the lower colorless and upper blue solutions. This is a positive reaction. A negative result is shown by the appearance of a distinctly blue ring. It is not the simplest matter to distinguish between the various shadings which occur, so that one must not make his decision without having had some experience in differentiating the colorations obtained with pure protein and peptone solutions.

(*b*) *The Triketohydrinden Hydrate Reaction.*—This reagent occurs in colorless crystals readily soluble in water. It may be obtained under the trade name of ninhydrin. Its formula is $C_6H_4\begin{matrix}CO\\ \diamond \\ CO\end{matrix}C(OH)_2$.

It is of especial importance that every precaution be taken to prevent error when this reagent is used, as reactions may rise from the presence of substances which are not at all associated with the hydrolytic products of protein material. This reagent is not by any means specific, even for albumin, peptone or amino-acids, although it was formerly believed that it reacted only with substances containing an amino and a carboxyl group, the former especially in the *a* position. It has been shown that there are a large number of compounds which are not in a chemical sense combinations with amino and carboxyl groups and which, nevertheless, give very characteristic reactions. Among these we find: amins; amino-aldehyds; urea derivatives; amino-sulphonic acids; ammonium derivatives of certain organic acids, dicarbonyl compounds and halogen-aldehyds; ammonium compounds of thiosulphuric, oxysulpharsenic and selenic acids; ammonium formate, ammonium thiolactate, etc. Of special importance is the fact that a very small amount of basic products of putrefactive origin will give a decided reaction (hence the importance of using every means to prevent decomposition of the tissue).

Certain further precautions are essential. If any of the original protein is left on the outside of the dialyzing thimble, a very obvious error will arise. If the serum used contains amino-acids, the amount must be determined by depth of color with the ninhydrin. One must avoid the presence of acid or ammoniacal fumes in the laboratory. Strong alkalis cause, in themselves, a coloration with the reagent, while dilute alkalis may decolorize the solution. Acids prevent the appearance of the blue color and will destroy the color already formed, even in the presence of a large amount of reacting material. It must be insisted, therefore, that the fluid to be tested be absolutely neutral. Further, all vessels and pipets must be absolutely clean and the water used must be free from bacteria.

Remove 10 c.c. of the dialysate by means of a pipet dipping below the toluol and place this in a large test-tube. Add 0.2 c.c. of a 1 per cent. aqueous solution of triketohydrinden hydrate. Heat rapidly to the boiling point and keep the mixture boiling for 1 minute. If the reaction is negative, the solution remains colorless or becomes, at most, light yellow. If the reaction is positive, a deep blue color will appear either immediately or on allowing the tube to stand for a short time. After use, the thimbles should be washed thoroughly in running water and then placed in boiling water for not over 15 seconds.

The reaction is carried out in the same way with the control tubes. Tube 2 should show a distinct positive reaction, while Tube 3 should give a negative result. The tube with serum alone should show a negative reaction, but occasionally it is positive, owing to the presence of a large amount of amino-acids in the serum as drawn. If the controls are all positive, the test is, of course, valueless, as some factor has been imperfectly controlled.

4. Test of Claude, Baudouin and Porak for Hyperthyroidism.—These observers [6] have described the use of the extract of the posterior lobe of the pituitary in the diagnosis of latent hyperthyroidism. This extract was obtained by the action of alcohol at 70° upon the pituitary powder, which was dried and freed from fat. The alcohol was evaporated and the residue dissolved in normal salt solution. The strength of the solutions used was such that 1 c.c. was equal to one-half of a posterior lobe of a beef's pituitary, corresponding to 0.5 gram of pituitary powder.

They found that the subcutaneous injection in normal cases produced a marked reaction, pallor, glycosuria, diarrhea, and acceleration of the heart. The blood-pressure remained the same or was lowered. When injected into cases of hyperthyroidism the results were the same, with the exception of the cardiovascular effects. In normal cases the acceleration of the pulse commenced two or three minutes after the injection and reached a maximum in 10 to 15 minutes. Then the increased frequency diminished, and in about twenty minutes the pulse-rate was normal. In the cases of hyperthyroidism the pulse, which was rapid before the injection of the pituitary extract, decreased very quickly from eight to ten beats. This decrease was reached in about two minutes, sometimes four or six, or rarely ten, and lasted from seven to eight minutes, returning, as a rule, to a rate markedly below the rate previous to the injection.

This bradycardia effect they think is due to stimulation of the vagus nerve, while its effect in the cases without existing hyperthyroidism is on the accelerator sympathetic. In the cases of hyperthyroidism there exists a tachycardia due to hyperexcitation of the sympathetics, and on account of this state of hyperexcitation these nerves do not react to the pituitary extract. The nerve endings of the vagus which are not excited feel the full effect of the pituitary stimulation and the heart is slowed.

5. Metabolism Studies as a Test for Hyperthyroidism.—It has

long been known that there exists a markedly augmented catabolism in cases of hyperthyroidism.[7] The respiratory interchange shows an increase of 50 per cent., 70 or 80 per cent., in the amount of oxygen consumed.[8]

Du Bois [9] has found that the increase in metabolism is strictly proportional to the severity of the clinical symptoms.

The development of apparatus for measuring the respiratory exchange of man has proceeded along two lines. In one type the subject is completely enclosed in a chamber; in the other, the subject is attached to the respiration apparatus by means of some breathing appliance. The chamber type includes the respiration apparatus of Pettenkoffer and Voit,[10] Sondén and Tigerstedt,[11] Jacquet,[12] and Grafe,[13] the Atwater-Benedict [14] respiration calorimeter and the apparatus of the Nutrition Laboratory.[15] The open-circuit apparatus are represented by the apparatus of Speck,[16] Zuntz-Geppert,[17] Tissot [18] and Douglas.[19] The closed-circuit apparatus include the two types of the Benedict [20] apparatus, Rolly's [21] modified Benedict apparatus and that of Krogh.[22]

The respiration calorimeter used at Bellevue Hospital has been described by Lusk [23] and by Riche and Soderstrom.[24]

(*a*) *Clinical Respiration Apparatus of Benedict.*[25]—The clinical respiration apparatus in its finished form consists of: (1) a respiration chamber, suitably illuminated and ventilated, in which the subject may lie comfortably upon a cot; (2) a universal respiration apparatus with a rotary blower for ventilating the chamber, an absorbing system for purifying the air of carbon dioxid and water, and a suitable oxygen supply; (3) accessory apparatus in the form of thermometers, barometer, and apparatus for recording the pulse-rate and the degree of muscular repose.

In this description of the clinical respiration apparatus, it is advantageous to consider, first, the universal respiration apparatus.

(*b*) *Universal Respiration Apparatus.*—The universal respiration apparatus, which was developed in the Nutrition Laboratory at Boston, has been used in a considerable number of researches. In principle the respiration apparatus is designed to supply the chamber with a moving volume of air, absorb the carbon dioxid in the outcoming air, replace the oxygen consumed by the subject, and return the air again to the chamber with a chemical composition not materially unlike that of atmospheric air.

In the process of purification the carbon dioxid in the air is completely absorbed, the amount excreted by the subject being determined by the increase in weight of the absorbing vessels. Thus gas analyses, with their attendant difficulties of technic, are unnecessary. The oxygen consumption is quantitatively determined directly by noting the amount it is necessary to introduce into the respiration chamber in order to secure the same volume of air in the chamber at the beginning and end of the experiment, due allowance being made for changes in temperature, pressure, and volume of water-vapor.

The air, as it leaves the chamber, contains the carbon dioxid and

water given off by the subject and is also deficient in oxygen, owing to the oxygen required for combustion in the subject's body. The outcoming air is forced by the positive rotary blower into the absorbing system and passes first into an empty glass Williams bottle, which serves as a trap to prevent any back suction of acid in case of an accident. The air then passes into a large Williams bottle, containing concentrated sulphuric acid, and from there into a smaller Williams bottle, also filled with acid, which acts as a control. The water-vapor is thus completely removed, but the carbon dioxid still remains. On reaching the valve, the air passes into either one of two sets of purifying systems, consisting of soda-lime and sulphuric-acid bottles. The carbon dioxid is completely absorbed by the soda-lime and the water given off by the moist soda-lime is also removed by the sulphuric acid in the second vessel. In certain instances it is desirable, particularly in long experiments, to insert two soda-lime bottles.

After the air leaves the carbon-dioxid absorbing system through the valve, it passes through a can containing sodium bicarbonate, to remove the small unweighable traces of acid vapor, which might otherwise irritate the nose and throat of the subject, and then continues into the respiration chamber.

Theoretically, oxygen may be admitted at almost any point in the air-circuit, but here it is represented as being introduced from a large cylinder through a meter at a point in the pipe, connecting the spirometer or tension-equalizer with the chamber. Variations in the volume of the air in the chamber are corrected by means of a spirometer. In describing the various parts of the universal respiration apparatus more in detail, it seems desirable to follow the course of the ventilating current.

Blower.—A most satisfactory blower, under the specification No. O.-D. Rotary Compressor, can be secured from the manufacturers in a surrounding iron box which is suitable for an oil immersion bath. It is a positive blower, in that the air withdrawn from the chamber may be forced through a considerable number of layers of sulphuric acid and soda-lime contained in suitable vessels. The rotary blower is connected by a leather belt to a small electric motor, and can be provided with a safety clutch to prevent the reversing of the wheel through carelessness and the drawing over of sulphuric acid from the water-absorbers. This latter feature has been found of advantage, although the safety trap has usually prevented the drawing over of the acid into the blower. The speed of the blower may easily be altered by a simple lamp resistance, these blowers usually giving a suitable ventilation—not far from 35 liters per minute—when rotating at the speed of 270 revolutions per minute.

Acid Trap.—To prevent the possibility of drawing back strong sulphuric acid into the delicate mechanism of the blower, an empty glass bottle is inserted in the system. While almost any form of bottle can be used for this purpose, it has been convenient to employ an empty reversed Williams bottle.

Water Absorbers.—The air leaving the respiration chamber contains

a large amount of water-vapor from the lungs and skin of the subject. Before the carbon dioxid produced by the patient is absorbed, it is important to remove this water-vapor entirely from the air. The current is therefore first passed through two or more bottles containing sulphuric acid. Usually one large-sized Williams bottle is sufficient to collect nearly all of the moisture, but this is followed by a second bottle, which retains the last traces of water-vapor. To facilitate handling and to prevent breakage, each bottle is usually enclosed in a small wire basket with a handle, by means of which it may be suspended directly from a hook on the arm of the balance. When these two Williams bottles are used, it is possible to retain the first one in the circuit until the acid and water have so increased in volume as to render them liable to be carried over mechanically into the second bottle. As much as 100 or 200 grams of water-vapor may be absorbed before a change is required; it is fundamentally important, however, to note that this second Williams bottle, as well as the water absorber following the carbon-dioxid absorber, must not increase in weight more than 10 grams before being renewed, and should be controlled by frequent weighing.

Tubing and Piping.—The Williams bottles, as well as the soda-lime bottles for absorbing the carbon dioxid, are fitted with short lengths of rubber tubing of good quality, to which are attached respectively male and female parts of ordinary garden hose couplings of the standard ¾-inch size (approximately 19 mm. internal diameter). The couplings are, therefore, interchangeable with different forms of apparatus. With a standard rubber hose gasket, the couplings can be made air-tight by a simple twist of the hand. All of the piping throughout the apparatus is of standard ½-inch (approximately 15 mm. internal diameter) pipe.

Two-way Valve.—In order to deflect the main air-current from one set of purifiers to the other, it is necessary to have a two-way valve. All of the valve connections are of this type. A long steel rod connects the two valves in such a way that by throwing the handle at one valve, both are simultaneously turned and the air-current instantly deflected from one set of purifiers to the other.

Carbon-dioxid Absorbing System.—The most effective absorbent for carbon dioxid is slightly moist soda-lime. The soda-lime containers are wide-mouthed glass bottles. Each bottle contains 2 kilograms of soda lime, capable of absorbing not less than 75 grams of carbon dioxid, and weighs, when filled, about 4 kilograms. The dry air in passing through the moist soda-lime absorbs moisture, and it must, therefore, be dried again, which is done by passing it through the Williams bottle.

Either series of carbon-dioxid absorbers may be used as desired, for if the air-current has been passing through the first series for a given experimental period, the air can be instantly deflected through the other, by turning valves simultaneously. The combined increases in weight of the absorbers represent the amount of carbon dioxid absorbed. It is possible that the amount of water-vapor given up by absorbers to the dry air passing through it may be actually more than the amount of carbon dioxid absorbed, so that the bottle may lose in weight. On the

other hand, the water-vapor given up is immediately absorbed, and hence the algebraic sum of the weight of the two bottles gives the weight of the carbon dioxid absorbed. Usually both bottles are weighed on a balance at the same time.

The moisture in the soda-lime is essential to the efficiency of the absorbent. The amount of water absorbed by the air-current from the soda-lime and collected in the Williams bottle may be determined by weighing the Williams bottle separately. If the soda-lime is re-moistened with the same amount of water that has been lost, the absorbent may be considerably regenerated. It has been found practicable to add the water through a funnel inserted in the intake of the soda-lime bottle at the end of each day's experimentation. By the next morning the experiment can be carried on as usual.

Although moist soda-lime is a most efficient absorber of carbon dioxid, yet it has been thought desirable to test the completeness of absorption by the insertion of a small flask containing a solution of barium hydroxid, in such a manner that, by opening the pet-cock, a portion of the air from which the carbon dioxid has presumably been absorbed may be deflected through the barium hydroxid solution, where the slightest trace of carbon dioxid is indicated by a turbidity.

As the amount of carbon dioxid given off by the subject is determined by noting the increase in weight of the soda-lime vessels with its attendant Williams bottle, the degree of absolute moisture in the air when it enters the soda-lime bottle and leaves the Williams bottle should be identical. If, however, the sulphuric acid in the Williams bottle which follows the soda-lime container, is allowed to accumulate water to such an extent that its efficiency as a water-absorber is somewhat less than that of the Williams bottle, preceding the soda-lime container, it is obvious that there would be a loss of water from the system as a whole and the amount of carbon dioxid thus measured would actually be too small by the amount of water escaping absorption. Conversely, if the air is not so dry before it enters the soda-lime bottle as when it leaves the Williams bottle following, there will be an undue increase in the weight of the carbon-dioxid absorbing system, owing to the excess water absorbed. If the routine with the Williams and the soda-lime bottles is carried out as previously outlined, no difficulty is experienced, but it is advantageous occasionally to test the efficiency of the apparatus for absorbing carbon dioxid and water-vapor. Consequently in blank tests, i.e., with the apparatus in operation but with no subject inside, it is advisable to weigh the sulphuric-acid and soda-lime vessels separately, and continue passing the air through the system for half an hour. Under these conditions, the loss in weight of the soda-lime vessel should, of course, be exactly counterbalanced by the increase in weight of the accompanying Williams bottle.

Sodium-bicarbonate Can.—In order to absorb the unweighable traces of acid fumes which may remain in the air after it has been carried through the Williams bottles, it is necessary to insert in the air-circuit a small can filled with dry sodium bicarbonate and cotton batting. This

completely removes the acid fumes and does not affect the determination of the carbon dioxid or of the oxygen in any way. The sodium bicarbonate does not need frequent renewal, the amount used remaining efficient for approximately a year's experimenting.

Oxygen Supply.—The point at which the oxygen is introduced may, of course, be varied according to the conditions under which the apparatus is to be used. The direct determination of the amount of oxygen absorbed by the subject may be made either by introducing it from a small cylinder of the gas and noting the loss in weight during the experiment, or by passing the oxygen through an exceedingly delicate and accurate gas meter. These small cylinders weigh, when filled, about 3 kilograms, and contain about 150 grams of oxygen with a purity of about 98 per cent.

One of the greatest difficulties in using these cylinders has been the selection of a suitable valve, that furnished on the cylinder by the manufacturer being difficult to utilize, owing to the high pressure under which these cylinders are filled. Formerly recourse was had to one of the numerous types of reduction valves, but a thorough test of these showed no valve which would functionate properly for a long period. One or two types of needle valves have been found which are much less expensive and give a satisfactory closure. Such a needle valve is coupled to the exit of the cylinder, then closed, and the main valve on the cylinder is opened to its fullest extent. The issuing gas may then be very delicately regulated by means of the needle valve. With so high a pressure it is obvious that the packing around the main valve stem should be excellent so as to give no opportunity for leakage of air. The valves may be tested by immersing the cylinder and valve in water or by weighing the cylinder carefully on a balance, and then again an hour later; any loss of oxygen between the two weighings will be instantly apparent.

Extended experience in respiration experiments has shown that the respiratory exchange is absolutely unaffected by increased oxygen percentages and even by the respiration of pure oxygen, but if the oxygen percentage is lowered to 11 or 12 per cent., respiratory disturbances are apt to appear.

Gas Meter.—From many standpoints, the use of a small weighable cylinder of oxygen is to be recommended. On the other hand, there are certain advantages in using an accurately calibrated gas meter under such conditions as to preclude excessive temperature fluctuations. Benedict and his associates generally employed a large cylinder of oxygen with a needle valve, conducting the gas through a carefully calibrated meter of the type devised by Bohr. This meter registers 5 liters for each complete revolution of the drum and may be read directly to 50 c.c. Constructed of britannia metal, it may, without injury, be completely immersed in water in a large aquarium vessel and so leveled as to be easily read. The corrections for temperature changes are minimized by this immersion in water. It is not possible, of course, to control the barometric fluctuations and the meter readings should, therefore, be corrected not only for the average of the temperature fluctuations obtain-

ing throughout the experimental period, but also for the average changes in the barometer. For relatively short periods, this can best be done by using the temperature readings taken at the beginning and end of the period, and the barometer readings taken at the same time.

The meter is calibrated by the method of weighing the gas delivered from an oxygen cylinder. Many tests of this type of meter show that, when properly installed, it gives admirable results, and when a long series of experiments is contemplated, its use is strongly recommended. A small, weighable cylinder of oxygen is required in either method, since such a cylinder is necessary for the calibration of the gas meter.

The oxygen, leaving the cylinder, first passes through the small bottle of water, which is immersed in the tank containing the gas meter. This serves to saturate the oxygen with water, for in its compressed state it is extremely dry.

Spirometer or Tension-Equalizer.—Although an absolute temperature control is theoretically possible with this apparatus, thus securing a constancy in the apparent volume of the air in the closed system, it is practically impossible to prevent slight temperature fluctuations, and these, together with the unavoidable and uncontrollable fluctuations in the barometric pressure, indicate the necessity for some form of tension-equalizer which will insure atmospheric pressure in the chamber. For this purpose a small spirometer is used. When a mouthpiece or nose-pieces are employed with the universal respiration apparatus instead of the chamber, the entire air-current passes through the spirometer, and sundry devices are attached to it for tracing graphically the volume of each respiration and for indicating the total ventilation of the lungs. When the respiration chamber is used, however, it is not necessary for the air-current to pass through the spirometer; consequently, the three-way valve is closed to the spirometer and direct connection is made between the spirometer and the respiration chamber, by means of a pipe.

As commonly used on the universal respiration apparatus, the spirometer has a content of about 2½ liters. During the development of the clinical chamber a considerably larger spirometer was employed which held approximately 5½ liters, but more recent testing of the apparatus has shown that the standard size is amply sufficient for the purpose. With a small spirometer there is always a possibility that a patient may suddenly make a violent or extended muscular movement inside the chamber, such as in turning over, throwing off the bed clothes, or unduly moving the arms or legs, which would produce an expansion of air that would lift the bell of the spirometer above its normal limits and out of the water, thus causing a leakage of air. The danger of such an accident may, of course, be avoided by the use of a larger spirometer, but only rarely would even a large subject make such extraneous muscular movements as would produce this effect. Two practical methods may be employed for preventing it, even with a small spirometer. One is to place a weight of 80 grams on the bell of the spirometer when it is rapidly rising, thus immediately lowering it; the weights can be removed when the conditions inside the chamber have again become normal. An-

other expedient is to place a bar across the top of the spirometer guide supports in such a manner as to prevent the bell from rising high enough for air to escape. With the use of such devices when necessary, we feel confident that a spirometer with the ordinary volume of 3½ liters is sufficient for use with the clinical chamber. Furthermore, experimental periods in which the muscular activity was sufficient to expand the air unduly would be of little, if any, value. With the use of the small spirometer, however, the operator should take care that no air is lost through an undue elevation of the spirometer bell or the admission of an unnecessarily large amount of oxygen.

The exact height of the bell should be recorded at the beginning and end of each experimental period by noting the position of a pointer attached to the counterpoise and traveling over a vertical millimeter scale. The spirometer bell is delicately counterpoised so as to give zero pressure at approximately a middle point of the scale. There is no particular compensation device used in connection with this spirometer to allow for the variations of the metal displaced as the bell enters or leaves the water; consequently there are—theoretically at least—slight alterations in the tension with the different positions, so that it is advantageous to have the bell in nearly the same position at the beginning and end of each experimental period. The oxygen supply is then shut off and the bell gradually sinks. It is highly desirable that at the end of each period the bell should always be sinking, and thus in part compensate for the slight alteration in tension. More recently we have found it advantageous to move the counterpoise rod up or down by hand at the exact end of the period, until the very delicate petroleum manometer indicates that there is no pressure. At this point the reading is taken.

Manometer.—The small oxygen consumption and the large volume of the respiration chamber with its accessory parts make the influence of slight changes in temperature and pressure of great moment in measuring the total oxygen consumption. Consequently it is essential to note the exact pressure inside the chamber. This is assumed to be atmospheric, but it is possible that the spirometer does not respond instantly to slight changes in pressure; accordingly it is more efficacious to use a very delicate manometer. This manometer is of the type employed by Pettersson and Sonden in their gas-analysis apparatus and indicates the slightest alteration in atmospheric pressure. It consists of a glass tube bent in the form of an arc and containing a drop of petroleum oil.

Balances.—The carbon-dioxid elimination is determined with this apparatus, not by means of gas analyses, as is customary, but by accurately weighing the soda-lime bottle and its accompanying Williams bottle. These two absorbers have a combined weight of not far from 5 kilograms, and since approximately 10 to 15 grams of carbon dioxid may be produced in a half-hour period, it is necessary to weigh these two vessels to within 0.05 gram.* The balance, covered with a glass case

* The specifications for the Sauter Precision Balance are as follows: Patent Precision Balance Nos. 7, 11, 10 kilos., with aluminum beam and iron support, black enameled. The sensitivity with full load is usually 0.01 gram, which is much better than the manufacturers claim.

to protect it from drafts of air, is placed in a suitable location in the laboratory and used for weighing both the soda-lime bottles and Williams bottles, also the small oxygen cylinders required when calibrating the gas meters.

Barometer.—One of the most important factors in the accurate measurement of the oxygen consumption is a knowledge of the temperature and of the barometric conditions obtaining inside the large respiration chamber at the end of the experimental period. From a consideration of the volume of the chamber, amounting to about 550 liters, it will be seen that a change of 0.1 millimeter in pressure corresponds to a variation in volume which, as will appear later, affects the measurements of the oxygen consumption by about 72 c.c. Accordingly we recommend the use of the highest grade standard barometers, with special illumination and lens attachment to read to 0.05 mm. Indeed, it can be said that one perequisite of the successful use of the respiration chamber is a barometer with an exceedingly high degree of accuracy. The necessity for an accurate barometer and the significance of the barometric measurements become greater, the shorter the experimental period. If one is dealing solely with periods of two or more hours, an ordinary barometer, reading to 0.1 or 0.2 mm., would be sufficiently exact for the purpose, but when one attempts to make the experimental periods as short as one-half hour, the most exact barometric measurements are necessary.

Connections of the Apparatus with the Respiration Chamber.—The universal respiration was primarily designed for the study of the respiratory exchange by using nosepieces or a mouthpiece. When the three-way valve is turned and the coupling is connected with the pipe at a point in the line, the universal respiration apparatus is ready for accurate tests with nose or mouth-breathing. All that remains is to turn the three-way valve at the proper moment so as to connect the mouth of the subject with the air-pipe. With the clinical respiration chamber, however, the apparatus is used in an entirely different manner. The main ventilating current of air, instead of passing continuously through the spirometer, is so deflected by a valve as to pass directly from the chamber to the rotary blower without passing through the spirometer. Furthermore, the returning air, instead of passing along to the spirometer, goes directly to the chamber. With this form of connection, therefore, the pipe serves simply as a lead from the chamber directly to the spirometer, which is no longer a part of the ventilation circuit, but has become a true expansion chamber connected with the large respiration chamber.

Respiration Chamber.—The respiration chamber is the new part of the clinical respiration apparatus, although here again this is, in a certain sense, but an elaboration of the smaller chambers used for small animals or, more especially, a modified form of the chamber of the bed calorimeter From the clinical standpoint the chamber is the most important feature of the apparatus.

In designing the respiration chamber an effort was made to secure

the perfect comfort of the subject, suitable illumination, absence of psychical unpleasantness or suggestion of confinement and, at the same time, as small a volume of extraneous air as is consistent with these prerequisites. It was necessary that it should be so arranged as to be quickly opened and closed, and that it should be capable of ample ventilation, not only as to the renewal of the air, but as to the movement of the air inside the chamber. Finally, it should be so constructed as to permit quick temperature control.

To meet these conditions a chamber was constructed of sheet copper, 0.5 mm. thick, in two parts—a base consisting of the bottom of the chamber together with a deep water seal, and the cover of the apparatus. The inside of the chamber is 200 cm. long and 65 cm. wide. The cover is curved and so constructed that it rests in the water seal; the closure between the cover and the base is therefore air-tight.* The highest point of the cover is 52 cm. from the bottom and the radius of curvature of the top is 34 cm. The pipe which leads directly to the spirometer or tension-equalizer, and provides for the sudden expansion or contraction of the air inside the chamber, enters through the cover near the ventilating fan.

The base is substantially mounted on wooden supports. Through the bottom are conducted the pipes for the ventilating air-current, including the intake pipe and the outgo pipe. Three small tubes also project through the bottom. These may be used for connection with the stethoscope, the pneumograph, or for any other purpose.

There are, in addition, several openings in the cover of the apparatus. A window of plate glass is placed in a recess of the cover and made thoroughly air-tight with physicist's wax. This may be easily tested at any time by pouring 1 c.c. of water over it. Provision is made for the insertion of the thermometers and psychrometer and for the insulated connections for the electric fan used inside the chamber. In the top of the cover is a small water-sealed opening, which may be used to pass in or take out small objects, such as a clinical thermometer, a glass of water, urine bottles, or similar articles. This small opening, or hand-hole, is of much practical benefit, also in removing the cover from the apparatus, for if this hand-hole is not opened, it is practically impossible to lift the cover of the apparatus out of the water seal, owing to the atmospheric pressure. By simply removing the cover of the hand-hole the entire cover of the apparatus may be lifted off in a few seconds without disturbing either the thermometers or the electrical connections. Experience has shown that it is possible to converse freely with the occupant of the chamber without a telephone, and the large window immedi-

* As the water seal has considerable depth, the apparatus may be adjusted for large subjects by lifting the cover at either end, or as a whole, by means of the pulley arrangement referred to later in giving the routine of an experiment, or by supports placed in the water seal. In thus increasing the volume of the chamber it is necessary only to make sure that the depth of water over the lower edge of the cover is sufficient for the complete exclusion of air. This will be found especially convenient in cases of obesity or for heart cases. Usually it will be sufficient to lift only the head end of the cover. The increase in the apparent volume of the chamber is readily corrected by a simple calculation.

ately above the subject gives an appearance of light and freedom, with absence of psychical disturbance, which is very much to be desired.

With this hermetically sealed chamber it is obviously necessary to provide for a ventilating air-current and oxygen supply, unless the principle of Kaufmann[26] is employed and the carbon dioxid is allowed to accumulate. This respiration chamber lends itself admirably to tests by the latter method, if they are desired, as the spirometer which is directly connected with the chamber allows for an expansion of the air without loss of carbon dioxid or a diminution of air. Such an experiment is, of course, limited by the increment of the carbon-dioxid percentage. In actual practice, however, we invariably ventilate the chamber by means of the universal respiration apparatus already described. Final adjustment of the pressure inside the chamber is obtained by means of the petroleum manometer.

Circulation of Air Inside the Chamber.—To provide a gentle movement of the air, which shall aid in the temperature control, the air in the chamber should be allowed to mix thoroughly, and comfortable conditions for the subject should be maintained. A rotary air impeller, of a standard type, should be installed inside the cover of the chamber. The discharge from the blower is directed toward the curvature of the top in such a way as to blow directly on the wet bulb thermometer and provide for the maximum air movement and equalization of both temperature and composition of air. It is necessary to operate this blower a half-hour before the experiment begins so that it may be thoroughly heated to its ordinary running temperature.

Temperature Measurements and Control.—An accurate and rapid temperature control for this chamber is of fundamental importance, even more important, in a sense, than the comfort of the subject; for to obtain an exact measurement of the oxygen consumption, the difference between the average temperature of the air inside the chamber at the beginning and that at the end of an experimental period must be accurately known. Theoretically an electrical resistance thermometer is to be preferred for obtaining these temperatures. As it was undesirable to complicate the apparatus further by the addition of an electric equipment of this type, four good mercury thermometers, graduated in 0.1° C., have been used and found to serve the purpose admirably. These are placed at different points in the chamber. The average readings of these four thermometers give a most satisfactory measurement of the average temperature of the air in the whole chamber, particularly when the subject has been in muscular repose for at least ten minutes prior to the final reading.

To control the temperature normally, the temperature environment of the room is held at such a point as to allow for a rate of normal heat radiation which will keep the temperature of the air inside the chamber not far from 22° to 23° C. In winter this is readily accomplished by a simple adjustment of the windows in the room. In summer it is more difficult, but for the greater part of the year it has been found practical to reduce the heat when necessary by placing a

piece of moist cheesecloth over a portion of the top and directing the current of an ordinary electric fan over the surface. In all but the hottest days of the summer this method has proved most satisfactory. In any event, the rapid movement of a current of air over the outside surface of the chamber, either with or without the use of the damp cheesecloth, assists greatly in the temperature regulation. As will be seen later, however, the distribution and sensitivity of the thermometers are such as to compensate for considerable temperature change without affecting the measurements.

Method of Recording the Pulse-rate.—An accurate record of the pulse-rate is of fundamental importance in all metabolism experiments. The usual method employed for such measurements, and the method which is used in the experiments with the clinical respiration apparatus, is for an observer to listen to the heart-beat through a Bowles stethoscope attached to the chest of the subject. Connection with the ear piece used by the observer is easily made without change in the air-content of the chamber, by means of one of the small tubes passing through the bottom of the apparatus.

Methods of Testing the Apparatus.—The universal respiration apparatus and chamber just described, although extremely simple in principle, nevertheless has certain complexities. For experiments with patients, therefore, it is necessary to test completely the feasibility of the apparatus for measuring or indicating the several factors. Tests for the efficiency of the absorbing vessels have already been described in connection with the detailed description of the universal respiration apparatus. Since this method of measuring the respiratory exchange involves a closed ventilating air circuit and depends upon the measurement of the amount of oxygen introduced, any leakage of air either into or out of the chamber would vitiate the whole experiment. It is, therefore, of prime importance to know that the apparatus is absolutely air-tight so that when the cover is properly in place, no air can enter or leave the circulating air-current. Fortunately such tests are very readily made with this type of apparatus.

Tests for Tightness.—It will be seen that the entire ventilation current is in a closed circuit, the tension-equalizer or spirometer allowing the volume to expand or contract according to the variations in temperature, pressure, or actual volume of air inside the system. By reading the millimeter scale over which the pointer on the spirometer bell passes, variations in the apparent volume of the air in the chamber may easily be noted.

To test the tightness of the apparatus, the various parts are connected as in an experiment with a subject, and the ventilating air-current started. After the first few minutes, during which the air throughout the whole system will be attaining equilibrium, the bell on the spirometer should reach a constant level, and thereafter the volume of air should remain absolutely constant unless affected by changes in temperature or atmospheric pressure, these being indicated by the readings of the barometer and the several air thermometers. If the changes

in the position of the spirometer bell cannot be accounted for by temperature or barometric changes, there is obviously a leakage of air into or out of the system, usually the latter. This may be found by testing the universal respiration apparatus and the chamber separately.

To test the efficiency of the universal respiration apparatus, especially when trying to locate a leakage of air, a water manometer, consisting of two glass tubes connected at the bottom by a short bit of rubber tubing and attached to a suitable standard, is found advantageous. With this device the slightest leak in any individual portion of the apparatus can readily be detected by applying pressure with a bicycle pump. When the respiration apparatus has been properly installed, with accurately fitting rubber gaskets and connections, and suitable inspection is given from time to time, there is no occasion for leakage, and such an occurrence can invariably be ascribed to faulty technic.

To find whether or not the defect is in the chamber, the two ventilating pipes may be shut off, leaving the chamber connected only with the spirometer. Under ideal conditions, with no temperature or barometric changes, the volume of the spirometer will remain constant for an indefinite length of time. To hasten the detection of a leak, one may place approximately 80 grams in weights upon the top of the spirometer bell and thus produce a slight pressure in the chamber. With the semicircular window used in the earlier development of the chamber, leaks were occasionally found between the wax and the sheet of celluloid used for closure, but none were detected at any other point in the cover. In the first base constructed, a leak was found by pouring gasoline into the base and noting where it escaped. The openings were so small that they could not be detected by the use of water, as it would not pass through them. Under ordinary conditions, if properly constructed, the chamber remains absolutely tight indefinitely, but it is desirable to inspect occasionally the wax closure of the window.

In tests for leakage, temperature and barometric changes are invariably to be expected, and hence it is necessary to compute the volume of the chamber and make corrections for these changes. The volume of the chamber may be found by simple computations based upon the dimensions of the chamber or by the chemical method of introducing a weighed amount of carbon dioxid from a small bottle of the liquefied gas and then computing the volume of the chamber from an analysis of the gas. For all practical purposes the method of computing the volume from the dimensions of the chamber is sufficiently accurate.

In testing the respiration chamber, weights have been placed not only upon the top of the spirometer bell, but likewise on the counterpoise of the spirometer, so as to produce a negative pressure; indeed, tests have been made with weights in both positions. Even when there is an increased pressure inside the chamber there is no interchange of air between the enclosed volume in the chamber and the outside air, thus showing that, when properly constructed and tested, the apparatus is independent of external atmospheric conditions.

In actual experimenting, the spirometer is connected in series with

the chamber and delicately counterpoised, so that the air in the chamber is practically under atmospheric pressure. Indeed, measurable differences between the pressure in the chamber and the atmospheric pressure are not detected save by the extremely delicate petroleum manometer.

The significance of a leak should be especially emphasized, for if the volume is reduced by the loss of air out of the system, oxygen is added to take the place of the air thus lost. As the oxygen introduced is measured by the meter and is considered as having been consumed by the subject, it will be seen that such loss of air vitiates the accurate measurement of the oxygen consumption. Conversely, if air leaks into the system the amount of oxygen added and measured is too small. So far as the respiration chamber itself is concerned one may confidently state that, with proper construction, neither of these disadvantages may be expected.

Control Tests.—Having tested separately the universal respiration apparatus and the respiration chamber, and having found them tight, it is necessary to demonstrate further the efficiency of the apparatus for measuring the respiratory exchange, and particularly the respiratory quotient, by making a series of control tests. The control tests used for the clinical respiration apparatus are, in the main, of two types, either chemical tests in which definite quantities of alcohol or ether are burned inside the chamber, or physiological tests with animals either fasting or after surfeit feeding.

Alcohol Check Tests.—The use of ethyl alcohol of known composition for testing the accuracy of respiration chambers is of long standing. The alcohol test may be used in two ways: When the respiratory quotient is alone of interest, alcohol may be burned in the chamber, and from the ratio between the carbon dioxid produced and the oxygen consumed, the accuracy of the apparatus for studying the respiratory quotient is determined. Thus the theoretical respiratory quotient of alcohol is 0.667. In other words, for every liter of oxygen absorbed in the combustion of alcohol there should be produced 667 c.c. of carbon dioxid. In this type of test it is unnecessary to note the weight of alcohol burned, as only the ratio between the oxygen and the carbon dioxid is desired. This test is made very rapidly, and if there is a leak in the chamber or an error in the oxygen introduction or in the absorption of carbon dioxid, the respiratory quotient of the alcohol will be distinctly affected.

The second type of alcohol test includes the measurement of the amount of alcohol introduced. From the weight of alcohol used, together with its specific gravity and percentage composition, the theoretical value for the carbon dioxid produced and the oxygen consumed may then be computed, and these values in turn compared with those actually found by measurement with the respiration chamber.

Determination of the Carbon-dioxid Production.—The determination of the carbon-dioxid production by this apparatus is theoretically extremely simple, since it is based solely upon weighing the carbon-dioxid-absorbing vessels before and after the experimental period. That

the entire carbon-dioxid production of a man is thus found rests upon two assumptions: first, that the change in weight of the bottles is due solely to the carbon dioxid given off; and second, that the quantities of carbon dioxid remaining in the air inside the chamber are the same at the beginning and end of each period.

In considering the first assumption we should emphasize the fact that the technic must be such as to make sure that the air is dried to the same degree before it enters the carbon-dioxid absorbers as it is when it leaves the following Williams bottle or water-absorber. Extensive experience in the laboratory work in the past has shown that errors of this nature are absent, with proper attention to the amount of water allowed to accumulate in the Williams bottle.

With regard to the second assumption, namely, the constancy in the amount of carbon dioxid remaining in the air in the chamber, it will be seen that this depends in large measure upon the relationship between the carbon-dioxid production and the rate of ventilation of the chamber. With a normal ventilation of 35 to 45 liters per minute and with a regular carbon-dioxid production, repeated tests, based upon accurate analyses made in connection with control tests of this apparatus as well as of the bed calorimeter, show that the variations in the residual carbon-dioxid in the chamber are practically negligible. If, on the other hand, the subject is more or less restless and there are material differences in the amount of carbon dioxid produced from hour to hour, residual analyses are essential. Complicating the experimental procedure by residual analyses is, however, to be avoided, if possible.

In a large majority of the experiments, the subject is in complete muscular repose, and comparison experiments should be made only under these conditions. With the subject in complete muscular repose, there is but little variation in the metabolism from hour to hour, and the assumption that the residual carbon dioxid remains constant holds true. Accordingly, if there are changes in the residual carbon-dioxid, these are occasioned by such muscular activity as would exclude completely the use of the results for any practical purpose.

It is perhaps especially fortunate, however, that so far as the determination of the respiratory quotient is concerned, material variations in residual carbon-dioxid are essentially without effect. If, for example, there is an increment of one-half liter of carbon dioxid inside the chamber during the experimental period, obviously the absorption of carbon dioxid in the soda-lime bottles will be one-half liter too small. On the other hand, the space occupied by the additional carbon dioxid in the chamber would normally have been filled by the admission of one-half liter of oxygen, and hence there is a similar deficit of one-half liter in the oxygen measured, the two errors essentially compensating. For all practical purposes, therefore, the measurements of the residual carbon dioxid may be entirely neglected without sacrificing in any way the accuracy in determining the respiratory quotient, even if complete muscular repose is not secured.

Determination of the Oxygen Consumption.—While the determination

of the carbon-dioxid production is very simple in theory and reasonably simple in practice, the determination of the oxygen consumption requires a much more complex procedure. Theoretically, nothing is simpler than measuring the oxygen admitted to the chamber as the subject lies breathing normally, but practically the criterion of the amount of oxygen admitted is the position at which the spirometer bell remains. Thus at the end of an experimental period the aim is to have the same apparent volume inside the chamber as at the beginning of the period, but even though the apparent volume remains constant, there are several factors which influence this volume. Those of temperature and pressure are obvious, but correction can readily be made for them from the temperature readings and accurate barometer readings provided for. It has already been pointed out in the preceding section that any changes in the carbon-dioxid residual in the chamber reduces the oxygen admitted, but it is important to note that even this substitution of carbon dioxid for oxygen is without material effect upon the respiratory quotient. We have also to consider the possible variations in the measurement of the tension of aqueous vapor inside the chamber.

Measurement of the Tension of Aqueous Vapor.—The volume occupied by the water-vapor inside the chamber may fluctuate with the changes in temperature and, indeed, with any changes in the amount of water vaporized from the lungs and skin of the subject, even during repose. These variations are not best expressed in cubic centimeters of water-vapor inside the chamber, but, for the purposes of computation, they are more advantageously designated as tension of aqueous vapor in millimeters of mercury, the amount of this tension being deducted from the observed readings of the barometer. Instead of determining the tension of aqueous vapor by the gravimetric method, i.e., by absorbing water vapor in U-tubes containing sulphuric acid, we depend upon an accurate psychrometer comprised of wet and dry bulb thermometers, graduated in 0.1 degrees C. and capable of being read with a lens to 0.01 degree C. By means of the small blower suspended on the inner side of the cover of the chamber, the air is blown with considerable velocity directly over the bulbs of the thermometers. The degree of depression of the temperature of the wet bulb over that of the dry bulb is carefully noted and then, from standard psychrometric tables, the tension of aqueous vapor is computed.

The validity of this method of measuring the water-vapor was tested in several ways: first by introducing a second wet and dry bulb psychrometer in the main ventilating air-current as it left the chamber; and finally by removing definite volumes of air and passing them over U-tubes containing sulphuric acid and pumice stone. While it was obviously impossible to secure absolutely the same degree of humidity at each point of measurement, nevertheless, when fluctuations occurred, the curves were all parallel and showed that the psychrometer, as installed, gave accurate results. Before each experiment the wet-bulb thermometer was removed and thoroughly drenched with

distilled water with which its reservoir was filled. Under these conditions most satisfactory results were obtained.

Temperature Measurements.—While theoretically an electrical resistance thermometer with a series of coils distributed in various parts of the cover would be ideal, tests made with a large number of accurate thermometers placed at different points in the chamber showed that average temperature conditions could be obtained by reading the temperature recorded by the thermometers. Even with the subject lying inside the chamber, when the small blower on the interior wall was running at a moderate speed, the temperature conditions throughout the chamber, as indicated by the thermometers suspended at different points, seemed to be constant. We have in one case the warm body surface of the subject, which has a temperature of approximately 32° C., and in the other the copper cover, which is in immediate contact with the room air and has a temperature of not far from 20° C. Since, in many instances, local cooling on the surface of the chamber is necessary, this extraneous temperature may be as low as 18° or even 17° C. In spite of these measurable differences in temperature at different points of the apparatus, all of the thermometer readings showed essentially the same curves for temperature fluctuations and proved that the average temperature changes in the chamber from the beginning to the end of the period were accurately given with the four thermometers noted; supplementary thermometers did not in any way alter the values of these differences.

From a rough calculation it can be seen that since the chamber has a capacity of approximately 550 liters, a difference of 1° C. would correspond to an increase in the volume of about 2 liters. As the temperatures are read and the differences computed to 0.01° C., we may therefore assume that the average error for temperature variations is probably not far from 20 to 30 c.c. of air. This, of course, applies directly to the measurement of the oxygen consumed, and is a recognized error in the measurement of this factor. On the other hand, when an experimental period continues for 30 minutes, which is at present the minimum length, this probable error amounts to but 1 c.c. per minute, or about 0.5 per cent. of the total oxygen consumption.

Measurement of Barometric Pressure.—By the same method of computation as that outlined in the preceding paragraph, it can be seen that each millimeter change in the barometer corresponds to approximately 700 c.c of air. Accordingly barometric measurements should be made with the very greatest care, preferably to 0.05 millimeter, this corresponding to an error of approximately ± 35 c.c. for each individual period. Obviously the temperature of the barometer with the correction, therefore, is recorded each time, and from the barometer readings is deducted the value for the tension of aqueous vapor obtained from the readings of the psychrometer as computed from the psychrometric tables.

Summary of Errors in the Measurement of the Oxygen Consumption.—The measurement of the oxygen consumption is, therefore, sub-

ject to the following errors: errors due to the variations in the residual amounts of carbon dioxid in the chamber at the end of the period; errors due to changes in the temperature and the barometer; and errors in the determination of the tension of aqueous vapor.

As we have already seen, the errors due to variations in the residual amounts of carbon dioxid in the chamber are negligible and play no rôle in the computation of the respiratory quotient, our main objective. The barometer can be read to 0.05 mm.; taking into account the volume of the chamber (550 liters), this error of reading will correspond to a possible error of $\pm$ 35 c.c. in the measurement of the oxygen. The thermometers are read and recorded to 0.01° C. as the average of the several thermometers, the possible variation corresponding to a like variation in the oxygen measurement of $\pm$ 20 to $\pm$ 30 c.c. Finally the psychrometer is also read to 0.01° C., and with the best psychrometric tables we are assured of an accuracy of but 0.05 mm., giving us still another possible error of $\pm$ 35 c.c. Should a plus error occur in all three factors, namely, the readings of the barometer, thermometers, and psychrometer, a maximum error of not far from 100 c.c. in the measurement of the oxygen consumption for the period would be conceivable. It is obvious that in actual practice there will be the usual compensation.

It will, therefore, be seen that the longer the experimental period and the greater the amount of oxygen under consideration, the less will be the influence of these errors upon the measurement of the oxygen consumption. And yet, as has been frequently demonstrated with a subject in complete muscular repose, remarkably regular metabolism measurements—including not only the measurement of the carbon-dioxid production and the oxygen consumption, but likewise the determination of the respiratory quotient—may be obtained in one-half hour periods. We may fairly say, therefore, that with a quiet subject results obtained in half-hour periods are perfectly reliable.

Routine of an Experiment.—The subject should be lying upon the bed of the respiration chamber not less than ten minutes before the cover is put in place. This is especially necessary if the subject has been unusually active or has walked about considerably prior to the experiment. The stethoscope should be attached and records of the pulse-rate begun immediately, as the best index of the quiet condition of the subject is the pulse-rate, which should have practically reached a level before the experiment is begun. The ideal condition for a subject is complete relaxation and muscular repose, with a minimum, regular pulse-rate. The subject may lie either on his back or his side as, in this type of apparatus, a fixed position of the head is not necessary. Inasmuch as there is always a slight warming of the ventilating blower inside the chamber, due to the passage of the electric current through it, the blower should be started as soon as preparations are begun for the experiment, as the longer the blower runs before the experiment begins, the better will be the results obtained. The subject should be fully dressed, including stockings and shoes; in addi-

tion, a pair of woolen socks should be drawn over the shoes, for it has been the experience of most subjects that if any discomfort is felt it is from a sensation of coolness about the feet, ankles, and the lower part of the legs. This is due to the fact that the air inside the chamber is always moderately dry, i.e., with a relative humidity of about 60 per cent., and is kept in rapid circulation by the fan blower. The sensitivity of the movable bed may be determined either by pressure with the hand or, more accurately, by dropping a known weight from a certain height upon the bed and noting the amplitude of the marking upon the kymograph record. After the soda-lime bottles and the Williams bottles have been examined to make sure that they have not become exhausted or will not become exhausted before the experiment is over, the cover is lowered with the hand-hole open. It has been found practical to suspend the cover with two cords running through pulleys and to counterpoise it in part, to assist in lowering it into place. When the cover is in position, the counterpoise weight is removed so that the cover rests solidly in the water seal on the base of the chamber; the cover of the hand-hole is then put into place.

As it requires some time for the atmospheric conditions inside the chamber to become constant, it has been found advantageous to delay starting the ventilation throughout the air-circuit for approximately 15 minutes. During this time the carbon dioxid accumulates inside the chamber until it amounts to approximately 0.3 to 0.4 per cent. of the volume of air. The moisture likewise accumulates to some extent. At the end of 15 minutes the rotary blower is set in motion and the air-current begins to circulate. The several thermometers and the psychrometer are then read. The temperature of the room should be adjusted by opening the doors or windows, so as to establish temperature equilibrium as soon as possible, for with a quiet, resting person the difference between the temperature of the room and that in the chamber is approximately 3° C. As soon as temperature equilibrium has been obtained—and this is usually inside of 15 or 20 minutes after the ventilation has been started—temperature readings are taken, a simultaneous reading of the barometer is made, and the position of the spirometer bell is accurately recorded. The valves are then turned.

While the spirometer is so counterpoised as to give 0 in one position, it is not an exact counterpoise, and slight differences occur. It is desirable, therefore, for the operator, while reading the position of the spirometer bell, to hold the counterpoise of the spirometer lightly in the hand in such a manner that the petroleum in the manometer registers exactly 0 at the time that the spirometer is read. If this adjustment is made at the end of each period, the slight differences in the position of the spirometer bell are compensated. From the average temperature readings, the barometer readings and the record of the position of the spirometer bell, the apparent and real volume of the chamber can be computed, the weight of the subject being taken into consideration, and due allowance made for the displacement of air by the body of the subject.

During the entire time the motor is running, carbon dioxid is being removed from the chamber in both the preliminary period and the main experimental period. This removal of the carbon dioxid causes a diminution in the volume of air in the chamber. In the preliminary period this may be compensated by an increase in the temperature and the spirometer bell will thus remain in essentially the same position as at the beginning or, as is usual, oxygen is introduced to maintain the spirometer bell in a median position. It should be noted, however, that no measurement of the oxygen introduced during the preliminary period is necessary, as only the amount added during the actual experimental period to maintain the spirometer bell at or about its original position is of importance. The amount of air residual in the chamber at the end of each period, as determined by the measurements of the temperature, the barometer, the psychrometer and the actual height of the spirometer bell, is taken into consideration in the final computations. The manipulation of the universal respiration apparatus has been so frequently and fully discussed that it seems unnecessary to give further details here.

The length of the experimental periods may vary considerably. Theoretically the longer the experimental period, the more reliable will be the results, for all errors in the readings of the thermometers and the barometer are minimized when extended over a long period. Practically, however, it is very desirable, especially with subjects in the postabsorptive condition, i.e., without food, to shorten the experimental periods as much as possible. Our observers have proved repeatedly that with quiet subjects, especially when they are asleep, determinations with a high degree of accuracy can be made in one-half-hour periods. In a large number of cases, after a preliminary period of 35 to 40 minutes, it is possible to secure three successive one-half-hour periods with the greatest uniformity in results. If the subjects are restless—and particularly if the restlessness occurs toward the end of the experimental period—the temperature and moisture are materially affected, and the experimental periods must, in consequence, be extended. While it is always a question whether it is advisable to attempt any observations with patients as restless and conditions as disturbed as in such cases, yet as a rule patients, as well as normal individuals, almost always become drowsy during an experiment and are inclined to fall asleep. The air inside the chamber is constantly in motion, the atmosphere is cool and pleasant, there is no odor, and the psychical conditions are, in general, most beneficial and satisfactory. Benedict has recently described a new form of portable respiration apparatus for clinical use. Pearce has also described a clinical method for determining the respiratory exchange.[27]

6. The Acetonitril Test of Reid Hunt for Hyperthyroidism.—Reid Hunt[28] found that mice, when fed upon thyroid gland, developed an increased resistance to acetonitril or methyl cyanid (CH_3CN). This substance is toxic, chiefly on account of slowly liberating hydrocyanic acid in the body. Hunt found that this reaction was specific

for thyroid and more delicate than any chemical test, and on this basis suggested this increased resistance to acetonitril as a delicate test for thyroid substance and as a means of determining whether there is an increased amount of thyroid secretion in the blood in cases of hyperthyroidism.

He applied the test to three cases of hyperthyroidism. The blood in one of these cases had a marked effect in increasing the resistance of mice to acetonitril, indicating an excess of thyroid secretion. The second case was doubtful, and the third negative.

The test is carried out by giving to mice 1 or 2 c.c. of blood made up with meal in the form of cakes, for 9 or 10 days before testing with acetonitril, using controls. One-fourth mg. of acetonitril per gram of body weight of mouse may be fatal to a normal animal in a few hours.

Hunt's findings have been corroborated by Trendelenburg[29] and Ghedeni.[30] Trendelenburg and also Lussky[31] claim that the blood of cats gives the test, even after removal of the thyroids.

This method has not been sufficiently tested as yet to be used clinically. A complicating factor is the variations in the natural resistance of animals and the possible variations in the amount of thyroid in the blood under normal conditions.

7. The Epinephrin Mydriasis Test of Loewi for Hyperthyroidism.—Loewi found in pancreatectomized animals, in human diabetes, and in cases of hyperthyroidism that the instillation of 1:1,000 solution of epinephrin produced a dilatation of the pupil. He proposed this procedure as a test for hyperthyroidism, and claimed that the internal secretion of the thyroid and suprarenal are synergistic, both acting by stimulating the sympathetic nervous system; therefore, in cases of hyperthyroidism the sympathetic system is in a state of increased irritability and the dilator fibers of the iris governed by the sympathetic respond abnormally to the action of epinephrin. Falta and Zak have corroborated this finding. Eppinger, Falta, and Rudinger found an increased epinephrin mydriasis in dogs fed on thyroid, and Eppinger and Hess reported the test positive in cases of hyperthyroidism. De Laet has also studied this test.[32]

8. The Skin Reaction of Goetsch for Hyperthyroidism.[33]—In this test 8 minims of a 1:1,000 solution of epinephrin are diluted with an equal quantity of sterile water and injected hypodermically into the arm. There is formed at once an area of blanching around the point of injection, and about the margin of this usually a red areola gradually shading off into the surrounding tissue. In about half an hour the center of the white area becomes bluish-gray to lavender, and at the end of about a half hour to two hours the red areola takes on the bluish or lavender color, while that in the center disappears. This lavender areola remains for about four hours from the time of injection and is the most characteristic part of the test. Accompanying the local reaction there may be an increase in pulse-rate, with palpitation of the heart and an exaggeration of the tremor and nervous symptoms in general.

Tests for Hypothyroidism.—Therapeutic Test for Hypothyroidism.—The diagnosis of a state of hypothyroidism is easy in typical cases, but the latent cases present a more difficult problem. The therapeutic test at present is the only one available and consists in the administration of thyroid extract. It is best carried out by giving tablets of desiccated thyroid gland, containing 1½ to 5 grains of the gland. The dose is given three times a day, and gradually increased, care being taken not to produce tachycardia, sweating, diarrhea, or nervousness, which are signs of intolerance. If the case is one of hypothyroidism, the symptoms will disappear or show improvement in about two weeks.

Pituitary Body

Tests for Hyperpituitarism.—1. Diagnosis of Hyperpituitarism by Means of the Increased Gas Exchanges.—The respiratory exchanges in cases of acromegaly have been studied by Magnus-Levy, Salomon, Bernstein and Falta. Falta claims that the cases so far studied do not show a consistent increase in gas exchanges in acromegaly as is the case in hyperthyroidism. Magnus-Levy, Salomon and Falta all agree that if the hyperpituitarism is uncomplicated by disorder of other glands of internal secretion (as thyroid) there is no increase in the gas exchanges.

2. Demonstration of Spontaneous and Alimentary Glycosuria as a Test for Hyperpituitarism.—Marie, who first described that condition of hyperpituitarism called acromegaly, showed that it is often accompanied by temporary or permanent glycosuria. Borchard, in an analysis of 176 cases, found spontaneous glycosuria reported in 63 and alimentary glycosuria in 8. In Falta's 8 cases there was spontaneous or alimentary glycosuria in 5.

The test for provocative alimentary glycosuria is carried out as described under functional diagnosis of diseases of the liver, page 94. The glucose test has been most frequently used in testing the tolerance for carbohydrates in cases of suspected hyperpituitarism.

Tests for Hypopituitarism.—In the functional diagnosis of hypopituitarism the opposite condition in relation to gas exchanges and glycosuria obtains, as compared with hyperpituitarism. In hypopituitarism the same tests are applied as for hyperpituitarism. The results are the opposite, i.e., the gas exchanges will be diminished, and glycosuria—spontaneous and alimentary—will be negative.

Adrenal Glands

Tests for Hypo-adrenalism.—1. Sergent's White Adrenal Line as a Test for Adrenal Insufficiency.—Emil Sergent [34] has described this vasomotor phenomena as a test of adrenal insufficiency. He selects the skin of the abdomen, and traces on it a geometrical figure—a rectangle, triangle or cross—thus doing away with possible confusion

with lines caused by folds of the skin, etc. The rounded end of a fountain pen is a good instrument for tracing the figure. The figure should be made by a simple superficial stroking; one must not bear down or scratch the abdomen. The motion should be deliberate and not rapid. Sergent claims that a too early appearance of an outline is always a sign of clumsiness, as such treatment strikes and suppresses the vasomotors, thus interfering with the reaction.

When the tracing has been made, all movement on the part of the patient is prohibited. After half a minute a pale line or band begins to be noticed, following the course of the pen. Gradually this becomes more and more distinct and white, at the same time becoming larger, so that eventually the line exceeds in size the actual area touched by the pen. This white line attains its maximum clearness in the course of about one minute, and persists for one, two or even three minutes before being gradually obliterated. This constitutes the reaction in well-defined cases of adrenal insufficiency. Sergent finds the lighting plays an important part in the technic, as in bright daylight or sunlight, or even in bright electric light, it is sometimes difficult to see the white line. For this reason, after making the tracing, the sheet or clothing should be drawn in such a way as to cast a light shadow. On account of the influence of the presence of clothing and such, before making the test, the patient should be put at rest for at least 15 minutes with the abdomen free and but lightly covered.

Sergent considers his so-called "ligne blanche surrenale" as due to the hypotension brought about by the hypo-adrenia. It is known in arterial hypotension that there is present a peripheral vasodilatation; when a slight stimulation of the skin is produced, vasoconstriction replaces the vasodilatation, with the resulting white line.

Massalongo [35] has studied the reaction produced, not by scratching the skin with the nail, but by rubbing it lightly with the finger tip or some blunt rounded instrument. Out of 400 cases of various diseases he found the white line in an unequivocal form in 30 cases, and even then often in a transitory and irregular manner; and of these, 22 were cases of typhoid and paratyphoid fever, which constituted 120 of the whole number. His observations led him to the conclusion that diminution of the angiotonic function and cardiotonic power may exist without the presence of the white line and of other symptoms of suprarenal inadequacy, and that arterial hypotension, asthenia, and collapse are not the exclusive function of this inadequacy, since they occur in the course of acute infective diseases independently of it. He found the presence of the line always associated with arterial hypotension and frequently with other symptoms attributable to suprarenal derangement, which, on the other hand, may exist without its presence. Hence by its rarity, inconstancy, and transiency, by its presence or absence in equal proportion in cases where changes in the suprarenal capsules were indisputable, the white line can only be credited with a relative diagnostic importance and significance and cannot be considered as pathognomonic. It has, however, some value from a prognostic point of

view, since its maximum frequency is met with in cases of serious import, with almost invariably a fatal issue.

2. Test for Hypo-adrenal Function by Means of Increased Sugar Tolerance.—It was shown by Eppinger, Falta and Rudinger [36] that cases of Addison's disease (hypo-adrenalism) had an increased tolerance for sugar. Polak [37] found that 2 mg. doses of epinephrin did not produce glycosuria in a case of Addison's disease, while it did so in normal persons. On these findings this method for determining the presence of hypo-adrenalism has been developed.

The methods for determining the sugar tolerance may be found on page 94, in the section on Liver Function Tests.

Tests for Hyperadrenalism.—1. The Deviation of Complement as Applied to Diagnosis of Hyperadrenalism.—The complement-fixation test has been applied by Polito and Corelli [38] to the diagnosis of hyperfunction of the suprarenal glands, using an alcoholic extract of the suprarenal gland as an antigen. Their results were not conclusive.

2. The Ehrmann-Meltzer Reaction as a Test for Hyperadrenalism.—Meltzer and Auer,[39] Wessely,[40] and others found that when epinephrin is applied to the frog's eye dilatation of the pupil is produced. Ehrmann [41] suggested this reaction as a test for epinephrin. He found it acted upon the dilator fibers of the iris in a strength of 1 to 20,000,000. Recent investigations have shown that other substances than epinephrin, contained in blood-serum, will produce the same reaction. For this reason the reliability of this test has been questioned.

3. Tests for Hyperadrenalism by Means of Sugar Content of the Blood.—It is well known that a hyperglycemia is present in cases of hyperadrenalism. Therefore, the presence of an excess of sugar in the blood, provided that other causes of hyperglycemia can be excluded, is suggestive of hyperadrenalism. It is also known that in cases of hyperadrenalism that the subcutaneous injection of 1 or 2 milligrams of epinephrin is followed in one-half to two hours by a glycosuria lasting three hours and accompanied by a hyperglycemia.

During the past few years several methods have been published that are applicable for the clinical estimation of sugar in blood. We shall describe the Bang method, the Lewis-Benedict method, and the Epstein method.

(*a*) *Micromethod of Bang for Estimation of Sugar in Blood.—Principle.*—Two or three drops of blood are transferred to a small, weighed piece of blotting paper, and the paper again weighed to determine the amount of blood. The paper is then treated with boiling acidified KCl solution, which coagulates the protein and allows the sugar to diffuse out. The sugar solution thus obtained is boiled with alkaline cupric chlorid solution. The amount of cuprous chlorid formed by the reducing action of the sugar is determined by titration with standard iodin solution.

Procedure.—Small pieces of good absorbent paper, about 16 x 28

mm. in size,* weighing about 100 mg. and held by a small spring clip, are used. To one of these previously weighed ** pieces of paper transfer 2-3 drops (about 120 mg.) of blood obtained by piercing the cleansed finger. Weigh again immediately and determine by subtraction the weight of the blood taken.

Coagulation of Blood-protein.—Transfer the piece of paper to a test-tube and add 6.5 c.c. of boiling acid-potassium chlorid solution† and let stand half an hour. The clear solution containing the sugar is poured into a 50 c.c. Jena flask, the flange of which has been removed. Wash the paper and tube again with 6.5 c.c. of hot salt solution and transfer washings to the flask. Cool.

Reduction of Cupric Chlorid.—Attach to the mouth of the flask a piece of tight-fitting rubber tubing about 2 inches long, provided with a clamp which permits of shutting off the contents of the flask from the outside air. Now add to the flask 1 c.c. of the cupric chlorid solution.‡ Heat so that the solution is brought to a boil in 1 minute and 30 seconds (an error of 5 seconds may be disregarded). Allow to boil for exactly 2 minutes. At the end of this time tighten the clamp over the mouth of the flask; at the same time remove from the flame and cool at once under the tap for about a minute.

Titration of Cuprous Chlorid Formed.—The titration is made with N/200 iodin solution§ run in from a very accurate buret (preferably a 2 c.c. buret graduated in 1/50 c.c.). Two or three drops of starch solution (preferably soluble starch†† are added as an indicator. During the titration air must be excluded to prevent reoxidation. This is done by running a slow stream of carbon dioxid from a generating bottle through a small tube which extends nearly to the bottom of the flask. The titration should be carried out against a white background and the end point taken when the blue color persists for 20-30 seconds.

Calculation.—The copper and other solutions used in the test bind about 0.12 c.c. of the iodin solution. This amount must hence be subtracted from the reading. The corrected reading is then divided by 4 to obtain the number of milligrams of glucose in the sample.

* Suitable pieces of paper, weighed, ready for use, and with clip attached, are purchasable. Unless specially prepared, the paper should be repeatedly washed with large volumes of hot water, acidified with acetic acid to remove impurities.

** The weighing is preferably made on a special torsion microbalance which, as well as the other apparatus used in this method, may be obtained from either of the firms mentioned in the preceding footnote. The weighing must be done in a few seconds and with an accuracy of about 1 mg.

† Consisting of 1,360 c.c. of saturated KCl, to which is added 640 c.c. of water and 1.5 c.c. of 25 per cent HCl.

‡ Copper solution. Introduce into a 1,000 c.c. flask 700 c.c. of boiled and cooled water. Warm to about 30° C. and add 160 grams of pure potassium bicarbonate in powder form. When dissolved, add 66 grams of pure KCl. Cool and then add 100 grams potassium carbonate. Finally add 100 c.c. of 4.4 per cent. solution of pure crystalline copper sulphate. Let stand a short time, then bring to the mark with boiled water. Allow to stand a day or so before using.

§ N/200 I solution, made fresh each day. Dilute N/10 I solution 20 times, or make as follows: Introduce into a 100 c.c. flask 2 grams KI, 1-2 c.c. of 2 per cent. KIO_3 solution and 5 c.c. of N/10 HCl. Bring to the mark with boiled and cooled distilled water.

†† A 1 per cent. solution of Kahlbaum's soluble starch in a saturated KCl solution.

Example.—If 0.68 c.c. of N/200 I solution were required, $\frac{0.68-0.12}{4}=$ 0.14 mg. glucose in the amount of blood used. If 140 mg. of blood were taken for analysis the per cent. of glucose in the blood would be $\frac{1000}{140} \times 0.14$ mg. $= 0.1$ per cent. glucose.

The results obtained by this method are a little higher than those obtained by other reliable methods due to the presence of certain I-binding substances in blood. As these appear to be nearly constant in amount, a correction may be applied. To obtain true value for glucose of the blood, therefore, 0.015 per cent. should be subtracted from the value obtained as above: 0.1 per cent. — 0.015 per cent. = 0.085 per cent. glucose.

To secure accurate results the method of Bang must be rigidly controlled, all new solutions and absorbent papers being checked up against pure 0.2 per cent. glucose solutions. Taylor and Hulton [42] also suggest the following precautions: A blank check must be made on the reagents each day an estimation is made. 0.010-0.15 gram of blood should be taken and must spread smoothly on the paper. The proteins are best coagulated by heating the blood-impregnated papers in the hot air oven at 100° C. (as recommended by Gardner and McLean [43]) for 5 minutes with the corks of flasks inverted. The solution should be boiled 4 minutes for complete reduction. The iodin solution must be fresh daily and checked each day. Determinations should be made in triplicate. Results cannot be depended upon to be more accurate than to 0.005 gram glucose in 100 c.c. blood. Other authors have recommended that an hour instead of half an hour be allowed for the diffusion of the blood sugar, the fluid being brought to the boiling point twice during this period or kept in a bath at 40° C.

(*b*) *Lewis-Benedict Method* [44] *of Estimation of Sugar in the Blood.*—*Principle.*—The red color obtained by heating a glucose solution with picric acid and sodium carbonate is employed as the basis of the colorimetric determination. The blood protein is removed by precipitation with picric acid.

Procedure.—Two c.c. of blood are aspirated through a hypodermic needle * and a piece of rubber tubing into an Ostwald pipet, a little powdered potassium oxalate in the tip of the pipet preventing clotting. The blood is drawn up a little above the mark and the end of the pipet is closed with the finger. After the rubber tubing and needle are disconnected, the blood is allowed to flow back to the mark and is discharged at once into a 25 c.c. volumetric flask containing 5 c.c. of water. The contents of the flask are shaken to insure thorough mixing and the consequent hemolysis of the blood. Then 15 c.c. of saturated aqueous solution of picric acid are added, as well as a drop or two of alcohol to dispel any foam, and the contents of the flask are brought up to the

* It may be more convenient to draw about 5 c.c. of blood directly into a test-tube containing a little finely powdered potassium oxalate and to remove 2 c.c. portions of this with the Ostwald pipet.

mark with water and then shaken. After filtration, 8 c.c. aliquots are measured out into large Jena test-tubes for duplicate determinations. Two c.c. of saturated picric acid and exactly 1 c.c. of 10 per cent. sodium carbonate are added (as well as two glass beads and 2 or 3 drops of mineral oil), and the contents of the tube are evaporated rapidly over a direct flame until precipitation occurs. About 3 c.c. of water are added; the tube is again heated to boiling to dissolve the precipitate; the contents of the tube are transferred quantitatively to a 10 c.c. volumetric flask,* cooled, brought up to the mark, shaken, and then filtered through cotton into the chamber of a Dubosecq colorimeter. The color is compared at once with that obtained from 0.64 mg. of glucose, 5 c.c. of saturated picric acid, and 1 c.c. of 19 per cent. sodium carbonate, when evaporated to precipitation over a free flame and diluted to 10 c.c. as was the unknown, or with the picramic acid standard mentioned below.**

Calculation.—If directions are followed exactly the calculation is as follows:

$$\text{Milligrams glucose in unknown} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \text{milligrams}$$

of glucose in standard.

(*c*) *Pearce's Modification of Lewis-Benedict Method.*[45]—This modification entails the use of an autoclave instead of the free flame and has the advantages of decreasing danger of loss and making it possible to carry out a large number of estimations at one time. Proceed exactly as in the Lewis-Benedict, but use 6 c.c. of the picric acid filtrate instead of 8 c.c., and instead of heating over the free flame introduce into an autoclave for 15-30 minutes at about 20 pounds pressure to the square inch. Compare with standard in a colorimeter. The standard recommended by Lewis and Benedict may be diluted one-fourth or the difference allowed for, since 6 c.c. of filtrate are used in place of 8 c.c.

(*d*) *Epstein Method of Estimation of Sugar in the Blood.—Principle.*—This method [46] is a modification of the Lewis and Benedict procedure, being based on the same principle but making possible the determination of reducing sugar in finger blood (0.1-0.2 c.c.) with a sufficient degree of accuracy for clinical purposes, and with little ex-

* In case of hyperglycemia the final volume of the reaction fluid is made 25 c.c. or 50 c.c., and the results are accordingly multiplied by 2.5 or 5.0.

** *Permanent Standard.*—A solution of picramic acid makes a very satisfactory permanent standard. The color is identical in quality with that formed in the method above and its solution keeps perfectly. The formula of the permanent standard is:

Picramic acid	0.064	gram
Sodium carbonate (anhydrous)	0.100	gram
Water to make	1000.0	c.c.

Dissolve the picramic acid with the aid of heat in 25 to 50 c.c. of distilled water which has been made alkaline with sodium carbonate. Cool and dilute to 1 liter. This solution has the same intensity of color as that obtained by the proposed method with 0.64 mg. of sugar, when the final volume of the reaction fluid is made 10 c.c. The solution should be standardized against pure glucose.

penditure of time. Instead of a Duboscq colorimeter the less expensive Sahli-Gower hemoglobin colorimeter or Küttner colorimeter is recommended.

Procedure.—The apparatus * and the following reagents are necessary:

1. Picric acid, saturated solution.
2. Sodium carbonate, 10 per cent. solution.
3. Sodium fluorid or potassium oxalate, 2 per cent. solution.

Put 1 or 2 drops of the fluorid or oxalate solution into the graduated test-tube. By means of the blood pipet 0.2 c.c. of blood is obtained from the tip of the finger or the lobe of the ear and is discharged into the tube containing the fluorid solution. The pipet is rinsed 2 or 3 times with distilled water and the washings added to the blood in the tube. Distilled water is then added to the 1.0 c.c. mark. After laking of the blood has taken place, picric acid is added to this (a few drops at a time) up to the 2.5 c.c. mark, the tube being gently shaken on each addition of the acid. Precipitation of the blood-proteins takes place; the sugar, together with an excess of picric acid sufficient for the reaction, stays in solution. The tube is finally shaken vigorously (the end of the tube being covered with the finger) and the contents filtered through a small filter, or, better still, centrifuged for 1 or 2 minutes.

One c.c. of the filtrate or the clear supernatant fluid obtained on centrifugalization is withdrawn, put into the plain test-tube, and heated carefully over the naked flame. The contents of the tube are boiled until all but 2 or 3 drops of the solution is evaporated. One-half c.c. of the 10 per cent. sodium carbonate solution is then added and the tube heated again until the contents are concentrated to a small volume equal to about 2 or 3 drops. The color of the fluid changes from yellow to deep red or reddish-brown and the reaction is completed.

Three or four drops of distilled water are added and the tube warmed gently. The contents are then transferred to the graduated tube of the hemoglobinometer. The boiling tube is rinsed several times with water (only 3 or 4 drops at a time being used). The tube is warmed with each rinsing before transferring of the contents to the graduated tube. The volume of fluid is then brought up to the mark 50 on the scale.

The color of the resulting solution is compared with that of the two standard tubes, A and B, which accompany the instrument. If it is darker than Standard A (representing 0.05 per cent. of sugar) and lighter than Standard B (representing 0.1 per cent.), the first standard

* The tubes belonging to this hemoglobinometer are not all equally calibrated. With some the 50 per cent. mark represents a volume of 1.0 c.c.; with others, 1.0 c.c. of fluid reaches up to the 43, 45, 46 or 47 per cent. mark. The error in the calibration is generally below the 10 per cent. mark; the graduations above this mark are usually correct. By means of the standard 1.0 c.c. pipet one can readily determine whether or not a given tube is properly calibrated. In order to facilitate a direct reading of the percentage of sugar on these hemoglobinometer tubes, it is essential to have 1.0 c.c. of fluid stand at mark 50. To overcome a discrepancy (if any exists) in the calibration of a given tube, one may put one, two or three small glass beads in the bottom of the tube, of such size as to raise the menicus of 1.0 c.c. of fluid up to the 50 per cent. mark.

is used for comparison. In either case the solution in the graduated tube is diluted gradually with water (just as is usually done in hemoglobin estimations) until the colors match.

The percentage of the sugar in the blood is then computed thus: Using the lighter standard A, the figure on the scale, divided by 1,000, represents the percentage of sugar in the blood. For example, the tube reads 86; then the result is

$$\frac{86}{1000} = 0.086 \text{ per cent.}$$

When Standard B is used for comparison, the figure on the scale is multiplied by 2 and divided by 1,000. For example, the tube reads 73; then the percentage of sugar is

$$\frac{73 \times 2}{1000} = 0.146 \text{ per cent.}$$

With the instructions given, the above formulas may be used for direct computation of the percentage of sugar only, when 0.2 c.c. of blood is used in the determination. When, however, only 0.1 c.c. of blood is used, the formulas apply as well, but the value obtained must be multiplied by 2.

It is better, in cases in which a high sugar content in the blood is suspected (in diabetes, for example), to use only 0.1 c.c. of blood for the determination. In all other cases 0.2 c.c. of blood should be used.

The Parathyroid, Pineal and Thymus Glands and Gonads

As yet there is no method known of experimentally estimating the functional activity of the parathyroid, pineal and thymus glands, or gonads.

REFERENCES

Tests for Hyperthyroidism

1. Kocher. Verhandl. d. deutsch. Gesellsch. f. Chir., 1910, 396.
2. Krecke. München. med. Wchnschr., 1911, lvii, 1601, 1676.
3. Marinesco. Ztschr. f. Nervenh., 1911, xli, 268.
4. Roseo. Biochim. e terap. sper., Milano, 1912, iv, 1.
5. Lampe. München. med. Wchnschr., 1913, 26.
   Lampe and Papazolu. *Ibid.*, 28.
6. Claude, Baudouin and Porak. Bull. et mém. Soc. méd. d. hôp. de Paris, 1914, xxx, No. 22, 1904.
7. Müller. Deutsch. Arch. f. klin. Med., 1893, li, 335.
8. Hirschlaff. Ztschr. f. klin. Med., 1899, xxxvi, 200.
   Magnus-Levy. Berl. klin. Wchnschr., 1895, xxxii, 650; Ztschr. f. klin. Med., 1897, xxxiii, 296.
   Salomon. *Ibid.*, 1904, xxiv, 635.
   von Noorden. Handb. d. Path. d. Stoffwechs., 1907, ii, 352.
9. Du Bois. Arch. Int. Med., 1916, xvi, 915.

10. Pettenkoffer and Voit. Ann. d. Chem. u. Pharm., 1882, II Supp. Bd., p. 52.
11. Sondèn and Tigerstedt. Skandin. Arch. f. Physiol., 1895, vi, 1.
12. Jacquet. Verhandl. d. Natur. Gesellsch. in. Basel, 1903, xv, 252.
13. Grafe. Ztschr. f. physiol. Chem., 1910, lxv, 1.
14. Atwater and Benedict. Carnegie Inst. Wash. Pub., No. 42, 1905.
15. Benedict and Carpenter. *Ibid.*, No. 123, 1910.
16. Speck. Phys. des mensch. Athmens nach eigenen Untersuch., Leipsic, 1892.
17. Magnus-Levy. Arch. f. d. ges. Physiol., 1894, lv, 1.
18. Tissot. Jour. de physiol. et de path. gén., 1904, vi, 688.
19. Douglas. Jour. Physiol., 1911, xxxii, Proc. Physiol. Soc., p. xvii.
20. Benedict. Amer. Jour. Physiol., 1909, xxiv, 345; Deutsch. Arch. f. klin. Med., 1912, cvii, 156.
21. Rolly and Rosiewicz. Deut. Arch. f. klin. Med., 1911, ciii, 58.
22. Krogh. Skandin. Arch. f. Physiol., 1913, xxx, 375.
23. Lusk. Arch. Int. Med., 1915, xv, 793.
24. Riche and Soderstrom. *Ibid.*, 805.
25. Benedict. Physical Review, 1906, xxii, 294.
——— Deutsch. Arch. f. klin. Med., 1912, cvii, 156.
——— Jour. Biol. Chem., 1915, xx, 301.
Benedict and Homans. Jour. Med. Research, 1912, xxv, 409.
Benedict and Talbot. Carnegie Inst. Wash. Pub. No. 201, 1914, 31.
——— *Ibid.*, Am. Jour. Dis. Child., 1912, iv, 129, 1912.
——— *Ibid.*, 1914, viii, 1.
——— Carnegie Inst. Wash. Pub. No. 201, 1914, 43.
Benedict and Tompkins. Boston Med. and Surg. Jour., 1916, clxxiv, 857, 898.
Carpenter. Carnegie Inst. Wash. Pub. No. 216, 1915, 37.
26. Kaufmann. Arch. de Physiol., 1896, viii, 329.
27. Benedict. Boston Med. and Surg. Jour., 1918, clxxviii, 667.
Pearce. Jour. Lab. and Clin. Med., 1918, iii, No. 7.
28. Hunt, Reid. Amer. Jour. Physiol., 1899.
——— Proc. Soc. Exper. Biol. and Med., 1905, iii, 15.
——— Jour. Biol. Chem., 1905, i, 33.
——— Jour. Amer. Med. Assn., 1906, xlvii, 790.
——— Hyg. Lab. Bull., 1907, No. 47.
——— Jour. Amer. Med. Assn., 1907, xlix, 240; 1911, lvii, 1032.
Hunt and Seidell. *Ibid.*, 1908, li, 1385.
——— Jour. Pharmacol. and Exper. Therap., 1910, ii, 15.
29. Trendelenburg. Biochem. Ztschr., 1910, xxix, 396.
30. Ghedeni. Wien. klin. Wchnschr., 1911, xxiv, 736.
31. Lussky. Amer. Jour. Physiol., 1912, xxxii, 63.
32. Loewi. Wien. klin. Wchnschr., 1907, xx, 728.
——— Arch. f. exper. Path. u. Pharmacol., 1908, lix, 83.
Falta. Wien. klin. Wchnschr., 1907, xx, 1559.
Lak. Verhand. d. 25 Kong. f. innere Med., 1908, 292.
Epinger, Falta and Rudinger. Wien. klin. Wchnschr., 1908, xxi, 251.
Epinger and Hess. Verhand. d. 26 Kong. f. innere Med., 1909, 385.
De Laet. Arch. méd. belges, 1918, lxxi, 481.
33. Goetsch. New York State Jour. Med., 1918, xviii, 259.
Hoskins. Endocrinology, 1918, ii, 460.

Tests for Hypo-adrenalism

34. Sergent. The white adrenal line: its production and diagnostic significance. Endocrinology, 1917, i, 18-24.
35. Massalongo. Riforma med., Aug. 24, 1918.
36. Falta. Erkrankungen der Blutdrüsen. Vienna, 1913.
37. Polak. Wien. klin. Wchnschr., 1909.

Tests for Hyperadrenalism

38. Polito and Corelli. Nouv. Riv. Clin. e terap., 1911, xiv, 482.
39. Meltzer and Auer. Centralbl. f. Physiol., 1904, xviii, 316.
40. Wessely. Ztschr. f. Augenh., Aug. 13, 1905, xiii, 310-320.
41. Ehrmann. Arch. f. Exper. Path. u. Pharmakol., 1905, liii, 96.
42. Taylor and Hulton. Jour. Biol. Chem., 1915, 22, 63.
43. Gardner and McLean. Bio.-Chem. Jour., 1914, viii, 391.
44. Lewis and Benedict. Jour. Biol. Chem., 1915, xx, 61. For modification, see Myers and Bailey. *Ibid.*, 1916, xxiv, 147.
45. Pearce. *Ibid.*, 1915, xxii, 525.
46. Epstein. Jour. Am. Med. Assn., 1914, lxiii, 1667.

CHAPTER VI

KIDNEY FUNCTION TESTS

In his learned lecture on "Some Phases of the Nephritis Problem," Professor Henry A. Christian made several observations which we shall do well to quote here:

"In all the earlier functional studies of patients with nephritis the desire to make an anatomical diagnosis has been prominent; sometimes it has been the acknowledged goal, at other times, though not so stated, it is evidently the aim of the investigator. Little by little it has become recognized that such an attainment has not been approached with any closeness, and I think that by most investigators it is now regarded as improbable that we will ever be able to correlate closely postmortem anatomical appearance with the functional disturbances of the kidney during life, at least so long as present pathological technic and classification continue in use. Improved methods of studying renal lesions, of course, may change these conditions at any time. In a number of patients we have had the opportunity to carry out a group of functional renal studies, and on the death of the patient have submitted the kidneys to pathological examination. In these patients there was no evident relation to be made out between anatomical changes in the kidney and antecedent functional disturbances in any selective sense that would justify an anatomical classification. In our experience in a functional sense, patients with nephritis do not separate themselves into distinctive

groups; rather is it indicated that there is a progressive increase in functional disturbance with advance of the lesion, though there is an undoubted tendency for certain cases to show continuously a much more marked impairment of function as measured by one set of tests than by another, indicating that functional disturbances depend on selective excretory activity, and that, were not the various renal structures pretty generally involved in nephritis, a more definite classification based on tests of renal function could be made.

"However, at the present time, tests of renal function are of more value for indicating the presence of a renal lesion, for measuring its extent and for indicating its management than as a means of classification of cases. For these purposes they add greatly to the value of our clinical study of patients with nephritis. Out of the very numerous methods of testing renal function certain ones have survived, either by reason of ease of application or by reason of yield of information in proportion to the amount of labor they require. Some have been discarded because the same information may be obtained from simpler procedures or from ones requiring less complicated and expensive apparatus or occupying less time in their carrying out. Others have been given up because they caused more discomfort and inconvenience to the patient than some other one yielding the same information. Those that are still in use, though they yield much valuable information, are not thoroughly satisfactory, and better ones probably can be worked out with our increasing knowledge of renal function under varying conditions in man and animals."

Barker gives the following classification of the tests used in determining renal sufficiency:

I. Physical—Chemical Methods.
   1. Determination of quantity and specific gravity of urine.
   2. Determination of osmotic pressure.
   3. Determination of freezing-point of urine.
   4. The "water experiment" and "polyuria test."
   5. Hyposthenuria—the amount of urine and its specific gravity after a renal test-diet.

II. Methods which determine the power to excrete specific chemical substances.
   1. Quantitative study of excretion of normal constituents.
      (*a*) Nitrogenous substances.
         i. Total non-protein nitrogen.
         ii. Urea.
         iii. Uric acid.
         iv. Creatinin.
      (*b*) Non-nitrogenous substances.
         i. Sodium chlorid and water.
         ii. Diastase.

2. Excretion of substances foreign to the body after ingestion or after injection into the blood, the muscles, or the subcutaneous tissues.
   (*a*) Phenolsulphonephthalein test.
   (*b*) Potassium iodid.
   (*c*) Lactose.
   (*d*) Methylene blue, indigocarmin or rosanilin.
   (*e*) Diuretics (theocin, diuretin, etc.).

III. Comparative analysis of blood and urine.
1. Nitrogenous substances.
   (*a*) Total non-protein nitrogen of blood and urine.
   (*b*) Urea of blood and of urine.
   (*c*) Ambard's coefficient.
   (*d*) McLean's index of urea excretion.
2. Non-nitrogenous substances.
   (*a*) Sodium chlorid content of blood and urine.
      i. The NaCl threshold.
      ii. The NaCl secretion constant.
   (*b*) The total electrolytes of the blood and urine.
      i. The hemorenal index.
3. Nitrogenous and non-nitrogenous substances.
   (*a*) Total nitrogen and total chlorids.
   (*b*) The nitrogen and chlorids of blood and urine, together with the water excretion and the specific gravity after a renal test.

IV. Other methods.
1. Bouchard's urotoxic coefficient.
2. Urea content of cerebrospinal fluid.
3. Determination of fatigability of the secretory power of the kidneys.
4. Determination of blood acidity in renal disease.
   (*a*) Measurement of CO_2 tension of blood.
   (*b*) Determination of the amount of $NaHCO_3$ necessary to render the urine alkaline.

V. Methods for determining functional capacity of each kidney separately.

We shall discuss the tests under the following headings

1. The chromocystoscopic tests.
2. The composition of the urine: after a special diet or after general diet.
3. The composition of the blood.
4. Urea content of the cerebrospinal fluid.

I. Chromocystoscopic Tests

The method of chromocystoscopy, that is to say, the administration by mouth, or preferably subcutaneously, of coloring matters, such as are readily excreted by the kidneys, is of greater or less practical value. It serves at least in localizing the ureteral orifices.

Methylene Blue Test.—Methylene blue was introduced for this purpose by Achard and Castaigne. The drug is given by mouth in ¼-grain doses, or preferably 15 minims of a 5 per cent. solution are administered by hypodermic injection. In health the drug will dye the urine in about one-half hour, while in the presence of disease of the kidneys this is delayed. Methylene blue is of little value, however, in estimating the functionating capacity of the kidneys, because it is slowly eliminated, and, therefore, requires observation for a long period of time. It has been estimated that only about 80 per cent. of the drug is excreted normally in the urine. It does not lend itself, moreover, to accurate colorimetric estimation.

Walker has shown that in obstruction of the lower urinary tract the excretion of methylene blue is retarded. In hypertrophy of the prostate the dye frequently does not appear for three or three and one-half hours, and is often excreted for a period of 8 or 10 days following the injection. In several instances it did not appear at all.

Underhill and Closson have shown that methylene blue is not a chemical entity but is a mixture of methylene blue and methylene azure. The appearance of the drug in the form of a chromogen necessitates additional manipulations when readings are being made, and affords an opening for speculation and difference of opinion and doubt as to the interpretation of the findings. Chauffard and Cavasse, Oulmont and Raymond consider that some significance is attached to the relations between the amount of chromogen and the amount of dye excreted.

The drug undergoes unknown chemical changes in the body, only a part (50 per cent.) being normally excreted in the urine. Occasionally it is completely destroyed in the body, even in health, and cannot be demonstrated at all in the urine, as has been described by Pugnaud and Revilliod, Walker and others (Rowntree and Geraghty).

Indigocarmine.—Indigocarmine was first used by Heidenhain in his investigation of the physiology of the kidneys. He showed that this drug was excreted by the epithelial cells of the convoluted tubules. Voelcker and Joseph, assistants of Czerny of Heidelberg, proposed the use of this dye for the purpose of testing the renal function. After an intramuscular injection of 20 c.c. of a 4-10 per cent. solution, the drug should appear in the urine of a healthy individual in less than one-half hour. It is delayed in the presence of disease. The delay in its appearance and the diminished intensity of the colors of the stream ejaculated from the ureters, as revealed by the cystoscope, is supposed to afford an indication of the relative amount of destruction of the secreting epithelium of the convoluted tubules. This dye has the advantage of being more readily eliminated than the methylene blue, but

has the decided disadvantage of being decolorized by purulent alkaline urine. It does not lend itself to colorimetric estimation, and only about 25 per cent. is eliminated by the kidneys.

Rosanilin.—Rosanilin (rosanilin trisulphate of soda), first introduced by Lépine, has not attained any popularity. One c.c. of a 1 per cent. solution injected subcutaneously usually makes its appearance in less than one-half hour. From 65 to 95 per cent. is recovered in 24 hours.

Phenolsulphonephthalein Test.—Phenolsulphonephthalein, which was first described by Remsen, is a bright red crystalline powder, somewhat soluble in water and alcohol, readily soluble in the presence of alkalis. The drug, as determined by Abel and Rowntree, is non-irritant locally, and is excreted practically entirely by the kidneys and with extraordinary rapidity, appearing in the urine normally within a few minutes of injection. In alkaline solution it presents a brilliant red color which is ideally adapted for quantitative colorimetric estimation.

This drug has been utilized by Rowntree and Geraghty to determine the functional capacity of the kidney in disease. By means of the test which they have introduced it is possible to determine accurately the condition of the kidneys, i.e., whether they are diseased, and in case they are, to determine the extent. This test permits one to determine whether the kidney disease, if chronic, will be apt to prove rapidly fatal, whether uremia is apt to develop, or whether any given case is suitable for surgical interference from the renal point of view. The technic of the test is as follows:

Technic.—Twenty minutes to one-half hour before administering the test, the patient is given 200 to 400 c.c. of water, in order to insure free urinary secretion; otherwise delay in time of appearance may be due to lack of secretion.

Under aseptic precautions a catheter is introduced into the bladder and the bladder completely emptied, or the patient is allowed to voluntarily empty the bladder. Noting the time, 1 c.c. of a carefully prepared solution of the phenolsulphonephthalein, containing 6 mg. to the c.c., is accurately administered subcutaneously, intramuscularly or intravenously by means of an accurately graduated syringe. The urine is allowed to drain into a test-tube in which has been placed a drop of 25 per cent. sodium-hydroxid solution, and the time of the appearance of the first faint pinkish tinge is noted. In patients without urinary obstruction the catheter is withdrawn at the time of the appearance of the drug in the urine, and the patient is instructed to void into a receptacle at the end of one hour, and into a second receptacle at the end of the second hour.

A rough estimate of the time of the appearance can be made by having the patient void urine at frequent intervals without the use of the catheter. In prostate cases it is wise to have the catheter in place until the end of the observation. The catheter is corked at the time of the appearance of the drug in the urine and the cork removed at the end of the first hour and at the end of the second hour, the bladder being thoroughly drained each time. In the case of many of the

patients of this type whom the author has observed, a retention catheter has been in use as part of the routine treatment, on account of the residual urine. Before the catheter is to be employed it is well to put the patient under the influence of hexamethylenamin.

Each sample of urine is measured and the specific gravity taken. Sufficient sodium hydroxid (25 per cent.) is added to make the urine decidedly alkaline, in order to elicit the maximum color. The color displayed in the acid urine is yellow or orange, and this immediately gives place to a brilliant purple-red color when the solution becomes alkaline. This solution is now placed in a liter-measuring flask and distilled water added to make, accurately, 1 liter. The solution is then thoroughly mixed and a small filtered portion taken to compare with the standard, which is used for all these estimations.

When the Dubosecq colorimeter is used, the standard solution used for comparison consists of 3 mg. of phenolsulphonephthalein (or ½ c.c. of the solution used for injection) diluted to 1 liter and made alkaline by the addition of only one or two drops of 25 per cent. NaOH solution. This is a beautiful purplish-red solution retaining its intensity of color for weeks or for an indefinite period. The one solution, therefore, serves for an immense number of tests.

One cup of the colorimeter (right) is half filled with this standard solution which has just been described, used for comparison, and the plunger lowered so that the indicator reads at 10. A varying quantity (depending upon the intensity of the color) of the diluted urine is placed in the other cup and the plunger manipulated until the two halves of the field are of an identical intensity of color. The indicator of the left plunger is now read, the fraction, as indicated by the Vernier scale, being taken into account. The estimation of the quantity present is then a question of simple arithmetic. For instance, the left side reads at 20, the standard being placed at 10. In other words, it takes a column of fluid twice as long to give the same intensity of color as that of standard, which, of course, shows that the solution contains only half as much dye. To obtain the percentage of dye excreted in the urine compared with the amount in the standard solution used for comparison, it is necessary to multiply the reading of the standard by 100 and divide by the reading indicated for the solution containing the urine. To return to our example, we have $\frac{10 \times 100}{20} = 50$ per cent. as much drug in the urine as in the standard solution used for comparison.

The 3 mg. of the standard for comparison has been chosen arbitrarily because of the beautiful pink color which is obtained when the indicator stands at 10. The amount of drug used for injection is 6 mg. We have compared the amount of drug in the diluted urine with that of the standard for comparison, but, if we wish to estimate the amount of drug excreted as compared with the amount of drug administered, we must compare the amount excreted with 6 mg. rather than 3 mg., which is

present in the solution for comparison. In the example given above we would have 50 per cent. of the 3 mg. or 25 per cent. of the 6 mg., which was the amount injected; so that the excretion is 25 per cent. of the amount administered.

Recently the Autenrieth-Königsberger colorimeter has been modified and utilized for the quantitative estimation of phthalein (Fig. 38). A standard alkaline solution, 6 mg. of phthalein to the liter, is placed in the wedge-shaped cell. The urine, collected as for the other method, is diluted to a liter and a small filtered portion poured into the rectangular cup. The wedge-shaped cell is now manipulated by means of the

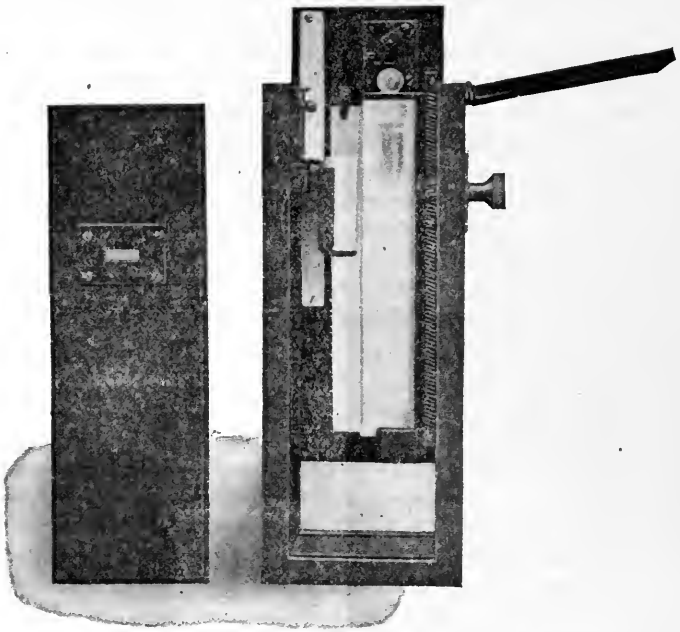


FIG. 38.—HELLIGE COLORIMETER, ROWNTREE AND GERAGHTY MODIFICATION.

screw, until the two sides of the color field are identical in intensity. The percentage is now read by the position of the indicator on the scale. This instrument is well adapted for the purpose, is approximately accurate, and is much cheaper than the Dubosecq colorimeter.

A very simple apparatus devised by Dunning, known as the Dunning Colorimeter, may be used for making approximate estimations. This instrument comprises 13 sealed ampules and 1 open ampule, and a box fitted with opaque glass, so arranged as to be used as a background for comparing colors. The sealed ampules contain the standard alkaline solution of phthalein, 6 mg. to the liter, and 80, 60, 50, 40, 35, 25, 20, 15, 10, 5 per cent. dilutions. Each ampule is marked with a figure indicating percentage strength, that containing standard solution carrying 100. The open ampule is intended for the urine specimen which should be collected and prepared as for the other methods of estimation. The

open ampule, containing the specimen, is placed in a colorimeter box and compared with the other ampules. When the specimen is matched, the percentage of excretion is indicated by the number on the test ampule selected.

Fairly accurate estimations, however, can be obtained by means of graduated cylinders—equal quantities of the standard solution and the diluted urine being used in separate cylinders and the denser solution being diluted until the colors become identical. The amount of drug in the solution being known, the amount in the urine can be readily calculated.

When the collected urine has been made strongly alkaline it is neces-

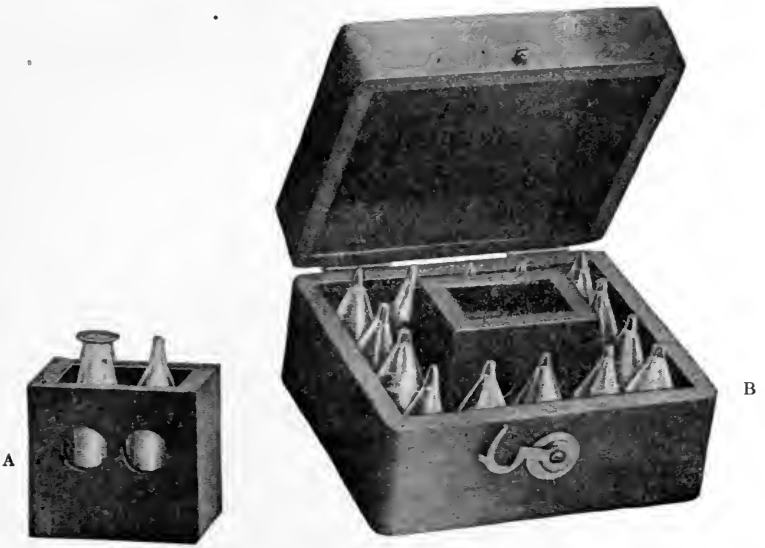


FIG. 39.—DUNNING COLORIMETER.
A, colorimeter box; B, complete apparatus.

sary to estimate the phthalein within a few hours, as the red fades gradually under these conditions. When it is desirable or necessary to defer the estimation for some hours or days, it is better to make the urine distinctly acid, under which condition the phthalein remains unchanged. It should, of course, be made alkaline again when the estimation is made.

The method, heretofore utilized in connection with other tests, of determining the time necessary for total elimination, is erroneous for the following reasons: Whereas, in the case of phthalein, a normal kidney excretes the greater part of the dye injected within 2 hours of the time of administration, and then only a small trace for the next 2 hours, the moderately diseased kidney secretes a fair amount within the first 2 hours—say 50 per cent. of that excreted by the normal kidney —but the concentration in the blood still being high, it continues to excrete a fair amount in the following two hours, so that at the end of

4 hours little difference may exist in the total work accomplished. One-hour and, at most, two-hour observations are therefore recommended. In cases in which only slight changes in function exist this can be most accurately demonstrated by a one-hour collection following the use of an intramuscular (lumbar) injection.

The excretion has been studied in several hundred normal individuals. In the earlier work subcutaneous administration was used exclusively, the drug appearing in the urine in from 5 to 11 minutes, 38 to 60 per cent. (average 50 per cent.) being excreted in the first hour after its appearance in the urine, and 60 to 85 per cent. for 2 hours. In health the elimination is practically complete in 2 hours, only a trace being present during the third and fourth hours.

Intramuscular and intravenous injections have also been employed. The time of appearance following the intramuscular administration is practically the same as that after the subcutaneous, but the output averages 5 to 10 per cent. more for the first hour. Following the intravenous injection, the drug normally appears in from 3 to 5 minutes, and from 35 to 45 per cent. of the drug is eliminated in the first 15 minutes, 50 to 65 per cent. in the first half hour, and 63 to 80 per cent. during the first hour. This rapidity of excretion following intravenous administration, is exceedingly striking and when this method is employed, observations for a quarter-hour or half-hour period only should be employed. For general use, however, we advocate the lumbar intramuscular method (the latter particularly when edema is present), as the technic involved is much simpler and the results obtained are reliable. The technic of the test is exceedingly simple. The injection is given, time of appearance noted, and collection of urine made for 1 or 2 hours. To each sample sufficient sodium hydrate is added to insure alkalinity and maximum intensity of color; then the urine is diluted to 1 liter, a small amount is filtered, the reading made, and the percentage of drug excreted is calculated.

Conclusions.—The summing up of the phenolsulphonephthalein test from Goodman's study is as follows:

1. In clinical influenza the small output of phenolsulphonephthalein is not in accordance with the findings in other general diseases.
2. The general series of diseases show a good output of phenolsulphonephthalein as a rule when there is clinically no evidence of kidney involvement.
3. The findings in regard to the value of this test, both from a diagnostic and a prognostic standpoint, in nephritis, confirm former conclusions in this respect and also the statement of Rowntree and Geraghty, that it reveals the degree of functional derangement, whether the nephritis be acute or chronic.
4. In several of Goodman's cases this test has revealed a degree of renal insufficiency, of which the clinical condition of the patient gave no evidence, but the existence of which has been confirmed by the fatal outcome of the case.

5. The test has served to demonstrate renal insufficiency in instances in which operation was contemplated and in which, though clinical and microscopic examinations were negative, subsequent developments confirmed the existence of the renal insufficiency.

6. In cases of ureteral or renal obstruction Goodman's findings are again in line with those of Rowntree and Geraghty in that he found a marked improvement, as indicated by the phenolsulphonephthalein test, following the removal of the obstruction.

7. In unilateral and bilateral disease of the kidney, the test has revealed the functional capacity of each kidney, and to such a satisfactory degree that, in some instances, it has assisted me to determine on the course of operative procedure. An absence or a very small output of the dye from one kidney, with an increased output from the other side, indicates a seriously diseased kidney on the one side, with a compensatory hypertrophy of the other kidney.

Tracy employed the test in about 300 cases, the material for this paper being based on the observations of the first 100 cases. He says that it does not seem possible to work out the minimum percentage of phthalein output with which it will be safe to undertake surgical operations, nor is it safe from the phthalein test to determine what cases should or should not be subjected to operation. He believes it will never be possible to determine this point from the phthalein test, as the functional activity of a kidney varies under numerous circumstances and at different times. In determining whether or not a patient should be subjected to operation, the history, clinical symptoms, and physical examinations are of much greater value than any renal functional test ever devised. The phthalein test used in conjunction with the clinical symptoms, history, and physical examination is of value. A small percentage of output should put the surgeon on his guard and cause him to study the patient most carefully before undertaking an operation. The phthalein test should be used only as one of the many methods of investigation in ascertaining the condition of the patient.

Goodman and Kristeller summarize the following advantages of this test:

1. The drug does not readily decompose in solution and can be sterilized by boiling.

2. The dose required is small, 1 c.c. of solution containing .006 gram of the dye.

3. The injection is painless, and is not followed by irritation if the solution is sufficiently alkaline.

4. The drug is excreted entirely by the kidneys.

5. The drug can be demonstrated in the urine in from three to ten minutes after the subcutaneous injection.

6. From 50 to 70 per cent. is excreted during the first two hours.

7. The drug lends itself to accurate colorimetric measurement.

8. The quantity of dye recovered in a specimen within a given time is not influenced by the volume of urine.

9. The presence of pus, phosphates, bile, and indican does not interfere with the colorimetric estimation of this drug.

Geraghty and Rowntree obtained the following results with the phenolsulphonephthalein test:

"The excretion has been studied in several hundred normal individuals. In our earlier work subcutaneous administration was used exclusively, the drug appearing in the urine in from five to eleven minutes, 38 to 60 per cent. (average 50 per cent.) being excreted in the first hour after its appearance in the urine, and 60 to 85 per cent. for two hours. In health the elimination is practically complete in two hours, only a trace being present during the third and fourth hours.

"*Influence of Amount of Urinary Secretion.*—The excretion of the drug does not run parallel to the excretion of urine. It is immaterial, as far as the excretion of the drug is concerned, whether the urinary output is 50, 200, 400 or 500 c.c. Similarly, the output does not seem to be much influenced by the previous administration of the different diuretics. Experimentally on animals, it was found that those diuretics (caffein, urea, dextrose, phlorizin, calomel) which are thought to exert a stimulating influence on the cells of the renal tubules, slightly increase the phthalein output, whereas those diuretics (hypertonic sodium chlorid, potassium nitrate and digitalis) which act entirely by mechanical factors, as by changes in osmotic tension, or changes in blood-pressure, slightly decrease or cause no change in the excretion of phthalein. No definite influence was noted in man, following the dose usually employed for diuresis, one-hour estimations being made.

"Heretofore, functional tests have not been considered of any great value to the clinician in relation to nephritis. In fact, hyperpermeability to methylene blue, indigocarmin and rosanilin has been shown to exist in acute and in chronic parenchymatous nephritis, while, on the other hand, decreased permeability with slow appearance and prolonged excretion has been demonstrated in the chronic interstitial variety.

"*Acute Nephritis.*—An opportunity to study only five cases of acute nephritis has presented itself.

"While no conclusions can be drawn from five cases, it is suggestive that in none of them was there increased permeability, but that, on the contrary, the permeability was markedly decreased when the condition was considered clinically grave.

"*Chronic Parenchymatous Nephritis.*—In all, twenty-one cases belonging to the clinical type of parenchymatous nephritis have been studied. These cases represent different grades of severity and the duration of the disease varies from a few weeks to seven years.

"In two very mild cases of short duration showing only slight edema, with albumin and casts, but with normal urinary output, the time of appearance of the drug and the amount excreted was normal. In one of these cases the time of appearance was eight minutes, and the output 52.5 per cent. for one hour. The second patient was a student who considered himself perfectly well, but in whose urine albumin and casts

were discovered by chance. On close inspection, a slight edema about the eyes was detected. No other evidence or suggestion of the disease could be found. In this instance 53 per cent. for the first hour and 8.6 per cent. for the second hour was excreted, following subcutaneous injection.

"In cases of longer standing or those in which disease is of ordinary severity, the time of appearance has always been delayed slightly (ten or twenty-five minutes) and the amount excreted is definitely below normal.

"In the most severe grades of chronic parenchymatous nephritis, or those in which the disease is of long standing and associated with secondary sclerotic changes, the output is reduced very markedly, and in some instances no trace of the drug can be found in the urine. Here also, as in the interstitial type, the absolute failure of excretion, or the excretion of a mere trace, has been followed within a short time by death from renal failure.

"Although the number of cases of chronic parenchymatous nephritis has not been very large, sufficient data have been collected to indicate that the test is of decided value in revealing the functional efficiency of the kidney in this condition. In the mild cases very little disturbance of function is indicated, and it may be impossible from the test alone to differentiate this condition from albuminuria. When there is a marked decrease in the phthalein output, marked renal changes are present, and when only excreted in traces, or not at all, a grave prognosis should be given, though no signs of uremia exist.

"*Chronic Interstitial Nephritis.*—Twenty-two cases of the type clinically classed as chronic interstitial nephritis have been under observation. In many of these cases, previous to the administration of the phthalein test, no accurate idea of the degree of involvement of the renal function could be ascertained even after the most careful clinical study. The phthalein test has proved itself of immense value in revealing the degree of destruction of the renal substance, and has demonstrated itself to be of extreme importance from the standpoint of both diagnosis and prognosis.

"In most of the cases of this series the time of appearance has been markedly delayed and the output of phthalein markedly decreased; where the output is lowest, the delay in appearance is most pronounced. The time of appearance, however, is not so important as the amount of excretion.

"*Uremia.*—In twenty-one cases under study, uremia has been present. Of this number, in fourteen uremia was grave; the patients exhibited nausea, vomiting, drowsiness or coma, and in several instances convulsions. In the remaining seven, mild symptoms only were present and had persisted over prolonged periods. Nine of the fourteen patients with grave uremia died during the attack. In all of these cases the phthalein elimination was zero or a faint trace only for two hours.

"In two of the five patients recovering from their uremia, the out-

put was 20 per cent., the uremia being the result of an acute exacerbation of a chronic nephritis. In two the output was 14 per cent.; in both of these the uremia was precipitated by a double pyelonephritis. The fifth case was one of acute exacerbation of chronic pyelonephritis in a man previously having had nephrectomy. This last patient has greatly improved, and at present has a two-hour excretion of 13 per cent.

"In the seven mild cases, exhibiting slight but persisting symptoms of uremia, the excretion respectively was as follows: 10 per cent. in one; 7 per cent. in three cases; a trace in two; 2 per cent. in the other, for two hours. Five of these patients died within three months of the performance of the test. Those living are still exhibiting evidences of chronic uremia, four months having intervened in one instance.

"Of four patients not exhibiting uremia at the time of the test, but in which the phthalein output was below 8 per cent. for two hours, one excreting 6 per cent. died within two months, another within three months, and the other two are still living, in one two months, and in the other three weeks intervening, but both are exhibiting evidence of chronic uremia.

"*The Study of Cardiac and Cardiorenal Cases.*—An attempt has been made to differentiate, by means of this test, between those cardiac cases with broken compensation or with passive congestion associated with the presence of albumin and casts in the urine and those cases in which cardiac insufficiency is associated with varying grades of true nephritis. In this connection thirty-three cases have been studied. From this study there appears to be no doubt, but that decrease in function accompanies marked passive congestion of the kidneys in the absence of any true nephritis. As the cardiac condition improves, however, the passive congestion becoming less marked and edema subsiding, the output of phthalein increases—and in one case rose from 16 per cent. to normal in the course of one week—the patient in the meantime losing 70 pounds in weight with the disappearance of a general anasarca. We feel that the phthalein test will prove of value in determining what degree of renal efficiency exists in this class of disease, and subsequently with improvement in the cardiac condition and the disappearance of edema, a continued low phthalein excretion will indicate with considerable certainty the presence of permanent organic changes in the kidney. We feel, however, that a much larger series should be studied clinically and at autopsy before very definite conclusions can be drawn.

"*Miscellaneous Cases.*—A large number of miscellaneous medical diseases have also been studied from the standpoint of phthalein excretion, among the number being ten cases of lobar pneumonia. In pneumonia the output is little if any decreased, and does not run parallel with the chlorid excretion. Three cases of persistent albuminuria have shown a normal output. In no disease other than renal, so far studied, has a marked reduction of the phthalein excretion been encountered.

"*The Relation of Phthalein Output to Blood-pressure, to Changes in the Eye-grounds and to the Blood-picture.*—In the majority of cases of chronic nephritis in which the blood-pressure has been high, the

phthalein elimination has been markedly decreased, but no exact parallelism exists, inasmuch as not a few instances have been encountered in which the systolic pressure has been over 200 mm. Hg., and the phthalein output one-half of normal, while on the other hand there have been instances in which the blood-pressure has been normal while the phthalein output has been zero or nearly so, the patients shortly after dying in uremia. While a high blood-pressure, when present, is considered of diagnostic and prognostic value, taken in conjunction with other clinical data, yet many patients died of renal insufficiency and exhibited a blood-pressure which was normal or practically so. Nor is the blood-pressure, even when high, increased in inverse proportion to the decrease in renal function.

"While in some instances marked changes in the eye-grounds, choked disk, tortuous vessels, hemorrhages, etc., have been present coincident with a very low phthalein output, in many cases, even of the most advanced and even fatal nephritis, no changes whatever in the eye-grounds could be detected, the patient at the same time failing to eliminate the phthalein.

"Moderate or rather severe grades of secondary anemia in the absence of disease of the kidneys can be present without any diminution in the phthalein elimination; for instance, two patients, one with 2,500,000 red cells, and hemoglobin 30 per cent., the other with hemoglobin 30 per cent., eliminated 61 and 57 per cent. for two hours respectively."

Rowntree and Geraghty also studied the value of the test *from a surgical standpoint.*

"As a result of obstruction in the lower urinary tract, pathologic changes may occur in the ureters and kidneys, dilatation of the ureters, varying grades of hydronephrosis, and as a result of the continued high pressure, atrophy of the parenchyma of the kidney. Not infrequently, infection occurs with the development of a pyelitis, a diffuse or localized pyelonephritis, or pyonephrosis. The occurrence of these complications is often difficult of recognition, and is often overlooked, particularly in the absence of symptoms of renal inadequacy. A large proportion of these cases of urinary obstruction have cystitis associated with albuminuria. The presence of casts in the urine is no contraindication to operation. The urinary output may be normal, also the urea and total solids, and yet the patient may be on the verge of renal failure and disastrous results may follow surgical interference.

"The test has been used in 100 cases of urinary obstruction, mostly cases of prostatic hypertrophy. The technic involved in these cases necessitates the use of a catheter; otherwise it does not differ from that described above.

"In the majority of cases, the test indicates a more or less degree of renal impairment, and taken in conjunction with the clinical condition it is of more value than the study of urine output, total solids, total nitrogen, and urea estimations combined.

"A marked decrease in the amount excreted invariably means severe

derangement of renal function, which may be of either a temporary or permanent character. Under such conditions one should proceed with extreme caution, and no surgical intervention should be attempted without further study, together with preliminary treatment. Under this régime, repeated functional tests will demonstrate eventually the nature of the derangement, for in true interstitial nephritis the output will continue low, whereas if the derangement is purely functional or secondary to pyelonephritis, usually improvement will follow as a result of the treatment and will be indicated by a decrease in the time of appearance of the drug and simultaneously an increase in the amount eliminated.

"The functional derangement due to infection in these cases is a much more dangerous condition than is the presence of even a fairly advanced condition of interstitial nephritis. The use of the test enables one to select the most favorable time for operation. In cases exhibiting a continued suspiciously low output, the use of nitrous-oxid gas, or spinal anesthesia, is suggested as preferable to ether in order to protect the kidneys. When only a trace of the drug continues to be excreted, prostatectomy should not be attempted, even though the patient presents no evidence of uremia."

In their original paper Rowntree and Geraghty stated that a dropping phthalein output was a contra-indication to operation, except in cases of necessity. This decrease in function usually means some change in the renal condition and in most cases it has been caused by the development of a pyelonephritis or an exacerbation of an old process. It is obviously wise to wait until the kidneys have recovered from this acute shock before subjecting them to further injury through operation.

The test can be used to equal advantage preliminary to any surgical procedure, when it is deemed important to know the true functional capacity of the kidneys.

The Phthalein Test as Applied to the Function of the Individual Kidney.—Functional tests have already demonstrated their value in this connection. But they have, at most, been able to determine only the relative working capacity of each kidney and have shed very little light on the absolute functional capacity of each organ.

The phthalein test, in association with ureteral catheterization, has been made in seventy cases of unilateral or bilateral disease.

Twenty minutes previous to examination 600 to 800 c.c. of water are given to the patient in order to insure a free flow of urine. The ureters are then catheterized.

As it is essential to collect all the urine secreted by each kidney during a definite period of time, in order to do accurate quantitative work, a form of ureteral catheter especially devised for this purpose has been used. The flute and catheter of Albarran, No. 6, or preferably No. 7, has been found to be most satisfactory. The catheters which have only side openings and no end opening cannot be depended upon for this purpose.

The catheters are passed up into the ureters to a distance of four inches. The cystoscope is then withdrawn, leaving the ureteral catheters in position. A small ureteral catheter is now passed into the bladder and the bladder thoroughly emptied, so that leakage, should it occur, can be detected. The time of injection is recorded, as is also the time of the appearance of the drug on each side. Starting from the time of appearance, the collection is continued for one hour, following subcutaneous or intramuscular injection. The amount of drug in each specimen is then estimated.

In normal cases the time of the appearance of the drug from the two sides has been almost always the same, and in the majority of cases this has been five to ten minutes following subcutaneous and three to five minutes following intravenous injections. The time will vary somewhat with the rate of urinary secretion. Normally the amount excreted by each kidney will be practically the same. The series of cases studied include tubercular or pyogenic infection, unilateral or bilateral, calculi, hydronephrosis, hypernephromata, etc.

Unilateral or Bilateral Surgical Disease of the Kidney.—It has been demonstrated that the time of appearance and the percentage of output is practically the same for the two healthy kidneys. When one kidney only is diseased, the time of the appearance of the drug is delayed on the diseased side, and the amount excreted is not only relatively but absolutely decreased. The amount of delay in the time of appearance is comparatively of little value. Reliance is to be placed only on the quantity excreted during a period of one-half or one hour, depending upon the method of administration.

Although in the majority of these cases of unilateral disease the combined output is equal to that of two normal kidneys, the greater part of the excretion is shown to be performed by the healthy kidney. In proportion to the decrease in function on the diseased side, there is approximately a proportionate increase in the function on the healthy side. In such cases following nephrectomy the remaining kidney eliminates, after the lapse of two or three weeks, an amount of drug which is normally excreted by two healthy kidneys. In all cases studied, the output from the remaining kidney has been greater than the combined output from two kidneys prior to operation.

In bilateral disease it has been found possible to determine the individual function (absolute or relative) of each kidney. It is in this class of cases particularly that the shortcomings of other functional tests have been most apparent, as one kidney may have been doing twice or three times the amount of work of the opposite kidney, and still be unable to assume the additional work of the other kidney. It may be doing the major part of the work at the expense of all, or nearly all, of its reserve power, but the phthalein test determines whether the kidney has a functional capacity which is normal, less than or greater than normal, and to what degree. In three cases of double renal tuberculosis, in which the amount of pus from each side was practically the same, the test permitted it to be determined that one kidney in each

TABLE 1.—TESTS TO SHOW EFFECT OF TISSUES ON PHENOLSULPHONEPHTHALEIN

Tissue	Phenolsulphonephthalein Gm.	C.c.	*Treatment*	Phenolsulphonephthalein Recovered (Per Cent.)
Sheep's liver	50	1	Undiluted + liquid petrolatum	0
Sheep's liver	50	1	Undiluted; exposed to air	Trace
Sheep's liver	50	1	150 c.c. 0.8% NaCl + liquid petrolatum	47
Sheep's liver	50	1	150 c.c. 0.8% NaCl; exposed to air	78
Sheep's liver	50	1	150 c.c. 0.8% NaCl; aërated	90
Human liver	25	1	40 c.c. 0.8% NaCl	10
Human liver	25	1	39.5 c.c. 0.8% NaCl + 0.5 c.c. 3% H_2O_2	30
Human liver	25	1	35 c.c. 0.8% NaCl + 5 c.c. 3% H_2O_2	30
Human liver	25	1	25 c.c. 0.8% NaCl + 15 c.c. 3% H_2O_2	44
Human liver	25	1	40 c.c. 0.8% NaCl + 0.5 gm. glucose	9
Human liver	25	1	40 c.c. 0.8% NaCl + 5 gm. glucose	20
Human liver	25	1	40 c.c. 0.8% NaCl + 15 gm. glucose	33
Human liver	25	1	39.5 c.c. 0.8% NaCl + 0.5 c.c. n/10 NaOH	11
Human liver	25	1	35 c.c. 0.8% NaCl + 5 c.c. n/10 NaOH	25
Human liver	25	1	25 c.c. 0.8% NaCl + 15 c.c. n/10 NaOH	77
Human liver	25	1	40 c.c. 0.8% NaCl + boiling	100*
Human liver	25	1	40 c.c. 0.8% NaCl; allowed to become putrid by standing 4 days	30†
Human kidney	25	1	40 c.c. 0.8% NaCl	90
Human kidney	25	1	39.5 c.c. 0.8% NaCl + 0.5 c.c. 3% H_2O_2	90
Human kidney	25	1	35 c.c. 0.8% NaCl + 5 c.c. 3% H_2O_2	94
Human kidney	25	1	25 c.c. 0.8% NaCl + 15 c.c. 3% H_2O_2	90
Human kidney	25	1	40 c.c. 0.8% NaCl + 0.5 gm. glucose	96
Human kidney	25	1	40 c.c. 0.8% NaCl + 5 gm. glucose	90
Human kidney	25	1	40 c.c. 0.8% NaCl + 15 gm. glucose	94
Cat's blood	50	1	Perfused through liver for 30 min. before addition of phenolsulphonephthalein	100‡
Dog's blood	50	1	Aërated with CO for 15 hours	95–96
Cat's blood and liver	whole	1	Blood perfused through liver 2 hours	45
Cat's blood and kidneys	whole	1	Blood perfused through kidneys 2½ hours.	95
Rabbit liver	32	1	64 c.c. 0.8% NaCl + 0.25 gm. glucose	79
Monkey liver	26	1	50 c.c. 0.8% NaCl + 0.25 gm. glucose	39
Beef liver	30	1	Undiluted at 5 C	52
Beef liver	30	1	Undiluted at 25 C	33
Beef liver	30	1	Undiluted at 35 C	26
Beef liver	30	1	Undiluted at 25 C. + 0.5 gm. glucose	25
Beef pancreas	30	1	Undiluted	82
Cat's muscle	25	1	Undiluted mash	38
Cat's intestine	25	1	Undiluted mash	34
Cat's liver	25	1	Undiluted mash	0
Sheep's spleen	50	1	Undiluted mash	90

* Enzyme entirely destroyed.
† Enzyme partially destroyed.
‡ Blood + phenolsulphonephthalein stood 30 hours.

instance had a function greatly in excess of the other, indeed sufficient functional capacity to allow of successful nephrectomy, marked improvement in general condition occurring subsequently in each case.

In six cases out of seventy Rowntree and Geraghty have found that the catheters caused some inhibition of function, as was also observed by Keyes, Jr., and Stevens. This influence can readily be estimated by taking the total function either before or after catheterization.

Kendall, in his investigations of the fate of phenolsulphonephthalein when injected into the animal organism, found factors other than the kidney influencing its retention. Injections of glucose into animals

seem to increase the excretion of the dye, whereas injections of amino-acids seem to retard its excretion. The various tissues seem to influence the dye, so that some of it is destroyed in the body. The preceding table (Table 1) is self-explanatory.

A state of acidosis also seems to retard the excretion of this substance by the kidneys.

Bookman found that fevers markedly influence the functional capacity of the kidneys.

II. The Composition of the Urine: after a Special Diet or after General Diet

1. **Renal Function as Measured by the Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine.**—Hedinger and Schlayer have recently proposed a quantitative test of the mode of urinary function, as measured by specific gravity, salt and water excretion in two-hourly periods. These authors show how the urinary response to a full dietary containing a reasonable amount of fluids, salt and purins varies in health and disease. They found that the normal and the nephritic individuals differ very markedly from one another in the results obtained with the so-called "nephritic test-meal." Not only can the absence or presence of renal function be determined, but likewise its intensity.

Mosenthal records the results of studies carried out along lines suggested by this work. The test-meal has been simplified somewhat, and it appears that the entire procedure, or a part of it, may very well become a valuable routine test for the general practitioner. He carried out this test in more than one hundred cases. The only patients not investigated, in whom renal function should have been ascertained, were those suffering from acute nephritides; of these, such as had been treated in the wards of the hospital during the past winter were too sick to take food in any quantity, or were so unmanageable as to preclude the proper collection of specimens. It has been ascertained that the nephritic test-meal, when duplicated on the same patients, yields identical results, provided clinical condition has not changed. In several instances triplicate and quadruplicate observations have been made.

Nephritic Test-meal.—The directions for the nephritic test-meal are contained in the following memoranda, given to the nurse in charge of the case in mimeographed form (Mosenthal):

For..... Date.....

All food is to be salt-free food from the diet kitchen.

Salt for each meal will be furnished in weighed amounts.

All food or fluid not taken must be weighed or measured after meals and charted in the spaces below.

Allow no food or fluid of any kind except at meal times.

Note any mishaps or irregularities that occur in giving the diet or collecting the specimens.

Breakfast, 8 A. M.:

Boiled oatmeal		100 grams
Sugar		½ teaspoonful
Milk		30 c.c.
Two slices bread		(30 grams each)
Butter		20 grams
Coffee	160 c.c.	200 c.c.
Sugar	1 teaspoonful	
Milk	40 c.c.	
Milk		200 c.c.
Water		200 c.c.

Dinner, 12 NOON:

Meat soup		180 c.c.
Beefsteak		100 grams
Potato (baked, mashed or boiled)		130 grams
Green vegetables		as desired
Two slices bread		(30 grams each)
Butter		20 grams
Tea	180 c.c.	200 c.c.
Sugar	1 teaspoonful	
Milk	20 c.c.	
Water		250 c.c.
Pudding (tapioca or rice)		110 grams

Supper, 5 P. M.:

Two eggs, cooked in any style		
Two slices bread		(30 grams each)
Butter		20 grams
Tea	180 c.c.	200 c.c.
Sugar	1 teaspoonful	
Milk	20 c.c.	
Fruit (stewed or fresh)		1 portion
Water		300 c.c.

No food or fluid is to be given during the night or until 8 o'clock the next morning (after voiding), when the regular diet is resumed.

Patient is to empty bladder at 8 A. M. and at the end of each period, as indicated below. The specimens are to be collected for the following periods in properly labeled bottles:

8 A. M.-10 A. M.; 10 A. M.-12 NOON; 12 NOON-2 P. M.; 2 P. M.-4 P. M.; 4 P. M.-6 P. M.; 6 P. M.-8 P. M.; 8 P. M.-8 A. M.

Specimens are to be left in the ward until called for at 8:30 A. M. by the attendant from the chemical laboratory.

The above dietary contains approximately 13.4 grams of nitrogen, 8.5 grams of salt, 1,760 c.c. of fluid, and a considerable quantity of purin material in meat, soup, tea and coffee. All these substances act as diuretics, and it is on the mode of excretory response to such stimuli

that the present study of renal function depends. Spaces are provided to chart the amounts of food not eaten by the patient, and corresponding allowances can be made in calculating the food intake. It is in no way essential that all the meals should be taken in their entirety, nor that the food should be exactly as indicated. The bill-of-fare here presented has been designed to adapt itself to the daily food supply furnished by the hospital. In private practice it would only be necessary to ask the patient to eat three full meals a day and write down the approximate quantities, as—1 cup of coffee, two slices of toast, two tablespoonfuls oatmeal, etc.—in order to be certain that the diet for the day contained a sufficient quantity of the diuretic materials of our ordinary food to make an adequate demand on the kidneys to test renal function. It is extremely desirable to insist upon the fact that, since the food as found in most households suffices to carry out these tests, and the procedure is not a complicated one, it need not be confined to hospitals and patients who can afford private nurses.

A wide variation may be permitted in the above-mentioned directions. Certain others, however, must be followed slavishly, in order to make the outcome of the test yield its maximum result. The urine must be collected punctually every two hours. No solid food or fluid of any kind must be taken between meals, and especial care must be observed that nothing of any kind is eaten nor drunk during the night, and that the night specimen is completed before breakfast is touched. The reason for this is that the normal kidney responds rapidly to fluids, ingested, so that within a few hours a marked diuresis occurs. The following observation may serve as an illustration of this previously well-established fact:

Time Interval	*Urine Volume*	*Fluid Ingested*
6 P. M.- 8 P. M.	84	7:30 P. M. supper, with
8 P. M.-10 P. M.	590	1,000 c.c. of water
10 P. M. -8 A. M.	361	

In this instance, within two and one-half hours of drinking 1,000 c.c. of water, over 590 c.c. were eliminated, while during the eight-hour period following the diuresis, only 361 c.c. of urine were voided.

Mosenthal makes the following summary of his findings:

"The nephritic test-meal, as suggested by Hedinger and Schlayer, and elaborated in this paper, has not only proved itself to be an admirable test for renal function, but also in many cases has been of great value in diagnosing cardiac, renal and other conditions. Much pleasure and profit may be derived from a study of diseases of the kidney from this point of view, since it forms a basis for a rational therapy and a stimulus toward keener clinical operation."

The test is a quantitative one of the mode of urinary function as measured by the specific gravity, salt, nitrogen and water excretion in two-hourly periods during the day and for a twelve-hour period at night. The *normal individual* yields specimens with specific gravity

figures which vary ten points or more from the highest to the lowest; a night urine is high in specific gravity, 1.018 or more, high in its percentage of nitrogen, above 1 per cent., and small in amount, 400 c.c. or less. The quantities of water, salt and nitrogen excreted approximate the intake. When kidney function becomes involved, the first signs are usually demonstrated in the night urine, the quantity becomes increased; the specific gravity and the nitrogen concentration are lowered. One or all of these changes from the normal may occur. In severe cases of *chronic nephritis*, an advanced degree of functional inadequacy of the kidney is indicated by a markedly fixed and low specific gravity, a diminished output of both salt and nitrogen, a tendency to total polyuria and a night urine showing an increased volume, low specific gravity and low concentration of nitrogen. Such functional pictures, however, are not confined to nephritis. They are found regularly in many other conditions: pyelitis, cystitis, hypertrophied prostate, marked anemia, pyelonephritis, polycystic kidney, and diabetes insipidus. The cause of diminished renal function, it is clear, must be sought for in many directions—the urinary passages, the blood or the kidney itself. Prognosis and therapy will depend largely upon the cause of the fundamental impairment and not upon its degree. A divergence between the degree of functional renal involvement and the intensity of the signs and symptoms of nephritis is frequently found, and accentuates the lack of parallelism that there may be between functional and anatomical lesions.

In *chronic diffuse (parenchymatous) nephritis,* the condition of renal function is characterized by its variability. In these instances, the results of the test-meal have proved to be extremely valuable in giving an idea of the status of the salt, nitrogen and water excretion, besides the picture of renal efficiency as a whole. The findings in myocardial insufficiency vary according to the activity of the heart. Distinct differences are found in myocardial decompensation and the accumulation of edema, in the period of eliminating edema, and subsequently, when the cardiac compensation is again fully established; it requires some time before the kidney resumes its normal activity. This intervening period is indicated by a tendency to a low, fixed specific gravity and a nocturnal polyuria. During the period of full myocardial decompensation the results of kidney activity are very characteristic, the specific gravity is markedly fixed at the level of about 1.020, the salt output is diminished, that of nitrogen is high—in marked contrast to the salt—and there is oliguria. When chronic nephritis and cardiac decompensation coexist, as they so often do in hypertensive nephritis, the urine may exhibit the characteristics due to either lesion. The determining factor is probably to be found in the chronic nephritis which may or may not be so far advanced as to present an unchanging barrier to the influence of renal congestion.

O'Hara found that, in general, salt excretion is impaired before there is much disturbance of water and nitrogen excretion. In most patients salt and water excretion behave very similarly; the nitrogen

excretion is greatly impaired, as a rule, only in the severe cases. Salt, water and nitrogen excretion show some disturbance in even the very mild cases in which phenolsulphonephthalein excretion is normal and there is no increased blood nitrogen. These dietary tests cannot be used in all cases of chronic nephritis. They cannot be carried out in those that are very severe. The methods involving the determinations of the indices of excretion of urea and salt do not have a number of the difficulties met with in carrying out the dietary tests. These indices were determined in fifteen cases in which both dietary tests were carried out, and the indices seem to give as much information as the other tests and to possess distinct advantages, inasmuch as they can be determined for practically every patient and require considerably less time and less labor in their execution. According to the author, the great advantage of all three of these tests is that they give information as to disturbance of renal functions in those mild cases in which phenolsulphonephthalein excretion is normal and the blood-urea-nitrogen is not increased.

Griessmann has made exact studies of the excretion of water, sodium chlorid and nitrogen in a small series of nephritis. The patients were placed on a diet of rice, condensed milk, and raspberry juice. The diet was analyzed for its contents in the above-mentioned constituents and the patients were kept on it until they had reached a state of equilibrium. Each experiment was divided into four periods: (1) The preliminary period in which the patient was kept on a milk or milk-rice diet until there was equilibrium in nitrogen, sodium chlorid, and water. (2) During the second period, the patient received the standard diet plus 20 grams of sodium chlorid. The additional salt dissolved in 400 c.c. of water was given only on the first day of this period. (3) In the third period, one or two liters of water were added to the standard diet. The extra water was also given only on the first day of the period. (4) In the fourth period, the patient received the standard diet plus 20 grams of urea dissolved in 250 c.c. of water on the first day. Two of the patients had markedly contracted sclerotic kidneys, as autopsy proved. Two suffered from arteriosclerotic renal changes, while the fifth had chronic glomerulonephritis. The changes in water excretion were the least noticeable. Defect in the excretion of sodium chlorid was found in all of the cases, being especially marked in one of the cases of interstitial nephritis. In the other cases there was a moderate delay in excretion. The urea excretion was studied in only three cases. A marked delay was noted in one of these also, a patient with contracted kidneys. The experiments show, Griessmann says, that as a rule disturbances in excretion of sodium chlorid and nitrogen are combined. Nevertheless, there are cases in which the disturbance of function chiefly affects only the salt or the urea. Thus the classification of renal diseases on the basis of excretion of salt and urea (Widal, Müller) seems justifiable.

The accompanying tables (Tables 2-14) are taken from Mosenthal's work:

TABLE 2.—NORMAL REACTION TO NEPHRITIC TEST-MEAL.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	c.c.	Sp. Gr.	Per Cent.	Grams	Per Cent.	Grams
8–10	153	1.016	1.32	2.02	0.89	1.26
10–12	156	1.019	1.25	1.95	0.74	1.15
12– 2	194	1.012	0.64	1.24	0.59	1.14
2– 4	260	1.014	0.77	2.00	0.56	1.46
4– 6	114	1.020	0.99	1.13	0.95	1.08
6– 8	238	1.010	0.43	1.02	0.52	1.235
Total day	1,115	.....	.....	9.36	.....	7.32
Night, 8–8	375	1.020	0.63	2.36	1.23	4.61
Total, 24 hours	1,490	.....	.....	11.72	.....	11.93
Intake	1,760	.....	.....	8.5	.....	13.4
Balance	+270	.....	.....	—3.22	.....	+1.47

[1] Impression: Normal reaction to the nephritic test-meal. Note the variations occurring in the fluid output, and the specific gravity, which are in inverse ratio; the night urine, which is small in amount and shows a high specific gravity and a high percentage of nitrogen; and the approximately normal output of water, salt and nitrogen in twenty-four-hours.

TABLE 3.—COMPARISON OF NIGHT AND DAY URINES IN NORMAL INDIVIDUALS.[1]

Case	Night Urine (12 Hours)			Day Urine (12 Hours)
	Specific Gravity	Nitrogen (Per Cent.)	Volume (c.c.)	Volume (c.c.)
1	1.020	1.23	375	1,105
2	1.017	1.20	352	1,796
3	1.027	2.07	290	634
4	1.019	1.12	350	1,032
5	1 018	1.03	390	1,945
6	1.018	1.43	361	1,413
7	1.019	1.14	355	2,156
8	1.018	1.08	402	2,446
9	1.026	1.42	277	866
10	1.030	1.58	210	1,496
11	1.029	1.85	213	468
12	1.025	1.23	248	861

[1] The nitrogen percentage, specific gravity and volume for the night urines of normal individuals on nephritic test-diet. Note the high percentage of nitrogen, high specific gravity and the small volume of urine, as compared to the day specimen.

TABLE 4.—SPECIFIC GRAVITY OF URINES COLLECTED IN TWO-HOURLY PERIODS.

Case	Specific Gravity[1]						Degrees of Variation in Readings
Normal (Table 2)	16	19	12	14	20	10	10
Incipient primary contracted kidney	09	14	09	10	14	06	8
Incipient primary contracted kidney	18	09	16	22	13	10	11
Advancing primary contracted kidney	18	17	13	13	13	15	5
Advancing primary contracted kidney	19	20	20	20	21	20	2
Advanced primary contracted kidney	11	11	10	11	11	11	1
Advanced primary contracted kidney	12	11	11	11	12	13	2
Advanced primary contracted kidney	10	09	10	09	09	10	1
Advanced primary contracted kidney	05	06	07	08	..	08	3
Incipient chronic diffused nephritis	25	..	24	33	28	30	9
Incipient chronic diffused nephritis	09	16	15	17	12	07	10
Advanced chronic diffused nephritis	12	11	14	11	13	11	3
Secondary contracted kidney	09	10	12	10	12	10	3
Congested kidney; myocardial decompensation marked	18	20	19	18	20	21	3
Congested kidney; moderate myocardial decompensation	25	24	24	25	24	21	4
Congested kidney; cardiac compensation; losing edema	12	15	10	15	13	10	5
Congested kidney; cardiac compensation; edema disappeared	05	06	11	09	09	10	5
Polycystic kidney	10	10	10	11	10	10	1
Marked anemia	10	10	10	10	10	11	1
Diabetes insipidus	04	04	06	04	04	04	2
Cystitis, pyelitis, prostatic hypertrophy	10	10	10	10	11	11	1
Pyonephrosis	11	12	12	13	12	12	2

[1] Variations of specific gravity in the day urines collected in two-hourly intervals. Note the fixed specific gravity of the severe cases of nephritis. Only the last two figures of each reading are given.

TABLE 5.—SHOWING THE CONSTANCY OF NOCTURNAL POLYURIA AND LOW SPECIFIC GRAVITY IN THE URINE OF A CASE OF ADVANCED CHRONIC DIFFUSED NEPHRITIS.

Volume of Urine (c.c.)		Specific Gravity of Urine[1]	
Day	Night	Day	Night
1,390	560	12	10
935	710	12	11
1,010	760	11	10
1,122	705	10	10
790	790	10	10
908	1,110	11	10
880	1,184	11	10
1,020	1,360	12	09
1,075	1,120	11	10
1,140	1,255	10	10
1,375	730	12	11
1,600	1,160	12	10
1,525	1,090	12	11
1,400	1,260	11	10
1,146	1,100	11	12
1,940	1,060	10	09
1,280	1,520	10	09
1,640	1,400	10	10
1,370	1,370	11	10
1,480	1,480	18	18
1,340	1,680	19	17
1,410	1,340	10	10
1,480	1,410	12	10
1,185	1,610	10	08

[1] Only two last figures are given.

TABLE 6.—NEPHRITIC TEST-MEAL IN A CASE OF CHRONIC DIFFUSE (PARENCHYMATOUS) NEPHRITIS.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	c.c.	Sp. Gr.	Per Cent.	Grams	Per Cent.	Grams
8–10	32	1.025	.....	.....	.....	.....
10–12	0	.....	.....	.....	.....	.....
12– 2	54	1.024	.....	.....	.....	.....
2– 4	64	1.033	.....	.....	.....	.....
4– 6	64	1.028	.....	.....	.....	.....
6– 8	66	1.030	.....	.....	.....	.....
Total day	280	.....	0.18	0.50	1.91	5.34
Night, 8–8	595	1.016	0.08	0.48	0.93	5.53
Total, 24 hours	875	.....	....	0.98	.....	10.87
Intake	1,760	.....	.....	8.50	.....	13.40
Balance	+885	.....	.....	+7.52	.....	+2.53

[1] Impression: There is marked salt and water retention; the excretion of nitrogen is adequate. The variations in specific gravity distinguish this case from one of cardiac insufficiency, and indicate that renal function as a whole is not seriously impaired.

TABLE 7.— NEPHRITIC TEST-MEAL IN THE SAME CASE AS PREVIOUS TABLE SOME TIME LATER.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	c.c.	Sp. Gr.	Per Cent.	Grams	Per Cent.	Grams
8–10	137	1.015	0.82	1.12	0.70	0.95
10–12	148	1.016	0.82	1.11	0.80	1.18
12– 2	108	1.016	0.78	0.84	0.79	0.85
2– 4	184	1.015	0.57	1.04	0.79	1.46
4– 6	96	1.015	0.48	0.46	0.79	0.76
6– 8	470	1.004	0.30	1.45	0.25	1.18
Total day	1,143	.....	.....	6.02	.....	6.38
Night, 8–8	960	1.010	0.34	3.26	0.38	3.65
Total, 24 hours	2,103	.....	.....	9.28	.....	10.03
Intake	1,760	.....	.....	8.50	.....	13.40
Balance	—343	.....	.....	—0.78	.....	+3.37

[1] Water and salt are excreted in excess in contrast to previous meal. The polyuria, both during the day and night and the rather low fixed specific gravity, are due to the fact that this patient is eliminating edema, and must not be confounded with similar curves obtained in case of high hypertensive nephritis, where they would indicate a considerable degree of impaired renal function. On physical examination, the two conditions can very easily be distinguished from one another.

TABLE 8.—NITROGEN, SALT AND WATER EXCRETION IN ADVANCED UNCOMPLICATED INTERSTITIAL NEPHRITIS.[1]

Case	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
1	776	1.014	0.37	2 88	0.65	5.08
2	1,282	1.013	0.29	3.69	0.52	6.71
3	1,276	1.012	0.42	5.41	0.49	6.28
4	1,535	1.009	0.35	5.34	0.42	6.51
5	1,873	1.011	0.41	7.64	0.14	2.59
6	1,541	1.010	0.30	4.70	0.30	4.64
7	1,665	1.009	0.12	2.00	0.09	1.50

[1] The approximate intake was 1,760 c.c. of fluid, 8.5 grams of salt and 13.4 grams of nitrogen in each case.

TABLE 9.—TYPES OF CASES OTHER THAN CHRONIC INTERSTITIAL NEPHRITIS, WHOSE URINE GIVES EVIDENCE OF ABNORMAL NOCTURNAL EXCRETION.

Disease	Night Urine		
	Volume (c.c.)	Specific Gravity*	Per Cent. Nitrogen
1. Chronic diffuse nephritis during			
(*a*) Water retention (edema formation)	595	16	0.93
	553	16	0.62
	367	20	0.88
	270	30	....
	106	28	....
(*b*) Elimination of edema	960	11	....
	960	10	0.47
	950	14	0.73
(*c*) Water balance (edema eliminated)	865	13	0.75
	490	14	1.01
	400	17	1.17
2. Heart disease during			
(*a*) Myocardial decompensation	275	21	1.85
	172	21	1.67
	119	25	1.82
	91	20	1.68
(*b*) Elimination of edema	1,340	11	0.51
	990	13	0.56
	850	10	0.28
	720	07	0.35
(*c*) Water balance (edema eliminated)	520	14	0.84
	515	11	0.53
	438	12	0.67
3. Hypertensive nephritis, complicated by myocardial decompensation	815	18	0.94
	546	17	0.68
	435	16	0.51
	405	18	1.07
	350	19	1.12
	108	18	1.09
4. Severe anemia	1,250	10	0.51
	680	10	0.52
	256	11	0.57
	180	16	0.39
5. Cystitis and hypertrophied prostate gland	1,395	09	0.43
	1,146	10	0.36
	775	11	0.44
6. Pyelonephritis	950	10	0.45
7. Polycystic kidney	1,290	10	0.45
8. Diabetes insipidus	594	07	0.20

* Last two figures only.

TABLE 10.—NEPHRITIC TEST-MEAL IN AN INDIVIDUAL WITH A SLIGHT GRADE OF ALBUMINURIA, A FEW CASTS, BUT NO OTHER SIGNS OF NEPHRITIS.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
8–10	65	1.020	.....	.....	.....	.....
10–12	67	1.021	.....	.....	.....	.....
12– 2	90	1.020	.....	.....	.....	.....
2– 4	126	1.019	.....	.....	.....	.....
4– 6	146	1.015	.....	.....	.....	.....
6– 8	172	1.013	.....	.....	.....	.....
Total day	666	.....	0.64	4.26	1.05	6.99
Night, 8–8	660	1.011	0.52	3.43	0.71	4.69
Total, 24 hours	1,326	.....	.....	7.69	.....	11.68
Intake	1,760	.....	.....	8.50	.....	13.40
Balance	+434	.....	.....	+0.81	.....	+1.72

[1] The concentration of urine, as indicated by the specific gravity and the percentage figures of sodium chlorid and nitrogen, is satisfactorily high. The total amounts of fluids and solids eliminated may also be considered normal. However, the urinary volume and specific gravity do not vary after the ingestion of meals as they should, and the night urine shows a distinct nocturnal polyuria with a low specific gravity. The whole picture, therefore, represents a moderately impaired renal function.

TABLE 11.—REACTION TO NEPHRITIC TEST-MEAL IN ADVANCED HYPERTENSIVE NEPHRITIS.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
8–10	133	1.010	0.36	0.48	0.35	0.47
10–12	176	1.009	0.36	0.63	0.34	0.60
12– 2	156	1.010	0.32	0.50	0.35	0.55
2– 4	212	1.009	0.36	0.76	0.34	0.72
4– 6	164	1.009	0.38	0.62	0.36	0.59
6– 8	104	1.010	0.33	0.34	0.33	0.34
Total day	945	.....	.....	3.33	.....	3.27
Night, 8–8	590	1.010	0.34	2.01	0.38	2.24
Total, 24 hours	1,535	.....	.....	5.34	.....	5.51
Intake	1,510	.....	.....	5.80	.....	12.20
Balance	—25	.....	.....	+0.46	.....	+6.69

[1] There is very marked fixation of the percentage figures for nitrogen and salt concentration and the specific gravity. There is evident nitrogen retention. The salt intake is too low to make it certain that a diminished ability to excrete salt does not exist.

TABLE 12.—REACTION TO NEPHRITIC TEST-MEAL IN EXTREME INTERSTITIAL NEPHRITIS.[1]

Time of Day	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
8–10	24	1.005	.....	.....	.....	.....
10–12	106	1.006	.....	.....	.....	.....
12– 2	82	1.007	.....	.....	.....	.....
2– 4	83	1.008	.....	.....	.....	.....
4– 6	0	.....	.....	.....	.....	.....
6– 8	230	1.008	.....	.....	.....	.....
Total day	525	.....	0.12	0.63	0.25	1.28
Night, 6–8	1,140	1.007	0.12	1.37	0.20	2.27
Total, 24 hours	1,665	.....	.....	2.00	.....	3.55
Intake	1,850	.....	.....	6.00	.....	13.00
Balance	+185	.....	.....	+4.00	.....	+9.45

[1] Note the low fixed specific gravity, the retention of salt and nitrogen, and the night urine, which is increased in amount, shows a low specific gravity and a low nitrogen concentration.

When there is passive congestion of the kidney due to myocardial insufficiency, the amounts of water and sodium chlorid eliminated are much diminished, while the nitrogen excretion remains approximately normal. An extreme case of this kind was found in L., who, on autopsy, proved to have cardiac decompensation, brought on by a severe grade of myocarditis, thrombosis of the coronary arteries and infarcts in the myocardium; the kidneys showed congestion only; urinary tests in this patient yielded the results shown in Table 13.

TABLE 13.—RESULTS OF URINARY TESTS IN MARKED MYOCARDIAL INSUFFICIENCY (PATIENT L.)

Day	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
1	850	1.022	0.02	0.17	1.76	14.9
2	770	1.022	0.04	0.31	1.74	13.4
3	545	1.025	0.03	0.16	1.56	8.5
4	420	1.025	0.02	0.08	1.56	6.6

In such instances, the minimal concentration of salt and the high percentage of nitrogen indicate the very limited ability of the kidney

to eliminate sodium chlorid, while the power to excrete nitrogen remains normal, provided a reasonable amount of urine is secreted.

Milder grades of myocardial decompensation yield similar results, though the sodium-chlorid elimination is not diminished to the same extent. These and two similar cases may be summarized in Table 14.

TABLE 14.—SUMMARY OF URINE TESTS IN CASES OF MYOCARDIAL DECOMPENSATION.[1]

Case	Urine		Sodium Chlorid		Nitrogen	
	Volume (c.c.)	Specific Gravity	Per Cent.	Grams	Per Cent.	Grams
1.....	540	1.020	0.25	1.34	1.44	7.78
2.....	573	1.019	0.28	1.62	1.76	10.08
3.....	484	1.022	0.52	2.53	1.58	7.64
4.....	446	1.018	0.61	2.72	1.38	6.15

[1] The low percentage of salt and the oliguria, as compared with the high concentration of nitrogen, are very strikingly shown in the above table.

DETERMINATION OF CHLORIDS.—Chlorids may be determined in the urine by the following methods as described by Hawk:

(*a*) *Volhard-Arnold Method.—Principle.*—The urine is acidified with nitric acid and the chlorids precipitated with a measured excess of standard silver-nitrate solution. The silver chlorid form is filtered off and the filtrate, the excess silver nitrate, is titrated back with standard ammonium thiocyanate solution. Ferric ammonium sulphate is used as an indicator. A red color due to the formation of ferric thiocyanate indicates that an excess of thiocyanate is present and that the end point has been reached.

Procedure.—Place 10 c.c. of urine in a 100 c.c. volumetric flask; add 20-30 drops of nitric acid (sp. gr. 1.2) and 2 c.c. of a cold saturated solution of ferric alum. If necessary, at this point a few drops of 8 per cent. solution of potassium permanganate may be added to dissipate the red color. Now slowly run in a known volume of the standard silver-nitrate solution (20 c.c. is ordinarily used) in order to precipitate chlorin and insure the presence of an excess of silver-nitrate. The mixture should be continually shaken during the addition of the standard solution. Allow the flask to stand 10 minutes, then fill it to the 100 c.c. graduation with distilled water and thoroughly mix the contents. Now filter the mixture through a dry filter paper, collect 50 c.c. of the filtrate and titrate it with standardized ammonium-thiocyanate solution. The first permanent tinge of red-brown indicates the end point. Take buret reading and compute the weight of sodium chlorid in the 10 c.c. of urine used.

Calculation.—The number of c.c. of ammonium-thiocyanate solution used indicates the excess of standard silver-nitrate solution in the 50 c.c. of liquid filtered. Multiply this reading by 2, inasmuch as only one-half

of the filtrate was employed, and subtract this product from the number of c.c. of silver nitrate (20 c.c.) originally used, in order to obtain the actual number of c.c. of silver nitrate utilized in the precipitation of the chlorid in the 10 c.c. of urine employed.

To obtain the weight in grams of the sodium chlorid in the 10 c.c. of urine used, multiply the number of c.c. of the standard silver-nitrate solution actually utilized in the precipitation by 0.010. If it is desired to express the result in percentage of sodium chlorid move the decimal point one place to the right.

In a similar manner the weight, or percentage of chlorin, may be computed, using the factor 0.006 instead of 0.010.

Standard silver-nitrate solution may be prepared by dissolving 29.042 grams of silver nitrate in 1 liter of distilled water. Each c.c. of this solution is equivalent to 0.010 gram of sodium chlorid or to 0.006 gram of chlorin.

This solution is made of such a strength that 1 c.c. of it is equal to 1 c.c. of the standard silver-nitrate solution used. To prepare the solution dissolve 13 grams of ammonium thiocyanate, NHSCN, in a little less than a liter of water. In a small flask place 20 c.c. of the standard silver-nitrate solution, 5 c.c. of the ferric alum solution and 4 c.c. of nitric acid (sp. gr. 1.2); add water to make the total volume 100 c.c., and thoroughly mix the contents of the flask. Now run in the ammonium-thiocyanate solution from the buret until a permanent red-brown tinge is produced. This is the end-reaction and indicates that the last trace of silver nitrate has been precipitated. Take the buret reading and calculate the amount of water necessary to use in diluting the ammonium thiocyanate in order that 10 c.c. of this solution may be exactly equal to 10 c.c. of the silver-nitrate solution. Make this dilution and titrate again to be certain that the solution is of the proper strength.

(*b*) *Volhard-Harvey Method.—Principle.*—This procedure differs from the Volhard-Arnold method in that the excess of silver nitrate is titrated directly without filtering, and hence in the presence of the silver chlorid. The procedure is thus more rapid but the exact end point is more difficult to determine.

Procedure.—Introduce 5 c.c. of urine into a small porcelain evaporating dish or casserole and dilute with about 20 c.c. of distilled water. Precipitate the chlorids by the addition of 10 c.c. of standard silver-nitrate solution and add 2 c.c. of acidified indicator.* Now run in a standard ammonium-thiocyanate solution from a buret until a faint red-brown tint is visible throughout the mixture. This point may be determined readily by permitting the precipitate to settle somewhat. Calculate the sodium-chlorid value, as indicated below.

* This is prepared as follows: To 30 c.c. of distilled water add 70 c.c. of 33 per cent. nitric acid (sp. gr. 1.2) and dissolve 100 grams of crystalline ferric ammonium sulphate in this dilute acid solution. Filter and use the filtrate, which is a saturated solution of the iron salt. This single reagent takes the place of the nitric acid and ferric alum, as used in the Volhard-Arnold methods, and insures the use of the proper quantity.

(If a red tint is produced when the first drop of thiocyanate is added, an additional 10 c.c. of the standard silver-nitrate solution must be introduced. The titration should then proceed, as above described, and proper allowance made in the calculation for the extra volume of silver nitrate employed.)

Calculation.—Since 2 c.c. of the ammonium-thiocyanate solution * is equivalent to 1 c.c. of the silver-nitrate solution, divide the buret reading by 2 and subtract the quotient from 10 c.c., the quantity of silver-nitrate solution taken. This value is the number of c.c. of silver-nitrate solution actually used.

Experimental Polyuria.—(*a*) *Strauss-Greenwald Method* (*Barton*). —The patient takes no nourishment after 7 P. M. At 6.30 A. M. the following morning a pint of water is ingested. The night urine is collected; also that voided at 7, 8, 9, 10, and 11 A. M. The amounts and specific gravity of each portion are recorded. The patient remains in a reclining position during the time of the test. In normal cases an amount of urine is passed in the first 3 hours, equal to that which was drunk. That is, by 10 A. M. at least a pint is voided. At 8 A. M. the specific gravity is lowest. Variations in the amount voided, time required, and specific gravity will indicate abnormal renal function.

(*b*) *Water Experiment of Kovesi and Roth-Schulz.*—At noon the patient is allowed to drink 1.8 liters of Salvator water in the course of one hour. The amount of urine is then measured and its freezing point determined in the fractions voided every half hour during the next 5 hours.

In acute nephritis the quantity of urine generally diminishes considerably at the onset of the disease, and usually at the same time when edema is developed. This is most obvious in scarlatinal nephritis, in which the primary seat of the lesion is glomerular. In these cases it is only the incapacity of the kidneys that is responsible for the rapid diminution of diuresis. There is neither molecular nor hydrostatic pressure of the blood. Moreover, the oliguria is found to be independent of the supply of fluid. In the first severe stages of the disease this supply increases the edema, but not the diuresis, or only to a very insufficient extent. It is, therefore, quite useless to give the patient large

* This is a solution of ammonium thiocyanate of such a strength that 2 c.c. is equivalent to 1 c.c. of the silver-nitrate solution. First make a concentrated solution by dissolving 13 grams in 1 liter of water. To determine the requisite dilution to make such a solution that 2 c.c. shall be equivalent to 1 c.c. of a silver-nitrate solution, proceed as follows: Introduce 10 c.c. of the silver-nitrate solution into a small porcelain evaporating dish or casserole; add 30-50 c.c. of distilled water, 2 c.c. of the acid indicator; and titrate, as described above, with the ammonium-thiocyanate solution. The total volume of the concentrated thiocyanate solution excluding that used in the titration is divided by 10, and the result multiplied by the difference between this buret reading and 20 c.c. This will give the volume of distilled water which must be added to the concentrated thiocyanate solution to render 2 c.c. equivalent to 1 c.c. of the silver-nitrate solution used in the precipitation of the chlorids. As 1 c.c. of the silver-nitrate solution is equivalent to 0.01 gram of sodium chlorid, the number of c.c. of silver-nitrate solution used, multiplied by 0.01 gram, will give the weight of sodium chlorid in the 5 c.c. portion of urine used. The weight of chlorin may be computed by using the factor 0.006 instead of 0.01.

quantities of fluid in order to wash out the kidneys. Such a measure unnecessarily irritates an organ which is already impaired (von Noorden). The spontaneous increase of diuresis is the first and surest sign of convalescence, or of transition into a chronic condition. Edematous fluid is excreted, and the kidneys at the same time begin again to react to the amount of liquid given.

Example.—A girl of fourteen, with acute scarlatinal nephritis, has taken daily 1,500 c.c. of milk. To this was added 1 liter of weak tea on the third and seventh days of the observation.

Day	Quantity of Urine	Extras
	c.c.	
1	550	..
2	320	..
3	350	1 liter tea
4	300	..
5	820	..
6	1,460	..
7	2,310	1 liter tea
8	1,670	..

"When the excretion of water and the reaction to the intake of water are lessened in cases of acute nephritis, it generally happens that the output of solid substances is also lowered (we are not here considering albumin). In chronic interstitial nephritis, on the other hand, it is frequently found that this parallel does not hold good. Thus, as long as oliguria persists in acute nephritis the valency figure (depression of the freezing-point in a sample of the total quantity of urine passed during twenty-four hours) is considerably below normal while

Table 15.—Freezing-point of Urine.

	Average Hourly Diuresis		Freezing-point Lowered	
	Spontaneous	After 1,800 c.c. Salvator Water During Three Hours	Spontaneous	After 1,800 c.c. Salvator Water During Three Hours
	c.c.	c.c.		
Normal	52	723	1.33°–2.17°	0.09°–0.75°
Acute nephritis	91	103	0.60°–0.75°	0.53°–0.87°

the molecular concentration of any one sample may remain within the normal limits. The defective reaction to the amount of fluid taken is all the more striking when it is a question of the physical analysis of the urine. The freezing-point of the urine—that is, its molecular concentration—varies hardly at all, whereas in healthy people that is not the case. Two examples given by G. Kovesi and W. Roth-Schulz illustrate this" (von Noorden).

Von Noorden considers that clinical experience justifies these authors in affirming that the defective adjustment of the kidneys to a varying water supply is an important clinical symptom. It is more characteristic of, and it is found more invariably in, cases of acute nephritis than either oliguria, hyposthenuria or oligochloruria.

(*c*) *Water Experiment of H. Strauss.*—In the evening the patient is allowed to take a pint of milk soup (unsalted). The following morning he is told to drink 500 c.c. of water, on an empty stomach. The bladder is emptied before the beginning of the experiment, about 10 P. M., and again at 5 A. M. For 5 hours following the ingestion of water at 6 A. M., no food or fluid is permitted. The amount of urine, the lowering of the freezing-point, and the sodium-chlorid content are determined.

Under normal circumstances, the polyuria appears within the first half hour, reaching its maximum at this time, and quickly sinking. The content of solids becomes less, and the depression of the freezing-point ($\triangle$) becomes less. With an incapacitated organ the excessive excretion of the water is delayed or does not take place and the cryoscopic determination will be altogether abnormal.

(*d*) *Opsiuria Test.*—Opsiuria, designating retarded urine excretion, is determined by the opsiuric test. The patient has his supper at 7 P. M., after which he may not take any more fluid. At 7 A. M. he empties his bladder, after which he takes 500 c.c. of ordinary drinking water, but nothing else (either to eat or drink) until 12 o'clock (noon). Punctually at 8, 9, 10, 11 and 12 o'clock the patient is asked to urinate. The amount of urine voided at each micturition is measured. At noon the test is finished. Normally, the urinary excretion increases pretty rapidly until the end of the second hour, after which it decreases rather quickly. Szollosy's observations showed that the opsiuric test is not only of value in the recognition of disturbances in the portal circulation —for the testing of which Gilbert and Lereboullet had especially introduced this method—but that it possesses a certain diagnostic value in other respects. By its employment the motor insufficiency of the stomach may be determined; the method may also be of value in recognizing certain disturbances of the circulation, particularly in beginning decompensation. It is of import in functional renal diagnosis.

2. Physical Methods for Testing Composition of the Urine.—For the determination of the freezing-point the following process may be used (Hawk).

FREEZING-POINT (*Cryoscopy*).—The freezing-point of a solution depends upon the total number of molecules of solid matter dissolved in it. The determination of the osmotic pressure by this method has come to be of some clinical importance, particularly as an aid in the diagnosis of kidney disorders. In this connection it is best to collect the urine from each kidney separately and to determine the freezing-point in the individual samples so collected. By this means, considerable aid in the diagnosis of renal diseases may be secured. The fluids most frequently examined cryoscopically are the *blood* and the *urine.* The freezing-

point is denoted by Δ. The value of the freezing-point for normal urine varies ordinarily between —1.3° and —2.3° C., the freezing-point of pure water being taken as 0°. Δ is subject to a very wide fluctuation under unusual conditions. For instance, following copious water, or beer drinking, the freezing-point may have as high a value as —0.2° C., whereas on a diet containing much salt and deficient in fluids the value may be lowered to —3° C. or even lower. The freezing-point of normal blood is generally about —0.56° C. and is not subject to the wide variations noted in the urine, because of the tendency of the organism to maintain the normal osmotic pressure of the blood under all conditions. Variations between —0.51° and —0.62° C. may be due entirely to a dietary condition, but if any marked variation is noted it can, in most cases, be traced to a disordered kidney function.

Freezing-point determinations may be made by means of the Beckmann-Heidenhain apparatus (Fig. 40) or the Zikel pektoscope. The Beckmann-Heidenhain apparatus consists of the following: A strong battery jar or beaker (C) furnished with a metal cover, which is provided with a circular hole in its center. This strong glass vessel serves to hold the freezing-mixture, by means of which the temperature of the fluid under examination is lowered. A large glass tube (B) designed as an air-jacket, and formed after the manner of a test-tube is introduced through the central aperture in the metal cover, and into this air-jacket is lowered a smaller tube (A) containing the fluid to be tested. A very delicate thermometer (D), graduated in hundredths of a degree, is introduced into the inner tube and is held in place by means of a cork, so that the mercury bulb is immersed in the fluid under examination but does not come into contact with any glass surface. A small platinum wire stirrer serves to keep the fluid under examination well mixed while a larger stirrer is used to manipulate the freezing mixture. (Rock salt and ice in the proportion 1:3 form a very satisfactory freezing mixture.)

In making a determination of the freezing-point of a fluid by means of the Beckmann-Heidenhain apparatus proceed as follows: Place the freezing mixture in the battery jar and add water (if necessary) to secure a temperature not lower than 3° C. Introduce the fluid to be tested into tube A, place the thermometer and platinum wire stirrer in position, and insert the tube into the air-jacket, which has previously been inserted through the metal cover of the battery jar. Manipulate the two stirrers in order to insure an equalization of temperature and observe the course of the mercury column of the thermometer very carefully. The mercury will gradually fall, and this gradual lowering of the temperature will be followed by a sudden rise. The point at which the mercury rests after this sudden rise is the freezing-point. This rise is due to the fact that, previous to freezing, a fluid is always more or less over-cooled and the thermometer temporarily registers a temperature somewhat below the freezing-point. As the fluid freezes, however, there is a very sudden change in the temperature of the liquid, and this change is imparted to the thermometer and causes the rise, as

indicated. It occasionally occurs that the fluid under examination is very much over-cooled and does not freeze. Under such circumstances, a small piece of ice is introduced into it by means of the side tube noted in the figure. This so-called "inoculation" causes the fluid to freeze instantaneously.

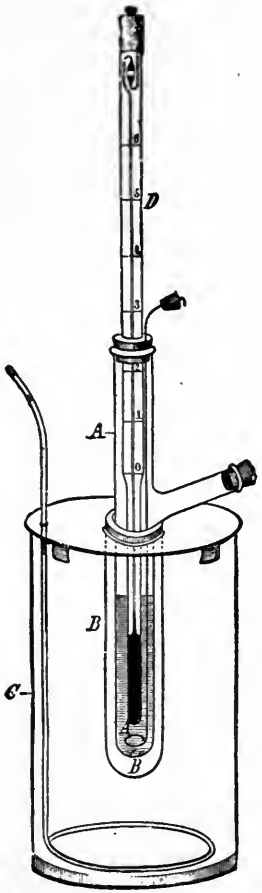


FIG. 40.—BECKMANN-HEIDENHAIN FREEZING-POINT APPARATUS (Long).

A, the tube in which the mixture to be observed is placed; B, the outside or air-mantle tube; C, the containing jar; D, thermometer. Two stirrers are shown, one for the cooling mixture in the jar and one for the experimental mixture.

According to Bugarsky a certain relation exists between the freezing-point depression and the specific gravity, namely:

$$K = \frac{\Delta}{S-1} = 75$$

The equation, where S represents specific gravity, has no general application, and, according to Steyrer, is only approximate for normal urines. The validity of the relation found by Bugarsky between the

electrical conductivity and the ash content of the urine seems also to require further proof.

Cryoscopy of the Urine (*Method of Dreser and of Koranyi*).—The method for the determination of the depression of the freezing-point ($\triangle$) of the urine has already been described (*see* p. 276).

Von Noorden says: "The hopes aroused by Dreser's and Koranyi's admirable work have vanished within a few years."

The urine contains organic and inorganic molecules in true solution. As a result, its freezing-point is lower than that of water. The difference may be expressed by the sign $\triangle$. In the mixed urine of twenty-four hours, the lowering of the freezing-point was found in healthy persons to vary between —0.87° and —2.43°. Examination of individual samples showed even greater variations. In consequence of the uniform relations which exist between the number of dissolved molecules in a solution and the freezing-point, the latter affords a convenient test for its molecular concentration. If this is increased, the condition is termed *hypersthenuria,* and if lowered, *hyposthenuria.* If the amount of the lowering of the freezing-point is multiplied by the quantity of the urine, a value for the total molecular output results. This product ($\triangle$ quantity of urine) is termed "valency value," as suggested by Strauss. In healthy people it varies considerably—from about 1,000 to 3,500. Pathological deviations, above or below, are denoted by the terms *polyvaluria* and *oligovaluria* respectively. It is convenient for many purposes to reckon the valency value in terms of its sodium-chlorid equivalent.

The quotient $\frac{\text{valency value}}{61.3}$ enables one to determine approximately how many grams of sodium chlorid must be contained in the urine in order to produce the given freezing-point. If this estimate has been arrived at, and the true sodium-chlorid content of the urine determined, it is possible by subtraction (sodium-chlorid equivalent — sodium-chlorid content) to arrive at the sum-total of the other urinary substances in solution.*

Quantity of urine..... = 1,500 c.c.
$\triangle$..... = 1.40°
Valency ..... = 2,100
NaCl equivalent ..... = 2,100 ÷ 61.3 = 34.2 grams
NaCl in urine..... = 10.8
NaCl equivalent of the other dissolved constituents ..... = 34.2 — 10.8 = 23.4 grams

* The doubtful element in estimating the NaCl equivalents lies in the fact that all urines have the same degree of dissociation of a 1 per cent. solution of sodium chlorid (A. Steyrer) for all their inorganic molecules. Urine which has been diluted to one-tenth of its usual density promises more accurate values as regards the determination of the freezing-point and the estimation of its products because, with this degree of dilution, the dissociation of all salts has reached a definite constant (Zangemeister). The formula would then read $\frac{\triangle \times \text{total quantity of urine} \times 10}{0.613}$

Very concentrated urine must be diluted from fifteen to twenty times, and the multiplication figure altered correspondingly. The figures 0.613 in the divisor indicate the lowering of the freezing-point in a 1 per cent. NaCl solution.

In normal urine the determination of the freezing-point is hardly more instructive than the specific gravity. The latter also depends upon the degree of concentration, and this fact and the quantity of urine may be very important factors in determining the total excretion of solid substances (Haser's Coefficient). If, however, the urine contains albumin the position is altered. The specific gravity will be greatly affected by the large and heavy albumin molecules, but the freezing-point hardly at all—e.g., 2 per cent. of urea lowers the freezing-point of water by 0.616°, 2 per cent. of albumin by only 0.0037°. Hence cryoscopy is a better test than the specific gravity of the output of extractives (salts and organic products of metabolism) in urine containing albumin. But cryoscopy has also its dark side, and is a fruitful source of error. This is due to the dissociation which takes place in watery solutions of salts. The greater the dilution, the greater the dissociation of salts. The dissociated NaCl molecule acts, not as one, but as two units; the dissociated Na_2SO_4 molecule not as one, but as three units, on the osmotic pressure (freezing-point). In dilute urine the freezing-point will be relatively lower than in concentrated urine, and though the amounts of solid substances may be absolutely equal (urea NaCl, and other salts, etc.), the product of the depression of the freezing-point multiplied by the quantity of urine (the important valency value) will be considerably higher in dilute than in concentrated urine. Although Koeppe has already pointed out the sources of error, practical cryoscopy has not taken them into consideration. They account for many apparent contradictions. Zangemeister has recently recommended that each specimen of urine should be diluted until the dissociation degree has reached a definite and approximately constant maximum before the cryoscopic determination is made. For this, dilution of from 10 to 15 times the volume of distilled water is sufficient.

The following example shows the effect of this method: For undiluted urine (1,216 c.c.) a valency value of 1,012 was found, and after dilution one of 1,189 was found. Zangemeister's suggestion is well worth considering. At all events, he excludes the most important source of error in cryoscopic determinations.

The Hemorenal Index.—The electrical conductivity of the urine in health and disease was first studied by Turner, who has given the name "hemorenal index" to the relation of the concentration of total electrolytes in the urine to that in the blood. Normally this relation is as 2.08 : 1, and the hemorenal index is, therefore, 2.08. According to Bromberg, there is a disturbance in the excretion of electrolytes in renal disease which is apparent early in its course. A hemorenal index under 2 is significant of early disease of the kidneys. He has suggested this method as a means of determining the functional sufficiency of the individual kidney by ureteral catheterization.

In 1913, Bromberg reported that ureteral catheterization is superfluous. When the hemorenal index is 2, the kidneys are either healthy, or only one of them is diseased. This fact can be determined by cystoscopy or chromocystoscopy. If the index is less than 2, bilateral

disease of the kidney exists. If the index is less than 1.5, whatever the condition is, it is inoperable (Barker).

(1) *Apparatus and Method in General* (Fig. 41).—The method of

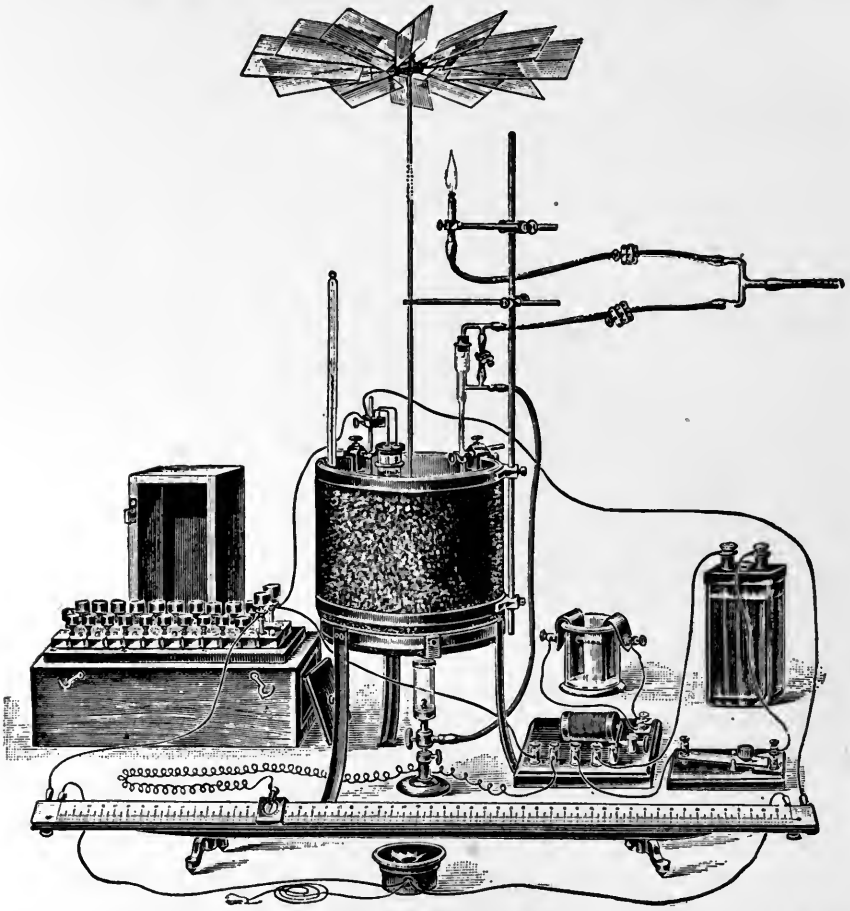


FIG. 41.—APPARATUS FOR THE DETERMINATION OF THE CONDUCTIVITY OF ELECTROLYTES (DI-ELECTRIC CONSTANT). (Kohlrausch-Ostwald.)

Conductivity Vessel.	Storage Battery.
Constant Temperature Bath.	Contact Key.
Support for Conductivity Vessels.	Slide Wire Bridge.
Thermometer.	Telephone.
Induction Coil.	Resistance Box.
Condenser.	

Kohlrausch * depends upon the use of alternating currents, in connection with the Wheatstone bridge.

The element, E, (Fig. 42) is connected with the induction coil, J, the wires of which are fastened to the measuring bridge, B, by means of

* Bibliography, The hemorenal index, Kohlrausch, 1879 (1), 1885 (9), 1893 (14); Ostwald, 1888 (6).

binding screws. The circuit of the bridge is made complete by means of a metallic wire, which passes through each of the binding screws and finally to the resistance solution, W, and the comparison resistance, R. To the binding screw between W and R is fastened a telephone wire, the other end of which leads to a movable metallic slide on the bridge.

By closing the circuit the element is set in operation, and the slide can be placed at such a position on the bridge that no current will pass through that part of the conductor in which the telephone is introduced.

The interposed telephone furnishes a means of determining the proper adjustment, instead of the galvanometer or dynamometer, in that when

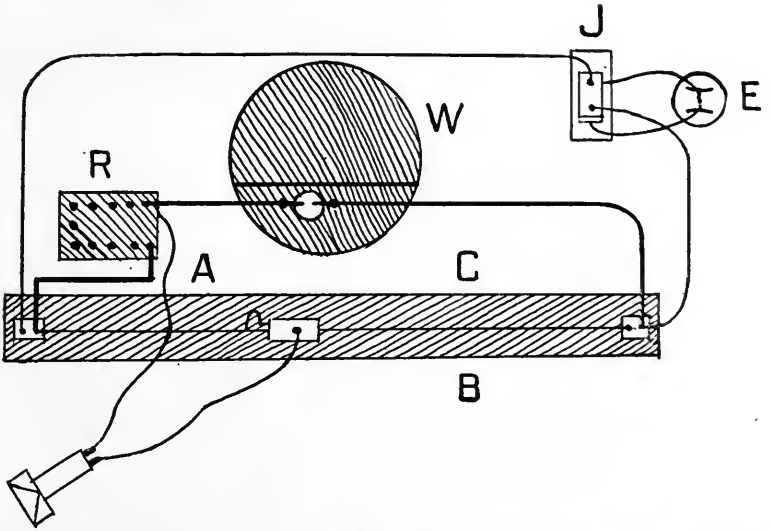


FIG. 42.—DIAGRAM OF APPARATUS FOR THE DETERMINATION OF CONDUCTIVITY OF ELECTROLYTES.

the slide is at the proper position the telephone is either silent or produces a minimum tone which is augmented by moving the slide in either direction on the scale of the bridge.

From the position of the slide corresponding to the minimum tone, we deduce, by means of Kirchhoff's laws,* the proportion $W : R = a : b$.

W is the resistance of the liquid and the electrode vessel; R is the interposed comparison resistance; a and b represent the number of divisions on the scale of the bridge, to the left and right of the slide when properly adjusted. The unknown resistance of the liquid or the reciprocal value, the electric conductivity, may be calculated from the above proportion.

(2) *The Apparatus in Detail.—The Element* (Fig. 43).—A small Bunsen chromic-acid element is sufficient. For the preparation of 1 liter.

* Bibliography, The hemorenal index, Ostwald, 1885 (1); Kohlrausch, 1895 (18).

of solution, 92 grams of pulverised potassium bichromate (or a corresponding quantity of the more soluble sodium salt) and 94 c.c. of concentrated sulphuric acid are rubbed together to a uniform pasty liquid; 900 c.c. of water are then carefully added, while stirring.

The Induction Apparatus.—According to Ostwald, the smallest induction coil used for medical purposes is best adapted to this work. The rapidity of the vibration of the interrupter is increased still further

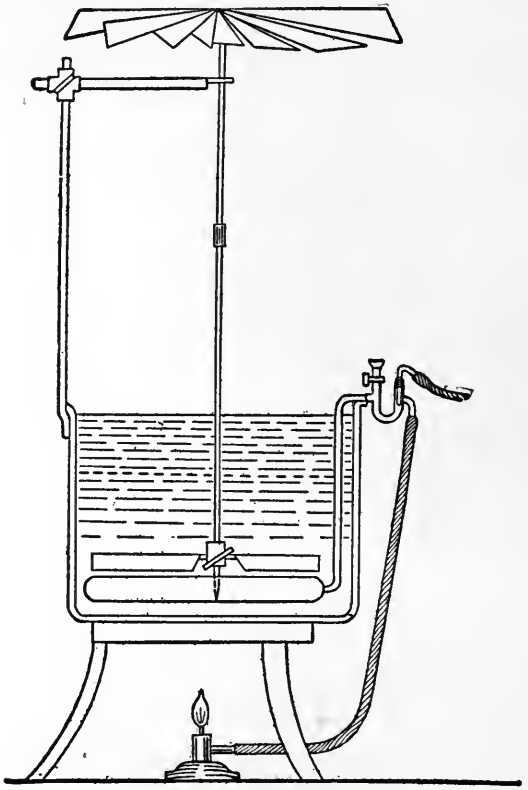


FIG. 43.—DIAGRAM OF CONSTANT TEMPERATURE BATH.

by filing the little block of iron on the spring down to rather small dimensions (1 or 2 mm.). The minimum tone of the telephone is sharper if the sliding brass tube is removed from the apparatus.

The sharpness of the telephone depends largely upon the nature of the induction apparatus used,* especially on the speed with which the current is alternated. If, therefore, the tone-minimum is not sharp, different induction coils should be tested, and the most suitable selected.

The Measuring Bridge.—Instead of the cylindrical bridge ** proposed by Kohlrausch, the bridge which is ordinarily employed may be

* Bibliography, Kohlrausch, 1879 (3).
** Bibliography, Kohlrausch, 1880 (8).

used. The simplest form * of the same (Fig. 44) is constructed as follows:

A paper (or wooden) scale, graduated in millimeters, is fastened on to a dry board of 110-120 cm. in length and 4-6 cm. in breadth. A metallic wire is stretched along the scale by means of the screws (A) on the ends of the board. Two brass plates are fastened by means of the screws (B) on the board, perpendicular to the scale, so that their inner edges pass exactly through the divisions 0 and 100 on the scale. The screws (B) serve also for fastening the wires from the induction apparatus and the resistances.

The bridge wire, which is about 0.2 mm. in diameter, should be made of perfectly clean German silver, or of platinum containing iridium. The German silver wire, owing to its gradual oxidation in the chemical laboratory, gives rise to irregular sounds in the telephone; the wire should therefore be renewed from time to time.

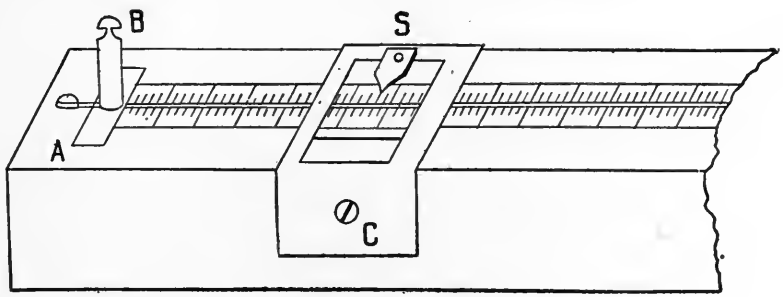


Fig. 44.—The Measuring Bridge.

For all exact measurements the uniformity of the wire should be tested and the wire calibrated before use. This may be done by the method of Strouhal and Barus.

The slide S of the measuring bridge, in its simpler form, consists of a rectangular, bent-down, metal frame with a small binding screw (C), with which the telephone is connected. It is adjusted to the bridge so that it may be easily moved. In the middle of the frame and in contact with the bridge wire, is a flexible German silver needle, the flattened point of which is so constructed that it is always in perfect contact with the wire. The sensitiveness of the telephone depends on the kind of contact.**

The Telephone.—Care should be exercised in the choice of the telephone. The Bell Telephone is well adapted to this work. Ostwald recommends the telephone of Ericsson, in Stockholm, as being very sensitive.

The sounds proceeding from the induction apparatus are annoying at first to the unpracticed. A small bulb of some wadding placed in

* Bibliography, Ostwald, 1888 (7); Wiedemann and Ebert, 1890.
** Bibliography, Elsas, 1891 (1).

the ear will remove this inconvenience. One learns very quickly, however, without this, to distinguish between the two sounds.

The Comparison Resistance.—Three resistances of 10, 100, and 1,000 units (Ohm or Siemens) are sufficient. More appropriate, however, and far more convenient is the use of a complete resistance-box, the greatest resistance of which amounts to 2,000 Siemens-units. By using greater resistances, one obtains better results; it is better to work with different electrode distances. The best results are usually obtained with resistances of from 100-1,000 units.

The resistances should be wound * bifilarly, and should be compared,

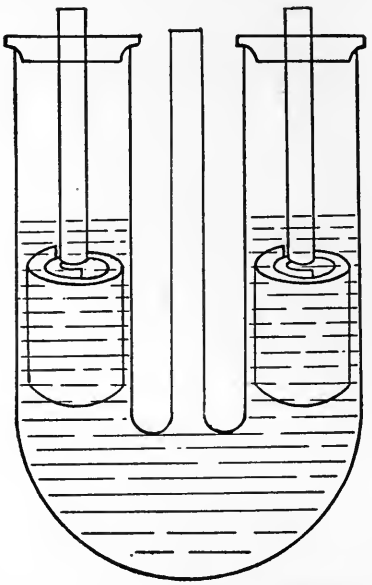


FIG. 45.—ELECTRODE VESSEL FOR LIQUIDS OF HIGH CONDUCTIVITY.

at least twice a year, with a standard resistance; at all events, one should satisfy one's self by testing the apparatus, from time to time, with solutions of known resistances.

As the resistance varies with the temperature, it is necessary, for accurate determinations, to make correction for this variation.

The Electrode Vessels.—Three different vessels are used for this purpose, according to the nature of the liquid to be investigated.**

(*a*) For Liquids of Rather High Conductivity (Concentrated aqueous solutions of salts, mineral acids and bases).—For such solutions, use may be made of the vessels employed by Kohlrausch† (Fig. 45).

* A new kind of winding by Chaperon. *See* Bibliography, Elsas, 1891 (2), Kohlrausch, 1893 (15).

** See also Kohlrausch, Wied. Ann., 1894, li, 346, for special forms of electrodes and vessels.—Tr.

† Bibliography, Kohlrausch, 1879 (2).

Two cylindrical beakers, which are reduced in size at the lower end, are joined together by means of a tube of about 9 mm. in diameter. The vessel contains from 12 to 25 c.c. of liquid. The electrodes consist of platinum, and are soldered to a copper wire which is introduced through a cover of hard rubber.

(*b*) For Liquids of Moderate Conductivity (Dilute aqueous solutions of salts, mineral acids and bases, about 1/32 to 1/1024 normal; also concentrated solutions of many organic acids and bases).—The resistance vessel best adapted to such solutions is that proposed by Arrhenius (Fig. 46).

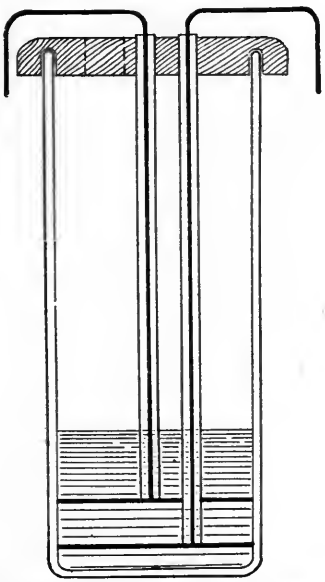


FIG. 46.—ELECTRODE VESSEL FOR LIQUIDS OF MODERATE CONDUCTIVITY.

Two circular plates of heavy sheet-platinum from 3 to 4 cm. in diameter are soldered, by means of silver solder and borax, to heavy copper wires; the distance between the plates should be about 1.5 cm. The wires are enclosed in narrow glass tubes which are carefully filled with thick liquid asphaltum. The joint between the platinum and the glass should be well covered with the asphalt glue. The wires are introduced through a cover of hard rubber which is fastened by means of a groove to the glass cylindrical vessel. It is advantageous to have two such vessels with the electrodes 1 and 2 cm. apart.

(*c*) For Liquids of Low Conductivity (Most dilute solutions of the organic acids and bases, especially aqueous solutions of neutral organic compounds, as well as homogeneous organic liquids and their mixtures).—For such liquids use is made of very narrow electrodes with greater surfaces; the apparatus of Pfeiffer * is well adapted to this work.

* *See* Bibliography, Pfeiffer, 1885.

Two glass tubes, R and R′, of 3 and 3.6 cm. in their outer diameters, are covered to a length of 13 cm. with platinum foil, P′ and P, the smaller tube being covered on the outside and the larger tube on the inside. The platinum-foil must form complete immovable cylinders. The larger platinum cylinder (soldered at the seam by means of silver) is inserted in the wider tube. The smaller glass tube, after being fused together at the top, is then placed into the larger, so that the two platinum surfaces form concentric cylinders. These cylinders are

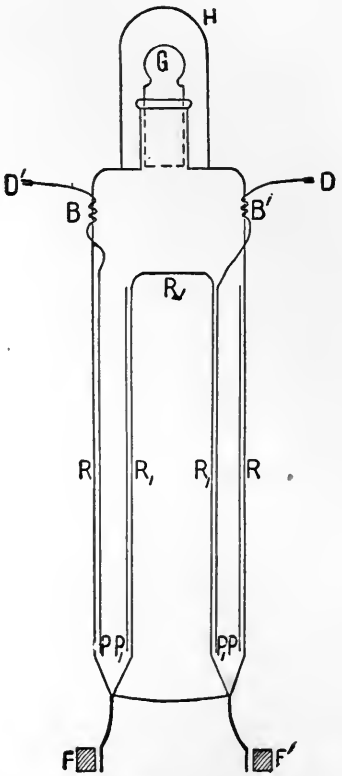


Fig. 47.—Electrode Vessel for Liquids of Low Conductivity.

fastened to the outer glass surface by means of platinum wires. On account of the large area, the surface of the platinum need not be covered with platinum-black.

The two glass tubes are then melted together all around their edges at the bottom, as shown in Fig. 47; and at the opposite end the outer tube is drawn out into a narrow neck, in which can be placed a glass stopper, G, which, in turn, is covered by the glass covering, H. D and D′ are the two electrodes leading to the platinum wires fused into B and B′. The whole vessel (30 cm. high) is supported by means of the glass tubes fused in at the bottom, which are fastened to the strong brass feet, F, F′.

If a resistance vessel is to be used, it is best to restrict one's self to the form proposed by Arrhenius, which, by the application of larger resistances, is sufficient for the majority of liquids.

The electrodes must always have a sufficiently large surface. A surface which is too small is accompanied by too great a current density, which frequently produces the phenomenon of polarization; the sharpness of the minimum tone depends largely upon the frequency of the replatinizing of the surface.*

For this purpose the electrodes are immersed in a dilute solution of platinum chlorid acidulated with hydrochloric acid, and the current of a single cell is conducted through, the direction of the current alternating frequently between the electrodes; or a small piece of zinc is brought in contact with the platinum surface until the plates are covered with a coating of black, spongy platinum.

According to Grotrian,** the well-platinized electrodes should be saturated with hydrogen by immersing them as cathodes in dilute sulphuric acid. It is especially necessary that the electrodes be well platinized.

The Thermostat.—On account of the great influence of temperature on conductivity,† accurate measurement of temperature is important.

Although Kohlrausch‡ has observed an influence of liquid baths on the minimum tone, and therefore made his determinations in air at the temperature of the room, yet the following, essentially the arrangement proposed by Ostwald, is to be preferred for convenience.

The electrode vessel is supported in the iron water-bath of 10 to 20 liter capacity, by means of a half-circular wooden cover.

A temperature regulator constructed in the ordinary manner, is fastened to this water-bath as shown in Fig. 41.

The regulator consists of a U-tube in which is fastened, by means of a rubber stopper, a cylindrical tube (Fig. 48, A). This tube is filled with oil or a concentrated solution of calcium chlorid, and then connected with the U-tube. A sufficient quantity of mercury is then conducted in through the funnel, after which, by suitably inclining the U-tube, the air in the part between the mercury and the funnel is completely replaced by oil or calcium-chlorid solution. The inflow and outflow of gas is indicated by the direction of the arrows.

To increase the sensibility, the inner portion of the gas-inlet tube is cut off at right angles. A very small side-opening in the same prevents the putting-out of the flame. By conducting in or removing liquid, by means of the funnel, the position of the top of the mercury column near the gas-inlet tube can be so regulated that widely varying temperatures may be obtained.

As a motor, the windmill of Ostwald§ may be used. It is advan-

* Bibliography, Kohlrausch, 1873 (20 and 21).

** *See* Bibliography, Wershofen.

† For most electrolytes, a change of one degree Celsius changes the conductivity about 2 per cent.

‡ Bibliography, Kohlrausch, 1885 (12). Kohlrausch also observed that the telephone, if too near the apparatus, was influenced by the induction coil. See also Kohlrausch, 1893 (16).

§ *See* Bibliography, Ostwald, 188 (8).

tageous in this case to have the fans made of thin aluminum plates.

The use, however, of Raabe's turbine seems, in general, to be more appropriate. The everywhere purchasable turbine, after thorough oiling of the parts of the apparatus, is connected by means of a cork to a double wheel, which turns on a brass axis. From here the motion is transferred to a smaller wheel, or directly to the stirrer, which consists of a glass rod fastened to the wheel and provided at the lower end with a glass or wooden paddle; the glass rod rotates in a wider, well-oiled glass tube. By employing this thermostat, it is possible to maintain the temperature constant to 0.05° C. For temperatures of 50° C. and over,

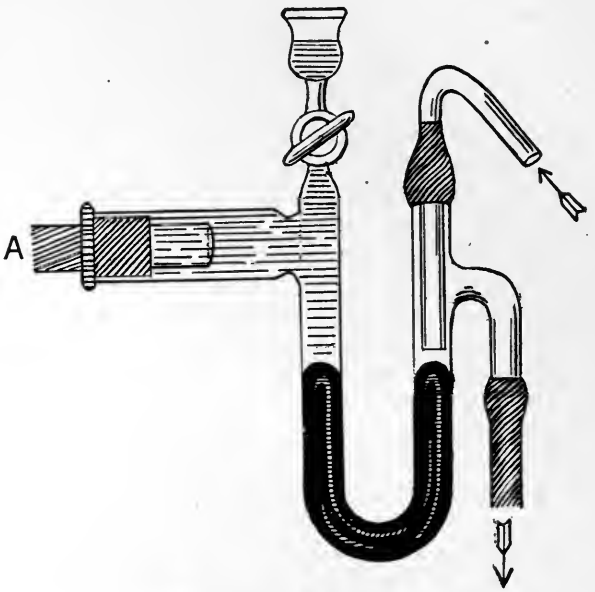


FIG. 48.—THE REGULATOR.

the water is covered with a layer of paraffin; for temperatures above 80° C. an altered thermostat is used as described by Bersch.*

The Contact.—The connection of the different parts of the apparatus is made by means of strong copper wires, which are as short as possible and fastened to suitable metallic clamps. A perfect contact is, above all, very important. The places of contact of the wires and the binding screws are carefully cleaned from time to time with a file and sandpaper; the binding screws should be well tightened, the stopper placed firmly in the rheostat, and the needle of the slide brought in good contact with the wire of the bridge. The contact with the electrode wires is made by means of small cups of mercury, which, like the electrode vessel, are placed in the opening of the wooden cover of the thermostat. The copper wire of the electrodes should be carefully cleaned, moistened with hydrochloric acid and immersed in the mercury.

* *See* Bibliography.

(3) *Method of Operation.*—As soon as the liquid takes on the temperature of the thermostat, the element is set in operation and the slide given such a position that the telephone is either silent or produces a minimum tone, the intensity of which increases with the least movement of the slide in either direction. In general, the adjustment is made so sharply that the two points on the scale at which an increase of tone may be distinctly recognized are not more than 2 mm. apart. Midway between these two points is the correct position, which, after some practice, can easily be determined accurately to from 0.2 to 0.3 mm. The resistances are so inserted that the slide, when adjusted, will lie near the middle of the measuring bridge; an error of 0.3 mm. in the adjustment here will produce an error of about 0.1 per cent. in the value of the conductivity.

Each determination should be repeated by inserting different resistances, and the mean value taken as the basis of the calculation.

An indistinctness in the minimum tone may, as already suggested, be due to very different causes. The difficulty is easily removed by replatinizing the electrodes; sometimes an increase in the electrode surface is necessary, varying according to the concentration and nature of the solution, as well as the amount of resistance inserted; frequently, also, a more rapid alternation of the current is necessary, and in many cases the cause lies in the amount of resistance. A resistance of from 100 to 1,000 mercury units gives, in general, the best results.

After a little practice, 10 to 12 solutions may easily be investigated in half an hour.

(4) *Calculation.*—The measurements for chemical purposes are limited almost wholly, in recent times, to the determination of the molecular conductivity represented by the constant μ* (or λ, according to Kohlrausch).

It is calculated from the formula:

$$\mu v = \gamma \frac{vb}{ra}$$

or, for aqueous solutions, taking into account the conductivity of the water employed, according to the formula:

$$\mu v = v\gamma \left(\frac{b}{a\ r} - \frac{b}{a_w\ r_w} \right)^{**}$$

where μ represents the molecular conductivity at the dilution v; v, the volume in liters, which contains 1 gram equivalent† of the electrolyte; a and b, the lengths of the wire to the left and right of the slide; a_w and b_w, the corresponding lengths for water; r and r_w, the re-

* For the meaning of this constant, *see,* in Bibliography, Ostwald, 1893 (2) and Kohlrausch, 1879 (6), 1885 (10).

** The formula follows directly from the proportion above. The correction for the conductivity of water is somewhat uncertain; for solutions of low conductivity the value of μv is doubtful.

† It is better to refer the conductivity to a gram-molecule of the substance, as it is impossible to separate acids, bases, salts, etc., from indifferent substances.

sistances in mercury units of the solution and the water; γ, the resistance capacity of the measuring vessel.

In order to determine the resistance capacity of the vessel, i.e., the resistance which a liquid with unit conductivity would show in the vessel, a liquid is used whose conductivity is accurately known. A 1/50 normal calcium-chlorid solution which has kept for a long time unchanged has, according to Kohlrausch, a molecular conductivity at 18° C. of 112.2, and at 25° = 129.7. This value in the above formula will give the value of the constant γ. For liquids of low conductivity and narrow electrodes, it is better to take a more accurately investigated solution of low conductivity—e.g., a solution of tartaric acid.

The molecular conductivity μ at 25° is, according to Ostwald:*

ν	16	32	64	128	256	512	1024	2048
μ	11.40	16.03	22.47	31.28	43.50	59.51	81.64	109.50

The reduction of the specific conductivity, k, to terms of μ follows from the relation $\mu = 10^7 \cdot k \cdot \nu$, where μ and ν have the meaning given above.**

The ratio $\frac{a}{b}$ is given for a wire 1 m. long in the table of Obach.

If the resistance of the wire is not uniform, the wire must be calibrated, and a corresponding correction applied to the above value.

The resistance, r, also requires a small correction. For most ordinary German-silver wires, an increase of 1° C. in temperature increases the resistance on an average of 0.0004 parts of the whole value. The necessary reduction to mercury units at 0° C. is easily made.

If one wishes (which is not always permissible) to establish the value, μ^{∞}—i.e., the conductivity at infinite dilution—it is determined indirectly, inasmuch as it does not result from direct observation.† For the calculation of the Ostwald constant ‡:

$$K = \frac{m^2}{(1-m)\,v}\,100$$

The value of μ is usually determined at the temperature 25° (the measurements of Kohlrausch were referred to 18° C.). On the temperature influence or the calculation of the temperature coefficient, *see* Bibliography, Kohlrausch, 1879 (4) and 1885 (13). If the determinations are made at higher temperatures, the error due to the dissolving of the glass must be taken into account. It is therefore advantageous to boil water in the electrode vessel several times before use.§

For the calculation of the velocity of ions and the transport numbers from the conductivity, *see* Bibliography, Kohlrausch, 1879 (7), 1885 (11); Lob and Nernst, 1888; Kistiakowsky, 1890; Bein, 1892.

* Bibliography, Ostwald, 1889 (10).
** Bibliography, Ostwald, 1886 (3).
† *See* Bibliography, Ostwald, 1888 (9).
‡ *See* Bibliography, Ostwald, 1888 (5).
§ Bibliography, Arrhenius, Krannhals and Sack.

3. **Renal Function as Determined by Excretion of Ingested Substances.**—Lactose Test.—Schlayer and Takayasu, in 1911, found that delayed excretion of lactose indicated disease of the renal blood-vessels —glomerulonephritis. It had been demonstrated by Voit, in 1897, that normal kidneys excreted lactose, ingested subcutaneously, quite promptly, and De Bonis, in 1907, had shown that this was accomplished exclusively by the renal glomeruli.

The *technic* of the test is as follows:

Twenty-five grams of lactose (absolutely pure, and free from moulds) are dissolved in 25 c.c. of distilled water, and pasteurized at 75°-80° C. for four or five hours on each of three successive days. Twenty c.c. of this solution are injected intravenously, aseptically. The urine is collected hourly and tested by Nylander's solution until the reaction ceases to be positive. Mishaps have occasionally occurred, such as chill and fever, but with a pure lactose these can be avoided.

Wechselmann has applied this test in the last two and a half years in many thousands of cases, and since he has been using an absolutely pure article he has had no mishaps or by-effects with it. His experience has confirmed its value as an index of the functioning of the glomerular apparatus. The elimination of salvarsan depends to such an extent upon the integrity of this apparatus, that tests of its functional capacity are of fundamental importance before giving salvarsan. Wechselmann finds that the value of the lactose test has been universally recognized, and adds that recent research by Schwarz and Pulay on it has opened new prospects for it. The by-effects which have deterred a number from continuing to use it have been traced to contamination of the lactose with molds.

Rowntree and Fitz have demonstrated that not only the glomerulus but also the kidney tubule takes part in the lactose excretion, and they think that lactose excretion is quite a satisfactory index of the vascular function of the kidney.

Bismuth Reduction Test (Nylander).—To 5 c.c. of sugar solution in a test-tube add 1/10 its volume of Nylander's reagent and heat for five minutes in a boiling water-bath. The solution will darken if reducing sugar is present, and upon its standing for a few moments a black color will appear.

This color is due to the precipitation of bismuth. If the test is made on urine containing albumin this must be removed, by boiling and filtering, before applying the test, since with albumin a similar change of color is produced. Glucose, when present to the extent of 0.08 per cent., may be easily detected by this reaction. (Rabe claims that 0.01 per cent. sugar may be so detected.) Uric acid and creatinin, which interfere with the Fehling test, do not interfere with the Nylander test. It is claimed by Bechold that the bismuth reduction tests give a negative reaction with solutions containing sugar when mercuric chlorid or chloroform is present. Other observers have failed to verify the inhibitory action of mercuric chlorid and have shown that the inhibitory influence of chloroform may be overcome by raising the tem-

perature of the urine to the boiling-point for a period of five minutes previous to making the tests. Urines rich in indican, urochrome, uroerythrin or hematoporphyrin, as well as urine excreted after the ingestion of large amounts of certain medicinal substances, may give a darkening of Nylander's reagent similar to that of a true sugar reaction.

Nylander's reagent is prepared by digesting 2 grams of bismuth subnitrate and 4 grams of Rochelle salt in 100 c.c. of a 10 per cent. potassium-hydroxid solution. The reagent is then cooled and filtered.

Potassium-Iodid Test.—In 1867, Duckworth suggested this test as an estimation of the renal function. Schlayer and Takayasu have suggested this as a quantitative test. The patient receives 0.5 gram of potassium iodid, in solution, by mouth, and the urine is tested every two hours for iodin. Iodid is rapidly excreted, since 65-80 per cent. of the amount ingested is eliminated in 24 hours. According to Antem, it takes 40 hours for the complete excretion of 0.5 gram KI, whereas others found that it requires 2 days for the elimination of this quantity (Monakow).

According to Schlayer and Takayasu, the iodid is excreted by the tubular epithelium. This has, however, not been confirmed.

Rowntree and Fitz found this test of doubtful value.

Wovschin obtained the results given in Table 16 after administration of potassium iodid by mouth and rectum. He found that the latter means of administration may be made use of in comatose patients.

Potassium Iodid in the Urine.—*Qualitative Determination.*—To the urine in a test-tube add a little chloroform, a few drops of sodium nitrite solution, and a few drops of dilute sulphuric acid. Shake well, and the free iodin will be dissolved in the chloroform. In the presence of starch solution, a blue color will be formed, if the urine is treated as above.

The technic employed for the *quantitative analysis* is the following: N/50 solution of sodium thiosulphate ($Na_2S_2O_3$) is prepared and carefully standardized against a definite quantity of potassium iodid. The standardization is performed as follows:

An exact 10 per cent. solution of potassium iodid is used. One c.c. of this 10 per cent. solution is diluted up to 100 c.c. with distilled water and introduced into a separating funnel. To this is added 5 c.c. of a mixture composed of strong nitric acid plus 5 per cent. of nitrous acid, and well shaken. This is allowed to stand 5 minutes and then 10 c.c. of chloroform are added. This should be well agitated to wash out the liberated iodin. This is allowed to stand for 5 or 10 minutes and the purple colored chloroform drained off from the bottom of the separating funnel into a beaker. The iodin extraction is continued and repeated with several fresh, small portions of chloroform and all of the chloroform used for the washing is collected in the same beaker. The washing should be continued until the returning chloroform is colorless.

Next the chloroform-iodin contents should be carefully titrated with

TABLE 16.—RESULTS OF DETERMINATION OF POTASSIUM-IODID ELIMINATION IN URINE (Wovschin)

Clinical Diagnosis	1 Gm. of Potassium Iodid Given by						
	Blood-Pressure	Mouth (*Day*)			Rectum (*Day*)		
	Mm. Hg	1st	2nd	3rd	1st	2nd	3rd
Cardionephritis	S. 200 / D. 50	0.25	0.01	..	0.32	0.1	..
Influenza	S. 115 / D. 80	0.51	0.1	Trace	0.3	0.05	..
Emphysema	S. 160 / D. 75	0.4	....	..	0.6	0.06	Trace
Bronchial asthma	S. 120 / D. 90	0.32	0.01	..	0.52	0.08	Trace
Rheumatic endocarditis	S. 125 / D. 80	0.5	0.15	..	0.65	0.02	..
Carcinoma of intestines	.....	Not given	Not given	..	0.75	0.15	..
Typhoid abscess of lung	S. 130 / D. 80	0.29	0.01	..	0.45	0.1	..
Peliosis rheumatica	S. 120	0.75	....	..	Not given	Not given	..
Nephritis	S. 180 / D. 80	0.3	0.03	..	0.28	0.08	..
Nephritis	S. 145 / D. 80	0.2	....	..	0.32	0.06	..
Nephritis	S. 165 / D. 85	0.39	0.01	..	0.45	0.06	Trace
Nephritis	S. 155 / D. 95	0.18	0.04	..	Not retained	Not retained	..
Uremia	S. 260 / D. 155	0.1	....	..	0.24	0.1	..
Uremia	S. 185 / D. 95	0.2	....	..	0.35	0.06	..
Nephritis	S. 160 / D. 90	0.09	....	..	0.28	Trace	..
Acute nephritis	S. 120 / D. 85	0.36	0.15	..	Not retained	Not retained	..
Arteriosclerosis	S. 145 / D. 85	0.35	0.02	..	0.45	0.06	..
Rheumatic endocarditis	.....	0.65	0.09	..	Not retained	Not retained	..
Pneumonia	S. 130 / D. 80	0.5	....	..	Not given	Not given	..
Pneumonia	S. 140 / D. 95	0.32	....	..	Not given	Not given	..
Pneumonia	S. 135 / D. 90	0.3	....	..	Not given	Not given	..
Pneumonia	S. 130 / D. 90	0.42	....	..	Not given	Not given	..
Influenza	.....	0.52	0.04	..	0.48	0.09	..
Auricular fibrillation	.....	0.5	0.2	..	0.42	0.08	Trace

the fiftieth normal sodium-thiosulphate solution until the purple chloroform becomes completely colorless. Having thus determined the amount of sodium-thiosulphate solution necessary to decolorize the iodin of 1 decigram of potassium iodid, its equivalent for 1 c.c. of N/50 $Na_2S_2O_3$ is calculated, using the obtained figure as the standard.

HIPPURIC ACID TEST.—It is stated by Jaarsveld and Stocvis, Stocvis and von der Velde that in cases of parenchymatous nephritis the synthesis of benzoic acid and glycocoll in the kidneys is only imperfectly accomplished, and that only a small proportion of ingested benzoic acid is found as hippuric acid in the urine of these patients. Von Schroeder and Schmiedeberg do not consider these theories as established, since Stocvis used a method which was not reliable.

On the other hand, Kronecker observed defective formation of hippuric acid in nephritis; but, unlike Stoevis, he found it more defective in cases of interstitial than in parenchymatous nephritis. Recent experiments with better methods, and where the nature of the diet was very carefully considered, are not favorable to Stoevis's views, but go to show that spontaneous excretion of hippuric acid is not more difficult than that of any other substance. The addition of benzoic acid to the diet yielded varying results, as in the case of healthy persons. The question has lost much of its interest since it has been recognized as probable that other organs besides the kidneys are able to synthesize hippuric acid. This is not the case with carnivora. At the same time, the matter deserves further inquiry. It is now evident that glycocoll may be considered as a normal constituent of the urine (whether free or combined is not yet fully determined). This fact was not known to earlier investigators.

Phlorizin Test.—Achard and Delamare suggested this glucosid injection as a test for renal function. It is known from the work of von Mering that the injection of phlorizin into animals induces a marked glycosuria without a hyperglycemia, and it has been proven that the effect of the glucosid is on the epithelial cells of the kidney.

An aqueous solution of 0.005 gram of phlorizin is injected subcutaneously. The urine is collected at frequent intervals and analyzed for glucose. Normally the maximum elimination takes place in an hour.

Krotoszyner and Stevens especially investigated this test with the results shown in Table 17.

Casper and Richter described the application of this test in the form of hypodermic or intramuscular administration of the drug in conjunction with ureteral catheterization. In this way (to quote them) they were able to study the action of the drug separately on each kidney, and to utilize the test as a comparative index of functional renal capacity in unilateral kidney lesions. According to their statement, the phlorizin test measures the quantity of functionating renal parenchyma and, by these means, that of renal capacity, the percentage of excreted sugar corresponding to the amount of functionating parenchyma of the kidney. When these statements failed to be entirely corroborated by personal observations of various authors, and particularly after Israel's demonstration of kidneys that had been found barren of phlorizin glycosuria, though possessing functionating parenchyma, Casper and Richter modified their views, stating that absence of phlorizin glycosuria showed an advanced degree of renal incapacity, while its quantity was directly proportional to that of the functionating parenchyma of the kidney.

The test was soon recognized as one of our most important means for estimating relative kidney function. Israel, who always maintained a skeptical attitude toward the real value of functional kidney tests, designated the test as a welcome addition to our diagnostic armamentarium, and Albarran concedes to the method real usefulness. Barth considers the test particularly important for the diagnosis of difficult cases, and Kapsammer claims for the phlorizin test first rank among

TABLE 17.—COMPARATIVE PHLORIZIN AND PHENOLSULPHONEPHTHALEIN TESTS, WITH REFERENCE TO QUANTITY OF RENAL URINES IN PATHOLOGIC CASES. (Krotoszyner and Stevens.)

No.	Age	Diagnosis	Phlorizin			Phenolsulphonephthalein					Quantity of Renal Urines Compared
			Per Cent.		Values Compared	Quantity		Per Cent.		Values Compared	
			R.	L.		R.	L.	R.	L.		
1	28	Bilateral pyelonephritis	0.8	0.56	1 : 0.7	1.5	6.5	2	9	1 : 4.5	1.0 : 4.3
2	30	Right-sided renal tuberculosis	0.37	2.4	1 : 6.5	10	30	Trace	17	0.1 : 1.7	1.0 : 3.0
3	27	Right-sided hydronephrosis	0.3	1.7	1 : 5.5	14	8	4	6	1 : 1.5	1.75 : 1.0
4	30	Right-sided renal neoplasm	0.03	1.47	1 : 5	3.5	13	Trace	13	0.1 : 1.3	1.0 : 3.7
5	56	Bilateral nephrolithiasis	0.18	0.12	1.5 : 1	16	7.5	3.5	8	1 : 2	2.1 : 1.0
6	33	Right-sided pyonephrosis	1.3	2.5	1 : 3	16	3.5	7.5	8	1 : 1	4.7 : 1.0
7	46	Right-sided hydronephrosis	0.35	1.9	1 : 5	14	7	14	6	2.3 : 1	2.0 : 1.0
8	30	Right-sided pyelonephritis	0.9	1.1	1 : 1.2	6	2	13	4	3.0 : 1	3.0 : 1.0
9	25	Right-sided ureter stone	1.2	0.88	1.5 : 1	26	39	10	14	1 : 1.5	1.0 : 1.5
10	30	Right-sided hydronephrosis	0.36	2.0	1 : 5.5	3.5	10	Trace	9	0.1 : 9	1.0 : 2.9
		Average	0.58	1.56	1.1 : 3.4	11.0	12.6	5.4	9.4	1.6 : 5.1	1.8 : 2.0

renal functional methods. Greene and Brooks designate the test the most practical of functional methods, a view from which they have not departed in the latest edition of their textbook. Krotoszyner and Stevens' results with the test were most gratifying, and careful comparison with the results from other functional tests, especially those from phenolsulphonephthalein, elicited this statement: The phlorizin, phenolsulphonephthalein and urea tests show in normal cases almost identical values for both kidneys, and exhibit in pathologic cases a striking parallelism of diminished functional values. These authors modified the test by injecting the drug intravenously. By means of this test they were able to study the total functional renal capacity, as well as the capacity of the individual kidneys. Their procedure is as follows:

The *bladder test* for determination of total renal permeability by means of intravenous phlorizin injection is performed in the following manner: The bladder is emptied by introduction of a catheter which is left *in situ,* and 1 cg. of phlorizin is injected into one of the arm veins. Testing for sugar is begun at once by letting a few drops of urine flow into test-tubes containing a few cubic centimeters of heated Fehling solution, until typical sugar reaction occurs.

In cases with normal renal permeability sugar appeared in about seven minutes and in cases with pathologic or reduced renal capacity in not less than fifteen minutes. In both types of cases, sugar excretion was at its acme during the first fifteen-minute period, was markedly reduced during the second, and was very low or had disappeared during the third period.

As a test for comparative renal function, the intravenous phlorizin test gives more accurate, and thus more reliable, results, while its technic is simple and less time-consuming than that of the phenolsulphonephthalein test, according to the results of Krotoszyner and Stevens.

Urotropin Test.—Falk and Sugiura recommended the following test for kidney sufficiency. One gram of hexamethylenetetramin (urotropin), dissolved in a glass of water, was administered about fifteen minutes before the evening meal, which included fluid to the amount of 500 c.c. or more. All the urine voided thereafter, including the morning specimen, was collected and tested for urotropin. The administration of a purgative at the same time as the hexamethylenetetramin would naturally invalidate the results. If a negative result is obtained with the iodin test (*see below*), and if formaldehyd is absent, the pressure of traces of hexamethylenetetramin may be detected by treating 1 to 2 c.c. of urine with 1 drop of concentrated sulphuric acid, warming the solution for a few minutes, and testing for formaldehyd with the phloroglucin reagent. Falk and Sugiura tested the urines in every case for free formaldehyd with this reagent. In only very few were marked red colors obtained. These indicate formaldehyd concentrations of 1 in 10,000 to 1 in 5,000 for the deepest red obtained. If the formaldehyd was liberated from the urotropin after the passage of the latter through the kidneys, it would be necessary to correct the quantity of urotropin eliminated by a corresponding amount of formaldehyd, but

TABLE 18.—UROTROPIN TEST. (Falk and Sugiura.)

Diagnosis	Urinary Findings		Urotropin		Phthalein Test	Non-protein N	Uric Acid	Mosenthal Tests	
	Albumin	Casts	Sp. Gr. of Urine	Per Cent. Recovered				Variation of Sp. Gr. of Urine	Vol. Day Urine / Vol. Night Urine
Normal.....	0	0	1.014	49	Per Cent. 80	Mgs. per 100 c. c. 28.0	Mgs. per 100 c. c. 2.2	18	6.7
Normal.....	0	0	8	31	70	28.6	2.4	16	6.0
Nephritis.....	+	+	8	0	39	40.1	5.0	13	0.54
Nephritis.....	+++	++	10	0	57	48.9	4.3	4	1.03
Nephritis.....	+	+	15	0	20	68.0	5.5	3	0.65
Nephritis.....	++++	+	15	0	34	37.0	4.4	12	0.68
Nephritis.....	—	—	18	0	31	40.0	5.6	8	0.49
Nephritis.....	++++	++	10	0	—	85.0	5.1	3	1.05
Nephritis.....	++++	++	9	0	—	72.0	4.3	3	1.29
Nephritis.....	+	+	2	0	30	34.7	4.3	7	1.07
Nephritis.....	+	+	14	0	25	40.5	6.0	10	0.61
Nephritis.....	+++	++	12	0	—	58.0	6.7	3	0.92
Nephritis.....	++	—	4	0	25	67.0	5.2	3	1.05
Nephritis.....	++	++	13	0	41	34.7	4.9	4	1.88
Nephritis and gout	+	+	16	0	10	42.6	6.8	3	0.80
Gout.....	0	0	7	36	50	34.0	6.2	9	3.7
Gout.....	0	0	—	64	45	33.0	4.4	17	6.6
Gout.....	+	0	11	49	50	35.0	4.7	8	1.21
Cirrhosis of liver.	—	—	6	0	88	21.0	1.0	6	1.0
Cirrhosis of liver.	—	—	13	0	79	25.6	1.1	9	0.71

it may have decomposed before passing through the kidneys, and the formaldehyd have been eliminated as such.

Falk and Sugiura contrasted the urotropin test with the phenol-sulphonephthalein test, the non-protein nitrogen of the blood, the uric acid of the blood, and the Mosenthal-Schlayer test-meals. From the

accompanying table (taken from Falk and Suguira) one may judge of the value of this test. Normally from 30 to 50 per cent. of the urotropin is eliminated in the urine. In cases of nephritis none is excreted.

Quantitative Test for Urotropin.—To 50 c.c. of the albuminous urine, 10 to 15 c.c. of alumina cream are added, and stirred slowly. After the protein and alumina have settled, the mixture is filtered through a folded paper, the precipitate on the paper being allowed to drain thoroughly. A known excess of iodin solution (iodin 3.6 grams, 95 per cent. ethyl alcohol, 100 c.c.) is then added from a buret, and stirred constantly. The amount needed may be judged by the formation of the precipitate as the iodin solution is added; or the mixture is filtered and treated with more iodin solution; or an approximate determination is made first and then a more accurate one with a new solution. After all the iodin has been added (as a rule 5 to 8 c.c. are required), the mixture is allowed to stand about 10 minutes, with occasional stirring and filtering with suction through a Gooch crucible containing an asbestos mat. Refiltration through the same mat is advisable. The precipitate is washed 5 to 10 times with cold water. The precipitate and crucible may be dried in a vacuum desiccator over calcium chlorid to constant weight. Better than weighing the precipitate is the titration of the iodin present with M/25 sodium-thiosulphate solution. For this purpose, the precipitate and crucible are transferred to a beaker, and 50 c.c. of water and 3 c.c. of glacial acetic acid added. Starch is used as an indicator.

Tests for Formaldehyd.—(*a*) *Phloroglucin Method.*—Prepare the reagent by dissolving 1 gram phloroglucinol and 20 grams sodium hydroxid in sufficient water to make 100 c.c. To 10 c.c. of urine in a test-tube, add with a pipet 2 c.c. of this reagent, placing the end of the pipet on the bottom of the tube in such a manner that the reagent will form a separate layer. A bright red coloration (not purple) is formed at the zone of contact if formaldehyd is present.

(*b*) *Phenylhydrazin-sodium-nitroprussid Test.*—This test consists of adding to the suspected fluid, 3 drops of a 5 per cent. aqueous solution of phenylhydrazin-hydrochlorid and then 3 drops of a 5 per cent. aqueous solution of sodium nitroprussid. An excess of a saturated aqueous solution of sodium hydroxid is added. It is important that the solutions to be tested, as well as the sodium hydroxid, should be slightly warmed to a little more than the body temperature. When formaldehyd is present in solution of 1 to 20,000 or stronger, there follows an intense blue color which gradually changes to green and then after a few minutes to brown. In solutions of less than 1 to 20,000 the first color is the intense green.

Creatinin in the Urine.—According to Neubauer the creatinin elimination is a good test of renal function. The urine is examined for creatinin in six-hourly periods on 3 successive days. The first day is called the "fore-period." The second day the patient receives 1.5 grams creatinin with his diet. The third day is called the "after-period." The creatinin in the urine is determined by Folin's method.

Folin's Colorimetric Method (Hawk).—*Principle.*—This method is based on the characteristic property possessed by creatinin, of yielding a certain definite color-reaction in the presence of picric acid in alkaline solution.

Procedure.—Place 10 c.c. of urine in a 500 c.c. volumetric flask; add 15 c.c. of a saturated solution of picric acid and 5 c.c. of a 10 per cent. solution of sodium hydroxid; shake thoroughly and allow the mixture to stand for 5 minutes. During this interval pour a little N/2 potassium bichromate solution into each of the two cylinders of the colorimeter (Duboscq) and carefully adjust the depth of the solution in one of the cylinders to the 8 mm. mark. A few preliminary colorimetric readings may now be made with the solution in the other cylinder, in order to insure greater accuracy in the subsequent examination of the solution of unknown strength. Obviously the two solutions of potassium bichromate are identical in color and in their examination no two readings should differ more than 0.1-0.2 mm. from the true value (8 mm.). Four or more readings should be made in each case and an average taken of all of them, exclusive of the first reading, which is apt to be less accurate than the succeeding readings. In time, as one becomes proficient in the technic, it is perfectly safe to take the average of the first two readings.

At the end of the five-minute interval already mentioned, the contents of the 500 c.c. flask are diluted to the 500 c.c. mark, the bichromate solution is thoroughly rinsed out of one of the cylinders and replaced with the solution thus prepared and a number of colorimetric readings are immediately made.

Ordinarily 10 c.c. of urine is used in the determination by this method, but if the content of creatinin is above 15 mg. or below 5 mg. the determination should be repeated with a volume of urine selected according to the content of creatinin. This variation in the volume of urine, according to the content of creatinin, is quite essential, since the method loses in accuracy when more than 15 mg. or less than 5 mg. of creatinin is present in the solution of unknown strength.

Calculation.—By experiment it has been determined that 10 mg. of pure creatinin, when brought into solution and diluted to 500 c.c., as explained in the above method, yields a mixture, 8.1 mm. of which possesses the same colorimetric value as 8 mm. of a N/2 solution of potassium bichromate. Bearing this in mind, the computation is readily made by means of the following proportion in which y represents the number of millimeters of the solution of unknown strength equivalent to the 8 mm. of the potassium bichromate solution:

$$y : 8.1 :: 10 : x \text{ (mg. of creatinin in the quantity of urine used).}$$

This proportion may be used for the calculation, no matter what volume of urine (5, 10 or 15 c.c.) is used in the determination. The 10 represents 10 mg. of creatinin which gives a color equal to 8.1 mm., whether dissolved in 5, 10 or 15 c.c. of fluid.

Barker writes: "Normally, examined in six-hourly periods, the creatinin excretion goes on in the form of a horizontal curve. The amount excreted, if excessive meat eating be avoided, is independent of the food intake, so that the maintenance of a rigidly monotonous diet is superfluous. If 1.5 grams of creatinin be taken in sweetened water by a normal person, about 60 to 90 per cent. is excreted in the first period, and in the second period 8 to 30 per cent. more, so that in the twelve hours, 70 to 100 per cent. of the amount swallowed is already eliminated. When the renal function is disturbed, there is a delay in the excretion, which may even extend over into the next day. Disturbances of creatinin excretion are especially marked in bilateral renal disease.

"The method does not permit one to draw conclusions regarding a definite lesion, and so it cannot be used for the classification of different nephropathies. Newbauer found delayed elimination sometimes in cases of arterial hypertension when he had no other clews pointing to disease of the kidneys. Excretion was also delayed in cases of gout, in which there were no certain signs of renal disease demonstrable. He thinks, therefore, that the early stages of a gouty kidney may, perhaps, be recognizable by this test. In chronic passive congestion of the kidney, disturbances of creatinin excretion were also observed. The method may be applied to the diagnosis of unilateral affections on ureteral catheterization, since differences greater than 20 per cent. between the elimination on the two sides point to pathological changes."

Urea in the Urine.—The elimination of ingested urea has been suggested as a test for kidney sufficiency. According to Pirondini, this is an excellent method.

The principle of intensification of the natural and spontaneous elimination of a substance which is a normal ingredient of the urine, he thinks, is an ideal method for a test. Experimental polyuria is less instructive because both renal and extrarenal factors are involved in this. The urea output is less independent of the kidney functioning. The urea ingested in the test is eliminated in a curve which resembles that of the water in testing polyuria. It shows less influence from position and from cirrhosis of the liver, but the curve reflects unerringly the condition of the kidneys. It may reveal impairment of function when the test polyuria seems to indicate satisfactory work on the part of the kidneys. It is most instructive in cases of disease of the bladder or prostate with secondary disturbance in kidney functioning; also in investigating the capacity of each and both kidneys with surgical kidney, and for testing the functioning of the remaining kidney after nephrectomy.

To perform the test the patient is given 30 grams urea dissolved in 4 to 6 ounces water, and the urine examined for urea by any of the reliable quantitative methods. The urine is examined 2 hours before the test and every 2 hours for 24 hours after the test. Under normal conditions there is a sharp rise in the urea excretion in the second

2-hour period. In kidney disease, this abrupt rise is absent or delayed (McKasky).

Diastase or Amylase in the Urine.—Pirondini in 1914, Geyelin and Geraghty, Rowntree and Cary have studied the elimination of diastase in the urine as a means of determining the functionating power of the kidney. According to these authors, the test is in agreement with the results obtained with phenolsulphonephthalein.

In cases of marked hematuria or polyuria, or in pancreatic disease this test gives discordant results.

The amylase may be examined by Wohlgemuth's method:

Quantitative Determination of Amylolytic Activity: Wohlgemuth's Method (as Given by Hawk).—Arrange a series of test-tubes with diminishing quantities of urine, introduce into each tube 5 c.c. of 1 per cent. solution of soluble starch, and place each tube at once in a bath of ice-water. When all the tubes have been prepared in this way and placed in the ice-water bath, they are transferred to a water-bath or incubator and kept at 38° C. for from 30 minutes to an hour. At the end of this digestion period, the tubes are again removed to the bath of ice-water in order that the action of the enzyme may be stopped.

Dilute the contents of each tube to within about ½ inch of the top, with water; add one drop of a N/10 solution of iodin and shake the tube and contents thoroughly. A series of colors ranging from dark blue through bluish-violet and reddish-yellow to yellow, will be formed. The dark blue color shows the presence of unchanged starch, the bluish-violet indicates a mixture of starch and erythrodextrin, whereas the reddish-yellow signifies that the erythrodextrin and maltose are present and the yellow solution denotes the complete transformation of starch into maltose. Examine the tubes carefully before a white background and select the last tube in the series which shows the entire absence of all blue color, thus indicating that the starch has been completely transformed into dextrins and sugar. In case of indecision between two tubes, add an extra drop of the iodin solution, and observe them again, after shaking.

Calculation.—The amylolytic activity of a given solution is expressed in terms of the activity of 1 c.c. of such a solution. For example, if it is found that 0.02 c.c. of an amylolytic solution, acting at 38° C., completely transformed the starch in 5 c.c. of a 1 per cent. starch solution in 30 minutes, the amylolytic activity of such a solution would be expressed as follows:

$$D\ ^{38^\circ}_{30^m} = 250$$

This indicates that 1 c.c. of the solution under examination possesses the power of completely digesting 250 c.c. of 1 per cent. starch solution in 30 minutes at 38° C.

4. **Pharmacological Tests.**—Diuretic Tests.—Attempts have been made to test the function of the kidneys by noting their response to pharmacological stimulation with caffein, diuretin, urea, theocin, euphyl-

lin, etc. None of these tests are, however, specific. Erich Meyer, in his researches on diabetes insipidus, is convinced of the efficacy of theocin in stimulating the concentrating function of the kidneys.

Bouchard's Urotoxic Coefficient.—Bouchard considers that he has experimentally proved that a healthy person produces an approximately constant amount of urinary toxins per kilogram of body-weight every twenty-four hours. He goes so far as to estimate a definite percentage of toxic effect for each individual constituent of the urine—water, salts, pigments, urea, organic bases, etc. He proceeded to determine, by experiment, how many c.c. of urine injected intravenously were necessary to kill an animal (rabbit), and expressed this in terms of the weight of the animal. From this he deduced the toxin production of the individual per diem and per kilogram ("toxin coefficient"). One of the first and most important conclusions he established was the diminution of toxic effects in renal disease, and especially in uremia. He concluded from this that, in consequence of the incapacity of the kidneys, the body does not excrete but retains both the poisonous products of metabolism and those toxic bodies which are absorbed from the intestines. This retention he regards as the cause of uremia and of death. Unfortunately, the method of this gifted investigator has not fulfilled all that it promised. The results of the experiment depend upon so many factors—one might say even upon so many chances—that even the most careful procedure cannot ensure uniform results. Many physical and chemical factors which had nothing to do with the uremic intoxication undoubtedly contributed to the death of the animal injected with urine. It is only an extremely poisonous or a non-poisonous state of the urine that is worthy of consideration. It is certainly not necessary to make use of this elaborate method in order to prove auto-intoxication in cases where there is a diminution of the toxins in the urine, accompanied by lessened excretion, or far-advanced nephritis, or general uremic symptoms. But even in these cases the method often fails. Instances have been described by experienced observers where, in spite of obvious uremia, Bouchard's method showed a high toxic value of the urine (Bernard). Apart from extreme cases, which are of little interest from the point of view of diagnosis and prognosis, the method has not helped us to arrive at a quantitative determination of the elimination of poisons. In spite of many adherents, belonging mostly to the Bouchard's school, the method has been subjected to severe criticism.

Von Noorden states: "I am in full agreement with Ewald and Ascoli who, in a recent critical study, and in spite of the contention of Claude and Balthazar, express the view that Bouchard's proposal is unsatisfactory as a test for determining the incapacity of the kidneys or the retention of toxins, or as affording theoretical insight into the nature of uremia. I expressed a similar view more than twelve years ago in my text-book on 'The Pathology of Metabolism.' In certain medical circles, however, and especially in Paris, the theory of the 'urotoxic coefficient' is awarded a high place, and whether toxic re-

tention is present or not, no analysis of nephritic urine is considered complete which has not been obtained by estimating this coefficient. This can only be regarded as pseudo-scientific humbug, which has nothing in common with the atmosphere of scientific seriousness which surrounded Bouchard's painstaking studies."

Cawadias injected the blood-serum of uremic patients into the peritoneal cavity of rabbits and produced immediately such phenomena as convulsions, paralyses, dyspnea, hypothermia, even death. Normal serum will produce toxic symptoms, but much larger doses are necessary than of the serum of a uremic patient. Even the sera of uremic patients vary in toxicity, the serum of a patient with convulsions being high in the scale. Cawadias emphasizes the fact that the serum of a well person differs from that of a uremic patient only in degree of toxicity. The results of injection are the same if only the right amount is used. The conclusion is drawn that in uremia there is no new poison, but a preëxisting toxic substance whose action becomes more intense.

III. The Composition of the Blood

Epstein, whose work on nephritis is a departure from the "respectable," currently accepted theories of this disease, has advanced a new conception of renal disturbances. His work has as yet not been corroborated. It is interesting, in any event, and we shall quote from him:

"From a comprehensive study of the blood, of effusions, and of the urine, in this and other forms of renal disease some light was gained concerning the morbid processes of this peculiar affection. In a series of 193 cases representing various renal conditions, which I have had occasion to observe in the past few years, the type of disease under discussion has been encountered in fifteen individuals. It appears from these observations that the pathologic and chemical basis of the cases now termed "chronic nephrosis" is that of a nutritional or constitutional disorder. Furthermore, the evidence acquired also points to the probability that certain cases of diffuse nephritis represent a form of renal disease in which some of the morbid phenomena correspond to those found in chronic nephrosis, whereas others are secondary to or associated with inflammatory changes in the kidneys.

"The most striking phenomenon in this group of renal diseases is the intense and persistent albuminuria. The quantity of albumin excreted in the urine is often great. Its daily output for weeks and months may amount to as much as 50 grams. The source of the albuminous substances in the urine is the blood-serum or plasma. In view of the fact that the total quantity of protein in the blood-serum, in a normal individual of average weight and size, is approximately 210 grams, the daily loss incurred by the blood in this manner may constitute a large percentage of that present in the circulation.

"Whether the excretion of these substances is due primarily to an altered protein utilization, or is the result of an unusual permeability of the renal epithelium, is a question which cannot be answered at the

present time. The fact remains, that as a result of the intense and prolonged albuminuria, profound changes take place in the composition of the blood. The development of a state of malnutrition is evidenced by a number of facts. Whereas normally the blood-serum contains from 6.5 to 8.3 grams of protein to the hundred c.c., of which a little more than one-third is globulin; in disease the quantity of protein may be much reduced and the globulins show a relative increase. The characteristic change in the blood-serum in cases of chronic nephrosis is a reduction in the total protein content with a marked relative increase in the globulin, which in certain instances constitutes nearly all of the protein present.

TABLE 19.—TYPES OF COMPOSITION OF BLOOD-SERUM IN CHRONIC NEPHROSIS

Total Protein (Gm.)	Incoag. Nitrogen (Gm.)	Per 100 c.c.		Cholesterol (Gm.)	Globulin in Protein Per Cent.
		Globulin (Gm.)	Chlorid (Gm.)		
3.611	0.065	2.038	0.404	0.760	59.0
2.731	0.101	2.598	0.390	1.226	95.0

"It is known that infections and intoxications of various sorts lead to an increase in the proportion of globulin in the blood. Hurwitz and Meyer have found that the increase in globulins may result from the toxic action of infecting agents on the body tissues. On the basis of other changes which occur in the blood, such as the accumulation of fatty substances, the increase in the globulin content indicates a tissue disintegration. The quantity of fats and lipoids which accumulate in the blood in this disease is extraordinary.

"In no other condition of renal disease are such changes in the blood-serum encountered. What concerns other substances in the blood, namely, non-protein nitrogen and the chlorids, it might be said that these often range within normal limits or may be slightly elevated. One reason for the low concentration of nonprotein nitrogen in the blood may be a large distribution of this constituent throughout the body in the edematous tissues. That this is so is illustrated by the following clinical observation:

"'The blood of a patient suffering from a severe nephritis, with symptoms of impending uremia (visual disturbances, violent headaches, vomiting) and a generalized edema, was examined and found to contain 0.070 grams of non-protein nitrogen per hundred c.c. Ten days later, when many of the symptoms disappeared and the edema partly subsided, the blood contained 0.210 grams of nonprotein nitrogen per hundred c.c. Finally two weeks after the second examination, when the patient was much improved and the edema had entirely subsided, the non-protein nitrogen of the blood was 0.70 per hundred c.c.'

"It is usually stated that, in cases of chronic nephrosis, the urine may have a high specific gravity; that it may contain normal quantities of nitrogenous urinary substances, but that it is deficient in chlorids. According to Widal this is one of the characteristic features of the disease.

"In discussing the classification of this group of nephritic conditions, I stated that there are certain cases of diffuse nephritis in which some of the manifestations resemble those found in chronic nephrosis, while others are referable to inflammatory changes in the kidneys. Examples of this type are found particularly in two groups of conditions: first, during or after pregnancy, and second, in association with diabetes mellitus. The first occurs in relatively young women who, in the course or directly after a pregnancy, develop renal disease. I have had occasion to observe six cases of this type. Not only do such cases show evidence of malnutrition as proved by the changes in the blood, but they also indicate the occurrence of disturbances in certain of the internal secretions. As in the cases of chronic nephrosis, the albuminuria is very intense; but formed blood elements may occasionally be found. The urine is scanty and of varying specific gravity; it is deficient in chlorids and may also show diminution in the nitrogen output. The blood shows changes similar to those observed in chronic nephrosis; namely, the reduction in the protein content of the serum, the relative increase in the globulins and an excessive increment in fatty material. Extensive edema develops; notwithstanding this fact, the non-protein nitrogen of the blood in these cases may be considerably elevated.

"The blood-pressure in the latter group of cases rises very high (240 mm. of mercury). The other clinical features which suggest a disturbance of the internal secretions are the cessation of menses (in four out of six cases), or the development of menorrhagia; loss of hair, pigmentation of the face (light coffee brown), exophthalmos, and varying degrees of enlargement of the thyroid.

TABLE 20.—TYPES OF COMPOSITION OF BLOOD-SERUM IN CHRONIC DIFFUSE NEPHRITIS (MILD AND SEVERE CASES)

Total Protein (Gm.)	Incoag. Nitrogen (Gm.)	Per 100 c.c.		Cholesterol (Gm.)	Globulin in Protein (Per Cent.)
		Globulin (Gm.)	Chlorid (Gm.)		
4.919	0.130	3.440	0.397	0.460	70.00
3.958	0.084	2.594	0.404	1.150	66.00

"The kidney of pregnancy, as stated before, is believed to represent a typical example of nephrosis. The cases just mentioned stand in etiologic relationship to pregnancy, and from the character of the changes in the blood they resemble the cases of chronic nephrosis; but, unlike those, they show a high blood-pressure. This circumstance, however, does not militate against the assumption that the intrinsic nature of

the disturbance is similar to that of chronic nephrosis. The early cessation of the menses in the majority of the cases suggests that the rise of blood-pressure may, to a certain extent, be the result of the associated disorder of the internal secretions, like that which occurs in the normal menopause.

"The other pathologic condition, as stated before, with which this type of renal disease is sometimes associated is diabetes mellitus. I have observed three such cases, two of which occurred in men. The ages of the three persons were 52, 58 and 59 years, respectively. In two of the cases the glycosuria and the albuminuria were discovered at the same time. In the remaining one, the albuminuria antedated the diabetes by three years. In these cases, some of the clinical findings differed from those of the preceding group.

"From a consideration of the facts presented, it seems probable that the morbid processes in chronic nephrosis and in some special cases of chronic diffuse nephritis represent a general constitutional disturbance, and not merely a disease restricted entirely to the kidneys. No other variety of renal disease gives rise to such changes as those found in this group of cases.

"Barring the special cases in which renal disease and diabetes coexist, another striking clinical manifestation in this group of cases is the edema. According to Widal, whose views are now generally accepted, the edema is the result of a failure on the part of the kidneys to eliminate salt and water. On a previous occasion, I discussed the question of the causation of edema, and offered evidence in support of the view that the retention of salt and water is not necessarily the result of a disturbed elimination of these substances by the kidneys. The forces which prevent their excretion are extrarenal. The hypothesis is briefly this:

"The loss of protein incurred by the blood-serum through the continuous albuminuria causes a decrease in the osmotic pressure of the blood. Through this condition and the additional circumstance that large quantities of fats and lipoids accumulate therein, the physicochemical state of the blood is disturbed to such an extent that it loses much of the power which it normally possesses to withdraw fluids from the tissues. Consequently deposition of fluid in the tissues occurs. In other words, the edema in this type of nephritis is not necessarily the result of a lessened permeability or diminished functional power of the kidneys to eliminate salt and water, but is the result of a change in the character of the blood. It is noteworthy that the edema fluid in these cases, unlike that present in any other condition, is composed almost exclusively of inorganic and nitrogenous salts and water. The view here presented is amply supported by experimental and clinical facts."

1. **Ambard's Coefficient.**—Lamy and Meyer endeavored to compare the concentration of urea in the blood with the rate of excretion in the urine. They did not recognize the importance of the rate of blood flow, and consequently were not able to find any relation between the two values. Five years later Ambard and Moreno announced their

laws of renal function. They were three in number, and reduced the study of kidney activity to a physicochemical basis. The blood-urea was regarded as a stimulus acting on the renal cells. The rate of excretion of urea was the response of the kidney to that stimulus. In their opinion the rate of circulation through the kidney was the chief factor governing the concentration of the urine. A dilute urine was a sign of a high rate of blood-flow, while a diminished blood supply was shown by the increase in the concentration of the urine and a consequent diminished output of water and of urea.

The first law dealt with the relation of the rate of output of urea to the concentration of urea in the blood. The rate of output was found to vary directly with the square of the concentration of urea in the blood, if the concentration of urea in the urine remained constant. In other words, if the quantity of urea in the blood were doubled, the amount excreted in a given time would be quadrupled.

According to the second law, the rate of excretion of urea varied inversely with the square root of the concentration of urea in the urine, if the blood-urea remained constant. Under these conditions a quadrupling of the concentration would result in a halving of the rate of output.

The third law was a combination of the first and second. If the concentration of the urea in the blood and urine varied simultaneously, then the rate of output would vary directly as the square of the concentration of urea in the blood, and inversely as the square root of that in the urine.

The following formula, used in calculating the coefficient, is derived from the third law by the addition of correction factors for the patient's weight and for a standard urinary concentration of 25 grams of urea per liter:

$$K = \frac{Ur.}{\sqrt{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}}$$

K = Coefficient of urea excretion.
Ur. = Grams of urea per liter of blood.
D = Output of urea in grams per twenty-four hours.
P = Weight of the patient in kilograms.
C = Grams of urea per liter of urine.
70 = Standard weight.
25 = Standard concentration of urea in the urine.
All weights and concentrations are compared to the standards of 70 and 25.

The normal value of the constant is from 0.06 to 0.09. With a decreasing kidney efficiency there is a rise in the constant, and with an increasing function the coefficient falls.

The analytical methods used by the French had been so inaccurate that this work remained practically unknown in this country until

McLean and Selling repeated the work, using more accurate methods, and found the coefficient to have the same degree of constancy that Ambard had found four years previously.

It has been claimed that the mathematical form of the coefficient is the real basis of its constancy and that the laws of function play absolutely no rôle in the attainment of this end. Addis and Watanabe claim that Ur. is a relatively constant value and that it is the dominant factor in keeping the coefficient constant. On the other hand, D and C are the factors which are subject to considerable variation. The effects of these variations are minimized by the fact that the greater variant is present as its fourth root, and the lesser as its square root. While their assumption is partly true, the fact remains that the blood-urea is not always a constant value. In a series of nephritics here presented there are instances of enormous variations in the blood-urea, without any corresponding change in the coefficient of urea excretion. While, strictly speaking, the laws of function may be mathematically inaccurate, and the formula from which the coefficient is calculated may tend to hide these inaccuracies, still there is a very definite relationship between the concentration of the urea in the blood and in the urine and its rate of excretion. Under similar conditions of urine concentration the rate of urea excretion is always greater with a high concentration of blood-urea than with a low one. Similarly, in the presence of a stationary blood-urea concentration the amounts of urea excreted under conditions of polyuria and low urinary concentration are greater than when the volume is small and the concentration high.

In spite of the lack of mathematical accuracy, the normal coefficient of urea excretion falls within comparatively narrow limits.

Objection has been made to the original form of the coefficient because the normal value is not a whole number, and because a diminishing renal function causes a rise in the coefficient. Balavoine and Onfray have advocated the use of a formula, giving the normal coefficient a value of 1, and so arranging the equation that a diminution in renal function would cause a drop in the constant. Their suggestion was not well received in France.

Ambard has suggested a means of calculating the functional capacity of a damaged kidney in terms of percentage of the normal. For example, if, after the injection of urea, a normal individual presents a blood-urea of 0.7 gram per liter, his rate of output should be 100 grams per twenty-four hours, at the standard concentration of 25 per 1,000. This gives a coefficient of 0.07. If a nephritic with a similar concentration of blood-urea excretes only 25 grams at the standard concentration, his rate of output is reduced to a quarter of the normal. His coefficient under these conditions would be 0.14, because

$$K = \frac{\text{Blood-urea}}{\sqrt{\text{Rate of Output}}} = \frac{0.7}{\sqrt{25}} = 0.14$$

Again, if the rate of output were reduced to one-ninth the normal, or 11.11 grams, the coefficient would be

$$\frac{0.7}{\sqrt{11.11}} = 0.21$$

The absolute functional value of the kidney can also be obtained from the coefficient by the use of the following equation:

$$\frac{(\text{Normal coefficient})^2}{(\text{Pathologic coefficient})} = \text{functional value of the kidney in terms of normal.}$$

If we apply this formula to the first case: $\left[\frac{0.07}{0.14}\right]^2 = 0.25$, that is, the functional capacity of the kidney is reduced to one-fourth of the normal value. This corresponds to the theoretical diminution in the rate of output. The same applies to the second case: $\left[\frac{0.07}{0.21}\right]^2 = 0.11$, or one-ninth of the normal value.

Ambard gives 0.07 as the normal value of the coefficient, with normal range of from 0.06 to 0.08. In a series of 107 determinations, McLean finds the maximum normal range of the coefficient to be from 0.05 to 0.09 (index 235-80). He considers any coefficient above 0.09 (index below 80) distinctly abnormal, unless the reduced rate of urea excretion can be accounted for. An insufficient water intake is a possible cause of increased values of the coefficient in quite normal persons.

Lewis gives his findings of Ambard's coefficient and McLean's index in the accompanying tables. The findings in normal cases are given in Table 21. The results in pathological cases are found in Tables 22-25.

Lewis, from whose excellent work we have largely drawn, thus summarizes his observations:

1. The laws of function are not followed with mathematical exactness in young and active individuals, but under routine conditions they are remarkably accurate. They are correct in principle.
2. The coefficient of urea excretion is subject to certain variations in normals, but any value below 0.06 or above 0.09 should be regarded as abnormal unless the excessive variation can be readily explained.
3. The coefficient is absolutely independent of the blood urea concentration. Its level is governed by the condition of renal function.
4. The coefficient is depressed in fever, in hyperthyroidism, in hypertension with early changes in the renal arterioles, and in early chronic diffuse nephritis. The depression is an evidence of increased renal activity due to irritation.
5. The coefficient is raised in myxedema.
6. There is an increase of the coefficient in myocardial insufficiency. Opinions are divided as to whether this is the effect of an extrarenal factor (the circulation) or whether there is a definite anatomic lesion in the passively congested kidney.

TABLE 21.—AMBARD'S COEFFICIENT AND MCLEAN'S INDEX IN NORMAL CASES. (Lewis.)

Case No.	Urea N. (Mg. per 100 c. c. Blood)	Ambard Coefficient	McLean Index	Phthalein Output (Per Cent. 2 Hours)	Diagnosis
301	9	0.053	228	55	Psychoneurosis
302	12	0.054	220	..	
303	14	0.056	204	..	Primary anemia
304	20	0.058	190	..	
305	10	0.062	167	62	Psychoneurosis
306	13	0.062	167	60	
307	10	0.066	147	..	
308	7	0.058	190	..	
309	16	0.063	161	..	Primary anemia
310	14	0.064	156	57	Cerebral arteriosclerosis
312	10	0.067	143	56	Neurasthenia
314	14	0.072	124	66	
315	14	0 072	124	76	Syphilis, tertiary
316	17	0.073	120	..	Primary anemia
317	16	0.074	117	60	Gastroptosis
319	19	0.075	114	65	Diabetes mellitus
320	11	0.076	111	47	Colloid goiter
321	16	0.077	108	67	Multiple sclerosis
322	14	0.077	108	..	Syphilis
323	11	0.077	108	..	Primary anemia
325	20	0.078	105	..	
326	13	0.079	102	50	Neurasthenia
327	18	0.079	102	60	Syphilis, tertiary
329	23	0.081	97	66	
330	14	0.082	95	51	Obesity
332	11	0.082	95	57	Pyloric stenosis
333	13	0.083	93	55	Neurasthenia
335	16	0.085	89	60	Chronic appendicitis
336	10	0.087	85	67	
337	17	0.087	85	55	General paresis
339	17	0.092	76	55	Intestinal parasitism
340	20	0.092	76	79	Psychoneurosis

TABLE 22.—PRIMARY HYPERTENSIVE CARDIOVASCULAR DISEASE. (Lewis.)

Case No.	Gram Urea per Liter of Urine, C.	Corrected Rate of Output per 24 Hours $D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}$	Gram Urea per Liter Blood Ur.	Urea Nitrogen (Mg. per 100 c.c. Blood)	Ambard Coefficient	McLean Index	Phthalein Output (2 Hours)
..	21.3	15.1	0.193	9	0.05	257	76
..	9.	13.1	0.171	8	0.048	278	70
10	....	....	...	9	0 05	256	60
14	....	....	.....	12	0.051	246	63
1	5.4	14.5	0.193	9	0.051	246	70
5	8.2	14.8	0.214	10	0.055	212	58
6	16.2	17.8	0.235	11	0.056	204	53
12	30.6	50.9	0.406	19	0.057	197	58
7	4.9	6.7	0.15	7	0.058	190	64
18	11.3	10.5	0.193	9	0.059	181	85

TABLE 23.—EARLY CHRONIC NEPHRITIS. (Lewis.)

Case No.	Gram Urea per Liter of Urine, C.	Corrected Rate of Output per 24 Hours $D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}$	Gram Urea per Liter Blood Ur.	Urea Nitrogen (Mg. per 100 c.c. Blood)	Ambard Coefficient	McLean Index	Phthalein Output
11	....	....	.....	10	0.052	237	69
2	29.4	37.9	0.321	15	0.053	227	65
3	7.11	18.9	0.235	11	0.054	220	78
8	4.6	12.3	0.193	9	0.055	212	71
..	4.6	6.3	0.15	7	0.059	184	..
..	14.7	14.5	0.193	9	0.051	246	67
..	3.2	8.9	0.15	7	0.05	256	..
4	19.6	21.8	0.257	12	0.055	212	56
..	....	....	.....	8	0.058	190	69
9	7.3	6.2	0.171	8	0.068	138	70
11	8.9	9.3	0.257	12	0.07	130	69

TABLE 24.—ACUTE NEPHRITIS. (Lewis.)

Case No.	Non-protein Nitrogen (Mg. per 100 c.c. Blood)	Urea Nitrogen (Mg. per 100 c.c. Blood)	Percentage of Total Non-protein as Urea Nitrogen	Ambard Coefficient	McLean Index	Phthalein Output (2 Hours)
...	...	34	....	0.116	...	40
...	...	16	....	0.062	...	..
...	...	11	....	0.069	...	55
160	...	45	....	0.13	38	..
...	...	39	....	0.104	59	..
162	...	44	....	0.134	36	15
...	...	22	....	0.079	108	50
85	44	27	61.5	0.172	21	40
...	20	10	50.	0.063	163	65
95	60	46	76.7	0.232	12	..
...	60	45	75.	0.203	16	48
...	57	36	63.2	0.162	24	..
...	49	27	55.1	0.124	42	..
...	...	24	....	0.107	56	44
...	...	22	....	0.119	45	..
...	30	22	73.3	0.111	52	50
...	19	12	63.2	0.074	117	..
...	14	9	64.3	0.054	219	58
...	16	9	56.1	0.061	172	63
132	129	117	90.8	0.4	4	15
...	...	45	....	0.179	3	23
119	178	148	83.2	1.09	0.5	..
...	170	141	83.	1.36	0.3	T.
...	125	102	81.6	0.55	2.1	11
...	78	55	70.5	0.41	3.8	..
...	42	23	54.8	0.186	18	27
...	26	11	42.4	0.124	42	35
...	22	8	36.4	0.064	156	45
...	22	11	50.	0.12	53	..

TABLE 25—CHRONIC NEPHRITIS. (Lewis.)

Case No.	Total Non-protein Nitrogen (Mg. per 100 c.c. Blood)	Urea Nitrogen (Mg. per 100 c.c. Blood)	Percentage of Total Non-protein Nitrogen Present as Urea Nitrogen	Gram Urea per Liter of Urine C	Corrected Rate of Output per 24 Hours $D \times \frac{76}{P} \times \frac{\sqrt{C}}{\sqrt{25}}$	Gram Urea per Liter of Blood Ur.	Ambard Coefficient	McLean Index	Phthalein Output (2 Hours)	Diagnosis
23	28	17	60.8	15.4	14.9	0.364	0.094	72	50	Chronic diffuse nephritis; secondary contracted kidney.
..	35	21	60.	18.6	24.6	0.45	0.091	78	..	
24	25	15	60.	13.5	10.7	0.321	0.097	70	35	Primary contracted kidney; myocardial insufficiency.
..	..	14	....	4.1	5.6	0.299	0.126	40	32	
26	33	25	75.8	....	....	0.535	0.098	67	41	Chronic diffuse nephritis; secondary contracted kidney.
28	30	15	50.	9.1	10.7	0.321	0.098	67	42	Chronic diffuse nephritis; secondary contracted kidney.
..	..	17	....	7.7	8.7	0.364	0.123	42	57	
35	26	17	65.5	12.1	12.5	0.364	0.103	60	40	Chronic diffuse nephritis; secondary contracted kidney; uremia.
..	23	13	56.5	4.9	6.9	0.278	0.106	57	34	
..	16	7	43.7	4.	1.3	0.15	0.129	38	..	
..	..	19	....	14.8	8.7	0.406	0.137	34	32	
39	50	33	66.	19.3	45.1	0.706	0.105	58	55	Chronic diffuse nephritis.
..	30	20	66.6	12.7	25.	0.428	0.085	89	..	
..	29	18	62.	23.4	40.7	0.385	0.061	175	..	
..	26	15	57.7	13.6	36.2	0.322	0.053	228	65	
44	23	17	69.5	6.1	9.3	0.364	0.112	53	55	Arteriosclerosis; arteriosclerotic kidney.
52	20	12	60.	8.9	4.9	0.257	0.116	48	66	Primary contracted kidney; myocardial insufficiency.

7. The coefficient is above normal in nephritis with renal insufficiency. This increase is more evident in chronic diffuse nephritis than in the vascular type, due to the greater frequency of renal insufficiency in the former cases. The coefficient shows an increase long before there is any evidence of nitrogen retention in the blood. The coefficient gives an excellent means of following the changes in renal function and of measuring the rate of progress of the disease.

8. There is a marked uniformity in the results of the phenolsulphonephthalein test and the coefficient in all stages of nephritis. In the later stages there is also a close agreement between the non-protein nitrogen of the blood and the coefficient.

9. In a few severe cases the coefficient varies without there being any evident change in the clinical condition; the causes of these variations have been discussed.

10. The prognostic value of the coefficient is considerable. Values above 0.2 are seen only in the severe cases, while constants persistently above 0.3 are found only in persons with a maximal impairment of renal function. A coefficient above 0.2 has a graver import in vascular nephritis than in that of the chronic diffuse type.

11. For an accurate prognosis, repeated determinations of the coefficient are of greater importance.

TABLE 26.—COMPOSITION OF NORMAL BLOOD AND OF THE BLOOD IN CERTAIN PATHOLOGICAL CONDITIONS.

(Mg. per 100 c.c.)

	Normal	Chronic Nephritis	Uremia
Total solids, per cent	20.0	13–19	12–18
Total N., per cent	3.0	2.5–3.0	1.7–2.7
Non-protein, N	25–35	35–90	90–350
Urea N	12–23	16–70	70–300
Uric acid	1–3	1–4	4–27
Creatinin	1–2	1–3	4–33
Creatin	5–9	.....	5–30
Amino-acid N	4–5	.....	6–16.0
Ammonia N	0.1–0.2	0.1–0.2	0.2–1.0
Chlorids as NaCl, per cent	0.65	0.55–0.75	0.45–0.65

Kholzoff has been giving a thorough trial to Ambard's formula for estimating the work of the kidneys by the proportion between the urea in the blood and in the urine. Kholzoff thinks that the total amount of urea and chlorid eliminated by the kidneys during the twenty-four hours is of greater importance than the percentage in urine or blood. At the same time he lauds the Ambard index as accurate and reliable, giving a better idea of the renal function than any other method. He says the method is not reliable for determining the function of each

kidney separately, because the technic of collecting urine from each kidney is not perfect. Yet it is highly important to collect the whole amount of urine without any losses. Therefore, this method can be only of limited use, as, for instance, for the purpose of determining the function of both kidneys, or when there is but one kidney due to congenital deformity or to disease.

Legueu has obtained very favorable results with this method. The uremic constant in case of a tuberculous process in the kidney is modified by the extent of the functional disturbance entailed by the morbid process, also by the concomitant or consecutive inflammation in the kidney, and by the extent of the compensating hypertrophy of the sound mate or of parts of the diseased kidney. This sound mate in time may become so functionally capable that it may compensate entirely the diseased kidney; in this case the uremic constant would indicate normal conditions in regard to the secretion of urine, and the diseased kidney could be removed without the slightest hesitation (Legueu).

Legueu found that in every case in which nephrectomy was done on the basis of a normal uremic constant—about 0.07—the ultimate course confirmed the correctness of the premises. A number of typical cases are described in detail out of the 70 in which the formula was calculated in advance of the nephrectomy. In 22 cases it proved impossible to introduce the ureter catheter and here the uremic constant was almost the sole reliance. Only one of the 70 patients died from renal insufficiency, and this was the only case in which the findings of the uremic constant had been disregarded; for certain special reasons the operation was attempted contrary to the indications. The fatal outcome of the nephrectomy in this case sustains anew the diagnostic importance of this method of estimating, by mathematical formula, the work the kidneys are doing in each individual.

2. McLean's Index of Excretion of Urea and Chlorid (Fig. 49).—In 1915, McLean, basing his studies on the work of Ambard and his collaborators, formulated an index for the study of kidney function. As Ambard's coefficient expresses changes in urea excretion by variations in the value of the constant, K, these variations must be expressed on an arbitrary scale. Values for K increase directly with increase in Ur. (the urea concentration in the blood), other factors remaining the same. Changes in K, then, reflect changes in the blood urea, which changes occur as the square root of changes in the rate of excretion.

In order to express the changes in rate of excretion in a manner mathematically correct and based on a scale of 100 for the sake of comparison, McLean used a formula adapted from the laws of Ambard, which he has called the Index of Urea Excretion. An index of 100, corresponding to a value for Ambard's coefficient of 0.080, is the standard normal index, and variations are expressed directly in terms of the normal. Thus an index of 50 indicates a rate of excretion of 50 per cent. of normal under the conditions of concentration in the blood and

urine. The index is based on a standard normal Ambard's coefficient of 0.080. The derivation of the index is as follows:

$$\text{Index} = \frac{(\text{Rate of excretion found})}{(\text{Standard Normal Rate})} \times 100$$

(under the same conditions of weight and concentration in blood and in urine)

From the law of Ambard:

$$(1) \qquad K = \frac{Ur.}{\sqrt{\text{Rate}}}, \quad \therefore \quad \text{Rate} = \left[\frac{Ur.}{K}\right]^2$$

Similarly (2), $0.080 = \dfrac{Ur}{\sqrt{\text{Normal Rate}}}$

$$\therefore \text{Normal Rate} = \left[\frac{Ur.}{0.080}\right]^2$$

Therefore (3), $\dfrac{\text{Rate}}{\text{Normal Rate}} = \dfrac{\left[\frac{Ur.}{K}\right]^2}{\left[\frac{Ur.}{0.080}\right]^2} = \left[\frac{0.080}{K}\right]^2$

and (4), $\text{Index} = 100 \times \left[\frac{0.080}{K}\right]^2 = \left[\frac{0.80}{K}\right]^2$

Substituting for K (Ambard's Coefficient) and simplifying

$$\text{Index} = \frac{\text{Gm. Urea per 24 hours} \sqrt{\text{Gm. Urea per liter urine} \times 8.96}}{\text{Wt. in Kilos} \times (\text{Gm. Urea per liter of blood})^2}$$

When K. = 0.080, the standard normal index, I, = 100.

In this form the index offers a means of measuring the rate of excretion, under the conditions found at any given time, directly in terms of the normal, and does not require the use of an empirical scale for comparison of pathological cases with the normal (McLean).

Ambard and Weill also applied laws to the excretion of sodium chlorid in the human subjects. They found that the same general laws (discussed under Ambard's Coefficient) were applicable here, with the important exception that, while excretion of urea occurs, no matter how low its concentration falls in the blood, there is a threshold for chlorid excretion, and when the concentration in the plasma falls below the threshold value, excretion of chlorid practically ceases. In view of the fact that there is a wide difference in chlorid content of the corpuscles and plasma, plasma alone, as the fluid part of the blood, has been studied. Ambard and Weill, partly by direct experiments and partly by plotting curves, established the normal threshold value for sodium chlorid as 5.62 grams per liter of plasma. Therefore, the sodium chlorid above 5.62 grams per liter determines the rate of excretion, and the law may be expressed as for urea:

$$\text{Constant} = \frac{\text{Excess of NaCl over 5.62 grams per liter of plasma}}{\sqrt{\frac{\text{NaCl in 24 hours}}{\text{Wt. in Kilos}}} \times \sqrt{\text{NaCl per liter of Urine}}}$$

For practical use it appears best to calculate the plasma sodium chlorid from the rate of excretion, and to compare the calculated concentration with that actually found. The formula, as derived with the use of values actually found for the constant in the above formula, reads:

$$\text{Plasma NaCl} = 5.62 + \sqrt{\frac{D \times \frac{70}{\text{Wt.}} \times \sqrt{\frac{C}{14}}}{79.33}}$$

This in the simplest form reads:

$$\text{Plasma NaCl} = 5.62 + \sqrt{\frac{\text{Gm. NaCl per 24 hours} \times \sqrt{\text{Gm. NaCl per liter Urine}}}{4.23 \times \text{Wt. in Kilos}}}$$

The constancy of this formula depends upon two factors: (1) the constancy of the threshold, and (2) the constancy of the rate of excretion of NaCl above the threshold. Assuming that the laws for rate of excretion of NaCl over the threshold remain constant in normal individuals, one may calculate the threshold by subtracting the calculated excess from the NaCl actually found in the plasma, by the following formula:

$$\text{Threshold} = \text{Plasma NaCl} \sqrt{\frac{D\sqrt{C}}{4.23\ \text{Wt.}}}$$

This formula is subject to error if the rate of excretion over the threshold varies.

The principles of the laws of urea and chlorid excretion are illustrated by McLean in a very simple way. If we imagine a vessel into which water flows at a constant rate, escaping through an outlet at the bottom, the water will seek the level in the vessel at which the pressure is such that the rate of outflow is exactly equal to the intake. If we then increase or decrease the rate of flow, the level will change to meet the new conditions. The change in level of the fluid in the vessel may be regarded as a compensatory change. Under physiological conditions fluctuations in the level of the blood-urea compensate for the changes in the rapidity of formation of urea, and changes in the level of chlorid in the plasma compensate for fluctuations in chlorid intake. Under pathological conditions, changes in the level of urea and sodium chlorid in the blood also occur to compensate for changes in the outlet, in

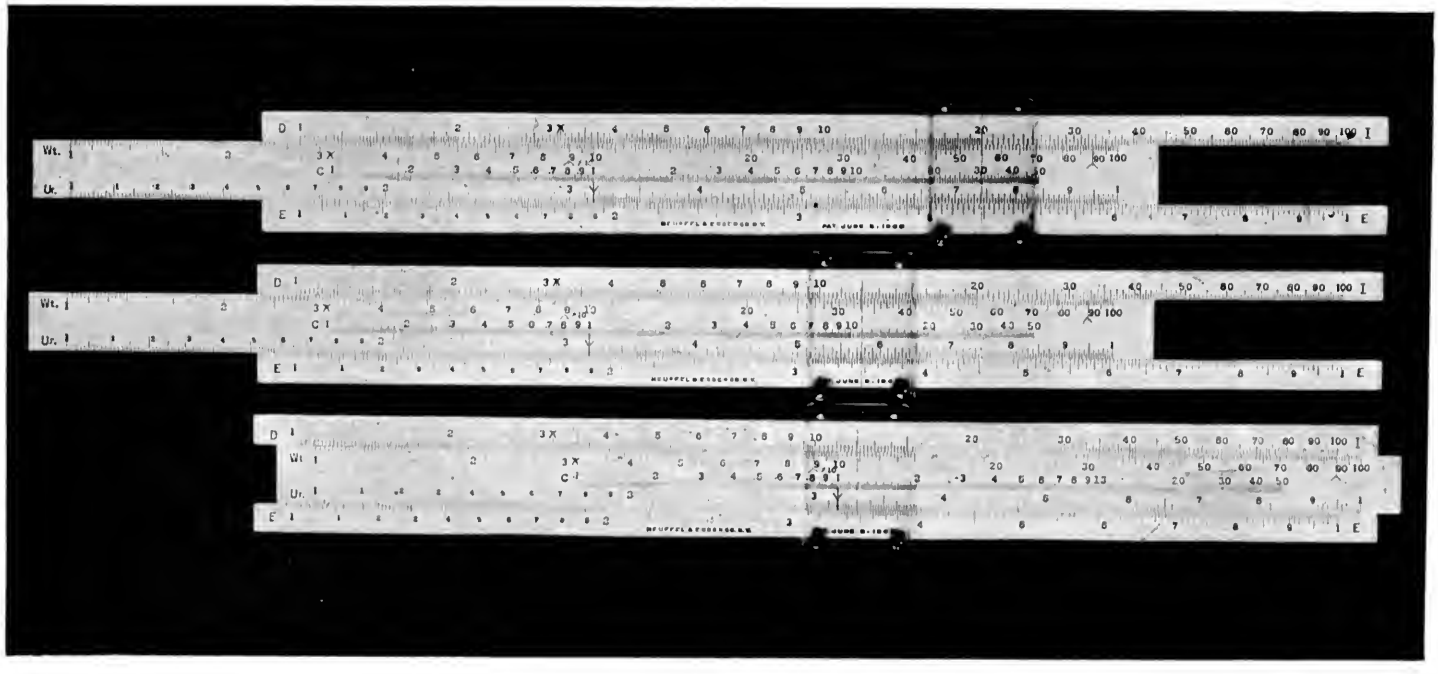


FIG. 49.—TEN-INCH SLIDE RULE ADAPTED TO THE CALCULATION OF THE INDEX OF UREA EXCRETION. (McLean, Journal of Experimental Medicine.)

Example:

Grams urea excreted per 24 hours, D = 20.0
Grams urea per liter of urine, C = 11.0
Grams urea per liter of blood, Ur = 0.330
Body weight, in kilos, Wt = 55.0

$$\text{Index} = \frac{D\sqrt{C} \times 8.96}{\text{Wt.} \times \text{Ur}^2} = \frac{20.0\sqrt{11.0} \times 8.96}{55.0 \times (.330)^2}$$

1. 55.0 on Wt. scale is set opposite 20.0 on D scale, first position.

2. Hair line on runner is moved to 11.0 on C scale, second position.

3. Slide is moved so that 3.30 on Ur scale is at hair line on runner, third position.

Reading is now made at the arrow which points to scale I and is at 100. Therefore Index, I = 100.

the form of the diseased kidneys. In the case of chlorid the outlet of the vessel must be considered as being at some distance from the bottom, and in such a case, only the level of fluid above the outlet would play any part in determining the rate of outflow, which would cease when the level of the fluid fell to the level of the outlet. Similarly, only the chlorid above the threshold determines the rate of excretion, which practically ceases when the threshold value is reached.

The *method of investigation followed by McLean* is as follows: One-half hour after the patient drinks 150 to 200 c.c. of fluid the bladder is emptied and the subject takes no further fluid or food until a carefully timed period, usually of 72 minutes, is ended. The urine excreted during this period is collected, and at the middle of the period about 10 c.c. of blood are withdrawn from an arm vein, clotting being prevented by a small amount of powdered potassium oxalate. The choice of a period of 72 minutes is merely for the sake of convenience, 72 minutes being 1/20 of 24 hours. A 1 or 2-hour period may, of course, be used, all calculations in any case being made on a basis of 24 hours. In case an error of a few minutes is made in the time of collection of the second specimen, the calculation should be made on the basis of the time actually elapsed between the voiding of the first and second specimens. The amount of urea in the whole blood, the total chlorids, estimated as sodium chlorid, in the oxalated plasma after centrifugalization, are determined. Both urea and chlorids are determined in the urine. By substituting the values obtained in the proper formulas the relationship of the rate of excretion of these substances to their concentration in the blood is determined. The index for urea and chlorid is calculated from the formula given above as follows:

"The results from the determinations are calculated to the form in which they are to be expressed in the formula. The weight of the individual, without clothing, should be known to within one kilogram. The four variables are substituted in the formula, and the index calculated. Calculation has been rendered simple by the use of a special calculating device, illustrated in Figure 49. With this device, which is a modified 10-inch slide-rule, one needs to remember neither the formula nor the mathematical principles involved. With a little practice in reading the scales, calculation becomes purely mechanical, and requires only a few seconds. Without this slide-rule, calculation is most easily performed with an ordinary slide-rule, or by the aid of logarithms."

McLean asserts that: "An index below 80 is to be considered as abnormal, though not necessarily seriously so. In renal disease an index below 50 is indicative of a considerable degree of impairment of functional ability. The amount of damage to the kidneys, it is believed, is increasingly greater as the index is lower, and tends to approach zero. But a low index may be only temporary, as in the passive congestion of heart-failure or in acute nephritis, and may return to normal on improvement of the condition which is responsible for impaired function. The actual figure obtained for the index should be of value in prognosis in renal disease, though renal function alone is

TABLE 27.—ILLUSTRATION OF INDEPENDENCE OF INDEX AND NITROGEN INTAKE*. (McLean.)

Date, 1915	Weight, Kg.	Grams Nitrogen in 24 Hours					Urea Excretion, 72 Minute Period			
		Intake	Output			Difference	Gm. per Liter Blood, Ur.	Gm. per Liter Urine, C.	Gm. per 24 Hours, D.	Index of Urea Excretion, I.
			Urine	Stools	Total					
Nov. 5....	65.0	19.2	16.1	3.15	19.25	—.05	0.858	9.87	37.5	22.0
Nov. 12....	64.8	9.6	7.92	1.61	9.53	+.07	0.499	10.68	12.8	23.2
Nov. 16....	64.8	9.6	6.6	2.92	9.52	+.08	0.411	7.06	11.0	24.0
Nov. 30....	65.4	4.8	3.48	1.37	4.85	—.05	0.211	4.37	3.32	21.4

* Case 1.—C. A. P., man, aged 45. Diagnosis: general arteriosclerosis, chronic interstitial nephritis.

TABLE 28.—ILLUSTRATION OF THE COMPARISON BETWEEN THE UREA INDEX AND PERCENTAGE OF PHENOLSULPHONEPHTHALEIN EXCRETED IN TWO HOURS. (McLean.)

Subject	Diagnosis	Blood Urea, Grams per Liter	Urea Index	Phenolsulphonephthalein, Per Cent. in Two Hours
1	Mitral stenosis	.132	250	83
2	Parenchymatous nephritis	.216	139	66
3	Mitral stenosis	.196	105	65
4	Acute nephritis (convalescent)	.383	92	46
5	Heart-block	.480	91	58
4	Acute nephritis (convalescent)	.395	89	51
4	Acute nephritis (convalescent)	.317	68	41
6	Chronic nephritis	.388	60	45
4	Acute nephritis (convalescent)	.406	56	34
7	Chronic nephritis	.446	52	41
8	Chronic nephritis	.437	47	38.5
7	Chronic nephritis	.454	44	33
7	Chronic nephritis	.546	39	42
8	Chronic nephritis	.400	37	27
4	Acute nephritis (convalescent)	.634	33	25.5
9	Mercuric chlorid poisoning	.610	19	15
10	Chronic nephritis	.488	18	25
11	Chronic nephritis	.977	8.1	8
11	Chronic nephritis	1.016	7.9	8.5
12	Chronic nephritis	1.850	7.4	7
4	Acute nephritis	1.320	7	6.5
4	Acute nephritis	.966	6.1	13.2
13	Chronic nephritis	1.110	4.2	2.5
14	Chronic nephritis	2.147	1	Trace
11	Uremia	3.430	0.27	0

often not the determining factor in prognosis. In our experience a low index has at times been the first indication of a serious kidney involvement. When the condition is stationary, life may be maintained for some time with a low index. For example, we have seen patients with chronic nephritis survive for several months with an index of from 5 to 8, and we have seen recovery from acute nephritis after a number of weeks, during which time we have obtained the same figures. In another instance, the patient survived for about a month after the index was as low as 1.2. Such figures, in chronic disease, certainly determine a grave prognosis. But, unless the condition is known to be progressive, it is difficult to give a prognosis as to the duration of life. Other aspects of the disease must be considered in attempting a prognosis.

"Progressive decrease in the index, usually associated with a corresponding increase in the concentration of urea in the blood, is of serious import. Two cases have recently been under observation in which such a fall in the index was followed by death within a short time. In one case the fall in the index was the first indication of impending uremia, although the first symptoms did not occur until ten days after the discovery of a change in the index.

"Use of the index as a guide to dietetic or other treatment of nephritis must depend on further studies. It is doubtful whether the diminished urea content of the blood which follows a diminished nitrogen intake has any direct beneficial effect. Whether a long continued rest of the urea function will improve that function remains to be demonstrated. On general principles, an impaired function should not be overtaxed, and a restricted protein intake should be advised in cases with a markedly lowered index of excretion. But brutal restriction of nitrogen intake to below the nitrogen requirement of the body does not seem to be indicated in cases which are capable of excreting normal amounts of urea, though the blood nitrogen may be high and the index low.

"The influence of diuretic drugs also requires further study. Here the findings will depend on the type of case studied, and it is necessary to have a satisfactory method for grouping them. Study of diuretic drugs, involving also their effect on chlorid function, is in progress.

"Application of the index is seriously interfered with when water excretion is greatly diminished, as in passive congestion or in some forms of nephritis. The laws of urea excretion depend on a sufficiently rapid rate of urine excretion, and they fail to apply when the water output is greatly diminished. We make it a general rule not to attempt to apply the index when a rate of urinary outflow equal to at least 500 c.c. in 24 hours cannot be attained."

3. Physical Methods for Determining Composition of the Blood.—Freezing-point, Refractive Index and Specific Gravity of Blood-serum.—In order to differentiate nephropathies, cardiopathies and allied conditions, Butterfield, Erdwurm and Braddock made extensive studies of the physical and chemical properties of the serum.

It is well known that the delayed elimination of certain substances in the usual forms of nephritis results eventually in the retention of

these substances in the body. At present there is a lack of definite information concerning the quantitative distribution of the retained substances throughout the body fluids and tissues. It is known, however, that an increased concentration of certain substances in the blood-serum occurs in many cases of nephritis. Furthermore, the blood-serum is the most accessible material to study for evidence of changes in concentration, which would result from retention of sufficient quantities of the substances in question.

Through the work of Widal and his collaborators, of H. Strauss and P. von Monakow, the existence of two definite forms of nephritic retention has been established. In one form water and salt are retained, while in the other, there is retention of nitrogenous substances. In some cases both forms of retention occur together. While this information has been derived chiefly from metabolism studies, it would seem logical to suppose that a sufficiently prolonged retention would be followed by characteristic changes in the blood-serum. The detection of these changes in the serum would depend largely on a fortunate selection of methods. Chemical methods alone would not always yield the information desired. A determination of the concentration of sodium chlorid, for example, would reveal little or nothing if both water and salt were retained in corresponding amounts. Nevertheless, the resulting dilution of the serum with aqueous salt solution could be detected by physical methods. H. Strauss made use of the refractometer for this purpose, and thereby called attention to the hydremic state of the blood-serum, estimated by the low refractive index, in cases of nephritis with edema.

Other physical methods may be used to detect different changes in nephritic blood-serum. The freezing-point, for example, may be taken as a measure of the concentration of relatively simple molecules, irrespective of their chemical nature. Although the freezing-point method has been more or less abandoned of late, previous reliable work has shown that there is an increased depression of the freezing-point of the blood-serum in most cases of uremia. Specific gravity determination has also been used to detect changes in the composition of blood-serum, and hydremic sera have been recognized previously by this method.

The numerical values of the freezing-point, refractive index, and specific gravity not only deviate from the normal, but may also vary independently of one another. These independent variations represent changes in the concentration of entirely different groups of substances. Consequently, the systematic use of these methods would seem especially applicable to the problem of nephritic retention, and should yield valuable results in the diagnosis of cardiopathies and nephropathies. This is in fact the case, and it has been possible with these methods to establish characteristic serum pictures for (1) chronic nephritis with edema, (2) chronic nephritis with uremia, and (3) chronic nephritis with both edema and uremia. Furthermore, chronic nephritis with edema may be differentiated from cardiac decompensation with edema by analysis of the serum. Finally, it is possible to recognize a group of cases which cannot be classified either as primary cardiopathy or neph-

ropathy. These cases show persistent hypertension and an extremely concentrated serum. In the absence of more detailed knowledge they may be designated provisionally as primary arteriosclerotic hypertension.

Butterfield and his collaborators, in their study of the physical changes of blood and serum, determined the freezing-point, refractive index, and specific gravity and supplemented them by determination of the protein content and dry residue of the serum. While these supplementary determinations do not add materially to the results, they furnish a striking demonstration of the relatively large variations possible in the solid constituents of the blood-serum in different diseases.

The freezing-point was determined with the Beckmann apparatus.

The refractive index was determined with the Zeiss immersion refractometer. The expression Δ Nd $\times 10^3$ represents the difference between the refractive indices of serum and water at the same temperature, multiplied by 1,000. This makes the numerical results more striking to the eye without affecting their validity in any way.

The specific gravity was determined with an Ostwald pyknometer. The figures given are corrected for temperature and buoyancy of air. The specific gravity is designated as "sp. gr." in the tables.

The protein content was determined gravimetrically after precipitation by alcohol. The dry residue was determined in the customary manner. The results of the determinations of the protein content and dry residue are expressed as percentage weight in volume.

Ten to 15 c.c. of serum are sufficient for a single determination of the freezing-point, refractive index, specific gravity, protein content and dry residue.

Butterfield, Erdwurm and Braddock draw the following conclusions from their work:

The results obtained (Table 29) demonstrate the practical value of this system of analysis in differentiating the cardiopathies from the nephropathies and in classifying the nephropathies. The diagnostic results thus obtained should correspond closely to the results obtained by a study of the metabolism balance. Determination of the freezing-point, refractive index, and specific gravity of blood-serum is a simple procedure and requires, at most, two hours, while a study of the metabolism balance covering the same ground would take at least a week. Analysis of serum by these methods possesses other advantages over the metabolism method. Retention of nitrogen or of water and salt is common to a variety of conditions, and is not necessarily indicative of nephritis. Serum analysis enables one to differentiate the cases in which retention is the result of defective renal elimination from those in which retention is due to other causes.

On the other hand, there are limitations to the serum work. It is obvious that the retention of any substance must attain a certain threshold figure with respect to time and quantity before any changes can be detected in the serum by these methods. It is conceivable that a slight retention could be detected by the metabolism method before definite changes occurred in the serum. The metabolism method

TABLE 29.—ANALYSIS OF THE BLOOD-SERUM.

Group	Case	Freezing point	$\triangle Nd \times 10^3$	Specific Gravity	Dry Residue	Protein Content
I. Normals.....	1 F	0.55	16.8	1.027	9.5	7.9
	2 B	0.56	17.0	1.026	....	7.7
	3 R	0.59	17.8	1.026	9.9	8.5
	4 E	0.57	17.9	1.027	....	....
	5 F	0.57	18.0	1.027	10.0	8.2
II. Cardiac Decompensation with Edema..	1 H	0.55	19.2	1.030	10.8	9.2
	2 L	0.58	17.3	1.026	9.7	8.0
	3 C	0.56	17.0	1.025	9.5	7.8
	4 H	0.56	16.4	1.024	9.1	7.4
	5 Z	0.53	16.0	1.024	9.1	7.4
	6 H	0.54	16.0	1.024	9.0	7.5
III. Chronic Nephritis with Edema.....	1 H	0.59	11.0	1.015	6.3	4.3
	2 H	0.53	11.3	1.016	6.6	5.0
	3 F	0.53	11.5	1.016	6.9	5.1
	4 T	0.55	12.6	1.017	7.1	5.6
	5 K	0.57	12.8	1.020	7.2	6.1
	6 F	0.56	12.8	1.021	....	5.7
	7 D	0.54	12.9	1.020	7.3	....
	8 R	0.55	12.9	1.020	7.2	5.6
	9 G	0.56	13.0	1.019	7.6	5.7
	10 V	0.59	13.2	1.017	7.5	....
	11 P	0.56	13.5	1.019	8.8	5.7
	12 R	0.54	13.5	1.018	7.7	6.0
	13 M	0.55	14.2	1.021	8.0	6.2
	14 R	0.55	14.2	1.022	8.4	6.6
	15 G	0.56	14.3	1.023	8.0	6.3
	16 T	0.55	14.5	1.023	8.1	6.7
Sept. 28	17 G	0.57	13.1	1.022	17.3	5.8
Oct. 7	17 G	0.55	14.3	1.022	8.1	6.6
July 27	18 K	0.55	13.8	1.020	8.1	6.3
Oct. 7	18 K	0.56	14.8	1.022	....	6.7
IV. Chronic Nephritis with Uremia.....	1 V	0.76	18.9	1.030	10.8	8.3
	2 P	0.68	18.9	1.030	10.7	8.4
	3 C	0.67	18.3	1.027	....	...
	4 L	0.66	18.2	1.030	11.4	9.0
	5 K	0.62	17.1	1.028	....	....
	60 S	0.61	16.5	1.027	11.1	7.8
V. Chronic Nephritis with Edema and Uremia.....	1 G	0.61	15.4	1.025	8.5	7.8
	2 P	0.63	14.4	1.022	8.2	5.9
	3 C	0.62	13.3	1.022	7.4	5.3
VI. Arteriosclerosis with Hypertension.....	1 G	0.59	20.8	1.033	12.2	9.8
	2 H	0.56	20.7	1.031	11.4	9.4
	3 W	0.56	19.7	1.030	11.6	9.3

and serum analysis should be used to supplement each other until sufficient comparative data have been obtained.

It should also be borne in mind that the serum findings merely give a picture of the state of the serum at the time of examination. In pathological cases this picture may change from time to time with the progression or regression of the correlated symptoms. A uremic attack may clear up after several weeks, either spontaneously or as a result of bleeding and restriction of protein in the diet, and the freezing-point may then return to normal. Similarly a hydremic serum may gradually change to normal during the disappearance of nephritic edema.

Serum analysis, as well as the metabolism method, also furnishes valuable indications as to the course of dietetic therapy to be followed in the different form of nephropathies. In the uremic type a restriction of protein to the minimum necessary for maintenance is clearly indicated; at the same time the general nutrition should be kept up by increasing the fat and carbohydrate in the diet. In the hydropic form of nephropathy restriction of salt is indicated. Bleeding is beneficial in either uremic or hydropic cases. The restriction of salt or protein in the diet relieves the kidney of unnecessary work and rests the damaged function. No one would expect such treatment to remove scar tissue from the kidney or restore destroyed structures. It may be possible, however, to prevent a progression of the destruction of renal substance and to restore the function of partially damaged structures. At present it is impossible to foresee the beneficial results which may be derived from the dietetic treatment of early cases of chronic nephritis. It is not unreasonable to suppose that similar results would be obtained as in the rational dietetic treatment of diabetes.

Krotoszyner and Hartman investigated the value of blood cryoscopy and blood urea in a comparative study of renal function. They discuss their results in the following words:

"A comparative study of the clinical importance of blood urea and blood cryoscopy must necessarily be based on the consideration of the significance of the two tests. Blood urea estimates renal function by quantitative determination of retention in the blood of a single, though admittedly the most important, constituent of urine; in renal insufficiency the test indicates pathologic accumulation of end-products of nitrogen metabolism, and, therefore, does not so much register the degree, course and variability, as merely the final stage of disturbed function. Blood cryoscopy, on the other hand, does not depend on retention of a single urinary constituent, but on the total sum of retained molecules; or, in other words, it measures renal secretory activity in its totality. On account of the narrow margin of freezing-points between normal and pathologic values, the rise or fall of renal functional capacity can be followed by repeated cryoscopy, and the test assumes, by this means, a prognostic significance which is of the utmost importance for surgical purposes, since on its findings indications for rational therapeutic measures may be based. Blood urea, on the other hand, because simply demonstrating abnormalities of renal metabolism,

to which the organism has been subjected in the past, and on account of its very broad range for normal values, is a less delicate index of variations in renal activity, and, therefore, lacks in prognostic finesse.

"Thus, in a patient with prostatic cancer, who died a few days after prostatectomy, freezing-points gradually rising to 0.580 were found, while the corresponding blood urea points ranged between the normal figures of 165 and 420. In a comparative study of a fatal case, of similar type, in which gradual deterioration of renal function was ascertained by freezing-points rising from 0.540 to 0.611, the corresponding blood urea points varied between 288 and 542, values which are considered to lie still within normal limits.

"In contrast to these findings, five patients with chronic nephritis, with death in uremic coma, showed for both tests high or pathologic points, thus giving parallel results.

"The greatest objection which has been made to blood cryoscopy is the limited range of normal freezing-points, which lie within 0.02 of 1 degree C., and on account of which the slightest inaccuracy in technic may vitiate the result. While this objection does not obtain with blood urea, because of its very broad range up to 550 mg., the drawbacks of the test, as pointed out above, are not offset by the technical difficulty of cryoscopy. After a long practical experience with blood cryoscopy we must admit that its technic is exceedingly delicate, and that correct determination of the end-point is only feasible after long and continuous practice; since differences exceeding 0.005 of 1 degree C. may considerably change interpretation of end-results. During the course of the test a great many precautions must be continuously observed in the handling of the apparatus, thorough skill in which can only be acquired by constant application of an intelligent operator with laboratory training. Everything in the performance of the test, therefore, depends on attention to detail, which, if neglected in the slightest, may entirely upset the end-result. As soon, though, as these technical difficulties have been mastered, the results assume a surprisingly uniform aspect and the test becomes, in experienced hands, a finer and more exact index of renal function than any other method of determination of total renal function. In determination of blood urea, on the other hand, the technic is rather gross and is easily learned and mastered, so that the average intern, after some experience, may obtain reliable results. We can, nevertheless, only partly agree with Hinman's statement, which designates blood urea as the simplest, most definite and most practical of tests of retention. It is certainly simpler, and therefore possibly more practical, but not more definite than cryoscopy, the technic of which is not so difficult as not to be within reach of every surgical assistant."

4. Chemical Composition of the Blood.—The Non-Protein Nitrogenous Constituents of the Blood.—Ever since the time Prevost and Dumas who, in 1823, first demonstrated an increase of the urea of the blood, after extirpation of the kidneys in animals, the total non-protein nitrogen and the urea of the blood have been the subject of repeated investigations and have been accorded considerable importance in the

diagnosis and prognosis of Bright's disease. Owing, however, to the fact that the methods employed have been various and more or less subject to error, the results obtained have been conflicting. This may be readily seen when we find that the total non-protein nitrogen in the normal person is given as anywhere from 25 to 60 mg. per hundred c.c. of blood. The brilliant methods recently devised by Folin render possible the accurate estimation of these substances in a small amount of blood, from 2 to 5 c.c. sufficing for all the analyses. The increase in accuracy depends upon an improved method of removing the proteins from the blood, and the use of Nessler's solution makes it possible to work with small quantities of blood (Tileston and Comfort).

The term "non-protein nitrogen" explains itself. It includes all the nitrogenous substances remaining after the removal of proteins by precipitation, in the case of Folin's method, by means of methyl alcohol and later zinc chlorid. Other names for it are "incoagulable nitrogen," "filtrate nitrogen," "rest" or "retention nitrogen."

Folin's method for the total non-protein nitrogen is essentially a "Micro-Kjeldahl" process, in which the ammonia, after neutralization of the products of digestion, is blown over into a collecting vessel by a current of air instead of by distillation, and is estimated by the use of Nessler's solution and the colorimeter, as in water analysis. His method of urea depends upon the quantitative breaking down of this substance to ammonia at a temperature of 150° C., and its subsequent estimation in the same manner as the total nitrogen. The figures obtained represent urea estimated as nitrogen, and include the ammonia nitrogen, which, however, is so small in normal blood, and presumably in most forms of disease, as to be negligible.

Hohlweg, in association with Meyer, was among the first to demonstrate the value of determining the rest N of the serum in the diagnosis and prognosis of severe nephritis. He reported observations on the rest N of the serum of patients with unilateral and bilateral kidney disease, before and after operation. He found normal values in pure unilateral disease and no increase following nephrectomy, if the remaining kidney was normal. In patients with one diseased kidney and a toxic injury in the other, the rest N is increased. Values up to 75 mg. for 100 c.c. of serum do not contra-indicate operation and in general indicate that the process in the good side is still reparable, or at least capable of improvement. Values of 100 mg. contra-indicate operation. The rest N may increase just after operation but in 4 to 6 weeks it returns to its original level and may go lower than normal.

Folin and Denis, working with the new methods, found in a series of 16 healthy adults that the total non-protein nitrogen varied within narrow limits, and from 22 to 26 mg. per 100 c.c. of blood, while the urea nitrogen was exactly half as much—from 11 to 13 mg. The blood was taken in the forenoon, from 3 to 6 hours after breakfast. The figures given by previous investigators, as already mentioned, are all too high by reason of faulty methods.

Tileston and Comfort studied 142 cases of one sort or another. For

the purpose of classification, they have been divided into 11 groups, as follows: (1) chronic nephritis; (2) other diseases of the kidneys and of the genito-urinary tract; (3) lead-poisoning; (4) the complications of pregnancy; (5) acute intestinal obstruction; (6) diseases of the heart and aorta; (7) the acute infections; (8) syphilis; (9) tuberculosis; (10) diseases of the nervous system; (11) miscellaneous diseases. These will be taken up in order.

For practical purposes they considered figures for the nitrogen below 30 mg. normal; those from 30 to 35, slightly increased; from 35 to 50, considerably increased; and from 50 to 100, greatly increased. One hundred mg. or more constitute a very dangerous elevation of the waste nitrogen. In the case of urea nitrogen, anything over 16 mg. is probably abnormal, and above 25 mg., considerably increased.

Tileston and Comfort concluded from their investigations that:

1. In the fasting healthy adult the total non-protein nitrogen varied between 22.9 and 25 mg. per 100 c.c. of blood, and the urea nitrogen between 12 and 14 mg.

2. The effect of a full meal with meat, in the case of the healthy adult, was a rise of total non-protein nitrogen averaging 4.7 mg., and of urea, averaging 2.5 mg.

3. In both chronic interstitial and chronic diffuse nephritis the cases without symptoms of uremia showed normal or moderately elevated values; the uremic cases, with one possible exception, showed a great increase in both nitrogen and urea.

4. The excretion of phenolsulphonephthalein was roughly proportioned to the degree of retention; the cases with 100 mg. or over of total nitrogen all showed 5 per cent. or less phenolsulphonephthalein excretion. Many cases, however, with a considerable impairment of phenolsulphonephthalein excretion showed no signs of retention, and a moderate amount of retention of waste nitrogen often occurred with no impairment of the elimination of phthalein.

5. The proportion of urea nitrogen to the total non-protein nitrogen in disease varied from 32 per cent. to 85 per cent. Where the nitrogen was normal the urea usually was about one-half the total nitrogen; where it was elevated, the urea usually, but by no means always, constituted about 70 per cent. of the whole. No reason could be found for these variations. The determination of the total non-protein nitrogen alone is therefore more valuable than that of the urea alone.

6. The estimation of the non-protein nitrogen is of the greatest value in the diagnosis of uremia. Amounts of 100 mg. or over were encountered in only two conditions besides uremia, namely, acute intestinal obstruction and profound anemia from hemolysis. Only 1 case of uremia without marked increase in nitrogen was encountered out of a total of 8 cases.

7. The determination of the total non-protein nitrogen is a great aid in the prognosis of chronic nephritis. Patients showing over 100 mg., with one exception, did not live more than 35 days.

8. The results of blood analysis furnish the best guide as to the diet to be given in nephritis. Cases with a considerable retention require a restriction of protein, and by this means a return to normal figures may be brought about, if the azotemia is not too pronounced. In cases of outspoken uremia, however, no marked reduction of the azotemia has resulted from a protein-poor diet. Nephritis with a normal amount of non-protein nitrogen does not call for any marked decrease of protein diet.

9. In chronic passive congestion of the kidneys there is little or no retention of nitrogenous waste products.

10. In pyelitis the presence of azotemia probably indicates involvement of the parenchyma of the kidney.

11. A marked elevation of the non-protein nitrogen of urea renders the patient a poor operative risk, and the azotemia should be overcome by diet, if possible, before an operation is attempted, in all cases in which delay is permissible. In hypertrophy of the prostate, for example, a low-protein diet may be combined with drainage of the bladder as a preliminary to operation.

12. Chronic lead-poisoning was accompanied by evidence of retention in all cases examined.

13. The eclampsia of pregnancy seldom shows a marked increase in non-protein nitrogen and urea. It is therefore distinct from uremia. Analysis of the blood will usually serve to distinguish between uremia and eclampsia.

14. In acute intestinal obstruction a tremendous increase in the nitrogenous waste products was found in all of the three cases examined. A return to normal took place in the two which recovered.

15. Compensated valvular disease of the heart—aortic aneurysm, acute pericarditis with effusion, and acute endocarditis in the absence of disease of the kidneys—all showed normal values.

16. In acute lobar pneumonia a considerable increase was seen in the majority of cases, reaching its maximum toward the crisis, but bearing no relation to the time at which resolution took place. Typhoid fever, acute rheumatism, and uncomplicated scarlatina showed normal figures.

17. Syphilis showed a considerable degree of retention in 36 per cent. of the cases examined, evident in all stages of the disease.

18. In cerebral hemorrhage, hysteria and neurasthenia, no increase was found.

19. Severe anemia due to hemolysis showed a marked retention, reaching in one case 100 milligrams of nitrogen.

20. In uncomplicated diabetes the values were normal; both cases examined in coma showed retention.

21. The administration of thyroid extract in two cases of myxedema caused an increase in both nitrogen and urea. Both cases were complicated with chronic nephritis.

22. In exophthalmic goiter the blood-nitrogen and urea were normal in amount.

23. No changes were met with in malignant disease which could not be ascribed to a complication with renal disease.

24. In a case of acute yellow atrophy the proportion of urea-nitrogen to the total non-protein nitrogen was decreased, although there was a considerable degree of azotemia.

Folin, Denis and Seymour state: "It would seem from these results as though the direct determination of the non-protein nitrogen (and urea) in the blood furnishes a more reliable guide to what might be called the protein tolerance of patients than can be obtained from any 'direct' test of kidney efficiency, for of all tests yet devised for this purpose the phenolsulphonephthalein test of Rowntree and Geraghty is admittedly the best."

The methods for the determination of non-protein nitrogen, urea, creatinin, etc., in the blood have been described (*see* p. 119).

An elaborate investigation of the non-protein nitrogen of the blood recently published by Bang of Lund, Sweden, has brought a confirmation of some of the earlier chemical statistics of the blood as well as an addition to the known facts. Thus the average figure for the non-protein nitrogen is placed, as the result of numerous new analyses, at 25 mg. per hundred grams of blood; out of this an average of 15 mg. is apportioned to urea. These values correspond fairly closely with those first established in this country for man by Folin and Denis.

It has been demonstrated that both amino-acids and urea, representing food and waste, respectively, from the standpoint of nitrogenous metabolism, occur in the corpuscles as well as in the plasma of the blood, the formed elements being permeable to such compounds. An analogous behavior is known in the case of blood-sugar. According to the newest analysis of Bang, both the corpuscles and the plasma of human blood, as a rule, contain practically the same amount of total residual nitrogen, urea and amino-acids. In several instances Bang has observed an increase in the urea content of the blood without any comparable change in the other non-protein nitrogenous constituents dur-

TABLE 30.—ANALYSIS OF BLOOD: NORMAL.*

Age	Total Nitrogen (mg.)		Urea Nitrogen (mg.)	
	After Fasting 12 Hours	2½ Hours After Heavy Meal	After Fasting 12 Hours	2½ Hours After Heavy Meal
24	22.9	25.9	12.0	13.6
26	25.0	27.9	13.6	14.5
27	23.7	29.5	12.6	15.1
29	23.8	32.3	14.1	20.9
29	23.2	26.4	12.3	13.1

* In this and the following tables, the figures for total nitrogen and for urea nitrogen represent milligrams per 100 c.c. of blood.

TABLE 31.—ANALYSIS OF BLOOD: NEPHRITIS

Sex	Age	Diagnosis	Systolic Blood-pressure (mm. Hg)	Urine		Blood	
				Phenol-sulphone-phthalein Per Cent. in 2 Hours	Diet	Total N (mg.)	Urea N (mg.)
M	41	Chronic interstitial.	180	66	Mixed.....	23.0	11.4
F	62	Chronic interstitial.	195	..	Mixed.....	23.2	11.1
F	52	Chronic interstitial.	210	40	Mixed.....	27.4	13.4
F	75	Chronic interstitial.	210	38	Mixed.....	28.2	14.6
F	66	Chronic interstitial.	220	38	Moderate protein	29.0	16.1
					Poor protein, 1 day.....	25.3	.....
M	65	Chronic interstitial.	200	..	Mixed.....	29.7	16.1
F	64	Chronic interstitial.	205	42	Mixed.....	31.5	16.4
F	46	Chronic interstitial.	180	50	Soft.....	31.7	15.4
F	61	Chronic interstitial.	250	..	Mixed.....	31.7	16.7
F	63	Chronic interstitial, with albuminuric retinitis.....	185	..	Mixed.....	32.0	17.7
F	53	Chronic interstitial.	240	..	Mixed.....	35.3	.....
F	24	Chronic interstitial, with cholelithiasis.....	...	80	Soft.....	57.5	27.7
M	48	Chronic interstitial, with uremia.....	240	22	Low protein, 6		
				26	days.....	24.2	12.5
M	25	Chronic interstitial, with albuminuric retinitis.....	212	0.0	Mixed.....	79.5	55.1
						102.2	79.5
						70.5	40.4
M	55	Chronic interstitial, with uremia....	210	0.5	Milk.....	87.5	53.1
						151.2	109.4
M	22	Chronic interstitial, with uremia.....	180	0.5	Mixed.....	188.7	141.0
						286.2	191.0
M	25	Chronic interstitial, with uremia.....	175	0.0	Low protein.....	220.0	143.0
					5 days mixed....	232.5	184.0
					3 days.....	324.0	237.0
F	20	Chronic diffuse....	132	43	Liquid.....	313.0	205.0
M	25	Chronic diffuse....	146	5	Soft.....	26.7	10.7
			210	20.0	Salt free 5 weeks.	22.6	8.0
					Mixed.....	55.2	31.5
						67.0	49.0
						140.6	113.1
						173.7	131.3
M	44	Amyloidosis.....	...	55	Mixed.....	33.9	15.3

ing starvation. This was demonstrated, however, to be associated with a lack of water, and disappeared as soon as a suitable intake of water was assured. The ingestion of protein did not lead, in Bang's experience, to any noteworthy concentration of amino-acids in the blood unless the intake was inordinately large. Evidently a renal loss of amino-

acids is thus averted so long as the blood content does not rise to an excretion level. The urea content may be decidedly increased, however, thus showing the speedy conversion of amino-acid nitrogen into its characteristic end-product of nitrogenous waste.

From the work of Tileston and Comfort, whom we have quoted above, the accompanying tables are extracted, showing their results in normal (Table 30) and pathological (Tables 31 and 32) subjects.

TABLE 32.—ANALYSIS OF BLOOD: OTHER DISEASES

Sex	Age	Diagnosis	Systolic Blood-pressure (mm. Hg)	Urine	Diet	Blood	
				Phenol-sulphone-phthalein Per Cent. in 2 Hours		Total N (mg.)	Urea N (mg.)
M	48	Acute nephritis.....	85	48	Soft.....	69 7	57.7
						29.5	15.7
M	58	Chronic passive congestion.....	128	70	Karell.....	24.6	13.7
M	54	Chronic passive congestion.....	170	49	Karell.....	25.0	13.8
M	58	Chronic passive congestion.....	120	50	Soft.....	30.6	16.9
M	45	Chronic passive congestion.....	90	58	Mixed.....	31.7	15.1
M	47	Chronic passive congestion.....	115	53	Karell.....	34.4	17.5
M	21	Chronic passive congestion.....	110	75	Soft.....	42.2	25.4
F	16	Chronic passive congestion.....	...	..	Soft.....	67.7	43.9
F	27	Pyelitis..	118	80	Soft.....	26.5	14.9
F	19	Pyelitis.....	112	30	Liquid.....	70.7	51.0
					Soft.....	40.2	29.2
					Mixed.....	33.5	18.2
M	47	Hypernephroma.....	125	..	Liquid.....	91.2	61.2
M	62	Stricture of urethra, with acute ascending infection.....	180	..	Soft.....	209.0	172.0
M	63	Stricture of urethra..	...	..	Soft, with meat once daily....	31.4	19.1
M	69	Tuberculosis of genito-urinary tract...	88	10	Liquid.....	50.0	37.6
M	86	Hypertrophied prostate with retention.	85	..	Mixed.....	48.7	37.6
					Low protein....	30.5	17.2
M	77	Hypertrophied prostate with retention.	142	60	Mixed.....	41.0	24.3
M	64	Carcinoma, prostate and bladder.....	110	10	Soft.....	58.2	45.5
M	70	Carcinoma, bladder.	...	0.0	.....	99.5	69.7

From the work of Folin, Denis and Seymour we copy the following blood findings in nephritis (Table 33):

TABLE 33.—RESULT OF BLOOD TESTS IN NEPHRITIS AS INFLUENCED BY DIET. (Folin, Denis and Seymour).

Case	Name	Age	Diagnosis	Blood									Phenol-sulphone-phthalein Excretion Per Cent. in 2 Hours
				Ward Diet			High Protein Diet			Low Protein Diet			
				Non-protein Nitrogen (Mg.)	Urea Nitrogen (Mg.)	Uric Acid (Mg.)	Non-protein Nitrogen (Mg.)	Urea Nitrogen (Mg.)	Uric Acid (Mg.)	Non-protein Nitrogen (Mg.)	Urea Nitrogen (Mg.)	Uric Acid (Mg.)	
13	[1]Patrick M.....	63	Chr. interstitial neph..	92	70	3.5	124	109	4.5	..	..	...	8
14	Mary K. O....	75	Chr. interstitial neph..	42	34	2.5	61	41	3.5	..	..	...	54
15	Hannah J.....	64	Chr. interstitial neph., aortic roughening, aneurysm of arch...	50	34	2.1	58	40	2.5	36	16	2.4	32
16	Mary K.....	81	Chr. interstitial neph., cardiac hypertrophy, aortic roughening, mitral regurgitation.	35	19	1.9	39	25	1.9	30	16	2.0	30
17	[2]Chas. M.....	60	Chr. interstitial neph., epilepsy.....	34	19	2.6	69	55	2.0	23	11	...	25
18	Dennis O'B....	31	Chr. interstitial neph., albuminuric retinitis alcoholic.....	58	41	2.0	[3]75 [4]80	52 62	2.1 2.5	44	20	2.1	6

[1] This patient died in "uremia" while on high protein diet two days after last sample of blood was taken.

[2] This patient was confined to bed during almost the whole period of experiment. Urinary nitrogen determinations made March 4 to April 4 showed nitrogen excretion varied from 22 to 15 grams in twenty-four hours. Went on low protein diet, as given in first twelve cases, April 23. During a five-day period nitrogen excretion averaged in twenty-four hours was 5 grams.

[3,4] Taken one week apart.

TABLE 34.—URIC ACID, UREA NITROGEN AND CREATININ OF BLOOD IN INTERSTITIAL NEPHRITIS (Chase and Myers)

Case	Age	Diagnosis	Condition	Mg. per 100 c.c. of Blood			Phthalein 2 Hrs., Per Cent.	Systolic Blood-pressure	Urine	
				Uric Acid	Urea N	Creatinin			Albumin	Casts
H. L.	23	Pulmonary tuberculosis	Unchanged	6.5	16	2.7	58	130	++	+
E. H.	41	Pericarditis	Unchanged	5.6	13	2.1	45	150	—	—
F. D.	45	Interstitial nephritis	Unchanged	5.5	12	2.5	37	185	—	+
B. D.	35	Diffuse nephritis	Unchanged	9.6	19	2.4	45	175	+	+
J. J.	65	Early interstitial nephritis	Unchanged	9.5	25	2.5	13	185	+	+
D. S.	56	Early interstitial nephritis	Unchanged	6.6	24	3.3	26	185	—	+
D. D.	52	Early interstitial nephritis	Unchanged	8.7	20	3.6	20	100	+	+
C. M.	54	Early interstitial nephritis	Unchanged	6.3	31	2.0	23	150	—	—
L. P.	57	Moderately severe chronic interstitial nephritis		8.0	80	4.8	0	240	++	++
			Improved	4.9	17	2.9	10	170		
J. P.	34	Moderately severe chronic diffuse nephritis	Improved	8.3	72	3.2	25	238	+++	++
				5.3	21	1.9	43	145		
W. C.	49	Moderately severe chronic diffuse nephritis	Improved	9.5	44	3.5	38	210	++	++
				2.5	19	1.9	52	120		
E. C.	50	Typical fatal case of chronic interstitial nephritis	Died	22.4	236	16.7	0	210	++	Pus
T. D.	34	Typical fatal case of chronic interstitial nephritis	Died	15.0	240	20.5	2–3	225	++	+
S. H.	37	Typical fatal case of chronic interstitial nephritis	Died	14.3	263	22.2	0	220	++	+
J. W.	34	Typical fatal case of chronic interstitial nephritis	Died	8.7	144	11.0	Trace	225	+	+

* Normal findings: uric acid from 2 to 3 mg.; urea nitrogen, from 12 to 15 mg.; creatinin, from 1 to 2.5 mg. per 100 c.c.

It would seem from these results as though the direct determination of the non-protein nitrogen (and urea) in the blood furnishes a more reliable guide to what might be called the protein tolerance of patients than can be obtained from any "direct" test of kidney efficiency, for of all tests yet devised for this purpose the phenolsulphonephthalein test of Rowntree and Geraghty is admittedly the best.

Garnier and Gerber studied the character of kidney function during infective jaundice. In very severe cases, oliguria and even absolute anuria indicated the inaction of the kidneys: the urea content of the blood increased up to the moment of death and attained exceptionally high values—as high as 9.2 grams per liter. The absolute value of the urea content of the blood is, however, not determinative of the prognosis, inasmuch as patients have recovered after showing contents of 5.50 and 5.93 grams of urea per liter of serum, and others succumbed after showing maximum contents of only 2.87 and 3.23 grams urea per liter. The Ambard coefficient attained very high values in severe cases —up to 1.22 and 1.28. Urea ceased to be retained in the blood stream, and the Ambard coefficient returned to normal in 7-15 days following the beginning of the jaundice.

Chase and Myers drew the following conclusions from their study of their blood chemistry in nephritis:

"An increase in the uric acid of the blood would appear to be of considerable value as an early diagnostic sign of incipient nephritis.

"The urea of the blood has been found very valuable as a guide to the treatment of moderately severe cases of nephritis, since any change in the patient's condition is quickly perceptible.

"As a prognostic test the blood creatinin has been found of very great service, over 5 mg. to 100 c.c. having invariably proved fatal after the lapse of a comparatively short period of time. During the terminal stages of the disease the concentration of the creatinin gradually rises, reaching 15 to 30 mg. in most cases at death.

"The determination of the carbon dioxid combining power of the blood plasma according to the method of Van Slyke is a valuable index to the acidosis of nephritis, from the viewpoint of both diagnosis and treatment."

We cite a table (Table 34) from Chase and Myers to show their analytical data.

Gettler and George examined the blood of 600 nephritics. Their results are given in the following table:

Table 35.—Range of Values in Nephritis, taken from 600 Determinations

Non-protein nitrogen	From 40 to 460 mg. in 100 c.c. of blood.
Urea nitrogen	From 20 to 375 mg. in 100 c.c. of blood.
Creatinin	From 2 to 42 mg. in 100 c.c. of blood.
Uric acid	From 3 to 17 mg. in 100 c.c. of blood.
Sugar	From 75 to 160 mg. in 100 c.c. of blood.
Alkali reserve	From 40 to 75 per cent.

From their work, they arrive at the following conclusions:

1. All the waste nitrogen products, nonprotein nitrogen, urea, creatinin and uric acid, are present in increased amounts in cases of true nephritis, and generally, but not invariably, present in greater concentration in the blood of those cases which are primarily considered as chronic interstitial nephritis (retention nephritis). This finding is in agreement with the results of practically all workers.

2. The degree of retention (when taking into account the functional efficiency of the cardiac muscle) is a direct criterion of the severity of the lesion.

3. They agree with Hopkins and Jonas that the sugar content in the blood is similarly increased in nephritis, and, at least in our cases, more marked in the patients suffering with the chronic parenchymatous form of the disease.

4. The alkali reserve is a valuable index of the degree of acidosis present. In their table, it runs considerably lower in the interstitial type of the disease. They would suggest that particular attention be paid to cases with low alkali reserve, as it becomes an important factor in the early recognition of uremia.

5. While they are not presenting figures, in attempting to correlate the clinical findings with the postmortem picture, they agree with Frothingham that there is no definite lesion of nephritis referable to a certain clinical picture. In other words, all the clinical and laboratory evidence in a nephritic patient may point to a certain form of the disease, but the postmortem examination is often not in agreement with the diagnosis made during life.

Frissel and Vogel made an effort to record graphically the course of renal function from the time of first observation to the time of death (see Fig. 50).

The figures on uric acid and creatinin, except during the last two weeks of life, are too few to be of much statistical value. At this period they are uniformly high. The average shown by the curves of phenolsulphonephthalein, non-protein and urea nitrogen, and the urea index, seems to indicate a method which, if checked by a large number of observations, might prove valuable in arriving at an average expectation of life for a given determination.

The curves for the non-protein nitrogen and urea nitrogen show a rapid rise during the three months preceding death, while during the earlier months there is a tendency for the curves to maintain a constant level. The elevation at the fifth month which disturbs the symmetry of the curves is caused by a single case in which the values were so high that the averages were disproportionately raised.

Mosenthal and Lewis found the relationship between tests of renal function and prognosis in nephritis to be very uncertain. The extrarenal factors, cerebral hemorrhage, myocardial insufficiency, intercurrent infections, etc., have caused a fatal termination so often that greater emphasis must be placed on the physician's clinical judgment

than on the interpretation of tests for renal function alone. In certain patients, degrees of impaired kidney activity are found which are ordinarily considered incompatible with life. These cases as well as others which exhibit uremia with apparently fair functional kidney processes, go far to show that uremia and renal lesions are not entirely dependent one on the other.

The most striking example of this kind in the series of Mosenthal and Lewis is a case with the diagnosis, polycystic kidneys. In December,

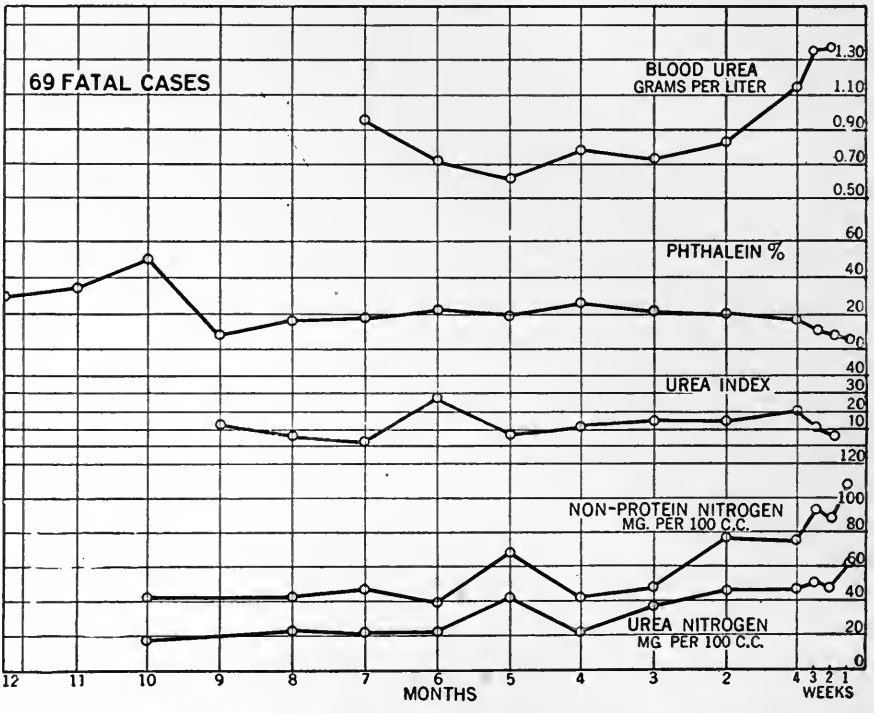


Fig. 50.—Chart on Renal Function Based on 685 Determinations. (Frissel and Vogel.)

1914, the total non-protein nitrogen of the blood was 118 mg., the excretion of phenolsulphonephthalein yielded only a faint trace in two hours (Ambard's coefficient, if determined, would undoubtedly have been very high), and the test-meal showed a maximal impairment of function (maximal specific gravity, 1.011; variation from the highest to the lowest specific gravity, 1 degree; a night urine of 1,290 c.c.). Fifteen months later (March, 1916), the phenolsulphonephthalein and test-meal were unchanged, the blood nitrogen was 112 mg., and Ambard's constant 0.67. One and one-half years after tests which indicated an extreme impairment of renal function, he is working at his office every day.

"A high non-protein nitrogen (above 90 mg.) or a high urea nitrogen

(of 65 mg. or higher) has been found to be the most reliable prognostic sign. Of eighteen such patients, fourteen died in the hospital or shortly after leaving it; of the other four patients, one is still in the hospital in a precarious condition, one is the case of polycystic kidney referred to above, one is a case of mercuric chlorid poisoning with recovery, and the last is a case of secondary contracted kidney who has followed therapeutic directions so closely that he may almost be said to have outlived his day. It is clear to the physician coming in contact with these cases of nitrogenous retention that a sharp line can be drawn between those suffering with increased protein catabolism and those who are not. An example of this kind has been given in detail. This factor when present almost invariably foreshadows an unfavorable prognosis; a retention, on the other hand, not coupled with an abnormal degree of protein destruction, may do very well (as the case of polycystic kidney mentioned previously). In the earlier stages of nephritis, the nitrogen of the blood is very likely to lag behind the other tests and it has been shown how Ambard's coefficient is more reliable under these circumstances as a test of renal function.

"In nephritis, whether of the chronic diffuse or of primary contracted type, an Ambard's coefficient of 0.20 or higher has usually been associated with a fatal issue within a few months at the most. Of 87 patients with an index less than 0.2, 63 were traced and 15 died, 7 of uremia and 3 of cerebral hemorrhage. Of 36 patients with an index above 0.2, 30 were traced and 24 died, 12 of uremia, 9 of myocardial insufficiency, and 3 of cerebral hemorrhage. It is only to be expected that the patients with a higher Ambard's coefficient should show a more unfavorable prognosis. However, here, as in the case of other functional tests, an infallible guide to prognosis is not demonstrated. The test-meal usually reaches a point of maximal intensity some time before the other tests. Uremia, or death from some other cause, may occur at these periods, and thus lend an aspect of greater prognostic importance to the test-meal than it actually possesses. It may be used as a valuable guide to prognosis in conjunction with the other procedures, but an opinion of maximal impairment of function should not be given without them.

"The phenolsulphonephthalein test in this series has yielded results which have not varied from those reported previously. An output of less than 10 per cent. has usually, but not invariably, warranted an utterly bad prognosis; occasional instances of death from uremia have taken place with an excretion of the drug in the neighborhood of 50 per cent." (Mosenthal and Lewis).

Mosenthal and his co-workers recommend charting the results of the various tests and comparing them with the nitrogen intake as shown in the accompanying chart (Fig. 51).

From their experiments, Mosenthal and Lewis draw the following conclusions:

"1. Such a graduation (as in the accompanying chart) calls to the attention of the clinician the relative degree of involvement as shown

by different procedures. Inasmuch as each of them has a significance apart from the others, comparison according to this method is an extremely valuable aid in the treatment and prognosis of diseases of the kidney.

"2. The level of the non-protein and urea nitrogen of the blood must be estimated largely as the resultant of three factors—kidney

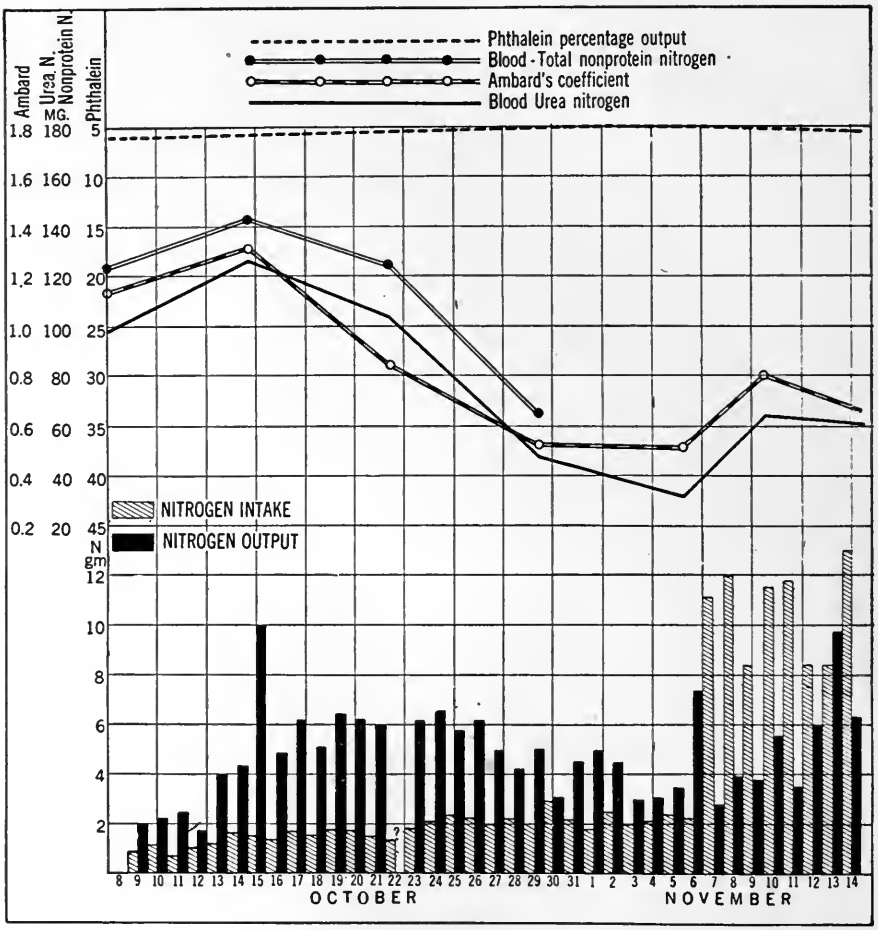


Fig. 51.—Chart from Case of Secondary Contracted Kidney, Indicating the Processes of Protein Destruction and Assimilation and Their Relation to Tests of Renal Function.

efficiency, diet and protein destruction. In judging of prognosis, when these substances are high in the blood of nephritics, due regard must be given as to whether their accumulation is brought about by retention alone or through retention coupled with protein destruction. The former offers a comparatively better prognosis than the latter.

"3. The coefficient of Ambard is a better method of determining

the ability of the kidney to excrete urea than the level of this substance in the blood.

"4. The progress of renal disease is probably followed most minutely by means of the phenolsulphonephthalein excretion and Ambard's coefficient, as these tests furnish figures in which even small variations are of significance.

"5. The test-meal for renal function, of the tests employed, gives the earliest indication of diminished kidney efficiency. It likewise reaches the maximum degree of impairment before the others.

"6. Each test for renal function covers only a limited range of the kidney's activities. It is, therefore, a mistake to speak of any test as measuring renal function as a whole. The aim should be to develop a proper interpretation of the old tests and easily applied new ones, in order to obtain a true guide to the treatment of diseases of the kidney."

Fitz investigated the renal functional capacity in diabetic patients. In the majority of cases the urea index tended to be normal or abnormally high, due, in part, to the rapid rate of water elimination which characterized many of the cases. In six cases of fatal diabetic coma, the urea index was abnormally low. In diabetes the blood-plasma chlorid is usually lower than would be calculated from the chlorid excretion according to the formula of Ambard and Weill.

Atchley after a very painstaking study of eight cases of acute nephritis comes to the conclusion, that "one must express the feeling that the investigation of this disease has been hindered by an interest too closely restricted to the kidneys. A broader study of the chemical balances of the body as a whole may demonstrate that the kidney is of secondary importance."

Creatinin in the Blood.—Lampert has made a study of the excretion of creatin and creatinin in diabetics and nephritics. He employed the Autenrieth and Müller method, and all patients were on a meat-free and bullion-free diet during the periods of observation. In healthy adults, he found the daily average excretion of creatinin to be between 0.9 and 2.4 grams, the majority being between 1.2 and 1.5 grams. Creatin was usually absent; when present, it was found only in traces (0.02 mg.). In diabetics, the values for creatinin were usually lowered or at the lower limit of the normal. With few exceptions creatinin was found to an amount less than 1 gram, though in several cases 1 to 1.2 grams were found. In diabetes gravis with a high grade of acetonuria, creatin was practically always found in the urine, usually more than 0.2 gram (on two occasions 1 and 1.2 grams). In diabetes levis with traces of acetone, creatin was lacking in 2 cases or present only in small amount (less than 0.3 gram). In nephritis (5 cases), the author found a decrease in creatinin constantly, even when there was a diuresis and only slight impairment of function. Creatin was found in only 1 case, in which there was marked renal insufficiency, and then only in small quantity (maximum 0.15 gram). Two cases of diabetes complicated by chronic interstitial nephritis were character-

ized by an especially low creatinin value. Low creatinin values were also observed in a case of cancer of the liver with cholemia; creatin was also present in small amount (0.1 gram or less). Lampert thinks it is plausible to attribute the large quantities of creatin found in the urine of diabetics with acidosis to a disturbance of metabolism and the diminished output of creatinin in nephritics to renal insufficiency. The generally accepted view that acidosis is the result of deficient oxidation of the acetone bodies suggests the idea that the increase in creatin is also due to a disturbance of intermediary metabolism. The findings in the nephritics suggest the use of this method in functional renal diagnosis.

Myers and Lough found that estimation of the creatinin of the blood in nephritis was valuable as a diagnostic and prognostic test. The increase of creatinin in the blood is considered a safer index of the decreased permeability of the kidneys than that of urea or uric acid because creatinin on a meat-free diet is entirely endogenous in origin and its formation is very constant, whereas the formation of urea and uric acid is subject, even normally, to great fluctuations. In the authors' 63 cases the creatinin was estimated by a modified Folin method, oxalated and laked blood being saturated with picric acid, filtered, treated with sodium hydroxid, and then compared colorimetrically with a standard creatinin solution of known strength, to which the alkali has also been added. A rise in the creatinin above 2.5 mgs. to 100 c.c. of blood was found to signify renal involvement almost invariably. Creatinin values of from 2.5 to 3 mgs. are to be viewed with suspicion; from 3 to 5 mgs. as decidedly unfavorable, and over 5 mgs. as probably indicating an early fatal termination.

Orlovius found that creatinin excretion is retarded in pregnancy when the kidneys are affected. The more retarded the excretion the more extended is the pathological process in the kidneys. Pronounced retardation of the creatinin excretion may be of import in determining the indication for an interruption of pregnancy.

For the determination of creatinin in the blood the following methods are used:

(*a*) *Methods of Folin—Preformed Creatinin* (Hawk).—Measure 10 c.c. of blood into a 50 c.c. volumetric flask or, better, into a 50 c.c. shaking cylinder which can be closed with a glass stopper. Fill to the 50 c.c. mark with saturated picric-acid solution and shake a few times. Add about 1 gram of dry picric acid to the mixture and shake for 5 minutes. Transfer the mixture to centrifuge tubes, throw down the sediment, and precipitate and pour the supernatant liquid through a filter. This is the most economical process where but little blood is available. If desired, however, double quantities of blood and reagents may be taken and filtration carried out without preliminary centrifugation. This process removes the protein materials and leaves the creatin and creatinin in the filtrate which is a saturated picric-acid solution. Then determine the preformed creatinin colorimetrically. For this purpose a standard solution of creatinin is necessary for comparison. Prepare this from

the standard creatinin stock solution as used in the analysis of urine by diluting an amount of this solution equivalent to 1 mg. of creatinin to 500 c.c. with saturated picric-acid solution. We have then a standard solution containing 0.2 mg. of creatin in 100 c.c. of saturated picric-acid solution.

Take 20 c.c. portions each of the filtrate and of the standard solution. To each solution then add exactly 1 c.c. of 10 per cent. NaOH from a buret. (If the blood filtrate becomes turbid on addition of alkali it must be centrifuged or filtered.) Allow to stand for 10 minutes and compare the colors directly in the colorimeter without further dilution. The standard creatinin solution may be set advantageously at 20 mm., although this is not necessary.

Calculation.—Since the blood was diluted 5 times in the precipitation procedure and as the standard for comparison contains 0.2 mg. of creatinin per 100 c.c., it is merely necessary to divide the reading of the standard by the reading of the unknown to obtain, without further calculation, the number of milligrams of creatinin in the 100 c.c. of blood.

(*b*) *Creatin Plus Creatinin.*—To determine the total creatin plus creatinin in the blood, carry out the preliminary precipitation with picric acid just as in the determination of creatinin above. Take 10 c.c. of this filtrate for the determination. Transfer it to a small Erlenmeyer flask or large test-tube. Cover the flask or test-tube with tin foil, transfer to an autoclave, and heat to about 120° C. for about 20 minutes. The autoclave should not be opened until the temperature has fallen below 100° C. Cool the solution to room temperature, rinse into a 25 c.c. volumetric flask with saturated picric-acid solution. Add 1.25 c.c. of 10 per cent. NaOH for the development of the color.

On account of the variations in the creatin content of normal blood two standard creatinin solutions are used. In working on pathological cases a third standard is desirable. These standards contain 0.5, 1, and 2 mg. of creatinin respectively per 100 c.c. of saturated picric-acid solution. To 20 c.c. of each of these solutions in measuring cylinders add 1 c.c. of 10 per cent. NaOH and allow to stand for 10 minutes. By inspection determine which standard corresponds most nearly in color with the unknown and use this for comparison. The standard is usually set at 10 mm. in the Duboscq colorimeter.

Calculation.—Multiply the reading of the standard by 125 and by 0.5, 1, or 2, according to which standard is used, and divide by the reading of the unknown in millimeters. The result gives the number of milligrams of creatin—creatinin in 100 c.c. of the blood examined.

Uric Acid in the Blood.—*Estimation of Uric Acid.*—Using the Kuttner colorimeter, the method (reliable for clinical purposes) is as follows: Two standard color tubes are used. No. 1 equals 0.01 mg. uric acid, and No. ½ equals 0.005 mg. uric acid. The reagents needed are acetic acid 1 per cent., colloidal iron 5 per cent., a saturated solution of sodium acetate, a saturated solution of sodium carbonate, and the Folin-Macallum uric acid reagent.

The test can be performed with 1 c.c., 0.5 c.c. or less of fresh non-hemolized serum. It is measured with a pipet, transferred into a short wide mouth test-tube suitable for boiling, the pipet rinsed several times with water and the rinsings also added. It is then further diluted with water and reagents so that every c.c. of the dilution contains 0.1 c.c. of serum. One c.c. of serum is diluted at first with about five volumes of water; one drop of a 1 per cent. acetic acid solution and two drops of a saturated sodium acetate solution are added for every 0.1 c.c. of serum used. This is boiled and the tube with contents is rapidly cooled by holding it in running tap water. The cooled mixture is poured into a graduated test-tube, a few drops of a 5 per cent. colloidal iron solution added, shaken and made up to 10 c.c. with water. This is centrifuged or filtered. Of the clear filtrate or supernatant fluid, which should be colorless, enough is taken with a pipet and transferred into the calibrated tube to reach the 50 mark. One drop of the uric acid reagent is added, then five drops of the saturated sodium carbonate solution, mixing well after each addition. The color should be allowed to develop for one minute and the estimation then made. For comparison two standard color tubes are used. No. 1 represents 10 mg. of uric acid per 100 c.c. of serum, that is, every ten divisions on the scale equal 1 mg. The second color standard called No. ½ represents 5 mg. of uric acid or 1 mg. for every twenty divisions of the scale for 100 c.c. of serum.

When the solution in the calibrated tube is darker than standard color tube No. 1, this tube must be used for the comparison. After diluting drop by drop with water until the colors match, the height which the meniscus has reached on the scale is noted. This figure divided by 10 represents the number of milligrams of uric acid in 100 c.c. of serum. Using this tube with the meniscus reaching 75 on the scale, the computation is as follows:

$$\frac{75}{10} = 7.5 \text{ mg. uric acid per 100 c.c.}$$

Should the color be lighter than standard color tube No. 1, then tube No. ½ must be used, but the reading must be divided by 20, thus:

$$\frac{75}{20} = 3.75 \text{ mg. uric acid per 100 c.c.}$$

When the color is too light to be compared to the standard color tube No. ½, indicating a very low amount of uric acid, 2 c.c. or more of the clear filtrate may be concentrated by boiling, but must be rapidly cooled, before the reagents are added, and compared with the standard, as already described, allowance being made for any additional quantity used by dividing by the proper factor.

One to 3 mg. of uric acid in 100 c.c. are normally present in human serum.

5. Acidosis in Renal Disease.—The acidosis of chronic nephritis is the result of inefficient renal excretion. In uremia, acidosis is probably a very constant feature, but only in a small number of cases is it of sufficient grade to cause clinical symptoms similar to those seen in advanced cases of diabetes. In cases of chronic nephritis of a mild type,

where the phenolsulphonephthalein test shows normal renal function, there is, as a rule, little or no acidosis. Advanced cases of chronic nephritis in which the phenolsulphonephthalein output is moderately or extremely decreased may show an acidosis by the "alkali tolerance" test, but the alveolar carbon-dioxid tension may be diminished. Only in very advanced cases is the acidosis so marked as to cause a decrease in the alveolar carbon-dioxid tension.

Marriott and Howland determined the inorganic phosphate in the serum of patients with acidosis occurring in nephritis. In every instance there was an increase in the phosphorus to many times the normal amount, that is, to from 8 to 23 mg. per 100 c.c. of blood. Simultaneous determinations of the combined carbon dioxid of the serum showed that in certain instances the phosphoric acid was combined with twice as much of the available base as was carbonic acid, in striking contrast to the normal conditions in which the base combined with phosphoric acid equals only from one-tenth to one-fifteenth of that combined with carbonic acid. The retention of acid phosphate (for approximately 90 per cent. of the phosphate in an average urine is acid phosphate) would seem to be sufficient to account for the degree of acidosis observed.

Porges and Leimdorfer have investigated the carbon tension of the blood in kidney disease, and have found that it varies with the degree of renal function.

To determine the degree of acidosis of a patient, the following methods may be used:

(1) Determination of hydrogen-ion concentration of the blood.
(2) Determination of the CO_2 tension of the blood.
(3) Determination of the CO_2 tension of the alveolar air.

(1) Hydrogen-Ion Concentration of the Blood (Levy, Rowntree and Marriott).—Heretofore the indicator method has not proved of great value in the studies of hydrogen-ion concentration of the blood, although the reaction of inorganic solutions may be determined quite accurately by this means. Different indicators show their color changes at varying degrees of hydrogen-ion concentration; for example, the color of methyl orange changes from pink to yellow as the pH of its solution changes from 3 to 5. At intermediate points, various colors may be obtained and a certain color indicates a definite pH. Similarly, phenolphthalein changes from colorless to pink between pH8 and pH10 and can be used for the measurement of H-ion concentrations between these two points. In carrying out the indicator method, it is necessary to have a series of standard solutions of known pH and an indicator exhibiting easily distinguishable color changes at hydrogen-ion concentrations approximating that of the solution under consideration. It is then simply necessary to add equal amounts of indicator to the standard solutions and to the solution being tested and to determine which of the colors in the standard solutions most closely matches that of the unknown solution.

This method has been successfully used on the urine by Henderson and by Walpole. As proteins interfere with the colors of many indicators, and as both blood and serum possess color, it has been impossible to apply the method directly to the blood. It seemed probable that the indicator method might be utilized for blood, provided coloring matters and proteins could be excluded by means of dialysis. If blood is dropped into collodion sacs and dialyzed for five minutes, the dialysate is free from proteins and coloring matter, but contains salts, and is well adapted to the use of indicators.

Since phenolsulphonephthalein exhibits definite variations in quality of color, with very minute differences in hydrogen-ion concentration between pH6.4 and 8.4, it was adopted as the indicator in this method.

Preparation of Standard Colors.—Standard phosphate mixtures are prepared according to Sorensen's directions as follows:

1/15 mol. acid or primary potassium phosphate. 9.078 grams of the pure recrystallized salt (KH_2PO_4) is dissolved in freshly distilled water and made up to 1 liter.

1/15 mol. alkaline or secondary sodium phosphate. The pure recrystallized salt ($Na_2HPO_4.12H_2O$) is exposed to the air from 10 days to 2 weeks, and protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $Na_2HPO_4.2H_2O$ is obtained; 11.876 grams of this are dissolved in freshly distilled water and made up to 1 liter. The solution should give a deep rose-red color with phenolphthalein. If only a faint pink color is obtained, the salt is not sufficiently pure.

The solutions are mixed in the proportions indicated below to obtain the desired pH:

Preparation of Solutions

pH.....	6.4	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	8.0	8.2	8.4
Primary Potas. Phos., c.c.	73	63	51	37	32	27	23	19	15.8	13.2	11.0	8.8	5.6	3.2	2.0
Secondary Sodium Phos., c.c.	27	37	49	63	68	73	77	81	84.2	86.8	89.0	91.2	94.4	96.8	98.0

Three c.c. of each of the solutions are placed in suitable small test-tubes (100 x 10 mm., inside measurement). Five drops of an aqueous 0.01 per cent. solution of phenolsulphonephthalein are added to each tube. The tops are sealed off. The series of colors, representing different concentrations of hydrogen-ions, constitute the standards for comparison of color in carrying out the determination.

Preparation of Sacs.—One ounce of collodion (Anthony's negative cotton) should be dissolved in 500 c.c. of a mixture of equal quantities of ether and ethyl alcohol, with occasional gentle shaking; a small amount of brown sediment appears at first. The solution should stand for at least 3 or 4 days, after which the clear supernatant liquid is

ready for use. A small test-tube (120 x 9 mm. inside measurement) is filled with this mixture, inverted, and half the contents poured out. The tube is then righted, and the collodion allowed to fill the lower half again. A second time it is inverted and rotated on its vertical axis, the collodion being drained off. Care must be taken to rotate the tube, in order to secure a uniform thickness throughout. The tube is clamped in the inverted position and allowed to stand for 10 minutes, until the odor of ether finally disappears. It is then filled 5 or 6 times with cold water, or soaked 5 minutes in cold water. A knife blade is run around the upper rim to loosen the sac and a few c.c. of water run down between the sac and the tube. By gentle pulling, the tube is extracted, after which it is preserved by complete immersion in water.

The Salt Solution Used in the Method.—The blood or serum is dialyzed against an 0.8 per cent. sodium-chlorid solution.

Before applying the test, it is necessary to ascertain that the solution is free from acids other than carbonic. To determine this, a few c.c. of the salt solution are placed in a Jena test-tube and 1 or 2 drops of the indicator added, whereupon a yellow color appears. On boiling, carbon dioxid is expelled, and the solution loses its lemon color and takes on a slightly brownish tint. In the absence of this change, other acids are present, and the salt solution is therefore not suitable. If, on the other hand, on adding the indicator, pink at once appears, the solution is alkaline and hence cannot be used.

Technic of Method.—The work must be done in a room free from fumes of acids or ammonia.

A needle is inserted into a vein and blood drawn with as little loss of CO_2 as possible. This is accomplished by drawing it under paraffin oil or by a specially devised tube. The tourniquet should be applied just before inserting the needle.

Approximately 3 c.c. of blood is run, by means of a blunt-pointed pipet, into a dialyzing sac, which has been washed inside and outside, with normal (0.8 per cent.) salt solution, and which has been tested for leaks by filling with the salt solution. The sac is lowered into a glass tube of 100 by 10 mm. inside measurements, containing 3 c.c. of the salt solution, and kept there until the fluid on the outside of the sac is as high as it is on the inside. From 5 to 10 minutes are allowed for dialysis. The collodion sac is then removed and .2 c.c. of the .01 per cent. phenolsulphonephthalein indicator solution thoroughly mixed with the dialysate. The tube is then compared with the series of standards until the corresponding color is found, which indicates the hydrogen-ion concentration present in the dialysate.

Comparison of Tubes with Standards.—For this, a good light (natural or artificial) and the white glass background of the examination box are requisites. Readings must be made immediately after the dialyzation. The two tubes most nearly approximating the color of the specimen are selected and one placed on each side of the specimen. These are critically inspected against the white background. If the relative

position of the tubes is changed, the differences in the colors are made more apparent at times.

(2) Van Slyke Method for the Determination of the Carbon-dioxid Combining Power of Blood-plasma.—Having centrifuged the fresh oxalated blood, pipet off the clear plasma and place in a separatory funnel of about 300 c.c. capacity. Slight hemolysis does not affect results appreciably, but hemolysis should be avoided as much as possible by immediate centrifugalization. In order to determine its alkaline reserve, saturate the plasma with carbon dioxid at alveolar tension. In other words, the operator blows vigorously through a bottle containing glass beads into the separatory funnel. If one blows directly into the separatory funnel, enough moisture condenses on the walls of the funnel to appreciably dilute the plasma. Close the funnel at the stop-cock just before the stream of breath stops, and shake for one minute in such a manner that the plasma is distributed as completely as possible about the walls. After the shaking has lasted a minute, blow a fresh portion of the alveolar air through the beads into the funnel and shake for one minute.

The CO_2 apparatus (Fig. 52) is held in a strong clamp, W, which is lined with rubber, and the lower stop-cock is supported by an iron rod, which is also covered with soft rubber tubing. The apparatus is completely filled with mercury. Care should be taken that capillaries A and F, which are above the upper stop-cock, are also filled with mercury. There should be no air bubbles within the apparatus. Six dropping bottles, which contain the following solutions, should be at hand:

1. Distilled water.
2. Phenolphthalein (1 per cent. in 95 per cent. alcohol).
3. Normal ammonium hydroxid.
4. Caprylic alcohol.
5. Normal sulphuric acid.
6. Mercury.

The mercury leveling bulb, H, should be hung by wire, I, on extension, N, about on the level with the lower cock, J. The apparatus must be thoroughly clean before the determination is started. The apparatus can be tested by allowing the mercury to run down and then forcing it up by raising and lowering bulb, H. The air is forced out and the mercury is caught in a bottle. (This is done until there is not a single air bubble in the apparatus.) Add. 1 drop of phenolphthalein to the upper cup, B, and a drop or two of the ammonium hydroxid. Now dilute this with about ½ c.c. of distilled water and draw off all except about 2 drops of the alkaline solution.

Now introduce 1 c.c. of the saturated plasma into the cup and allow it to flow under the alkaline solution, so that none of the carbon dioxid escapes. Turn stop-cock, C, so that E and Z are connected, and allow the plasma to run in until capillary, F, is exactly filled. Add 0.5 c.c. of distilled water to cup, B, and then allow to run down to capillary, F. Repeat this, taking care that no air enters the apparatus with the liquid.

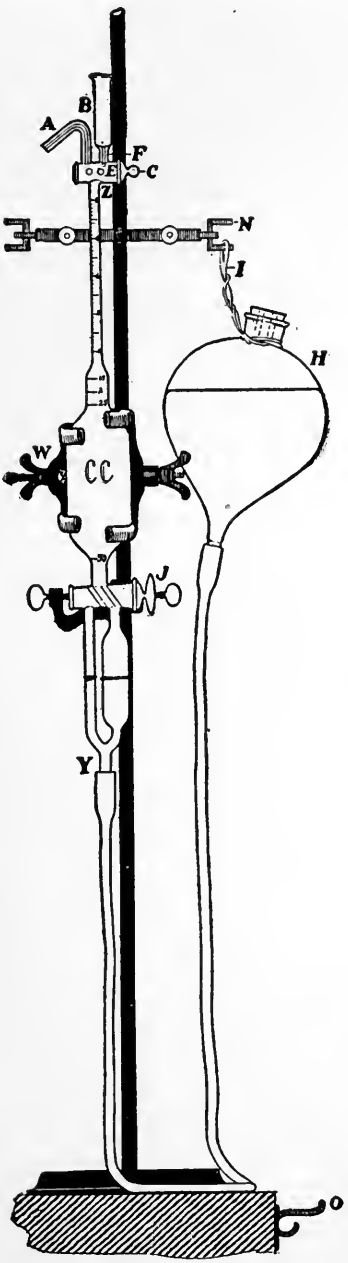


FIG. 52.—VAN SLYKE CARBON DIOXID APPARATUS. (Blaivas Modification.) (From "Newer Methods of Blood and Urine Chemistry," Gradwohl and Blaivas, C. V. Mosby Co.)

Now admit into capillary, F, 1 drop of caprylic alcohol to prevent foaming, and pour about 1.5 c.c. N/10 sulphuric acid into the cup. Admit

enough of the acid into the apparatus, carrying the caprylic alcohol along with it, so that the total volume in the apparatus is exactly 2.5 c.c. Draw off the excess sulphuric acid. Now place a few drops of mercury in cup, B, and allow to flow down to capillary, F, in order to seal the latter and make it capable of holding an absolute vacuum. During this whole operation, the lower stop-cock, J, should remain open, and when the apparatus is set up, it should be in such adjustment that, if the wire, I, which is connected to bulb, H, is lowered to hook, O, the mercury will run to the mark, X, on the figure, care being taken that the mercury will not run into fork, Y. Place wire, I, on hook, O, and allow the mercury to fall until the meniscus of the mercury has dropped to the 50 c.c. mark on the apparatus. This is controlled by stop-cock, J. The bubbles of CO_2 are now seen escaping.

In order to completely extract the carbon dioxid, remove the apparatus from the clamp and shake by turning it upside down about a dozen times. (The thumb should be placed over cup, B, so as not to lose any of the mercury.) Then replace the apparatus, the mercury leveling bulb, H, still being at the lower level, O, and allow the solution to flow into the small bulb below the lower stop-cock (right side). Drain the solution out of the portion of the apparatus above the stop-cock, J, as completely as possible, but without removing any of the gas (the last drop being allowed to remain above). Now raise the mercury bulb, H, in the left hand, and with the right hand immediately turn the lower stop-cock, J, so that the mercury is admitted to the upper part of the apparatus through the left-hand entrance of the stop-cock without readmitting the watery solution. Hold the leveling bulb, H, beside the apparatus so that its mercury level corresponds to that in the apparatus, and the gas in the latter is under atmospheric pressure. A few hundredths of a c.c. of water will float on the mercury in the apparatus, but this may be disregarded in leveling. The calculation of the result into terms of volume percentage of carbon dioxid, bound as carbonate by the plasma, is quite complicated and we consequently use the direct reading from the apparatus, minus .12.

Plasma of normal adults yields 0.65 c.c. to 0.90 c.c. of gas, which is the direct reading on the apparatus. If .12 were subtracted, the normal figures would be 53 to 78 in terms of volume per cent. of carbon dioxid chemically bound by the plasma. Figures lower than 50 per cent. in adults indicate acidosis. The exact calculation of the result into terms of carbon dioxid bound as carbonate by the plasma is quite complicated, and consequently the worker is advised to subtract 0.12 from his reading on the apparatus. The result thus obtained gives approximately (within 2 to 3 per cent.) the volume per cent. of carbon dioxid bound by the plasma.

Example.—Reading on the Van Slyke apparatus is 0.74 minus 0.12, which equals 0.62 per cent. of carbon dioxid bound by 1 c.c. of plasma. For 100 c.c. of plasma multiply 0.62 per cent. by 100, which equals 62 per cent. (normal).

(3) Determination of Alveolar Carbon-dioxid Tension (Marriott).

—*Collection of Alveolar Air.*—The method of collection is essentially that of Plesch, as modified by Higgins. A rubber bag of approximately 1,500 c.c. capacity is connected by means of a short rubber tube to a glass mouthpiece. About 600 c.c. of air are blown into the bag with an atomizer bulb, and the rubber tube clamped off by a pinchcock. The subject should be at rest and breathing naturally. At the end of a normal expiration, the subject takes the tube in his mouth; the pinchcock is released and the subject's nose closed by the observer. The subject breathes back and forth from the bag four times in twenty seconds, emptying the bag at each inspiration. The observer should indicate when to breathe in and out. Breathing more frequently will not greatly alter the results. At the end of twenty seconds, the tube is clamped off and the air analyzed. The analysis should be carried out within 3 minutes' time, as carbon dioxid rapidly escapes through rubber.

The foregoing procedure applies to patients who are capable of cooperating to some extent. In the case of comatose patients, the initial amount of air in the rubber bag must be greater (1,000 c.c. at least), and the period of rebreathing prolonged to 30 seconds. This is necessary, as it is not feasible that the bag be completely empty of air at each inspiration; and therefore a longer time is required for the carbon-dioxid tension in the bag and in the lungs to become equal. The initial amount of air in the bag should be such that it is at least one-half and preferably as much as two-thirds emptied at each inspiration. Since comatose patients cannot hold the mouthpiece, some form of mask is necessary. This may be a gas anesthetic mask or such a device as described below for use with infants.

A special mask has been devised for the collection of alveolar air from infants. It is made from the nipple of a wide-mouth (Hygeia) nursing bottle and a piece of thin rubber tissue (dental dam). A sheet of the tissue (8 by 10 inches) is perforated in the center by a piece of hot metal or glass tubing of large bore. The whole is stretched and pulled over the nipple and slipped down to the lower rim. A small amount of rubber cement is applied to hold the tissue and nipple together. A strip of adhesive plaster three-eighths inch wide is applied around the rim of the nipple so as to overlap the rubber tissue and hold it firmly in place. The extreme tip of the nipple is cut off and a short glass tube, three-eighths inch in diameter, inserted.

"In making a collection of alveolar air from infants a rubber bag of 500 c.c. capacity is connected with the mask and partially filled with air by means of an inspirator bulb. The neck of the bag is closed off by a pinchcock or with the fingers, the mask placed over the nose and mouth of the infant and the rubber tissue closely drawn around the face, so as to prevent the escape of air. The mask should, if possible, be placed over the face just at the end of expiration. Respirations are allowed to continue for from 28 to 32 seconds, and at the end of an expiration, the neck of the bag is closed off and the mask removed from the face. We have found that it is necessary that the infant should be breathing quietly for one minute previous to the

collection of the air sample, as vigorous crying, just before the mask is put on, leads to a lowering of the carbon-dioxid tension, as determined, by several millimeters. Crying during the collection of the sample almost invariably occurs and facilitates mixing of the gases. The effect is to raise the tension somewhat if crying is very vigorous, but not to such an extent as to be significant. The initial amount of air in the bag must be such that during inspiration the bag is from one-half to two-thirds empty, but never completely collapsed. The amount of air required for infants under 1 year of age varies from 250 c.c. to 400 c.c." (Marriott.)

The mask described above for use with infants may be very conveniently used for the collection of alveolar air samples from dogs. The animal's nose is inserted into the mask and the rubber tissue drawn closely around the muzzle. The time of a collection need not exceed 25 seconds.

For analysis of the air samples, the apparatus required comprises 8 test-tubes, containing standard phosphate solutions; a standard bicarbonate solution; a small test-tube; a glass tube or pipet drawn out to a capillary point, and a box for color comparison.

Principle of the Method of Analysis.—The method depends upon the fact that if a current of air, containing carbon dioxid, is passed through a solution of sodium carbonate or bicarbonate until the solution is saturated, the final solution will contain sodium bicarbonate and dissolved carbon dioxid. The reaction of such a solution will depend upon the relative amounts of the alkaline bicarbonate and the acid-carbon-dioxid present. This, in turn, will depend upon the tension of carbon dioxid in the air with which the mixture has been saturated and will be independent of the volume of air blown through, provided saturation has once been attained. High tensions of carbon dioxid change the reaction of the solution toward the acid side. Low tensions have the reverse effect; hence the reaction of such a solution is a measure of the tension of carbon dioxid in the air with which it has been saturated.

The reaction of such a solution may be determined by adding to it an indicator, such as phenolsulphonephthalein, which shows, over a considerable range of reaction, definite color changes. A certain color indicates a certain reaction.

Solutions of a given reaction may be prepared by mixing acid and alkaline phosphates in definite proportions. Such solutions can be kept unaltered for long periods of time and may be used as standards for comparison.

Preparation of Standard Phosphate Solutions.—Fifteenth molecular acid potassium phosphate: Of the pure, recrystallized salt (KH_2PO_4), 9.078 grams are dissolved in distilled water, 200 c.c. of 0.01 per cent. phenolsulphonephthalein solution are added, and the whole is made up to 1 liter.

Fifteenth molecular alkaline sodium phosphate: The pure, recrystallized salt ($Na_2HPO_4.12H_2O$) is exposed to the air for from 10 days

to 2 weeks, and protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $Na_2HPO_4 . 2H_2O$ is obtained. Of this salt, 11.876 grams are dissolved in water, 200 c.c. of 0.01 per cent. phenolsulphonephthalein solution are added, and the whole is made up to 1 liter. The solutions are mixed in the proportions indicated in the table.

Proportions in Which Solutions are Mixed

Mm	10	15	20	25	30	35	40	45
Acid Potassium Phosphate, c.c.	17.8	25.2	31.0	35.7	40.5	45.0	47.0	40.2
Alkaline Sodium Phosphate, c.c.	88.2	74.8	69.0	64.3	59.5	55.0	53.0	49.8

The solutions thus made are put into small test-tubes (a convenient size is 10 by 75 mm.) and stoppered or sealed off. These standard tubes should be kept in a dark place when not in use.

The standard bicarbonate solution is prepared either by weighing out 0.530 gram of desiccated sodium carbonate or by measuring accurately 100 c.c. of tenth normal sodium hydroxid into a 1 liter volumetric flask. Two hundred c.c. of 0.01 per cent. phenolsulphonephthalein are added, and the whole is brought up to the mark with distilled water. Carbon dioxid from a cylinder or from the lungs may be passed through this solution to convert the alkali into bicarbonate, or the solution may simply be used as it is, as the alveolar air that will be blown through the solution subsequently will accomplish the same purpose.

The comparison with the standard colors is conveniently made with a box similar to that used with the Sahli hemoglobinometer, but containing 3 instead of 2 holes. It is a small, flat box of blackened metal, 55 by 45 by 20 mm., backed with opal glass and with 3 slits on the front. By the use of this device, slight color changes may be easily detected, and the temperature of the tubes is not raised by the heat of the hands. In addition, the whole device, with tubes, may be immersed in water at a given temperature.

All of the standard solutions used in this method should be kept in glass which does not readily give off alkali, that is to say, such a glass as "Jena," "Non-sol" or "Pyrex." A small amount of toluene or thymol added to each solution serves to prevent the growth of molds.

Technic of the Method of Analysis.—In analyzing a sample of air, about 2 or 3 c.c. of the standard bicarbonate solution are poured into a clean test-tube of the same diameter as the tubes containing standard phosphate solutions, but from 100 to 150 mm. long. Air from the bag is then blown through the solution by means of a glass tube drawn out to a fine capillary point, until the solution is saturated, as shown by the fact that no further color change occurs. The tube is stoppered and the color immediately compared with that in the standard tubes.

In comparing, one can readily read to millimeters. Color changes are not quite so sharp above 35 mm. as at the lower end of the scale, but here changes are of less significance. In making the color comparisons, the solution being compared is placed between the two standards which it most nearly matches. When there is doubt as to whether the color of the solution is higher or lower than one of the standards, changing the order in which the tubes are placed in the comparison box will generally make the relationships clear.

The standard solutions described are so prepared as to give correct results when the determination is carried out at a temperature of from 20° to 25° C. (from 68° to 77° F.). When the room temperature is considerably higher or lower than these points it is advisable to immerse the tubes in water at approximately 25° C. during the blowing. They may be removed from the water for the color comparison, however, provided this is quickly made. The differences due to the ranges of temperature occurring under ordinary circumstances are practically negligible.

No correction for barometric pressure is required, as, from the nature of the determination, barometric fluctuations are self-corrective. Variations in the temperature of the subject are never great enough to affect the value as much as 1 mm., and therefore may be neglected.

6. Indicanemia as a Sign of Renal Disease.—Tschertkoff studied the indican of the blood in kidney affections. Irrespective of the nature of the diet, urea retention or indican is never encountered in the serum of healthy or diseased individuals without renal insufficiency. In nephropaths who have considerable urea retention in their serum, indicanemia is a regular occurrence. In the presence of a urea content of about 1.5 per mille, indicanemia was never missing. In chronic nephritides indicanemia and urea retention of 1.5 per mille are unfavorable symptoms, indicating grave renal alterations. Indicanemia is the only sign of renal insufficiency in those cases in which acetonemia has attained a normal degree through alimentary influences.

Dorner studied the indican of the blood and other body fluids in nephritics with uremic symptoms and in other patients with convulsions, following the suggestion of Obermeyer and Papper, who believed they had established the fact that a marked increase of the pigment in the blood is practically diagnostic of uremia. The technic was as follows: 10 to 20 c.c. of blood obtained by venesection or syringe were precipitated with 5 times the volume of 95 per cent. alcohol, and filtered. The filtrate was evaporated and the residue taken up in water. This was precipitated by the addition of 2 to 3 drops of lead acetate solution, filtered and made up to 10 c.c. by the addition of water. A like amount of Obermeyer's solution was then added. The mixture was shaken in a water-bath with 1 c.c. of chloroform. A more or less intense blue color in the chloroform indicates a positive test. Dorner studied 26 patients; 7 of these had well-marked uremic symptoms and 2 suspected uremia. His conclusions were: (1) The presence of indican in the blood in large quantity is characteristic of outspoken uremia. (2) When

indican is present in the blood in considerable amount it is also found in the pleural exudate and anasarca fluid, but not in the spinal fluid. (3) No toxic effect can be attributed to the indican in uremia.

Rosenberg has been studying to determine the extreme limit of normal indicanemia, and tabulates the figures found in 40 healthy persons or patients with apparently sound kidneys, in 32 with kidney disease but no uremia, and in 29 with urea in the blood up to 1.5 per cent., 1,000. Jolles's test is too delicate to be dependable, but the Obermeyer-Tschertkoff test begins to give a positive response at just about the point where the high indican content of the blood corresponds to actual insufficiency on the part of the kidneys.

IV. Urea Content of the Cerebrospinal Fluid

Soper and Granat studied 29 cases of nephritis and 56 cases of non-kidney disease with regard to the urea content of the cerebrospinal fluid. They concluded that a urea content of more than 0.2 per cent. points to severe uremia and a rapid, fatal termination; a urea-content of 0.1 to 0.2 per cent. is of grave prognostic import and may be followed by uremia; below 0.1 per cent. no significance can be attached to the findings.

Canti studied the urea content of the cerebrospinal fluid with the following results: Examinations of the spinal fluid, both antemortem and postmortem, from a considerable number of cases with and without uremia gave some valuable facts upon which to base prognosis. The normal urea content of the spinal fluid was found to lie below 0.05 per cent., as determined by the hypobromite method. Among the clinical cases of uremia, all those showing a high urea content in the spinal fluid were fatal. Most of those with low urea content survived, and in several the diagnosis was found to have been erroneous. Among cases not clinically classed as uremic there were two groups, one having a high urea content and being complicated with disease other than renal, which masked the signs suggestive of uremia; the second, those with a low urea content, although not cases of uremia, included many different forms of disease, showing that a high urea content is usually absent in the presence of uremia. A certain proportion of uncompensated cardiac cases were diagnosed as uremia but they revealed low urea content. The treatment of such cases along lines adapted to uremia was not well tolerated, but treatment directed to the heart gave good results.

BIBLIOGRAPHY

General

Albarran. Ann. d. mal. d. org. génito-urin., 1905, ii, 1332.
Ambard. Physiologie normale et pathologique des reins, 1914.
Austin. Pennsylvania Med. Jour., 1913, xvii, 394.
Baetjer. Arch. Int. Med., 1913, xi, 593.
Barker. Monographic Med., 1916, Vol. III, 863.

Bauer and Nyiri. Ztschr. f. urol., 1915, ix, 81.
Blum. Nierenphysiologie und funktionelle Nierendiagnostik, 1913.
Braasch and Thomas. Jour. Am. Med. Assn., 1915, lxiv, 104.
Cabot. New York Med. Jour., 1906, lxxxiii, 962.
Christian. Boston Med. and Surg. Jour., 1913, clxix, 468.
Fitz. *Ibid.*, 384.
Folin and Denis. Jour. Biol. Chem., 1915, xxii, 321.
Foster. Arch. Int. Med., 1913, xii, 452.
Frothingham. Am. Jour. Med. Sc., 1915, cxlix, 808.
Gaudy and Jacques. Ann. Soc. belge d'urol., 1913, xiii, 34.
Geraghty. Johns Hopkins Hosp. Bull., 1915, xxvi, 155.
Janeway. Tr. Congr. Am. Phys. and Surg., 1913, 14.
Jungmann and Meyer. Arch. f. exper. Path. u. Pharmakol., 1913, lxxiii, 49.
Machwitz, Rosenberg and Tschertkoff. München. Med. Wchnschr., 1914, lxi, 1268.
von Monakow. Deutsch. Arch. f. klin. Med., 1914, cxv, 47, 224.
Quinby. Tr. Am. Urol. Assn., 1914, viii, 138.
Quinby and Fitz. Arch. Int. Med., 1915, xv, 303.
Rowntree. Am. Jour. Med. Sc., 1914, cxlvii, 352.
Thevenot. Province méd., 1913, xxiv, 162.
Zondek. Ztschr. f. klin. Med., 1915, lxxxii, 78.

Methylene Blue Test

Achard and Castaigne. Gaz. hebd. de méd., Paris, 1897, No. 37, 433.
Bard. *Ibid.*, 433.
Bard and Bonnet. Arch. gén. de méd., Feb., 1898, 129.
Chauffard. Presse méd., 1898, i, 13.
Chauffard and Cavasse. *Ibid.*, 129.
Czylharz and Donath. Wien. klin. Wchnschr., 1899, xxiv, 649.
Müller. Deutsch. Arch. f. klin. Med., 1899, lxiii.
Oulmont and Raymond. Presse méd., 1899, 201.
Pugnaud and Revilliod. Arch. gén. de méd., 1902, viii, 19.
Rowntree and Geraghty. Jour. Pharmacol. and Exper. Therap., 1909-1910, i, 584.
Underhill and Closson. Amer. Jour. Physiol., xiii, 358.
Voisin. Gaz. hebd. de méd., 1897, 433.
Walker. The renal function in urinary surgery, 1908.

Indigocarmine and Rosanilin Tests

Dreyfus. Thése de Lyon, 1898.
Heidenhain. Handbuch der Physiologie.
Kapsammer. Nierendiagnostik und Nierenchirurgie, 1907.
Lepine. Lyon méd., 1898, lxxxvii, 251, 573.
Oppenheimer. Verhandl. d. deutsch. Ges. f. Urol., 2nd Kongress, 1909, 289.
Voelcker and Joseph. München. med. Wchnschr., 1903, 2081.

Phenolsulphonephthalein Test

Abel and Rowntree. Jour. Pharmacol. and Exper. Therap., 1909, i, 231.
Austin, Pearce, and Eisenbrey. Jour. Exper. Med., Oct. 14, 1912, xvi, 375.
Boyd. Jour. Am. Med. Assn., 1912, lviii, 620.
Cabot and Young. Boston Med. and Surg. Jour., Oct. 12, 1911, clxv, 549.
Deutsch. Wien. klin. Wchnschr., Aug. 8, 1912, xxv, 1217-1223.
Eisenbrey. Jour. Exper. Med., Nov. 14, 1912, xvi, 375.
Geraghty. Jour. Am. Med. Assn., 1913, lx, 191.
Goodman. *Ibid.*, lxi, 184.
Goodman and Kristeller. Surg. Gynec. and Obst., 1911, xii, 56.

KAHN. *Ibid.*, 1917, xxiv, 458.
KEYES. Am. Jour. Urol., 1912, 601.
KEYES AND STEVENS. New York Med. Jour., June 1, 1912.
——— Am. Jour. Urol., Oct. 7, 1912.
REMSEN. Am. Chem. Jour., vi, 280.
ROWNTREE AND GERAGHTY. Jour. Am. Med. Assn., 1911, lvii, 811.
——— Arch. of Int. Med., 1912, 284.
——— Jour. Pharmacol. and Exper. Therap., 1910, i, 593.
SCHMIDT AND KRETSCHMER. Am. Jour. Urol., Aug., 1912, 399.
SEHRT. Centralbl. f. Chir., Aug. 17, 1912, No. 33, xxxix, 1121-1124.
SOHON. Am. Chem. Jour., xx, 257.
WHITNEY. Jour. Am. Med. Assn., 1912, lviii, 936.

Renal Function as Measured by Elimination of Fluids, Salt and Nitrogen, and the Specific Gravity of the Urine

VON SZOLLOSY. Ztschr. f. exper. Path. u. Therap., 1916, xvii, 115.

Cryoscopy of the Urine

DRESER. Exp. Arch., 1892, xxix, 303.
KOEPPE. Berl. klin. Wchnschr., 1901, 736.
KORANYI. Ztschr. f. klin. Med., 1897, xxxiii; 1898, xxxiv, 1.
NAGELSCHMIDT. *Ibid.*, 1901, xlii, 274.
VON NOORDEN AND RITTER. *Ibid.*, 1891, xix, 197.
STRAUSS. *Ibid.*, 1902, xlvii, 337.
ZANGEMEISTER. Berl. klin. Wchnschr., 1903, 1198.

The Hemorenal Index

ARRHENIUS. Ztschr. f. physik. Chem., 1889, iv, 96.
BEIN. Wied. Ann., 1892, xlvi, 29.
BERSCH. Ztschr. f. physik. Chem., 1891, viii, 384.
BROMBERG. Jour. d' Urol., 1913, iv, 739.
——— Deutsch. med. Wchnschr., 1913, xxxix, 1358.
——— *Ibid.*, xl, 118.
BUGARSKY. Arch. f. d. ges. phys. d. Menschen, 1897, lxviii, 389.
ELSAS. Wied. Ann., 1891, xliv, 668.
——— (2) *Ibid.*, 675, 678.
ENGELMANN. Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1903, xii, 396.
KISTIAKOWSKY. Ztschr. f. physik. Chem., 1890, vi, 105.
KOHLRAUSCH, F. (1) Wied. Ann., 1879, vi, 1 and 145.
——— (2) *Ibid.*, 1879, vi, 6.
——— (3) *Ibid.*, 1879, vi, 8.
——— (4) *Ibid.*, 1879, vi, 14.
——— (5) *Ibid.*, 1879, vi, 49 and 50.
——— (6) *Ibid.*, 1879, vi, 152.
——— (7) *Ibid.*, 1879, vi, 160.
——— (8) *Ibid.*, 1880, xi, 653.
——— (9) *Ibid.*, 1885, xxvi, 161.
——— (10) *Ibid.*, 1885, xxvi, 163.
——— (11) *Ibid.*, 1885, xxvi, 170.
——— (12) *Ibid.*, 1885, xxvi, 172 and 184.
——— (13) *Ibid.*, 1885, xxvi, 222.
——— (14) *Ibid.*, 1893, xlix, 225.
——— (15) *Ibid.*, 1893, xlix, 233.
——— (16) *Ibid.*, 1893, xlix, 242, 247.
——— (17) *Ibid.*, 1894, li, 346.
——— (18) Prakt. Physik., 1895, vii, 255.

KOHLRAUSCH, F. (19) *Ibid.*, 1895, vii, 304.
——— (20) Pagg. Ann., 1873, cxlviii, 143.
——— (21) *Ibid.*, 1893, xlix, 235.
KRANNHALS. Ztschr. f. physik. Chem., 1891, v, 251.
LOB AND NERNST. Ztschr. f. physik. Chem., 1888, ii, 948.
OSTWALD. (1) Allgem. Chem., 1885, i, 537.
——— (2) *Ibid.*, 2e Aufl., 1893, xi, 640.
——— (3) Jour. f. prakt. Chem., 1886, N. F. xxxiii, 353.
——— (4) Ztschr. f. physik. Chem., 1885, i, 537.
——— (5) *Ibid.*, 1888, ii, 278.
——— (6) *Ibid.*, 1888, ii, 560.
——— (7) *Ibid.*, 1888, ii, 562.
——— (8) *Ibid.*, 1888, ii, 565.
——— (9) *Ibid.*, 1888, ii, 843.
——— (10) *Ibid.*, 1889, iii, 272.
PFEIFFER. Wied. Ann., 1885, xxv, 233.
SACK. Wied. Ann., 1891, xliii, 212.
STEYRER. Beitr. z. chem. Physiol. u. Path., 1902, ii, 312.
TURNER. British Med. Jour., 1906, ii, 1196.
WERSHOFEN. Ztschr. f. physik. Chem., 1890, v, 486.
WIEDMANN AND EBERT. Phys. Pract., 1890, i, 385.

Lactose Test in Renal Function

DE BONIS. Gior. internaz. d. sc. med., 1907, xxix, 446.
NUSSBAUM. Arch. f. d. ges. Physiol., 1878, xvi, 179; 1879, xvii, 580.
ROWNTREE AND FITZ. Arch. Int. Med., 1913, xi, 258.
SCHLAYER AND TAKAYASU. Deutsch. Arch. f. klin. Med., 1911, ci, 333.
SCHWARZ AND PULAY. Ztschr. f. exper. Path. u. Therap., 1915, xvii, 383.
VOIT. Deutsch. Arch. f. klin. Med., 1897, lvii, 547.
WIDAL AND VALLERY-RADOT. Semaine méd., 1914, xxxiv, 325.

Potassium Iodid Test

ANTEM. Arch. f. exper. Path. u. Pharmak., 1902, xlviii.
DUCKWORTH. St. Bartholomew's Hosp., Rep., 1867, iii, 216.
VON MONAKOW. Deutsch. Arch. f. klin. Med., 1912, cii, 248.
QUETSCH. Berl. klin. Wchnschr., 1884, xxi, 353.
ROUX. Thése de Paris, 1890.
ROWNTREE AND FITZ. Arch. Int. Med., 1913, xi, 258.
SCHLAYER AND TAKAYASU. Deutsch. Arch. f. klin. Med., 1911, ci, 333.
STUDENI. Dissertation. Zurich, 1897.
WOVSCHIN. Jour. Am. Med. Assn., 1915, lxv, 1101.

Hippuric Acid Test

JAARSVELD AND STOCVIS. Exper. Arch., 1879, x, 268.
KRONECKER. *Ibid.*, 1883, xvi, 344.
LEWIN. Ztschr. f. klin. Med., 1901, xlii, 371.
VON NOORDEN. Metabolism and Practical Medicine, 1903, Vol. II, p. 452.
SCHMIEDEBERG. Exper. Arch., 1881, xiv, 388.
VON SCHROEDER. Ztschr. f. physik. Chem., 1879, iii, 323.
SERTOLI. Gazz. d. osp., 1898.
SIRECI: *Ibid.*, 1896.
STOCVIS AND VON DER VELDE. Exper. Arch., 1883, xvii, 189.

Phlorizin Test

ACHARD AND DELAMARE. Bull. et mém. Soc. méd. d. hôp. de Paris, 1899, 379.
ALBARRAN. Exploration des fonctions rénales. Paris, Masson, 1905.

BARTH. Arch. f. klin. Chir., 1903, lxxi.
GREENE AND BROOKS. Diseases of the genito-urinary organs. Philadelphia and London, 1917, p. 85.
ISRAEL. Berlin klin. Wchnschr., 1902.
KAPSAMMER. Nierendiagnostic und Nierenchirurgie, Vol. I, p. 92, 100.
KROTOSZYNER. Am. Jour. Dermat., xi, 9.
VON MERING. Centralbl. f. d. med. Wissensch., 1885, 531.
STEVENS, W. E. The comparative value of modern functional kidney tests. Jour. Am. Med. Assn., May 16, 1914, 1544.

Urotropin Test

FALK AND SUGIURA. Jour. Pharm. and Exper. Therap., 1917, ix, 241.
——— *Ibid.*, 1916, viii, 39.
——— Biochem. Bull., 1916, v, 17.

Tests for Formaldehyd

MARSHALL AND WALKER. Jour. Am. Chem. Soc., 1913, xxxv, 820.

Creatinin in the Urine

BARKER. Monographic Medicine, 1916, Vol. III, 870.
DETWILLER AND GIFFITH. New York Med. Jour., 1915, cii, 798.
NEUBAUER. München. med. Wchnschr., 1914, lxi, 857.
VAN HOOGENHUYZE. Tijdschr. v. inland. Geneesk., 1913, lvii, 1594.

Urea in the Urine

PIRONDINI. Policlinico, 1915, xxii, 404.

Diastase or Amylase in the Urine

GERAGHTY, ROWNTREE AND CARY. Ann. Surg., 1913, lviii, 800.
GEYELIN. Arch. Int. Med., 1914, xiii, 96.
PIRONDINI. Policlinico, 1914, xxi, 157.

Diuretic Tests

BECK. Therap. Gaz., 1915, xxxix, 533.
FITZ. Arch. Int. Med., 1914, xiii, 945.
HERRINGHAM AND TREVAN. Quart. Jour. Med., 1913, vi, 505.
MOSENTHAL. Boston Med. and Surg. Jour., 1914, clxx, 245.

Bouchard's Urotoxic Coefficient

ALBU. Arch. f. Path. Anat. und Phys., 1901, clxvi, 77.
ASCOLI. Uraemia, Jena, 1903.
BECK. Arch. f. d. ges. Phys. d. Menschen, 1889, lxx, 560.
BERGH. Ztschr. f. klin. Med., 1898, xxxv, 52.
BERNARD. Les fonctions du rein dans les nephrites chroniques, 1900.
BOUCHARD. Autointoxications, 1877, p. 118.
CAWADIAS. Compt. rend. Soc. de biol., 1910, lxviii, 976.
EWALD. Die Autointoxikationen. Deut. Med., 1901, xix, 72.
GUMPRECHT. Centralbl. f. inn. Med., 1897, 569.
MÜLLER. Verhandl. d. Kongress f. Inn. Med., 1898, 163.
VON NOORDEN. Metabolism and Practical Medicine, 1907, Vol. II, p. 480.
——— Therapie der Gegenwart, 1889, p. 243.
POSNEI AND VERTUM. Berl. klin. Wchnschr., 1900, 75.
STADTHAGEN. Ztschr. f. klin. Med., 1889, xv, 383.
WOLFF. Deutsch med. Wchnschr., 1884, 627.

The Composition of the Blood

EPSTEIN, A. A. Jour. Exper. Med., 1912, xvi, 719; 1913, xvii, 444; 1914, xx, 334.
——— Concerning the causation of edema in chronic parenchymatous nephritis. New York Med. Record, 1917, xci, 128.
EPSTEIN AND ROTHCHILD. Am. Jour. Physiol., 1917, xlii, 586.
HURWITZ AND MEYER. Jour. Exper. Med., 1916, xxiv, 515.
ROWE, A. H. The albumin and globulin content of human blood-serum. Arch. Int. Med., Sept., 1916, 455.
WIDAL. Jour. méd. français, 1911, v, 18.

Ambard's Coefficient and McLean's Index of Excretion of Urea and Chlorid

ACHARD AND LEBLANC. Bull. et mém. Soc. méd. d. hôp., Paris, 1914, xxx, 337.
AMBARD. Compt. rend. Soc. de biol., 1910, lxix, 411, 506.
——— Physiologie normal et pathologique des reins, Paris, 1914.
——— Rev. de méd., 1913, xxxiii, 474.
AMBARD AND CHABANIER. Arch. urol. de la clin. de Necker, 1913, i, 248.
——— Jour. d. physiol. et de path. gén., 1912, xiv, 753.
——— Semaine méd., 1912, xxxii, 217.
AUSTIN. Med. Rec., 1913, lxxxiii, 1064.
AUSTIN AND MILLER. Jour. Am. Med. Assn., 1914, lxiii, 944.
BAUER AND HABETIN. Ztschr. f. Urol., 1914, viii, 353.
BAUER AND VON NYIRI. *Ibid.*, 1915, ix, 81.
BAYNE-JONES. Arch. Int. Med., 1913, xii, 90.
BONANOME. Policlinio, 1913, xx, 1065.
BRET AND BONLUD. Jour. d'urol., 1913, iv, 185.
BUTTERFIELD AND BRADDOCK. Proc. Soc. Exper. Biol. and Med., 1914, xii, 73.
CHABANIER AND LOBO-ONELL. Arch. urol. de la clin. de Necker, 1913, i, 235.
COURMOUNT, BONLUD, SAVY, AND BLANC-PERDUCET. Bull. et mém. Soc. méd. de hôp., Paris, 1913, xxix, 259.
FARR AND AUSTIN. Jour. Exper. Med., 1913, xviii, 228.
FROTHINGHAM AND SMILLIE. Arch. Int. Med., 1915, xv, 204.
KHOLZOFF. Russk. Vratch, 1914, xiii, 165.
LEGUEU. Urol. and Cutan. Rev., 1914, ii, 117.
LEWIS. Arch. Int. Med., 1917, xix, 1.
MARSHALL. Jour. Biol. Chem., 1913, xiv, 283.
MCLEAN. Am. Jour. Physiol., 1915, xxxvi, 357.
——— Jour. Exper. Med., 1915, xxii, 212, 366.
MCLEAN AND SELLING. Jour. Biol. Chem., 1914, xix, 31.
MCLEAN AND VAN SLYKE. *Ibid.*, 1915, xxi, 361.
PEABODY. Jour. Exp. Med., 1913, xvii, 71.
WIDAL, AMBARD AND WEILL. Semaine méd., 1912, xxxii, 361.
WIDAL AND JAVAL. Compt. rend. Soc. de biol., 1904, lvi, 301.

Physical Methods for Determining Composition of Blood

BUTTERFIELD, ERDWURM, AND BRADDOCK. Am. Jour. Med. Sc., 1915, 63.
HINMAN. Surg. Gynec. and Obst., 1914, xix, 482.
STRAUSS. Ztschr. f. klin. Med., 1906, cx, 501.
VEIL. Deutsch. Arch. f. klin. Med., 1913, cxii, 504
——— *Ibid.*, 1914, cxiii, 226.

The Non-protein Nitrogenous Constituents of the Blood

FARR AND AUSTIN. Tr. Coll. Phys., Philadelphia, 1913, xxxv, 167.
——— Jour. Exper. Med., 1913, xviii, 228.
FOLIN AND DENIS. Jour. Biol. Chem., 1912, xi, 527.
——— *Ibid.*, 1913, xiv, 33.

FOLIN AND DENIS. *Ibid.*, 1916, xxvi, 473.
FOLIN, DENIS AND SEYMOUR. Arch. Int. Med., 1913, xii, 223.
FOSTER AND DAVIS. Am. Jour. Med. Sc., 1916, cli, 49.
FROTHINGHAM, FOLIN AND DENIS. Arch. Int. Med., 1913, xii, 245.
HOHLWEG. Deutsch. Arch. f. klin. Med., 1911, civ, 216.
——— Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1915, xxviii, 459.
HOPKINS AND JONAS. Arch. Int. Med., 1915, xv, 964.
KARSNER AND DENIS. Jour. Exper. Med., 1914, xix, 259.
PEARCE. Jour. Lab. and Clin. Med., 1916, ii, 130.
PREVOST AND DUMAS. Ann. de chim. et phys., 1823, xxiii, 90.
THAYER AND SNOWDEN. Am. Jour. Med. Sc., 1914, cxlviii, 781.
TILESTON AND COMFORT. Arch. Int. Med., 1914, xiv, 620.

Creatinin in the Blood

LAMPERT. Ztschr. f. klin. Med., 1914, lxxx, 498.
MYERS AND LOUGH. Arch. Int. Med., 1915, xiv; 536-546.
ORLOVIUS. Ztschr. f. Geburtsh. u. Gynäk., 1916, lxxvii, No. 213.

Acidosis in Renal Disease

ADLER AND BLAKE. Arch. Int. Med., 1911, vii, 479.
DORNER. Arch. f. klin. Med., 1914, cxiii, 342.
HIGGINS, PEABODY AND FITZ. Jour. Med. Research, 1916, xxiv, 263.
HIGGINS AND MEANS. Jour. Pharmacol. and Exper. Therap., 1915, vii, 1.
HOWLAND AND MARRIOTT. Am. Jour. Dis. Child., May, 1916.
LEVY, ROWNTREE AND MARRIOTT. Arch. Int. Med., 1915, xv, 389.
MARRIOTT. Jour. Am. Med. Assn., 1916, lxvi, 1594.
——— Arch. Int. Med., 1916, xvii, 840.
PALMER AND HENDERSON. *Ibid.*, 1915, xv, 109.
PEABODY. *Ibid.*, 1917, xiv, 236.
——— Am. Jour. Med. Sc., 1916, cli, 184.
PORGES AND LEIMDORFER. Ztschr. f. klin. Med., 1913, lxxvii, 464.
ROSENBERG. München. med. Wchnschr., 1916, lxiii, 117-120.
TSCHERTKOFF. Deutsch. med. Wchnschr., 1914, xl, 1713-1715.

Urea Content of the Cerebrospinal Fluid

SOPER AND GRANAT. Arch. Int. Med., 1914, xiii, 131.

INDEX