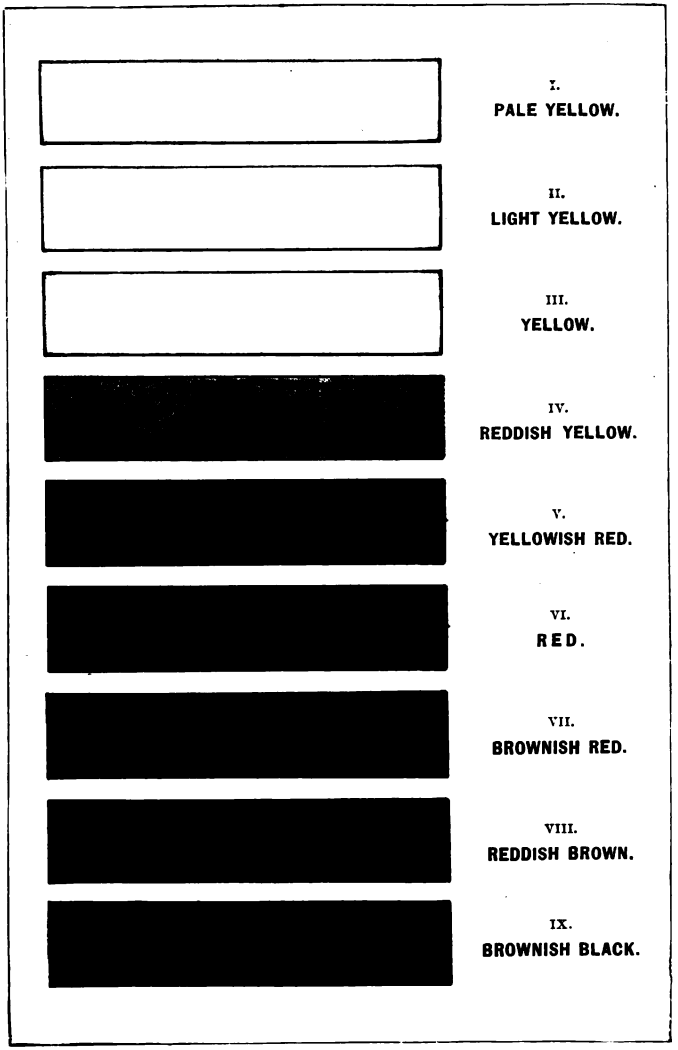
Katherine Drinker

PLATE I



SCALE OF URINARY COLORS, ACCORDING TO VOGEL.

EXAMINATION

OF

THE URINE

A MANUAL FOR STUDENTS AND PRACTITIONERS

BY

G. A. DE SANTOS SAXE, M.D.

Instructor in Genito-urinary Surgery, New York Post-graduate Medical School and Hospital; Assistant Genito-urinary Surgeon, Bellevue Hospital, Out-patient Department; formerly Assistant Pathologist to the Columbus Hospital; Member of the American Urological Association; Fellow of the New York Academy of Medicine, Etc.

Second Edition, Revised

With Text Illustrations and Colored Plates, a Number of Them Original

PHILADELPHIA AND LONDON

W. B. SAUNDERS COMPANY

1909

Set up, electrotyped, printed, and copyrighted September, 1904. Reprinted April, 1906. Revised, reset, electrotyped, printed, and recopyrighted October, 1909

PRINTED IN AMERICA

PRESS OF
W. B. SAUNDERS COMPANY
PHILADELPHIA

TO

THE MEMORY OF

My Father

IN WHOSE LABORATORY I GAINED MY FIRST KNOWLEDGE OF EXPERIMENTAL SCIENCE

THIS BOOK IS DEDICATED

PREFACE

The object of this manual is to furnish a concise guide to the examination of urine for the practitioner in his daily work and for the student in his laboratory course, as well as in his study for examinations.

In accordance with this aim, an effort has been made to treat the important and practical parts of the subject as fully as possible, while theory, discussion of moot-points, and cumbersome analytic details have been either omitted or treated in the briefest possible manner.

The theory of urinary secretion and the methods of functional examination of the kidneys have been given a rather more prominent place than has been done in other text-books, for the reason that a knowledge of these themes, while still of little practical use, adds materially to a grasp of the modern status of uranalysis and of the directions in which advances may be expected in this science.

Special attention has been paid to technics and to the interpretation of findings. In this connection some hints as to the methods of working that developed in my own experience have been inserted, which I trust may prove helpful to the beginner.

In a text-book of this scope, it is not necessary to

mention in detail the authorities consulted, and this has not been done here, except in a few instances. I take occasion, however, to acknowledge my especial indebtedness for material to the works of Neubauer and Vogel, Hammarsten, Von Jaksch, Ogden, F. C. Wood, Simon, Purdy, Heitzmann, and Tyson, and to express my sincere thanks to Dr. Henry T. Brooks, Pathologist to the Postgraduate Hospital, for many valuable hints in the preparation of this book.

The metric system is used in the greater part of the book, but the old system of weights and measures has been retained in some places for the sake of convenience in working, and in order to keep intact the original formulas of certain authorities.

Unless otherwise specified, the reagents and solutions are supposed to correspond to the standard of the United States Pharmacopœia.

If this little volume will contribute towards a clearer and more precise understanding of the subject of uranalysis on the part of the student or practitioner, my task shall not have been fruitless.

G. A. DES. S.

PREFACE TO THE SECOND EDITION

TIME was when those engaged in laboratory work were bidden to "stick to their lasts" when they attempted to come into contact with patients. To-day we see on every hand a clearly defined change in the relations of the laboratory man and the bedside man. In fact, to attain the highest standing in clinical medicine, in surgery, or in any of the specialties, it is now necessary for a physician to be thoroughly trained in laboratory work and to be able to apply laboratory methods in his daily routine, as well as in his search for the unknown in the particular branch in which he is interested. The highest places in the gift of our great universities in the department of clinical medicine and in some of the specialties are now given to men who have made reputations in laboratory research as well as in clinical study. As was well said recently by Dr. Christian, Dean of the Harvard Medical School, "The future of medicine in this country lies in a perfect co-ordination of the laboratory and the ward, both of which should be under the same head."[1]

Written by one who is primarily devoted to clinical work in a branch of surgery in which the greatest dependence is placed upon urine examinations, this book is, therefore, addressed to the clinical worker, the practitioner, who aims to make the laboratory his

[1] Quoted from memory by the author.

daily guide and counselor. Whether he himself examines the specimens, or whether he is fortunate enough to have this work done for him, the practitioner will require a thorough knowledge of the urine when he comes to interpret the findings of the microscope or of the test-tube.

In the new edition the clinical side of urine analysis has been still more markedly emphasized than in the first. The book has not only been revised and amplified, but many of the chapters have been entirely rewritten, to meet the important advances in the field of physiologic chemistry and chemical pathology in the midst of which we live to-day. In many respects this second edition also represents the fruits of individual experience in urinalysis and in clinical work in urinary diseases, covering now a period of ten years, as well as the results of the author's published researches on urethral shreds, prostatic and vesicular elements in the urine, etc.

Among the chapters which have been practically rewritten are those on acidity, albuminuria, albumoses, mucin, nucleo-albumin, indican, phosphates, sulphates, and the nitrogenous bodies, including uric acid. A number of new subjects have been introduced—*e. g.*, a short section on the pentoses, a brief account of Cammidge's reaction, a detailed description of the methods of preserving and staining urinary sediments and of preparing sediments for bacteriologic examinations. New chapters have also been inserted on urethral shreds, vesicular sago-bodies, etc.; on diabetes, and on the toxemias of pregnancy. A complete yet concise account of the present-day methods of functional renal diagnosis has been incorporated.

In every instance tests which have proved valueless have been dropped. Few new tests have been admitted, and only such as have proved useful in the author's own work. A number of new and original illustrations have been added. In order to make the volume compact, in spite of the numerous additions, smaller type has been used for the parts that are not apt to be required for daily reference.

The favorable reception of the first edition and its adoption as a text-book in a number of the medical schools of this country leads the author to hope that this edition will prove even more useful than its predecessor to the student and the practitioner.

G. A. DES. S.

130 WEST 71ST ST., NEW YORK CITY.
October,, 1909.

CONTENTS

PART I—GENERAL CONSIDERATIONS

PART II—CHEMIC EXAMINATION OF THE URINE

ORGANIC CONSTITUENTS

PROTEIDS

CARBOHYDRATES

NITROGEN GROUP: UREA, URIC ACID, ETC.

ACETONE GROUP: INDICAN AND OTHER ETHEREAL SULPHATES; DIAZO-REACTION

COLORING-MATTERS; BILE; BLOOD; OTHER ORGANIC CONSTITUENTS

INORGANIC CONSTITUENTS

PART III—MICROSCOPIC EXAMINATION

PART IV—URINARY DIAGNOSIS

PART V—FUNCTIONAL RENAL DIAGNOSIS

APPENDIX

EXAMINATION OF THE URINE

PART I

GENERAL CONSIDERATIONS

CHAPTER I

INTRODUCTION. COMPOSITION OF URINE IN HEALTH

A KNOWLEDGE of the characters of the urine in health and disease is of the greatest importance to the physician, inasmuch as this fluid constitutes one of the most valuable aids to both medical and surgical diagnosis. Next to the pulse and the temperature, the urine offers the greatest possibility for an insight into the workings of the human system. Aside from its purely clinical value, urinalysis is essential to the study of the physiologic processes of the body and the determination of changes in its metabolism produced through normal or abnormal influences.

The urine is a watery fluid secreted by the kidneys, and in health contains a large variety of constituents which are derived from the wasting and decomposition of the fluids and tissues of the body and from certain elements of the food.

The constituents of normal urine are difficult to classify

systematically on account of their complexity, but a useful division of the dissolved elements of the urine (modified from Hoppe-Seyler) is as follows:

1. Gases	Oxygen, nitrogen, and carbonic acid (very small quantities; dissolved).
2. Inorganic salts	(*a*) Chlorids of sodium and potassium. (*b*) Potassium sulphate. (*c*) Sodium, calcium, and magnesium phosphates. (*d*) Calcium carbonate. (*e*) Silicic acid. (*f*) Ammonium compounds.
3. Urea and its congeners	Urea, uric acid, xanthin, creatinin, allantoin, oxaluric acid, sulphocyanic acid, etc.
4. Aromatic substances	The ethereal sulphates of phenol, cresol, pyrocatechin, indoxyl, and skatoxyl; hippuric acid and aromatic oxyacids.
5. Fatty and other non-nitrogenous substances	Fatty acids; oxalic, lactic, glycerophosphoric acids, and a very minute amount of certain carbohydrates.
6. Other organic substances	Pigments; ferments, especially pepsin; mucoid substances.

The following table (Parkes) gives an idea as to the character and general quantitative relations of the urinary constituents, but it cannot serve as a clinical guide for the interpretation of urinary analyses. For this reason other tables (pp. 19, 20) have been compiled by the author, as exhibiting the chief points necessary for judging the results of quantitative estimations in urine. The figures given in these tables correspond with results of analyses carried on in the author's laboratory, and in the main are in accordance with the figures given by other writers on urine analysis.

The composition of normal urine, according to Parkes, is as follows:

COMPOSITION OF THE URINE

Amount of Urinary Constituents Passed in Twenty-four Hours (Parkes)

Constituents.	Average Man, Weight, 66 Kilograms.	Per Kilogram of Body-weight.
Water	1500.00 grams	23.000 grams
Total solids	72.00 "	1.100 "
Urea	33.18 "	0.500 gram
Uric acid	0.55 gram	0.008 "
Hippuric acid	0.40 "	0.006 "
Creatinin	0.91 "	0.014 "
Pigments and other organic matters	10.00 grams	0.151 "
Sulphuric acid	2.01 "	0.030 "
Phosphoric acid	3.16 "	0.048 "
Chlorin	7–8.00 "	0.126 "
Ammonia	0.77 gram	
Potassium	2.50 grams	
Sodium	11.09 "	
Calcium	0.26 gram	
Magnesium	0.21 "	

ANALYSIS OF NORMAL URINE FROM THE CLINICAL VIEWPOINT

I. General Characters of Normal Urine

Transparency.—Clear or faintly cloudy.

Color.—Amber-yellow.

Specific Gravity.—1015 to 1020 at 15° C.

Reaction.—Acid (depending on diet, etc.).

Acidity (total) may be expressed in one of the following ways (see p. 35):

1. Equivalent to from 3 to 5 cc. $\frac{1}{10}$-normal NaOH in 10 cc. of urine.
2. Equivalent to from 2 to 4 gm. of oxalic acid in twenty-four hours.
3. Equivalent to from 1.15 to 2.3 gm. of HCl.
4. Equivalent to from 0.4 to 20 parts per thousand of acid phosphates.

Amount voided in twenty-four hours (average):

1200 to 1500 cc. (50 fluidounces).

24.0 cc. of urine in twenty-four hours per each kilo of body-weight; a kilo being equivalent to 2.3 pounds.

II. Normal Constituents of Urine

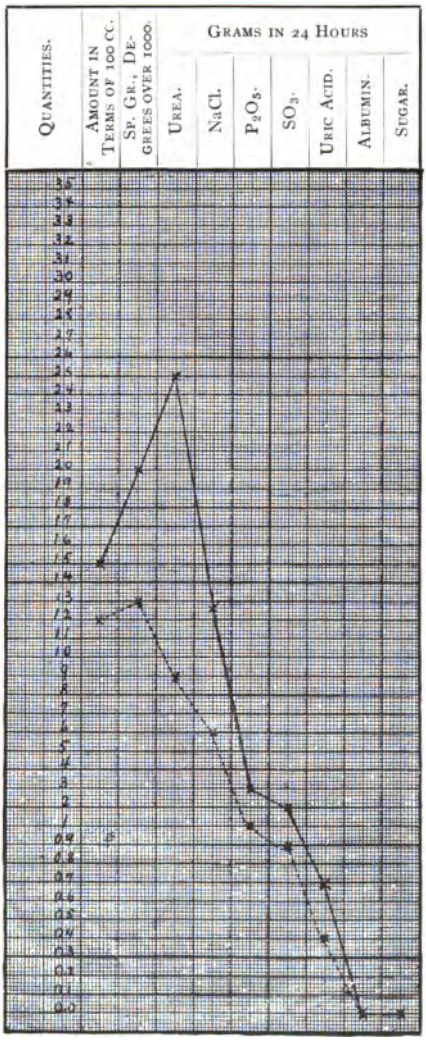
	Per Cent.	Parts in 1000 (Grams in Liter).	Grams in Twenty-four Hours.	Grains per Ounce (Approximate).
Total solids	3.49–4.66	34.95–46.60	61.14 (average)	16.80–22.08
Chlorids (NaCl)	0.6–1.0	6.6–10.0	10.0–15.0	2.88–4.80
Phosphates (P_2O_5) (total)	0.17–0.26	1.7–2.6	2.5–3.5	0.8–1.25
{ ⅓ earthy			1.0–1.5	
{ ⅔ alkaline			2.0–4.0	
Sulphates	0.1–0.2	1.0–2.0	1.5–3.0	0.48–0.96
Urea	1.5–2.0	15.0–20.0	20.0–30.0	7.20–9.60
Uric acid	0.016–0.083	0.16–0.83	0.2–1.25 (average 0.7)	0.048–0.384
Indican	trace		0.0045–0.0195	

III. Graphic Expression of Quantities in Urine

For comparing normal and abnormal conditions of excretion, there is nothing more convincing than the graphic method. A simple chart for this purpose is shown herewith. The *solid* line shows the proportions of urea, chlorids, phosphates (phosphoric acid), and the specific gravity in *normal urine*. The *dotted* line shows an *abnormal urine* plotted in the same way (a case of cancerous cachexia). (See Plate 2.)

The urea, chlorids, phosphates, sulphates, and uric acid are expressed in grams per twenty-four hours; the specific gravity is shown by the two last figures of the hydrometer reading, the normal average being taken as 1020. The quantity voided in twenty-four hours is indicated "in terms of 100 cc."—*i. e.*, by the number of times 100 cc. are voided.

PLATE 2



GRAPHIC EXPRESSION OF QUANTITIES IN THE URINE.

Solid line, normal urine; dotted line, an example of pathologic urine in a case of cancerous cachexia.

CHAPTER II

SELECTION OF A SPECIMEN OF URINE

For accurate quantitative work it is absolutely necessary to obtain the total amount of urine passed in twenty-four hours or, at least, a sample obtained after mixing thoroughly the entire quantity so collected. In the latter case the quantity eliminated in twenty-four hours must be measured and made known to the examiner. A perfectly clean bottle, holding about ½ gallon, should be used for collecting the twenty-four-hour quantity. It should be well corked after each addition, and should be kept in a cool, dry room to avoid decomposition. It is important that no foreign matter whatever, such as dust, feces, sputum, etc., be introduced into the sample, and the urine should never be allowed to stand in open or dirty vessels.

The reason for collecting the **twenty-four-hour quantity** is that the proportion of the constituents and the amount of albumin, etc., vary a good deal at different hours of the day and night, and that the object should be to obtain the average composition of the urine in the twenty-four hours.

The **best time** to obtain a sample of urine when we do not want the twenty-four-hour quantity, but merely wish to test qualitatively for sugar, albumin, etc., is during the day, about three hours after a meal. It is a mistake to take the morning urine, as is so commonly done; for in slight albuminuria the urine passed in the morning is the least likely to contain albumin, and the same is true of

sugar in glycosuria. Sometimes, when a case requires careful study, a comparison of the day urine and the night urine is desired; in that case we collect the urine from 7 A. M. to 7 P. M. in one bottle, and from 7 P. M. to 7 A. M. in another bottle, each being appropriately marked.

If a more concentrated urine is desired than is obtained in a given case with ordinary diet, it may be advisable to

Fig. 1.—Stoppered graduate.

Fig. 2.—Cylindric graduate.

reduce the amount of water and other fluids ingested for a day or so, and thus a urine with greater concentration and more abundant sediment may be obtained for microscopic examination.

The urine is subject to rapid **decomposition** after being voided, as the result of the action of molds and other micro-organisms, and also as the result of reactions between its various constituents. On standing, a normal urine precipitates, as a rule, first, some amorphous urates,

then some uric-acid crystals, and sometimes calcium oxalate. If allowed to stand open, and if exposed to high temperature, as in hot weather, the urine becomes clouded from a large number of bacteria and fungi. At the end of a few days it becomes ammoniacal—that is, the urea is gradually transformed into ammonium carbonate, according to the following formula:

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3.$$

In consequence of this the urine grows alkaline and the sediment contains ammonium urate, triple phosphate, and amorphous masses of calcium phosphate. Urines of normal concentration and acidity do not decompose so rapidly as urines of low density and slight acidity.

While this series of changes is known as *alkaline fermentation*, there is another form that has been known as *acid fermentation*. This change is sometimes seen as a preliminary stage of alkaline fermentation. It consists of an increase in acidity, a darkening in color, and the deposition of uric acid and urates, and occasionally of calcium-oxalate crystals, together with many yeast fungi and bacteria. This change is probably caused by the mucus, which acts as a ferment and induces an acetic-acid or lactic-acid fermentation. The reason for this increase of acidity has been also attributed to a reaction between the biurates and MH_2PO_4, dihydrogen phosphate (Hammarsten).

Preservation.—It is very important, therefore, that the urine be examined in as fresh a state as possible. In order to preserve it during the collection of the specimen or during the transportation to the laboratory, etc., a number of substances have been used as antiseptics. The most practical is the addition of a crystal or two of thymol,

or an ounce of cold saturated solution of boric acid to the quart of urine. With thymol, however, the urine may give a test suggesting the presence of bile. Chloroform (a few drops) will preserve urine well for chemic analysis (save that it gives a deceptive precipitate with Fehling's solution), but it makes the sediment unfit for microscopic examination. The use of formalin, of which 2 or 3 drops are added to the urine, has been advocated of late, but it is apt to cause a reduction of Fehling's solution which may be taken for glucose; besides, it is apt to affect the organized sediment, shrinking the cells and distorting the casts. The addition of formalin to urine also obscured the microscopic examination, in the author's experience, by the production of innumerable gas-bubbles if the urine was allowed to stand for any length of time. The addition of 5 grains of salicylic acid to 4 ounces of urine is favored by some, and is said to be very efficient. In warm weather urines should be kept on ice.

QUESTIONS ON CHAPTERS I AND II

Name the principal groups of constituents in the normal urine.

What gases are found in it?

What inorganic salts? What organic substances?

What is the average amount of total solids in urine in twenty-four hours? The average amount of chlorids, phosphates, sulphates, urea, uric acid? Describe a graphic method for recording quantitatively the constituents of urine.

Give the rules in selecting a specimen of urine.

How is the specimen collected?

Why do we insist upon the twenty-four-hour quantity?

At what time should urine be taken if it is not possible to obtain the twenty-four-hour quantity, and why?

Describe the processes of decomposition occurring in urine on standing.

What constitutes alkaline fermentation?

What is known as acid fermentation?

How should urine be preserved from decomposition?

CHAPTER III

PHYSICAL PROPERTIES OF THE URINE

Amount.—The amount of urine passed during twenty-four hours by a healthy adult is from 1200 to 1600 cc., the average being about 1500 cc. or about 50 fluidounces. Women pass smaller quantities than men, and small-sized persons void less than large-sized individuals. One kilogram of body-weight produces on the average 1 cc. of urine in the course of one hour. The average amount is obtained by multiplying the body-weight in kilos by 24 ($60 \times 24 = 1440$ cc.). Children eliminate 28.0 cc. per 5 kilograms per hour. The drinking of large quantities of liquids increases, while the taking of violent exercise, through free perspiration, diminishes, the average amount. The physiologic limits are 800 to 3000 cc. In health the largest amount is usually passed in the afternoon, a moderate amount in the forenoon, and the smallest amount during the night.

In warm weather considerably smaller quantities are passed than during the cold months. In disease the quantity of urine may be diminished or increased, or the urine may be suppressed through obstruction, or retained in the bladder through an impediment in the region of its neck or in the urethra.

Oliguria is a term used for diminished quantity of urine (below 800 cc. in twenty-four hours). *Anuria* is used to designate cases in which no urine, or a very small quantity, is passed—*i. e.*, when there is a partial or complete *sup-*

pression of urine. *Polyuria* is a term used for a markedly increased quantity of urine (over 3000 cc.).

The increase or decrease must, however, be constant, as attested by several examinations, and must be independent of ingested fluids to justify the terms polyuria or oliguria. Polyuria must be distinguished from pollakuria (frequent urination) and refers only to the quantity passed, not the frequency of voiding the bladder.

Diminution in quantity is noted in fevers; in shock after anesthesia or after operations on the genito-urinary organs; in stasis in the kidney due to heart disease, etc.; in acute congestion of the kidney; in acute nephritis and in the acute phases of chronic nephritis; in diseases accompanied by diarrhea and vomiting, such as cholera or yellow fever, and in all diseases in the last stages before death.

Increased quantity is observed in diabetes mellitus and insipidus, in some diseases of the nervous system, such as hysteria and convulsions, and in cerebral hemorrhage; in convalescence from acute and inflammatory diseases; in convalescence from acute congestion and from acute diffuse nephritis; in chronic nephritis, both interstitial and diffuse, and in amyloid kidney; in hypertrophy of the heart and in all conditions causing increased blood-pressure; also in cases in which diuretics have been freely used or a large amount of water drunk.

With the exception of the chronic diffuse and interstitial varieties of nephritis, in which there is an increase, there is a tendency to diminution in all forms of Bright's disease. A marked diminution in the urine in chronic nephritis, if accompanied by low specific gravity, is a very grave sign and usually precedes death.

Color.—The normal color of urine is of a pale-yellow, straw, or amber tint, but even in health it may vary considerably according to the amount of water drunk, etc. Dilute urines are usually pale; concentrated urines are deeply colored. The action of the skin in perspiration, the quantity of water drunk, and sometimes the quality of the food may have to do with changing the color of the urine in health. Color is not a very trustworthy sign in urine examinations.

In disease the color of urine may be changed, owing to the increase or diminution of the normal coloring-matters or the addition of abnormal pigments. The general rule, stated before, about the proportion of color to concentration usually holds good in disease. An exception, however, is the urine of diabetes mellitus, which is characteristically pale, but has a high specific gravity owing to the presence of sugar. *Very pale-colored* urine occurs in diabetes insipidus, hysteria, interstitial nephritis, etc. *Highly colored* urine occurs in acute fevers and in inflammations, and is due to concentration and also to the presence of uroerythrin (see p. 186). *Reddish* urine is always due to the presence of abnormal coloring-matters, usually blood. A *brown* or *reddish* color is seen in urines after ingestion of rhubarb and senna; a *yellow* color, after santonin. A *dark-brown* urine may be a sign of hemorrhage from the kidney, and be due to the presence of methemoglobin or hematin. *Smoky* and *dark* urine is often seen after carbolic acid has been used internally or sometimes after its external use, as well as after the taking of large doses of salol or guaiacol. These urines show the black color, especially on standing, owing to the formation of hydroquinon as the result of decomposition of the

phenol (see Phenol, p. 181). A dark urine is also seen in the presence of alkapton (see p. 127).

Bile-pigments, when present in excess, give a dark color with a yellowish-green foam, and on standing the urine becomes *greenish*. In cholera and typhus the urine may be *blue*. In melanotic cancer the urine is almost *black*, especially after standing, owing to the presence of melanin. A *greenish-blue* urine is eliminated after methylene-blue has been taken. A greenish tint is also sometimes seen after the use of a large quantity of milk, also in chronic Bright's disease and in diabetes with large amounts of sugar.

Odor.—The odor of normal urine is peculiar, aromatic, sometimes styled "urinous." It is due to the presence of volatile acids—phenylic, taurylic, and damaluric—in very minute quantities, and partly also to urea. The odor is strongest in concentrated urine. On standing, normal urine acquires a disagreeable odor which is both putrid and ammoniacal. The putrid odor is due to decomposition of mucus or other organic matters. The ammoniacal odor is due to the formation of ammonium carbonate. It is important to note the odor of *freshly passed urine*, for if it is putrid or ammoniacal the urine must have decomposed within the body—*e. g.*, in the bladder. A urine which contains a large amount of pus or albumin may suffer decomposition of its proteid matter to such an extent as to evolve an odor of sulphuretted hydrogen. A fecal odor is noted when there is contamination of the urine by the feces through a fistula, etc.

Certain *vegetable foods* and certain *drugs*, when taken internally, very quickly produce a peculiar odor in the urine. Thus, turpentine produces the odor of violets;

cubebs, copaiba, sandalwood oil, asparagus, etc., produce their characteristic odors in the urine.

In disease the only abnormal odors to be noted are the increased odor, usually styled "strong," in concentrated urine; the sweet or fruity smell of diabetic urine; the foul odor of cystitis, and the "sulphuretted" odor of urine containing much pus. A peculiar odor, intense in its foulness, is noted in pyelitis. A sulphuretted odor is noted in the presence of cystin in decomposing urines containing this substance.

Consistence.—Normal urine is always of the consistence of water. When the urine contains much pus or mucus, especially if it has become alkaline, it becomes very thick and stringy. Urine containing much sugar or albumin tends to froth when shaken. Chylous urine which contains fat in fine emulsion is thickened in consistence.

Transparency.—Normal freshly voided urine is always perfectly clear, but on standing a few minutes there is generally a faint cloud which floats usually in the center of the urine or settles at the bottom if the urine is dilute enough. This cloud or "*nubecula*" consists of mucus, epithelium, bacteria, and débris of cells. It is much more pronounced in women on account of the admixture of vaginal mucus, which becomes washed away from the genitals in the passage of the urine from the urethra. In catarrhal conditions of the genito-urinary tract, especially in cystitis, prostatitis, and urethritis, the normal mucous cloud is markedly increased, so that the urine appears cloudy soon after being voided, and the cloud sinks more rapidly to the bottom. The mucous cloud may be distinguished from other causes of turbidity by the fact

that it tends to float in the center of the fluid, and is precipitated on the addition of an excess of acetic acid.

A cloudy urine may be the result of the presence of bacteria, phosphates, urates, or pus.

Bacteria, when present in large numbers (bacteriuria), give rise to a faint cloud which tends to float in the middle of the vessel and does not settle, even when the urine stands for a long time. This cloud, unlike the mucous cloud, remains unchanged on the addition of acetic acid.

Phosphates may give rise to cloudiness when there is an excess of the earthy or triple salts and when the acidity of the urine is lowered, thus lessening the solubility of the phosphatic elements. It is difficult to mistake turbidity due to phosphates for anything else, as it does not form a cloud, but pervades the urine uniformly, settling slowly on standing. A few drops of acetic acid, by correcting the lowered acidity, will almost instantly clear up the urine in such cases.[1]

Urates, if present in excess in normal acid urines, may cause turbidity or may give a sediment of urates—of sodium, potassium, calcium, and magnesium—if the urine be allowed to stand in the cold. This deposit settles quickly, is white, pinkish, dirty-yellow, or brick-red in color, and often settles on the sides of the vessel. It is well to remember that a sediment occurring in acid urine can be composed only of urates or of organized elements (pus-cells, casts, etc.). The distinguishing test for the cloudiness due to urates consists in heating the urine gently over the flame, which quickly dispels the cloudiness and clears the urine.

[1] With a deposit of phosphates there usually is a certain amount of carbonates which add to the cloudiness. They are also dissolved on the addition of acetic acid.

Pus in the urine shows its presence by cloudiness immediately after passing, and on standing a few minutes by the deposit of an opaque, amorphous, yellowsh-white sediment, which sinks to the bottom much more rapidly than mucus. In the presence of an admixture of pus and mucus the opacity of the sediment and the rapidity of its sinking grow less as the mucus is increased. In an alkaline or ammoniacal urine the pus-deposit occurs as a tenacious, gelatinous-looking mass which adheres to the bottom and to the sides of the vessel. On repeatedly shaking the urine, it is impossible to disintegrate this mass completely, and if it be allowed to settle and the urine be then decanted into another vessel, the gelatinous pus is often dislodged in a lump and precipitated into the second vessel.

To test a cloudy urine for pus, as distinguished from mucus, urates, etc., *Donné's reaction* is of value. It consists of allowing the sediment to settle in a conic glass, decanting the urine which floats over it, and adding a solution of potassium hydrate drop by drop until the gelatinous, glairy mass described above is formed, adheres to the bottom of the vessel, and slips out from it in a lump. This same mass is formed spontaneously in purulent urine by alkaline fermentation. The same test may be performed more rapidly by adding equal parts of the KOH solution to the urine and observing the appearance of the gelatinous precipitate.

Donné's test is conclusive only in acid urines. If the urine is alkaline, it may fail to show pus, which is then best looked for with the microscope. According to Goldberg, Donné's test is sensitive to 1000 pus cells per cubic millimeter of urine.

The table on page 32 shows in a condensed form how

DIFFERENTIATION OF THE PRINCIPAL CAUSES OF TURBIDITY IN URINE

I. *Heat the urine for a few seconds:*

Turbidity increases or precipitate forms:
= Bacteria, mucus, phosphates, or pus.[1]

Turbidity disappears:
= Urates.

II. *Add acetic acid:*

Turbidity unchanged:
= Bacteria.

Increased, or precipitate:
= Mucus or pus or both.

Dispelled at once:
= Phosphates.

III. *Add potassium hydrate:*

Turbidity or precipitate is changed to gelatinous coagulum:
= Pus.

[1] If the turbid urine contains albumin, the precipitate will increase with heat and with acetic acid, but will disappear on the addition of an excess of alkali (soluble alkali albumin). Albumin is omitted from this table because in itself it does not cause turbidity in urine.

the source of turbidity may be detected in a few seconds in a urine with the use of only two reagents and heat.

Chyluria may be mistaken for pyuria. The former depends upon the presence of a parasite—*Filaria sanguinis hominis*—in the blood, and is characterized by urine which is a fat-emulsion and is milky in appearance. This urine will stand for days without settling, is milky, yellowish-white in color, and shows a film of fat or of creamy flakes on the surface. The fat is dissolved on shaking with ether and the urine is cleared. When there is much fat, chylous urine may coagulate on standing.

Reaction.—The reaction of normal urine is faintly acid to blue litmus-paper, but occasionally it is faintly alkaline to red litmus and faintly acid to blue litmus—in other words, amphoteric. The mixed quantity of twenty-four hours is always acid normally. The acidity is due to the presence of acid sodium phosphate (monosodic acid phosphate—NaH_2PO_4) and not to free acid. The degree of acidity of the urine depends upon the amount of the acid phosphate as compared with that of the di-sodium or alkaline phosphate. The urine is neutral or amphoteric when the disodium salt is present in a larger quantity, although not enough to neutralize the mono-sodium or acid salt, so that both blue and red litmus-papers give a reaction (Huppert).

In *testing the reaction of urine* strips of litmus-paper are used—the blue paper turns red in acid urine; the red paper turns blue in alkaline urine, and if the urine be amphoteric, both reactions take place. In testing, the paper should be dipped to about half its length into the urine.

Real and Apparent Acidity.—A great deal of work has been done during the past few years by both physiologists and chemists in solving the numer-

ous problems arising from the peculiar behavior of the urine toward litmus-paper and other indicators. The question was asked for a long time how it was possible that an acid fluid could be excreted from the alkaline blood. Heidenhain and other physiologists attributed the change in reaction to some vital process which goes on in the cells of the kidney. Experiments showed, however, that no mysterious vital process was necessary to produce an acid fluid from an alkaline. As early as 1876, Maly and Posch showed that when a solution of disodic phosphate (Na_2HPO_4), commonly known as "alkaline phosphate," and a solution of monosodic phosphate (NaH_2PO_4), commonly known as "acid phosphate," were mixed and dialysed through parchment, the resultant dialysed fluid had an acid reaction. As both of these salts occur in the blood, it is easily seen that no vital phenomena are necessary to effect the change from alkalinity to acidity during the process of urinary excretion. Analogous experiments with other sets of alkaline salts by Koeppe (1901) showed that by mixing a solution of neutral salts with a solution of alkaline salts an acid fluid could be produced. The explanation of this phenomenon is based upon the fact that the molecules in the mixture of salts become dissociated and that during the process of solution new combinations may form, resulting in the formation of acid salts.

According to modern physical chemistry an alkaline reaction is due to the presence of the *ions* OH, while an acid reaction is due to the presence of the *ion* H. Titration, no matter how accurate, cannot determine the actual number of OH *ions*, on the one hand, or of H *ions* on the other, which *may be formed* in a solution. In other words, while formerly chemists understood by "degree of acidity" the amount of hydrogen that could be replaced by the sodium of the sodium hydrate solution, regardless of whether these hydrogen ions were already dissociated or not, modern physical chemistry regards as acidity *the number of dissociated hydrogen ions in a given volume of fluid*. The most accurate way to estimate the dissociated *ions* is by electrochemic measurements. Thus Rohrer[1] found by this method that the presence of the hydrogen ions in the normal urine is exceedingly low. *The actual acidity of the urine by accurate methods is about* $\frac{1}{10,000}$ *of that indicated by titration.*

The most difficult problem in titrating the urine for total acidity is the choice of an indicator. The reason of this is that the acidity of the urine (in the ordinary sense of the word) depends upon a variety of acid salts. Each of these has a separate end-reaction point. Litmus-paper, which was formerly used as an indicator, is the poorest of all acid-detecting substances for this purpose. As there is no phosphate neutral to litmus-

[1] Archiv. für die gesammte Physiologie, vol. lxxxvi, p. 586, 1901.

paper (the acid phosphates give an acid reaction; while the normal or monacid phosphate is alkaline), litmus is worthless as an indicator in urine (see Oxalic Acid Method).

The difficulty with other indicators is that it is impossible to watch accurately the end reaction. Phenolphthalein is preferred in acidimetry on account of the sharpness of the end reaction which it gives, and because it is in itself but very feebly acid as compared to other indicators. The presence of ammonium salts, however, materially interferes with its action. For clinical purposes, however, phenolphthalein is sufficiently accurate, at least, for comparison.

Finally, according to Lieblein, there is still another source of error, namely, the fact that the addition of an alkaline solution, such as is used in titration, induces a combination of the alkaline phosphates with the calcium chlorid of the urine, resulting in a calcium phosphate of variable constitution. The phosphoric acid is not neutralized in constant proportion, therefore, in any of the titration methods with sodium hydrate solution. The amount of phosphoric acid remaining non-neutralized will depend upon the amount of calcium and magniesum salts in the urine. For these reasons Folin's method (see p. 37) is the most accurate of the procedures thus far devised for acidimetry of urine.

It is questionable whether results obtained by titration are sufficiently accurate to repay the time taken in carrying out the method. The results, at best, are valuable only as a means of comparison.

Methods of Determining Total Acidity.[1]—*The "Oxalic Acid" Method with Litmus.*—The so-called "oxalic acid" method, in which the total acidity is expressed "in terms of oxalic acid," is not trustworthy and is no longer used by the author. It is as follows:

Take 100 cc. from a twenty-four-hour specimen, and titrate it in a flask or beaker with a decinormal solution of sodium hydrate, using strips of sensitive litmus-paper as indicators, until a faintly alkaline reaction is produced. The number of cubic centimeters of the sodium hydrate solution multiplied by 0.0063 will give the percentage of acidity in terms of

[1] It is needless to say that the acidity of the urine should always be tested in fresh samples of twenty-four-hour urine, for the estimation would otherwise be interfered with, both by the possibility of alkaline fermentation and by the so-called acid fermentation, which at times precedes alkaline decomposition.

oxalic acid. Normally, the total acidity corresponds to 0.189 per cent. oxalic acid, or 1.9 gm. oxalic acid per liter, or from 2 to 4 gm. of oxalic acid daily.

The Phenolphthalein Method.[1]—The solutions required are a decinormal sodium hydrate solution and a 1 per cent. alcoholic solution of phenolphthalein.

Fill the buret with the $\frac{1}{10}$-normal NaOH solution and note the amount (reading at lower meniscus, after allowing the fluid in the buret to stand for two or three minutes). Place 10 cc. of urine in a beaker and add 2 drops of phenolphthalein solution. Add NaOH solution from the buret drop by drop, until the red color which forms as we approach the point of neutralization no longer disappears on shaking. A uniform pale red color now tinges the urine, indicating that the acidity is neutralized. The number of cubic centimeters of NaOH solution used, multiplied by 10, will show the acidity in 100 cc. or, in other words, the "percentage of total acidity." Normally, from 3 to 5 cc. of $\frac{1}{10}$-normal sodium hydrate are used for 10 cc. of urine—*i. e.*, the acidity is from "30 to 40 per cent." In the twenty-four-hour quantity (say, 1500 cc.) it is normally from between "20 and 30 per cent." According to Emerson, the error is at least from 4 to 8 per cent.

The disadvantage of this method is the uncertainty of phenolphthalein as an indicator under the conditions which exist in the urine (see above).

Freund and Töpfer's Method.—This is somewhat more accurate than the preceding, alizarin being used as an indicator.

Ten cc. of urine are placed in a beaker and from 2 to 4 drops of a 1 per cent. alizarin solution are added. In the presence of free acids a pure yellow tint is developed, while combined acids give rise to a deep violet color. If neither of these colors appear, there are probably combined acids and disodic (alkaline) phosphates. The amount of decinormal hydrochloric acid solution added to this urine, required to produce a pure yellow color, represents the amount of *alkaline* phosphates present,

[1] Naegeli, Zeitschrift für Physiol. Chem., 1900, xxx, 313.

while the amount of decinormal NaOH solution required to produce a deep violet color represents the amount of *acid* phosphates. Normally, the acidity corresponds to from 4.0 to 20.0 per cent. *acid phosphates* (Serkowski). When over 20 per cent., there is hyperacidity.

Folin's Method.—In order to rule out the error which arises with phenolphthalein from the presence of ammonium compounds and of calcium phosphate (see above), Folin[1] adds an excess of potassium oxalate. He measures with a pipet 25 cc. of urine into a 200-cc. Erlenmeyer flask, adds 1 or 2 drops of 0.5 per cent. phenolphthalein solution, and 15 to 20 gms. of potassium oxalate. After shaking the flask thoroughly for one minute, the contents is immediately titrated with the decinormal NaOH solution, the shaking being continued. The sodium hydrate is added until a faint, yet distinct, color is obtained.[2] The calculation in "percentage" is the same as with the phenolphthalein method of Naegeli (see p. 36).

Clinical Significance of Reaction and Acidity of Urine.—In drawing conclusions from the quantitative estimation of acidity, the diet should be considered as a prime factor. The more proteid material is assimilated, the higher the acidity will be, while a vegetable diet will reduce the acidity or cause the urine to become amphoteric.

Normally, the acidity of the urine *varies at different times of the day*, there being an ebb and flow of the acidity tide corresponding with the meals. The acidity begins

[1] American Journal of Physiology, 1903, ix, 265.

[2] *To calculate the acidity* of the urine, obtained by any of the titration methods with sodium hydrate, in terms of hydrochloric acid, multiply the number of cubic centimeters of decinormal NaOH used by the coefficient 0.00365. One cubic centimeter of the decinormal soda solutions corresponds to 0.0365 gm. of hydrochloric acid. Normally, the acidity of the twenty-four-hour urine corresponds to about 1.15 to 2.3 gm. HCl.

to diminish soon after a meal, and reaches its lowest point in about three or four hours, the urine even becoming alkaline at such times. The urine is less acid after a vegetable *diet*, and is increased in acidity after a red-meat diet. The *discharge of hydrochloric acid* by vomiting, washing out the stomach, or through a fistula, or the presence of an excess of HCl in the stomach may cause an alkaline reaction. It is on this account that the acidity should never be tested in other than twenty-four-hour urines. The urine may become alkaline after *hot baths* and *free perspiration*. Alkaline salts or vegetable acids given internally as drugs or taken with the food in large amounts will decrease the acidity of the urine, the acids being converted into carbonates in the blood, passing into the urine as such. The patient should not take any of these drugs while under observation for the acidity of his urine.

An *increase of acidity* is seen in persons with increased proteid exchange. It has been noted that in certain neuroses of the urinary tract there may be an increase of acidity, and the same takes place in diabetes mellitus when diacetic and oxybutyric acids are present. An increased acidity is also said by some writers to be characteristic of excessive intestinal auto-intoxication. The increase of indican and of the other ethereal sulphates is a better measure for the intensity of intestinal putrefaction (see p. 174) than is the acidity of the urine. There may be increased acidity without indicanuria, in which case it is improbable that an increased putrefaction exists in the intestine. It should be remembered that the *so-called* percentage of acidity *varies with the specific gravity of the urine*, and that urine of high specific gravity is usually highly acid, while by simply making the patient drink more

water we can reduce the "percentage of acidity." It has been claimed for a long time that the acidity of the urine is quantitatively increased in persons with the *uric-acid diathesis*, but at present little importance is attached to the question of the reaction of the urine in this clinical condition. Uric acid in solution never influences the urinary acidity directly, although it may increase the acidity indirectly by transforming neutral into acid phosphates (Croftan). There is a limit to the possible increase in acidity in the urine set by the resistance of the body against an acid intoxication. The amount of ammonia excreted is increased whenever an acid intoxication is present, the body thus protecting itself against loss of alkaline mineral material.

The urinary acidity is always increased in the presence of fever, for the reason that febrile conditions are accompanied by an increased oxidation of proteids. During this process of oxidation sulphuric and phosphoric acids become free from the sulphur and the phosphorus which form part of the proteid molecule.

Decreased acidity may occur after the absorption of a serous exudate or transudate or after the absorption of blood into the intestine, the blood salts entering into the circulation and rendering the urine alkaline. In some cases of pneumonia, in typhoid fever, and in diseases of the central nervous system there may be alkaline urine, and a marked alkalinity has been noted by Emerson in certain cases of nephritis, especially of the chronic parenchymatous type accompanied by much edema.

Obstruction and inflammation of the lower portion of the urinary tract or in the renal pelvis may also render the urine alkaline. The alkalinity of any urine, therefore, is

only important *when noted in a freshly voided specimen*, although the rapidity with which a urine grows alkaline on standing may be noteworthy in cases of cystitis, stone, etc., provided the temperature be kept moderately low and protection of the urine against bacterial contamination be carefully maintained.

To test for the presence of *volatile alkali*, which is present in urines which have undergone alkaline fermentation, as distinguished from the *fixed alkali* present in urine alkaline in virtue of a change of diet, etc., the red litmus-paper should be watched while it is drying. When there is fixed alkali this litmus-paper retains the blue color where it has been dipped into the alkaline urine. When there is volatile alkali, the blue color fades on drying, the paper returning to a red color.

Specific Gravity.—The specific gravity of normal urine is between 1012 and 1024 when the amount passed is normal. The standard normal average is 1020 at 60° F. (15.5° C.). In disease it varies between 1002 and 1060. In hot weather it may reach 1035, owing to abundant perspiration. The specific gravity is also increased by muscular exertion. In children it averages 1021. It is proportionate to the amount of total solids in the urine, and these in turn vary according to the time of the day, the meals, the amount of exercise, the amount of fluid drunk, and the total amount of urine passed daily. A scientific determination of specific gravity cannot be made, therefore, without a specimen from the mixed twenty-four-hour quantity. Determinations with specimens obtained at various times may be necessary, but they are of no value unless the conditions of diet, exercise, temperature, etc., are known.

Determination.—The specific gravity of urine is usually determined clinically by the use of a special hydrometer or *urinometer* (Fig. 3), which is sufficiently accurate for ordinary work. The best type is that of Squibb. The urinometer is constructed for a scale from 1000, which is marked at the top of the stem, to 1060, its principle being that the weight of the instrument is constant and that the denser the fluid the less deeply will the instrument sink in it. A small cylinder holding about 2 ounces, sometimes made with flutings, intended to lessen the amount of urine required, is supplied with each instrument, with a thermometer showing the temperature of the fluid tested.

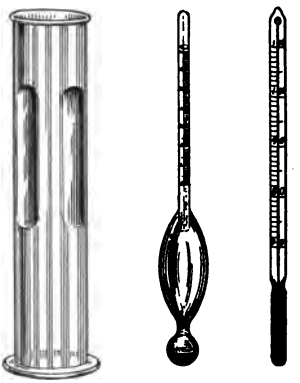


Fig. 3.—Squibb's urinometer with thermometer and cylinder.

Every urinometer must be tested with distilled water at 60° F. (15.5° C.), in which it should sink to 1000, or zero. (Squibb's urinometers are standardized at 22.5° C. or 77° F., a more convenient temperature for clinical work.) Carefully made urinometers have accurate division of the fractions of degrees, which gradually approach one another as they get nearer the bulb, as allowance is made for the weight of the stem above the water. The best instruments have been tested by the makers and bear the correction for variations in temperature on the container.

In determining the specific gravity a sufficient amount of urine is poured into the cylinder, which should not be too small in proportion to the urinometer, as the latter must

not, on any account, be allowed to impinge against the side of the cylinder. If this occurs, the influences of friction and of capillary attraction tend to prevent the instrument from sinking. The urinometer should be dried before immersion. The foam should be removed from the urine by means of filter-paper, and the instrument should be lowered gently with a slight spinning turn, which will prevent it from being attracted to the sides of the vessel. The reading should be taken when the instrument is completely at rest and is not touching the sides of the cylinder at any point. The urine will be found rising in a small hillock around the stem (Fig. 4), over the level of the fluid, and in reading care must be taken not to read at the top of the hillock, but at the level of the lower portion of the meniscus formed at the contact of the urine with the stem.

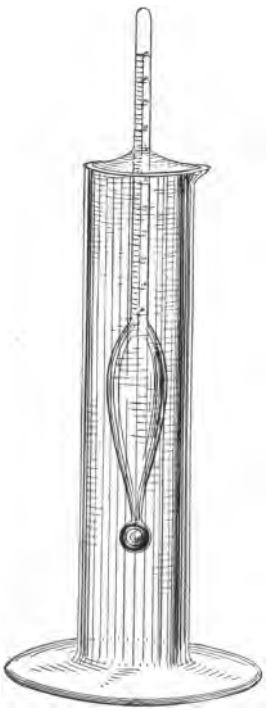


Fig. 4.—Urinometer immersed, showing effect on the surface of the urine.

The specific gravity of urine should never be taken in a specimen freshly voided, as the latter is apt to be at or near the temperature of the body—*i. e.*, 98.6° F. (37.2° C.)—while the urinometer is made for about 60° F. (15.5° C.). If an immediate examination is required, the specimen must be cooled rapidly to the proper temperature by immersing the

cylinder in cold water. A correction for temperature may be roughly made with sufficient accuracy for clinical purposes by adding 0.001 to the specific gravity read for every 3° C. that the urine is below the point at which the urinometer is standardized, or subtracting the same amount for every 3 degrees that the urine is above that point.

Other and more accurate methods for estimating specific gravity are the Westphal-Mohr balance, the pyknometer, Sprengel's tubes, etc.

The *Westphal balance* is a beam scale, finely constructed, which has a small glass thermometer suspended by a fine wire from the balance end in the urine to be tested. The weight of this thermometer in urine, as compared to its weight in distilled water at standard temperature, gives the specific gravity of the urine. These balances are very accurate, but their price is somewhat high and the urinometer is sufficiently accurate for the practising physician.

The methods for determining the specific gravity by means of the pyknometer, Sprengel's tubes, etc., may be found in text-books on physics. They require an accurate analytic scale, and depend upon the principle of comparing the weight of a very accurately measured volume of distilled water with exactly the same volume of urine of the same temperature, the specific gravity being deduced from the ratio of these two weights. They constitute the most accurate methods for taking specific gravity, but are useful only for strictly scientific work.

In taking the *specific gravity of small amounts of urine*—*i. e.*, amounts less than the 30 cc. which are required to float a Squibb's urinometer—we are compelled to resort to some expedient other than the ordinary method. The use of *test-tubes* instead of cylinders is not to be counte-

nanced, inasmuch as the readings cannot be accurate, the instrument constantly adhering to the wall of the tube by capillary attraction. The method of *diluting* the urine with water a certain number of times and multiplying the specific gravity of the diluted urine by that number is, if anything, still less accurate, for the error is multiplied in proportion to the dilution, and no urinometer is made accurate enough to read such exceedingly low gravities as are shown by diluted specimens. The use of the Westphal balance enables us to read the specific gravity of 15 or 20 cc., and the pyknometers and Sprengel's tubes may be used with still smaller quantities, but all these are inaccessible to the physician on account of the high price of the apparatus they require.

The Author's Method for Small Amounts of Urine.—A special method devised by the author for the estimation of specific gravity in the smallest amounts of urine without recourse to expensive appliances or complicated manipulation consists in the use of a *urinopyknometer*, which is a new form of hydrometer that determines with clinical accuracy the specific gravity of about 3 cc. of urine.

In this instrument the urine is placed in the hydrometer instead of the hydrometer being floated in the urine. The urinopyknometer is a little smaller than the ordinary urinometer, and consists of a small flask with a well-fitting glass stopper, the head of which bears a tiny bead of mercury (Fig. 5, D–E). This flask is attached to a small spheric bulb (Fig. 5, C), over which is the stem (A–B) of the instrument, graduated in reverse order as compared to the ordinary urinometer—that is, the mark 1060 is at the top of the stem and the mark 1000 at the bottom. When the flask is filled with distilled water up to the mark M, and when the

instrument is closed and immersed in distilled water, it reads 1000 at the level of the distilled water. When urine is poured into the flask to the same mark, the instrument sinks in distilled water in proportion to the specific gravity of that urine, which is then read on the scale. The same precautions in reading are taken in using this instrument as with the urinometer, and, in addition, the flask must be dry and perfectly clean (washed with alcohol or ether). The urine must be poured in accurately with a small dropper until it reaches the mark, so that the lower meniscus touches it as the flask is held at the level of the eye. The urinopyknometer is of special value in cases in which very small amounts are furnished for analysis, as in infants, in catheterizing the ureters, and in emergency work,[1] when but small quantities are voided by the patient.

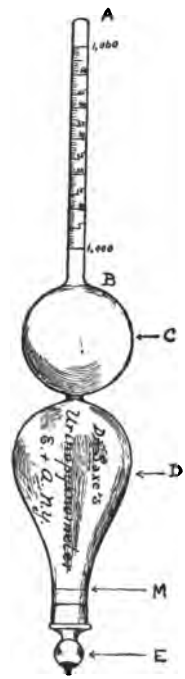


Fig. 5.—The author's urinopyknometer.

Pathologic urines often show an increase or a diminution in specific gravity, but inasmuch as the latter varies normally to a considerable extent with the quality of meals, the amount of exercise, etc., an abnormal specific gravity is of value only when obtained from a twenty-four-hour specimen *the volume of which is known.*

The specific gravity is *increased* in the beginning of acute fever, after prolonged surgical operations, especially

[1] New York Med. Journal, Oct. 17, 1903.

with ether anesthesia, on account of the hemorrhage and the perspiration, in acute Bright's disease in its early stages, and in other conditions accompanied by blood in the urine. In the presence of large amounts of sugar in diabetes the specific gravity may be very high and sometimes reaches 1050. When a large amount of urine is passed of a high specific gravity we suspect diabetes, but absence of sugar must not be inferred from a low specific gravity.

A *diminished* specific gravity occurs in diabetes insipidus, in hysteria, and in chronic interstitial nephritis. In all forms of Bright's disease, except in the acute in its first stage, and in stasis in the kidneys of heart disease, there is a tendency toward a lower specific gravity as well as a lessened percentage of urea. If no albumin and no sugar be present, the rule is that a lower specific gravity usually means less urea. A marked decrease in specific gravity is a serious symptom in chronic Bright's disease.

Total Solids.—The determination of the total solids eliminated in the urine during the twenty-four hours is useful clinically, inasmuch as it gives an insight into the relations of quantity and specific gravity. The general rule is that the quantity of urine is small when the specific gravity is high; in other words, that the specific gravity varies in proportion to the concentration—*i. e.*, in proportion to the amount of solids dissolved in the fluid. In certain diseases there is a noteworthy diminution of the total solids excreted, reflected in a lower specific gravity, and it is useful to know whether a lower specific gravity in a given case depends largely or materially upon an increased amount of the watery element of urine or upon a markedly decreased amount of total solids.

For clinical purposes, the amount of total solids in the

twenty-four-hour urine is determined approximately by multiplying the two last figures of the specific gravity by *Haeser's coefficient*, which is 2.33, and thus obtaining roughly the number of grams of solids in 1000 cc. (1 liter) of urine (33.8 oz.). This number, multiplied by the number of cubic centimeters passed in twenty-four hours and divided by 1000, will give the amount of solid constituents eliminated in twenty-four hours.

Example: The specific gravity of urine is 1020. Multiply the last two figures by 2.33: $20 \times 2.33 = 46.6$ grams in 1000 cc. of urine; therefore, $\frac{46.6 \times 1500}{1000} = 69.9$ grams of solids in twenty-four hours.

This method is sufficiently accurate for clinical purposes, but for scientific work a given amount of urine is evaporated in a previously weighed porcelain dish, the residue dried, allowed to cool, and weighed repeatedly until there is no further loss of weight from drying. The variations between the solids found by weighing and those found by multiplying by 2.33 are, on the average, very slight. The *average excretion of solids* is 61.14 grams (945 gr.) in twenty-four hours for a person weighing 145 pounds avoirdupois (66 kilos). The excretion of solids varies according to age, weight, and height.

Parkes lays down the rule, which he worked out by means of the combined observations of a number of authors, that 10 per cent. must be deducted from the average solids in persons between forty and fifty years of age; 20 per cent. between fifty and sixty; 30 per cent. between sixty and seventy; and 50 per cent. above seventy. The normal standard applies to an ordinary diet of mixed foods and to the ordinary exercise of a healthy man in daily work. In persons who have fasted for two days or longer, as in fevers, etc., one-third should be deducted from the average solids; one-eighth, if the diet be spare; one-tenth, if somewhat below that of health. For perfect rest one-tenth should be deducted; for comparative rest, one-twentieth.

It should be noted that no deductions can be drawn as to the amount of *any particular solid* from the weight of the total solids, and especially that although urea normally constitutes about one-half of the solids excreted, an estimation of the urea present by dividing the amount of total solids by two is of no value, as the *proportions* of the various constituents vary markedly.

QUESTIONS ON CHAPTER III

Amount.—What is the normal amount of urine passed by an adult? What is the average? How does it vary according to the sex and size of the person? According to the amount of liquid drunk and the amount of exercise? According to the time of day in health? According to season?

Define oliguria; anuria; polyuria; pollakuria.

When is diminution in quantity noted?

When is an increase noted? What is noted in Bright's disease, as a rule, as regards the quantity of urine?

Color.—What is the normal color of urine? On what does it depend in health? In disease? In what diseases do very pale urines occur? Highly colored urines? To what is a reddish urine due? A dark-brown urine? A "smoky" urine? A black urine? A yellowish-green foam? A blue urine? A greenish-blue urine?

Odor.—What is the odor of normal urine? What is it due to? What does an ammoniacal odor in a freshly passed urine indicate? An odor of sulphuretted hydrogen? A fecal odor? An odor of violets? A drug odor? A fruity odor?

Consistence.—When does urine become thick and stringy? When does it tend to froth? What is the consistence of chylous urine?

Transparency.—Describe normal urine as regards transparency. What is the nubecula? How is the nubecula noteworthy in women? When is it increased and how does the cloud behave then? How is this cloud distinguished from other causes of turbidity? What are these other causes? How does a cloud due to bacteria behave? A cloud due to phosphates? To urates? How are these clouds distinguished from one another? What is the behavior of cloudiness due to pus? What is Donné's reaction for pus? How is chylous urine distinguished? Give in tabular form the tests distinguishing all these causes of cloudiness.

Reaction.—What is the normal reaction of urine? What is it due to? When is urine neutral or amphoteric? How does its acidity vary nor-

mally? When is freshly passed urine alkaline? How do you test reaction in urine?

What is the best way to obtain an idea as to the *total acidity* of the urine? What do we mean by acidity "in terms of oxalic acid"? In terms of HCl? In terms of decinormal NaOH solution? Under what condition is acidity increased? Decreased?

What is the normal *specific gravity* of urine? How does it vary and why? What is needed for accurate determinations? Describe the urinometer and its mode of application. What other methods may be used? What is the Westphal balance? Describe the urinopyknometer and its uses. What precautions should be taken in reading the specific gravity with any urinometer? When is the specific gravity of urine increased? When is it diminished? In what disease is a marked diminution of the specific gravity a grave symptom?

What is the clinical value of a knowledge of the *amount of total solids?* How is this amount calculated approximately? What is the average amount of solids excreted in twenty-four hours? What changes must be made in this figure to allow for age? For exercise and diet?

PART II

CHEMIC EXAMINATION OF THE URINE

A. ORGANIC CONSTITUENTS

I. PROTEIDS

CHAPTER IV

SERUM-ALBUMIN

THE most common proteid in the urine in disease is serum-albumin, although pathologic conditions of the kidneys, of the blood, or of other organs may also result in the excretion of other proteids, such as serum-globulin, fibrin, hemoglobin, albumose, peptone, nucleo-albumin, etc. By *albuminuria* we usually mean the presence of serum-albumin and serum-globulin, with due regard to the possibility and probability of the presence of other proteid constituents of minor importance.

It has been shown (Leube, Fürbringer, Senator, and Posner) that a healthy kidney may excrete a certain amount of albumin, although very careful experiments have proved that not every urine contains albumin (Leube and Winternitz). According to Grainger Stewart, the minute quantities of albumin in normal urines are due to epithelial and

other débris. At any rate, there is no doubt that occasionally serum-albumin and serum-globulin in small quantities occur in the urine without any changes in the kidney, probably owing to a sudden disturbance of the circulation (von Jaksch). Recent studies by Ott and others have shown that so-called nucleo-albumin is present in all healthy urines, and that, consequently, it is very important to distinguish this proteid from serum-albumin. (See Mucin, Mucoid, Nucleo-albumin, p. 87.)

Albumin, like other proteids, is a colloid which does not crystallize and does not penetrate through animal membranes. So long as the animal membrane—in this case tubular epithelium—remains intact; so long as the proteid itself remains unchanged; so long as the pressure in the blood-current and in the excretory channel remains normal, no albumin is excreted by the kidney (see Secretion of Urine, p. 389). Albuminuria may be due, therefore, to one of three factors, namely: (1) Changes in the kidney which affect the excretory epithelium; (2) changes in the blood which render its serum-albumin more diffusible; (3) changes in the blood-pressure. Serum-albumin in noticeable amounts is never found in healthy urine, and its presence is always an important clinical symptom.

Albuminuria.—By albuminuria, in the clinical sense, we have come to understand the presence of amounts of albumin which are detected by the ordinary tests, and not the presence of minute traces discernible only with very delicate methods. Hofmeister lays down a good rule when he says that whenever Heller's ring test is positive within three minutes after contact, albuminuria is present.

Albuminuria may be subdivided into false and true albuminuria. *False albuminuria* (accidental albuminuria,

spurious albuminuria, pseudo-albuminuria) is dependent upon an admixture of albuminous substances (mucus, pus, blood, semen, prostatic fluid) with the urine during its transit from the kidney downward. This condition occurs in pyelitis, ureteritis, cystitis, prostatovesiculitis, spermatorrhea, etc., and in the presence of fistulous openings into the urinary or genito-urinary tract, through which proteid exudates, etc., may enter the urine. These albuminurias are distinguished by microscopic examination of the sediment, by catheterizing the ureters and examining the urine direct from the kidneys, and by the fact that other proteids than serum-albumin are, as a rule, relatively increased. Fifty thousand pus-cells can give one part in a thousand of albumin. The false or extra-renal albuminurias are especially noteworthy in connection with life insurance examinations.

True or *renal albuminuria* is dependent upon the excretion of serum-albumin (and usually of serum-globulin, *q. v.*) by the renal tubules. This form of albuminuria, however, may occur *without any anatomic lesions of the kidney*. The following classification of true albuminuria is intended to show the various clinical types which are distinguished with reference to their origin or to some special features, such as periodicity, etc.

I. **Functional Albuminurias.**—*Physiologic Albuminuria.*—Properly speaking, this term should be applied only to the excretion of the minute quantities of albumin occurring normally in the urine. According to Senator, any amount over 0.4–0.5 gm. in twenty-four hours cannot be physiologic. Hofmeister considers any quantity as pathologic which gives a ring with Heller's test within three minutes. Traces shown by such delicate tests as Spiegler's are not considered pathologic.

A group of conditions at times accompanied by albuminuria are sometimes classed as "physiologic." They are, more properly speaking, *functional* albuminurias occurring under special conditions:

1. *After Severe Muscular Exertion.*—A comparatively slight, temporary albuminuria, sometimes with casts, occurs in young men who indulge in *unaccustomed* physical exertion. This is seen in soldiers, football players, bicycle riders, mountain climbers, etc. The tendency to albuminuria diminishes as these persons become accustomed to the exercise.
2. *After Eating an Excess of Proteid Food.*—"Alimentary albuminuria," also slight and transient, may occur in young robust subjects (soldiers, etc.) after a heavy proteid meal.
3. *Following Nervous Shocks and Other Vasomotor Changes.*—Prolonged cold baths have given rise to transient albuminuria. The same is true of mental shock, mental strain or fatigue, and after faradization. The amount of albumin in these cases does not exceed 0.5 per cent.
4. *During Labor.*—Women in childbirth frequently have albuminurias which disappear during the first forty-eight hours of the lying-in period. This albuminuria is independent of renal lesions and is probably due to muscular fatigue, to changes in the circulation, or possibly to the excretion of toxins after labor.
5. *Newborn Children.*—During the first week or ten days of life a slight albuminuria (at times with casts) may occur, without any special significance.

II. **Essential Albuminurias.**—The word "essential" is here used for want of a better name to designate a group of comparatively rare albuminurias the cause of which is unknown, but which are not physiologic nor apparently purely functional, yet which are not accompanied by any renal lesions:

1. *Cyclic albuminuria* (Pavy) or *orthostatic (postural) albuminuria; albuminuria of adolescence* (Leube).

(*a*) By *cyclic* albuminuria is meant one which recurs periodically at certain hours in the twenty-four (usually between 12 and 1 P. M. and again from 7 to 11 P. M.—double cycle).[1]

(*b*) *Orthostatic (orthotic) or postural* albuminuria occurs only when the patient is standing, and disappears when he goes to bed. (These albuminurias are, of course, also cyclic if they come on regularly in the daytime and disappear at night.)

These albuminurias occur for the most part in young anemic and neurasthenic subjects (male and female), amount to about one part per 1000, and are probably the result of abnormal conditions of the circulatory apparatus (Serkowski). As

[1] The term "intermittent" is discarded as confusing.

these cases occur chiefly during the years of growth (puberty, adolescence) it has been surmised that the kidney does not seem to be able to keep pace with the patient's bodily development.

3. *Albuminuria Minima* (Lecorché and Talamon).—This occurs in adults, and is a very slight albuminuria, which persists after infectious and debilitating diseases. The amount never exceeds 0.5 per 1000. These cases may go on with a mere trace of albumin for years or may develop into chronic nephritis. According to Albarrant a persistent minimal albuminuria may be the precursor of renal tuberculosis. Some French authors regard it as a premonitory sign of gout. It may be due to a healed acute nephritis which has left some small part of the kidney badly functionating. These cases are on the borderline, close to the nephritic group.

III. **Traumatic Albuminurias.**—These may be *transient*, due to slight injuries to the kidney and pelvis (massage of the kidney will produce it—Menge), to movable kidney, and to injuries to the brain, apoplexy, etc. When the kidneys are seriously injured healing carries with it a chronic (interstitial) nephritis with albumin and casts.

IV. **Hematogenous Albuminurias.**—A number of changes in the blood can cause albuminuria without renal lesions. Among them are severe anemia; purpura, scurvy, cholemia, diabetes, leukemia, severe wasting diseases, and after the use of anesthetics (ether, chloroform).

V. **Nervous Albuminurias.**—Insanity, conditions of mental depression, psychoses, paralyses of certain parts of the brain, epilepsy, delirium tremens, etc., may be accompanied by an albuminuria (up to 0.6 per cent. —Serkowski).

VI. **Albuminuria of Renal Stasis.**—In conditions of passive congestion—*e. g.*, in cardial, pulmonary, and hepatic diseases in the presence of mechanical pressure (stones, tumors)—albuminuria may occur (not over 0.1–0.2 per cent.), with casts and usually a few red blood-cells.

VII. **Toxic Albuminurias.**—Among the *toxic causes* of albuminuria may be mentioned irritants (cantharides, turpentine), poisoning with arsenic, mercury, phosphorus, lead, antimony, alcohol, and mineral acids. Phosphorus, turpentine, and cantharides may, however, produce true renal lesions. In syphilis there may be albuminuria in the early second-a ries (later there may be a nephritis due to syphilitic lesions in the kidneys). Albumin and casts may also occur during the administration of balsamic drugs (sandalwood oil, etc.).

The *febrile diseases*, especially those of infectious character, are fre-

quently accompanied by transient albuminuria during the height and the decline of the fever. These albuminurias may or may not be followed by nephritis and are known as *febrile albuminurias.*

VIII. **Nephritic Albuminurias.**—This class is characteried by actual more or less permanent lesions in the kidneys. It must be remembered that in some forms of nephritis (chronic interstitial) the urine is often free from albumin. The albuminuria of nephritis may also be intermittent, disappearing for a time with rest, diet, etc., reappearing under the provocation of indiscretions in diet, etc.

The following table shows the amount of albumin which may be expected in various conditions of the kidney:

Disease.	Usual Amount.	Highest Amount.
Renal congestion.	Traces.	0.5 per 1000.
Acute parenchymatous nephritis.	1 to 5 per 1000 (globulin high).	Usually not over 10; rarely 15 per 1000 (enormous amount, 85 per 1000 in early nephritis of syphilitics).
Subacute parenchymatous nephritis.	4 to 8 per 1000.	Rarely 10 per 1000.
Chronic parenchymatous nephritis.	Traces.	1.0 per 1000
Chronic interstitial nephritis.	Faint traces or none.	Traces seldom over 0.5 per 1000.
Amyloid kidney.	Small amount usually.	Large quantity, especially globulin, in some cases.
Uremia.	Trace, or disappears before attack.	Often increases during convulsions.

It will be seen, therefore, that the mere fact that the urine contains albumin does not mean that the patient has nephritis, although this was the general view formerly, and many of the older practitioners still cling to it to-day. No diagnosis of Bright's disease can be made from the presence of albumin alone. Another point to be remembered is that the quantity of albumin present is not a measure of the extent or advancement of a renal lesion. Indeed, in the worst

cases of chronic Bright's disease—interstitial nephritis—albumin is frequently present in very small quantities and often temporarily or permanently absent. In the presence of an acute inflammatory process in the kidney or of a chronic parenchymatous nephritis the variations in the daily amount of albumin, taken together with the amount of urea, the number of casts, etc., may assist in determining the prognosis of a case.

The physician must be on his guard against the false alarms of accidental albuminurias and the comparatively less important types of albuminuria grouped under the heading of circulatory, febrile, hematogenous, and intermittent. A careful study of all the clinical features in the case, especially as to the periodicity of the symptom, the quality of the proteid substance found, the condition of the circulation, and the presence of microscopic features indicating renal disease, is the basis of accurate diagnosis in such cases.

METHODS OF TESTING FOR ALBUMIN

Precautions.—In testing for albumin, it is most important to have a fresh specimen of urine and to have a perfectly clear urine. Decomposition gives rise to an abundant bacterial growth, which in itself may give a faint albumin reaction in some cases. The urine should be filtered through several thicknesses of the better quality of white filter-paper, such as is used for chemical analysis. Loosely textured filter-paper of the cheaper grades is useless. Even with the best of care, filtration may not remove bacteria. An asbestos filter may also be used or the urine may be shaken with a small amount of heavy calcined magnesia. If the latter is used, the urine should

be rendered acid by the addition of a few drops of nitric acid before it is tested. Fine sand may also be used for filtering, but care should be taken to have it perfectly free from organic matter.

If the urine is concentrated, it should be diluted before testing, as dilution eliminates the action of certain bodies, such as urea, phosphates, etc., which interfere with albumin tests.

The best time to detect small amounts of albumin is in the evening, especially in walking patients. In the morning, after a night's rest, the urine of such patients is frequently free from albumin.

Heat Test.—With Nitric Acid.—A test-tube is filled with clear urine[1] and the upper portion of the fluid is heated to boiling. If the heated portion becomes cloudy, even in the slightest degree, as we can see by holding the tube against a black surface, the turbidity is due either to albumin or to earthy phosphates. If the cloudiness is due to the latter, it disappears on adding a few drops of nitric acid; if it is due to albumin, it remains. If no precipitate appears or if the precipitate redissolves on shaking upon the addition of the acid, two or three more drops of the acid are added, and if a flocculent precipitate results, albumin is present.

Under favorable conditions heat will detect 1 part of albumin in 100,000 parts of urine, but there are many sources of error in the test by heat. The effects of the presence of earthy phosphates have already been spoken of. Another source of error is the fact that if there is very little albumin, the amount of acid will be in excess and the al-

[1] When the urine is not clear, it should be filtered before applying any of these tests.

bumin will dissolve; on the other hand, if both phosphates and albumin are present and too little acid be used, the acid is taken up by part of the phosphates, while the remainder of these salts unite with the albumin to form soluble alkaline albuminates, the urine remaining clear.

Very highly acid urine from natural causes may fail to precipitate albumin by heat on the addition of acid, owing to the fact that a soluble acid albumin is formed under these conditions. The latter may be precipitated on the further addition of nitric acid or on neutralizing with potassium hydrate. There is a danger, however, of converting the albumin into soluble alkali albumin with the least excess of alkali, and so redissolving the precipitate. Highly alkaline urine converts the albumin into a soluble alkali albumin which is non-coagulable by heat, like the acid albumin. In this case the addition of a few drops of dilute acid will convert the alkali albumin into serum-albumin, which will be precipitated by heat.

If the test-tube is not clean and happens to contain a drop of nitric acid, boiling may fail to cause a cloud on account of the conversion of serum-albumin into non-coagulable acid albumin. A flocculent precipitate formed *before* the addition of acid in the heat test may be calcium phosphate, which is soluble in an excess of acid. Such a precipitate occurring *after* the addition of acid is albumin. The urine should not be heated after the addition of the acid, as the coagulated albumin is soluble in the heated acid, and this is the reason why this test is not good for very small amounts of albumin, as the latter may be dissolved upon the addition of the acid if the fluid is hot enough.

Another source of error is the presence of uric acid or

urates, which may give rise to precipitates that are crystalline (not flocculent) and which do not appear if the urine be diluted with three parts of water before the test. The precipitate of uric acid is dark brown in color and does not fall until the specimen begins to cool. The presence of resinous acids in cases in which copaiba, cubebs, turpentine, benzoin, sandalwood oil, or Peru or tolu balsams have been taken gives a precipitate which dissolves in alcohol, while albumin is precipitated by this substance. Urines full of bile-pigments at times give a precipitate of biliverdin, but this is soluble in alcohol.

With Acetic Acid and Salt.—The heat test may be performed in the same manner as has been already described, with acetic instead of nitric acid. Formerly, the urine was rendered slightly acid with a few drops of 2 per cent. acetic acid, and the upper portion of the test-tube was boiled. The precipitate which results may be either serum-albumin or it may be partly composed of nucleo-albumin and partly of serum-albumin. As we shall see later, nucleo-albumin (or a substance thus designated) occurs very frequently in normal urine, and it is exceedingly important to eliminate the possibility of a mistake resulting from the appearance of a precipitate of nucleo-albumin.

The elimination of nucleo-albumin in the test with acetic acid is best secured by the use of a sufficient amount of a saturated solution of sodium chlorid, which when added to the urine prevents the precipitation of nucleo-albumin.

The principle underlying the use of salt solution is the fact that nucleo-albumin is coagulated by heat in urine only when the urine is poor in neutral salts. This fact has been known for some time, but it was due to Cohn-

heim[1] that it has been so systematically applied to the differentiation of proteids. If saturated sodium chlorid solution is added to the urine any lack of neutral salts that may have been present in the urine as voided is balanced by this addition. The salt solution, therefore, is used simply to make sure that there are enough neutral salts in the urine to keep the nucleo-albumin from coagulating when the acetic acid and heat test is applied.

Method of Performing the Test.—Purdy[2] gives the following method of performing this test: A test-tube is filled about two-thirds full of the previously filtered urine and about one-sixth of this volume of a saturated sodium chlorid solution is added. Next, from 5 to 10 drops of acetic acid (50 per cent.) are added, and the upper portion of the contents of the test-tube is boiled for one-half minute, giving a precipitate if albumin be present.

Hastings performs the test as follows: To the urine one-fifth volume of a saturated sodium chlorid solution (30 per cent.) is added. The upper third of the tube is heated. If a cloud appears it may be due to phosphates. From 2 to 5 drops of acetic acid (50 per cent.) are now added, and the upper portion of the tube is again heated. If the cloud was due to phosphates, it will disappear on the addition of the acid, and the upper portion of the tube will clear up, while the phosphates in the lower portion, which by this time have become heated, will cloud the tube until the acid permeates the entire liquid, when the contents will be cleared. If the cloud persists at the top of the fluid, after the addition of acetic acid and after heating again,

[1] Chemie der Eiweisskorper, Braunschweig, 1900.

[2] Practical Urinalysis, Philadelphia, 1900, fifth edition, p. 72.

serum-albumin is present (sometimes associated with serum-globulin).

This test, while somewhat less delicate than the heat and nitric acid test, is very satisfactory for routine use. In the author's hands it has given very accurate results in upward of 3000 urines tested by the modification of Hastings just mentioned. When very faint traces are present, however, the test should be confirmed by some of the other methods, notably the nitric acid ring test and the ferrocyanid test.

Great care should be taken in the use of the acid and heat tests to have the urine perfectly clear (as has already been mentioned). A test for albumin with any of these methods is absolutely worthless with cloudy urines unless there be a large amount of proteids present. The practice of testing the urine without filtering, as is done in laboratories which employ the "quick lunch" methods of examination and which handle a very large number of urines in a short time, is to be especially deprecated. No reliance can be placed upon findings under these conditions, for nearly every urine will be found to contain faint traces of albumin unless the tests are performed with all due precautions against error.

The Nitric Acid Test (*Heller's Test*).—This test is exceedingly sensitive, and for all practical purposes is probably the best. The urine should be filtered, even though it appears perfectly clear. A small amount of pure, colorless nitric acid—HNO_3 (c. p.)—is poured into a small test-tube; the latter is held obliquely in the left hand, while the urine is allowed to trickle down the side of the tube from a pipet consisting of a glass tube 6 or 8 inches long and drawn to a point. The urine will overlie the acid if it

has been poured in gently enough. If albumin is present, a distinct, sharp, white zone will appear at the junction of the two fluids, varying in thickness—not according to the amount of albumin, but according to the rapidity with which the urine is poured and also according to the effervescence that follows the addition of the urine (Ogden). The band may vary from $\frac{1}{32}$ to $\frac{1}{2}$ inch in width, and in the presence of large quantities it is narrow, but very dense. If minute traces are present, it takes the shape of a hazy ring or cloud, which becomes more distinct after a few minutes.

Another way of performing this test is to pour the urine into the tube first and to allow the acid to pass down the side and under the urine. This manner of using nitric acid is not so convenient as the first.

Still another method involves the use of a small wineglass. This is filled one-half full of the filtered urine, the glass is inclined so that the urine reaches nearly to its edge, and the nitric acid is poured under the urine, as slowly as possible, from a small bottle, until the acid reaches about one-third the volume of the urine. The objections to this method are that it requires more acid and is not so convenient as the test-tube method.

Precautions in Using Heller's Test.—When nitric acid is placed in contact with normal urine, a brown ring appears which increases on standing and is due to the action of the acid on the coloring-matters. In urine with much coloring-matter the albumin, if present, will also be colored brown, or if there is much indican, the albumin ring will assume a reddish or violet tinge; if much blood-coloring matter, a brownish-red; if biliary pigments, a green color.

The overlying of nitric acid with urine precipitates the albumin as acid albumin, since the latter is not soluble in the excess of acid at the point of contact. Mucin is also precipitated, but is dissolved by the excess of nitric acid at the point of contact, and, therefore, does not form part of the ring. Peptones are not precipitated in this test, but albumoses and resins are. The differentiation from resins is best made by using some other test which does not precipitate the latter (sulphosalicylic acid). Nucleo-albumin[1] gives a ring which is fainter and is about 1 cm. above that due to albumin. A white zone is also formed by the action of nitic acid on the mixed urates if these are in excess. This zone of acid urates is found over the zone of contact between the acid and the urine; it tends to diffuse rapidly into the urine above and is dissipated on the cautious application of heat. If uric acid is present in large amounts urea nitrate precipitates in crystals, but this can occur only in very concentrated urines.

If there is a large amount of carbonic acid and ammonium carbonate, as after alkaline fermentation, a vegetable diet, etc., the contact of urine with nitric acid produces so much effervescence that the test cannot be used. In this condition the following method (Hoffmann and Ultzmann) may be used: Add to the urine about one-quarter part of potassium hydrate solution, warm the mixture, and filter; if the fluid is still cloudy and cannot be cleared by ordinary filtration, add 1 or 2 drops of the magnesium solution (see p. 218), warm again, and filter. The urine thus cleared will show albumin with Heller's test.

In the presence of resins, coloring-matters, etc., rings

[1] Or (as we shall see later), more accurately, *euglobulin*, etc.

of precipitate which are soluble in alcohol will occur (compare under Heat Test with Nitric Acid, p. 57).

Other Tests for Albumin.—The heat tests and Heller's test are the most important modes of detecting albumin. They are usually sufficient, but in case of doubt and when we desire extreme delicacy, the tests which follow may be used. In practice it is a much better plan to use one or two tests as a matter of routine, to know these tests with all their possibilities and fallacies, and not to burden one's self with too many tests and reagents. Many of these less important tests are very sensitive, but clinically extreme delicacy is not desirable, as it leads to confusion when we remember that normal urine contains very faint traces of albumin. In the following enumeration of tests the order of preference is shown, the first four described being the most important and most satisfactory to use for general work. These four tests are in constant use in the author's laboratory as confirmatory tests for albumin.

Potassium Ferrocyanid Test.—To 10 cc. of urine add 5 drops of strong acetic acid. If a precipitate appears, it is due to nucleo-albumin and should be filtered off. Then a few drops of a 5 per cent. solution of potassium ferrocyanid are added, and the turbidity, varying from slight opalescence to a denser cloud, shows the presence of albumin. An excess of the reagent will dissolve the precipitate, which is best observed by holding the test-tube in front of a black surface and by comparing it with a test-tube of normal urine. Albumose and nucleo-albumin give precipitates, the former dissolving with heat, the latter, on the addition of a few drops of lead acetate solution. An excess of this solution will also dissolve the albumin.

Nitric-magnesium Test (*Roberts*).—The solution is

composed of 1 part of strong nitric acid and 5 parts of saturated solution of magnesium sulphate, making a mixture safe to handle. It is applied by the usual contact method. It is more sensitive than Heller's test. In the author's experience with this reagent, which has been extensive, its delicacy has been found rather confusing clinically. It shows a double or triple ring, the lowest and densest of which is due to albumin, the upper to nucleo-albumin or urates, or both. Urine which does not show a ring with Heller's test occasionally shows a faint reaction, especially on standing, with Roberts' test. Unless the lower ring is sharply defined, dense, and appears almost instantly, clinical albuminuria cannot safely be diagnosticated with Roberts' test. It is most conveniently employed in the simple glass instrument known as the horismascope or albumoscope (Fig. 6). The urine is poured into the large end of the tube, and Roberts' solution is then poured down the small funnel until the level of the solution reaches about the center of the black background in the larger tube. The rings are seen very distinctly against this background. The instrument can also be used with Heller's and with many other tests.

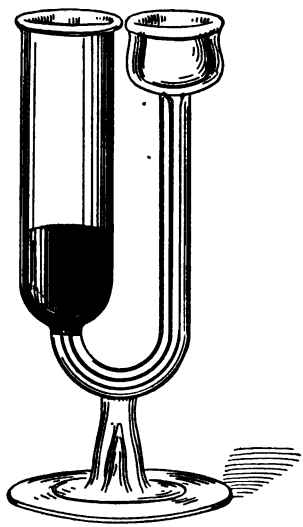


Fig. 6.—The horismascope.

Mercuric Chlorid Test (*Spiegler*).—The reagent consists of 8 gm. of mercuric chlorid, 4 gm. of tartaric acid, and 20 gm. of glycerin in 200 cc. of distilled water. It is used by the contact method, like Heller's test. This test reacts with globulin and albumoses, but not with peptone. Spiegler's test has recently been modified by Jolles, with the result of rendering it much more delicate. Jolles recommends $HgCl_2$, 10 grams; succinic acid, 20 grams; sodium chlorid, 10 grams; water, 500 cc. A very sharp white ring is produced by albumin. The urine must first be filtered and acidified with acetic acid and filtered again to remove nucleo-albumin.

Salicylsulphonic Acid Test (*Sulphosalicylic Acid Test, Roch and MacWilliams*).—Add 1 or 2 drops of a saturated solution of the reagent, or more if the urine is alkaline, to about 20 drops of the urine in a small test-tube. On shaking, opalescence or turbidity immediately appearing, shows albumin. Turbidity occurring slowly implies minute traces. On boiling, the precipitate is due to albumin or globulin.

Picric Acid Test (*Johnson*).—Into a test-tube 6 inches long pour 4 inches of filtered urine. Hold the test-tube obliquely and gently pour an inch of a saturated solution of picric acid over the surface of the urine. To make the reagent add 6 or 7 grains of picric acid to 1 ounce of boiling distilled water. In the portion of the tube where the reagent mixes with the urine a yellow turbidity of coagulated albumin will appear. If the amount of albumin is small, the upper part of the tube may be heated to increase the turbidity. Picric acid precipitates, besides albumin, urates, peptone, albumose, the vegetable alkaloids and mucin, all of which, except mucin, are dis-

solved with moderate heat. There must be an actual mixture of the urine with the reagent, not a mere surface contact.

Potassio-mercuric Iodid Test (*Tanret*).—This is very sensitive and is prepared as follows: Mercuric chlorid, 1.35 gm.; potassium iodid, 3.32 gm.; acetic acid, 20 cc.; distilled water, enough to make 100 cc. The two salts should be separately dissolved in portions of the water and the solutions mixed. The reagent is heavy, and is used by the contact method without previously acidulating the urine. It precipitates the same substances as picric acid, and the precipitates are also dissipated by heat or alcohol; they reappear on cooling. According to Oliver, the precipitate of nucleo-albumin is not dissolved by heat if a large excess of the reagent is used. Albumin gives a white, sharply defined zone.

Trichloracetic Acid Test (*Raabe, Grosstern, and Fudakovsky*).—A saturated solution of the crystals of trichloracetic acid (½ ounce of the crystals to 2 drams of water) is made and used by the contact method. It is claimed by some not to precipitate peptone or nucleo-albumin, and is esteemed very highly as a delicate test. The trouble with it is that it is too delicate, as it precipitates mucin and, therefore, cannot be regarded as trustworthy. It also precipitates albumoses, alkaloids, and sometimes uric acid, all these disappearing on the addition of heat.

Potassium Sulphocyanid Test (*Zouchlos*).—A few drops of a mixture of 100 cc. of potassium sulphocyanid solution (10 per cent.) and 20 cc. of acetic acid are added to urine. A cloud or precipitate occurs if albumin is present, and the test is said to detect 0.007 per cent. of the

proteid (Schick), but Huppert classes it among the less delicate tests.

Phenic-acetic Acid Test (*Mêhu and Millard*).—The formula for this is: Glacial phenic acid, 2 dr.; acetic acid (c. p.), 6 dr.; potassium hydrate solution, from 6 dr. to 2 oz. Apply by the usual contact method. It reacts with peptone, nucleo-albumin, albumoses, and alkaloids, all of which precipitates are said to disappear with heat. It is supposed to detect 1 part of albumin in 200,000 parts of urine.

Mercuric Chlorid and Citric Acid Test (*Stütz and Fürbringer*).—A mixture of the double salt of mercuric chlorid and sodium chlorid with citric acid is put up in gelatin capsules. A solution of this reagent is not so sensitive as Tanret's, does not precipitate alkaloids, but sometimes gives opacity in urines that do not contain albumin. Concentrated urines which contain much uric acid must be diluted before using this reagent.

Resorcin Test (*Carrez*).—One gm. of resorcin is dissolved in 2 cc. of distilled water in a test-tube, and the urine is poured upon the surface. A white ring shows albumin. Peptone also gives a white ring, but it disappears on immersing the tube in boiling water.

Betanaphthol-sulphonic Acid Test (*Riegler*).—Ten gm. of the crystalline reagent are dissolved in 200 cc. of distilled water and filtered. Five cc. of urine are treated with 20 or 30 drops of the solution. Turbidity indicates the presence of albumin. Boiling will not make the precipitate disappear if it was due to albumin. If it was due to albumose or urates, boiling will redissolve the cloudiness.

Other Tests.—A great variety of substances besides

those named have been employed to precipitate albumin. It would be useless to enter into a description of all these tests. Examples are: Tannin (Almén); sodium sulpho-antimoniate (Schlippe); potassium antimoniate (Palm); phosphomolybdic acid (Sonnenschein); chromic acid (Kirk); iodin and potassium iodid (Cohen); calcium chlorid and hydrochloric acid (Jolles); formalin (Iatrona); formalin and mercuric chlorid (Polacci).

Comparative Delicacy of Albumin Tests.—The order of delicacy of the tests described above is a matter still under discussion, the opinions of various authorities differing somewhat on the subject. In the following table the author has endeavored to give the order which has the largest number of indorsers and which corresponds to his own results. It is essentially the order given by Huppert, who bases his list on the researches of Lecorché and Talamon, Vass and Ott:

	Reagent and Author.	Detects Albumin in—
* 1.	Mercuric chlorid (Spiegler; Jolles' modification)	1: 350,000 parts urine.
* 2.	Trichloracetic acid (Raabe, etc.)	1: 75,000 " "
* 3.	Salicylsulphonic acid (Roch and MacWilliams)	1: 60,000 " "
4.	Phenol acetic acid (Millard).	
5.	Potassiomercuric iodid (Tanret).	
* 6.	Heat and nitric acid	1: 50,000 " "
* 7.	Heat and acetic acid (with NaCl).	
* 8.	Potassium ferrocyanid	1: 40,000 " "
* 9.	Nitric acid (Heller)	1: 40,000 " "
10.	Betanaphthol sulphonic acid (Riegler)	1: 40,000 " "
11.	Resorcin	1: 30,000 " "
*12.	Magnesium and nitric acid (Roberts)	1: 15,000 " "
13.	Potassium sulphocyanid (Zouchlos).	
14.	Metaphosphoric acid	1: 1000 " "
15.	Picric acid (Johnson).	
16.	Mercuric chlorid citric acid.	

The most delicate test is not necessarily the most desirable in practice. The tests marked with an asterisk in the table are most important, and are practically the only ones a physician need use.

QUANTITATIVE TESTS FOR ALBUMIN

Gravimetric Method.—This is the only accurate method of determining the amount of albumin, but is entirely too elaborate for clinical work. It consists of boiling a certain volume of urine, to which enough acid has been added to render it distinctly acid in reaction. The coagulum is collected upon a dry, weighed filter; washed, dried, and weighed again. The increase in weight in the filter corresponds to the amount of albumin by weight in the given volume of urine.

Another method is to remove the albumin by coagulation, to determine the diminution in specific gravity in the supernatant fluid, and to multiply this difference in specific gravity by 400 (Zahor's coefficient).

A third method is to determine the total nitrogen in a urine containing albumin (by Kjeldahl's method), and to find the difference in nitrogen in the same volume of the urine, deprived of albumin by coagulation, multiplying the difference by 6.3 (Van Nuyes and Lyons). A fourth method is to determine the difference in refraction between the urine containing albumin and the same urine after coagulation. The error is said to be very small (Ehlenger).

APPROXIMATE CLINICAL ESTIMATION

The simplest and least accurate mode of estimating the percentage of albumin consists in boiling the urine with acid, allowing the coagulum to settle, and then measuring the comparative bulk of the precipitate as compared with the total volume of urine. It is this method that gave rise to the confusing expressions of 25, 50, 75, or even 100 per cent. of albumin, and its use has been encouraged by the introduction of graduated tubes devised for the purpose of

indicating the relative bulk of the coagulum. Such quantities as 75 per cent. are obviously impossible when we remember that urine cannot possibly contain more than 5 per cent. of albumin by weight, the usual amounts measured being less than 1 per cent. This method of estimating the bulk of the coagulum is, therefore, very misleading and should never be used.

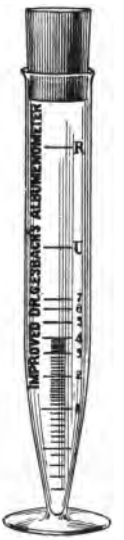


Fig. 7.—Esbach's albuminometer.

Esbach's Method.—This is the most convenient method of quantitative estimation of albumin, and if carefully performed is sufficiently accurate for clinical purposes. A graduated glass tube, or albuminometer (Fig. 7), with walls rather thicker than the ordinary test-tube and provided with a rubber stopper, is the apparatus used. This tube is filled with urine to the letter U; then Esbach's reagent is added up to the mark R. This reagent consists of 10 parts of picric acid and 20 parts of citric acid in 1000 parts of distilled water. The tube is closed with the stopper, and the urine and reagent are mixed by inverting the tube several times. It is then allowed to stand for twenty-four hours in a vertical position. The number at the level of the precipitate shows the number of grams of albumin contained in 1 liter of urine—that is, the number of parts per thousand. Each part per thousand represents $\frac{1}{10}$ of 1 per cent. by weight, so that 5 gm. per liter means 0.5 per cent. of albumin. It is best to use the latest form of Esbach's tube (Fig. 7), which has a foot and graduations in small fractions of 1 gram at the

lower part, so that minute quantities of albumin can be estimated. For urines with large quantities of albumin, exceeding 0.7 per cent., which is the highest mark on the tube, we must use dilutions with equal parts of distilled water. The same procedure may be used when very small quantities of urine are available, the reading being, of course, multiplied by the number of times the urine has been diluted.

Precautions in Using Esbach's Method.—Esbach's method has a limit of accuracy, but in most cases it is accurate within approximately 0.1 per cent. The error is greater in very large and in very small quantities. In fact, very small amounts will not even settle, but will remain as a diffuse cloud in the tube. This is especially the case in concentrated febrile urine. If the urine is not strongly acid, it must be made so with acetic acid before the test is used. The tube should be inverted and not shaken while mixing the urine and reagent. The divisions are calculated for room temperature, and the tube should be allowed to remain at about that temperature. If the room is too warm the sediment will settle much more rapidly. If the patient has been taking balsamics or resins, such as sandal-wood oil, Esbach's method cannot be used, because the resinous acids are precipitated by picric acid.

Tsuchiya's Method.—Tsuchiya[1] has worked out a method which is said to be more accurate than Esbach's.[2] The reagent consists of a solution of 1.5 gm. of crystalline phosphotungstic acid in 100 gm. of 96 per cent. alcohol and 5 gm. of concentrated hydrochloric acid.

The urine must be diluted to a specific gravity of 1006 to 1008, and still further if it contains more than 5 or 6 parts per 1000 of albumin. The diluted urine is placed

[1] Centralblatt für Innere Medizin, 1908, No. 5.

[2] Attention is called to the fact that phosphotungstic acid precipitates in acid solutions a large variety of other bodies besides albumin, including uric acid, xanthin, etc. These, however, are present in very small amounts in the dilution used in this method.

in an Esbach tube up to the mark U and the reagent is filled to the mark R. The closed tube should be inverted cautiously at least ten or fifteen times and should be allowed to stand for twenty-four hours, whereupon the amount of albumin may be read on the scale. This method gives no precipitate with normal urine, as is sometimes the case with Esbach's reagent. The precipitate of albumin settles more regularly and uniformly than with Esbach's solution. The method is more accurate and is applicable to urines with small amounts of albumin, especially in febrile urine, in which Esbach's reagent gives merely a cloud.

Goodman and Stern's Modification.—Goodman and Stern [1] make use of Tsuchiya's solution as a basis for a titration method. They determined the amount of albumin necessary to cause the first sign of cloudiness. They found that the solution precipitated exactly 0.0001 gm. albumin, and worked out the following method: First the Heller test is made, and if much albumin is present, the urine is diluted ten times (1: 10). If not, undiluted urine is used. Five cc. of the phosphotungstic acid solution (see p. 72) are put in a test-tube, and then, with a 2-cc. pipet, graduated in tenths of a cubic centimeter, the filtered urine is added to this, and shaken after the addition of each tenth, and urine added again until a whitish cloud appears. The number of tenths of a cubic centimeter is then read off. The calculation is then as follows: If it takes 1 cc. of diluted urine (1 : 10) there is 0.0001 gm. of albumin in 0.1 cc. of undiluted urine, or in 100 cc. there is 0.1 gm. albumin, and in 1000 cc. there is 1 gm. of albumin. If 0.7 cc. diluted urine (1 : 10) is used, then 0.07 cc. undiluted urine contains 0.0001 gm. albumin, 7 cc. diluted urine contain 0.01 gm. albumin, and 700 cc. contain 1 gm. albumin. The following equation gives the percentages:

700 : 1.0 : : 100 : x or 0.142 per cent. or 1.42 gm. per thousand.

Centrifugal Method (*Purdy*).—For this purpose a centrifuge revolving at a uniform speed of 1500 revolutions a minute and a special percentage-tube, holding 15

[1] Jour. Amer. Med. Assoc., December 12, 1908.

cc., are employed. To 10 cc. of the urine add 3 cc. of a 10 per cent. solution of potassium ferrocyanid and 2 cc. of 50 per cent. acetic acid. Allow to stand for ten minutes to insure the entire precipitation of the albumin. Place the percentage-tube in a centrifuge with a radius, with tubes extended, of exactly $6\frac{3}{4}$ inches; revolve at 1500 revolutions a minute for three successive periods of five minutes each, and read off the bulk-percentage. This method is simple, fairly accurate, and convenient, provided it be performed exactly as prescribed. According to Purdy, each $\frac{1}{10}$ cc. of precipitate represents 1 per cent. bulk measure of albumin. Ogden found that in order to find the percentage of albumin by weight from this bulk measure we must divide the latter, expressed in $\frac{1}{10}$ cc., by 6. The great source of error in this method is an excess of urates, which adds to the bulk of the precipitate. When this is known, it is better to centrifuge the precipitate, decant the supernatant fluid; to add hot water, which dissolves the urates, and to centrifuge again. The most important part of this method is to allow the precipitate to settle thoroughly before centrifuging and to employ a centrifuge of the proper arm-length and proper uniform speed.

The table on page 75 from Purdy will serve to convert bulk-percentage into weight-percentage.

Vassilieff's Method of Titration.—This consists in titrating the urine with a solution of sulphosalicylic acid until all the albumin is precipitated, and then obtaining an end-reaction for the excess. The urine, which is rendered slightly acid with acetic acid, is measured off in a 10-cc. pipet and diluted with water to 50 cc. Two drops of a watery 1 per cent. solution of a yellow dye, known as "Echtgelb," are added, and the whole is titrated with a

PURDY'S QUANTITATIVE METHOD FOR ALBUMIN IN URINE (CENTRIFUGAL)

Table showing the relation between the volumetric and gravimetric percentage of albumin obtained by means of the centrifuge with radius of six and three-quarter inches; rate of speed, 1500 revolutions per minute; time, three minutes.

Volumetric Percentage by Centrifuge.	Percentage by Weight of Dry Albumin.	Grains per Fluidounce Dry Albumin.	Volumetric Percentage by Centrifuge.	Percentage by Weight of Dry Albumin.	Grains per Fluidounce Dry Albumin.	Volumetric Percentage by Centrifuge.	Percentage by Weight of Dry Albumin.	Grains per Fluidounce Dry Albumin.
¼	0.005	0.025	13½	0.281	1.35	31½	0.656	3.15
½	0.01	0.05	14	0.292	1.4	32	0.667	3.2
¾	0.016	0.075	14½	0.302	1.45	32½	0.677	3.25
1	0.021	0.1	15	0.313	1.5	33	0.687	3.3
1¼	0.026	0.125	15½	0.323	1.55	33½	0.698	3.35
1½	0.031	0.15	16	0.333	1.6	34	0.708	3.4
1¾	0.036	0.175	16½	0.344	1.65	34½	0.719	3.45
2	0.042	0.2	17	0.354	1.7	35	0.729	3.5
2¼	0.047	0.225	17½	0.365	1.75	35½	0.74	3.55
2½	0.052	0.25	18	0.375	1.8	36	0.75	3.6
2¾	0.057	0.275	18½	0.385	1.85	36½	0.76	3.65
3	0.063	0.3	19	0.396	1.9	37	0.771	3.7
3¼	0.068	0.325	19½	0.406	1.95	37½	0.781	3.75
3½	0.073	0.35	20	0.417	2.	38	0.792	3.8
3¾	0.078	0.375	20½	0.427	2.05	38½	0.801	3.85
4	0.083	0.4	21	0.438	2.1	39	0.813	3.9
4¼	0.089	0.425	21½	0.448	2.15	39½	0.823	3.95
4½	0.094	0.45	22	0.458	2.2	40	0.833	4.
4¾	0.099	0.475	22½	0.469	2.25	40½	0.844	4.05
5	0.104	0.5	23	0.479	2.3	41	0.854	4.1
5½	0.111	0.55	23½	0.49	2.35	41½	0.865	4.15
6	0.125	0.6	24	0.5	2.4	42	0.875	4.2
6½	0.135	0.65	24½	0.51	2.45	42½	0.885	4.25
7	0.146	0.7	25	0.521	2.5	43	0.896	4.3
7½	0.156	0.75	25½	0.531	2.55	43½	0.906	4.35
8	0.167	0.8	26	0.542	2.6	44	0.917	4.4
8½	0.177	0.85	26½	0.552	2.65	44½	0.927	4.45
9	0.187	0.9	27	0.563	2.7	45	0.938	4.5
9½	0.198	0.95	27½	0.573	2.75	45½	0.948	4.55
10	0.208	1.	28	0.583	2.8	46	0.958	4.6
10½	0.219	1.05	28½	0.594	2.85	46½	0.969	4.65
11	0.229	1.1	29	0.604	2.9	47	0.979	4.7
11½	0.24	1.15	29½	0.615	2.95	47½	0.99	4.75
12	0.25	1.2	30	0.625	3.	48	1.	4.8
12½	0.26	1.25	30½	0.635	3.05			
13	0.271	1.3	31	0.646	3.1			

Test.—Three cc. of 10 per cent. solution of ferrocyanid of potassium and 2 cc. of 50 per cent. acetic acid are added to 10 cc. of the urine in the percentage-tube and *stood aside for ten minutes*, then placed in the centrifuge and revolved at rate of speed and time as stated at head of table. If albumin is excessive, dilute the urine with water until volume of albumin falls below 10 per cent. Multiply result by the number of dilutions employed before using the table.

25 per cent. solution of sulphosalicylic acid until the mixture takes on a permanent brick-red color. The amount

of albumin can be computed by multiplying the number of cubic centimeters of sulphosalicylic acid used by 0.01006. This is a convenient and accurate method.

To Remove Albumin from Urine.—Hofmeister's method may be used when other tests are to be made with which albumin would interfere. About 10 cc. of a 40 per cent. sodium acetate solution are added to the urine, and then some concentrated ferric chlorid until a red color is produced throughout the fluid. The urine is rendered neutral or faintly acid and is boiled. The precipitate of basic ferric acetate throws down all albumin and the filtrate is clear and free from proteids. This method cannot be used if sugar is present. When this is the case the urine is simply boiled and acetic acid is added until the precipitate is flocculent and the filtrate no longer clouds. For quantitative work the urine should be restored to the original volume before further tests are made.

CHAPTER V

OTHER PROTEIDS IN URINE

SERUM-GLOBULIN[1]

THE tests that have thus far been described for albumin make no distinction between serum-albumin and serum-globulin; for, when we speak clinically of "albumin" in the urine, we mean the precipitate of albumin and globulin which results on testing for proteids in the urine. As these two substances almost always appear together, a differentiation of globulin from albumin and a knowledge of their comparative amounts do not seem of great clinical value. There are some cases, however, in which the presence of globulin has a special significance. Serum-globulin constitutes a variable proportion of the total proteids in albuminurias (from 8 to 60 per cent.). It is noted in unusual quantities in the urine of catarrhal cystitis, in acute nephritis, and particularly in amyloid degeneration of the kidney and other conditions accompanied by considerable destruction of the renal epithelium. In chronic Bright's disease globulin is present in comparatively small amounts or is even absent. The distinction between serum-globulin and serum-albumin chemically is, therefore, a refinement of urinalysis which may sometimes be of value to the clinician.

[1] Under this heading it is intended to consider the entire globulin group of proteids occurring in urines. The differentiation of globulins into euglobulin, pseudoglobulin, and fibrinoglobulin (Hofmeister) has not been attempted.

Qualitative Tests.—1. A layer of faintly alkaline urine is floated over a saturated solution of $MgSO_4$, and the precipitate which results is globulin without the serum-albumin.

2. The urine is exactly neutralized, filtered, and saturated completely with magnesium sulphate at ordinary temperature, or with a saturated solution of ammonium sulphate. A white precipitate immediately forming is globulin. A precipitate appearing later, with the use of ammonium sulphate, may be ammonium urate.

3. If the globulin precipitate be filtered off, the same urine may be tested for serum-albumin by heating with a few drops of acetic acid.

4. A very simple method is that of Roberts. It depends upon the fact that the globulins are insoluble in water and are held in solution in the urine by the salts therein. A wineglass is filled with water and a few drops of albuminous urine are allowed to fall into it. If globulin is present in any quantity, each drop is followed by a milky streak, until the water assumes an opalescence, which disappears on adding acetic acid.

Quantitative Test.—The amount of globulin may be determined separately from the albumin by carefully neutralizing the urine with ammonia and by precipitating the globulin with an equal volume of saturated neutral solution of ammonium sulphate. The mixture is well shaken and allowed to stand for some hours. The precipitate is thoroughly washed with saturated magnesium sulphate solution, dried at 100° C., boiled with water, extracted with alcohol and ether, then dried, weighed, incinerated, and, finally, weighed again, the last weight being the amount of globulin.

FIBRIN

Fibrin is a product of coagulation of blood, lymph, transudates, or exudates. It is an elastic, white, stringy substance, insoluble in water, ether, and alcohol; soluble with difficulty in solutions of sodium chlorid (5 to 15 per cent.), of potassium nitrate (6 per cent.), and of magnesium sulphate (5 to 10 per cent.). When fibrin is dissolved in saline solutions, a globulin is found in the solution. Fibrin is coagulated by heat from its solutions, precipitated by saturating them in magnesium sulphate, and dialyzing the salts from these solutions. The addition of weak hydrochloric acid causes fibrin to swell up into a transparent jelly, while the addition of strong acids dissolves it, with the formation of acid albumin or syntonin and albumoses. Pepsin and hydrochloric acid digest it, as does the pancreatic juice, the results being albumoses and peptone. Fibrinogen, which is the predecessor of fibrin in circulating blood, has been found to possess properties resembling those of globulin.

Clinical Significance.—Fibrin in the urine usually means the presence of blood, the quantity depending upon the extent of the hemorrhage. Sometimes fibrin is present when there are no blood-corpuscles, and the urine may coagulate spontaneously on standing, or throw down a sticky sediment.

Detection.—It is important to distinguish between fibrin and large amounts of pus forming a gelatinous, sticky mass. In the latter case, the addition of water thins the urine and the addition of an alkali forms a white precipitate of alkaline albuminate. To test a fibrin coagulum it should be separated by filtration and washed with water. The residue should show the chemic characteristics al-

ready detailed. A part of the mass may be treated with a dilute solution of sodium hydroxid. If it is insoluble on standing for a long while, it is probably fibrin, since albuminous bodies dissolve in this solution. A fibrin clot, however, dissolves completely if warmed gently for several hours on a water-bath with a 1 per cent. solution of sodium carbonate, This solution is filtered and treated with Millon's reagent, which gives a deep-red color.

Millon's Reaction.—One part of metallic mercury is dissolved in 2 parts of nitric acid, evaporated to ½ volume, and 1½ parts of water are added. Allow to stand for twenty-four hours and decant. A drop of this reagent produces a precipitate which turns red on heating, or a red color on heating if the proteid is present in small amount.

ALBUMOSES

By "albumoses" are meant substances derived from the native albumins (serum-albumin, globulin), which constitute the first stage in the transformation of native proteid substances in the process of digestion with ferments (pepsin, trypsin). Albumoses may, however, also be derived from albumins or globulins under the influence of acids or alkalis, or in virtue of metabolic changes in tissue proteids (Cohnheim).

The albumoses, as a class, possess certain definite characteristics which distinguish them from the proteids thus far discussed. While they give the general reactions of the proteids, they are uncoagulable by heat and behave differently toward precipitating reagents than do albumin and globulin. They are precipitated with nitric acid and with potassium ferrocyanid and acetic acid, but the precipitates again dissolve on heating and reappear on cooling.

They are precipitated with phosphotungstic and tannic acids and respond to the biuret test.

A great deal of confusion existed formerly between albumoses and the so-called "peptones." Modern biologic chemistry accepts Kühne's definition of a peptone as a proteid body which cannot be precipitated by "salting out," *i. e.*, by complete saturation of the solution with ammonium sulphate. The peptones are the last stage of evolution of native proteids (albumin, globulin, etc.) in the process of digestion. When peptones are split up their products of decomposition no longer give any of the characteristic proteid reactions, but are substances of totally different compositions, *e. g.*, the amino-acids, etc. Judged by this definition of these bodies, peptones are not present as such in the urine, and the term "peptonuria," formerly used so frequently, has been changed to "albumosuria."[1]

The *definition of albumoses*, therefore, places them between serum-albumin and serum-globulin on the one hand and peptone on the other, in the sequence of proteolysis. Albumoses are soluble products of native albumins that do not coagulate with heat, and that are still precipitated by saturating with certain salts (ammonium sulphate, zinc sulphate in acid solutions). Peptones are not coagulated by heat, but no longer are precipitated by saturation with the salts mentioned.

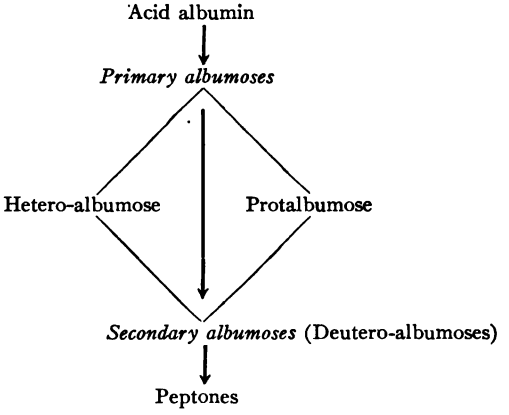
Presence of Albumoses in the Body-fluids.—It was formerly thought that albumoses (and peptones) exist normally in the different

[1] True peptone, however, occurs occasionally in the urine, *e. g.*, in lobar pneumonia, pulmonary tuberculosis, gastric ulcer, and during the puerperal period (Neumeister, Ito, Emerson). Furthermore, the pepsin which is always present in the urine digests albumoses into peptones (Neumeister), and these have been erroneously assumed to have been directly excreted.

tissues and fluids of the body. Neumeister, in 1888, however, showed that this is not true, and that albumoses and peptones had been formed artificially in the process of chemic analysis. He found that *normally no albumoses nor peptones exist* (in any appreciable amounts) anywhere in the body save in the digestive tract. The albumoses and peptones of the food are retransformed into albumins and globulins in the intestinal wall (Hofmeister). Pathologically albumoses are formed in the body in the course of pus formation and in the absorption of exudates. Buchner accounts for this formation of albumose by the action of peptic or tryptic ferments secreted by leukocytes or other body cells. In fevers albumose has been found in increasing amounts in the blood (Krehl), and an albumose is probably the cause of the febrile reaction of Koch's tuberculin. Albumoses occur pathologically in the urine in similar conditions as those just mentioned (Cohnheim).

Classification of Albumoses.—This is quite complex, but for our purposes the following explanation, containing the essential facts to be remembered in this connection, will suffice.

The scheme of classification for the purposes of urine analysis may be thus arranged:



Reading from above downward we note the successive stages in the transformation of acid albumin into peptone, and thus perceive the relative position of the albumoses.

The first step in the formation of albumoses from acid albumin is represented by the *primary albumoses*. These are precipitated by complete saturation of their solution with NaCl.

The *primary albumoses* are subdivided (Kühne) into: (*a*) The protalbumoses, which are precipitated by saturation with NaCl only from *neutral* solutions, and are soluble in water; and (*b*) the hetero-albumoses, which are completely precipitated by saturation with NaCl only from *acid* solutions, and are insoluble in water, but soluble in weaker salt solutions.

The *secondary albumoses* or *deutero-albumoses* are derived from the primary albumoses by further digestion (also artificially by boiling with acids or with superheated steam). These are precipitated by NaCl with acetic acid or by ammonium sulphate, most of them being thus affected in neutral, a few in acid or alkaline, solutions.

Secondary Albumoses (Deutero-albumoses).—These will be considered first, as they are by far the most frequent form of albumoses found in the urine (Hofmeister). In fact, if primary albumoses occur more frequently than is now supposed, they are changed rapidly to secondary albumoses by the pepsin always present in the urine.

Clinical Meaning.—Secondary albumoses occur in a great variety of conditions, but almost always associated with fever or with *breaking down of tissue elements, the absorption of exudates* or of toxins. Secondary albumose is present in about 90 per cent. of all febrile diseases, particularly in cases with an exudative inflammation (pneumonia, pleurisy), in suppurative conditions, such as abscess, appendicitis, empyema, osteomyelitis. It is also noted in infections (rheumatism, septicemia, typhoid, tuberculosis, [cavities, fever], erysipelas, measles, scarlet fever, smallpox), particularly when the toxins are being eliminated rapidly and the temperature falls (Emerson). Gangrene, cancer, and other conditions of direct tissue breakdown cause it. In pregnancy and in the puerperium albumosuria may indicate absorption of toxins.

The *destruction of many red blood-cells* may give rise to albumosuria, as in the absoption of large hemorrhages, in purpura, scurvy, leukemia, etc. In ulcers of the in-

testine (tuberculosis, typhoid) or of the stomach albumosuria may be present as the result of direct absorption of albumose from food into blood. Albumosuria also occurs in *diseases of the liver* (acute yellow atrophy, cirrhosis, catarrhal jaundice, etc.).

An important form of albumosuria is associated with *nephritis*, where it may occur with albuminuria. Albumosuria may be the first sign of an impending nephritis, or may remain as a vestige of a nephritis which has healed. It is especially marked in syphilitic nephritis.

Diagnostic Value.—The detection of albumose—and by albumose in the urine we mean secondary albumose (deutero-albumose), unless otherwise stated—is not of very great practical value. This body occurs in so many conditions that its presence cannot be regarded as especially characteristic of any disease. It does not usually occur in large amounts; on the contrary, usually in small quantities, and before we conclude anything as to its meaning we must eliminate three sources of error (Emerson):

1. The admixture of albumose to the urine from the genital or genito-urinary tract (spermatorrhea, prostatitis, postcoital urine).

2. The formation of albumose from albumin in the process of testing the urine.

3. The possibility of direct excretion of albumose from the food (milk) in nephritis.

These sources of error being excluded, a marked albumosuria may be of value in the diagnosis of abscesses (appendicitis) in inaccessible parts of the body; in the differentiation of cerebrospinal meningitis from tuberculous meningitis; and of gastric or intestinal ulcer from other conditions (excluding cancer) which may simulate these.

Detection of Secondary Albumoses.—1. *Hofmeister's Test.*—One-fifth volume of concentrated acetic acid is added to the urine. To the mixture phosphotungstic acid is added, and the whole is allowed to stand for half an hour. If no cloudiness appears, secondary albumoses are absent. If they are present, a milky opacity appears within a few minutes.

2. *Biuret Test.*—This test is universally used, but has the disadvantage of being obscured by the color of the urine if used directly upon urine. It consists in rendering the urine alkaline with an excess of NaOH and adding dilute copper sulphate (5 cc. saturated solution to 1000 cc. water) or by pouring the dilute copper solution upon the surface of the urine. In the former case the urine will turn reddish brown, in the latter a ring of the same color will appear.

The biuret test should be performed with the precipitated (or isolated) albumose. For this purpose the albumose may be precipitated with phosphotungstic acid (see Hofmeister's test), the sediment centrifuged and dissolved in a small amount of water. The biuret test will then give a characteristic *violet* color.

3. *Hammarsten-Bang Method.*—This is the best and most accurate method for the isolation and testing of albumose. Its object is to remove the urobilin which interferes with the final biuret reaction: Ten parts of urine are thoroughly mixed with 8 parts of saturated solution of ammonium sulphate. The mixture is heated to the boiling-point and kept there for a few seconds. While hot it is then centrifuged, until fairly solid sediment is formed, and the supernatant fluid decanted or pipeted off. The precipitate is shaken with alcohol (to remove urobilin), the residue is mixed with water, and the mixture heated to the boiling-point and filtered to remove albumin. The filtrate is shaken with chloroform (to remove any possible traces of urobilin) and the watery portion is tested with the biuret reaction, which should give a characteristic violet color.

Primary Albumoses (Bence-Jones' Body).—Primary albumoses in the urine are exceedingly rare. Practically the only form to be considered is a type of proteid excretion known by its discoverer's name—*Bence-Jones'* albumosuria.

Bence-Jones' Body.—This was classified as a primary albumose (a hetero-albumose), but recent researches have thrown considerable doubt as to the real character of the

so-called Bence-Jones' body. Some authors (Dechaume) consider it to be a mixture of several albumoses. For the present, for want of a better place, we continue to classify it under the primary albumoses, chiefly to distinguish it sharply from the secondary albumoses that are ordinarily found in the urine.

Clinical Significance.—Bence-Jones' body occurs very rarely in urine. It seems to be derived from the bone-marrow. There are at present not over 35 cases on record (Boston). It has been found in cases with multiple myeloma, with severe involvement of the bone-marrow, and in one case of lymphatic leukemia. The cases in which Bence-Jones' body was found were usually fatal in from one to two and a half years and ran a rather acute course. Males were found affected in 80 per cent. of cases. In 15 per cent. there was a history of severe traumatism to the bones or joints. appears in osteo-malacia.

The amount of this body in the urine in these cases ran as high as 7 per cent., but usually it was below 1 per cent. In some cases (Boston) the excretion was intermittent.

Detection.—1. *Acetic Acid Test.*—The urine is rendered acid with acetic acid. From 10 to 40 cc. are placed in a beaker and heated gently on a water-bath, noting the temperature. A slight cloud appears at 52° C.; a marked turbidity at 54° to 56° C.; a dense cloud at 56° to 60° C. As the urine approaches 90° C. a tough sticky coagulum forms, but when the urine is boiled for five minutes the coagulum dissolves and *reappears on cooling* (Boston).

A simpler way to perform this test is with a test-tube and a small flame. Warming causes the slight opacity; heating nearly to boiling, the precipitate, which redissolves on prolonging the boiling and reappears on cooling.

2. *Nitric Acid Test.*—To the urine nitric acid is added, drop by drop, shaking after each addition. A pinkish tint appears in the heated portion.

3. *Boston's Method.*—From 15 to 20 cc. of urine in a test-tube are mixed with an equal amount of saturated NaCl and well shaken. To this are added 3 cc. of 30 per cent. NaOH and the mixture shaken thoroughly. The upper portion of the urine is heated to boiling and 10 per cent. lead acetate solution is added, drop by drop, heating after each drop. A brown color, turning black, is obtained within one minute, and is due to lead sulphid, the sulphur being derived from that loosely bound in the albumose.

MUCIN (MOERNER'S MUCOID)

The mucins are proteid bodies which occur in the secretion of mucous glands, *e. g.*, in the saliva, the bronchial secretion, the intestines, the bile, and the secretions of the genito-urinary tract. Mucins are soluble in water, and their solutions when concentrated are syrupy, slimy, and viscous, and by addition of acetic acid are converted into thick, tenacious, gelatinous masses.

On adding acetic acid to dilute solutions of mucin a thick, stringy deposit is formed, not a flocculent precipitate. An excess of acetic acid does not redissolve mucin precipitates, or does so only with difficulty (Cohnheim). Mucins are not precipitated by boiling, and the addition of neutral salts (sodium chlorid) keeps mucin in solution even after acetic acid is added. (This addition of neutral salts serves to keep mucin in solution when heat and acetic acid are used in testing for albumin, in order to avoid confusion between mucin and albumin.)

Chemically, mucin belongs to that class of proteids known as the "glycoproteids" because it is a compound of a proteid molecule with a molecule resembling a carbohydrate (a glucosamin) and capable of reducing Fehling's solution.

Mucoid.—K. Moerner[1] found that the normal urine

[1] Skand. Arch. f. Physiol., 1895.

contains small quantities of a substance similar to mucin, which he classed as a "mucoid." The latter term was applied by Hammarsten to substances sharing some of the properties of mucins, but differing from them in other particulars.[1] Moerner's mucoid differs from true mucin in that *it dissolves fairly readily in an excess of acetic acid.* On boiling with acids or alkalis it is easily changed to an acid or alkali albumin, and, like mucin, contains a carbohydrate molecule.

Moerner's researches showed that the "nubecula" of normal urine, and also to a lesser degree the fluid itself, contained small amounts of "mucoid." In 260 liters of urine he was able to isolate but 4.3 gm. of this mucoid. This substance cannot be precipitated from urine with acetic acid under ordinary conditions, because it is held in solution by the urinary neutral salts. Moerner was able to obtain his mucoid only after treating the urine with acetic acid and extracting with chloroform.

Clinical Significance of Mucin or Mucoid.—For the present the terms "mucin" and "mucoid" may be used without distinction, preference being given to "mucoid," because in all probability all mucin in the urine is in the form described by Moerner, although this remains to be shown definitely. In normal urines small traces of mucoid occur which are not discernible clinically except by such methods as Moerner used. Ordinary tests do not reveal normal mucoid. In pathologic conditions (catarrhal inflammation) involving a hypersecretion of mucus anywhere along

[1] The line of demarcation between mucin and mucoid is, for the present, somewhat vague, but the term mucin is preferably applied to the proteid in the secretion of mucous glands, while the term mucoid is applied to substances resembling mucin which may occur in other parts of the body.

the urinary or genito-urinary tract an increase of mucin in the urine is to be expected. In marked cases the mucus appears as an increased form of the normal cloud or nubecula, floating in the urine or slowly sinking as a stringy mass. In less marked cases the mucus may be in solution and chemical tests will show its presence.

Detection.—The urine should be diluted with water to diminish the solvent action of its salts on mucin or mucoid, and acetic acid should be added in the cold, care being taken not to add an excess. The precipitate may be treated with a dilute mineral acid and the product tested with Fehling's solution, whereupon a reduction of copper will show that the precipitate was mucin or mucoid, while the absence of a reduction will exclude the precipitate from this class of proteids, and will point to their probable membership in the class to be spoken of next.

SUBSTANCES FORMERLY TAKEN FOR "NUCLEO-ALBUMIN"

So-called Nucleo-albumin (Moerner's Body, Euglobulin).—For many years it was believed that "nucleo-albumin" occurs normally in urine and is found very frequently also in disease. The fainter ring which appears at some distance *above* the true albumin ring in Heller's nitric acid test was regarded as due to "nucleo-albumin," as was also the precipitate obtained with diluted urine in the cold upon addition of acetic acid. The ring above the contact surface in Heller's test can be obtained in a very large proportion of urines, especially if these be diluted. The opalescence or precipitate with acetic acid in the cold due to the same body is not easily soluble in an excess of acetic acid (contrast with "mucoid").

The controversy as to the true nature of the proteid substance which gives rise to these phenomena in so many urines has been a long one, and various investigators have given different names to the substance thus detected. For example, Reissner called it "soluble mucin"; Hofmeister regarded it as a "mucin-like body"; F. Mueller, as a "globulin"; Huppert, as a "nucleo-albumin or a mucin-like body"; Obermeyer, as a "nucleo-albumin." Obermeyer's work had an important bearing on the question, as he found this body in 32 cases of jaundice, increasing with the intensity of this symptom, and called it a nucleo-albumin; henceforth precipitates with acetic acid in the cold were regarded as nucleo-albumin. The researches of Moerner and those of Matsumoto, however, have placed the whole matter in a different light:

(*a*) *Moerner's Work.*—K. Moerner,[1] in the memoir (already quoted on p. 87) in which he described urinary mucoid, also presented the results of very careful experiments with normal urines which were intended to solve the question as to the nature of the substance which had hitherto been called "neucleo-albumin," and which had so frequently responded to the tests mentioned in the preceding paragraph.

Moerner found that the *so-called* "nucleo-albumin" was in reality a compound of serum-albumin and of one or more of three organic acids which occur in the urine—chondroitin-sulphuric acid, nucleinic acid, and taurocholic acid. The first is always present in the urine, the two last mentioned, occasionally. The addition of acetic acid gives rise to a combination of the serum-albumin with the organic acids mentioned and a precipitate results. The precipitating acids are present in the urine in excess, but in variable amounts. The greater the amount of these precipitating bodies, the closer the resulting precipitate resembles nucleo-albumin, and this accounts for the fact that it had been so long taken for nucleo-albumin.

Moerner found his compound in a large number of normal urines, but as he worked with very large amounts of urine and found only small quantities, some of the workers who followed him failed to obtain his

[1] Skand. Arch. f. Phys., 6, 1895, p. 332.

results. Therefore, while Moerner's work is of great importance, there is some doubt as to the acceptance of his explanation of the precipitate obtained with acetic acid in the manner mentioned.

(*b*) *Matsumoto's and Oswald's Work* (1903–04).—These observers regard the body precipitated by acetic acid and giving the superimposed ring with Heller's test as a compound of euglobulin and fibrinogen (fibrinoglobulin). As such it is probably present in all normal urines in traces, and is increased in such conditions as cyclic and orthostatic albuminuria and in nephritis.

Clinical Significance.—Whether the proteid substance which reacts to these tests and is so often found normally is Moerner's body or whether it is a euglobulin, therefore, is not yet settled. The important facts to remember are that (1) *Small traces of a proteid the true nature of which is not yet known definitely may occur normally in the urine;* (2) *that the term "nucleo-albumin" should not be carelessly applied to this proteid;* (3) that this proteid is increased in non-nephritic albuminurias (cyclic, orthostatic), and may occur in increased amounts as a premonitory sign of impending nephritis. It is also increased in leukemia (Mueller), in jaundice (Obermeyer), in acute infections and fevers (typhoid, pneumonia, meningitis, erysipelas).

Detection of "Moerner's Body" or Euglobulin.—*Heller's test* is the most satisfactory clinical method for detecting this proteid. The ring appears about half a centimeter above surface of contact, but it may diffuse and merge with the acid on standing. Dilute urines give better results with this test. The urate ring should be excluded by gently heating or by still further diluting the urine.

Citric Acid Method.—L. Grimbert and A. Dufau[1] ap-

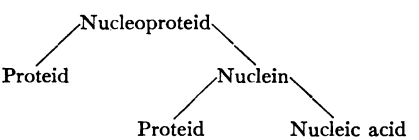
[1] Journ. de Pharm. et de Chimie, vol. xxiv, 1906, p. 193.

plied the method of Lecorché and Talamon[1] to the detection of Moerner's body. A solution of 100 gm. citric acid is made in 75 cc. of distilled water. The fluid is cooled. A small quantity of the solution is poured into a test-tube and the filtered urine layered over it. Moerner's body gives a white, nebulous ring at the zone of contact, and sometimes the cloudiness permeates the superimposed urine. Pathologic albuminuria (serum-albumin and globulin) give no ring or cloud with citric acid. This test is useful when Heller's test gives a ring which is doubtful as regards its origin from true albumin or from Moerner's body.

NUCLEOPROTEID

In the preceding section we dealt with a proteid body, hitherto *falsely* called "nucleo-albumin." We must now, for the sake of completeness, consider briefly the occurrence of *true nucleoproteid* in the urine. We use the term nucleoproteid as the more accurate term, in place of nucleo-albumin, for the two are not synonymous.

Definitions.—A *nucleoproteid* is a compound of nuclein with some proteid matter. A nuclein, in turn, is a combination of some form of proteid matter with nucleic acid (Chittenden). The following schema from Cohnheim shows this:



Nucleoproteids when digested with pepsin yield nuclein,

[1] Traité de l'Albuminurie et du Mal de Bright, Paris, O. Doin, 1888, p. 82.

and when boiled with dilute mineral acids yield purin bases (see below).

Nucleo-albumins yield, not nuclein, but pseudonuclein on digestion, and do not yield purin bases on cleavage with dilute acids (Hammarsten).

Clinical Significance.—Nucleoproteids do not occur normally in the urine. (The substances formerly taken for them have been considered on p. 89.) In certain pathologic conditions associated presumably with destruction of cell-nuclei in the kidney or elsewhere nucleoproteids have been found in the urine.

The presence of nucleoproteid in the urine may be regarded, for the present, an evidence of cell breakdown, either in the kidneys (acute nephritis, renal degeneration due to poisons, renal stasis) or along the urinary tract (inflammatory or suppurative desquamation or degeneration of epithelia and leukocytes, *e. g.*, in pyelitis). Unfortunately, however, we are still in the dark as to the exact relation of nucleoproteid to serum-albumin and globulin, and in the few cases in which thorough investigations were made an increase of nucleoproteid has not always been found when it should have been expected theoretically, *e. g.*, in the presence of certain suppurative conditions.

Detection.—The difficulty lies in the detection and identification of nucleoproteids. The acetic acid precipitate of nucleoproteid is insoluble in an excess of this acid. Magnesium sulphate added to urine to saturation also throws down the nucleoproteids. On boiling the precipitate, after treating it with dilute acids, *no reducing substance* is obtained which affects Fehling's solution (distinction from mucin). Nucleoproteids contain phosphorus, and in order to prove the precipitate to be such we must demon-

strate the presence of phosphorus. Furthermore, these products when digested with pepsin must yield nuclein. These tests are not only impossible of execution in a clinical laboratory but also are extremely difficult to perform in the urine, even for an expert chemist (Emerson).

QUESTIONS ON CHAPTERS IV AND V

Albumin.—What is usually meant by albuminuria? Can normal urine contain albumin? What proteids are present in normal urine constantly? What are the physical conditions for the excretion of albumin? What three factors determine albuminuria? What is meant clinically by "albuminuria"? What do we mean by "false albuminuria"? By true or renal albuminuria? How may renal albuminuria be subdivided? Define: Functional albuminuria; physiologic albuminuria; essential albuminuria; cyclic, orthostatic; albuminuria minima. Traumatic, hematogenous, nervous, circulatory, toxic nephritic albuminuria.

What amounts of albumin are usually excreted in (*a*) acute congestion; (*b*) acute, subacute, and chronic parenchymatous; (*c*) chronic interstitial nephritis; (*d*) amyloid kidney?

Describe the heat test with nitric acid. How is the cloudiness differentiated from that due to phosphates? What are the sources of error in this test? Describe the heat test with acetic acid. How do you distinguish nucleo-albumin in this test?

Describe Heller's test. What are the different methods of performing it? What precaution should be taken in testing with this method? What is the value of the other tests for albumin in practical work?

What does picric acid precipitate besides albumin? Describe the tests with potassium ferrocyanid and nitric magnesium. What is the comparative delicacy of these tests?

What is the most accurate method of determining the *amount of albumin* in the urine? What other methods may be used for this purpose? What is the value of the method by "bulk percentage"? Describe Esbach's method; Tsuchiya's method; Purdy's centrifugal method; Vassilieff's method of titration.

What chemic substances do we mean by "albumin" in the urine? In what conditions is it useful to differentiate *globulin?* Describe Roberts' simple method of testing for globulin.

Describe *fibrin* and its chemic characters. What does its presence in the urine indicate? How is it distinguished from pus precipitate?

What are *albumoses?* In what conditions have albumoses been found in the urine? Secondary albumoses? What are the chief chemic features of these two classes of bodies? What is Bence-Jones' body and how is it detected?

Define *mucin.* What is mucoid? What is the clinical significance of this body? How is it detected?

What substances were *formerly called nucleo-albumins?* How do they show in Heller's test? What is *nucleoproteid?* When does it occur in urine? How is it detected?

II. CARBOHYDRATES[1]

CHAPTER VI

GLUCOSE

($C_6H_{12}O_6$)

THE question as to whether glucose is present in normal urine has not been finally settled, although the latest researches of Wedenski apparently affirm this statement. For clinical purposes this question is not of importance, as the quantities of sugar that may be present in normal urine are so exceedingly small that they cannot be recognized by the ordinary tests such as are employed in routine examinations. Glucose is present in small quantities in normal blood, and this amount becomes greatly increased in some diseases, reaching its highest point—about 0.1 per cent.—in advanced diabetes.

Clinical Significance.—The presence of glucose in the urine is no more synonymous with diabetes than the presence of albumin is with Bright's disease, but diabetes is the condition which most commonly causes glycosuria. Diabetes is now assumed to be a disease of metabolism which prevents the conversion of the carbohydrates into their simpler elements. The glucose in the urine of diabetics is derived either from the food or, in severe cases, from the

[1] A number of carbohydrates are met with in the urine, including glucose, levulose, lactose, etc. The general properties of carbohydrates should be known to the student or may be studied in books on general chemistry. As in the case of proteids, there is one carbohydrate which is of great importance clinically, namely, glucose.

tissues. It does not originate only from the carbohydrates ingested, for some proteids may become split up, liberating a carbohydrate, *e. g.*, nucleins and mucin, which contain 30 per cent. each of a carbohydrate group. Glucose may also be formed synthetically from the decomposition product of proteids. Von Noorden and Rumpf found that sugar (glycogen) could be formed by the decomposition of fats. It is probable, therefore, that in advanced cases of diabetes with wasting, glucose is derived from several classes of tissue products, chiefly, however, from the proteids. The amount of glucose in the urine in diabetes mellitus varies from 0.5 to 12 per cent. (see p. 380).

Besides diabetes, a series of other causes may produce glycosuria: Thus, *alimentary glycosuria* is due to an excess of carbohydrates in the food; *medicinal glycosuria* is due to drugs, such as chloroform, amyl nitrite, phloridzin, the inhalation of illuminating gas, etc.; *secondary glycosuria* accompanies cirrhosis of the liver, severe injuries of the brain, apoplexy, paresis, and other nervous diseases; exophthalmic goiter, the removal of the thyroid, or the use of large doses of thyroid extract; a new growth in the pancreas or the occlusion of the pancreatic duct by a stone, followed by atrophy of the gland. Glycosuria may also occur during pregnancy, in the course of acute infectious diseases, such as diphtheria, and just before death in cases of chronic nephritis, possibly as the result of edema of the brain.

DETECTION OF SUGAR IN THE URINE

The Copper Tests.—These tests are most commonly used for the detection of glucose in the urine. They all depend upon the power of glucose to reduce copper oxid in an alkaline solution.

Trommer's Test.—This is the oldest of this group of tests, and while there are better methods for clinical use, the student should practice with Trommer's in order to familiarize himself thoroughly with the process of copper reduction in the urine. Emerson has lately emphasized the great importance of this test:

A test-tube is filled half full of urine, and about one-third volume of 10 per cent. potassium hydrate solution is added. To this mixture a 10 per cent. solution of copper sulphate is added, drop by drop, shaking after each addition until the copper hydrate which forms as the solution is added no longer dissolves. The upper portion of the mixture is now heated, and if sugar is present, a yellow or red precipitate will at once appear (copper suboxid). The heating should immediately be stopped and the tube allowed to stand, when the precipitate will spread downward.

The urine should be examined while fresh, and if there is much albumin, it should be previously removed (see p. 76). If the reaction is at all doubtful, it is better to perform the test without heating, and to allow the tube to stand for a day, when the precipitate of cuprous oxid will be found present. According to Neubauer, most of the other organic substances which reduce the salts of copper do so only when heated.

The explanation of the reaction is as follows (Emerson):

"If to pure water be added KOH and then the $CuSO_4$, the first drop of the latter will cause a precipitate of $Cu(OH_2)$ ($CuSO_4 + 2NaOH = Na_2SO_4 + Cu(OH)_2$). These flakes of $Cu(OH_2)$, on heating, will blacken, since $Cu(OH)_2 2CuO$ is formed. If the glycerin or the tartrates be added to the water, all of the $Cu(OH)_2$ is dissolved to a blue solution, which will not blacken on heating, as it does if undissolved. If, instead of these, glucose be added to the water, the same blue solution of the $Cu(OH)_2$ is obtained. This, however, on warming is reduced, and a

yellow or red precipitate falls. In the case of glucose the body giving the bright blue solution is $C_6H_{12}O_{65}Cu(OH)_2$.

"In performing the test it is particularly important that an excess of copper should not be added, since the black oxid will cover the precipitate of the cuprous salts. Normally, 3 to 5 drops of the $CuSO_4$ are sufficient to give a blue precipitate. In case sugar is present, however, the addition must continue until the first flakes of $Cu(OH)_2$ remain. The test is positive only when a yellow or red precipitate falls, yellow ($Cu_2(OH)_2$) in a relatively weak alkaline, red (Cu_2O) in a strongly alkaline, solution. In case under 0.2 per cent. sugar is present there will be no precipitate, and yet even then the test may be very suggestive, since the yellow solution will be of such a clear brilliant color. Again, the precipitate should occur under the boiling-point or when the urine is just brought to that point to exclude the reduction by those bodies normally present."

Precautions to Be Taken in All Copper Tests.—Certain precautions must be taken in all copper tests. These may be summarized as follows:

Albumin interferes with the reduction of the copper oxid and must always be removed if present in more than a faint trace. Concentrated urines, with high specific gravity, and especially urines with an excess of coloring-matters, urea, uric acid, and other nitrogenous bodies, must be diluted before testing with copper solution. An excess of copper sulphate or too strong solutions should never be used, and all copper tests are preferably to be performed with dilute reagents. The rules given in this respect under the different tests should be carefully observed.

Prolonged boiling should be avoided, thirty seconds being ample in the great majority of cases, for prolonged boiling reduces copper when other organic substances than sugar are present. A positive reaction is constituted by the precipitation of a yellow orange or red, heavy, dense precipitate, which spreads through the test-tube on standing, and eventually falls to the bottom. An amorphous, flocculent precipitate, pale grayish in color, is due to earthy phosphates. A flocculent precipitate may also be due to albumin or other proteids.

A change of color does not constitute a reaction, although in the presence of very faint traces of sugar, less than 0.1 per cent., the contents of the tube changes to a yellowish or greenish color. In these cases confirmatory tests are necessary if there is any doubt as to the cause of the change of color. In the majority of cases a change of color due to other substances than sugar can be avoided by using sufficiently dilute urine and by avoiding

excess of copper sulphate and prolonged heating. When these factors are disregarded, a change of color is produced with any of the copper tests, from blue to various tints of green, brown, and yellow, and in these cases is due to other organic compounds in urine.

It is well to remember that the compounds which may reduce copper besides carbohydrates in the urine are as follows: Uric acid, creatinin, hippuric acid, urate, hypoxanthin, mucus, indican, and the series of drugs which increase glycuronic acid. The latter are principally chloral hydrate, chloroform, camphor, menthol, morphin, phenol, resorcin, and thymol. Drugs which give rise to the elimination of pyrocatechin, hydroquinon (these urines are light colored when passed, but become dark on standing, or on the addition of KOH). Furthermore, a positive reaction may occur in some cases after the use of salol, salicylic acid, benzoic acid, phenacetin, sulphonal, rhubarb, santonin, crysophanic acid, oxalic acid, glycerin, copaiba, and other resinous drugs. It is to be noted, furthermore, that the use of saccharin in considerable amounts may interfere with the reduction of copper, a point of value in considering minimal glycosurias in patients who are on a sugar-free diet, but who use saccharin for sweetening their food.

The invariable rule to follow in testing for sugar by any of the copper methods is that a confirmatory test, preferably the bismuth test (Nylander's), or in case of further doubt, the phenylhydrazin test, or, finally, the fermentation test, should be employed in all cases in which there is any doubt as to the positiveness of the copper reaction. Patients under observation for glycosuria should, moreover, be kept free from any drugs which may influence the reaction for several days before the test is applied.

Fehling's Test.—This is by far the most popular test for sugar in the urine. It has the advantage of being easily applied and of being sufficiently delicate for clinical purposes. Its principle is that of copper reduction, but in order that the solution may hold dissolved as much copper as possible there is added to it sodium potassium tartrate (Rochelle salt). The addition of a tartrate, or of glycerin, or ammonia is, indeed, employed in several other copper tests (Purdy, Haines, Pavy) for the same purpose.

Fehling's solution is composed of two reagents which

must be kept separate in a dark place, in glass-stoppered bottles, the stoppers of which should be greased with a mixture of vaselin and paraffin to prevent adhesion to the neck of the bottle. The formulas for these solutions, according to the researches of Marshall, are as follows:

1. *Copper solution:*
   Copper sulphate.....34.652 gm.
   Distilled water, enough to make.....500 cc.

2. *Alkaline solution:*
   Sodium-potassium tartrate (Rochelle salt).....173 gm.
   Sodium hydrate solution (specific gravity 1.140)[1]..500 cc.

Ten cc. of the combined reagent (5 cc. of each reagent) require 0.05 gm. (50 mg.) of glucose to complete the reduction in this amount of solution. In other words, each cubic centimeter of the combined reagent corresponds to 0.005 (5 mg.) of glucose.

The calculations of Marshall, upon which the formula given above is based, showed that 5 molecules of crystallized cupric sulphate are reduced to cuprous oxid by 1 molecule of glucose, thus:

$$C_6H_{12}O_6 : 5CuSO_4 + 5H_2O :: \text{glucose} : \text{cupric sulphate}$$
$$180 : 1247.5 :: 5 \text{ gm.} : 34.652 \text{ gm.}$$

In other words, 34.652 gm. of cupric sulphate will be reduced by 5 gm. of glucose.

Method of Testing with Fehling's Solution.—Equal parts of each of the solutions should be diluted with about four times the amount of water, and the mixture should be boiled for a few seconds. The mixed reagent keeps for a day only. The separate reagents keep longer, but the preliminary

[1] The sodium hydrate solution, specific gravity 1.140, contains 77.0 gm. of caustic soda and enough distilled water to make 500 cc.

boiling of the diluted mixture should never be neglected as a test for the stability of the solution. If the solution remains clear on boiling, the reagents are to be regarded as fit for use. If cloudiness or a precipitate occurs, the reagents should be rejected and fresh solutions procured.

To the diluted mixed reagents add the suspected urine, drop by drop, boiling the mixture at the top of the tube only for a second or two after the addition of each drop. If sugar is present in considerable amounts, the first two drops will cause a heavy precipitate at the upper part of the fluid. If no precipitate occurs, urine may be added, drop by drop, boiling for a second after each drop, until we are satisfied that no reaction takes place.

A positive reaction is characterized by heavy, finely powdered, yellow, orange or red precipitate of copper suboxid. If the precipitate does not appear until five minutes or longer after the addition of the urine, the quantity of sugar is very small, less than 0.5 per cent. If no precipitate occurs within thirty minutes on standing, allow the test-tube to stand for a day, as traces of sugar may show after a few hours.

It cannot be emphasized too strongly that the use of insufficiently dilute Fehling's solution, the use of concentrated urine without previous dilutions, and prolonged boiling after the addition of the urine must be avoided in order to get accurate results with this test. All the precautions already spoken of above as applying to the copper tests in general must be observed in working with Fehling's test. In doubtful cases confirmatory tests, which will be considered further on, are necessary. The value of Fehling's test as a routine method is universally acknowledged, but it is trustworthy only if properly performed.

Pavy's Test.—The solution used in this test consists of:

Cupric sulphate	320 grains
Neutral potassic tartrate	640 "
Caustic potash	1280 "
Distilled water	20 fluidounces

This solution is made and used in the same way as Fehling's, but the proportions of copper in it are calculated on the assumption that the formula for grape-sugar is $C_6H_{14}O_6$ instead of $C_6H_{12}O_6$, as Fehling had it. Therefore, in Pavy's solution 100 minims correspond to 0.5 grain of glucose.

Haines' Test.—This is a modification of Fehling's. It is claimed that the solution prepared according to Haines' formula is stable and keeps indefinitely, while the ordinary Fehling's solution does not. In the author's experience, Haines' solution keeps well for several weeks if kept carefully stoppered in a dark place, but there is a tendency to deposit a film of copper upon the walls of the reagent bottle, as shown by a reddish color visible when the bottle full of reagent is looked at by reflected light. Later, a deposit of copper may appear at the bottom of the bottle. Haines' solution keeps far better than Fehling's when the latter is mixed, yet the former is not indefinitely stable. For qualitative tests Haines' solution is available even when there is a slight film of copper upon the walls of the bottle, but the solution should not be kept longer than needful.

The formula for Haines' solution is as follows:

Copper sulphate	30 grains
Distilled water	½ ounce
Pure glycerin	½ "
Potassium hydrate solution	5 ounces

The copper is dissolved in the water, the glycerin is mixed

thoroughly, and the potash solution is added. One part of this solution is diluted with 3 parts of water in a test-tube and the reagent is boiled. Six or 8 drops (not more) of the suspected urine are added and the mixture is boiled for a few seconds. If sugar is present, a yellow or yellowish-red precipitate appears.

The author's experience with this test has been very satisfactory and it may be recommended as an excellent routine test for practitioners. Its advantages are that it is effective with small amounts of urine and that it does not quite so readily cause confusion in the presence of other copper-reducing substances as does Fehling's solution. A worker of experience, however, will prefer Fehling's method.

The Bismuth Tests.—These depend upon the power of glucose to reduce the salts of bismuth, giving a black precipitate of the metallic base.

1. **Nylander's Test.**—This is one of the most valuable tests for glucose, especially as a confirmatory method in cases of doubtful reactions with the copper methods. The Nylander test, when negative, is conclusive proof of the absence of sugar. When positive, however, it should be confirmed by further tests.

The reagent consists of 4 gm. of Rochelle salt, dissolved in 100 cc. of a 10 per cent. solution of sodium hydrate. The solution is warmed and 2 gm. of bismuth subnitrate are added. One part of this fluid is added to 10 parts of urine and the mixture is heated. In from three to five minutes it turns black if sugar is present. The reaction demonstrates the presence of 0.1 per cent. of sugar.

Precautions.—The reagent should be perfectly clear (filtered when necessary) and should be kept in a dark bottle. The fluid in the test-

tube may be boiled vigorously, and if more than 0.1 per cent. of sugar is present the fluid will turn black *at once* or *soon after boiling* (formation of bismuth suboxid). When traces of sugar are present, the fluid will turn brown.

Reduction of bismuth *after the fluid has cooled* is not due to sugar. When no sugar is present there may be a white precipitate due to phosphates. Albumin may cause a red precipitate, and if present in excess may give rise to a black deposit which may be mistaken for that due to sugar. A positive reaction, which is usually partial, however, may occur in the presence of an excess of urinary coloring-matters, indican, glycuronic acid, hydrogen sulphid, and ammonium carbonate. Dark-colored urine should, therefore, be diluted, while decomposing urines (ammoniacal cystitis, pyelitis) should not be tested with this method. Practically the same drugs as interfere with Fehling's and other copper tests cause a reduction with Nylander's solution. In case of doubt, therefore, confirmatory tests are necessary (phenylhydrazin, fermentation, polariscope).

The other bismuth tests are not as satisfactory nor as delicate as Nylander's. They are mentioned here for the sake of completeness:

2. **Boettger's Test.**—Equal volumes of potassium hydrate solution (U. S. P.) and urine are mixed in a test-tube, and a pinch of bismuth subnitrate is added. The mixture is shaken and boiled. A black or gray precipitate appears in the presence of sugar. This test is used but rarely, as it is deceptive in the presence of traces of sulphur, which precipitates bismuth sulphid. Brücke modified it as follows:

3. **Brücke's Modification.**—To remove the sulphur compounds Frohn's reagent is used. It consists of 1.5 gm. of freshly precipitated bismuth subnitrate mixed with 20 gm. of water and heated to boiling, whereupon 7 gm. of potassium iodid and 20 drops of concentrated hydrochloric acid are added. Ten cc. of water are poured into a test-tube and the same bulk of the suspected urine is poured into another tube. To the water add a drop of Frohn's reagent, which will cause a precipitate. Then add, drop by drop,

concentrated hydrochloric acid until the precipitate is redissolved. In this way we ascertain approximately the amount of reagent to be added to the suspected urine. Add the same quantity of hydrochloric acid to the urine in the other test-tube, then add the bismuth solution until precipitation is complete, and filter. Further addition of hydrochloric acid or of the reagent should not render the filtrate turbid. The filtrate is then boiled with an excess of alkali, as in Boettger's test. If sugar is present, a gray or black precipitate appears. According to Brücke, this test will detect 0.4 per cent. of glucose in water.

Pseudoreactions for Glucose.—The following table shows briefly the conditions in which deceptive reactions resembling those for glucose occur with the copper and the bismuth tests:

G. BRÜCKNER'S TABLE[1] (SLIGHTLY MODIFIED) OF PSEUDOREACTIONS FOR GLUCOSE

I. Normal urinary constituents when present in sufficiently large quantities may give rise to deceptive reactions with:

Copper Tests.	*Bismuth Tests.*
Traces of carbohydrates, as dextrose, isomaltose, pentoses, animal gum, glycuronic acid compounds.	Uro-erythrin.
Pyrocatechin.	Urinary pigments (urobilin in particular) when present in greatly increased amounts cause a brownish discoloration of the phosphatic deposit.
Bile-pigment.	Indican.
Urinary pigment.	
Uric acid.	
Indican.	
Kreatinin.	
Urobilin.	
Urobilinogen.	

[1] After Sahli, Diagnostic Methods of Examination, Philadelphia, W. B. Saunders Company, 1905, p. 486.

Concentrated urines are particularly apt to effect reduction, and the same is true of urines containing a moderate or large quantity of formed elements (leukocytes, erythrocytes, epithelial cells).

II. Products of abnormal metabolism which effect reduction:

Copper Tests.	*Bismuth Tests.*
Homogentisic acid.	Hexoses (dextrose, levulose, isomaltose, lactose [in puerperal women]), pentoses, glycogen, increased quantities of glycuronic acid compounds.
Uroleucinic acid.	Blood-pigment.
	Increased quantities of hematoporphyrin.
	Uroleucinic acid (weak).
	Homogentisic acid (weak).

III. Reducing substances added to the urine as preservatives, which consequently make the reduction tests for sugar impossible:

Copper Tests.	*Bismuth Tests.*
Formaldehyd.	
Chloroform.	

IV. Drugs or the derivatives obtained from them as the result of metabolic change:

Copper Tests.	*Bismuth Tests.*
Acetphenetidin.	Antipyrin.
Antifebrin.	Arbutin.
Arbutin.	Benzoic acid.
Benzoic acid.	Benzosol.
Benzosol.	Quinin (large doses).
Copaiba balsam.	Chloral.
Chloral.	Eucalyptol.
Glycuronic acid compounds of drugs (see p. 127).	Glycuronic acid compounds of drugs (see p. 127).
Morphin.	Indican.
Phenacetin.	Kairin.
Saccharin.	Rheum (also frangula and cascara sagrada).
Salicylic acid.	Salol.
Salol.	Senna.
Sulfonal.	Sulfonal.
Turpentine.	Turpentine.
Thallin.	Trional.
Urethan.	

Other Tests for Glucose.—The first two of these are important confirmatory tests, but are somewhat too elaborate for every-day use.

Rubner's Test.—"Ten cc. of a concentrated solution of neutral lead acetate (1 part of lead acetate to 10 parts of distilled water) are added to 10 cc. of urine. The mixture is filtered and ammonia carefully added to the filtrate, drop by drop, until a cheesy precipitate remains. The mixture is then heated over a water-bath to 80° C. During the heating, if dextrose is present the precipitate will become a beautiful rose or salmon red. The chemistry of this reaction is not positively known. The test is reliable, very delicate, and, therefore, particularly appropriate when Fehling's test seems doubtful. If the precipitate is heated too much the color becomes brown, like café au lait, and is no longer characteristic. Milk-sugar gives a yellow-red to brown color" (Sahli). This test is too intricate for routine work. Glycuronic acid compounds may give a similar reaction, but with this exception Rubner's test is one of the most reliable we have.

Phenylhydrazin Test.—This test should be employed whenever there is doubt whether the reduction of copper or bismuth is due to the presence of glucose (or some other carbohydrate) or to the presence of creatinin, uric acid, hippuric acid, etc., in excess. It is based upon the principle that with glucose present in 0.01 per cent. or even smaller amounts, phenyl glucosazon ($C_{18}H_{22}N_4O_4$) is formed by this method, a compound which crystallizes in characteristic yellow needles recognizable under the microscope, and usually arranged in sunburst fashion (Plate 3). These crystals (glucosazon) are almost insoluble in water, but soluble in boiling alcohol and melt at 204° C. Similar

PLATE 3

CRYSTALS OF PHENYLGLUCOSAZONE (*after von Jaksch*).

compounds are formed with this method in the presence of lactose (galacosazon), levulose (levosazon), the pentoses (pentosazon), and glycuronic acid, but these differ in their melting-points or in other respects as shown in the following table (Zunz, quoted by Emerson):

The urine reduces Fehling's.

- I. Gives crystals of phenylhydrazin in urine itself.
  - A. Melting-point of crystals about 200° C.
    - (*a*) Fermentation positive.
      1. Dextrorotatory = GLUCOSE.
      2. Levorotatory = LEVULOSE.
    - (*b*) Fermentation negative. ..... LACTOSE.
  - B. Melting-point of crystals about 150° C.
    - (*a*) Orcin reaction positive. ..... PENTOSES.
    - (*b*) Orcin reaction negative. ..... ISOMALTOSE.
- II. Gives crystals with phenylhydrazin only after urine has been treated by heating with dilute H_2SO_4. — Glycuronic acid compounds.

Method of Testing with Phenylhydrazin.—Into a beaker 0.5 gm. of the colorless crystals of phenylhydrazin hydrochlorate and 1.5 gm. of sodium acetate are placed. Enough water is added to dissolve this mixture, when the beaker is placed over a water-bath and gently heated. Five cc. of urine are now added and the mixture is kept boiling for five minutes. It is then slowly cooled. In the presence of sugar the crystals will settle at the bottom. The fluid may then be centrifuged (especially if the crystals are scanty), and if desired the crystals may be separated by decanting, dissolving them in hot 60 per cent. alcohol, adding water, and boiling the alcohol away. Under the microscope the crystals appear as small, bright yellow needles, arranged in sunbursts, sheaves, fans, etc., or sometimes distributed singly. Lactosazon (with lactose) gives shorter, heavier crystals, often pointed at both ends.

The Melting-point of Crystals.—This may be necessary in differentiating pentoses, which of late have been more thoroughly studied and are assuming greater clinical importance. For this purpose the following simplified method is recommended by Emerson and has given satisfac-

tion in the writer's laboratory: Into a small flask filled three-quarters full of concentrated H_2SO_4, a test-tube, half full of the same acid, is fitted by means of a perforated stopper. The whole is held by a clamp to an upright support. Into the test-tube a thermometer (Centigrade) is carefully dipped, submerging the mercury bulb in the acid. To the lower part of the thermometer and parallel to it is fastened a pointed glass tube of small caliber, closed at the tip, containing the crystals (Fig. 8). This tube is attached to the thermometer by means of a rubber band (above the acid). The flask is warmed slowly with a Bunsen burner and the point noted at which the crystals melt. A temperature of about 200°–205° C. is characteristic for glucose, levulose, or lactose (see table), while pentoses give lower melting-points (159° to 160° C.). The crystals for the purposes of this test should be dissolved in hot (60° C.) alcohol, the solution poured into water, and the crystals redeposited by evaporating the alcohol. When impure the crystals obtained with the phenylhydrazin test show a lower melting-point than when pure.

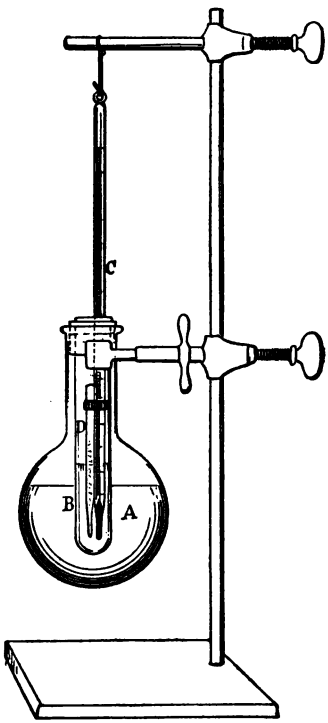


Fig. 8.—Melting-point of crystals: *A*, Flask; *B*, test-tube of sulphuric acid; *C*, thermometer; *D*, fine-bore tube for crystals. (From Emerson's "Clinical Diagnosis" by permission.)

QUANTITATIVE DETERMINATION OF SUGAR

For quantitative analysis with any method the entire amount of twenty-four hours should be collected, measured, and a sample of the thoroughly mixed urine should be examined. The amount

of sugar eliminated varies a great deal according to the time of day and the length of time after the meal. The urine should be kept as has been described in the introductory chapter, to prevent fermentation. Quantities of sugar are expressed usually in percentage, but the total number of grams of sugar eliminated must be calculated, as this is the final criterion. The progress of the disease and the result of treatment may be judged partly by comparative study of the quantity of glucose eliminated daily. The albumin should be removed before making quantitative tests for sugar:

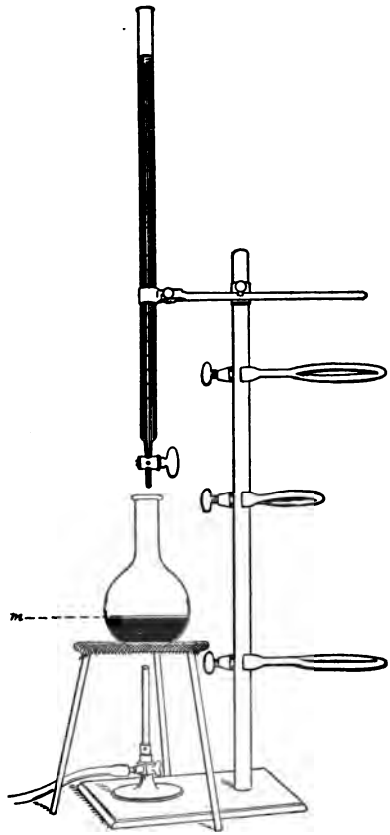


Fig. 9.—Apparatus for the quantitative estimation of sugar: *m*, Meniscus (Ogden).

Place 50 cc. of the urine in a porcelain evaporating dish, add 2 or 3 drops of dilute acetic acid, and boil thoroughly. If albumin does not appear, add acetic acid, drop by drop, stirring constantly, and heating until the co-

agulum forms. Filter and wash the precipitate once or twice with water. Collect the filtrate; wash with water into a graduate, and add enough water to make the original 50 cc. Mix the contents of the graduate thoroughly and use for quantitative tests.

Fehling's Method.—The principle of Fehling's method has already been explained above (p. 100). The quantitative application of it consists in titrating a known quantity of Fehling's solution in a flask with the (diluted) urine and heating the mixture till no more copper precipitates, and until the supernatant fluid is perfectly free from blue color.

The apparatus required is shown in Fig. 9. The burner should be one which can be carefully regulated; the flask should have a capacity of 100 cc., and the buret should be graduated in $\frac{1}{10}$ cc. The tripod should be about 20 cm. high and be covered with wire gauze or asbestos.

Diluting the Urine.—This is extremely important, as the best results are obtained with urines containing from $\frac{1}{2}$ to 1 per cent. of glucose. The specific gravity of the urine should be taken. The quantity of urine voided in twenty-four hours being known, the specific gravity of the urine is compared with that which would normally correspond to the quantity eliminated, *i. e.*, 2 liters of normal urine daily would have a specific gravity of 1015; 3 liters of the same urine, a specific gravity of 1010; 6 liters, a specific gravity of 1005. If a diabetic urine measures 3 liters and has specific gravity of 1030, it contains enough sugar to raise the specific gravity from normal (1010) to 1030. The difference is a specific gravity of 1020. The last two figures of this "corrected specific gravity" multiplied by 0.23 (in this example 4.6 per cent.) will give very roughly the percentage of sugar in the urine. The urine should, therefore, be so diluted that it contains 0.5 per cent., *i. e.*, ten times for a urine containing 5 per cent. glucose. The dilution should be accurately done by means of pipets and with distilled water. The dilution must be well shaken.

Five cc. of each of the two solutions are measured accurately and placed in the flask. To this 30 cc. of *distilled* water is added, and the mixture is heated to the boiling-point. The diluted urine (see above) is

poured into the buret accurately to the zero mark, allowing all the urine above zero adhering to the walls of the buret to settle on the surface of the liquid in the buret. (Waiting for two minutes is usually sufficient for this purpose.) The stopcock is opened and the urine is allowed to settle down to zero if needful. The buret is placed in position over the flask, and when the fluid in the latter has just come to a boil the diluted urine is added from the buret, the contents of the flask being kept gently boiling *until the faintest trace of blue color disappears.*

The chief difficulty with Fehling's method lies in determining this end-reaction. It is best, according to some authorities (Emerson), to add the urine from the buret and to heat the mixture, but to remove it from the flame as soon as it has begun to boil. An abundant red precipitate forms, which if allowed to stand in the air becomes reoxidized. For this reason we must allow this precipitate to settle only for a few moments, and then watch the color of the meniscus or clear uppermost layer in the flask. If there is still blue color in this meniscus, more urine must be added and the boiling repeated until all traces of blue disappear.

Calculation.—Inasmuch as 10 cc. of the combined solutions are reduced by exactly 50 milligrams (0.005) of glucose, the amount of urine required to reduce the solution in the flask will contain just 0.005 of glucose. From this the percentage of glucose in the urine can be easily calculated as follows:

If 15 cc. of diluted urine, 1: 10, were needed to reduce the copper in the flask, then 15 cc. of the diluted urine contained 1.5 cc. of undiluted urine. Hence, 1.5 cc. of urine contained 50 mgm. of glucose. The percentage is then calculated according to the following equation:

$$1.5 : 0.050 :: 100 : x, \text{ therefore, } x = 3.33 \text{ per cent.}$$

If the total amount of urine voided was 2000 cc. in twenty-four hours, then 3.33 per cent. multiplied by 2000 and divided by 100 equals 66.6 gm., the amount of sugar in twenty-four hours.

Although Fehling's quantitative method has been considered a standard for years, it has never been satisfactory, even in the hands of experts, owing to the great difficulty of determining the exact point of end reaction, *i. e.*, that point when all the copper had been reduced and the solution was perfectly colorless. For this reason

the writer has during the past few years ceased using and teaching this method, which, in his opinion, is most unsatisfactory for the practitioner and most trying for the laboratory worker.

Purdy's Method.—The most convenient and satisfactory modification of Fehling's method is that of Purdy. This method has the advantage of giving a sharper end reaction than does Fehling's, and for this reason gives more accurate results than the latter.

The solutions required are as follows:

I. Copper sulphate, pure, crystals..... 4.158 gm.
Distilled water, enough to make.....500.0 cc.

II. Rochelle salt..... 20.4 gm.
Potassium hydroxid, pure..... 20.4 "
Ammonia (sp. gr. 0.88).....300.0 cc.
Distilled water, enough to make.....500.0 "

Five cc. of each solution (10 cc. in all) are used in the test and correspond to 0.005 gm. of glucose. The method of using it and the method of calculating the percentage of sugar in the diluted urine are the same as described with Fehling's solution. The urine is very slowly added from the buret until no trace of blue color remains, the fluid in the flask being kept gently boiling.

Fermentation Test.—This test is very convenient for clinical use, but is not so accurate as the other quantitative methods. A very convenient method of performing it is with the aid of *Einhorn's saccharometer* (Figs. 10 and 11).

Two sets of the apparatus are used, one with the suspected urine, the other with normal urine as a control test. A small piece of yeast, which should be fresh, is mixed thoroughly with a definite quantity of the suspected

urine, measured in a marked test-tube furnished with the apparatus. The mixture is then poured carefully into the graduated tube, which resembles the Doremus urea apparatus, care being taken to expel all the air by slanting the tube so that the bubbles escape. The tubes are allowed to stand at a temperature of about 86° F. until fermentation has ceased—*i. e.*, for about twenty-four hours. The carbon dioxid resulting from the fermentation collects at the top of the tube and the percentage of sugar is read off at the level of the fluid. If the second tube also shows a small amount of gas, this is deducted from the reading of the first tube. The results are only approximate.

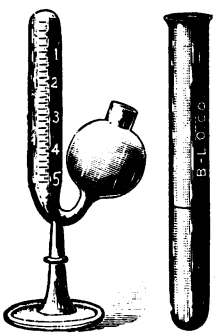


Fig. 10.—Einhorn's saccharometer.

Fig. 11.—Einhorn's saccharometer with stop-cock.

Lohnstein's Method.—A valuable fermentation saccharometer has been described by Lohnstein, which directly indicates the percentage of sugar from 0 to 10 per cent. without requiring dilution. According to a number of observers, this instrument is very accurate, although Riva-

rono, of Turin, found that comparative tests with polarimetry, Fehling's method, and Lohnstein's apparatus showed marked errors, which increased especially with the amount of albumin in the urine and the degree of dilution of the specimen.[1]

Lohnstein's saccharometer is intended to rectify the errors which are apt to enter in an estimation with Einhorn's apparatus, because in the latter no allowance is made for temperature, pressure, etc., as should be done in all volumetric gas analyses. Lohnstein's instrument consists of a U-shaped tube with a long arm, and another ending in a bulb. Mercury is poured into this bulb up to the zero mark on the scale of the long arm. A definite quantity of urine is poured over the mercury, a little yeast is added, and the apparatus is closed with a glass stopper greased in vaselin. The stopper is weighted with lead so as to prevent the escape of any gas. The apparatus is set aside at a fixed temperature (20° or 35° C.), and the mercury rises as the gas of fermentation develops. Its level is read directly on the scale of the tube in percentage of glucose. The temperature and tension in this method remain always the same.

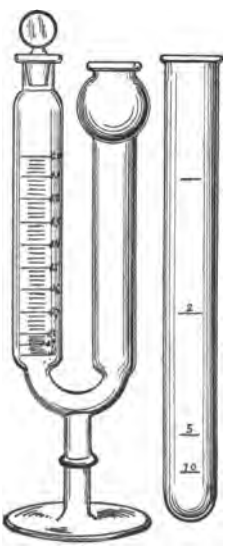


Fig. 12.—Lohnstein's saccharometer.

The disadvantages of all the fermentation methods are their inaccuracy and the fact that they require twenty-

[1] Riforma Medica, March 30, 1904, p. 343.

four hours for their completion. According to Ogden, the fermentation tests yield results that are about 0.5 per cent. lower than those obtained with Fehling's method.

Roberts' comparative density method is also approximate, but is preferred by some, as it requires no apparatus to speak of. Four ounces of the urine are placed in a 12-ounce bottle and a piece of compressed yeast is added.[1] The bottle is then stoppered with a nicked cork, to allow the escape of CO_2, and set aside in a warm place. A tightly corked 4-ounce bottle filled with the same urine without any yeast is placed next to it. Fermentation goes on for eighteen to twenty-four hours. The fermented urine is then decanted into a cylinder and its specific gravity is taken. At the same time the specific gravity of the unfermented urine is also taken and the loss of density is noted. According to Roberts, the loss of each degree of specific gravity corresponds approximately to 1 grain of sugar to the fluidounce. Thus, if the specific gravity of the urine was 1040, and after fermentation 1020, it contained 20 grains of sugar to the fluidounce. The two specimens of urine should be kept at exactly the same temperature.

Roberts' comparative density method may also be employed in an apparatus devised by Heinrich Stern, of New York, styled a *urinoglucosometer* (Fig. 13). This consists of a small and a large glass tube, united at their bases to form a U. The small tube is marked at 50 cc., the large one at 100 cc. Both are closed by means of perforated metal caps. The smaller tube is filled with urine to the mark, and is tightly closed with a rubber stopper. A small piece of compressed yeast is placed in the larger tube

[1] One-sixteenth of a cake of Fleischman's compressed yeast.

and urine is added up to the mark. A urinometer is then inserted into each tube, the metal caps are put over the tubes, and the apparatus is set aside at a temperature of 80° F. for from twelve to fifteen hours. The larger tube is opened and the contents stirred to liberate the CO_2. The flocculent mass is allowed to settle and the cap is replaced. Then the specific gravity of the urine in both tubes is read from the flat tops of the metal caps. Each degree of specific gravity lost equals 0.2196 gm. of glucose per 100 cc. of urine. The difference in specific gravity between the urines in the two tubes, multiplied by 0.2196, is the percentage of sugar present. The method is convenient and, though open to the objections of inaccuracy which obtain in all fermentation tests, is sufficiently exact for clinical purposes.

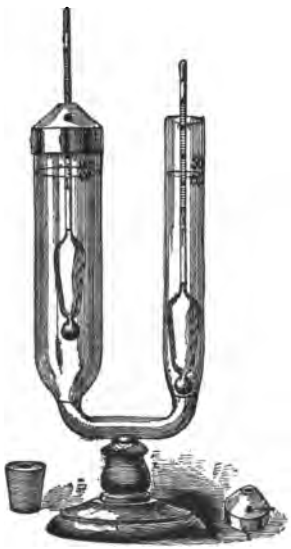


Fig. 13.—Stern's urinoglucosometer.

Schütz's Method.—An instrument devised by J. Schütz is styled an *areosaccharometer*, and its construction is based upon the difference in the density of urine before and after fermentation. The instrument consists of a vessel which is filled with urine and immersed in water, the long neck of the instrument being so graduated that the

divisions indicate the percentage of sugar corresponding to the difference in density before and after fermentation. The vessel is filled with urine to the proper mark and 1 gm. of compressed yeast is added. Enough shot is put in to allow the vessel to sink to 0. The contents are shaken well and are allowed to ferment at room-temperature for from twenty-four to thirty-six hours. The instrument is then immersed in water and the specific gravity and the percentage of sugar read. This method is said to be more accurate than Einhorn's, but the latter is more convenient for every-day work.

Polarization.—The polariscope offers the most accurate and quickest method of determining the amount of glucose when the quantity exceeds 1 per cent. The best instruments in the hands of experts can detect smaller amounts (0.01 per cent.), but, unfortunately, the apparatus is so costly that it is out of the range of the practitioner. The method depends upon the fact that glucose rotates polarized light toward the right, and that the degree of rotation varies in proportion to the percentage of sugar in the urine.

Although theoretically accurate for grape-sugar solutions, polarimetry is open to the objection, when used with urine, that the latter contains sometimes levulose, oxybutyric acid, etc., which rotate polarized light to the left.

A polariscope especially devised for clinical work by Ultzmann is made by Reichert of Vienna. It is very convenient and sufficiently accurate for all purposes, reading from 0.1 up to 25.0 per cent.

By means of this saccharimeter the percentage of sugar in urine may be read directly upon the Vernier scale of the instrument. The polariscopic tube is set upon a stand. A concave mirror illuminates the visual field. Fig. 14 shows the Ultzmann polariscope in section. In this figure *a* and *b* are a system of magnifying ocular lenses focusing the image at the

point *p*. The upper prism is marked *c*, the lower, *f*. The upper prism is connected with the movable part of the sliding scale; *p* is a transparent plate of quartz, polarizing to the right and left respectively. The scale is so arranged that each division corresponds to 1.0 per cent. glucose in the urine at 20° C. Tenths of 1 per cent. can be read with the aid of the

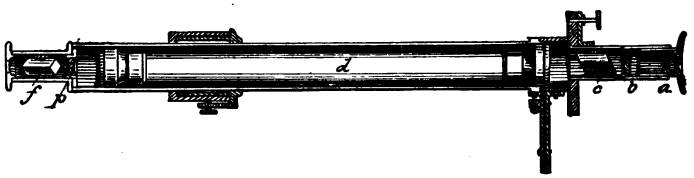


Fig. 14.—Ultzmann's polariscope.

sliding Vernier. To read the tenths of a degree it is necessary to see at which mark of the Vernier there is a perfect alignment with one of the marks of the percentage scale. In Fig. 15, the zero mark on the Vernier is between the fourth and fifth percentage degree on the right half of the scale. To determine how many tenths per cent. besides the 4 per cent. of sugar are present, we count the divisions on the small scale from 0, and

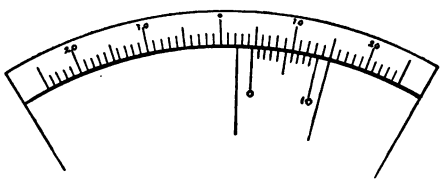


Fig. 15.—Vernier scale of Ultzmann's polariscope.

find that the sixth division is the first to be directly opposite one of the marks of the large scale. The reading then is 4.6 per cent. glucose in the scale as shown in the figure.

The urine to be examined is placed in a glass tube with metal screw ends, into which are fitted optically inactive glass disks. The lower end of the tube is closed, the fluid poured in, and, avoiding air bubbles, the upper glass disk is placed in position and screwed down. If the urine is very light colored and perfectly clear, it may be examined as such. If not, it must previously be decolorized and cleared by mixing it with a sufficient amount of powdered basic lead acetate, and filtering through several thicknesses of filter-paper till the fluid is perfectly clear. The addition of the lead acetate in bulk does not alter the volume of the fluid, and for this reason this method of clearing is preferable. In some cases, however, it is very

difficult to clear the mixture by filtration, and then it may be necessary to mix the urine with a solution of basic lead acetate before filtering. If this is done, the volume of the urine should be known and one-fourth of this volume of lead acetate solution (10 per cent.) should be added to the urine. Instantly a fine white precipitate of lead chlorid, sulphate, and phosphate will be thrown down, and the mixture should then be filtered through a dry filter. One-fourth of the amount of sugar read on the scale should then be added to get the true amount in the urine thus prepared.

When the glass tube is filled with water, or when it is filled with normal urine, the two halves of the disk *p* seen through the ocular appear of the same color. If a solution of glucose be placed in the tube, however, there will be a difference in the colors of the two sides when the zero mark of the Vernier is at zero of the scale, and only by moving the Vernier to the right can the two halves of the disk be made to appear of the same color. When this point is reached, the amount of glucose may be read upon the scale.

Albumin, if present, must be previously removed, the original volume being noted and allowances made for any changes due to the removal of the albumin. The reason for removing albumin is that it polarizes light to the left, thus neutralizing the action of sugar. The Ultzman polariscope is simple enough to be used by practitioners and is not a very expensive instrument.

QUESTIONS ON CHAPTER VI

Glucose.—What does its presence in the urine indicate? What is diabetes? What other causes may produce glycosuria? What class of tests are most commonly used for searching for glucose in the urine? On what principle do they depend? Describe Trommer's test. What precautions should be taken when using any of the copper tests? Name the principal substances other than sugar which reduce copper sulphate. Describe Fehling's qualitative test. What method is used with a more stable solution? Describe the phenylhydrazin test. On what principle are the bismuth tests based? Describe Boettger's test; Brücke's modification; Nylander's test; picric acid and potash test; Rubner's test.

How should a sample be collected for quantitative analysis of sugar, and why? What are the disadvantages of Fehling's method for quantitative analysis of sugar? Describe the technic of Fehling's quantitative method.

Describe Purdy's method. What are its advantages? Describe the fermentation test. Describe the comparative density method for sugar. What is the polarization test for glucose and what is its value? Describe the Ultzmann polariscope.

CHAPTER VII

OTHER CARBOHYDRATES IN THE URINE

LACTOSE

LACTOSE (milk-sugar) is found sometimes in the normal urine of nursing women, but usually it is present in such cases in very small quantities, although Ralfe reports a case in which as much as 3 per cent. occurred. It is more apt to occur near the end of gestation, and especially in cases of mastitis. In women who have weaned their children early it is also frequently seen for a few days or a week. Lactose has no pathologic significance in urine, and is important only because it is apt to be mistaken for glucose.

Lactose ($C_{12}H_{22}O_{11} + H_2O$) is characterized by crystallization in white or colorless four-sided prisms, with acuminated ends, bounded by four triangles, and by turning polarized light to the right with a rotating power of +59.3 degrees, while the rotation of grape-sugar is +53.1 degrees. It reduces salts of copper, but does not undergo alcoholic fermentation with yeast. Certain cleft fungi, however, convert milk-sugar into alcohol, and ferments induce lactic acid and butyric acid fermentation readily with lactose. It is quite soluble in cold and freely soluble in hot water; insoluble in alcohol and ether, and precipitated by acetate of lead and ammonia.

Lactose can be recognized with certainty only by isolating it, and the methods for doing this will be found in works

on physiologic chemistry. If the urine reduces Fehling's solution feebly, but does not ferment with yeast, and if it rotates polarized light strongly to the right, especially if the urine is that of a pregnant or nursing woman, lactose is probably present.

The phenylhydrazin test with lactose forms a crystalline body called phenyl-lactosazone, which occurs in yellow needles grouped in clusters, melting at 200° C.

LEVULOSE

Levulose (fruit-sugar) is rarely found in the urine and, when present, is usually associated with glucose. It rotates polarized light *to the left*, thus partly neutralizing the action of glucose, so that when present with glucose more sugar is found by chemic tests than by polarization. (It must be noted that other bodies, such as glycuronic acid, cystin, and beta-oxybutyric acid, etc., diminish the optic activity of urine.)

Levulose ($C_6H_{12}O_6$) is non-crystallizable when impure, but when pure forms long wavy needles. It reduces salts of copper much more feebly than does grape-sugar. It is detected by the polariscope in the following manner (see also Phenylhydrazin Test, p. 108): To distinguish left-handed rotation of light caused by substances other than levulose, the urine is subjected to alcoholic fermentation. If the rotation is due to the levulose, it disappears; if due to the other substances, it persists after fermentation.

LAIOSE

Laiose (Leo's sugar) was found by Leo, in 1887, in some severe cases of diabetes. Laiose ($C_6H_{12}O_6$) is left-rotating, reduces copper salts, and forms a compound with

phenylhydrazin. It does not ferment and is not sweet in taste. Its isolation is troublesome and of no consequence clinically, except that samples of urine that contain it show more sugar by titration than by polarization.

PENTOSES

Pentoses are sugars containing five atoms of carbon ($C_5H_{10}O_5$), and are products of hydrolysis of the other carbohydrates in the body. Pentoses form the carbohydrate molecule in the glycoproteids contained in the nuclei (see Nucleoproteids, p. 92) of such organs as the liver, spleen, pancreas, thyroid, thymus, and brain. Examples of pentoses are arabinose, rhamnose, and xylose. The last is of greatest importance.

[*Xylose* rotates polarized light to the right, and its osazone (see Phenylhydrazin Test) crystals melt at 150° to 160° C. Xylose reduces copper as well as bismuth solutions, and reacts with Rubner's test, but in contrast to glucose it does not ferment with yeast. *Arabinose* gives similar reactions, but reduces copper more readily than xylose.]

Clinical Significance.—There are three varieties of pentosuria (Janeway): (1) *Alimentary pentosuria*, which is of no clinical significance, but should be noted to avoid confusion with the other forms. Pentoses are excreted after the ingestion of beverages and foods containing large amounts of soluble and assimilable carbohydrates—*e. g.*, fruit syrups, malt liquors, etc.

(2) *Diabetic Pentosuria*.—Kültz and Vogel found pentoses in a number of diabetics. Diabetic pentosuria is rare and is confined to very severe forms of diabetes.

(3) *Idiopathic Pentosuria*.—This has assumed importance of late by the report of a collection of 24 cases by Janeway in 1906. Small amounts of pentose (0.05 to 0.6

per cent.) occur in these cases without clinical symptoms. The cause of this pentosuria is not known, but it is supposed that for some reason excessive quantities of pentoses are formed in the organism.

Detection.—Pentoses may be *suspected* when the ordinary sugar tests show the following peculiarities: (*a*) Fehling's or the other copper tests do not show a reduction while hot, but do so suddenly on cooling; (*b*) Nylander's test shows not a brown or black, but a gray precipitate; (*c*) the fermentation test is negative.

Orcin Test (*Bial's Method*).—The pentoses give a characteristic reaction with orcin. The following method was devised by Bial[1] as a modification of a test described by Salkowski and Blumenthal. It is the easiest of this group of methods and is said to be sufficiently accurate to differentiate glycosuria from pentosuria. If glucose is present also, it should be removed first by fermentation and the residue tested for pentoses.

"Bial's reagent consists of 500 cc. of 30 per cent. hydrochloric acid, 1 gm. of orcin, and 25 drops of the official liquor ferri sesquichlorati (German Pharmacopeia). Four to 5 cc. of this reagent are boiled in a test-tube, and, after removal from the flame, several drops or, at most, 1 cc. of urine are added. A green color should appear at once or almost immediately. If the test is carried out in this manner, Bial states that the small amount of heat employed is not sufficient to cause any reaction between the reagent and the most unstable glycuronates" (Sahli).

OTHER SUBSTANCES ALLIED TO GLUCOSE

There are a number of substances closely allied to glucose which are comparatively less important in urine analysis.

Inosite (*muscle-sugar*) ($C_6H_{12}O_6 + 2H_2O$) is occasionally found in conjunction with grape-sugar and also in the last stages of chronic nephritis, in tuberculosis, syphilis, and typhus fever, as well as after drinking large quantities of water. Properly speaking, inosite is not a sugar, but

[1] Deutsche med. Woch., 1903, No. 27.

should be placed among the aromatic compounds. It does not reduce the copper salts; does not ferment with yeast; is optically inactive, and does not combine with phenyl-hydrazin.

Its isolation and detection are cumbersome and not of sufficient clinical value to warrant further description here.

Saccharose (*cane-sugar*) ($C_{12}H_{22}O_{11}$) is very rarely found in urine and is of no clinical importance. It has been found after eating large amounts of cane-sugar and in urines contained in a bottle that had not been properly cleaned (syrup). It is at times added to the urine by persons who wish to deceive the physician. When traces are present, they are usually overlooked. When larger amounts are present, the urine may be boiled with dilute hydrochloric and sulphuric acid, and the cane-sugar is converted into dextrose and levulose, the solution rotating light to the left, while sugar alone rotates strongly to the right. Pure saccharose does not reduce Fehling's solution.

Maltose.—Maltose is found occasionally in the urine in health and in disease. Von Jaksch found it in cases of malignant tumors.

Dextrin.—Reichard has found dextrin in the urine of diabetes instead of grape-sugar. In such cases the urine behaves with Trommer's test just like a solution of dextrin, the fluid becoming first green, then yellow, and sometimes dark brown.

Gum.—A gum-like substance has been found in the urine by Landwehr, who calls it "animal gum" and considers it a normal constituent.

Glycuronic acid ($C_6H_{10}O_7$ or $CHO.(CH.OH)_4.COOH$) does not occur as such, but as a glucosid-like

combination with a variety of aromatic substances of the urine, especially indoxyl, phenol, etc. Its chief clinical importance is that it is apt to be mistaken for glucose. Glycuronic acid is dextrorotatory, but its paired combinations, which exist in normal urine, are levorotatory, so that Haas, Johannovsky, and others found that normal urine turns polarized light slightly to the left. It reacts to the reduction tests with copper and forms a crystalline compound with phenylhydrazin, but does not ferment with yeast. According to Zunz (see p. 109) glycuronic acid compounds form an osazone with the phenylhydrazin test only after the urine is heated with dilute H_2SO_4. In other respects glycuronic acid seems to act like the pentoses and is difficult to differentiate from these.

Glycuronic acid apparently has nothing to do with diabetes. It is increased after the administration of those compounds with which it forms combinations in the urine—*i. e.*, phenol (Kültz), benzol, naphthol (Nencki), menthol, camphor, turpentine, resorcin, hydroquinon, thymol, chloral, acetanilid, curare, morphin, chloroform, etc. With these it forms fixed compounds, as naphthol-glycuronic acid, etc.

Glycuronic acid, when pure, is a syrup soluble in water and alcohol, which, on boiling with water, forms an anhydrid (lactone), glycuron ($C_6H_8O_6$), which crystallizes in colorless plates. By the action of bromin it is converted into saccharic acid ($C_6H_{10}O_8$), and is regarded as intermediate between this acid and gluconic acid ($C_6H_{12}O_7$), obtained by the oxidation of glucose with bromin (Hammarsten).

Alkaptonuria.—A rare condition known as "alkaptonuria" is characterized by a dark urine which grows darker

(reddish-brown) on standing. The change is more rapid when an alkali is added, hence the name "alkapton." When a piece of cloth is dipped into such a urine it is dyed black. When voided the urine is highly acid. It reduces Fehling's solution, but fails to give Nylander's test. It does not polarize light, does not ferment, and gives no crystals with phenylhydrazin.

Formerly these urines were supposed to contain pyrocatechin, but alkaptonuria is now known to depend upon the presence of a number of other compounds, two of which have been isolated—homogentisic acid (hydroquinon-acetic acid) and uroleucinic acid. The former appears to be derived from tyrosin (*q. v.*).

Clinical Significance.—Alkaptonuria is due to an obscure disturbance of proteid metabolism and seems to be congenital. It occurs without symptoms and is discovered usually by accident. It is said to occur in members of the same family and especially in children of consanguineous marriages. Only 40-odd cases are on record.

CAMMIDGE'S REACTION

Before closing the consideration of carbohydrate bodies we must give a brief account of Cammidge's method for the detection of a glycerin-like compound which is excreted in the urine in some diseases of the pancreas.

Cammidge's reaction was introduced in 1904, but has not as yet gained universal acceptance. Further studies are needed to confirm its clinical value as set forth by its author. The reaction depends upon the fact that in pancreatitis and pancreatic necrosis glycerose is set free from the fat and enters the blood, whence it appears in the urine. It is converted into glycerose by boiling with min-

eral acids. The latest technic described by Cammidge is as follows:

The urine examined must be from a twenty-four-hour specimen or from the mixed morning and evening excretion. If albumin is present, the specimen must be faintly acidified, boiled, filtered, and made up to the original bulk with distilled water. If sugar is present, it must be removed by fermentation with yeast, and if the urine is alkaline, it must be made just acid before proceeding.

1. To 40 cc. of urine add 2 cc. of strong HCl, place in a small flask with a funnel in the mouth (to act as a condenser), boil for ten minutes, then cool the flask thoroughly in a stream of water, and make up the result to 40 cc. with distilled water.

2. Add slowly 8 gm. of powdered lead carbonate, allow the mixture to stand for a few minutes, and when the reaction is complete, cool and filter through a close-grained filter-paper until the fluid is perfectly clear. Ten cc. of this clear filtrate are diluted to 20 cc. with distilled water.

3. Shake this clear filtrate with 2 gm. of powdered tribasic lead acetate;[1] filter several times, getting as clear a filtrate as possible.

4. Add 2 gm. powdered sodium sulphate and bring mixture to a boil. Cool the flask in a stream of water and filter (to remove the lead as lead sulphate) to make 20 cc.

5. To 20 cc. of the filtrate add 0.8 gm. of phenylhydrazin hydrochlorid, 2 gm. of sodium acetate, and 1 cc. of 50 per cent. acetic acid. Boil the mixture on a sand-bath for ten minutes and filter while hot through a small funnel moistened with hot distilled water. If the result is less in volume than 15 cc., make up to that bulk with hot distilled water, thoroughly mixing the whole.

In case of pancreatic disease, a light yellow flocculent precipitate forms. This must be examined microscopically, and will be found to consist of thread-like crystals arranged in sheaves and bundles. They dissolve in ten to fifteen seconds on being irrigated with 33 per cent. sulphuric acid. Any precipitate other than the crystalline deposit mentioned is not to be regarded as evidence of a positive reaction.

6. A control test is carried out in exactly the same way, except that the preliminary boiling with HCl is omitted; this, of course, gives crystals if sugar is present, and, in that case, the test has to be repeated after fermentation with yeast.

[1] Tribasic lead acetate ($Pb(C_2H_3O_2)_2 2PbO$) must be used. Misleading results occur with the use of the solution of lead subacetate, U. S. P. (Schroeder).

Clinical Significance.—Cammidge claims that his reaction is positive in all cases of pancreatitis. It is said to be negative in healthy subjects, in 75 per cent. of cases of cancer, and "with very few exceptions" in non-pancreatic diseases.

The solubility of the crystals obtained by this method is claimed to be of diagnostic import. If the crystals dissolve (under the microscope) within one-half to one minute in 33 per cent. H_2SO_4, acute pancreatitis is present; if they take longer (two minutes), chronic pancreatitis; if five minutes, pancreatic cancer is probably present. These points are not generally conceded.

As yet Cammidge's reaction can be looked upon only in the light of a confirmatory sign. It has no negative value so far as the writer's experience with it is concerned, and its positive value must be checked by the clinical features of each case.

QUESTIONS ON CHAPTER VII

What is the clinical significance of lactose in the urine? What are its characteristic properties? How is it detected?

What are the properties of levulose and how is it detected?

What is meant by laiose?

Name a few of the other substances allied to sugar that may occur in the urine.

Define the pentoses. What is their clinical significance? How are they detected? What is the significance of glycuronic acid? How is it detected? What is meant by alkaptonuria? What are the characters of the urine in this condition and to what acids is it due?

What is Cammidge's reaction, and in what diseases is it supposed to aid in diagnosis?

III. THE NITROGEN GROUP: UREA, URIC ACID, ETC.

CHAPTER VIII

THE EXCRETION OF NITROGEN IN THE URINE: UREA

THE most accurate measure for the metabolism of the proteids is the output of urinary nitrogen. This output depends upon two factors: (1) The amount of nitrogenous foodstuffs (chiefly proteids) taken in, and (2) the burning up and wasting of the tissues of the body (the proteid constituents of cells, etc.). Formerly, the determination of the urea contained in the urine was regarded as all that was necessary to determine clinically the condition of nitrogenous excretion. Urea, however, forms from 85 to 90 per cent. of the total nitrogen excreted in the urine. The remainder is present in the form of ammonia (2 to 5 per cent.), as uric acid (from 1 to 3 per cent.), and as extractives (7 to 12 per cent.—Hammarsten) or "undetermined nitrogen." At least 10 or 15 per cent. of the total nitrogen, therefore, is due to substances other than urea. Of the total nitrogen eliminated by man, 95 per cent. is excreted in the urine and about 5 per cent. in the feces.

Unfortunately, the determination of the total nitrogen and the differential determination (so-called partition) of the several quantities of ammonia, uric acid, creatinin, etc., which contribute to the total nitrogen output necessitate

time, training, and apparatus quite beyond the limits of ordinary clinical work. For this reason, the technic of the elaborate methods of analysis which are required in this work has been omitted from these pages. The practitioner should be acquainted, however, with the value of nitrogen determination as well as with the importance of separate determinations of the quantities of urea, uric acid, ammonia, etc., entering into the total nitrogen output.

By the term *nitrogen balance* we mean the proportion between the intake and the output of nitrogen. When these are equal, as is the case when a patient is fed on a constant, sufficiently full diet, we say that he is in a state of *nitrogenous equilibrium*. Normally, the total amount of nitrogen in the urine on a mixed diet is from 10 to 15 gm. daily. The healthy body has a tendency of compensating or accommodating the nitrogen output during one period of twenty-four hours to the nitrogen intake during the preceding day. Even if the amount of nitrogen taken in varies from day to day, the average urinary nitrogen excreted during a period of days will equal the average of the nitrogen taken in during these days. For this reason, determinations of nitrogen must be made over a period of several days. The character of the food, the amount of water drunk, and the amount of exercise should also be carefully watched. The amount of nitrogen *intake* may be calculated with the aid of tables, showing the N. contents of various foods.

Normally, the total nitrogen is increased by anything that will increase the assimilation of proteids. The amount is greater upon a meat diet, after meals and after exercise, as well as after hot baths. By drinking considerable amounts of water the nitrogen excretion may also be increased.

In disease, the nitrogen output is *increased* in febrile conditions, in diseases accompanied by a rapid waste of tissues, in diabetes, arsenic, antimony, phosphorus, and other metallic poisoning. A nitrogen increase also takes place whenever there is a diminished absorption of oxygen, as in severe hemorrhages, suffocation, dyspnea, etc., and when exudates or transudates are absorbed.

The total nitrogen is *diminished* in convalescence from wasting diseases in myxedema and during pregnancy. After labor, the nitrogen again increases. Anything that interferes with the absorption or assimilation of proteids will diminish the nitrogen output. This is the case in very

severe depression of bodily vitality. When ascites or other exudates or transudates are forming and when water is retained in the body, nitrogen is also diminished. In nephritis the kidneys may cease to excrete urea, thus announcing the probable onset of uremia, and in such cases, of course, the nitrogen is very markedly decreased.

The relative proportion of nitrogen due to urea, to ammonia, and to amino-acids (the latter, the so-called "undetermined nitrogen") is disturbed markedly in *toxemias of pregnancy* and other toxic conditions. As this subject has assumed great importance of late years, a separate chapter has been devoted to it in another part of this book (see p. 386).

UREA

(CH_4N_2O.)

Urea is the principal nitrogenous organic constituent of urine, and is derived chiefly from the metabolism of the proteids. As has been said above, urea is but a partial index of the amount of nitrogen excreted, though, clinically, the test for the percentage of urea is the simplest method of determining the state of nitrogenous excretion. Urea, however, represents only about 85 per cent. of the total nitrogen of the urine, while 15 per cent. is represented by uric acid, the purin bodies, hippuric acid, ammonia, and by creatinin.

Furthermore, the quantity of urea excreted is very variable in health, according to the amount of nitrogen intake. The quantity of urea, as determined clinically, is to be regarded, therefore, not as an absolute criterion, but only as a factor, valuable in diagnosis only when the nitrogen intake is duly accounted for.

The quantity of urea changes not only with the amount and composition of the food but also with the rapidity of

tissue-waste in health and in disease. The amount of urea excreted fluctuates at times from day to day, even in health. It is considerably increased in fever, on account of the increased breaking down of the proteids of the body, and also in diabetes. Urea is diminished in diseases of the liver, such as acute yellow atrophy, its place being taken by leucin and tyrosin (see p. 201), and also in cirrhosis of the liver. It is also diminished in chronic diseases accompanied by wasting and in renal diseases characterized by interstitial changes. The use of thyroid extract is often followed by an increase, the use of alcohol in children by a decrease, of urea, and a diminution has been observed in chronic alcoholism in adults (see table on p. 135).

The **amount of urea** normally excreted with an average diet is from 20 to 40 gm. (308.6 to 617.2 grains) in twenty-four hours in adults, or from 1.5 to 2.5 per cent. With a milk diet or with a diet containing little nitrogen, 15 gm. a day may be excreted, while in fever 50 gm. are often found.

The usual standard amount of urea in the urine of a healthy adult on a mixed diet is taken as 2 per cent., or 20 gm. per liter, or 10 grains to the ounce.

Tests for Urea.—Urea (CH_4N_2O) is an isomere of ammonium cyanate, and may be prepared artificially by the action of ammonia on carbonyl chlorid, by the hydration of cyanamid, and from ammonium carbonate.

It is soluble in alcohol and water; insoluble in ether; odorless, with a salty taste and a neutral reaction in solution. It crystallizes in colorless four- or six-sided prisms with oblique angles, or in delicate, white, silky needles. On the addition of nitric acid it forms urea nitrate, which crystallizes in plates of an octahedral, hexagonal, or lozenge shape (Fig. 16). With oxalic acid it forms urea oxalate, in the

form of flat or prismatic crystals. If a drop of fluid is suspected to be urine, it is placed on a glass slide, and the latter is carefully warmed over the flame and set aside to crystallize after a drop of nitric acid has been added. Crystals of nitrate of urea will form and will be recognized under the microscope. This test is facilitated by the evaporation of the urine to a more concentrated form.

TABLE OF VARIATIONS IN THE AMOUNT OF UREA EXCRETED. (After Ogden.)

I. *The Amount of Urea is Increased:*

(*a*) *Normally:*
1. A plentiful diet of mixed character.
2. Increased exercise.
3. During the day as compared to the night.
4. After the administration of ammonium compounds.
5. After hot baths.
6. When metabolism is temporarily increased by drinking large amounts of water or by any other means.

(*b*) *Abnormally:*
1. In acute fevers, owing to increased metabolism, especially in the early stages. Exceptions: Cholera and acute intestinal diseases, acute nephritis, etc., with dropsy.
2. In dropsies when the fluid is becoming reabsorbed (temporarily).
3. Before a chill in malaria (diminished afterward).
4. In diabetes insipidus, urea high, but specific gravity low.
5. In diabetes mellitus, owing to increased metabolism.
6. In chronic gout.

II. *The Amount of Urea is Diminished:*

(*a*) *Normally:*
1. With a diet poor in nitrogen (vegetarians) and in starvation.
2. After very profuse perspiration.
3. In normal pregnancy (often), the average being 20 gm. per twenty-four hours.
4. After taking small doses of quinin (slightly).
5. Prolonged drinking of large amounts of water (for a short time, urea increased).

In most diseases, especially in diminished metabolism and lowered nutrition, principally:

(*b*) *Abnormally:*
1. In most renal diseases, especially in the chronic forms, interstitial and parenchymatous. Not so markedly in amyloid kidney. In acute nephritis, especially when dropsy is increasing.
2. In functional disturbances of the kidneys; congestion.
3. In acute fevers, after the acme and during convalescence, when metabolism is low.
4. In all dropsies while the fluid is increasing.
5. Shortly before death; usually a marked diminution.
6. After prolonged vomiting.
7. With marked diarrhea, urea being partly eliminated in the feces.
8. In all degenerative changes of the liver.

Quantitative Estimation of Urea.—Approximate Estimation.—As urea is the most important solid con-

stituent of the urine and is present in the largest amount, the specific gravity runs closely parallel to the amount of the urea, and this fact has been utilized for an approximate estimation of the amount of urea present by means of the specific gravity. This can be done only when there is no sugar present, when the albumin is less than 0.2 per cent., and when the chlorids are normal. Such a urine with a specific gravity of 1020 to 1024 and a daily quantity of 1500 cc. (50 ounces) will contain from 2 to 2.5 per cent.

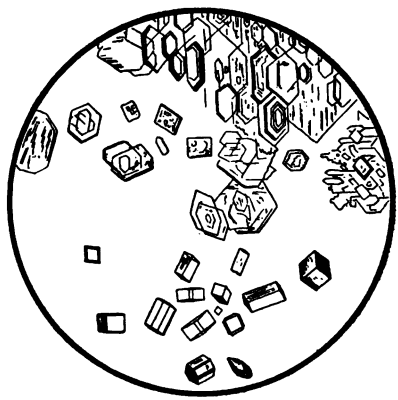


Fig. 16.—Crystals of nitrate of urea (upper half) and oxalate of urea (lower half) (after Funke).

of urea. A specific gravity of 1014 would indicate 1 per cent. of urea, and of 1028 to 1030, about 3 per cent.

This method, however, is very inaccurate, inasmuch as the chlorids fluctuate markedly and materially influence the specific gravity.

Hypobromite Method.—Clinically, this method, introduced by Knop, is the most readily available. It depends upon the principle that urea, when brought into contact with

sodium hypobromite, is decomposed into nitrogen, carbon dioxid, and water, according to the following formula:

$$CH_4N_2O + 3NaOBr = 3NaBr + CO_2 + N_2 + 2H_2O.$$

The amount of nitrogen disengaged is the measure of the urea. The carbon dioxid set free at once combines with the excess of sodium hydrate in the hypobromite solution used, forming sodium carbonate, which remains in solution. All methods of quantitative analysis of urea which involve the use of hypobromite solution depend upon the fact that 1 cc. of nitrogen at standard temperature and pressure equals 0.0027 gm. of urea; or, conversely, that 1 gm. of urea at 0° C. furnishes 370 cc. of nitrogen.

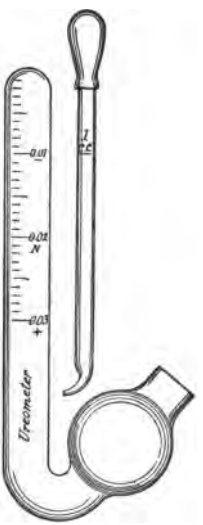


Fig. 17.—Doremus' ureometer.

Doremus' Method.—Various apparatus have been devised for the application of these methods of urea estimation, but Doremus' (Fig. 17) is most frequently used in this country. It is, unfortunately, exceedingly inaccurate, and its results should not be relied upon in cases in which the determination of urea is of vital importance.

The apparatus consists of a bulb with an upright graduated tube, and a small nipple pipet holding 1 cc. of urine. Each of the small divisions on the tube represents 0.001 gm. of urea in 1 gm. of urine, or $\frac{1}{10}$ of 1 per cent. Each of the large divisions represents 0.01 gm. of

urea in 1 gm. of urine, or 1 *per cent. of urea.* The apparatus is supposed to read correctly at average room-temperature and average pressure.

The bulb is filled with the hypobromite solution, the apparatus being inclined so as to remove the last air-bubble from the closed part of the tube. The pipet is then filled with urine accurately up to the 1 cc. mark. Care should be taken after filling the pipet with urine to wipe it dry, so as to remove all the adhering urine, and also to see that no drop escapes from the point before it is introduced into the bend of the tube. The point of the pipet is introduced into the bend of the tube just as far as it will go, and the nipple is slowly and gently compressed, so as to expel all the urine, but not to expel any air out of the nipple into the gas tube. The evolution of the nitrogen is allowed to proceed at the upper closed end of the tube, the apparatus being set aside until no more froth or bubbles are observed. The level of the fluid is then read upon the tube. Two forms of this apparatus are made—one graduated to read fractions of a gram per cubic centimeter of urine, the other to read the number of grains of urea per fluidounce of urine. In using the former, each of the large divisions corresponds to 1 per cent., each of the small divisions to $\frac{1}{10}$ per cent. of urea.

In using the Doremus apparatus the urine must be free from sugar and albumin, and the instrument should be allowed to stand a sufficient length of time after if has been filled until the level of gas remains stationary. The apparatus made with a base for support is the best.

Knop's solution of hypobromite is prepared by taking 70 cc. of a 30 per cent. solution of sodium hydrate, diluting it with 180 cc. of water, and then adding 5 cc. of bromin and stirring until all the bromin has been

dissolved. This solution keeps for about ten days if kept in a cool, dark place. The alkaline solution must be cold, and the bromin should be added out-of-doors or under a laboratory hood. Ammonia may be inhaled if necessary to neutralize the irritating fumes of bromin. Another way of preparing the hypobromite solution is by dissolving 100 gm. of caustic soda in 250 cc. of water and adding 25 cc. of bromin.

Rice's solutions for the hypobromite method offer a convenient means of obtaining the same result without the disagreeable necessity of handling pure bromin. These solutions are put up in separate bottles, one containing the alkali, the other, the bromin. Five cc. of each of these solutions and 20 cc. of water make up the fluid to be poured into the Doremus apparatus. These solutions keep well and are very useful for clinical work.

The formulas for Rice's solutions are:

(1)	Caustic soda	100 gm.
	Distilled water	250 "
(2)	Bromin	30 "
	Potassium bromid	30 "
	Water	240 "

Hinds' modification of Doremus' apparatus (Fig. 18) is more accurate and convenient than the original form, as the urine is measured more exactly and no nitrogen is lost by escape from the bulb.

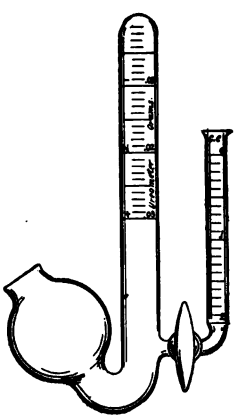


Fig. 18.—Hinds' modification of the Doremus ureometer.

A number of other methods have been devised for the determination of urea. Most of these methods are too elaborate for clinical work. Hüfner's method, however, is comparatively simple and far superior in accuracy to Doremus'.

Hüfner's Method.—The principle of this method is similar to that of the Doremus—*i. e.*, the decomposition of urea into nitrogen, carbon dioxid, and water by sodium hy-

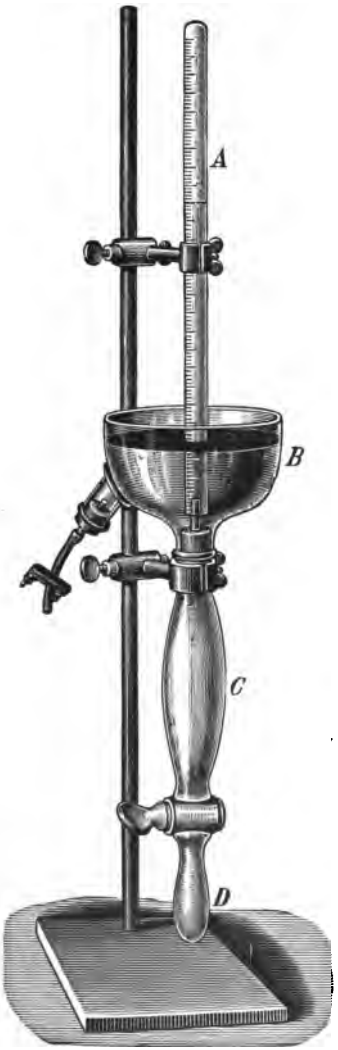


Fig. 19.—Hüfner's apparatus for estimation of urea.

pobromite. This is much more accurate than the Doremus method and is practically sufficiently exact for all clinical purposes, provided it be performed correctly and due allowances be made for barometric pressure, etc.

Hüfner's apparatus (Fig. 19) consists of a cylindric vessel (*C*), separated from a smaller receptacle below (*D*) by a glass stop-cock. An open dish (*B*) fits upon the upper end of *C* in such a way that the upper end of *C* protrudes into *B*, a rubber stopper making the joint water-tight. The eudiometer tube (*A*), which is about 40 cm. long and 2 cm. in diameter, is graduated in tenths of a cubic centimeter. This tube is held in position bottom up over the protruding upper end of *C* by a clamp, the whole apparatus being attached to a stand.

The solution required is the same as that used in the Doremus method (the first formula given on p. 139 should be used), or if desired, Rice's solutions may be employed with Hüfner's method.

Method of Estimation with Hüfner's Apparatus.—The urine is first diluted so that it roughly contains not more than 1 per cent. of urea. The dilution is carried out by estimating the urea probably present from the specific gravity (see p. 135). By means of a long pipet exactly 5 cc. of diluted urine are filled into the lower receptacle, carefully avoiding the entrance of any urine into the upper chamber. The pipet is carefully washed with distilled water and the washings are also allowed to flow into *D* until that receptacle is filled up to the stop-cock. The latter is now closed, and hypobromite solution is poured into the upper chamber *C*. The dish above it and the gas tube *A* are filled with saturated sodium chlorid solution and the tube inverted into the dish is placed in position exactly over the upper end of the hypobromite chamber with the aid of the clamp. The stop-cock is now opened, and as the hypobromite solution is heavier than the diluted urine, it flows into the lower receptacle, mixes with the urine, and develops CO_2 and N. The carbon dioxid is absorbed by the sodium hydrate and the nitrogen rises into the gas tube *A*. The apparatus is allowed to stand for about twenty minutes. The eudiometer tube is tightly shut with the thumb immersed into the dish, and is removed from the apparatus and placed in a deep receptacle with distilled water at room temperature, in which it is allowed to remain for fifteen minutes. The level of the gas is then read by holding the tube in the distilled water so that the level of the contents of the tube and that of the water are equal. The temperature of the water and the barometric pressure are noted. The volume of gas thus read must be reduced to standard temperature (0° C.), standard barometric pressure (760 mm.), and absolute dryness for exact determinations. For this purpose the tables on pages 142 and 143 (Jolles, quoted by Klopstock and Kowarsky) may be used.

While these tables were intended originally for use with Jolles' apparatus, they are equally useful with Hüfner's. They are the means of saving tedious and elaborate calculations, and render the readings of the apparatus more accurate than when corrections for temperature and pressure are neglected. The tables assume that 2.5 cc. of urine have been used in the test, and the volume N. read must be reduced first to correspond with this, as is shown on page 143.

TABLE FOR THE

Showing the grams of urea per liter represented by 1 cc. of nitrogen at

Height of Barometer.	10°.	12°.	14°.	15°.	16°.
700.....	0.934	0.943	0.926	0.922	0.917
702.....	0.945	0.937	0.929	0.925	0.920
704.....	0.948	0.940	0.932	0.927	0.923
706.....	0.951	0.943	0.934	0.930	0.926
708.....	0.954	0.945	0.937	0.932	0.928
710.....	0.957	0.948	0.939	0.935	0.931
712.....	0.959	0.951	0.942	0.938	0.933
714.....	0.962	0.953	0.945	0.941	0.936
716.....	0.964	0.956	0.948	0.944	0.938
718.....	0.967	0.959	0.951	0.946	0.941
720.....	0.970	0.962	0.953	0.949	0.944
722.....	0.973	0.964	0.956	0.951	0.947
724.....	0.975	0.967	0.958	0.954	0.950
726.....	0.978	0.970	0.961	0.957	0.952
728.....	0.981	0.973	0.964	0.959	0.955
730.....	0.984	0.975	0.967	0.962	0.957
732.....	0.987	0.978	0.969	0.965	0.960
734.....	0.989	0.981	0.972	0.968	0.963
736.....	0.992	0.983	0.975	0.970	0.966
738.....	0.995	0.986	0.977	0.973	0.969
740.....	0.998	0.989	0.980	0.975	0.971
742.....	1.000	0.992	0.982	0.978	0.974
744.....	1.003	0.994	0.985	0.981	0.976
746.....	1.005	0.997	0.988	0.983	0.979
748.....	1.008	0.999	0.991	0.986	0.981
750.....	1.011	1.002	0.993	0.989	0.984
752.....	1.014	1.005	0.996	0.992	0.987
754.....	1.017	1.008	0.999	0.994	0.990
756.....	1.019	1.011	1.001	0.997	0.992
758.....	1.022	1.013	1.004	0.999	0.995
760.....	1.025	1.016	1.007	1.002	0.998
762.....	1.028	1.018	1.010	1.005	1.000
764.....	1.030	1.021	1.012	1.008	1.003
766.....	1.033	1.024	1.015	1.011	1.006
768.....	1.036	1.027	1.017	1.013	1.008
770.....	1.039	1.029	1.020	1.016	1.011

To use the table, determine the temperature of the water in Centigrade and the barometric pressure at the time of observation. Find the figure in the proper column, giving the correction coefficient, and multiply this coefficient by number of cubic centimeters of nitrogen read upon the tube of the apparatus. If more or less than 2.5 cc. of urine has been used, make proper allowances. The result is the corrected amount of urea in grams per liter.

ESTIMATION OF UREA

various temperatures (Centigrade) and at different barometric pressures.

17°.	18°.	19°.	20°.	21°.	23°.	25°.
0.913	0.909	0.904	0.900	0.895	0.886	0.877
0.916	0.911	0.907	0.903	0.898	0.889	0.879
0.919	0.914	0.909	0.905	0.901	0.891	0.882
0.921	0.917	0.912	0.908	0.903	0.894	0.885
0.924	0.920	0.915	0.910	0.906	0.897	0.887
0.927	0.922	0.917	0.913	0.909	0.899	0.890
0.929	0.925	0.920	0.916	0.911	0.902	0.892
0.932	0.927	0.923	0.919	0.914	0.904	0.895
0.934	0.930	0.926	0.921	0.916	0.907	0.897
0.937	0.933	0.928	0.924	0.919	0.910	0.900
0.940	0.935	0.931	0.927	0.921	0.912	0.903
0.943	0.938	0.933	0.929	0.924	0.915	0.905
0.945	0.940	0.936	0.932	0.927	0.917	0.908
0.948	0.943	0.938	0.934	0.930	0.920	0.910
0.951	0.946	0.941	0.937	0.933	0.922	0.913
0.954	0.949	0.944	0.939	0.935	0.925	0.915
0.956	0.951	0.947	0.942	0.938	0.928	0.918
0.959	0.954	0.950	0.945	0.940	0.931	0.921
0.961	0.957	0.952	0.947	0.943	0.933	0.923
0.964	0.959	0.955	0.950	0.945	0.936	0.926
0.967	0.962	0.957	0.952	0.948	0.938	0.928
0.969	0.964	0.960	0.955	0.951	0.941	0.931
0.972	0.967	0.962	0.958	0.953	0.944	0.934
0.975	0.970	0.965	0.961	0.956	0.946	0.937
0.977	0.973	0.968	0.963	0.958	0.949	0.939
0.980	0.975	0.970	0.966	0.961	0.951	0.942
0.982	0.978	0.973	0.968	0.963	0.954	0.945
0.985	0.981	0.975	0.971	0.966	0.957	0.947
0.988	0.983	0.978	0.974	0.969	0.959	0.950
0.991	0.986	0.981	0.976	0.971	0.962	0.952
0.993	0.988	0.984	0.979	0.974	0.964	0.955
0.996	0.991	0.987	0.981	0.976	0.967	0.957
0.999	0.993	0.989	0.984	0.979	0.969	0.960
1.001	0.996	0.992	0.987	0.981	0.972	0.963
1.004	0.999	0.994	0.989	0.984	0.974	0.965
1.006	1.002	0.997	0.992	0.987	0.977	0.968

Thus:

Volume, of N. = 9.3 cc.
Dilution was 1 : 4 (1.25 cc. urine and 3.75 cc. water).
Hence 9.3 × 2 = 18.6 cc. N. in 2.5 cc. urine.
Temperature, 16° C. } Coefficient = 0.998.
Barometer, 760 }
0.998 × 18.6 = 18.56 grams urea per liter.

Absolute Analytic Methods.—For physiologic studies and for accurate scientific work urea is determined by more elaborate methods, which will be found described in the larger works. Of these, the Moerner-Sjöqvist method is probably the best. By the use of this method all the nitrogenous constituents of the urine except the urea and ammonia are precipitated by means of alcohol and ether after the addition of a solution of barium chlorid and barium hydrate, and finally the urea is determined in the concentrated filtrate by Kjeldahl's nitrogen method after driving off the ammonia. Kjeldahl's method consists in converting all the nitrogen of organic compounds into ammonia by the use of sulphuric acid and heat. The ammonia is distilled over and collected in standard sulphuric acid. These methods are not employed in ordinary clinical work.

QUESTIONS ON CHAPTER VIII

Nitrogen.—What does the total nitrogen output indicate? What do we mean by the "nitrogen balance"? What factors influence the nitrogen output?

Urea.—What is urea chemically? What is its physiologic significance? How does the amount of urea vary in health? In disease? What is the normal amount excreted in twenty-four hours in grains? In grams? What is the normal percentage of urea in urine? Describe a test for the presence of urea in a liquid. How can the amount of urea be approximately deduced from the specific gravity, and under what conditions?

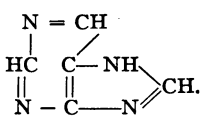
What is the principle of the hypobromite method?

Describe Doremus' apparatus. How is it used? What solution is employed? What precautions are observed in using it? What is Hinds' modification? Describe the method of Hüfner.

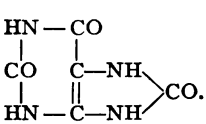
CHAPTER IX

THE PURIN BODIES: URIC ACID AND ITS CHEMICAL CONGENERS

NEXT to urea, the urine contains a group of nitrogenous compounds much more complex in structure, which are regarded as derived from a chemical group to which E. Fischer gave the name of "purin." According to this author the purin bodies (also known as the xanthin or alloxur bodies) are derived from a hypothetic, chemical molecule, "purin," $C_5H_4N_4$, containing carbon and hydrogen atoms arranged in a ring. The ring, which is called "the purin ring," is the same for uric acid and the other members of this group, the differences being merely the substitution of one or more of the hydrogen atoms of the ring by different radicals. The purin ring has the formula:



The purin bodies (including uric acid) are derived from this by the substitution of hydroxyl, amid, or alkyl groups for the various hydrogen atoms—*e. g.*, uric acid is—



Uric acid is the only constituent of this group that has slight acid properties. All the other compounds are bases known, therefore, as *purin bases* (also xanthin bases or alloxuric bases).

A. URIC ACID

Uric acid ($C_5H_4N_4O_3$) is a nitrogenous compound formed in the body by the decomposition of nucleins of the nuclei of both tissue cells and food-stuffs. Normally, small amounts of uric acid (0.7 to 0.75 gm. in twenty-four hours) are excreted in the urine. The substance crystallizes in the shape of rhombic, rectangular prisms, wedge or whetstone shape, of a color varying from the palest yellow to a deep yellowish red (Plate 4).

Pure uric acid is soluble in 16,000 parts of cold water and in 1600 parts of boiling water. Impure uric acid is more readily soluble in water. It is not acid in reaction in cold solutions when tested with litmus-paper. It is insoluble in alcohol and ether, but soluble in warm glycerin, insoluble in strong mineral acids, but soluble in alkalis. It is more soluble in solutions of urea than in water. On boiling it reduces alkaline solutions of copper, first giving a white precipitate of cuprous urate.

The Origin of Uric Acid.—As has already been said in the definition of uric acid, this body is a product of oxidation of the nuclein bases. Horbaczewski regarded it especially as derived from the nuclei of the leukocytes. It has been shown, however, that the other cells of the organism containing nuclei and, therefore, nucleins, may also be the source of uric acid, and that, in fact, the leukocytes give origin to a very small fraction of the uric acid output. The greatest source of uric acid are the nuclein or the purin

PLATE 4

URIC-ACID CRYSTALS; NORMAL COLOR (*after Peyer*).

compounds contained in the food. The amount of uric acid excreted is, indeed, to be measured only in the light of our knowledge of the amount of nuclein-containing food that is eaten.

The old idea that uric acid is derived from all proteids of the food is wrong, for when very large amounts of proteid food containing no nuclein are given, there is no increase of uric acid. On the other hand, when large amounts of nuclein are given in the food (organs containing many nucleated cells, like the pancreas, brain, etc.) there is always an increase in the excretion of uric acid.

The term *endogenous uric acid* is applied to the uric acid derived from the nucleins of body cells, while the term *exogenous uric acid* is used to designate that derived from the nucleins of the food. There is also a very small amount of uric acid formed synthetically in the body, as is the case in birds and in certain reptiles. In these animals uric acid is the chief nitrogen compound excreted in the urine, and is probably derived from urea by synthesis.

Two theories are held as regards the place where uric acid is formed: Garrod claims that uric acid is not only excreted, but *formed* in the kidneys, but the view generally held to-day is that it is formed in the *tissues*, especially in the *liver* and *spleen*, and merely *excreted* by the kidneys. The latter view is supported by the following facts: Uric acid is found in the blood normally and continues to be formed there after extirpation of the kidneys. It is greatest in amount during digestion, when the liver and spleen are most active, and it accumulates in the blood and tissues in gout and anemia, when the excretion is diminished.

Pathologic Significance.—A great deal of nonsense

has been written about uric acid and its pathologic significance. The cause for the widespread misapprehension of the real significance of uric acid lay in the misunderstanding of its formation, and in confusing really normal conditions with pathologic states, or in attributing to uric acid symptoms which had quite a different origin. In the light of modern knowledge, an increased elimination of uric acid has a far less serious significance than it had before modern research had demonstrated the mechanism of uric acid formation. No conclusion should be drawn from an increased excretion of uric acid without a previous knowledge of the patient's diet with reference to the presence of nucleins therein. Moreover, it has been shown (Croftan) that the kidneys can destroy uric acid and the urinary uric acid cannot always be considered as an index to the amount of uric acid in the circulation. The urinary uric acid merely represents the algebraic sum of the uric acid circulating in the blood and that destroyed by the kidneys.

Uric acid is *increased* in the following conditions (Serkowski):

(1) After a proteid diet rich in nucleins.

(2) After drinking coffee or caffein-containing beverages.

(3) After the use of salicylic acid or sodium salicylate.

Uric acid is *diminished:*

(1) After a vegetable diet.

(2) After eating yolks of eggs, milk, and dairy products.

(3) After the ingestion of cherries and fruit containing quinic acid.

(4) After the use of artificial proteid foods containing no nuclein.

(5) After drinking saline and alkaline mineral waters (increase of alkalinity of blood and of oxidation).

(6) After the administration of a number of drugs, among which are colchicum, citric acid, potassium iodid, urotropin, etc.

(7) In lead-poisoning.

Uric acid is increased in fevers and in all conditions in which there is rapid wasting of tissues. This increase is at the expense of the endogenous uric acid. After severe muscular exercise, great fatigue, and during the attacks in gout there may be also an increase of uric acid. Whenever there is a leukocytosis (as after a full meal or in septic infection), the destruction of leukocytes leads to an increase of uric acid. In leukemia there is a marked increase of uric acid (4.2 gm. in twenty-four hours, Bartels), and the same is true of cirrhosis of the liver (8 gm. in twenty-four hours, Bouchard). The cause of this increase may lie in the interference with the uric-acid-forming function of the liver. In pseudoleukemia there is no increase in uric acid.

An increase of uric acid may occur in neurasthenia, together with a phosphaturia. In pernicious anemia the uric acid is increased (due to diminished metabolism and to leukocytosis), while in mild forms of anemia, chlorosis, etc., there is a diminution of uric acid.

A decrease in the elimination of uric acid may be due to a retention of this product, owing to an insufficient excretion or to an imperfect oxidation. In chronic kidney diseases and other conditions diminishing the amount of solids eliminated, after prolonged drinking of large amounts of water, after chronic wasting diseases in which metabolism is lowered, the uric acid may also be diminished.

The **relation of uric acid to gout** has been placed in a doubtful position by recent investigations. Gout has

been considered for a long time to be due to the increase of uric acid in the blood, but it has been found recently that such an increase does not always take place. The amount of uric acid in acute gout may be high, but is more often low, and never so high as in leukemia. The amount of uric acid excreted in the urine is so variable that it is of practically little clinical importance, especially with a mixed diet. A meal composed of food rich in nuclein will increase the uric acid in the urine more than an attack of gout.

Apparent Increase.—It is important to apprehend properly what actually constitutes an increase of uric acid in the urine. A common error is to consider urine of high specific gravity, acid in reaction, which deposits uric-acid crystals and urates, as a specimen of increased elimination of uric acid. The presence of uric-acid crystals or of a deposit of urates does not by any means indicate necessarily an excess of uric acid; in fact, such deposits may occur when a urine is cooled and undergoes "acid fermentation." Urines of high concentration (in persons who drink little water, etc.) and of high acidity are very apt to deposit uric acid.

A *true increase* of the uric acid in the urine can be detected only by quantitative methods. While such an increase may be accompanied by a deposit of uric-acid crystals, this deposit alone is no evidence of an increased elimination of this substance.

The cause of this seemingly paradoxic fact is that under ordinary conditions nearly all the uric acid exists in the urine in combination with sodium and other bases (see Urates, p. 154) as urates. Of these urates, those known as the neutral salts are readily soluble in the urine, while

others, known as the acid urates, are much less soluble. Uric acid itself is almost insoluble in urine. As a comparatively slight influence—an increase in acidity—will convert the neutral urates into the acid, or even into uric acid, it will be seen that a deposit of urates or of uric acid depends not so much upon the amount of these substances as upon the solubility of the particular types found in a urine, a solubility affected by cold and the presence of high acidity.

Normal urine contains about 0.2 to 1.25 gm. of uric acid in twenty-four hours, the average being 0.7 gm. The proportion of uric aid to urea is about 1:45.

Detection.—(*a*) A few crystals of the material are placed on a slide and dissolved in dilute sodium hydrate. A drop of dilute hydrochloric acid is added under the cover-glass, and typical crystals of uric acid will form.

(*b*) *Murexid Test.*—A small quantity of the specimen is treated with nitric acid in a small porcelain dish, and gently warmed until a yellowish-red residue is left. A very small quantity of ammonia is added, when the color will change to a beautiful purple, changing to a deep blue on the addition of a little sodium hydrate. The color disappears on warming and does not reappear on evaporating. In the case of xanthin and guanin, the color returns on evaporation.

(*c*) *Silver Test.*—Uric acid is dissolved in dilute sodium carbonate and dropped upon a paper moistened with silver nitrate. A dark spot of reduced silver is produced.

(*d*) *Copper Test.*—Uric acid dissolved in dilute sodium hydrate and treated with Fehling's solution gives a precipitate of white cuprous oxid and, finally, of red cuprous oxid.

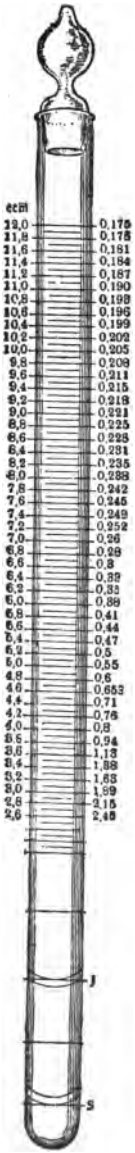


Fig. 20.—Ruhemann's uricometer.

Quantitative Estimation of Uric Acid.—There is no convenient clinical method for the estimation of uric acid which is at the same time accurate. The following methods are described because they are less troublesome than any others devised:

Heintz's Method.—To 200 cc. of filtered urine free from albumin add 10 cc. of hydrochloric acid. Allow this to stand for twenty-four hours in a cold place; collect the uric-acid crystals on previously dried and weighed filter-paper; wash once or twice in cold distilled water; dry the whole at about 100° C., cool, and weigh. By subtracting the weight of the filter-paper the weight of the uric acid in 200 cc. of urine is obtained. This method is only approximate.

Ruhemann's Method.—In 1902 Ruhemann suggested a colorimetric method of estimating the amount of uric acid in urine based on the titration of uric acid with iodin with the aid of carbon disulphid. A graduated tube with a glass stopper, called the *uricometer*, is used for this test. One cm. above the bottom of this tube is a line marked S, and 2.7 cm. over this is another line

marked J, the distance between them being divided into two by another line. Above the line J is a scale indicating parts of uric acid per 1000, beginning below at 2.45 and ending above at 0.175 (Fig. 20).

The tube is filled to S with pure carbon disulphid, the lower meniscus touching the line. The iodin solution (iodin, 1.5; potassium iodid, 1.5; absolute alcohol, 15; distilled water, 185) is poured over this up to the mark J, and enough urine is added to reach the mark 2.45, and then, drop by drop, closing the tube and shaking it after each addition. The carbon disulphid, absorbing the free iodin, becomes coppery-brown; the rest of the fluid assumes the color of urine. On the addition of further amounts of urine, drop by drop, the carbon disulphid becomes violet, pinkish violet, pink, pale pink, and finally milky white, the foam taking part in the color changes. The end-reaction is shown by a pale-pink color in the carbon disulphid. The stopper should be removed cautiously, and the foam got rid of by gently shaking the tube from side to side. The scale is read directly, showing the number of parts of uric acid per 1000 of urine, or grams per liter. If the urine has very little uric acid, then only half the amount of iodin solution should be used, the rest of the space up to J being filled with distilled water and the figures divided by two. If an excess of uric acid is present, twice the amount of iodin is used and the result multiplied by two. The urine must be acid and free from albumin. This method has been found in the author's hands to give a considerable percentage of error, but it is recommended by some clinicians. There are a number of substances in the urine capable of forming compounds with iodin and interfering

with this test, especially bile, albumin, blood, antipyrin, aspirin, and diacetic acid. Their presence should be excluded by appropriate tests.

Folin-Hopkin's Method.—To 100 cc. of urine are added 10 gm. of ammonium acetate, and ammonia is added, drop by drop, until a slight ammoniacal odor is perceived. The mixture is set aside for three hours and then filtered. The sediment is washed six to eight times with a saturated solution of ammonium carbonate and then transferred by means of hot distilled water to a beaker by means of a wash bottle. Then 15 cc. of concentrated H_2SO_4 are added (specific gravity 1,845) and the whole is titrated with $\frac{1}{20}$ potassium permanganate standard solution until the first appearance of pink, which diffuses throughout the liquid and persists for a few seconds. Each cubic centimeter of $KMnO_4$ used equals 3.648 mg. of uric acid, the total product representing the amount of uric acid in 100 cc. of urine. This is the most satisfactory method among the simpler ones thus far devised.

URATES

As has already been mentioned, nearly all the uric acid of the urine is in combination with sodium, potassium, ammonium, calcium, and magnesium, in the form of urates. These are very soluble at body-temperature, but are precipitated, on cooling the urine, in the form of amorphous granules.

Uric acid is dibasic, forming neutral and acid salts, and the acid salts are much less soluble than the neutral. They therefore form the bulk of the precipitate, while the neutral or less acid salts remain in solution. Urine which remains clear for some time on standing at room-temperature often contains a large proportion of the neutral urates.

When an acid is added to such urine, the urates become acid, consequently insoluble, and precipitate in a finely granular form, with the result that the urine becomes decidedly opaque. This is the cause of the opacity ob-

served with the nitric acid test for albumin and of the opaque zone occurring in Heller's test. Heat dispels this precipitate. If the urine mixed with acid be allowed to stand, the sediment falls to the bottom in the form of crystals of acid urates, and if the acid be allowed to act long enough, crystals of uric acid are deposited.

B. THE PURIN (XANTHIN OR ALLOXUR) BASES

We have already referred to the general chemical characters of these bases on p. 145. They are derivatives of the nucleins, form with uric acid a part of the purin bodies of E. Fischer, are present in very small quantities in normal urine, and have the following composition (Hammarsten):

Xanthin	$C_5H_4N_4O_2$
Heteroxanthin and 1-methylxanthin	$C_6H_6N_4O_2$
Paraxanthin	$C_7H_8N_4O_2$
Guanin	$C_5H_5N_5O$
Hypoxanthin	$C_5H_4N_4O$
Adenin	$C_5H_5N_5$
Episarkin	$C_4H_6N_3O$ (?)
Carnin	$C_7H_8N_4O_3$
Epiguanin	$C_6H_7N_5O$

Origin of the Purin Bases.—Of these, xanthin, guanin, hypoxanthin, and adenin are the most important and are known as "the real nuclein bases." These have been shown by Kossel to be cleavage products of the nucleins. They are, therefore, decomposition products of the nuclei of the body cells.

The other purin bodies mentioned in the above list have not been so thoroughly studied, but three of them (heteroxanthin, paraxanthin, and 1-methylxanthin: Albanese, Bondzynski, etc.) which form the bulk of the purin bases

in the urine are derived from the theobromin, caffein, and theophyllin, and their compounds occurring in food (tea, coffee, cocoa, etc.).

The term *endogenous* purin bodies is used to designate those derived from tissue metabolism, while *exogenous* purins are derived from the food.

Clinical Significance.—Inasmuch as the purin bases are closely related to the nucleins, they are increased in the urine in all conditions in which there is either a *greater*

Fig. 21.—Xanthin crystals (after the drawings of Horbaczewski, as represented in Neubauer and Vogel).

ingestion of nucleated tissue (certain foods containing many nuclei, see Uric Acid, p. 147) or a *breakdown* of nuclei in the body.

Normally, the purin bases occur in the urine in very small quantities (Flatow and Reitzenstein, quoted by Hammarsten), 15.6 to 45.1 mg. in twenty-four hours.

They are increased in healthy persons by feeding upon food rich in nuclei and food containing derivatives of caffein, theobromin, etc.

In diseases the purin bases are especially increased in leukemia (destruction of nuclei of leukocytes).

QUANTITATIVE TEST FOR PURIN BODIES (URIC ACID + PURIN BASES)

The methods of quantitatively measuring the amount of uric acid and of purin bases are far too complex for routine clinical work, and, therefore, we shall content ourselves in describing an approximate clinical method which has become popular during the past few years. The method cannot replace the more accurate processes of estimating uric acid and other purin bodies as such, but may be used for comparison. The method is chiefly of value in regulating the patient's diet, diminishing his purin intake (exogenous purin), and then watching the diminution of the purin output. If this does not diminish in proportion within a reasonable time, we must conclude that there is an increase of endogenous purins eliminated.

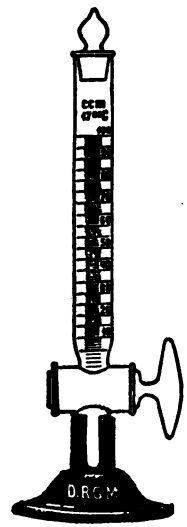


Fig. 22.—Purinometer.

Camerer's Method.—An apparatus is made under the name of *purinometer* (Fig. 22), in which the amount of purin bodies as a whole may be approximately estimated as follows (the apparatus consists of a graduated cylinder divided into two parts by a glass cock):

The total quantity of the urine must be known and well mixed. It is, if necessary, freed from albumin by boiling in slightly acid solution. With the tap at right

angles to the tube, 20 cc. of No. 1 solution are added to 90 cc. of urine. The phosphates are at once precipitated and the tap is turned parallel to the tube. In ten minutes the phosphates will have passed into the lower portion of the tube, and the tap is again turned at right angles and No. 2 solution added up to 100 cc. The resultant precipitate of silver-purin should be pale yellow. Incline the purinometer backward and forward until all the white silver chlorid is dissolved. If this does not occur, add a few drops of strong ammonia or use diluted urine. Place the apparatus in a cupboard away from the light, and read off the number of cubic centimeters occupied by the precipitate an hour later, although it is better to wait for twenty-four hours.

Solution No. 1 consists of:

Ludwig's magnesia mixture[1]	100 cc.
Ammonia (20 per cent.)	100 cc.
Talcum	10 gm.

Solution No. 2 consists of:

Silver nitrate	1 gm.
Ammonia (strong)	100 cc.
Talcum	5 gm.
Distilled water	100 cc.

A table is given with the apparatus for converting the reading in cubic centimeters to the amount of purin-nitrogen in twenty-four hours.

[1] Magnesium chlorid crystals..... 100 gm.
Water..... 1000 cc.
Ammonia, enough to give strong odor.
Ammonium chlorid, enough to dissolve the precipitate which forms.

This table shows the nitrogen percentage yielded by each cubic centimeter of the precipitate. Multiply this factor by the number of cubic centimeters contained in the twenty-four-hour urine divided by 100.

Cubic centimeters.	Percentage Purin-nitrogen.
4	0.0078
5	0.0097
6	0.0117
7	0.0136
8	0.0156
9	0.0175
10	0.0185
11	0.0195
12	0.0205
13	0.0218
14	0.0225
15	0.0234
16	0.0249
17	0.0260
18	0.0265
19	0.0270
20	0.0275
21	0.0283
22	0.0286
23	0.0299
24	0.0312
25	0.0325

Example.—The precipitate yielded 10 cc. This contains 0.0185 nitrogen. The total daily urine was 1120 cc. : 0.0185 × 11.2 = 0.2072 purin-nitrogen.

AMMONIA

The normal urine invariably contains small quantities of ammonia (NH_3), the amount averaging 0.7 gm. in twenty-four hours on a mixed diet, and representing from 3.5 to 5 per cent. of the total nitrogen (see p. 137).

Ammonia is normally present not only in the blood but also in the tissues, and is changed to urea in the liver and other organs. The relation of ammonia to the total nitrogen is a fairly constant one in health. In disease it is increased when the formation of urea is interfered with, as in cirrhosis of the liver. It furthermore seems to serve as the neutralizer of excessive acidity with which the body defends itself against the overproduction of acids (acidemia and acid intoxication), thus maintaining the alkalinity needed to sustain life.

The organic acids against which ammonia rises in this way are increased in persons living on a very abundant proteid diet (diabetics), in diseases accompanied with fever, in certain types of insanity, in diabetes (oxybutyric acid, diacetic acid), and in the toxemias of pregnancy (probably), especially the cases accompanied by persistent vomiting and those of "the pre-eclamptic state" and eclampsias. In all these conditions, therefore, there may be a more or less marked increase of ammonia, usually at the expense of the urea—*i. e.*, with a comparatively diminished urea percentage.

CREATIN AND CREATININ

Creatin and creatinin ($C_4H_9N_3O_2$ and $C_4H_7N_3O$) are found in normal urine, creatinin containing one molecule less of water than creatin. They are both derived from muscle-tissue of the body, [redacted]. The amount varies according to the waste of muscle and the meat ingested.

Normally, 0.6 to 1.5 gm. of creatinin are excreted daily, this amount being probably formed in the kidneys (Serkowski). To excrete 1.0 gm. of creatinin one must eat about 330 gm. of meat.

The amount of creatinin is increased in fever, [redacted]. It is

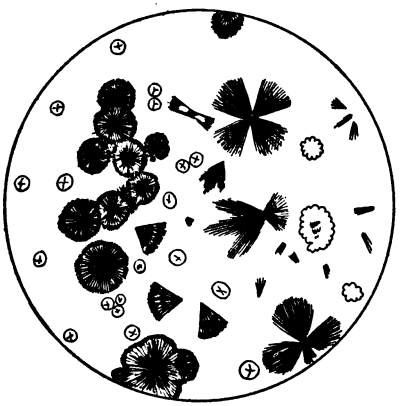


Fig. 23.—Crystals of creatinin-zinc chlorid (Salkowski).

diminished in cachexias, starvation, muscle-atrophy, and in chronic nephritis (Serkowski).

ALLANTOIN

Allantoin ($C_4H_6N_4O_3$) is found in the urine of newborn infants and also in adults, but in the latter in mere traces. It is increased by meat diet, by taking tannic acid, and in cases of diabetes insipidus and hysteria.

Allantoin is obtained from uric acid by oxidation—*e. g.*, with potassium permanganate—and is decomposed by heat and hydrochloric acid into allanturic acid and urea:

$$\underset{\text{Allantoin.}}{C_4H_6N_4O_3} + H_2O = \underset{\text{Allanturic acid.}}{C_3H_4N_2O_3} + \underset{\text{Urea.}}{CH_4N_2O}$$

NUCLEIC ACID

Mœrner found nucleic acid in very small quantities in the urine. Larger amounts appear in combination with

albumin as nucleo-albumin. These acids are interesting physiologically, as they are compounds of phosphoric acid, xanthin bases, and non-nitrogenous substances. They may contain as much as 9 or 10 per cent. of phosphorus. They do not give the reactions of proteids, but when some of them are boiled with dilute mineral acid, a carbohydrate substance is produced which gives the reduction test with copper.

HIPPURIC ACID[1]

Hippuric acid ($C_9H_9NO_3$) occurs in normal urine of man in quantities of from 0.1 to 1 gm. in twenty-four hours, varying largely according to the amount of vegetable food.

Fig. 24.—Hippuric-acid crystals (Jakob).

It is absent in the urine of carnivora and is very abundant in that of herbivora.

It crystallizes in fine needles or in four-sided prisms and pillars with ends beveled in two or four planes (Fig. 24).

[1] Hippuric acid is classified chemically as one of the aromatic group of substances in the urine. It is placed here for convenience of study.

The typical crystals are vertical rhombic prisms. Hippuric acid is soluble in water and alcohol, and its solutions are strongly acid in reaction. It combines with alkalis and alkaline earths to form soluble salts, but its silver, copper, and lead compounds are sparingly soluble in water. Strong acids precipitate it from solutions of its salts. On boiling with an alkaline hydrate hippuric acid decomposes into benzoic acid and glycocoll. This is clinically interesting because the same decomposition takes place in alkaline fermentation, especially in urine containing albumin, so that such urine is found to contain benzoic acid instead of hippuric. Hippuric acid is probably formed in the kidneys by the union of benzoic acid and glycocoll. Clinically it has no very great significance, its amount depending chiefly upon the diet. It is increased by vegetable diet and by the use of benzoic acid, salicylic acid, and their congeners; also in acute fevers, diseases of the liver, and diabetes.

Detection.—Add concentrated nitric acid to the urine and evaporate it to dryness. The residue heated in the test-tube gives the odor of bitter almonds, due to *nitrobenzol.* When an excess is present, hippuric acid may be separated in crystals by evaporating the urine to one-fourth its volume and adding some hydrochloric acid.

QUESTIONS ON CHAPTER IX

What are the purin bodies? What is the "purin" molecule? What purin body is an acid?

What is *uric acid?* Give an account of its origin. When is uric acid excretion increased? Diminished? What is its clinical significance? What can you say of the relation of uric acid to gout?

Give the general properties of uric acid. How is it detected? Describe Heintz's method; Ruhemann's method; Folin-Hopkins' method.

In what form does most of the uric acid occur in urine? What two varieties of uric acid salts occur in the urine? Which are the less soluble?

What causes the opacity observed on adding an acid to urine rich in neutral urates? How is the precipitate affected by heat?

What are the *purin bases?* Name some of the most important members of this group. What is their clinical significance? Describe the method of estimating the total amount of purin bodies.

How much *ammonia* appears normally in the urine? When is this amount increased? Decreased?

Whence are *creatin* and *creatinin* derived, and what causes their variations in urine?

Under what conditions has *allantoin* been found in the urine?

What element do the *nucleic acids* contain that is of interest, and in what amount? What do these acids form when boiled with dilute mineral acids?

What causes the variations in the amount of *hippuric acid?* What are the general properties of this acid?

Into what constituents does hippuric acid decompose on fermentation?

How is hippuric acid probably formed? When is it increased in the urine? How is it detected?

IV. ACETONE GROUP; INDICAN AND OTHER ETHEREAL SULPHATES; DIAZO-REACTION

CHAPTER X

ACETONE AND DIACETIC ACID

ACETONE

ACETONE (C_3H_6O) is a volatile compound belonging to the group of ketones, and may be present in large amounts in urine in disease. In health the urine contains traces of it, especially after the use of alcohol and of food rich in proteids.

Clinical Significance.—According to von Jaksch, pathologic acetonuria occurs as follows: (1) Febrile acetonuria, in scarlet fever, typhoid pneumonia, measles, small-pox, etc.; (2) diabetic acetonuria; (3) in certain forms of cancer, independently of inanition; (4) in starvation, especially in gastric ulcer and after the use of rectal feeding; (5) in mental diseases; (6) in auto-intoxication; (7) in derangements of digestion; (8) in chloroform narcosis.

Acetonuria is common in fevers. In diabetes its appearance shows an advanced stage, but it must be remembered that the predominance of nitrogenous food in diabetics tends to produce acetone. Cerebral irritation may give rise to persistent acetonuria.

Preparation of Urine for Acetone Tests.—The first requisite for accurate tests for acetone is that the urine be *freshly voided.* There should be no preservatives in such urine, especially *no thymol,* as this interferes with acetone reactions.

If the urine proves negative to the ordinary acetone tests it should be distilled and the distillate tested again. *Without testing the distillate no negative acetone reaction should be accepted as final.* This especially

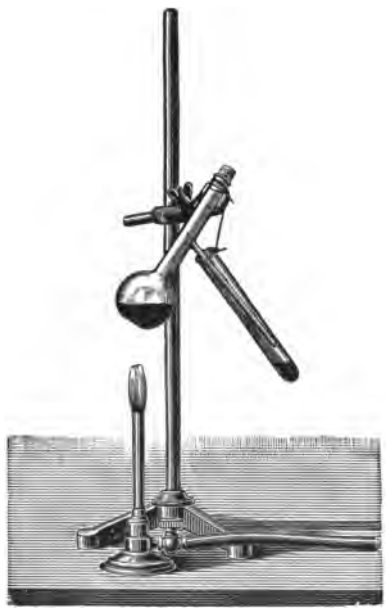


Fig. 25.—Simple apparatus for distilling urine (Sahli).

applies in cases of diabetes and of the toxemia of pregnant women. Small traces can be detected with the distillate when the urine proves negative. An easy method of distilling the urine is described by Sahli as follows, and can be used by the practitioner without any trouble:

"About 50 cc. of urine acidified with a little phosphoric acid (sufficient for a marked Congo reaction, to prevent foaming) are poured into a fractionation flask (Fig. 25) and heated to gentle boiling, preferably over a water-bath or over a wire gauze. A test-tube is then slipped over the

projecting arm for a receiver and fastened with a piece of string or wire. The upper, open end of the flask is closed with a cork. The distillate will now collect in the test-tube without any special cooling apparatus. Within a few minutes several cubic centimeters will have distilled over, and the acetone test can then be performed upon the distillate."

"Hoppe-Seyler claims that if diacetic acid is also present the distillation will produce acetone artificially, so that in such an event another method must be selected. Instead of being distilled the urine is rendered slightly alkaline, and then extracted with pure ether, the ether shaken with water, and the test performed upon the aqueous solution."

Detection.—Legal's Nitroprussid Test.—This is not a delicate test, but suffices for clinical purposes, as a rule. It should be repeated with the distillate if the urine is negative. To the urine or the distillate a few drops of concentrated watery solution of sodium nitroprussid are added. (The latter should be freshly prepared.) Then a solution of sodium hydrate is added till strongly alkaline. A ruby-red color changing to yellow is seen, which may be due to acetone or to creatinin. If due to acetone, the addition of glacial acetic acid to excess will turn the fluid to a purple red and finally to a violet color. If creatinin caused the reaction, the red turns to yellow and to green, finally to blue. The test is not positive for acetone unless a purple-red color is obtained.

Jackson-Taylor's Modification of Legal's Test.—Jackson-Taylor[1] modified the above test as follows: Equal parts of urine and of the sodium nitroprussid solution are mixed in a test-tube. Over this mixture strong ammonia is layered by pouring it along the side of the tube (see Heller's Albumin Test, p. 61). When no acetone is present there will be no ring, or only a faint orange-brown ring at the point of contact of the two fluids. The presence of acetone is evidenced by a magenta or petunia-

[1] "Lancet," 1907, iii, 23.

red ring, which may spread upward in the ammonia. This test has proved most satisfactory in the hands of the present writer, and is to be recommended for routine work. No acetic acid is used in this test.

Lieben's Iodoform Test.—This is best applied to the distillate of the urine. Half a liter of urine is treated with phosphoric acid or hydrochloric acid in the proportion of 3 to 100 cc. The mixture is partly evaporated into the distilling apparatus, and the process completed in a retort. The acid is added to prevent the evolution of gases. Lieben's test consists in adding a small amount of potassium hydrate solution to the distillate, and then a few drops of a solution of iodin and potassium iodid (iodin, 1; potassium iodid, 2; distilled water, 50). Acetone, if present, gives a yellowish precipitate of iodoform at once. Alcohol gives the same precipitate, but more slowly. For this reason Gunning uses ammonium hydrate and tincture of iodin, which react only with acetone, and not with alcohol. The crystals are recognized under the microscope by their characteristic shape (flat hexagons, often arranged in star-like groups—Fig. 26), and the odor of iodoform also helps in detecting acetone.

Gunning's Iodoform Test.—This is a modification of Lieben's, designed to avoid confusion of acetone with alcohol in the urine. An alcoholic solution (tincture) of iodin and a little ammonia water are added to the distillate or to the urine itself (the latter is not so satisfactory). If alcohol is present, no precipitate will occur, while acetone produces a precipitate of iodoform and of iodin, the latter falling as a black sediment even if there be no acetone present. A large amount of acetone in the urine will cause the disappearance of the black iodin sediment after a while,

but if there be little acetone, the iodin sediment is found at the bottom of the test-tube, with a layer of iodoform in yellow crystals lying over it in a thin stratum (Fig. 26). The mixture must not be warmed while there is any nitrogen iodid in it—*i. e.*, for twenty-four hours—on account of the danger of an explosion.

Reynold's Test.—Acetone dissolves freshly precipitated mercuric oxid. Yellow mercuric oxid is precipitated from a solution of mercuric nitrate in a test-tube by the addition

Fig. 26.—Crystals of iodoform.

of an alcoholic solution of potassium hydrate. The urine is added and the mixture shaken. The liquid is filtered until it passes through clear. To the filtrate ammonium sulphid is carefully added. If acetone is present, some mercuric oxid has been dissolved, and is shown in the filtrate by a dark ring of mercuric sulphid at the contact of the two liquids.

Frommer's Test.—Ten cc. of urine in a test-tube are treated with 1 gm. of potassium hydrate (the solid sub-

stance) and without waiting for a solution to take place from 10 to 12 drops of a 10 per cent. alcoholic solution of salicylaldehyd (salicylous acid) are added. The mixture is heated gently to 70° C. (not boiled). In the presence of acetone there appears a deep red ring at the bottom of the tube, at the line of contact of the two substances. This reaction is very delicate and does not take place with other substances than acetone.

DIACETIC ACID

Diacetic acid (aceto-acetic or ethyldiacetic acid, $C_6H_{10}O_3$) is probably derived from beta-oxybutyric acid (see p. 172). Diacetic acid is readily converted into acetone, alcohol, and carbon dioxid by the action of alkalis, a similar decomposition possibly taking place in the blood. It is a colorless liquid, which gives a deep-red color with ferric chlorid.

Clinical Significance.—Diacetic acid is now regarded as the most important body, clinically, of the acetone group. Folin[1] finds that diacetic acid is much more abundantly and more commonly present in urine than acetone, and that the tests for the latter really show diacetic acid. This acid should always be looked for in diabetes and in toxemias, in addition to, or even in preference to, acetone.

A mere trace of diacetic acid occurs normally in urine. It occurs also in starvation and in persons on a rigid non-carbohydrate diet. When carbohydrates are given, it disappears.[2]

The presence of diacetic acid is usually a serious symptom. It occurs in the urine in toxemias, and especially

[1] Journal A. M. A., May 2, 1908.

[2] This test of diet should be used in diabetics before drawing conclusions as to the significance of diacetic acid.

in diabetes and in fevers. In children with fever its presence is not of grave import. In diabetes its appearance is a poor prognostic sign. A form of auto-intoxication accompanied by diaceturia is sometimes rapidly fatal without any definite lesions.

Detection.—Von Jaksch's method is trustworthy. A solution of iron perchlorid is cautiously added to the urine. If phosphates precipitate, they are removed by filtering, and some more of the reagent is added. A characteristic Bordeaux-red color appears, but this color may be produced with the same reagent in the presence of salicylic acid, carbolic acid, and other substances. If the urine be previously boiled, diacetic acid does not give the reaction, or gives a much fainter color, while the other substances continue to give it. If the deep Bordeaux-red color develops, therefore, when a portion of the original urine is boiled, the reaction is negative.

Another portion of the urine may be acidulated with sulphuric acid and extracted with ether. The ether extract is shaken with water and $Fe_2Cl._6$ added. A violet color appears in the layer of water if diacetic acid is present. If the color pales after standing for one or two days, diacetic acid was present. If the color reaction with the ethereal extract does not fade, the substance is probably beta-oxybutyric acid (see below), of which diacetic acid is a further oxidation. The urine to be tested should be perfectly fresh, as diacetic acid decomposes readily into acetone, etc., in urine on standing.[1]

[1] This process is attributed to Gerhardt by Purdy.

BETA-OXYBUTYRIC ACID

Beta-oxybutyric acid ($C_4H_8O_3$) sometimes accompanies diacetic acid and acetone in urine. It is the substance from which diacetic acid is derived. Acetone, in turn, is formed from diacetic acid. It forms an odorless, colorless, transparent syrup with aqueous vapor, and rotates polarized light to the left. It gives no reaction with ferric chlorid, but is easily convertible into acetone and diacetic acid by oxidizing.

Clinical Significance.—This substance is met with in large amounts in severe forms of diabetes and in small amounts in scurvy, scarlet fever, measles, in the starving, the insane, in cancer patients dying from coma, and in persons living exclusively on meat and fat. In diabetes it is probably the cause of the acid intoxication which usually precedes and accompanies diabetic coma. Herter found that the persistent appearance of more than 25 gm. of this acid indicates impending coma.

It is found in the blood of diabetic patients and is a homologue of lactic acid. It is formed from diseased muscle as lactic acid is from healthy, but the steps by which it is formed are unknown. Its presence in the blood has given rise to the alkaline treatment of severe diabetes, which is supposed to neutralize the acid in the blood.

Detection.—There is no simple reaction for this member of the acetone group. If the urine is fermented with yeast and tested for its polarity, a strong left-hand rotation indicates the probable presence of this acid. A better way is to ferment the ether extract of urine, then to acidify the fermented fluid with phosphoric acid, to extract with ether, and to test the extract with the polariscope.

If levorotatory, β-oxybutyric acid is quite surely present (Emerson). The process of isolation is tedious.

QUESTIONS ON CHAPTER X

What is *acetone?* Is it present in healthy urines; if so, under what conditions? What is its significance in disease? How should urine be prepared for acetone tests?

Describe Legal's test for acetone; Jackson-Taylor's modification; Lieben's test; Gunning's test; Reynold's test; Frommer's test.

What is *diacetic acid* and when does it occur in the urine? What does it augur in diabetes? How is is detected?

How do we differentiate the iron reaction of diacetic acid from that of salicylic acid, etc.?

Describe *beta-oxybutyric acid.* What is its clinical significance in the urine? From what tissues is it probably formed? How is it detected?

CHAPTER XI

INDICAN AND OTHER ETHERAL SULPHATES; DIAZO-REACTION

INDICAN

Indican (indoxyl-potassium-sulphate, $C_8H_6NO.SO_2.KO$) is formed from indol (C_8H_7N), a product of intestinal putrefaction of albuminous substances. The indol is absorbed from the intestine and becomes oxidized to indoxyl (C_8H_6NO), immediately combining with potassium sulphate (and also to a slight extent with sodium), forming indoxyl potassium sulphate, which is eliminated in the urine.[1]

Indigo-blue is formed by the oxidation of indoxyl-potassium-sulphate, thus:

$$\underset{\text{Indoxyl potassium sulphate.}}{2C_8H_6NKSO_4} + O_2 = \underset{\text{Indigo-blue.}}{2C_8H_5NO} = \underset{\text{Potassium hydrate sulphate.}}{2HKSO_4}.$$

Indigo-red, which has the same formula as indigo-blue, is also a product of oxidation of indican.

Clinical Significance.—Putrefaction goes on constantly in the normal intestine, except in the newborn, and so indoxyl is a constituent of normal urine. The quantity separated from the urine as indigo-blue is between 0.005 and 0.0025 gm. in twenty-four hours in normal man on a mixed diet. A milk diet gives very small amounts,

[1] For further data on the chemistry of indican, see p. 224.

while a liberal meat diet gives the largest amounts excreted in health.

Indicanuria has been the subject of study since 1853, when it was first described by Hill-Cassel. Many confusing and conflicting statements concerning excessive excretion of indican have appeared and are still appearing in medical literature. In the following the author intends giving the student a concise summary of the important facts to be borne in mind in this connection.

An excess of indican merely shows that there is putrefaction of proteids somewhere in the body. Usually this putrefaction takes place in the intestines, but in a smaller number of instances it may occur in other parts of the body.

Indicanuria may, therefore, be at once divided into two groups: (1) *Intestinal* and (2) *extra-intestinal.* The latter, which is the less important, may be dismissed briefly. It may occur as the result of proteid putrefaction in such conditions as putrescent cancer, pulmonary gangrene, empyema, putrid bronchiectasis, pulmonary tuberculosis with cavities, etc. In these conditions indol is formed and absorbed into the blood and is excreted as indican.

Intestinal indicanuria is dependent upon the putrefaction of proteids by the bacteria of the intestine. Trypsin has also something to do with this favoring putrefaction; for when the pancreatic duct is obstructed, indican greatly diminishes or disappears from the urine.

The experiments of Jaffé and of Ellinger and Prutz showed that indican is formed largely if not entirely in the *small intestine,* especially in its terminal portion, and not in the large gut. These observers tied the end of the small intestine in animals and produced a rapid and marked indicanuria. When this small gut was tied high up, where

there is comparatively little bacterial putrefaction, indicanuria was absent. These observers also state that obstruction of the large intestine does not produce indicanuria unless indirectly affecting the small intestine. Basing their ideas upon these researches, Leube, Jaksch, Senator, and especially Nothnagel insist that there is an important difference between the large and the small intestine in regard to the production of indican, and that obstruction or even ligation of the large intestine does not produce indicanuria. The influence of trypsin probably has to do with this difference in the behavior of the small and the large intestines, for trypsin acts in the former, but is destroyed by the time the food remnants reach the large gut.

Indicanuria is, therefore, present in a variety of conditions associated with increased intestinal putrefaction (especially in the small intestine) and with occlusion of the bowel, such as mechanical obstruction of the intestines, ileus, acute and chronic peritonitis, appendicitis, ulcers of the intestine leading to scar-formation and contraction (tuberculosis, typhoid), gastroptosis, enteroptosis and lead-poisoning, cholera, intestinal auto-intoxication, etc.

Anything that will *increase intestinal putrefaction* will, therefore, give rise to indicanuria, especially if the putrefying material is *retained* sufficiently long to allow absorption of indol to take place. The study of intestinal putrefaction belongs to other treatises. In this connection we might mention two factors which have been brought forward as influencing intestinal putrefaction, the functions of the stomach and of the liver respectively.

It has been claimed by Carles and others that the *secretion of HCl* has something to do with indicanuria, and when HCl is low there is an increased bacterial putrefaction in the stomach. This has been shown to be incorrect by Enriquez and Binet, who claim that both hypo-acidity and hyperacidity may be accompanied by indicanuria.

The liver cells, when incapacitated by disease, are held reponsible for a type of indicanuria due to *hepatic insufficiency* by Gilbert and Weil. This was also proved false by Enriquez and Binet, who produced liver lesions in dogs and found no indicanuria. On the other hand, the *flow of bile*, when impeded or when diminished, will increase intestinal putrefaction and hence indicanuria may be due to biliary insufficiency or to a stenosis of the bile-duct. The bile, it will be remembered, acts both as an antiseptic and a stimulant of peristalsis.

The Relation of Constipation to Indicanuria.—This is a most important question. The older writers, especially Nothnagel, insisted that habitual constipation alone could not give rise to indicanuria. These authors pointed to the fact that indicanuria is often present in intestinal diseases accompanied by diarrhea. Recent observations have shown, however, that constipation, especially when chronic, may be the sole cause of indicanuria (Emerson, Enriquez, and Binet). Some observers even found that they could produce indicanuria by giving patients astringent drugs. On the other hand, indicanuria is rare in simple diarrhea, or even in typhoid fever accompanied by severe diarrhea. In false diarrhea, which indicates fecal retention, there is indicanuria, *e. g.*, in mucomembranous colitis, but not in conditions in which the diarrhea acts as a thorough evacuant of the tract. By giving purgatives, especially castor oil, an indicanuria due to constipation may be markedly diminished or made to vanish.

In interpreting indicanurias the student should, therefore, never fail to consider the presence of constipation. The patient's diet should also be inquired into. People "living high," on a rich proteid diet, who overeat habitually, are apt to have an excess of indican, which is easily removed by appropriate dietetic measures.

Detection.—Indican is in itself a colorless substance or a brown syrup easily soluble in water, alcohol, and ether, and having a bitter taste. With acids and heat it is easily converted into indigo-blue (Heller's uroglaucin) and indigo-red (Heller's urrhodin). This conversion is the basis of the tests for indican.

Ordinarily, indoxyl does not alter the color of fresh urine, but sometimes it may be partially or completely oxidized in the body, giving the urine a blue color, due to the deposit of indigo-blue. The same may take place outside of the

body on ammoniacal decomposition. Calculi of indigo have rarely been found in the urinary tract as the result of the separation of indigo.

Heller's Test.—This is a very convenient and practical test. Into a small conic glass or wineglass pour 4 cc. (1 dm.) of hydochloric acid (c. p.), and add one drop, or at most two, of pure nitric acid. The latter makes the test more delicate, but an excess of nitric acid is fatal to the test, as the oxidation will be so rapid that the reaction cannot be seen and a yellow color results. To these acids add fifteen drops of the urine and stir. Within from five to twenty minutes an amethyst color appears. Under these conditions the mixture may be colored a pale-yellowish or pinkish tint, showing that indican is diminished. A distinct amethyst color, which is not intense and which appears rather slowly, indicates a normal amount. A deep violet color appearing quickly indicates an increase of indican.

It is best to perform this test always in the same sized receptacle, and to use exactly the same proportions of urine and acids, although the quantities may be varied to suit the observer. Some definite rule should be observed always, frequently controlling oxidations with normal urine. In this way one can easily learn to distinguish increase of indican at a glance.

Jaffé's Test.—To about 10 cc. of urine in a large test-tube add an equal amount of fuming hydrochloric acid, and then, with constant shaking, a perfectly fresh saturated solution of calcium hypochlorite, drop by drop, until the blue color ceases to deepen. Instead of this solution a 0.5 per cent. solution of potassium permanganate may be used. Shake with some chloroform, which dissolves the indigo,

and allow to stand, the chloroform separating as a blue fluid, the color being more or less deep according to the amount of indican. Dark urines, whose other coloring-matters are decomposed by hydrochloric acid and hypochlorite, should first be decolorized with basic acetate of lead solution. This may be done by adding 1 cc. of lead acetate solution to 10 cc. of urine and filtering. The filtrate should be tested for indican.

Obermeyer's Test.—The reagent is composed of strong hydrochloric acid, to which are added 4 parts per 1000 of ferric chlorid, the combination forming a fuming yellow liquid which keeps indefinitely. The urine should preferably be decolorized with a small amount of lead acetate solution, as its pigments prevent the recognition of the blue color. Equal parts of urine and of Obermeyer's reagent are mixed in the test-tube, and a small quantity of chloroform is added. The tube is corked and inverted several times without shaking. The chloroform becomes blue in proportion to the indican present, increasing on standing. Normal urine becomes faint blue, while an increase of indican gives a deep blue color.

Approximate Quantitative Estimation.—*Robin's Method.*—The accurate methods of quantitative estimation of indican being too complex for chemical use, the following approximate method may be employed with advantage. The solutions required are: (1) Obermeyer's reagent (HCl, 1000; Fe_2Cl_6, 4); (2) a 25 per cent. solution of lead acetate; (3) a solution of potassium chlorate containing 1 per cent. of available Cl, or 34.6 gm. of the salt per liter.

"To 10 cc. of the urine add 1 cc. of the lead acetate solution, and filter through a double filter. Put 5 cc. of the filtrate into a test-tube, add 5 cc. of Obermeyer's reagent and 2 cc. of chloroform, and invert the tube about ten times, or until the color of the chloroform ceases to become more intense. The latter will assume a violet or blue color according to the amount of indican present. Now add from a dropper the potassium

chlorate solution, shaking the mixture after each addition until the blue color of the chloroform disappears. The potassium chlorate liberates chlorine in the presence of a strong mineral acid and oxidizes the indigo formed by the addition of Obermeyer's reagent. If the amount of indican is normal, one or two drops will cause discoloration. In recording results write: 'Indican = x gtt. $KClO_3$ solution'" (Boardman Reed).

This method has given very satisfactory comparative results in the hands of the present writer. The progress of an indicanuria can be conveniently watched by this simplified process.

Precautions in testing for indican with any of these tests should include the removal of albumin, as the latter develops a blue color with hydrochloric acid on standing for a long time. A red color obscuring the blue due to any indoxyl develops when iodin is present in patients who had been taking iodids. In this case a small quantity of a strong solution of sodium hyposulphite should be added to the test-tube and the mixture shaken. When the chloroform has settled again, the blue of the indigo will appear. It is important to note the specific gravity of the urine to be tested for indican. If an intense reaction is obtained with urine of low specific gravity, there is far more indican excreted relatively than when the same tint is obtained with a urine of high specific gravity.

Rosenbach's Burgundy-red Reaction.—This is a color-reaction observed in severe intestinal disturbances in which there are a high degree of decomposition and a large amount of indican. The urine is reddish in color to start with, and on the addition of nitric acid, drop by drop, and boiling, it gives a deep, Burgundy-red color.

OTHER ETHEREAL SULPHATES

Besides indican, there are several products of decomposition eliminated in the urine which appear in the form of sulphates of sodium and potassium. These, together with indican, are grouped chemically as the *ethereal sulphates*. The amount of these bodies in the urine varies with the extent of putrefaction in the intestine. It is said that the best criterion of the occurrence of the amount of putrefaction in the body is the relation of the ethereal sulphates to the total sulphates, the normal proportion being about 1 to 10, the relative amount of ethereal sulphates rising as putrefaction increases. The two ethereal sulphates besides indican that are of importance are skatoxyl potassium sulphate and phenol potassium sulphate. For further details, see Sulphates, p. 224.

Skatoxyl potassium sulphate ($C_9H_8NO.SO_2K$) is formed from skatol under the same conditions as indican is from indol. It is present in the urine as a colorless compound, and when oxidized gives a red color. When the indican test gives a purple-red color, the presence of skatol may be surmised. This red color cannot be removed by shaking the urine with chloroform, but it is extracted by amyl-alcohol. Normally it occurs in smaller quantities than indican, and clinically it is of little interest, except in connection with indoxyl.

Phenol Potassium Sulphate ($C_6H_5OSO_3.K$).—Phenol is a product of intestinal putrefaction, and is probably intermediate as to the place where it is developed between indol, which arises high up in the intestine, and skatol, which is formed low down in the tract. It is absorbed from the intestine, enters the blood, and combines with potassium sulphate to form an ethereal sulphate, phenol

potassium sulphate. This is present in normal urine in amounts averaging about 0.03 gm. in twenty-four hours, and constitutes the form in which all the phenol or carbolic acid of the body exists.

Clinical Significance.—When there is an excess of indican there is usually an increased amount of phenol, but the reverse is not true. In albuminous putrefaction in other parts of the body than the intestine—as, for example, in empyema, etc.—there may be an increase of phenol alone, indican remaining normal. The use of carbolic acid, lysol, and other phenol compounds may increase the amount of phenol potassium sulphate. When phenol compounds are taken, the urine becomes smoky, dark brown, or black on standing exposed to the air, as the result of the splitting up of phenol into pyrocatechin and hydroquinon. Clinically any condition that causes increase of indican also causes an increase of phenol, especially increased putrefaction in the lower part of the small intestine and in the upper part of the large intestine.

Detection.—To detect phenol, add some nitric acid to the urine and boil, when the urine will give an odor of bitter almond oil. After cooling, add some bromin water, causing a precipitate of tribronitrophenol. To another portion of the original test add sodium hydrate to excess, and observe an orange-red color, due to the formation of sodium nitrophenol. Other tests are too elaborate, as they require the distillation of the urine.

EHRLICH'S DIAZO-REACTION

In 1882 Ehrlich described a reaction in the urine, which has taken his name, and which depends upon the presence of certain aromatic substances which form anilin colors in

the presence of diazosulphobenzol. The latter is formed by the union of sulphanilic acid (amidosulphobenzol) and HNO_2. In order to obtain a fresh solution of diazosulphobenzol, a solution of sodium nitrite is added to a solution of sulphanilic acid containing 5 per cent. of hydrochloric acid. When the two solutions are mixed, HNO_2 is set free, and diazosulphobenzol is formed. The reagents used in Ehrlich's diazo-reaction, therefore, are as follows:

Solution 1:

Sulphanilic acid	1 part
Hydrochloric acid (c. p.)	50 parts
Distilled water	1000 "

Solution 2:

Sodium nitrite	1 part
Distilled water	200 parts

The solutions should be kept in separate well-stoppered bottles of amber glass, and should be mixed when needed by combining 50 cc. of No. 1 and 1 cc. of No. 2. The sodium nitrite solution does not keep well and should be prepared freshly at frequent intervals.

The test is performed by mixing equal bulks of the freshly voided urine and the mixed reagents; quickly adding one-tenth volume of ammonia water, and shaking.[1] A deep cherry-red color indicates a positive reaction, and if the reaction is marked, the foam will appear salmon-pink or even deep-red in color. On standing for twenty-four hours a green precipitate is formed, which is a further proof of the true reaction.

[1] Another way is to mix 15 cc. of urine and 15 cc. of solution No. 1, shaking gently, adding 5 drops of solution No. 2, and shaking well with ammonia.

Clinical Significance.—The chief clinical value of the diazo test is in the diagnosis of typhoid fever. It is present very constantly in severe types of typhoid fever, with an intensity running usually parallel to the severity of the infection (Wood). The clinical value of the diazo-reaction is, however, greatly lessened by several facts:

1. It often does not occur in the milder forms of typhoid fever until the acme of the disease has been reached, and at times is wholly absent in such cases.

2. It occurs in many other diseases, notably in tuberculosis, pneumonia, pleurisy, many acute fevers (measles, scarlet fever, diphtheria, erysipelas, etc.), in syphilis, cancer, septicemia, pyemia, rheumatism, etc. In practice the chief diseases in which it puzzles the diagnostician are tuberculosis and septicemia. In pulmonary tuberculosis its presence is of bad prognostic import and its persistence indicates an advanced stage of the disease.

3. The reaction occurs in the urine of persons who have been taking certain drugs—*e. g.*, naphthalin, chrysarobin, etc. This reaction is, however, distinguished from the true diazo-reaction by the absence of a green precipitate occurring on standing for twenty-four hours, and by the fact that the color does not disappear on the addition of acids.

Other drugs—*e. g.*, gallic and tannic acids and their compounds, iodin, and the iodids—inhibit the appearance of the reaction. This is especially to be noted in tuberculosis, where tannates are frequently given for the diarrhea.

QUESTIONS ON CHAPTER XI

What is *indican?* How is it formed?
How is indigo-blue formed?
What is the clinical significance of indican?

What does diminution of indican imply? When is it increased?

What weight should be given to indicanuria in clinical work?

What is the principle of all indican tests? Describe Heller's test; Jaffé's test; Obermeyer's test; Robin's quantitative method.

What precautions should be taken in testing for indican?

How does indican give the urine voided a blue color?

What are indigo calculi?

What are the *other chief ethereal sulphates* besides indican in the urine?

What does the relation of ethereal sulphates to the total sulphates indicate? What is the normal proportion?

What is skatoxyl potassium sulphate? How is it detected?

What is phenol potassium sulphate? Where is it formed probably?

Why does urine containing phenol turn dark on standing exposed to the air?

What is the clinical significance of phenol potassium sulphate in urine? How is its presence detected?

Describe *Ehrlich's diazo-reaction.* On what principle is it based? What is its clinical significance? What are its limitations? In what diseases does it occur?

V. COLORING-MATTERS; BILE; BLOOD; OTHER ORGANIC CONSTITUENTS

CHAPTER XII

URINARY COLORING-MATTERS

NORMAL COLORING-MATTERS

Two pigments are always present in normal urine —*urochrome* and *hematoporphyrin.* Two other pigments—*uroerythrin* and *urobilin*—may be present, especially on sanding. Urochrome gives the yellow color to the urine. Hematoporphyrin is present in small amounts, is red in color, and is considered under the head of Blood Coloring-matters. Uroerythrin gives the pink color to urate deposits and urobilin gives urine a dark-brown color. Of the normal pigments the only one that has any clinical significance is urobilin, an increase of which is noted in certain diseases.

Urobilin ($C_{32}H_{40}N_4O_7$; urophaëin, Heller; hydrobilirubin Maly) was first isolated by Jaffé in 1868. It is present in the urine chiefly as a chromogen—urobilinogen—and its color is set free only when this chromogen is decomposed. In some diseases there is an increased amount of free urobilin.

It is derived partly through the decomposition of the hemoglobin of the blood, but chiefly through the decomposition of the biliary pigments (bilirubin) which takes place in the intestine as the result of bacterial action. Urobilin may be formed either from bile-pigment or from

blood-pigment. *Conditions accompanied by destruction of red blood-cells give an increased amount of urobilin*, and this amount is a measure of the destruction of blood-pigment. Normal urine contains 0.08 to 0.20 gm. of urobilin in twenty-four hours, the excretion being greater in tropic climates.

Clinical Significance.—Urobilin is absent: (1) In newborn children. (2) In obstruction of the bile-ducts (jaundice). (3) In phosphorus-poisoning. (4) In severe nephritis.

Urobilin is increased (Serkowski): (1) In conditions of *intestinal putrefaction* (in which it is parallel to indican). (2) In *hemorrhages* into the organs or cavities of the body. If no excess of urobilin occurs there could not have been any bleeding. (3) In atrophic cirrhosis of the liver. (4) In scarlet fever. (It is not increased in diphtheria.) (5) In appendicitis. (6) In malaria. (7) In cancer.

Detection.—Urobilin is best detected by examining the urine directly by means of a small pocket-spectroscope.[1] The urine may have to be diluted if it is deeply colored. The characteristic absorption-band is between the green and the blue parts, between the lines *b* and *F* (Fig. 28). These bands are rendered sharper on adding a few drops of tincture of iodin to 10 cc. of urine.

When no spectroscope is available, an old test, which is very useful, the so-called *urophaëin test of Heller*, may be employed. To about 7 cc. of concentrated sulphuric acid add twice the amount of urine in a conic glass, pouring the urine into the acid from the height of about 4 inches. If the amount of urobilin is normal, a garnet-red color appears, which is so intense that but little light passes through

[1] For the method of using this instrument, see page 197.

the mixture; if increased, the mixture is opaque, and if diminished, it is transparent. This test is not of any value as a specific test of urobilin, for other coloring-matters and such abnormal constituents as bile, sugar, etc., give this reaction. It is frequently used, however, as a test for urobilin after excluding the presence of sugar, bile, etc.

Harley's Test.—Dilute the twenty-four-hour urine with water until it measures 1800 cc. (60 oz.), or, if the quan-

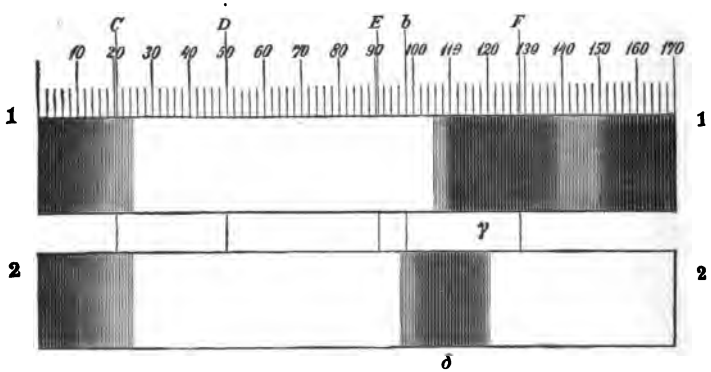


Fig. 27.—1, Acid urobilin; 2, alkaline urobilin (after Neubauer and Vogel).

tity exceed this amount, concentrate it to this measure. To 4 parts of it in a test-tube add 1 part of pure nitric acid, and allow the mixture to stand for some minutes. If the quantity of urobilin is normal, the mixture will change but slightly in color. If there is an excess, it will become pink, red, or purple, according to the amount. The acid liberates the coloring-matter which may be concealed, so that a pale urine often contains a large amount of pigment.

BILIARY PIGMENTS AND ACIDS

BILE-PIGMENTS

The presence of bile in the urine is represented by the occurrence of biliary pigments and biliary acids. Both groups will be considered here together for the sake of convenience.

Bilirubin.—Bile-pigments occur in the urine in the form of a combination of bilirubin with alkalis. When urine rich in bile-pigment is allowed to stand exposed to the air, bilirubin becomes oxidized to the green biliverdin. Bilirubin itself is orange colored and is an intermediate product in the body between hemoglobin and urobilin. The oxidation products of bilirubin are, in the order of importance, biliverdin, already mentioned (green), bilicyanin (blue), bilifuscin, biliprasin, and choletelin.

Bilirubin occurs in a free state in urine, but the color of a bile-containing urine, though almost always abnormally deep, varies greatly between a yellowish brown to a nearly pure green, according to the presence of oxidation products. On shaking, the froth is persistently yellow or greenish yellow. The urine permanently stains filter-paper a yellow color, and usually contains an excess of urobilin and of indican. A bile-containing urine always gives a reaction for albumin, especially for nucleo-albumin, and the nitric acid test with such urines is unsatisfactory on account of the masking of the white zone by the coloring-matter.

For this reason a urine containing much bile should be tested preferably by the *heat test* for albumin. The sediment of such urines often contains many epithelial cells from the kidneys, casts, blood-cells, and crystals of bilirubin. The organized sediment may be stained yellow.

Clinical Significance.—Bile-pigments occur in the urine in nearly all forms of jaundice. In the severer types bile-acids are also present, especially, however, in catarrhal jaundice or in obstruction of the bile-ducts. In congestion of the liver due to organic heart disease, in cancer of the liver (pressure on hepatic duct), acute yellow atrophy of the liver (with leucin and tyrosin). In hypertrophic cirrhosis the urine contains bile-pigments (and in severe cases, bile-acids), but in atrophic cirrhosis there may be no jaundice, consequently no bile in the urine, though both may be present in the later stages (Serkowski). Bile-pigments may also appear in severe infectious diseases, phosphorus-poisoning, and other conditions in which there is a destruction of red cells.

Detection.—On inspection the urine containing bile may show the characteristics already described as regards its tint, when viewed by reflected light, and the color of its foam.

Gmelin's test consists in overlaying concentrated fuming "yellow" nitric acid with the urine. This nitric acid, also known as crude or nitroso-nitric acid, contains HNO_2. It should not contain too much of this acid, but enough to show a distinct yellow color. By placing a small piece of wood (match-stick) in nitric acid and heating, HNO_2 is developed, and the fluid can be used for this test. There is always some resin in wood and a green ring appears at the point of contact; below this a blue ring, and next to this a red one. The green ring is characteristic of bile; the others may come from urobilin or from indican. At times a beautiful play of colors—green, blue, violet, red, and yellow, in the order named—is seen with this test. This test may also be applied by placing a drop of urine on a porcelain plate

and allowing a drop of fuming nitric acid to mingle gradually with the urine, giving the same play of colors.

Marêchal's Test.—About a dram of an alcoholic solution of iodin of moderate strength is poured into a test-tube, and the urine is allowed to flow down the sides of the inclined tube so as to underlie the reagent. A green color appears just below the point of contact of the two fluids.

Rosenbach's test is based on the fact that when a large amount of urine containing bile passes through white filter-paper, the bilirubin is retained in the paper, and when a drop of nitric acid is placed on the inner surface of the filter, a green spot is produced, changing to red.

BILE-ACIDS

Bile-acids can be divided into two groups—the glycocholic and the taurocholic acid groups. The former are acids containing nitrogen, but no sulphur, and on the addition of water can be split into glycocoll and cholic acid. The taurocholic acids contain nitrogen and sulphur and are split into taurin and cholalic acid.

Clinical Significance.—Bile-acids are found in the urine in the same conditions as are bile-pigments, but their determination is much more difficult than that of the pigments. According to von Leyden, the urine of hematogenous jaundice contains only bile-pigments, while that of hepatogenous jaundice contains both bile-pigments and bile-acids. This is not always true, however, although the presence of a considerable amount of bile-acids shows the existence of hepatogenous jaundice, while their absence does not show the absence of this form of icterus. The question as to whether bile-acids occur in normal

urine has not been settled. Bile-acids are present in hepatic congestion, cirrhosis, tumors of the liver, and severe acute catarrhal jaundice, anemia, scurvy, and splenic leukemia. Their clinical significance is important.

Detection.—The tests for biliary pigments are usually sufficient to show the presence of bile without testing for the acids separately. Bile-acids are not usually tested for in routine examinations. They must first be isolated by concentrating the urine and extracting the residue with strong alcohol, removing the alcohol by evaporation, and precipitating with basic lead acetate and a little ammonia. The precipitate is washed with water and again extracted with warm alcohol, evaporated to dryness after the addition of a few drops of sodium carbonate solution. The residue is extracted for the third time with alcohol, and the acids precipitated by the addition of ether to the alcoholic extract. The following test is then carried out with the precipitate:

Pettenkofer's Test.—A small amount of watery solution of bile-acids is treated in a test-tube with 5 drops of a 10 per cent. solution of cane-sugar, and this mixture carefully layered over concentrated sulphuric acid. A red or purple ring will appear at the point of contact. (The tube is dipped into cold water and shaken gently. A small amount of the purple fluid is poured into one tube containing glacial acetic acid, and into another containing alcohol. The spectrum of the first shows an absorption-band in green, while the second, after a few moments, becomes brown and then shows two bands, one between *D* and *E* and the other at *F*.)

BLOOD-PIGMENTS

The presence of blood in small quantities does not change the color of the urine, but larger amounts give rise to cloudiness and to a reddish sediment if the hemorrhage is recent. If the blood has been mixed with the urine for any length of time within the body, or if ammoniacal fermentation has occurred, the bloody urine is of a dirty brownish-red or dark-brown color, sometimes with a tinge of green.

The coloring-matters of the blood which occur in the urine are *hemoglobin* and its derivatives, *oxyhemoglobin*, *methemoglobin*, and *hematin*. *Hematin* is a reduced hemoglobin, and the latter is converted into hematin and a coagulated albuminous substance by means of heat. Methemoglobin is intermediate between hemoglobin and hematin. Oxyhemoglobin is obtained by shaking hemoglobin with air. These different substances are all distinguished by special absorption-bands in their spectra.

Blood coloring-matters can enter the urine either by direct excretion by the kidney or by the disintegration of the blood-cells after they have entered the urine from different sources. The color of such urines differs according to the amount of hemoglobin or methemoglobin, the former giving a bright color, the latter a dark brownish-red. Recent hemorrhages from the larger vessels give more hemoglobin, while capillary bleeding gives more methemoglobin.

Clinical Significance.—Hemoglobinuria means the direct passage of the blood coloring-matters into the urine without any red blood-corpuscles. This occurs in a large variety of general diseases, such as scurvy, purpura, scarlet fever, malarial poisoning, etc. The comparative absence

of blood-corpuscles and the presence of a small amount of albumin (or even the absence of albumin) distinguish hemoglobinuria from hematuria. It must be remembered, however, that blood-corpuscles are rapidly dissolved, especially in alkaline urine, and, therefore, that red cells may have been present in the specimen. Urine containing dissolved corpuscles is apt to be alkaline, while the urine of hemoglobinuria is acid and contains a much smaller amount of albumin. It is of lower specific gravity and deposits less sediment than the blood of hemoglobinuria.

Hematuria is a term applied to urine containing both blood-corpuscles and blood-pigments. The causes of hematuria are very numerous, for blood may come from the kidney, from the renal pelvis, from the ureter, bladder, prostate, or urethra. Blood from the kidney may be due to acute congestion, acute nephritis, or to acute axacerbations of chronic kidney disease. In acute parenchymatous and in chronic diffuse nephritis, in interstitial nephritis as the result of changes in the vessels, and in amyloid kidney hematuria is not uncommon.

Hematuria is very common in tuberculosis, tumors of the kidney, and stone in the kidney. It occurs also after the administration of certain drugs, such as turpentine, and following injuries to the kidney. In the tropics it is often caused by parasites, to which allusion will be made later on (p. 337).

Bleeding from the lower urinary tract may be due to tuberculosis, stone or tumor of the bladder; acute or chronic inflammations of the bladder; traumatism; acute urethritis, urethral chancre, or surgical operations for strictures, etc. (See Table of Causes of Hematuria, p. 278.)

Relation to Albuminuria.—The presence of blood coloring-matters in very minute quantities may be observed in urines that are in every way normal. Very small amounts of hemoglobin without any albumin may be found in scarlet fever just before the onset of albuminuria, thus giving warning of the impending danger to the kidney. The blood-pigment disappears when the albumin becomes copious, and reappears as it diminishes. Hemoglobin is not found, however, in albuminuria due to fevers; as in typhoid, pneumonia, etc.

Detection of Blood-pigments.—Guaiacum Test.—The reagents needed are: (1) A freshly prepared tincture of guaiacum. Take a penknife-pointful of the powdered guaiac resin and mix with 3 to 5 cc. of 95 per cent. alcohol; shake for a minute, allow to stand, and after a few minutes filter 5 drops into a test-tube; (2) to this add 20 drops "seasoned" oil of turpentine. This is made by allowing commercial turpentine to stand exposed in an open dish and in a light room till thick, and diluting with about five volumes of ordinary turpentine (Schumm). To about 5 cc. of urine add the mixed reagents (turpentine and guaiac) and shake repeatedly. A little alcohol now added brings out the color better. On standing a short time a blue or bluish-green color develops in the presence of blood. Or the urine to be tested is layered under this mixture, and a bluish-green and then a brilliant blue contact-ring is produced when blood-pigments are present. Pus also gives this ring, but in this case the ring disappears on heating the mixture. The blood ring, however, stays. Before using this test an alkaline urine must be made acid. This test is very delicate.

Benzidin Test.—A test similar to the guaiacum test, but still more delicate. The reagent consists of ½ cc. of a fresh solution of Merck's pure benzidin in glacial acetic acid and 2 or 3 cc. of 3 per cent. hydrogen peroxid. To 10 cc. of urine add 0.5 to 1.0 cc. of glacial acetic acid, and shake well. Then add one-third volume of ether and shake thoroughly. Allow to stand a little while and add 5 to 10 drops of absolute alcohol. Shake gently and pipet off the cleared ethereal layer. The latter is added to the benzidin reagent in another test-tube and the mixture is well-shaken. If small amounts of blood are present, a green color develops; if larger amounts, a blue color. The reaction is about twenty

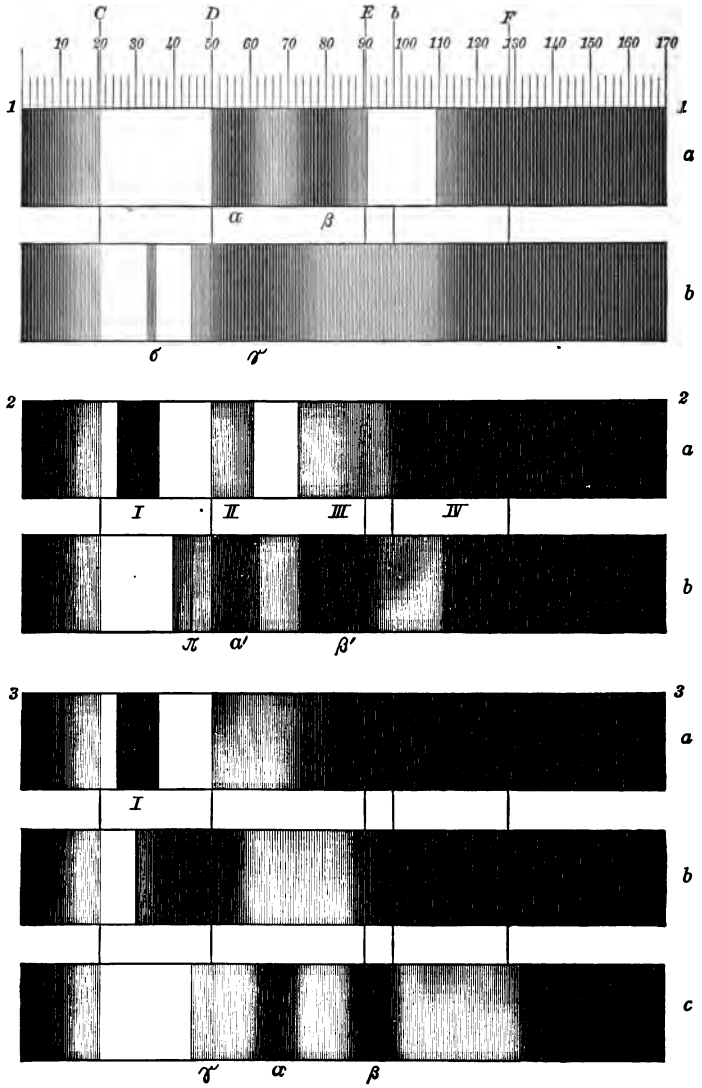


Fig. 28.—Spectra: 1 *a*, Oxyhemoglobin; *b*, oxygen-free hemoglobin. 2, Methemoglobin: *a'*, in neutral solution; *b'*, in alkaline solution. 3, Hematin in acid alcoholic solution; *b*, in ammoniacal solution; *c*, reduced hematin (after Neubauer and Vogel).

times more delicate than Heller's. It can be used both in hematuria and hemoglobinuria, and is not interfered with by pus, sugar, bile, iodide, senna, or rhubarb (Schlesinger and Holst).

Heller's Test.—The earthy phosphates are precipitated from the urine by means of caustic potash solution and gentle heat. The precipitate of earthy phosphates carries with it, as it sinks, the blood coloring-matters and appears not white, as in normal urine, but blood-red. In alkaline urine the phosphates may be precipitated by a few drops of the magnesium fluid (see p. 218) on the application of heat. Heller's test is not nearly so delicate as the above tests. It is open to the objection that it reacts with hematoporphyrin and with certain vegetable drugs, iodids, iron salts, and nitrites.

Teichmann's Hemin Crystal Test.—The precipitated earthy phosphates obtained in the preceding test are filtered out and dried on a slide. A minute granule of common salt is thoroughly mixed with the dried mixture of hematin and earthy phosphates. Any excess of salt is removed, the mixture is covered with a cover-glass, a hair is intersposed, and a drop or two of glacial acetic acid is allowed to pass under the cover-glass. The slide is carefully warmed until bubbles begin to appear. On cooling, crystals of hematin hydrochlorate will form. Gentle heat only should be used in precipitating the earthy phosphates with caustic potash (see Heller's Test) and the urine filtered quickly. Bubbles appearing under the cover-glass before heat is applied are carbonic acid, and should be allowed to pass away.

Spectroscopic Test.—The urine is placed in a test-tube or small trough with plate-glass sides, and examined with a small pocket-spectroscope. Water may be poured on the surface of the urine without mixing, thus obtaining a mixture graduated from pure water to pure urine. Observe the absorption-bands of oxyhemoglobin, two in number, between lines *D* and *E*; or the single line of reduced hemoglobin between *D* and *E*; or the four bands of methemoglobin, the latter giving a dark band in the red between *C* and *D* if the reaction of the urine is acid (Fig. 28).

The spectrum of hematin is rarely seen, and is difficult to distinguish from that of methemeglobin. If the urine is rendered strongly alkaline by ammonium hydrate, and if ammonium sulphid be added, the spectrum of reduced hemoglobin will appear with two bands between *D* and *E*, like oxyhemoglobin, only a little nearer to the green.

Hematoporphyrin ($C_{16}H_{18}N_2O_3$), discovered in 1871 by Hoppe-Seyler, is a derivative of hemoglobin and is present in traces in normal urine. It is identical with

iron-free hematin. A urine containing this substance is opaque and almost black, or, in a thin layer, reddish-brown. Clinically it is important when present in large amounts. It has been seen in increased quantities in leprosy, acute articular rheumatism, pulmonary tubercu-

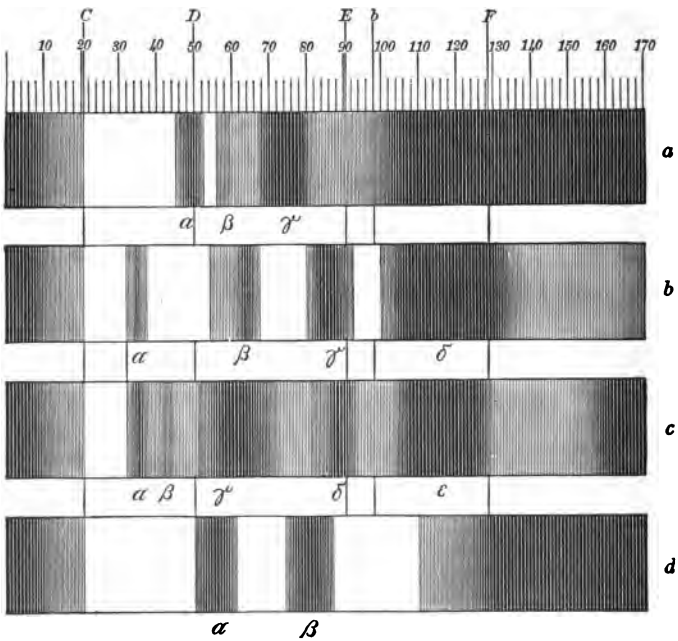


Fig. 29.—Spectrum of hematoporphyrin: *a*, Acid; *b*, alkaline; *c*, neutral; *d*, metallic spectrum.

losis, pleurisy with effusion, etc. It is increased by the use of large doses of sulphonal, trional, or tetronal; in lead-poisoning; in intestinal tuberculosis, and in certain nervous diseases. It can be detected only by the spectroscope, the alkaline solution presenting a four-banded spectrum which

is most characteristic (Fig. 29). Its isolation is troublesome.

Melanin is a pigment sometimes found in urine in cases of melanotic cancer or sarcoma. It occurs usually in solution of the urine or in small black particles. Urine containing this pigment is normal in appearance when freshly voided, but on exposure to air it becomes brown or black. The pigment is eliminated in the form of a chromogen—melanogen—which becomes oxidized to melanin, probably in the liver, although this is still a matter of discussion. Melanin has also been observed very rarely in severe wasting conditions and in chronic malaria. It must be noted that melanin may be entirely absent from the urine in cases of actively progressing new growths of the melanotic type.

Detection.—On adding nitric acid to the cold urine, the latter turns black. *Zeller's test* consists in the addition of bromin water, causing a yellow precipitate that gradually blackens. The addition of ferric chlorid will cause a gray precipitate soluble in an excess of Fe_2Cl_6, constituting *von Jaksch's test*, which is much more delicate than Zeller's and should always be used to detect melanin.[1]

QUESTIONS ON CHAPTER XII

What two pigments are always present in normal urine? What two other pigments are usually present and especially on standing?

What pigment gives the yellow color to urine? The dark-brown color? The pink color to the urates?

In what form is *urobilin* present in normal urine?

What are the theories of origin of urobilin?

[1] For other conditions causing black urine, see under alkapton, pyrocatechin, hydroquinon, phenol, etc.

What relation does the amount of urobilin bear to the destruction of the blood-pigment in the body?

In what conditions is urobilin increased?

How is *urobilin* detected by the pocket-spectroscope? By Heller's urophaëin test? What is the value of the latter test? Describe Harley's test.

What two classes of substances represent bile in the urine?

How do *bile-pigments* occur in the urine? What is their significance?

What is the difference between *bilirubin* and *biliverdin?*

What are the characteristics of a bile-containing urine? What albumin test should be used in such urines, and why?

In what conditions does bile appear in the urine? Describe Gmelin's test; Maréchal's test; Rosenbach's test.

What is said of *bile-acids* in jaundice?

In what conditions are bile-acids present in the urine?

What is Pettenkofer's test for bile-acids?

What is the appearance of urine containing fresh *blood?* Old or decomposed blood?

What coloring-matters from the blood may occur in the urine? How do they enter the urine?

What coloring-matter is found chiefly in recent hemorrhage?

Define hemoglobinuria; hematuria. How does the urine of the first differ from that of the second condition?

When does the hemoglobinuria occur?

In what diseases is hematuria seen?

What warning of an impending nephritis do we get in the urine of scarlatina?

What is the relation of albuminuria to hemoglobinuria in scarlatina? In typhoid and other fevers?

Describe the guaiacum test; the benzidin test; Heller's test; Teichmann's test; the spectroscopic test.

What is *hematoporphyrin?* What is its clinical significance?

What is *melanin*, and what does its presence imply?

What is Zeller's test for melanin? Von Jaksch's test?

CHAPTER XIII

LEUCIN, TYROSIN, FATTY MATTER, AND OTHER ORGANIC CONSTITUENTS

LEUCIN AND TYROSIN

($C_6H_{13}NO_2$ and $C_9H_{11}NO_3$)

THESE substances are products of retrograde changes of nitrogenous substances, and occur in certain fetid secretions of the skin, as in the axilla. They can be produced from the tissues of some glands, as the liver, pancreas, or spleen. They are found in the urine chiefly in diseases in the liver, especially in rapidly destructive processes, such as acute yellow atrophy or phosphorus-poisoning, and in smaller quantities in acute infectious diseases, such as typhus and smallpox. These urines always contain a large amount of biliary coloring-matter and albumin. When present in large amounts, leucin and tyrosin deposit spontaneously in the sediment. Leucin and tyrosin usually, if not always, occur together in the urine, and although some authors claim that they are present in minute traces in normal urine, their presence in any urine is very rare. The urea is very much diminished in such urines.

Detection.—If the crystals are not deposited spontaneously, the urine should be evaporated in order to display them. If this does not suffice to demonstrate them, the *method of Frerichs* may be used. A large quantity of urine is treated with basic lead acetate, filtered, the excess of lead removed from the filtrate by sulphuretted hydrogen, and the filtrate evaporated to a small volume over a water-bath. Tyrosin needles will crystallize in twenty-four

hours, but leucin spheres do not appear until later, as leucin is much more soluble. If a mixture of leucin and tyrosin is extracted with alcohol, the leucin will dissolve and leave a more or less pure tyrosin. After separating the two in this way the following chemic tests may be applied:

Tests for Tyrosin.—(1) *Hoffmann's Test.*—A bright pink or crimson color is produced when a solution of tyrosin that has been boiled in an excess of water is heated with Millon's reagent. (See p. 80.)

(2) *Piria's Test.*—Tyrosin heated with a few drops of sulphuric acid, diluted, and boiled with barium carbonate will give a filtrate which strikes a violet color on the addition of a dilute solution of ferric chlorid. An excess of iron should be avoided, as it destroys the color.

(3) A hot solution of tyrosin acidified with 1 per cent. acetic acid turns bright red on the addition of a little sodium nitrite.

(4) *Von Udránszky's Test (Furfurol).*—A small crystal of tyrosin is dissolved in 1 cc. of water; one drop of a 0.5 per cent. solution of furfurol is added. Underlie this with concentrated sulphuric acid, keeping the temperature of the mixture not over 50° C. The fluid is colored rose red.

Tests for Leucin.—(1) A little leucin heated on a platinum sheet with a little nitric acid melts and forms an oily drop which rolls about on the platinum and does not adhere to it.

(2) On the addition of a trace of quinon and a few drops of sodium hydrate to a cold aqueous solution of leucin, a deep-violet color appears. (This reaction occurs also with certain proteids.)

The microscopic appearances of leucin and tyrosin crystals are characteristic and will be found described on page 267.

FAT AND CHOLESTERIN IN THE URINE

A trace of fat is present in solution in normal urine, and abnormal quantities have been found in disease, as in cases of chronic nephritis which give fatty epithelium, fatty casts, and free oil-drops; in fatty kidney, phosphorus-poisoning, and diabetes mellitus, and in cases of cystitis and vaginitis in which fatty epithelium is present.

In *chyluria* (see p. 33) the fat in the urine is in the form of a molecular emulsion, while in *lipuria* it is present in the urine in the form of a clear fluid oil. Chylous urine may result from leakage of a lymph-vessel into some part of the urinary tract, as in filariasis. In this disease the lymphatic vessels in the bladder-walls become filled with the embryos or mature worms of *Filaria sanguinis* and rupture, allowing the escape of lymph into the urine. Chyluria may occur, however, without the presence of these parasites and without definite cause. Fat in the form of oil has been found in the urine in pregnancy, fractures of the long bones, eclampsia, diabetes, pulmonary tuberculosis, and after large doses of cod-liver oil (Roberts); in cystic cheesy degeneration of the kidney; in abscesses communicating with the ureter; in heart disease, and in calculous disease of the pancreas. The admixture of fat from lubricants of catheters or from fatty material used in the vagina for examinations must not be mistaken for fat in the urine.

Cholesterin ($C_{26}H_{44}O$) is a monatomic alcohol, normally present in bile, blood-corpuscles, nerve tissues, and elsewhere in the body. In disease it occurs in gall-stones, in

pus, in tuberculous and cheesy masses, in old transudates, tumors, etc. It may be present in urine under pathologic conditions, in such cases as cheesy cystic kidney, but this is of very rare occurrence. Extensive fatty degeneration of some part of the urinary tract, as subacute or chronic nephritis or the fatty stage of acute nephritis, may rarely give cholesterin crystals in the urinary sediment. Cholesterin crystallizes in large plates, the appearance of which

Fig. 30.—Cholesterin crystals (Jakob).

is characteristic (Fig. 30). It is insoluble in water, but readily soluble in alcohol, ether, chloroform, etc. Cholesterin is detected by means of the microscope.

OTHER ORGANIC CONSTITUENTS OF MINOR IMPORTANCE

Besides the organic substances already described, very small amounts of a number of organic bodies may occur in normal urine and are sometimes increased pathologically. They may be divided into five groups: (*a*) The non-nitrogenous organic acids, *e. g.*, oxalic, lactic, succinic, etc. (*b*) The fatty acids, including formic, acetic, butyric, and propionic. (*c*) The aromatic oxyacids—hydroparacumaric and paraoxyphenylacetic, etc. (*d*) Aromatic substances present as ethereal sulphates, *e. g.*, phenol, etc. (*e*) Ferments.

Oxalic acid ($C_2H_2O_4$) is probably present in very small quantities in normal urine. Whenever there is an interference with oxidation in the body, as in diabetes, diseases of the liver, heart, or lungs, it may be increased.

Most of the oxalic acid in the urine exists as *calcium oxalate*, a salt that crystallizes and is deposited in the urinary sediment when it is present in excess. The crystals are described on page 259, where the subject of oxaluria is further discussed.

Lactic acid ($C_3H_6O_3$) is not found in normal urine, but its salts have been found in advanced disease of the liver (acute yellow atrophy, cirrhosis), in phosphorus-poisoning, in diseases of the muscles (trichinosis), and after severe muscular efforts. In the latter case it occurs in the form of sarcolactic acid. The detection of lactic acid will be found described in the larger text-books.

Succinic acid ($C_4H_6O_4$) has been found at times in normal urine, usually in combination with sodium. It is increased by eating asparagus.

Glycerophosphoric Acid ($C_3H_7O_3.PO(OH)_2$).—A portion of the phosphoric acid which is present in the urine in organic combination is contained in the nucleic acid of this fluid. (See p. 161.) It is said by some authors (Sotnitschewsky, 1880) that another portion of the organic phosphoric acid is present as glycerophosphoric acid. The amount of phosphoric acid united to organic compounds in the urine is 1 per cent. of the total phosphoric acid, according to Lépine, Eymonet, and Aubert (1884). Glycerophosphoric acid is a dibasic substance, syrupy in consistence, and forms salts soluble in water.

Benzoic acid ($C_7H_6O_2$) has been at times found in normal urine, and is interesting as the mother-substance of hippuric acid (*q. v.*). It is increased by the ingestion of benzoic acid or benzoates, etc., and in decomposing urine it is derived from hippuric acid. In the human body, according to Baumann, benzoic acid is formed from the decomposition of proteids in the intestine.

Chondroitin-sulphuric acid ($C_{18}H_{35}NO_{13}$-SO_3.OH) was found constantly in normal urine by Moerner (1895). This substance has been mentioned when speaking of nucleo-albumin. Moerner considers the substance described as nucleo-albumin as a compound of a proteid with chondroitin-sulphuric acid, and also with nucleic and taurocholic acids, the latter being present especially in urine of jaundice.

Oxyproteic acid ($C_{43}H_{82}N_{14}SO_{31}$) is regarded as an intermediate oxidation product of the proteids and is said to be a normal constituent of human urine (Töpfer, Bondzynsky, and Gottlieb).

Sulphocyanic Acid (CN.SH.); **Hydrogen Sulphocyanid.**—Accord-

ing to Gscheidlen, sulphocyanids occur in normal urine as constant constituents, 0.035 gm. of potassium sulphocyanid being found per liter.

Volatile Fatty Acids.—Traces of acetic, butyric, formic, and propionic acids have been found in the urine. The amounts grow larger during alkaline fermentation, and are increased in some diseases of the liver; in fevers; in diabetes (von Jaksch). They have no special clinical significance, and are produced by the same conditions that cause acetonuria. Von Jaksch gave the name *lipaciduria* to the presence of an increased amount of volatile fatty acids.

Aromatic Oxyacids.—Normal urine contains small amounts of hydroparacumaric and paraoxyphenylacetic acid in the form of potassium salts. These substances are probably products of intestinal proteid decomposition, and occur in larger amounts whenever indican is increased. Paraoxyphenylglycolic and oxyamygdalic acids are two other substances of the same class (Huppert) that have been found in the urine, and still another set of acids of this sort are uroleucic and homogentisic (Kirk, Baumann and Wolkoff), which constitute *alkapton*, described separately on page 127, on account of its more marked clinical significance.

Aromatic Substances.—A series of aromatic substances occurring in the urine in combination with sulphuric acid are parakresol, pyrocatechin, and hydroquinon.

Pyrocatechin is said to occur very frequently in urine. These urines are light colored when passed, but become dark on standing or on the addition of KOH solution. *Hydroquinon* occurs in urine after carbolic acid poisoning, giving the urine its dark color. It is present as an ethereal sulphate.

There are methods for the isolation and quantitative estimation of all these ingredients, but these are too complex and not sufficiently important to be described here.

Ferments.—Pepsin and trypsin have been found in urine. Leo found that normal urine possesses digestive powers for fibrin, and Neumeister has shown that true pepsin occurs in urine. Trypsin has been found by Sahli, though its presence is doubted by some authors.

Accidental Organic Constituents.—There are a large number of organic substances which may be present in urine when they are taken internally or absorbed from the skin or mucous membranes. The discussion of these belongs properly to pharmacology and toxicology, but a few of the more important ones may be mentioned: alcohol, glycerin, chloroform, chloral, iodoform, sulphonal, salicylic acid, salol, resorcin, guaiacol, thymol, naphthol, copaiba, santonin, aloin, phenacetin, acetanilid, antipyrin, quinin, morphin, etc.

QUESTIONS ON CHAPTER XIII

What are *leucin* and *tyrosin?* When are these substances found in the urine? What is their relation to the occurrence of albumin, and of biliary pigments and of urea in the urine? What is the frequency of their presence in urine? How may the crystals be obtained? Which crystallizes first? How do we separate the two by means of a solvent? What is Hoffmann's test? Piria's test? The furfurol test? What is the platinum-foil test for leucin?

In what form does *fat* occur in the urine? What is lipuria?

What is *chyluria*, and what is it due to?

In what conditions has fat in the form of oil-globules been found in the urine?

What extraneous fat must be guarded against in urinary examination?

What is *cholesterin?* Under what conditions may it be present in the urine? What are its properties, and how is it detected?

Name some of the organic acids present in urine?

Where has phenol been found increased in the urine? Where pyrocatechin? What ferments occur in urine?

B. INORGANIC CONSTITUENTS

THE principal inorganic constituents of the urine are the chlorids, phosphates, and sulphates occurring in combination with sodium, potassium, ammonium, calcium, and magnesium. There are also traces of carbonates of the alkalis, and also minute quantities of iron, fluorin, and silicic acid, as well as free gases, including carbonic acid, nitrogen, and oxygen. The total amount of inorganic substances excreted in twenty-four hours is between 9 and 25 gm.

CHAPTER XIV

CHLORIDS

THE chlorids, next to urea, are the chief solid constituents of the urine. Most of the chlorin in the urine exists as sodium chlorid, and small amounts are combined with potassium and ammonium. The chlorids in the urine are derived from the food, and most of the salt ingested is eliminated in the urine as such. Normally, from 10 to 15 gm. of NaCl are excreted in twenty-four hours, but if salty food is eaten, the amount may reach 40 to 50 gm. In starvation the chlorids almost entirely disappear from the urine. The tissues need a certain amount of sodium chlorid, and if salt-containing food is given after a period of starvation, some of the salt is retained in the body until the tissue fluids have enough NaCl, when the original equilib-

rium is restored. An increased excretion of chlorids takes place when large amounts of water are taken. The amount of chlorids in the urine is lowered at night and increases in the afternoon, especially upon a vegetable diet (Serkowski). Usually the amount of chlorids eliminated is parallel to that of urea, but an exception must be made in excessive muscular work, when urea is increased and chlorids are diminished.

Clinical Significance.—The chlorids are *diminished* in all acute diseases, especially those in which there is a serous exudation or transudation (dropsy), vomiting, or diarrhea.

The test for chlorids is, therefore, often of considerable clinical value in determining the progress of an *exudative process* or of an *effusion* into one of the serous cavities. In pneumonia the chlorids are very low or even absent in the acute stage, but *as the exudate becomes absorbed* and convalescence sets in, the chlorids increase and may exceed the normal for a time. In differentiating between acute meningitis and typhoid fever this test is also useful, as the serous exudation in acute meningitis causes a marked diminution in the chlorids, while in typhoid these are only moderately diminished. Other acute diseases in which chlorids are diminished or absent are cholera, septicemia, pyemia, puerperal fever, and acute articular rheumatism. In the acute diseases mentioned, especially in pneumonia, a persistent decrease of chlorids is of bad prognostic import, while an increase is a good omen. The chlorids increase rapidly after the crisis in pneumonia. After operations Guyon found that a low percentage of chlorids was a bad prognostic sign and that patients with very low NaCl in the urine (less than 1.0 per liter) would probably not survive.

In chronic diseases accompanied by dropsy the chlorids may be absent from the urine, and if the fluid be absorbed they gradually rise to near the normal. When there is no exudation or transudation in chronic diseases, the amount of chlorids is in proportion to the amount of salt in the food—*i. e.*, the amount of chlorids is a measure of the appetite.

The following table (Serkowski) shows the conditions in which chlorids are increased or decreased:

Chlorids are *increased:*	Chlorids are *diminished:*
1. After a vegetable diet.	1. At night; during repose.
2. After massage.	2. In gastric diseases (insufficient absorption).
3. After chloroform anesthesia.	3. In fevers, especially lobar pneumonia.
4. In prurigo.	4. In nephritis (chronic).
5. In rickets.	5. In chyluria.
6. After taking digitalis.	6. In cancer (insufficient nutrition).
7. During the absorption of exudates and transudates.	7. In dropsy, exudates and transudates. (During the rapid formation of these.)
8. After the crisis in acute fevers.	8. In many cachexias, in anemia, and in starvation.
9. In tertian malaria (destruction of red cells).	9. In diarrhea or vomiting.
10. In cirrhosis of the liver.	
11. In poisoning by pyrogallol, etc. (destruction of red blood-cells).	

Detection.—Silver Nitrate Test.—Before applying this test, if more than a trace of albumin is present, it should be removed by heat, as albuminate of silver forms and interferes with the reaction. The test may be applied by acidulating the urine in a test-tube with nitric acid,

shaking, and adding a drop of a 1:8 silver nitrate solution. A solid flocculent mass of precipitate falling rapidly indicates normal or increased chlorid; a diffused cloudy precipitate indicates a diminution. The addition of nitric acid is necessary to prevent the formation of silver phosphate.

Quantitative Test.—If a more accurate determination is wanted, Volhard's method as modified by Salkowski should be used (quoted from Boston, Clinical Diagnosis, 2d ed., p. 180):

The *reagents required are:*

(1) Pure nitric acid, sp. gr., 1.2.

(2) Concentrated solution of double sulphate of iron and ammonia, free from chlorin (if necessary, free from chlorin by recrystallization).

(3) Saturated solution of silver nitrate (29.075 gm. of the crystals per liter). Of this 1.0 cc. corresponds to 0.01 gm. NaCl.

(4) Ammonium sulphocyanid solution $6\frac{3}{7}$ gm. in enough water to make 400 cc. Of this solution 2.5 cc. correspond to 10 cc. of the standard silver solution.

The following method may be employed for standardizing the sulphocyanid solution:

Ten cc. of the silver solution (3) are placed in a flask and 90 cc. of water added. Four cc. of nitric acid (1) are now added, and finally 5 cc. of the double sulphate solution (2). After the mixture is well shaken, the sulphocyanid of ammonium solution is carefully added from the buret until a slight red color appears. This process should be repeated often, noting each time the quantity of sulphocyanid solution necessary and the mean obtained. Based upon these results the sulphocyanid solution is diluted to a point so that 2.5 cc. correspond to 1.0 cc. of the silver solution. Should the reaction (red color) appear after 20 cc. of the sulphocyanid solution are added, the following formula serves to determine the quantity of water to be added to 1 liter;

$$= 20 : 25 :: 1000 : x. \therefore (x = 1250).$$

Therefore, 250 cc. of water are added in order that 25 cc. shall correspond to 10 cc. of the silver solution.

Method of Testing.—(I) Ten cc. of the urine, previously freed from albumin, are placed in a 100-cc. flask. (II) Fifty cc. of water are added. (III) Four cc. of the nitric acid (1) are added, and then an excess (15 cc.) of the silver nitrate (3) solution. (IV) The mixture is thoroughly shaken till the precipitation is completed and the fluid begins to clear, and then enough water is added to make 100 cc. (V) The resultant mixture is filtered through dry filter-paper into an 80-cc. flask. (VI) The 80 cc. of fluid obtained are now poured into a 250-cc. flask. (VII) Five cc. of the solution of the double sulphate of iron and ammonia (2) are added. (VIII) The sulphocyanid solution (4) is slowly added from a buret till the end reaction (red color) is obtained and does not disappear on shaking.

The excess of silver solution not needed to precipitate the chlorids from 10 cc. of urine is now estimated as follows: Of the sulphocyanid solution 2.5 cc. corresponds to 1.0 of silver solution. Therefore should, for example, 12.5 cc. of the sulphocyanid solution be required to produce the end reaction, then there were 5 cc. of the silver solution not employed in precipitating the chlorids from the 10 cc. of urine. Since 15 cc. of silver solution have been added, and 5 cc. were found to be in excess, it required 10 cc. of $AgNO_3$ solution to precipitate the chlorids from 10 cc. of urine. As 1 cc. of the $AgNO_3$ solution corresponds to 0.01 gm. of NaCl, 10 cc. of the $AgNO_3$ solution will correspond to 0.1 gm. of NaCl in 10 cc. of urine, or to 1.0 gm. of NaCl in 100 cc. of urine and of the total output of urine for twenty-four hours was 800 cc., the quantity of NaCl excreted in that period would be 8.0 gm.

Purdy's Centrifuge Method.—This has been described in general under the heading of Albumin Tests. The percentage tubes of the apparatus are filled to the 10 cc. mark with the urine. From 15 to 30 drops of nitric acid are added to prevent the precipitation of silver phosphate, the amount of acid varying with the specific gravity of the urine (the higher the latter, the more acid is used), and the tubes are filled to the 15 cc. mark with a 1:8 solution of silver nitrate. The tubes are closed and the contents thoroughly mixed. The tubes are placed in the standard centrifuge and revolved at the rate of 1500 revolutions a

minute for three successive periods of five minutes each, when the quantity of bulk-percentage is read off on the scale of the tube. This bulk-percentage is converted into weight-percentage of NaCl with the aid of the following table:

PURDY'S CENTRIFUGAL TABLE—CHLORIDS

Normally from ½ to 1 per cent., or 10 to 15 gm. in 24 hours.

Bulk Per Cent. $AgCl_2$.	Per Cent. of NaCl.	Gram per Liter NaCl.	Grains per Ounce NaCl.	Bulk Per Cent. $AgCl_2$.	Per Cent. of NaCl.	Gram per Liter NaCl.	Grains per Ounce NaCl.
¼	0.03	0.3	0.15	8	1.04	10.4	4.98
½	0.07	0.7	0.31	8½	1.1	11.0	5.29
¾	0.1	1.0	0.47	9	1.17	11.7	5.6
1	0.13	1.3	0.62	9½	1.23	12.3	5.91
1¼	0.16	1.6	0.78	10	1.3	13.0	6.22
1½	0.19	1.9	0.93	10½	1.36	13.6	6.53
1¾	0.23	2.3	1.09	11	1.43	14.3	6.84
2	0.26	2.6	1.24	11½	1.49	14.9	7.2
2¼	0.29	2.9	1.41	12	1.56	15.6	7.46
2½	0.32	3.2	1.56	12½	1.62	16.2	7.78
2¾	0.36	3.6	1.71	13	1.69	16.9	8.09
3	0.39	3.9	1.87	13½	1.75	17.5	8.4
3¼	0.42	4.2	2.02	14	1.82	18.2	8.71
3½	0.45	4.5	2.18	14½	1.88	18.8	9.02
3¾	0.49	4.9	2.35	15	1.94	19.4	9.33
4	0.52	5.2	2.49	15½	2.01	20.1	9.65
4¼	0.55	5.5	2.64	16	2.07	20.7	9.94
4½	0.58	5.8	2.8	16½	2.14	21.4	10.27
4¾	0.62	6.2	2.96	17	2.2	22.0	10.51
5	0.65	6.5	3.11	17½	2.27	22.7	10.87
5½	0.71	7.1	3.42	18	2.33	23.3	11.2
6	0.78	7.8	3.73	18½	2.4	24.0	11.51
6½	0.84	8.4	4.05	19	2.46	24.6	11.82
7	0.91	9.1	4.35	19½	2.53	25.3	12.13
7½	0.97	9.7	4.67	20	2.59	25.9	12.44

QUESTIONS ON CHAPTER XIV

What are the principal classes of inorganic constituents in the urine?
What inorganic substances occur in minute quantities?
What gases occur in the urine?
What is the chief group of solids in the urine next to urea?

Whence are the *chlorids* of the urine derived?

How much NaCl is eliminated normally in twenty-four hours?

What effect has starvation upon sodium chlorid elimination. Increased amounts of water drunk?

What does increase of chlorids mean in diabetes insipidus?

When are chlorids diminished? Of what value is this in diagnosis?

How does dropsy affect the amount of chlorids? Absorption of dropsical fluid?

How are the chlorids estimated approximately? How are they determined accurately?

Describe Purdy's centrifuge method for the estimation of chlorids.

CHAPTER XV

PHOSPHATES

THE phosphates of the urine are derived partly from the food and partly from the decomposition of proteids containing phosphorus, particularly nuclein and lecithin from the tissue cells, especially from the brain and nerves. Organic phosphorus compounds (from eggs, milk, etc.) are much more readily assimilated than inorganic. Insoluble compounds of phosphoric acid with earthy salts are formed in the intestines and are excreted in the feces without being absorbed. These compounds are formed especially when the diet is rich in calcium (and magnesium) salts.

Normally the average excretion of phosphates is represented by from 2.5 to 3.5 gm. of phosphoric acid (P_2O_5) in twenty-four hours, or 1.7 to 2.6 gm. per liter. Of this amount about two-thirds (2.0 to 4.0 gm. in twenty-four hours) are represented by the alkaline phosphates (Na,K), while the remaining one-third (1.0 to 1.5 gm. in twenty-four hours) is derived from the earthy phosphates (Ca,Mg).

Earthy phosphates are insoluble in water, but soluble in acids. In acid urines they occur as *acid* phosphates in solution, although occasionally a faint precipitate of acid calcium phosphate ($CaHPO_4 + 2H_2O$) is seen in acid urines. When urine becomes alkaline the acid phosphates are converted into *normal* phosphates and precipitate as a heavy, amorphous, whitish sediment. On heating a faintly acid, neutral, or alkaline urine (see Albumin Tests),

a similar precipitate is often obtained, because this converts the acid phosphates into normal phosphates, which are precipitated, and into superphosphates, which remain in solution.

$$4CaHPO_4 = CaH_42PO + Ca_32PO_4.$$

The addition of a few drops of acid quickly dissolves the earthy phosphate precipitate. When ammoniacal fermentation occurs, the ammonia combines with the magnesium phosphate to form ammoniomagnesium phosphate or "triple phosphate." (See Description of Crystals, p. 263.)

Alkaline phosphates are soluble in water and alkalis. The sodium compound is chiefly responsible for the acidity of the urine (monosodic acid phosphate, NaH_2PO_4). The alkaline phosphates are more abundant in the urine than the earthy, the proportions being usually as two is to one.

Clinical Significance.—The amount of phosphates in the urine varies normally with the amount of food absorbed containing phosphorus. On a mixed diet the amount of phosphoric acid excreted should not exceed 3.5 or, at most, 4 gm. in twenty-four hours. Normally the relative proportion of phosphoric acid to the total nitrogen is from 17 to 20 : 100 (average 18.9: 100). This ratio $\frac{N=100}{P_2O_5=18.9}$ is known as *Zueltzer's coefficient.*

1. *True Phosphaturia.*—A true excess of phosphoric acid excreted beyond the normal limit stated above (3.5 to 4.0 gm. in twenty-four hours) or an increase in the P_2O_5 as compared to the nitrogen (15 urea = 7 nitrogen [Sahli]) constitutes *true phosphaturia.* This is not a definite condition, but merely a *symptom* that may occur in a variety of conditions in which there is either (*a*) an increased amount of phosphorus absorbed in the food, or (*b*) an increased metabolism of the phosphorus-containing tissues of the body (brain, nerves, etc.).

2. *Clinical Phosphaturia.*—This is quite a different condition, and is the "phosphaturia" commonly spoken of when no qualification is made. This is an *apparent increase* of the phosphates which is due to a tendency on the part of the urine to precipitate phosphatic deposits. This precipitation is, in turn, due to a change in the acidity of the urine from acid to neutral or alkaline. The change depends either (1) upon alkaline fermentation in the urinary tract; (2) upon a lowered acidity as the result of a predominantly vegetable diet, or the drinking of alkaline mineral waters, or the ingestion of alkaline diuretics, etc. (We have already referred to the subject of phosphatic turbidity, etc., on p. 215.) Clinical phosphaturia, therefore, does not mean an increase in the amount of P_2O_5 excreted, but a diminished acidity or an increased amount of the earthy elements, especially calcium. Naturally, both clinical phosphaturia and true phosphaturia may coexist, but this is comparatively rare.

Clinical phosphaturia is especially typical of *neurasthenia.* In *hysteria* the proportion of earthy phosphates rises as compared to that of the alkaline phosphates, until the two groups may be as 1 : 1 (Gilles de la Tourette and Cattelineau).

Phosphatic Diabetes.—This is said to be an independent disease of metabolism (Teissier) in which large amounts (30 to 35 gm. P_2O_5 in twenty-four hours) of phosphates are excreted. It has nothing to do with true or glycosuric diabetes, but phosphatic diabetes (a badly chosen term, because confusing) may lead to or end in diabetes mellitus.

The following tabular statement sums up the clinical significance of changes in the amount of phosphates excreted. The conditions followed by a question mark are doubtful:

Phosphates *increased:*	Phosphates *decreased:*
1. In active metabolism.	1. In starvation (slightly).
2. With a diet rich in nuclein.	2. In chronic diseases with lowered metabolism.
3. In bone diseases, *e. g.*, rickets, osteomalacia(?).	3. In chronic renal diseases (renal insufficiency).
4. In destructive pulmonary diseases, *e. g.*, tuberculosis(?).	4. In pregnancy (formation of fetal bones).
5. In nervous and mental diseases, *e. g.*, insanity (mania); neurasthenia; organic nervous diseases; meningitis.	5. In gout (may be unchanged).

Phosphates *increased:*	Phosphates *decreased:*
6. In "phosphatic diabetes" (see text).	6. In acute yellow atrophy (phosphates disappear completely).
7. After mental strain, excitement.	
8. In diabetes mellitus (proteid diet, etc.).	

Detection.—Earthy Phosphates.—Render half a test-tube full of filtered urine alkaline with ammonia, and warm gently, causing the precipitation of earthy phosphates in the form of a whitish cloud that settles to the bottom of the tube. The precipitate is dissolved on the addition of acetic acid.

This test may be also made to serve approximately for quantitative estimation, according to Ultzmann: A test-tube 2 cm. wide is filled with the urine to the depth of $5\frac{2}{3}$ cm., and a few drops of strong ammonia are added. The mixture is warmed over an alcohol lamp until the earthy phosphates separate. The depth of the sediment is measured after standing for fifteen minutes. Normally, the layer will be 1 cm. high; a greater depth indicates an increase, while a less abundant precipitate means diminution.

Alkaline Phosphates.—After the earthy phosphates have been separated as shown above, the mixture is filtered. To the filtrate add one-third of its volume of magnesium fluid (magnesium sulphate, ammonium hydrate, ammonium chlorid, of each, 1 part; water, 8 parts). The white precipitate consists of alkaline phosphates. To make this test available for approximate estimation, according to Ultzmann, 10 cc. of the urine are treated with 3 cc. of the magnesium fluid. A precipitate of crystalline ammoniomagnesium phosphate is formed, together

with an amorphous mass of calcium phosphate. If a milky turbidity permeates the entire fluid, the alkaline phosphates are normal in amount. If an abundant precipitate gives the fluid the appearance of cream, they are greatly increased, and if a slight turbidity follows, or if the fluid remains transparent, they are decreased.

Estimation of Total Phosphoric Acid.—The following method is based upon the fact that, when a solution of a phosphate acidulated with acetic acid is treated with a solution of uranium nitrate or acetate, a precipitate of uranium phosphate occurs, and when a soluble salt of uranium is added to a solution of potassium ferrocyanid, a reddish-brown precipitate is formed. The solutions required are:

1. A *standard solution of uranium nitrate* or *acetate*, consisting of 35.5 gm. of pure uranic nitrate or acetate in 1000 cc. of distilled water. One cc. corresponds to 5 mg. of phosphoric acid (phosphoric anhydrid, P_2O_5).[1] As the salts of uranium are apt to be contaminated with uranium oxid, the following method (Tyson) is recommended in preparing the standard uranium solution:

Dissolve 20.3 gm. of yellow uranic oxid in strong acetic acid previously diluted to nearly a liter. To determine the strength of the solution place 50 cc. of the standard solution of sodium phosphate in a beaker with 5 cc. of the solution of sodium acetate and heat on a water-bath to 90° or 100° C. The uranium solution is then allowed to

[1] Standard solutions for quantitative analysis may be bought already prepared, and the rather high prices of these will often appear insignificant when the time required in making such solutions at one's office is considered. The standard solutions should preferably be made by a competent pharmacist or analytic chemist, as they require testing by titration methods which are often tedious.

run from a buret into the warm mixture until precipitation ceases.

Then a drop of the mixture is carried on a glass rod into contact with a drop of the potassium ferrocyanid solution or a porcelain dish, and if the reddish-brown uranium ferrocyanid does not appear, cautiously continue the addition of the uranium solution until the color responds to the test. The quantity used is then read off, being that which is sufficient to decompose sodium phosphate corresponding to 0.1 gm. of P_2O_5. From this is calculated the amount of distilled water to be added to make 1 cc. of the uranium solution correspond to 0.005 gm. of phosphoric acid.

2. *Sodium Acetate Solution.*—One hundred gm. of sodium acetate are dissolved in 900 cc. of distilled water, and to this 100 cc. of acetic acid are added.

3. A *saturated solution of potassium ferrocyanid* to be used as an indicator.

Method.—To 50 cc. of the urine in a glass beaker add 5 cc. of sodium acetate solution, and warm the mixture over a water-bath to 80° C. Drop the standard solution of uranium from a buret into the hot urine slowly, as long as the precipitate forms, or until a drop of the mixture, removed by means of a glass rod and placed on a portion of the plate, gives a distinct brown color with a drop of the indicator. This point indicates the end-reaction; the number of cubic centimeters used is read off and, multiplied by 0.005, gives the amount of phosphoric acid in 50 cc. of urine, from which the quantity in twenty-four hours is calculated. The end-reaction, shown by the brown color on the porcelain dish, takes place when the uranium solution has precipitated all the phosphoric acid and the mixture in the beaker contains pure uranium.

Cochineal tincture is also used instead of potassium ferrocyanid as an indicator. A few drops of it are added to the urine before heating, and the standard uranium solution is added until a faint but distinct and permanent green color appears—that is, until all the phosphoric acid has been precipitated and there is a slight excess of uranium.

Phosphoric Acid with Earthy Phosphates.—To determine phosphoric acid which is in combination with lime and magnesia, 200 cc. of urine are treated with ammonia, the precipitate is collected after twelve hours on a filter, and washed with ammonia water (1:3). The filter is broken at its point, the precipitate washed into a beaker, and dissolved while warm with as little acetic acid as possible. Add 5 cc. of sodium acetate solution, dilute to 50 cc., and treat as in the preceding method. The difference between the total phosphoric acid and that combined with the earths represents the quantity combined with the alkalis.

Purdy's Centrifugal Method.—Fill a Purdy centrifugal percentage tube to the 10 cc. mark with the urine and add 2 cc. of 50 per cent. acetic acid, shake, and add 3 cc. of a 5 per cent. solution of uranium nitrate. The tube is closed, inverted several times until the urine and reagent are well mixed, allowed to stand for three minutes to secure complete precipitation, placed in the Purdy centrifuge (a centrifuge with an arm measuring $6\frac{3}{4}$ inches is essential), and made to revolve at the rate of 1500 revolutions a minute for three minutes. The bulk-percentage of uranyl nitrate is read at the level of the precipitate and is converted into the corresponding per cent. of P_2O_5 (by weight) by the use of the following table, which is that

of Purdy, with the addition of a column showing the number of grams of P_2O_5 per liter of urine.

Purdy's method is a rapid and convenient one, and is sufficiently accurate for comparative clinical estimations:

PURDY'S TABLE FOR CENTRIFUGAL ANALYSIS—PHOSPHATES

Normally: 0.17–0.26 *per cent.,* P_2O_5*; or* 1.7–2.6 *grams per liter; or* 2.5–3.5 *grams in twenty-four hours.*

Bulk Per Cent. $H(UO_2)PO_4$.	Per Cent. P_2O_5.	Grams per Liter P_2O_5.	Grains per Ounce P_2O_5.	Bulk Per Cent. $H(UO_2)PO_4$.	Per Cent. P_2O_5.	Grams per Liter P_2O_4.	Grains per Ounce P_2O_4.
½	0.02	0.2	0.1	11	0.14	1.40	0.67
1	0.04	0.4	0.19	12	0.15	1.50	0.72
1½	0.045	0.45	0.22	13	0.16	1.60	0.77
2	0.05	0.5	0.24	14	0.17	1.70	0.82
2½	0.055	0.55	0.26	15	0.18	1.80	0.86
3	0.06	0.6	0.29	16	0.19	1.90	0.91
3½	0.065	0.65	0.31	17	0.2	2.	0.96
4	0.07	0.70	0.34	18	0.21	2.10	1.
4½	0.075	0.75	0.36	19	0.22	2.20	1.06
5	0.08	0.80	0.38	20	0.23	2.30	1.1
6	0.09	0.90	0.43	21	0.24	2.40	1.15
7	0.1	1.00	0.48	22	0.25	2.50	1.2
8	0.11	1.10	0.53	23	0.26	2.60	1.25
9	0.12	1.20	0.58	24	0.27	2.70	1.3
10	0.13	1.30	0.62	25	0.28	2.80	1.35

QUESTIONS ON CHAPTER XV

What is the origin of the urinary phosphates? In what amounts is P_2O_5 normally excreted?

In what condition is the amount of phosphoric acid increased?

What is phosphaturia? What is clinical phosphaturia? True phosphaturia?

What is phosphatic diabetes?

When is phosphoric acid diminished in the urine?

How do you detect and approximately estimate earthy phosphates? Alkaline phosphates? Total phosphoric acid?

What is the most rapid clinical method for the latter?

CHAPTER XVI

SULPHATES, CARBONATES, AND LESS IMPORTANT INORGANIC CONSTITUENTS

SULPHATES

THE sulphates of the urine are derived from two sources: (1) The food; (2) the tissues whose proteid molecules burn up, giving up their sulphur contents.

The sulphates in the urine occur in three distinct forms of compounds:

(1) The *mineral* or *preformed sulphates*, which occur as compounds of Na, K, Mg, and Ca; (2) the *conjugate, ethereal,* or *aromatic sulphates*, indol, skatol, phenols (cresol, pyrocatechin, hydroquinon), occurring usually as K or Na salts of the ethereal sulphates; and (3) the *neutral sulphates* (incompletely oxidized sulphur)—*e. g.*, cystin, taurin, thiosulphates, sulphocyanids, etc.

The relations between each of these groups and the total sulphur in the urine is shown by the following table from Croftan:

Total sulphur	*Acid sulphur compounds* (sulphates, 80 to 86 per cent.).	1. *Preformed* (mineral) sulphates. 2. *Conjugate* (ethereal, aromatic) *sulphates* (nominally one-tenth of total sulphates).
	Neutral sulphur compounds (suboxidized sulphur, 14 to 20 per cent.).	Cystin, taurin, thiosulphates, sulphocyanids, etc.

The *mineral* or *preformed sulphates* of the food form a small part of the total sulphur excreted in the urine. The bulk of the urinary sulphates is derived from the metabolism of proteids, both from food and tissues. It is not surprising to find, therefore, that the excretion of total sulphur varies usually as the excretion of nitrogen (the ratio of H_2SO_4 to N. being

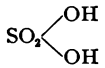
normally 1: 5), and that this ratio is a fairly constant one. [About four-fifths of all the sulphur in albuminous food is excreted in the urine, but some foods give off more sulphur than others, the average being 1 per cent. sulphur, so that 100 gm. albumin in food give off 2.5 gm. H_2SO_4 in twenty-four hours.]

The *total sulphate output* is, therefore, an index to proteid metabolism (though not as accurate as the nitrogen output). The amount of total sulphates by itself has little clinical value, but is useful in determining the different sulphate groups by subtraction (see below). Normally the total sulphur, as SO_3, is equivalent to from 1.5 to 3 gm. in twenty-four hours (= 2.5 gm., average, H_2SO_4) on a mixed diet.

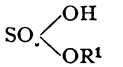
The total sulphates are *increased* in all conditions tending to increase proteid metabolism, and *diminished* in the reverse conditions. The following table sums up the principal causes of total sulphur variations:

Total sulphates *increased:*	Total sulphates *decreased:*
1. On a meat diet.	1. On a proteid-free diet.
2. After exercise.	2. In diminished metabolism.
3. In fevers.	3. In convalescence from fevers.
4. In acute diseases.	4. In nephritis (usually varies as urea).
5. In acute articular rheumatism.	5. In jaundice (relative increase of neutral sulphur, taurin, etc.).
6 In chorea.	6. In rickets (?).
7. In diabetes mellitus (meat diet).	7. In anemia, cachexia.
	8. In acute gastro-enteritis.
	9. In many skin diseases, *e. g.*, eczema.

The *ethereal sulphates* (conjugate sulphates, aromatic sulphates) have received partial consideration under the heading of Indican, etc. (p. 174). The constitution of these compounds is illustrated by the following graphic formulæ:



H_2SO_4 = Sulphuric acid.

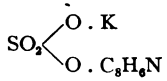


Ethereal sulphate.

The ethereal sulphates are, therefore, compounds of sulphuric acid in which an aromatic radicle is substituted for one of the hydrogen atoms. The principal compounds of this group are indol (the source of indican), skatol, phenol, cresol, pyrocatechin, and hydroquinon. They occur in the urine as the potassium or sodium salts of the corresponding ethereal

[1] R = aromatic radicle in ethereal sulphates.

sulphates. This means that the remaining H atom in the above graphic formula is substituted by an atom of K or Na. Indol and skatol, furthermore, are not excreted as such, but after oxidation, as indoxyl and skatoxyl respectively. The potassium salt of the ethereal sulphate of indoxyl (clinically known as indican) has been the following graphic formula:



Indoxyl-potassium sulphate (indican).

The *ratio* of ethereal sulphates to the total sulphates is said to be 1 : 10; but this is not at all constant, and this ratio has no clinical value, for the simple reason that the total sulphates represent largely albumin-breakdown in food and tissues, while the ethereal sulphates represent largely intestinal decomposition and putrefaction. The absolute amount of ethereal sulphates is of greater clinical value, and corresponds to from 0.15 to 0.3 gm. of SO_3 in twenty-four hours.

The amount of ethereal sulphates may be considered as a quite accurate indication of the *degree of intestinal putrefaction.* The following table[1] shows the principal causes affecting the amount of ethereal sulphates excreted:

Ethereal sulphates *increased:*	Ethereal sulphates *decreased:*
1. By a rich meat diet; by eating putrid food.	1. Starvation; milk diet (casein [?] inhibits intestinal putrefaction).
2. After taking drugs furnishing phenol and other aromatic compounds.	2. After taking calomel or other intestinal antiseptics.
3. In constipation (not constant).	3. In acute intestinal catarrh.
4. In chronic intestinal catarrh and conditions accompanied by same. (Carcinoma of liver; acute yellow atrophy, etc.)	4. After acid (HCl) medication; in hyperchlorhydria, and a diet rich in NaCl.
5. In typhoid fever, tuberculous enterocolitis, peritonitis, cholera.	
6. After alkaline medication; in hypochlorhydria, and a diet low in NaCl.	

[1] Arranged by the author from data furnished by Emerson, Clinical Diagnosis, 1908, p. 139.

The *neutral sulphates* (suboxidized or unoxidized organic sulphur) constitute from 14 to 20 per cent. of the total sulphur in health. The exact mechanism underlying the failure of this sulphur to become oxidized is obscure, but normally the neutral sulphur in the urine is derived chiefly from (1) the bile (taurocholic acid partly reabsorbed from bowel); (2) the saliva (sulphocyanids reabsorbed from bowel); (3) the putrefaction of albumin in the intestine, and (4) the incomplete oxidation of tissue albumins (Croftan).

Pathologically there may be an increase of neutral sulphur compounds in a number of conditions, the exact reason not being understood as yet in some of them.

Neutral sulphur is increased in inanition, in asphyxia, dyspnea (deficient oxidation). It is said to be increased after muscular fatigue and as a result of tissue waste, and after the ingestion of sulphur and its compounds, *e. g.*, sublimed sulphur, sulphonal, and also after taking chloral and inhaling chloroform. Large amounts of neutral sulphates are excreted in some febrile conditions, such as pneumonia and typhoid (in the latter, thiosulphates have been found).

The biliary origin of neutral sulphur (taurin, taurocholic acid) accounts for its increased elimination in jaundice (sometimes as high as 60 per cent. of total sulphur) and in biliary stasis (gall-stones, diseases of the liver, bile-ducts, etc.). (Neutral sulphur is diminished in bile-fistula.) A special condition known as *cystinuria*, in which neutral sulphur is markedly increased, will be discussed on page 268. This condition seems to be an unaccountable metabolic freak, the chief interest of which clinically lies in the danger of possible formation of cystin stones.

Detection.—For ordinary purposes the following test is sufficient: To 10 cc. of filtered urine add about 3 cc. of barium solution (barium chlorid, 4 parts; concentrated hydrochloric acid, 1 part; distilled water, 16 parts), a little at a time, shaking after each addition. When less sulphates than normal are present the fluid shows opalescence; when normal, it becomes opaque, milky; when in excess, it grows thick and creamy. The precipitate normally fills one-half of the concavity of the test-tube.

Quantitative Determination of Total Sulphates.—(*a*) **Purdy's Centrifugal Method.**—A convenient clinical

test for determining with a fair degree of accuracy the total sulphates is that of Purdy. (Compare: Phosphates, Chlorids, Albumin.) A graduated centrifuge tube is filled to the 10 cc. mark with urine; 5 cc. of a solution of barium sulphate are added. The solution is made up of 4 parts barium chlorid, 16 parts distilled water, 1 part HCl. Mix the contents of the tube well, inverting several times, and allow to stand for about five minutes, and centrifuge at a uniform speed of 1500 revolutions per minute for three minutes, employing a centrifuge with standard arm-length of $6\frac{3}{4}$ inches. The bulk-percentage of $BaSO_4$ is read off on the tube. The following table shows the equivalents in weight-percentage of SO_3, in grams per liter, and in grains per ounce.

TOTAL SULPHATES (After Purdy)

Normally: 0.1–0.2 per cent., or 1–2 grams per liter

Bulk Per Cent. $BaSO_4$.	Per Cent. SO_3.	Gram per Liter SO_3.	Grains per Ounce SO_3.	Bulk Per Cent. $BaSO_4$.	Per Cent. SO_4.	Gram per Liter SO_3.	Grains per Ounce SO_3.
$\frac{1}{8}$	0.04	0.40	0.19	$2\frac{1}{4}$	0.55	5.50	2.64
$\frac{1}{4}$	0.07	0.70	0.34	$2\frac{1}{2}$	0.61	6.10	2.93
$\frac{3}{8}$	0.1	1.	0.48	$2\frac{3}{4}$	0.67	6.70	3.22
$\frac{1}{2}$	0.13	1.30	0.62	3	0.73	7.30	3.5
$\frac{5}{8}$	0.16	1.60	0.77	$3\frac{1}{4}$	0.79	7.90	3.79
$\frac{3}{4}$	0.19	1.90	0.91	$3\frac{1}{2}$	0.85	8.50	4.08
$\frac{7}{8}$	0.22	2.20	1.06	$3\frac{3}{4}$	0.91	9.10	4.37
1	0.25	2.50	1.1	4	0.97	9.70	4.66
$1\frac{1}{4}$	0.31	3.10	1.49	$4\frac{1}{4}$	1.03	10.3	4.94
$1\frac{1}{2}$	0.37	3.70	1.78	$4\frac{1}{2}$	1.09	10.9	5.23
$1\frac{3}{4}$	0.43	4.30	2.06	$4\frac{3}{4}$	1.15	11.5	5.52
2	0.49	4.90	2.35	5	1.21	12.1	5.81

(*b*) The **gravimetric method,** according to Salkowski, consists in weighing the precipitate of barium sulphate, obtained by adding barium

chlorid to a known volume of urine. Fifty cc. of diluted or filtered urine are acidified in a beaker with 5 cc. of hydrochloric acid and heated to boiling for about fifteen minutes to break up the ethereal sulphates. Barium chlorid is then added until no more precipitation occurs. The precipitate is collected on a small filter the weight of whose ash is known, and washed with hot distilled water until no more barium chlorid is found in the filtrate—*i. e.*, until the filtrate remains clear after the addition of a few drops of sulphuric acid. The precipitate is washed with hot alcohol to remove resinous matter and then with ether. The filter is removed and burned with its contents in a platinum crucible. It is cooled over sulphuric acid in a drying-oven to 100° C., weighed, and the weights of the crucible and filter-ash are deducted.[1] The remainder is the weight of the barium sulphate formed, from which the sulphuric acid is calculated as follows: One hundred parts of barium sulphate correspond to 34.33 parts of SO_3.

Quantitative Estimation of Ethereal Sulphates.—Mix 100 cc. of urine with an equal volume of alkaline barium chlorid solution (BaCl saturated solution, 1 part; BaOH saturated solution, 2 parts) in a beaker, stir well, allow to stand for ten minutes, and filter until the filtrate reaches 100 cc. Add dilute HCl till strongly acid and boil on a water-bath till all the $BaSO_4$ precipitate has been deposited, leaving supernatant liquid clear. Filter off this sediment; wash on the filter with distilled water, dry, and weigh. Multiply the weight by 2 to get the amount of ethereal sulphates in 100 cc. of urine.

Quantitative Determination of Preformed or Mineral Sulphates.—The amount of preformed sulphates is obtained simply by subtracting the quantity of ethereal sulphates from that of total sulphates. (The presence of preformed sulphates is demonstrated qualitatively by acidifying some urine in a test-tube with acetic acid and adding BaCl solution, see above, p. 226.)

Quantity of Neutral Sulphur.—The amount of *total sulphur* is determined and from this the amount of *total sulphates* (total sulphuric acid, see above) is deducted, giving the amount of neutral sulphur. To determine the total sulphur the following method is used:

Fifty cc. (25 cc. if there is cystin) of urine are evaporated to dryness in a silver crucible. To the residue is added a mixture of potassium nitrate 4 parts, sodium carbonate 1 part (chemicals to be free from sulphur),

[1] To correct for a slight error due to the formation of a small amount of barium sulphid, a few drops of pure sulphuric acid are added after the platinum crucible has cooled, thus converting the sulphid into sulphate. Heat again to redness to drive off the excess of sulphuric acid.

and the resultant mass burned till white. The residue is thoroughly washed out with water and the watery solution carefully poured into a porcelain dish. In this the fluid is evaporated three times, adding HCl each time. The HCl is added to take up the nitric acid, all of which must be removed. The residue is dissolved in water and allowed to stand to see if any AgCl separates. If it does, filter (Emerson). In the filtrate the neutral sulphur is precipitated by adding BaCl. The precipitate of $BaSO_4$ is filtered out, dried, and weighed, as described under total sulphates.

CARBONATES

Minute quantities of carbonates and bicarbonates of sodium, ammonium, calcium, and magnesium are found in fresh urine of alkaline reaction. Ammonium carbonate may occur in large amounts, owing to alkaline decomposition. The carbonates in urine are derived from the food, especially from vegetable acids, such as lactic, tartaric, malic, succinic, etc.

They are, therefore, most abundant in the urine of herbivora. An excess of carbonates renders the urine turbid when passed or on standing, and, as a rule, the sediment is mixed with phosphates.

Detection.—On the addition of an acid the presence of carbonates is detected by the evolution of gas-bubbles, and this gas, when passed into baryta water, renders the latter turbid. The determination of the amount of carbonic acid will be found described in the larger text-books.

INORGANIC CONSTITUENTS OF MINOR IMPORTANCE

Gases in the Urine.—One liter of human urine normally yields about 100 to 200 cc. of gases, which consists of from 83 to 95 volumes of CO_2, 0.5 volume of oxygen, and from 6 to 16 volumes of nitrogen. These gases exist in solution and are separated by means of a vacuum-pump. They have no clinical significance.

Iron.—Traces of iron are found in the residue of urine after evaporation. The amount in a healthy urine varies between 0.003 and 0.0011 gm. in a liter. The exact combination in which iron occurs is still un-

known. The presence of iron is detected from the ash of the evaporated residue, which is dissolved in a little hydrochloric acid, and the solution divided into two parts: the first part is boiled with a drop of nitric acid and treated with some potassium sulphocyanid solution, which produces a blood-red color in the presence of ferric oxid. The other half of the solution is boiled with nitric acid and diluted. On addition of potassium ferrocyanid a precipitate of Prussian blue is formed after standing a while.

Hydrogen Peroxid.—Schönbein found this substance in the urine in very small amounts, but so far as is known it has no significance. Dilute indigo solution is bleached by hydrogen peroxid in the presence of a solution of iron sulphate. The urine must be perfectly fresh.

Fluorin.—Hydrofluoric acid was discovered in urine by Berzelius (London, 1812), but occurs in urine only in faint traces. Berzelius found enough, however, in a considerable volume of urine to etch lines on a glass plate.

Thiosulphuric acid does not normally occur in human urine (Salkowski, Presch), but was found in a case of typhoid fever by Strümpell. It is present in the normal urine of cats and dogs. Its isolation and detection will be found described in Neubauer and Vogel, 1898, page 20.

Hydrogen sulphid (sulphuretted hydrogen) is rarely present in freshly passed urine. It is not found in diseases accompanied by putrefaction, after the ingestion of sulphids, or after sulphur baths. It may pass into the urine through a rectal fistula. The chief importance of this gas lies, however, in the fact that it is formed during decomposition of purulent urine (pyelitis, cystitis). Hydrogen sulphid putrefaction has been ascribed (Müller, von Jaksch, etc.) to a special coccus, and, according to Salkowski, the unoxidized sulphur of albumin is the source of this sulphid in albuminous urine. The presence of hydrogen sulphid may be detected by its odor (see Odor of Urine, p. 28) and by hanging in the acid urine a strip of blotting-paper moistened with lead acetate solution and then with a drop of NaOH solution. The paper turns black, owing to a deposit of lead sulphid.

Silicic acid is present in normal urine in minute traces, and presents no clinical interest.

Nitric and Nitrous Acids.—According to Wulffius and Schönbein every normal urine contains small amounts of nitrates derived from food and drinking-water. Nitric acid was found in traces in normal urines freshly passed, while nitrous acid occurs in urines after long standing, through the reduction of nitric acid. It disappears as putrefaction increases.

ACCIDENTAL INORGANIC CONSTITUENTS

A number of inorganic substances may be present accidentally in the urine, which may otherwise be normal or may show evidences of disease.

Metals.—Mercury, arsenic, antimony, lead, silver, thallium, cadmium, and lithium have been found in urine under various circumstances. The study of their absorption, elimination, and detection belongs to toxicologic chemistry.

Halogens.—Iodids and bromids appear in the urine after they have been taken internally. They are of sufficient clinical interest to merit a few words as to their detection.

When the nitric acid test for albumin is performed, the elimination of *iodin* may be detected by the appearance of a reddish-brown zone (free iodin) at the border-line between urine and acid, the brown color gradually spreading downward into the acid.

Iodids are detected by adding chloroform to urine, then a few drops of yellow HNO_3, and shaking. The acid sets free the iodin and the chloroform becomes pink or purple.

Bromids are detected in the same manner, but more of the acid is added, and the chloroform is tinted a brownish-red.

QUESTIONS ON CHAPTER XVI

What varieties of *sulphates* are present in the urine?

What is meant by "preformed sulphuric acid"?

What is "conjugate sulphuric acid"? How much is excreted daily in health? What is meant by "neutral sulphur"?

Whence are sulphates in the urine derived?

What is the clinical significance of sulphates? With what other constituents do they run parallel usually?

When are sulphates increased? Decreased?

Describe the ordinary test for sulphates.

How is total sulphuric acid determined? What methods are used for determining the amount of ethereal sulphates? The preformed sulphates? The neutral sulphur?

What *carbonates* are found in fresh urine? In decomposing urine? Whence are they derived?

What is a simple test for carbonates?

How is the presence of *iron* detected?

In what amounts does healthy urine contain iron?

How is *hydrogen peroxid* in the urine detected?

CHAPTER XVII

URINARY CONCRETIONS

CONCRETIONS are termed sand, gravel, stones (or calculi), according to their size. They are either primary or secondary. Primary concretions are deposited from urine that has undergone no decomposition, either as a result of an excess of some normal constituents or as the result of some foreign additions to the urine. Secondary concretions are due to decomposition of the urine with the resulting precipitation of compounds of ammonia, etc., produced by such decomposition.

The classification into primary and secondary is, however, not practical and has no clinical value, as the presence of decomposition, inflammation, and suppuration in the urinary tract can always be detected from other elements in the examination of the sediment.

The most common calculi are those of—(1) Uric acid and its compounds; (2) calcium oxalate; (3) mixed phosphates. Rarer forms are made up of calcium carbonate, xanthin, cystin, and urostealith. Besides the urinary calculi, there are prostatic calculi and fibrin or blood concretions.

Uric-acid calculi are the most common. They are brown or some shade of red, usually smooth, but may be irregular. They leave only a trace of residue after ignition.

Calcium-oxalate calculi are quite common; are usually dark brown or dark gray, typically irregular, with more or less sharp points, and are sometimes called "mulberry

ON HEATING THE POWDER ON PLATINUM FOIL, IT

Does burn				Does not burn			
Without flame		With flame		The powder when treated with HCl			
The powder gives the murexid test	The powder when treated with KOH gives			Does not effervesce		Effervesces.	
				The gently heated powder with HCl			
					The powder when moistened with a little KOH		
	No noticeable ammonia reaction.						
Does not give murexid test. The powder dissolves in HNO_3 without effervescence. The dried yellow residue becomes orange with alkali, beautiful red on warming.	Strong ammonia reaction.	Flame pale blue; burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia.	Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether.		No NH_3 or, at least, only traces of NH_3. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous).	Effervesces.	Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol or ether. Soluble in KOH with heat. Precipitated herefrom by acetic acid and generation of H_2S.
					Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives a crystalline precipitate with ammonia.		
Uric acid.	Ammonium urate.	Xanthin.	Cystin.	Urostealith.	Fibrin.	Calcium carbonate.	Calcium oxalate.	Bone-earth (magnesium and calcium phosphate).	Triple phosphate (mixed with unknown amount of earthy phosphates).

calculi." They may be small and smooth ("hemp-seed calculi"). They give a considerable residue after ignition and are soluble in acids without effervescence.

Mixed-phosphate calculi or "fusible calculi" consist of calcium phosphate and of triple phosphates of ammonium and magnesium. Phosphates may form the outer layers of other calculi of various compositions, but rarely form the nucleus of a calculus alone. Mixed-phosphate calculi are white, very brittle, melt in the blow-pipe flame, and are soluble in acids, but insoluble in alkalis.

As a rule, calculi are of mixed composition if large in size, and show on section several concentric layers around a nucleus. The latter may consist of a foreign body, organic matter, such as blood-clots, or of a dense mass of calcium oxalate or uric acid.

Analysis of Calculi.—A portion of the calculus should be powdered, and when the stone is of any size it should be sawed across, so that each layer may be examined separately. A portion of the powdered calculus is exposed on a platinum foil to dull-red heat for a considerable time. The table on p. 233 from Heller is a convenient guide to the examination of calculi.

QUESTIONS ON CHAPTER XVII

What designations are applied to *concretions* according to their size?

What are primary concretions? Secondary?

What are the most common varieties? Name some rarer forms.

Describe uric-acid calculi; calcium-oxalate calculi; mixed-phosphate calculi.

What do most calculi show on section?

How is a calculus prepared for analysis?

PART III

MICROSCOPIC EXAMINATION

CHAPTER XVIII

GENERAL CONSIDERATIONS

THE microscopic examination of urinary sediments should form part of the routine analysis, and should supplement the chemic tests. Very often the microscopic findings give more important clues as to the clinical conditions present than does the chemic analysis.

The microscopic examination should be made as soon as possible after the urine has been voided. On standing, decomposition and fermentation take place, which alter the microscopic picture materially. The most important change which occurs in urine on standing, however, is the disintegration of casts, epithelia, etc. A specimen of urine should never be violently shaken, as this also contributes to the breaking up of casts and other delicate structures.

METHODS OF OBTAINING SEDIMENTS

By Gravity.—This consists simply in placing the urine in a suitable glass, covering with a glass plate in order to keep out dust and foreign matter, and allowing to stand, preferably in a dark cool place, for about twelve hours.

The edges of the glass should be ground flat and the cover should be made of ground glass.

The time required for the urine to settle is a serious objection to this method, and the decomposition which the urine undergoes on standing so long renders it unfit for microscopic examination. Casts may become disin-

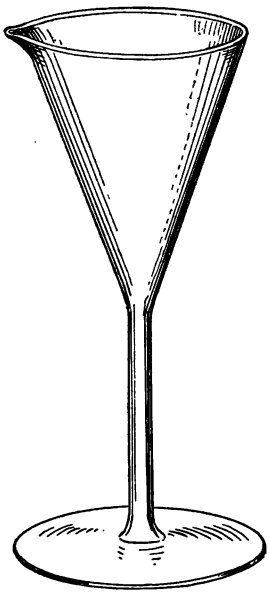


Fig. 31.—Urine or sediment glass.

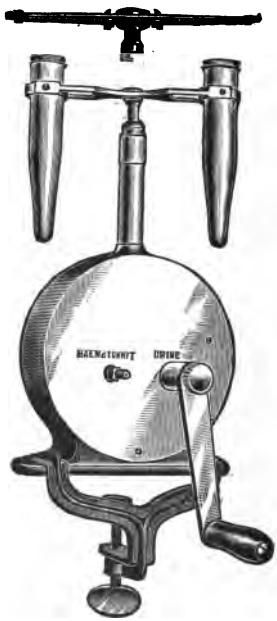


Fig. 32.—Hand centrifuge.

tegrated, chemic deposits altered and dissolved, and cells may change beyond recognition in decomposing urine. In order to prevent this, the addition of a crystal of thymol or of a small quantity of saturated solution of boric acid or a drop or two of formalin may be added to the urine. Of these, thymol is as satisfactory as any other preservative,

but some workers prefer chloral, salicylic acid, chloroform, etc. The writer would warn against the use of formalin, especially in excessive quantities, as it seriously interferes with the examination (see p. 24).

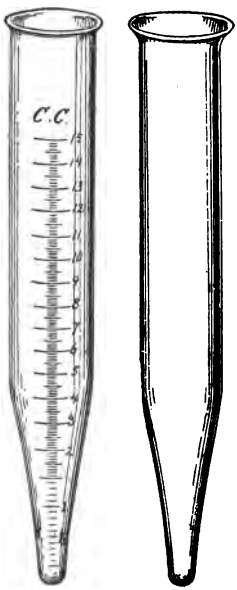
Centrifugal Method.—For accurate work, the use of a centrifuge for obtaining urinary sediments is almost indispensable. In this method the urine is placed in glass

Fig. 33.—Ball-bearing water-motor centrifuge.

Fig. 34.—The Purdy electric centrifuge.

tubes which are drawn out to a point, and revolved at high speed in a centrifugal apparatus, thus depositing all solids at the bottom of the tube in a few minutes. Immediate microscopic examination is then possible, and not only is there no danger of decomposition, but the sediment is more concentrated, and thus one is sure to have all the elements deposited, irrespectively of the specific gravity of the urine or the character of the sediment.

The simplest centrifuge is operated by hand (Fig. 32) and is satisfactory for ordinary purposes when only a few examinations are to be made daily. It gives a speed of 3000 revolutions a minute at the maximum. A water-power centrifuge which can be connected to the ordinary faucet is an excellent form of this apparatus (Fig. 33). It is very easy to operate, giving a smooth and rapid motion without much noise, and its price is moderate. The most satisfactory centrifugal machine is operated by electricity. There are numerous types, but Purdy's electric centrifuge (Fig. 34) is unquestionably the most serviceable. It can be operated either by batteries or with the street current, and is capable of all grades of speed, from 500 to 10,000 revolutions a minute. With this centrifuge from three to five minutes are sufficient to precipitate all the elements of a sediment, and approximately the same time is required with the simpler centrifuges worked by hand or water power. Purdy's apparatus has arms of such lengths that the tips of the tubes describe a circle of a standard diameter of 13½ inches (Fig. 34), and can also be used for quantitative estimations of chlorids, etc., that have been described in previous chapters. All these centrifuges have aluminum shields which carry the glass



a *b*

Fig. 35.—Purdy's tubes for the centrifuge: *a*, Percentage tube; *b*, sediment tube.

tubes and are supported on elastic cushions to prevent breakage during rotation. The tubes hold about 15 cc. each, and are made either plain or graduated (Fig. 35).

It is important that a worker who examines urine should become accustomed to using one centrifuge at a certain rate of speed for a certain length of time, so as to be able to compare the relative amounts of sediment and the relative proportions of the microscopic elements thereof in different specimens. A great difference as regards these points will be noted if different speeds are used for different lengths of time, or still more if the gravity method be used for some specimens and the centrifuge method for others.

METHOD OF EXAMINING THE SEDIMENT

The sediment having been obtained in one of the ways described, a drop of it is drawn into a pipet and deposited on a slide. The pipet should consist of a simple glass tube $\frac{1}{8}$ inch in diameter, drawn to a fairly fine point at one end. The opening at this end must not be too small, lest the tube be clogged. The upper end may have smoothed or ground edges, as the worker prefers. In drawing up the necessary sample of the sediment the pipet is to he held in the right hand, with the index-finger closing the upper opening firmly. It is passed into the urine glass or centrifuge tube, and should almost touch the bottom thereof, then be slowly withdrawn, the pressure of the index-finger being gently relaxed until the upper stratum of the sediment is reached. In this way the pipet will contain parts of every layer of the sediment. It must be remembered that the elements of a urinary deposit are thrown down in a certain order, the lightest coming last. Casts, mucus, and the smaller cells

are among the lightest elements. The sediment is often so dense that it is better to dilute it slightly with a few drops of urine, which are allowed to get into the pipet from the supernatant portion. After depositing the sediment upon the slide, the pipet should not be blown out with the mouth, as is commonly done. It should be preferably at once immersed in a solution of liquid soap and cresol and then rinsed in running water. A cylinder with the antiseptic solution mentioned can be kept for cleansing such pipet. Urine frequently contains typhoid bacilli and other pathogenic germs, and after the top of the pipet has been closed with the finger which has previously handled urine, the mouth should not be applied to the glass tube. Nipple pipets may be used, but are not so convenient.

Two or three slides should be in readiness, perfectly clean and free from grease and scratches. [Slides should be immersed at once after using in a solution of antiseptic soap in a wide-mouth glass-stoppered jar. They can be used over and over again (till they become scratched) for examining urine sediments and before using need only be rinsed in hot water and thoroughly dried on a soft cloth. The soap solution should be frequently renewed. For bacterial work (staining) slides should be taken out of the soap solution and washed with distilled water and then with alcohol, and passed through a flame repeatedly before applying the sediment.]

The sediment is deposited at first in the center of the slide and then spread in as thin a layer as possible over the entire slide by means of the pipet held flat against the glass. If too much urine is on the slide, the mass of fluid will bulge on account of the power of cohesion, which confines it to the

glass surface. In such cases it is better to drain off a drop or two from the slide, and so secure a thin layer of urine.

The next step is to place the slide on the stage of the microscope and to examine its entire surface with a low-power objective.

The low power enables one to search systematically, preferably with the aid of a mechanical stage, the surfaces of several slides in a few minutes, and suffices to detect all the larger elements, including casts, crystals, large epithelia, and pus. The higher power (Leitz No. 7) should be used only for the minute study of casts, renal cells, and red blood-cells, etc. The exclusive use of the high power frequently makes one miss casts and other important elements which are found on a general survey with the low power.

In using high power (No. 7 Leitz or $\frac{1}{6}$ Bausch and Lomb) a drop of the sediment is placed on the center of the slide and is carefully covered with a clean cover-glass. The excess of fluid must be absorbed with filter-paper, as the cover-glass should rest firmly, not float, on the slide.

In searching for casts the fine adjustment should be kept constantly changing by very slight turns of the screw. This brings different planes of a cast into view, and as casts are cylindric and often twisted, some parts are brought into focus in one position of the screw, while others are not seen until the focus is changed. If strong diffuse white light is available, the search for casts is facilitated by the use of the flat mirror; the reduction of the diaphragm aperture to the minimum, and the elimination of the Abbé condenser. In this way the faintest hyaline casts become visible. Ordinarily, however, the concave mirror and the Abbé condenser may be used for urinary examination by workers

who must content themselves with the comparatively uncertain light available in a large city.

As regards the microscope, no description is here needed, as the student is supposed to be familiar with its features. For urinary work a moderate-sized microscope of medium price is sufficient, and the objectives most useful are the Leitz's No. 3 or Zeiss' objective AA for low power, while Leitz's No. 7 or Zeiss' objective D is sufficient for the higher magnifications. An oil-immersion objective of $\frac{1}{12}$ inch diameter is not absolutely essential, but is necessary in bacteriologic work in connection with urinalysis.

The microscopes made by Bausch and Lomb and by Spencer are excellent for urinary work, and may be had at lower prices than the corresponding imported instruments. In these instruments eye-piece I, and objectives $\frac{2}{3}$ inch and $\frac{1}{6}$ or $\frac{1}{8}$ inch are the most useful for urinary work.

In microchemic reactions, as in testing pus-cells with iodin or acetic acid, a drop of the reagent should be dropped close to one edge of the cover-glass, and drawn under the latter by applying blotting-paper to its opposite edge.

PRESERVATION AND MOUNTING OF URINARY SEDIMENTS

In order to preserve a sediment we must prevent decomposition, the growth of bacteria, and other changes. This is done by allowing the sediment to settle thoroughly and by washing it in such media as will remove the soluble urinary constituents.

Crystalline sediments may be most conveniently and almost indefinitely preserved by the *dry method* recommended by A. S. Delépine. The sediment is first washed by placing the urine in a centrifuge tube,

thoroughly centrifuging the deposit, decanting the fluid portion. filling the tube with distilled water, and centrifuging again. The process of washing and centrifuging is repeated three or four times till all soluble parts of the urine have been removed. The sediment is then dried at a temperature below 40° C. on a water-bath. (If the sediment is insoluble in alcohol, it may first be washed in absolute alcohol and then quickly dried with moderate heat.) The dry sediment may be kept in sealed glass tubes. When needed for microscopic examination it may be mixed with a drop of water and covered with a cover-glass. In this way phosphates, calcium oxalate, uric acid, cholesterin, cystin, hematin, indigo, calcium carbonate, urates, etc., may be preserved.

The dry sediment may also be mounted in Canada balsam. This is done by placing a drop of water on a cover-glass, mixing a little dry sediment, and drying in the air. As soon as dry, the cover-glass is placed face downward upon a drop of thick Canada balsam in the center of a slide and gently pressed down. Enough water must remain in the crystals to prevent the balsam attacking them while it is still soft, so the drying must not be too complete (Delépine). This method preserves uric acid, cystin, hematoidin, indigo, calcium carbonate, and urates, but is not suitable for calcium, oxalate, or phosphate crystals.

These crystals (calcium phosphate, sulphate, oxalate, triple phosphate) may be preserved in cells of varnish made by "ringing" slides on a turntable with shellac varnish, covering with a cover-glass, and sealing with more of the varnish. The crystals are thoroughly washed in the manner described above, and the final washing is allowed to become perfectly saturated with the substance to be preserved. This is done by keeping the sediment in contact with a small amount of water for several weeks, then mounting a drop in a cell, as described. Triple phosphate is best mounted in a saturated solution in ammonia, which may be kept in a varnish cell for a year or two, but which ultimately attacks the varnish.

Casts, epithelia, and other organized sediments must be first treated with a preservative solution before mounting. The sediment is thoroughly centrifuged, the supernatant fluid decanted, and from five to ten times the bulk of a preservative solution is added.

For *casts* the best preservative is Müller's fluid (potassium bichromate, 2 parts; sodium sulphate, 1 part; water, 100 parts). This is diluted with an equal volume of water, the sediment is mixed (without shaking) with the dilute fluid, and is allowed to settle. The fluid is decanted and fresh fluid is added. This is repeated three times, when undiluted Müller's fluid is added. The sediment remains in this for two weeks, the fluid is decanted, and the deposit washed well with 50 per cent. alcohol. The lat-

ter is poured off and the following "glycerin solution" added: Alcohol, glycerin, and water, of each, 10 parts; carbolic acid, 1 part. In this the casts keep for years if the bottle is kept well closed and if only a small amount of glycerin solution is allowed to moisten the deposit. To mount the casts a drop of the deposit is mixed with a drop of fresh glycerin solution and is preserved in a cell, or simply examined under a cover-glass.

For *epithelia, pus, tissue fragments*, etc., the sediment may be simply washed four or five times with the glycerin solution and then mounted in the same medium.

STAINING OF SEDIMENTS

The staining of urinary sediments is difficult and usually unsatisfactory because of the interference of urinary constituents with the action of the stain.

The Author's Method.—In order to get the best results the sediment should be repeatedly washed with distilled water, with the aid of the centrifuge. The washed sediment is then spread upon slides and dried in the air. The slides are covered with equal parts of absolute alcohol and ether, and the fluid allowed to evaporate. If crystalline frost appears on the slide it should be rejected and the sediment washed more thoroughly. The sediment thus fixed is stained with dilute eosin-hematoxylin or with Unna's polychrome blue or with the following staining method employed in preference by the author:

Saturated alcoholic solution of eosin..... 1 part
Water.....4 parts

Stain for thirty seconds and wash thoroughly in distilled water; then add: aqueous solution of methylene-blue, 2 per cent. (or saturated alcoholic solution of methylene-blue 1 part to 4 parts distilled water). Stain for from two to five minutes, according to the thickness of the film. Wash well in distilled water. The nuclei of the epithelia and pus-cells appear blue or purple, the cell-bodies a faint pink, the bacteria a deep blue or purple.

Collodion Film Method.—Instead of fixation with alcohol and ether the film may also be fixed to the slide by pouring over it a very thin solution of collodion (collodion 2 drops to 3 ounces of alcohol and ether, equal parts) and allowing to dry. Staining may be carried out then as above, but the collodion film tends to take up the stain and thus to interfere with the microscopic picture. Collodion films are more useful, however, when the sediment has been stained in the centrifuge tube and then fixed on

the slide. This method has not proved satisfactory in the author's hands, and is too tedious for clinical investigation.

Centrifugal Staining.—The best method of staining sediments in the centrifuge is that of Elise Wolff.[1] The sediment is allowed to deposit spontaneously, the urine is decanted and replaced with 10 per cent. formalin solution, and after twenty-four hours the fluid is again decanted and replaced with alcohol. The latter is removed with the aid of the centrifuge, and dilute eosin-hematoxylin or some other suitable stain is added. The stain is allowed to act for twenty-four hours, the sediment centrifuged, the stain decanted, and the sediment spread upon a slide. Then the excess of dye is removed with alcohol, the film cleared with xylol, and mounted in Canada balsam.

Casts do not stain well with any of these methods and are best studied unstained, either fresh or in preserved sediments. The above methods are applicable chiefly to the study of bacteria, urinary epithelia, pus-cells, shreds, etc.

EXTRANEOUS MATERIALS IN THE SEDIMENT[2]

It is important to know and to recognize various extraneous matters occurring in the sediment, as they often lead to errors. The admixture of these matters is derived from exposure to the air, from unclean bottles, from the feces and the external genitals, as well as the clothing of the patient.

The *fibers of textile fabrics*—of cotton, linen, silk, and wool—are often found in the urine. Cotton fibers are coarse, wavy, or twisted, with edges more compact in the center; the center is wrinkled and shows irregular striations. They are highly refractive. Linen fibers are less refractive and are composed of finer fibrillæ. Irregular transverse breaks appear at different parts of linen fibers, and the outer fibrillæ are often broken off and branch irregularly

[1] Deutsche med. Wochenschrift., No. 24, 1906.

[2] I am indebted for most of the following data to Heitzmann's handbook, which contains a detailed description of the foreign materials found in urine.

from the main fiber. Silk fibers are smooth, shining, homogeneous, with jagged ends. Wool fibers are coarse

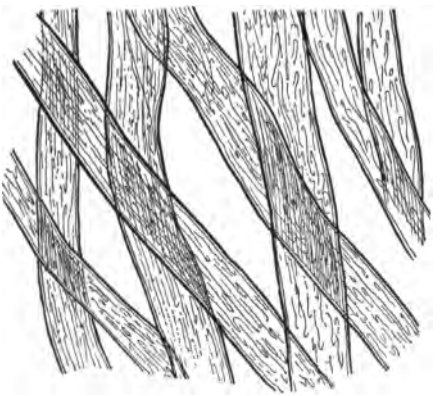


Fig. 36.—Cotton fibers.

and have serrated outlines, like the scales on some amphibia. They are finely striated.

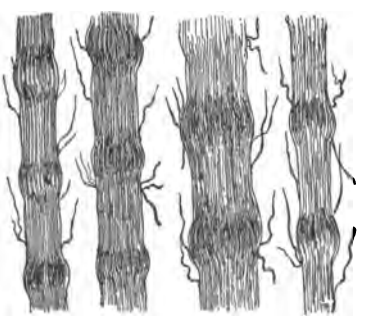


Fig. 37.—Linen fibers.

Hairs are often found in urine, and are distinguished by their pigment, their central medullary canal, and their

regular, straight outline. Particles of *feathers* show a characteristic formation, beginning with a fine quill from which branch the barbules composed of different sized

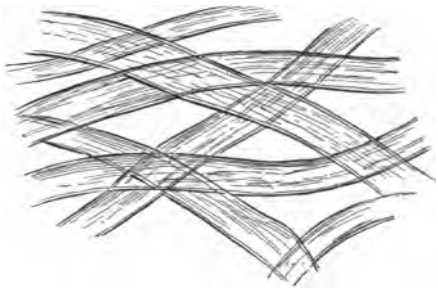


Fig. 38.—Fibers of silk.

links, gradually tapering toward the end. The *scales of insect wings* appear as delicate, transparent plates with little stems.

Fig. 39.—Wool fibers.

Starch globules are seen in the urine, owing to the use of starch powders for dusting purposes. They are oval or round, highly refractive, with a central hilum and con-

centric striations. Lycopodium, also used for dusting, consists of round shells filled with many spherical particles.

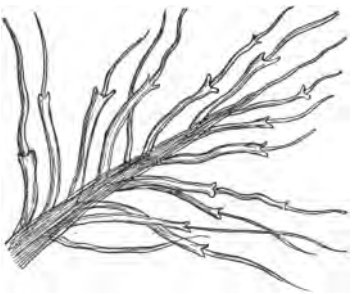


Fig. 40.—Feather.

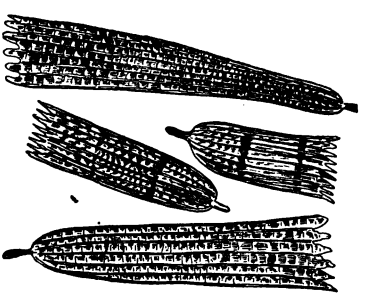


Fig. 41.—Scales from moth-wings.

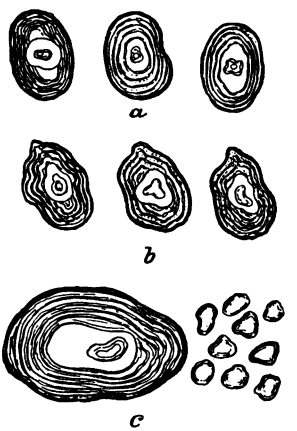


Fig. 42.—Globules of starch: *a*, Rice-starch; *b*, corn-starch; *c*, wheat-starch.

Cellulose appears usually in the form of a framework of cells with straight lines bounding the individual cells, which are angular or rectangular and contain large oblong nuclei.

Cork appears in yellowish or reddish-brown particles of irregular size, which are highly refractive and often grouped in masses.

Oil globules are yellowish, highly refractive, while *air bubbles* have sharply defined double contour and a bluish-black refraction. *Flaws in the glass*, scratches in the slide, etc., are faint blue in refraction, and do not move when the fluid under the cover-glass is set in motion by a gentle tap at the side of the microscope stage or by inclining the latter backward. Rust particles may resemble hematoidin crystals, but are more irregular.

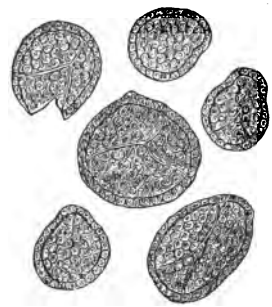


Fig. 43.—Globules of lycopodium.

Fecal matter varies greatly in appearance. It consists of different forms of vegetable matter, such as spiral fibers from the air-vessels of plants; vegetable fibers, hairs of plants, cellulose, starch-globules, fat-globules, and crystals, spores, etc. Partly digested muscle-fibers, yellowish or brown in color, with their striations, also appear in fecal matter. There may be also connective-tissue threads, mucus threads, epithelia, pus-cells, and crystals of triple phosphate. The epithelia are usually of a flat variety, but columnar epithelia are sometimes found. Various bacteria, fungi, yeasts, etc., from the feces will also be seen. The importance of recognizing fecal material lies in the possibility of a fistula between the rectum and the urinary tract. In a specimen of urine examined by the author the presence of fecal material and of masses of tumor cells and of pigmented intestinal epithelia has led to a diagnosis of a

rectovesical fistula due to carcinoma of the rectum. The patient was operated upon on the strength of the urinary

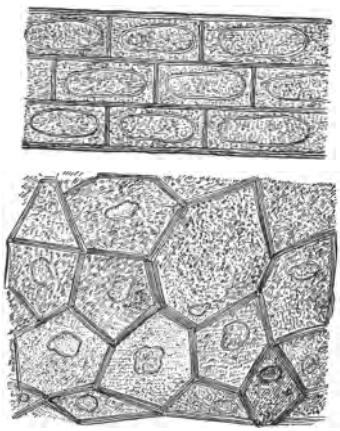


Fig. 44.—Cellulose.

Fig. 45.—Cork.

diagnosis and a cancerous tumor found which had ulcerated into the bladder.

CLASSIFICATION OF SEDIMENTS

The simplest classification of urinary sediments is into the unorganized and the organized. The following table from Tyson presents this classification in the most convenient form:

TABLE OF SEDIMENTS.—(*Tyson*)

UNORGANIZED.

I. Uric acid (crystalline).

II. Uric acid compounds:
- (*a*) Acid sodium urate (amorphous, occasionally crystalline).
- (*b*) Acid potassium urate (amorphous).
- (*c*) Acid calcium urate (amorphous).
- (*d*) Acid ammonium urate (crystalline).

III. Calcium oxalate (crystalline).

IV. Earthy phosphates:
  (*a*) Ammoniomagnesium phosphate (crystalline).
  (*b*) Calcium phosphate (amorphous and crystalline).
V. Calcium carbonate (crystalline).
VI. Calcium phosphate (crystalline).
VII. Leucin and tyrosin (crystalline).
VIII. Cystin (crystalline).

ORGANIZED.

I. Mucus and pus.
II. Epithelium.
III. Blood.
IV. Casts.
V. Spermatozoa.
VI. Fungi and infusoria.
VII. Elements of tumors.
VIII. Entozoa.

Another classification divides sediments into those occurring in acid urines and those present when urine becomes alkaline. Uric acid, urates, calcium oxalate, cystin, leucin, and tyrosin are usually found in acid urine; triple phosphate, calcium phosphate, ammonium urate, and calcium carbonate, in alkaline urine.

SEDIMENTS IN ACID AND ALKALINE URINES

The gross features of urinary sediments have already been considered in Chapter III, under the heading of Transparency, and the changes occurring in urine as the result of fermentation have been spoken of under the title of Selection of a Specimen of Urine, page 21. It is very important that the student should understand the chemistry of alkaline fermentation and of the so-called acid fermentation, so as to be able to interpret the sediments occurring in normal urine.

A *normal urine, freshly passed* and of acid reaction, contains no sediment save the faint cloud of mucus already spoken of (p. 29) and a few epithelial cells. An occasional leukocyte, a few cylindroids, and a few crystalline fragments, usually of the uric-acid type. In the urines

of women there are often a larger number of epithelia (from the vagina) and, as a rule, crystals are remarkably few in number. Urine of *alkaline reaction*, which is often normally seen three or four hours after a meal, may be more or less cloudy when passed, and may rapidly deposit flocculi of earthy phosphates, composed of amorphous granules which quickly disappear on the addition of a few drops of acetic acid.

Acid urines on standing, especially in the cold, precipitate granular amorphous matter of considerable bulk, whitish, pinkish, or reddish in color, readily soluble by heat, and consisting of urates of potassium, sodium, ammonium, calcium, and magnesium. The same urine may show, on standing still longer, crystals of uric acid of a yellow or yellowish-red color, and often the octahedral crystals of calcium oxalate. These deposits are caused by what is sometimes called *acid fermentation*. According to Scherer, this is caused by the action of the mucus of the bladder as a ferment, producing lactic and acetic acid from the coloring-matters of the urine. These acids combine with some of the bases of the neutral or alkaline urates which are in solution in normal urine, then first produce the more insoluble acid urates, which are thrown down, and later combine with the residue of the bases, leaving the crystalline uric-acid sediment.

Another explanation of the occurrence of urate deposits is that the excess of phosphoric acid in the acid sodium phosphate unites with the basic urates, rendering them acid, less soluble, and, therefore, precipitates them. The same process going on further, according to the length of time and the amount of acid sodium phosphate present, converts the acid urates into uric acid.

The acidity of the urine is diminished in the course of these changes, and it may become neutral or alkaline before the next stage—of alkaline fermentation—sets in.

If urine is allowed to stand still longer, or if it is kept in a warm place, especially in an open vessel, it undergoes *alkaline fermentation.* In this the urea is converted into ammonium carbonate through the action of decomposing mucus, which has a fermentative effect, or, according to

Fig. 46.—Deposit in ammoniacal urine (alkaline fermentation): *a*, Crystals of ammoniomagnesium phosphate (triple phosphate); *b*, crystals of ammonium urate (after Neubauer and Vogel).

some authors, through the action of a mould which multiplies within the urine and deposits with the salts in the form of a white sediment at the bottom of the vessel.

The results of the conversion of urea into ammonium carbonate are a great increase in alkalinity and a series of changes in the sediment. At the beginning of the reaction the uric-acid crystals begin to dissolve and to become fragmented, and prismatic crystals of sodium urate and dark,

spheric crystals of ammonium urate adhere to the fragments of uric acid. The latter disappear altogether as the urine grows alkaline; masses of granules of amorphous calcium phosphate and triangular prisms (coffin-lid shaped) of triple phosphate (ammoniomagnesium phosphate) crowd the field together with a number of opaque black spheres of ammonium urate, some of which show spicules. In addition there are numerous spores, bacteria, infusoria, and granular matter, fragments of cells, etc.

In pathologic conditions the sediment characteristic of either acid or alkaline urine may be deposited within the body, in the pelvis of the kidney, or in the bladder, and when urine freshly voided shows either of the sets of sediments described, we have to deal often with uric-acid gravel or stone, or with an obstructive inflammatory or suppurative condition somewhere in the urinary tract.

QUESTIONS ON CHAPTER XVIII

How are sediments obtained? Describe the gravity method; the centrifuge method.

Describe the method of preparing sediments for examination; the preservation and mounting of sediments.

What extraneous materials may be found in the sediment?

What is the importance of recognizing fecal elements?

What sediment is found in normal urine of acid reaction? Normal urine of alkaline reaction?

What is acid fermentation? What changes does it produce in urine?

What is alkaline fermentation and what sediment is found in the urine in this process?

What may be inferred when acid fermentation goes on in the urine before it is voided? If alkaline fermentation occurs so?

PLATE 6

SEDIMENT OF ALKALINE FERMENTATION (*after Hofmann and Ultzmann*).

CHAPTER XIX

UNORGANIZED SEDIMENTS

Uric Acid.—Uric acid occurs as a heavy sediment of small bulk, sinking to the bottom or sometimes adhering to the sides of the glass. The crystals are often large enough to be seen with the naked eye, and often form masses of yellowish-red color known as "gravel" or "sand." These crystals are frequently seen in normal urine, especially after a diet rich in meat and after exercise. They often occur at the end of the so-called acid fermentation (see p. 23); also in concentrated urine in fever, etc., and in any diseased condition in which there is an increase in the production of uric acid. It must be remembered that the occurrence of uric-acid crystals in urine is not necessarily a sign of the uric-acid diathesis, and before this diagnosis is made the diet of the patient and other conditions modifying the urine must be studied. The only safe basis for a conclusion as to the elimination of uric acid is in a quantitative chemic test.

Uric-acid crystals vary greatly in shape, but the typic forms are the rhombic or six-sided plates. Variations of this form are very often found (Fig. 47). Thus, whetstone-shaped crystals, alone or in stellate groups, and crystals resembling a comb with teeth on two sides, etc., are frequently met with. All these crystals are a more or less deeply tinted yellow, although perfectly colorless, diamond-shaped, and pointed crystals often occur. In cases of

uric-acid calculi, uric-acid crytals often occur in masses of considerable size and irregular form. Microchemically these crystals are distinguished by adding a small amount of alkali, such as potassium hydrate solution, while the specimen is under the microscope. The crystals will readily dissolve and will soon reappear if a drop or two of acetic acid be added. Another method is to use the murexid test (see p. 151).

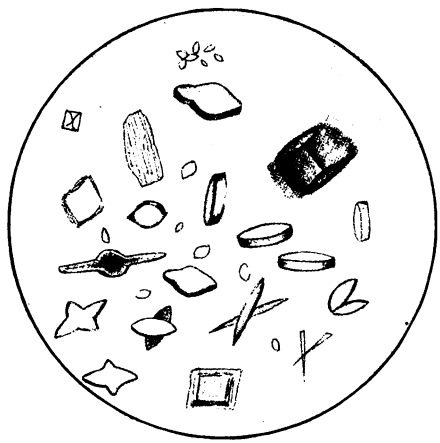
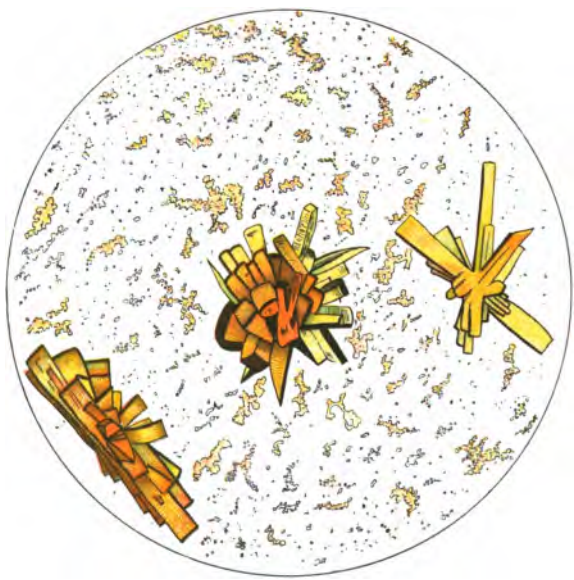


Fig. 47.—Various forms of uric acid.

In most instances, however, the characteristic shape and color of the crystals leave no doubt as to their character on microscopic examination.

Uric-acid Compounds.—Sodium Urate.—This is usually amorphous, and forms the greater bulk of the heavy powdery deposit of mixed urate known as "brick-dust" or "lateritious" sediment. The color of this sediment varies with the color of the urine from which it is deposited, pale urines giving an almost white sediment, while high-

PLATE 7



URIC-ACID CRYSTALS WITH AMORPHOUS URATES (*after Peyer*).

colored urines give a red sediment. Sodium urate and the other urates are found in the first stages of acid fermentation, also in urine that has stood in the cold, and in fevers; after physical and mental exertion; in disorders of the stomach and intestine; on the first day of menstruation, and in various conditions where there is defective oxidation or assimilation.

Most commonly sodium urate is seen under the microscope in the form of groups of light or dark brown, fine, amorphous granules, in moss-like masses which easily adhere to any larger elements of the sediment. Rarely, sodium urate occurs in pointed crystals (Fig. 48), which

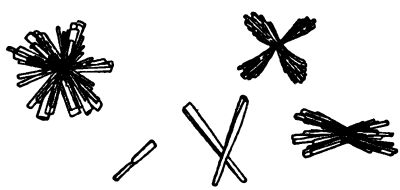


Fig. 48.—Acid sodium urate crystals (Ogden).

are fan shaped, pointed toward the center, and broader toward the periphery, or arranged like sheaves of wheat. These crystals are striated in a characteristic manner.

Potassium Urate.—This is found in acid urine in the form of amorphous granules, forming a part of the mixed urate sediment. This deposit is insoluble in cold, but soluble in hot water.

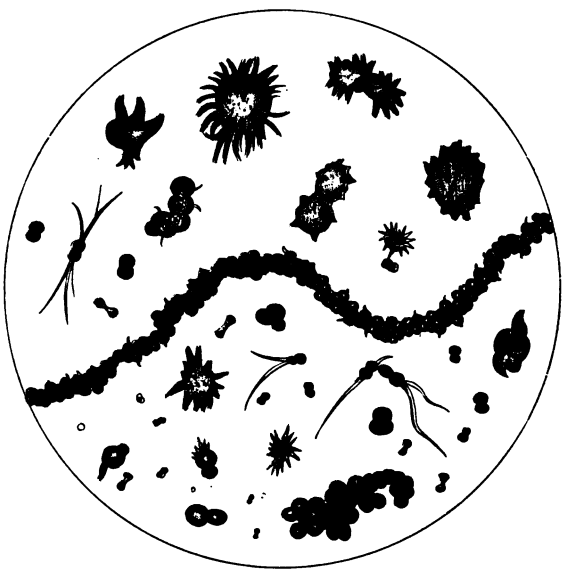
Calcium urate is rarely found, and usually in small amounts, in the amorphous deposit of urates in acid urine. It has the same solubility as potassium urate.

Ammonium Urate.—This occurs in the form of yellowish-red or dark-brown spherules studded with fine

sharp thorns, which have given rise to the term "thorn-apple crystals" and "hedge-hog" crystals. These thorns or spicules are sometimes curved or branched and vary in length. The salt also often crystallizes in clumps of needles arranged in sheaves. In the center of these a small spherule may be found. The crystals of ammonium urate are soluble in hot water and in acids. On addition of the latter they are transformed into uric-acid crystals. On addition of potassium hydrate the odor of ammonia is evolved. According to some observers, the spherules are really sodium urate, while the thorns are composed of uric acid (Beale, Hassall, and Thudichum). Still others claim that sodium urate undergoes a change in the urine, on standing, from the amorphous form into small dumb-bells, and finally into the globules of ammonium urate, the change marking the transition of the acid sediment into an alkaline. Rarely ammonium urate occurs in the shape of highly refractive colorless or yellowish needles or prisms. In old urines it may occur as tufts of needles, sheaves, or stars. On addition of HCl they dissolve and uric acid crystals appear, a reaction which distinguishes them from leucin.

Method of Dealing with Amorphous Urates.—A sediment, consisting largely of amorphous urates, is difficult to examine, as many of its other elements are obscured by the abundant granules of urates. The latter may be eliminated in the following manner: The urine is allowed to settle thoroughly, is decanted, and the amount of urine decanted from the sediment is replaced by an equal amount of warm water. This dissolves the urates, and the sediment may now be allowed to settle again or is centrifuged, and may be examined for elements other than urates.

PLATE 8



AMMONIUM URATE, SHOWING SPHERULES AND THORN-APPLE-SHAPED CRYSTALS (*after Peyer*).

The warm water also swells and renders colorless any normal blood-cells present. Boiling water should not be used for fear of coagulating any albumin present and thus rendering the sediment unfit for examination. A method which saves time, but which is not applicable to albuminous urines, is as follows: The sediment containing urates is *very gently* heated on a slide over an alcohol flame, and, if necessary, a few drops of warm water are added. The urates dissolve, leaving the other elements visible.

Clinical Significance of Urates.—Deposits of amorphous urates are often found in highly acid or highly concentrated urines, as, for example, in acute fevers. They are often seen also in diseases of the heart, liver, and kidneys. Ammonium urate is frequently found in the urine of newborn children. Before drawing any conclusions as to the meaning of a deposit of urates we must know the length of time the urine has stood and the temperature to which it has been subjected. Urine which deposits urates on standing in the cold may be perfectly normal, and ammonium urate is found in normal urine on alkaline fermentation. It is the only urate found in alkaline urine.

Calcium Oxalate.—Normally, calcium oxalate is kept in solution in urine by acid sodium phosphate. When this solvent has become transformed to a neutral phosphate, calcic oxalate becomes deposited. This substance is often found in crystals in acid urine, and occurs frequently in urine on standing twenty-four hours. Calcium oxalate crystals are often of very small size. They can be scarcely seen with the ⅔ objective and a high-power (No. 1) eye-piece. They are often mistaken for pus-cells when seen with this low power, and are distinguished only by careful focusing and by noting the square corners and the

×-figure in the center. Calcium oxalate crystals occur in two typical forms—the octahedral and the dumb-bell shapes—but there are several variations of these (Fig. 49). The octahedral crystals consist of two four-sided pyramids placed base to base, and when seen from the apex of one of the pyramids appear like squares crossed obliquely by two lines, something like the outline of a square envelope. If the long axis of the crystal is turned toward the observer, the crystal appears as a long and very sharply pointed

Fig. 49.—Calcium oxalate crystals (Jakob).

octahedron. If several octahedra are joined, they may give the appearance of an opened umbrella. These crystals are sometimes adherent to renal casts and sometimes coalesce into larger masses.

The dumb-bell shaped crystals of calcium oxalate are less frequently found, but are very characteristic (Fig. 50). They may be crossed, forming double dumb-bells, and should not be mistaken for the yellow and brown dumb-bells of uric acid and of ammonium urate. The latter are

easily soluble in alkalis, while those of calcium oxalate are soluble with difficulty. The dumb-bells of uric acid are insoluble in dilute hydrochloric acid, while those of calcium oxalate are soluble. Small circular fragments of dumb-bells of calcium oxalate are sometimes mistaken for red blood-cells. They are distinguished by the fact that they are highly refractive, colorless, and unaffected by acetic acid.

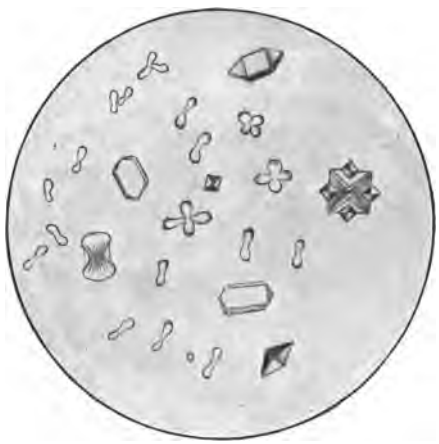


Fig. 50.—Atypical forms of calcium oxalate dumbells, crosses, rosets, multiple crystals, etc.

The deposit of calcium oxalate crystals may be *primary* —*i. e.*, formed inside the body—and in this case the larger octahedra and dumb-bells are found. After the urine has been standing, *secondary crystals* of smaller size but similar shapes are found, often accompanied by uric acid. The large crystals may be deposited secondarily on the addition of acetic acid to the urine.

Clinical Significance.—Calcium oxalate, when present in small amounts, has no clinical significance, as it occurs

in normal urines, especially after eating certain fruits and vegetables, such as apples, oranges, grapes, bananas, tomatoes, rhubarb, asparagus, spinach, and turnips, on account of the amount of oxalic acid contained in these substances. It is often increased in digestive disturbances, after an abundant diet of carbohydrates or of meat, and especially when the oxidizing power of the system is diminished. Oxalic acid is an intermediate product of metabolism between uric acid and urea, and when oxidation is diminished, *oxaluria* is the result. In diseases of the nervous system oxaluria is commonly observed, and some authorities claim that an excess of oxalic acid in the blood is poisonous and causes a train of nervous and other symptoms which constitutes the condition of "oxalic-acid diathesis." This is, however, a disputed point.

The occurrence of primary deposits of calcium oxalate, especially in masses of crystals and accompanied by blood or other evidences of irritation in the kidney, is often indicative of stone in the kidney or bladder. Caution should be used in applying the term *oxaluria* indiscriminately when the deposit of oxalate is secondary—*i. e.*, occurs on standing and decomposition. A large amount of calcium oxalate occurring in a fresh urine of high specific gravity justifies the term oxaluria. A clinical condition known by this name has been recognized, and gives the symptoms of neurasthenia and dyspepsia, hypochondriasis, and melancholia, with headaches, general malaise, and pains in the loins. In severe forms this condition has been incorrectly called "false Bright's disease," owing to the similarity of the symptoms to those of nephritis.

Phosphates.—The earthy phosphates are the only varieties that appear in sediments of the urine. They

consist of—(*a*) Neutral magnesium phosphate; (*b*) triple phosphate (or ammoniomagnesium phosphate); (*c*) calcium phosphate. They occur in very feebly acid, neutral, or alkaline urine, especially after alkaline fermentation. We have already studied the gross appearance of these deposits (p. 30).

Neutral Magnesium Phosphate.—This is a very rare crystalline sediment, found in concentrated alkaline urines which have not yet undergone ammoniacal changes. When the latter occur, triple phosphate crystals (see above) appear. Neutral magnesium phosphate was found in cases of gastrectasia by Stein. It occurs in large, elongated, highly refractive rhomboid plates with one or both ends obliquely cut off, or in square plates, with or without needles at their ends.

Ammoniomagnesium phosphate ($MgNH_4PO_4$), or triple phosphate, occurs in two forms—the "coffin-lid" and the "feathery" or stellate crystals. The former is the most common, and consists of a triangular prism with one of the three angles wanting. These crystals are very typical and are quite large in size. At times they are shortened into squares which may be mistaken for the octahedral crystals of calcium oxalate (Fig. 51). The stellate or feathery crystals are not so common, but are found in the urine on addition of ammonia. Various stages

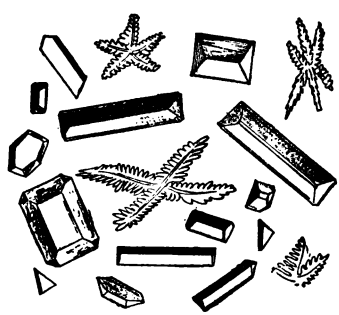


Fig. 51.—Crystals of triple phosphate (ammoniomagnesium phosphate).

of their evolution into triangular prisms are often seen. The shorter, square, triangular prisms are distinguished from calcium oxalate by the fact that there are larger crystals of more typical shape about them, for these atypical triple phosphate crystals never occur alone. Besides, the phosphate crystals are dissolved by acetic acid, while those of the oxalate are insoluble in this acid.

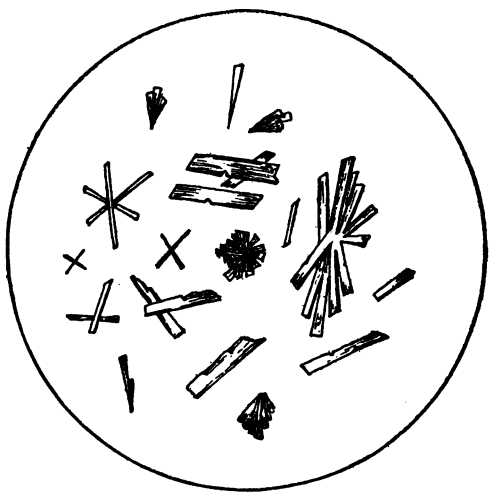


Fig. 52.—Acid calcium phosphate.

Calcium phosphate is either amorphous or crystalline. The amorphous form is often present after meals as a whitish, flaky deposit, and is precipitated by heat, but readily dissolved by acetic acid. In very feebly acid urine it is seen in small, highly refractive granules, arranged in clumps or adhering to other elements of the sediment. It is often seen with triple phosphate in neutral or alkaline urine.

The crystalline form is often seen in urine, and may be

mistaken for crystals of sodium urate. Crystals of acid[1] calcium phosphate are found in urines about to undergo alkaline fermentation, and which are still weakly acid. They are prismatic and occur either singly or in star-shaped, often in fan-like groups. They are distinguished from sodium urate crystals by adding acetic acid, which rapidly dissolves the phosphate crystals, while the urate dissolves more slowly and often is replaced by crystals of uric acid.

Clinical Significance.—By *phosphaturia* is meant, properly speaking, an excess of phosphates (phosphoric acid) in the urine, and not necessarily a precipitate of phosphates. The term has been applied incorrectly to cases where the urine was normal, but less acid than usual, owing to an excess of alkalis derived from the food, and in consequence showed a precipitate of phosphates. Normal urine two or three hours after a meal, especially under a vegetable diet, very often shows a deposit of amorphous or crystalline phosphates. These deposits, however, are not constant. Should the precipitate constantly be seen and be very abundant, phosphaturia may be suspected. In most cases phosphaturia shows a low state of nutrition and a condition of neurasthenia. The clinical diagnosis of phosphaturia should be made only when the deposits occur immediately after the urine is voided, and when a change of diet will not rectify the trouble. (For further data concerning Phosphaturia, see p. 216.)

Primary deposits of crystalline phosphates (deposited within the body) are often found in cases of inflammation and suppuration of the urinary tract, such as cystitis,

[1] This compound has long been erroneously called "neutral" calcium phosphate.

pyelitis, etc., especially when there is a decomposition of the urine within the tract as the result of an obstruction somewhere; for example, an enlarged prostate, a stricture of the urethra, etc.

Infection of the kidney, pelvis, or bladder may be accompanied by ammoniacal decomposition of the urine, which results in the deposit of triple phosphates.

Calcium Carbonate.—This is a very rare deposit in human urine, but is found in large amounts in horses and other herbivora. It occurs in alkaline urine on standing

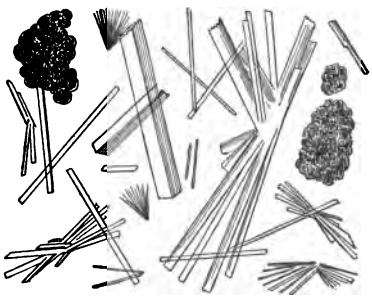


Fig. 53.—Calcium sulphate (von Jaksch).

for a long time, along with phosphatic deposit. It occurs either as an amorphous sediment or characteristically in small spherules, resembling ammonium urate, but smaller, colorless, usually in pairs (dumb-bells), often concentrically striated, and are distinguished by their effervescence (CO_2) upon addition of acetic acid.

Calcium Sulphate.—This is a very rare sediment and occurs in highly acid urine, of high specific gravity, in the form of needle-like prisms (Fig. 53), or in elongated plates with obliquely cut ends. They may be single or in sheaves or rosets. They are distinguished from cal-

cium phosphate by their insolubility in ammonia, acetic, and sulphuric acids, and their slight solubility in HCl (Délépine).

Leucin and Tyrosin.—These two substances have already been described under the heading of Chemic Examination (p. 201).

Leucin occurs in the shape of more or less yellow, highly refracting spheres which resemble oil-drops. By suitable illumination many of these spheres will be found marked

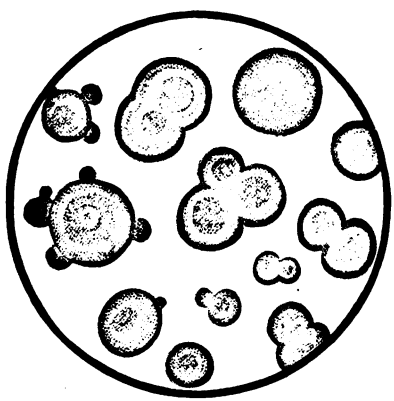


Fig. 54.—Leucin (Jakob).

with radiating and concentric stripes. They often are arranged in masses and chains, merging together at their edges.

The suspected urine should be evaporated slightly, and if leucin is present the crystals will usually be deposited. The spheres are, unlike oil-globules, insoluble in ether, but are soluble in alkalis and insoluble in cold mineral acid. They are distinguished from spheres of ammonium urate by the presence of spines on the latter and by the fact that the urate is soluble on being warmed.

Tyrosin.—Tyrosin crystallizes in the form of exceedingly fine needles arranged in sheaves or in rosets radiating from the center. These crystals are colorless, but when arranged in masses often look dark. They are tasteless, odorless, very sparingly soluble in cold water; more soluble in boiling water; almost insoluble in strong alcohol; insoluble in ether; readily soluble in acids, alkalis, and alkaline salt solutions. Tyrosin is recognized after obtaining

Fig. 55.—Tyrosin crystals (Jakob).

the crystals by one of the tests described under Chemic Examination (p. 202).

Cystin.—Cystin is a rare sediment in the urine. It occurs in crystals in the shape of hexagonal, colorless plates of moderate size and high refraction. They are often superimposed or may form more or less regular masses (Fig. 56). The sides of these plates are usually equal, but sometimes two sides are found shorter or longer than the others. Cystin also crystallizes in quadrilateral prisms or groups of prisms. The crystalline sediment

forms a whitish or yellowish-gray deposit, is met with in pale urine of acid reaction, and is gradually dissolved in alkaline urine, developing during decomposition the odor of sulphuretted hydrogen and of ammonia.

Cystin sediment is soluble in ammonia, in mineral acids, and in alkaline hydrates and carbonates, except ammonium carbonate. It is insoluble in alcohol, ether, and water, and is precipitated from alkaline solutions by acetic acid. Its solutions rotate the plane of polarized light toward the

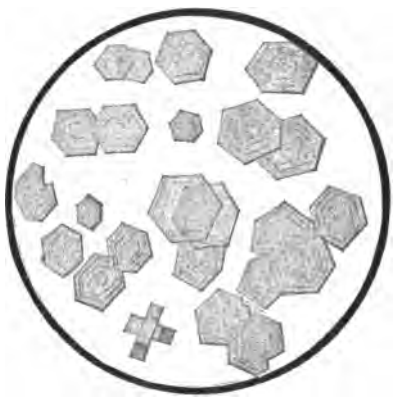


Fig. 56.—Crystals of cystin (Jakob).

left. Cystin is an amido-acid with the formula of $C_3H_6NSO_2$, and contains 26 per cent. of sulphur, which gives rise to the odor of sulphuretted hydrogen already spoken of. The crystals are not readily confounded with uric acid, but they may be treated with ammonia and the solution evaporated. If the crystals are uric acid, ammonium urate will form and will remain as an amorphous residue. Hydrochloric acid also readily dissolves cystin, but leaves uric acid unchanged. Acetic acid redissolves triple phos-

phate crystals, but leaves cystin unaltered. The presence of cystin should be suspected in all urines in which an odor of sulphuretted hydrogen is detected.

Clinical Significance.—Cystin is probably not present in normal urine. In disease the amount varies, and the daily quantity may reach as high as 1.5 gm., although ordinarily it is very much smaller. The cause of cystinuria is not definitely known. Brieger and others have found that certain products of intestinal putrefaction—the *diamins*—are eliminated in the urine and feces of persons with cystinuria. These diamins are said to arise only as the result of putrefaction due to specific germs, and cystinuria may be regarded as the result of specific intestinal infection. As yet no definite relation has been found between the formation of cystin and of diamins, although both occur under the same conditions. Heredity seems to play some rôle in cystinuria, as many cases have occurred in several members of the same family. Both infants and adults may eliminate cystin, but it rarely occurs in old age. It may not be connected with any symptoms, although present for years; but usually there are symptoms of irritation in the urinary tract. It has been observed in some cases of liver disease and of acute articular rheumatism.

Bilirubin and Hematoidin.—**Bilirubin** may be found in amorphous or crystallized form in the sediment of urine containing bile. The crystals occur as needles in stellate clusters, often adhering to cells, or in the form of minute rhombic plates varying in color from yellow to ruby red. They are soluble in sodium hydrate, and on the addition of nitric acid they show a green rim.

Hematoidin is a derivative of hematin, and was first found by Virchow in extravasated blood. Its crystals are

identical in shape and all other respects to those of bilirubin, and probably the two are identical. These crystals are found not uncommonly in urinary sediments after extensive hemorrhage, the evacuation of an abscess, or in pyonephrosis. They have been found in nephritis of pregnant women, in acute yellow atrophy, cirrhosis of the liver, severe persistent jaundice, phosphorus-poisoning, and cancer of the bladder.

Indigo.—Very small amounts may be present, except in intestinal obstruction, where larger quantities may be expected. Indigo occurs as stellate needles or rhombic or lanceolate crystals of blue color, insoluble in water, readily in chloroform. (See Indican, p. 174.)

Melanin.—This may be found in the sediment in the shape of black or dark-brown granules, free or within epithelia, casts, etc.

Fat-globules.—Fat occurs quite frequently in the urine, but we must be careful not to confound it with that from outside sources contaminating the specimen, which, as a rule, occurs in larger, more irregular, and more yellowish globules. When enough fat is voided to be seen by the naked eye, and when little or no albumin is present, the term *lipuria* may be applied. When urine presents a milky appearance with a creamy layer on top on standing, the term *chyluria* is used. The latter has already been discussed on page 203. Microscopically, fat globules and granules vary in size, and when the larger ones are found there may also be some slender needles of margaric acid lying between the globules or even within them. Fat globules are recognized by their dark contour and high refraction.

Aside from the cases of chyluria and lipuria (the latter

has been observed in health temporarily after a fatty diet, in pregnant women, and in phosphorus-poisoning), a small number of fat-globules is seen in a great many cases of chronic inflammation in the genito-urinary tract. These globules are probably a product of protoplasmic degeneration, as they are found both free and in masses within epithelial and pus-cells. They are more numerous in the chronic cases, and often the cells may be completely changed into masses of fat-granules. These globules are found in chronic nephritis, pyelitis, cystitis, prostatitis, urethritis, and vaginitis.

They are especially interesting in fatty casts (see p. 307).

Cholesterin is occasionally found in urinary sediments. It crystallizes in large, colorless, transparent plates whose angles and sides are often broken, and whose acute angles are often as small as 76 degrees. Larger masses of these crystals have a pearly lustre and a greasy feel. The occurrence of cholesterin and its chemic characters have already been considered (p. 203). It is readily detected in the urinary sediment by means of the microscope. If a mixture of 5 parts of sulphuric acid and 1 part of water be allowed to act upon the crystals, a bright, carmin-red color appears, which changes to violet. Another method of testing under the microscope is by adding dilute sulphuric acid and then a solution of iodin. The crystals will show a play of colors, beginning with violet, then bluish-green, and finally blue (see Fig. 30).

QUESTIONS ON CHAPTER XIX.

Name the principal forms of unorganized sediments.
How does *uric acid* occur in the urinary deposits?

What does the presence of uric-acid crystals indicate? What simple chemic tests may be used to distinguish these crystals?

Describe the appearance of deposits of sodium urate; ammonium urate; potassium urate; calcium urate.

How are urates eliminated from a urinary sediment?

What is the clinical significance of urates?

Describe the two forms of *calcium-oxalate* crystals. How are the dumb-bells distinguished from those of uric acid and of ammonium urate? From red blood-cells?

What is meant by a primary deposit of calcium oxalate?

What is the significance of calcium-oxalate crystals? What diet favors the appearance of these crystals in the urine?

In what conditions and diseases is oxalic acid increased?

When should we apply the term "oxaluria"?

What is meant by "oxalic-acid diathesis"? By "false Bright's disease"?

What *phosphates* occur in the urinary sediments? In what reactions of urine do they occur?

In what forms do triple-phosphate crystals occur? How are the square prisms distinguished from calcium oxalate?

What two forms of calcium phosphate are found in sediments? How is the crystalline form distinguished from sodium urate?

What is meant by *phosphaturia?* What does phosphaturia indicate?

When are primary sediments of phosphates found?

What *carbonate* compound is sometimes seen in sediments? How is it distinguished? What *sulphate*, and how does it occur?

Describe the appearance of deposits of *leucin;* of *tyrosin;* of *cystin.*

What odor does cystin develop during decomposition? How is this substance distinguished from uric acid? What is the clinical sgnificance of cystin?

Describe the crystals of *bilirubin* and *hematoidin*, and state their clinical significance.

What mistake is often made in connecton with *fat* in the urine?

What is meant by lipuria? By chyluria? When does lipuria occur?

What does a small number of fat-globules indicate? In what conditions are they found? Describe their appearance.

What is the appearance of *cholesterin* crystals? What reaction do cholesterin crystals give with H_2SO_4 and iodin?

CHAPTER XX

ORGANIZED SEDIMENTS

THE second group of sediments includes cells, fibers, and other formed elements of various parts of the urinary tract, some of which are normally present in the urine, while others indicate some disturbance or disease. The study of the organized sediments is the most important portion of the microscopic study of the urine, as upon the recognition of these elements very largely depend the localization of the diseases of the urinary tract and the determination of the nature and stage thereof.

BLOOD

With the exception of the urine of women during menstruation, etc., in which the presence of blood is unimportant, the occurrence of red blood-cells in urinary sediments is always abnormal. Red cells vary in appearance according to the part of the tract from which they come, according to the freshness of the specimen, and according to the concentration of the urine, the presence or absence of decomposition, etc.

Normal blood-cells—i. e., cells so unaltered that they look very much like cells in fresh blood—are very characteristic in appearance. They are biconcave disks of a yellow color, considerably smaller than the white cells, about $\frac{1}{3200}$ inch in diameter, without nuclei, granules, or other visible cell-contents. On careful focusing the rela-

tions of light and shadow at the center and periphery alternates as the objective is approached to the slide or removed from it. With the low power (objective $\frac{2}{3}$ eye-piece No. 1, B. and L.) the red blood-cells are visible as very minute faintly yellowish disks. They are best studied with the $\frac{1}{6}$ or $\frac{1}{8}$ objectives.

The first change that blood-cells undergo in the urine is the alteration in outline known as "crenated," irregular

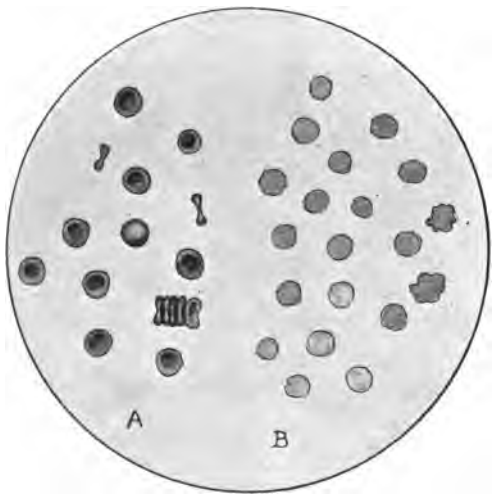


Fig. 57.—Red blood-cells in the urine: *A*, Normal; *B*, abnormal.

or "mulberry shaped." This may be found in urine with a high percentage of sodium chlorid.

Abnormal Blood-cells.—Blood-cells which have entered the urine within three or four hours begin to lose their color and swell until they are mere rings or shadows. Instead of being biconcave, they become almost spheric, very pale or almost colorless, and about two-thirds of the normal

cells in diameter. Any blood-cell which has lost its yellow color and its characteristic outline is "abnormal."

Normal blood, when present in large amounts, produces, in alkaline urine, a bright red color, and in highly acid urine, a brownish-red color. Abnormal blood, if present in sufficient amount, renders the urine brownish or smoky, and if in large amounts, almost black. Very commonly, however, the amount of blood is so small that it can be detected only microscopically. Urine containing blood always gives a reaction for albumin, even if the amount is minute.

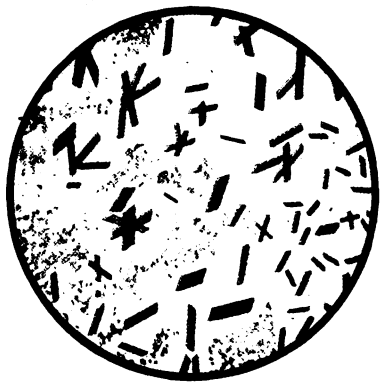


Fig. 58.—Teichmann's hemin crystals (Jakob).

Detection.—The chemic detection of blood by Teichmann's method has already been described on page 178. The crystals of hemin which are thus obtained are brown, rhombic plates, the proper chemic name of which is hematin iodid or chlorid. The crystals are very small, and often crossed or arranged in groups (Fig. 58). This test is useful when there is any doubt as to the presence of blood in the urine.

To Remove Blood from a Sediment.—The blood-cells may be so abundant in the sediment that they obscure everything else, and must be destroyed before examination. This is done as follows: The urine is allowed to settle slowly, the supernatant fluid is decanted, and a large amount of lukewarm water, with a few drops of dilute acetic acid, is added to the sediment. The mixture is stirred thoroughly, breaking up all clots, and allowed to settle again. The process is repeated until the water is free from color; the sediment is allowed to settle and examined. The blood-cells are washed out and almost invisible, while casts, etc., can be seen.

Clinical Significance of Blood.—We have already considered this subject under the heading of Blood-pigments (Chapter XII, p. 193). The distinction between hematuria, in which blood-corpuscles are present together with blood-pigments, and hemoglobinuria, in which the pigment alone appears, can be made with the microscope. The first purpose of microscopic examination in connection with blood is to locate, if possible, the source of the hemorrhage.

Blood from the Kidney.—As blood from this organ is usually in contact with urine for a considerable time before it passes out, it is generally abnormal in character and more or less dark brown or smoky. In injuries to the kidney, in acute congestion, etc., however, the blood may be normal and bright red. The reaction of the urine containing blood in the kidney is usually acid, although it may be alkaline if the amount of blood be large. A characteristic feature of blood from the kidney is that it is often accompanied by casts to which red cells adhere. This is the only positive evidence that blood comes from the kidney.

Blood-clots may be found in such urines, but are usually smaller than those derived from hemorrhage lower down in the tract.

GUIDE TO THE ORIGIN OF BLOOD IN HEMATURIA

(After Oertel, as quoted by Brooks)

Origin.	Quantity.	Differential points.	May occur in—
Kidney.	Usually comparatively small.	Clots usually absent. Associated with blood-casts, epithelial and hyaline casts, renal epithelium. Intimately mixed with urine. Many swollen (loss of hemoglobin) phantom corpuscles. Sediment slight (or abundant).	Acute and chronic nephritis. Malignant growths. Renal calculus, tuberculosis, embolism, abscess, acute febrile processes, hemophilia, and in filariasis (malaria) and distomiasis. Frequently in poisoning from turpentine, etc.
Pelvis of kidney and ureters.	Variable.	Absence of casts of any kind, or renal epithelium. Fibrinous molds of ureters may be present. Pus-cells in calculus.	Diseases of pelvis, calculus, etc.
Bladder.	Frequently large.	Blood-cells well preserved unless urine is ammoniacal; clots frequent. Heavy sediment (often scanty). Pus in cystitis. Shreds of tumor tissue in papilloma or malignant growths. If neck of bladder is involved, blood appears at end of micturition.	Stone, cystitis, tumors, varicose veins of vesical neck. Distoma hæmatobium, etc.'
Urethra.	Small.	May be expressed; first part of micturition.	Urethritis, trauma, etc.

The causes of bleeding from the kidneys include acute congestion and acute nephritis, in each of which the amount of blood varies considerably, according to the severity of the condition. In chronic nephritis a small number of red cells may be found, but during an exacerbation there may be an abundant bleeding, fresh blood-cells

appearing suddenly, with a diminution in the daily quantity of urine. In chronic interstitial nephritis blood is not infrequently found as the result of disturbances in circulation caused by heart disease or by diseased arteries. In amyloid kidneys hemorrhage occurs sometimes, owing to the infiltration around the small blood-vessels.

The three great causes of blood in the urine are tuberculosis of the kidney, tumors of the kidney, and stone in the kidney. In tuberculosis the bleeding may be intermittent or continued for a long period, and is accompanied by pus in considerable amounts. The diagnosis is made by finding tubercle bacilli in the sediment. In tumors of the kidney blood appears in considerable amounts at times, usually in attacks. Here, also, there is usually pus in the urine, and the sediment contains at times a number of cells which resemble the characteristic elements of the tumor. Stones in the kidney or in the renal pelvis give rise to either constant or intermittent bleeding. The urine may contain pus and fragments of stone or masses of crystals which hint at the presence of calculus. It must be remembered that in the early stages of tuberculosis, of tumors of the kidney, and of stone there may be no bleeding, but, as a rule, the appearance of blood in these conditions occurs sufficiently early to call attention to the possibility of one of these diseases, and unless their presence can be eliminated, the occurrence of attacks of hematuria is a sufficient basis for suspecting one of these three conditions. The study of the clinical symptoms, especially of the presence of pain, colic, and tumor, and the study of the epithelial elements and of pus-cells and crystals, as well as the examination for tubercle bacilli, will usually lead to a diagnosis.

Certain drugs which irritate the kidney, such as cantharides, turpentine, etc., may give rise to hematuria. Wounds, contusions, concussion of the kidney by indirect injury, and the invasion of the kidney by parasites (see p. 337) are also causes of bleeding. Other sources of hemorrhage in the kidney are cysts of that organ, purpura, and renal embolism.

Hemorrhage from the Pelvis and Ureter.—There is nothing microscopically characteristic about bleeding from the ureter, but the passage of thin, cylindric clots may help in the diagnosis. The presence of a large number of epithelial cells from the ureter may assist in localizing the trouble, although these epithelia are often difficult to differentiate. The causes of these hemorrhages include acute pyelitis, stone, acute ureteritis, injuries, etc.

Bleeding from the bladder is seen in moderate amounts in acute and chronic cystitis, but the most important causes of this bleeding are the same as in the case of the kidney—*i. e.*, stone, tuberculosis, or tumor of the bladder. Blood from the bladder is usually normal or comparatively unchanged; but if the urine is highly acid or alkaline, or if there is retention and the blood remains in the bladder for a long time, the blood-cells are abnormal. Bleeding from the bladder is characteristically associated with clots—at least more so than bleeding from the upper urinary organs. As a rule, pus is present in all conditions of the bladder accompanied by bleeding, with the exception of injuries, surgical operations, etc. In the diagnosis of the cause of the bleeding from the bladder the sediment must be examined for characteristic cells or fragments of a tumor, for crystalline masses or fragments of a stone, for tubercle bacilli, or other bacteria.

PUS

Pus-cells or leukocytes occur in the urine in the form of small, round, granular bodies about twice the size of normal red blood-cells. They contain one or more nuclei, which may or may not be seen in the specimen, but appear very clearly on the addition of acetic acid. As a rule, they can be easily recognized, but if any doubt arises as to their nature it may be dispelled by the addition of a little solution of iodin and potassium iodid. The leukocytes stain a deep mahogany-brown (glycogenic reaction), while epithelium, which may resemble them, assumes a light yellow color. In freshly passed urine leukocytes may exhibit active ameboid changes and assume irregular outlines. In dilute and highly alkaline urine they become swollen, globular, and hydropic, and the granulations in them become pale or disappear. In ammoniacal urines the pus-corpuscles may burst and coalesce into sticky masses.

The amount of granulation varies widely in pus-corpuscles, and the granules may be coarse or fine. In some cases minute fat-globules appear in the pus-cells, and the whole cell may be replaced by them. This is never found in acute inflammations, and the more fat-globules are found, the more chronic the process is, in all probability. Pus-cells may contain rust-brown crystals of hematoidin in the form of needles or flakes, especially in cases in which pyuria is accompanied by hemorrhage. Dark-brown pigment-granules may be found in these cells in chronic cystitis. Very rarely pus-cells may show delicate cilia, showing their origin from the epithelia of the uterus (endometritis).

The pus-cells in the urine may be derived from the kidney or its pelvis, the ureters, the bladder, the urethra,

or from the rupture of an abscess into some portion of the urinary tract. Pus-corpuscles in the urine are also often formed from the connective tissue and from the epithelia of the various organs of the urinary tract. For this reason they bear at times characteristics of the epithelium whence

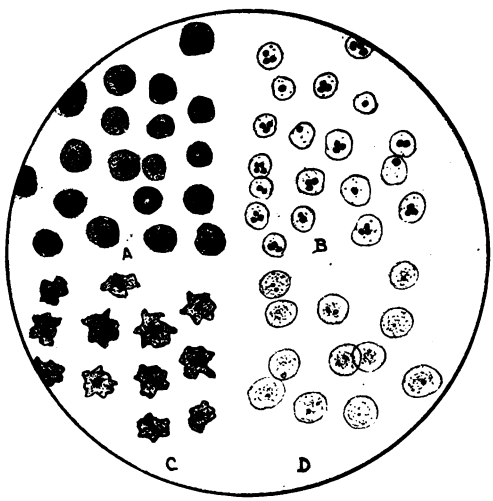


Fig. 59.—Pus-cells in the urine: *A*, Normal pus; *B*, same after adding acetic acid; *C*, pus-cells showing ameboid movements; *D*, pus-cells in ammoniacal urine altered by ammonium carbonate.

they come—as, for example, the pigmented pus-cells of chronic cystitis.

Clinical Significance.—A few leukocytes may be seen in perfectly normal urine. When present in moderate numbers, the presence of an inflammation somewhere in the tract may at once be suspected. When they are very numerous, the diagnosis of suppuration may be made, provided the other features of the urine corroborate it.

The presence of pus having been determined, the next step is to find its source, and the only way to do this is to study the other elements of the sediment, such as epithelia, casts, etc., which accompany the pus.

Pus from the kidney may be found in any inflammatory or suppurative condition of this organ. A few cells will be found in simple irritation and in non-suppurative nephritis, showing that the pus-cells are the product of an inflammation. In chronic nephritis pus-corpuscles containing fat-globules and granules are frequently observed. In suppuration of the kidney and pelvis (pyelonephritis), due to ascending infection from the bladder or to a stone, a tumor or a tuberculous process, the presence of pus is a prominent feature. Pus from the kidney or pelvis is also seen in urine from abscess of the kidney, suppuration of the pelvis (pyelitis), and pyonephrosis. In cases of abscess of the kidney there may be no pus before the abscess ruptures into the pelvis or into a pocket communicating with this part, when there will be a sudden appearance of pus. The same will be observed when there is a sudden removal of an obstruction in the pelvis or ureter.

Pus from the bladder is seen in acute and chronic inflammation and in stone, tumor, or tuberculosis of the bladder. **Pus from the seminal vesicles** is seen in seminal vesiculitis (spermatocystitis). **Pus from the prostate** may be present in prostatitis, prostatic abscess, and **pus from the urethra** in urethritis, especially of gonorrheal origin. In all the above-named conditions the localization of the origin of the pus can be made usually by noting the presence of epithelia (*q. v.*) from the corresponding organs. It is important to distinguish, in the urine of women, **pus which comes from the uterus or vagina,**

and if there is any doubt, the urine should be obtained by catheter or should be voided after a thorough vaginal douche.

CONNECTIVE-TISSUE SHREDS

These are found in the form of sinuous, highly refractive fibers, single, or more often in irregular bundles, and finely fibrillated or granular. Their thickness and length vary within wide limits. They are distinguished from mucus-threads by their high refraction, their fibrillated appearance, and wavy outlines. Linen fibers are coarser, not so wavy, and of higher refraction than connective-tissue shreds.

Connective-tissue fibers in the sediment indicate a new growth or an ulcerative, suppurative, and to a lesser degree a severe inflammatory lesion of the genito-urinary tract.

In chronic interstitial nephritis and in chronic diffuse nephritis connective-tissue shreds may be present in the urine along with other microscopic features. In tuberculosis of the kidney, in tumors, or in stone of the kidney these shreds may also be present and indicate an intense destructive or inflammatory condition. Tumors of the bladder, especially cancer and papilloma, may also be accompanied by the appearance of these shreds, in conjunction with more typic tumor elements. Connective-tissue shreds may also occur in company with pus in abscesses from various portions of the genito-urinary tract and in hypertrophied prostate.

Whenever these shreds appear in the urine they indicate a disintegration of connective tissue, consequently they are, as a rule, signs of deep-seated and serious lesions in the tract (Heitzmann).

EPITHELIUM

Epithelial cells from various parts of the urinary tract occur in both normal and pathologic urines. In normal urine the epithelia are few or moderate in number, and represent the shedding of the surface layers from the ordinary wear and tear of the parts. In pathologic conditions not only is their number increased, but the cells are altered and the elements characteristic of the deeper layers appear together with those from the superficial strata.

There is considerable divergence of opinion among modern authorities on urinary diagnosis as to whether it is possible accurately to recognize the sources of the various epithelia that may occur in the urine. The majority of authors take the negative side of this question. The reasons advanced for this view are twofold: First, that there is a great similarity between epithelia lining certain parts of the urogenital tract, and those derived from other quite distinct parts; and second, that even when there are marked differences between the epithelia belonging to distinct portions of the tract, these differences are largely destroyed by the distortion (swelling, obliteration of contour) which all epithelia undergo in the urine.

The writers who hold this view concede at most the possibility of recognizing with certainty the origin of large flat epithelia (bladder, vagina), and the smaller rounded columnar or caudate cells which may come from the upper parts of the tract. A few of these authors go so far as to recognize the probability of caudate cells coming from the pelvis and the possibility of distinguishing renal epithelia by their small size and rounded outline.

A more minute differentiation of epithelia from the various parts of the tract is regarded as feasible by a smaller

group of authors, who cling to the older views on this question which have been handed down to us by Ultzmann and his school. In Ultzmann's treatise on urinary diagnosis which appeared in 1871, there are excellent, though slightly conventionalized, illustrations of the epithelia from each of the different parts of the urinary tract. These illustrations were drawn for Ultzmann by Carl Heitzmann, who afterward went a step further than his teacher and studied these epithelia still more minutely. The elder Heitzmann's views are ably supported by his son, Louis Heitzmann, who is perhaps the most radical exponent of these views among modern writers, and to whom urinary diagnosis owes the present development of this subject.

An experience of ten years, covering the examination of many thousands of urines in private as well as in hospital work, has convinced the present writer that the recognition of the chief sources of epithelial desquamation is not only possible with practice, but is absolutely indispensable in urinary diagnosis. This conviction was reached after a study of a large number of cases of surgical affections of the urinary organs in which the writer not only studied the urine, but personally examined the patients with the aid of the cystoscope, the ureteral catheter, etc., and in many instances controlled the diagnosis after operation. It is only those who have the opportunity of seeing the clinical as well as the microscopic side of these cases that can judge with certainty as to the real value of the methods of carefully studying the epithelia of the urine. Like all diagnostic methods, this one has many pitfalls and is at times misleading, but after a moderate amount of experience one begins to realize its possibilities more and more, and soon learns to value it highly.

It may be said that every part of the urinary tract has theoretically its characteristic epithelium. *In practice, however, it is not always possible to say of a given cell in a urinary sediment just where it came from.* It is possible, however, to find enough cells bearing the characteristics of epithelia from a given part to form a judgment as to the portion of the tract which was the source of the desquamation. The farther away we get from the bladder, the more difficult is it to distinguish the epithelia of one part of the tract from those of another. While it is true that the epithelium, as found in urinary sediments, usually has entirely different outlines from that found histologically in the portion of the tract whence it came, the change which is due to the influence of the urine affects all classes of epithelia more or less in the same way, so that the relative appearance remains distinctive in most cases. The whole basis of distinguishing certain epithelia, from the kidney, for example, from others derived from other parts, is the *size* of the cells as compared to a standard. As a rule, the standard cell of a urinary sediment is the leukocyte or pus-corpuscle, because it varies but little Fig. 60.

Three general types of epithelia occur in the urine: The flat or squamous cells, the cuboid, and the columnar. These terms refer to the generic histologic types, and whenever they are used in the following descriptions, table, etc., it is understood that the terms "cuboid" and "cylindric" refer to cells which were originally of these shapes. Cuboid cells usually appear in the urine as round or slightly ovoid. Cylindric cells appear often as caudate, pear-shaped bodies, the angular outlines having been rounded off under the influence of the urine.

The cell bodies of the urinary epithelia are more or less markedly granular. The amount of granulation varies in different regions of the genito-urinary tract, and with the reaction of the urine. Particles of pigment and fat-droplets are sometimes found in the cell bodies. The latter usually indicate chronicity of an inflammatory process. In urine containing bile the granulations appear yellow.

The epithelia always possess one or more nuclei, but at times the nucleus drops out, leaving a vacuole. The prominence of the nucleus varies with the amount of granulation and the density of nuclear substance. It also depends on the reaction of the urine. On addition of acetic acid to the drop of sediment on the slide, the nuclei stand out and the granulations become indistinct. Urinary epithelia should be studied with a $\frac{1}{6}$ or $\frac{1}{8}$ objective and a 1-inch eye-piece. A compensating eye-piece is of advantage, but not essential.

In distinguishing epithelia the average size and average shape of a number found should be considered. Usually one or two types predominate in a specimen. Transitional types and sizes cannot be taken as criteria.

The table on p. 289 will prove useful to the student inexperienced in differentiating epithelia in the urine. The cells are classified here according to general shape in three columns. When cells are encountered of a given shape they may belong to one of a number of parts of the genito-urinary tract. By glancing at the proper column, the student at once will be able to know between what group of cells he must differentiate. He must next seek to determine the size of cells seen, by comparing them to the standard—the pus-cell. In the table the pus-cell from the urine under consideration in each case is taken as a

Reference table showing urinary epithelia in three morphologic groups, each arranged according to relative *longest* diameter, as compared to that of a pus-cell from the same urine. Sizes are average and approximate.

Order of Sizes.	Squamous (Flat).	Relative Diameter.	Cubcid (Round).	Relative Diameter.	Cylindric (Caudate).	Relative Longest Diameter.
I	.....	.....	*Kidney* (convoluted tubules) (12 to 25 μ).	1½	*Kidney* (straight collecting tubules).	1½
II a	.....	.....	*Prostate* (acini).	2	*Prostate* (ducts).	2
II b	.....	.....	*Ureter.*	2	*Ureter* (deepest layers).	2
II c	.....	.....	.....	.....	*Seminal vesicles* (irregular).	2.5
III	.....	.....	*Renal pelvis* (rare, except injury to lining).	3–4	*Renal pelvis* (common type).	3–4
IV a	*Cervix.*	3–5+	*Cervix.*	3–5+	*Cervix, uterine mucosa* (ciliated).	3–4
IV b	*Urethra, male* (fossa navicularis).	5	*Urethra* (both sexes).	3–5	*Urethra* (both sexes; *more frequent* in male).	3–5
IV c	*Urethra, female* (most frequent type).	3–5				
IV d	.....	.....	.....	.....	*Ejaculatory duct* (ciliated).	5+
V	*Bladder* (superficial layers, 30 to 60 μ).	5–7+	*Bladder* (middle layers).	5–7+	*Bladder* (deep layers).	5–7+
VI	*Vagina* (superficial layers).	7–8+	*Vagina* (middle layers).	7–8+	*Vagina* (deep layers).	7–8+

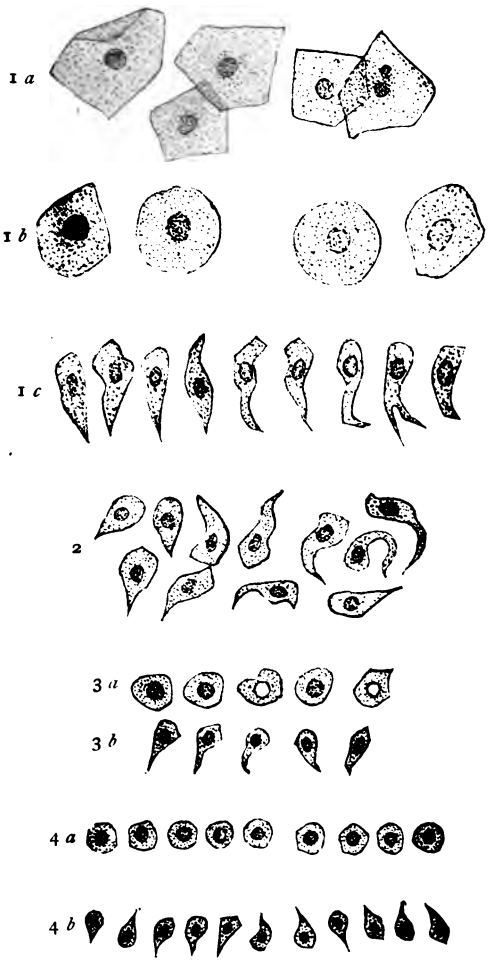
unit. The size of pus-cells varies in different urines, but the relative size of the epithelia varies to a smaller extent. The numbers on the left side of the table show the order of sizes, while on the right side of each column appears the diameter of each group of cells compared to the standard. The figures stand for an *average* size. Practice alone can enable a student to differentiate between cells with close ratio. In some instances the distinction is impossible—*e. g.*, between cells from the prostate and those from the ureter, between those from the cervix and the urethra.

The table and Plates 9 and 10 show, perhaps, better than any description the possibilities and the limitations of the method of differentiating epithelia in the urine.

In the following each of the groups of epithelia belonging to the different organs of the genito-urinary tract will receive separate consideration.

Epithelia from the Bladder.—The upper layers are flat, the middle layers cuboid or round, the deepest layers columnar or caudate. A small number of the flat epithelia appear in every normal urine and have no significance. If they appear accompanied by pus and with round vesical epithelia, they show disease in the bladder. The flat epithelia are found free or in pavement clusters of irregular size and shape. They are smaller than the cells from the upper layers of the vagina, but near the neck of the bladder they become quite large and may be confounded with vaginal epithelia. They do not contain bacteria, however, as the latter frequently do, and the nuclei of bladder epithelia are almost always larger in proportion to the cells than those of vaginal epithelia. Cuboid or round epithelia from the bladder, when present

PLATE 9



EPITHELIA IN THE URINE FROM THE BLADDER, URETER, RENAL PELVIS, AND KIDNEY. × about 500.

1. *Bladder:* (*a*) Superficial, (*b*) middle, and (*c*) deep layer. 2. *Renal pelvis.* 3. *Ureter:* (*a*) Cuboidal, (*b*) columnar. 4. *Renal tubules:* (*a*) Convoluted tubules, (*b*) straight collecting tubules.

in moderate or large numbers with the flat epithelia from the upper layers, indicate an acute cystitis. Cuboid or round epithelia alone, without the flat cells, indicate a chronic cystitis, and the presence of columnar or caudate epithelia from the deepest layer of the bladder indicates deep ulceration, tumors, or an intense inflammation. Cuboid and columnar epithelia may contan fat-granules, which indicate a chronic process. In the epithelia from the middle layers the formation of so-called endogenous pus-corpuscles may be observed—that is, pus-corpuscles are formed within the epithelia, especially in cases of hypertrophied prostate, of uterine tumors pressing on the bladder, or any other condition which keeps up irritation or pressure from the outside.

Epithelia from the Ureters.—These are especially seen in specimens obtained by the ureteral catheter, but may occur in urine mixed with pelvic epithelium. Two forms of epithelial cells from the ureter are seen: (1) Cuboid or round cells identical in size with those from the acini of the prostate and twice the diameter of a pus-cell. They are rarely numerous, and when present with renal and pelvic cells, the diagnosis between them and prostatic cells can be safely made. (2) Small caudate cells, smaller than the corresponding cells of the pelvis, from the deepest layers of the ureteral lining. These cells are distinguished from columnar cells in the bladder by the fact that they are much smaller. The ureter is rarely affected alone, except by irritation from stone, etc., and the presence of cells from the ureter is almost always accompanied by the signs of pyelitis or cystitis.

Epithelia from the Pelvis of the Kidney.—The epithelia in this part are very irregular in shape. The

characteristic form is the caudate, pear-shaped, or lenticular, but round and cuboid shapes, smaller than bladder epithelia, also occur. The pelvic cells are smaller than those from the bladder and larger than those from the ureter and often have curved or bifurcated tails. Their transverse diameter is a little larger than that of a pus-cell. Their long diameter is three to four times this diameter. They have distinct nuclei and well-marked granules. Their presence indicates irritation or inflammation in the renal pelvis, and they occur in large numbers, with pus, in pyelonephritis. The round-cells, which are less frequently found, closely resemble the renal cells, but are larger, being three to four times the diameter of a pus-cell.

Renal Epithelium.—The characteristic epithelial cell from the tubules of the kidney is small, round, more or less granular, with a single nucleus. These epithelia are the most important of all the cells found in the urine, and at the same time are the most difficult to recognize and the most frequently overlooked. Two forms are distinguished—the cuboidal (round), from the convoluted tubules, and the columnar, from the straight collecting tubules. Renal epithelia are much smaller than those of the pelvis of the kidney or of the ureter in the same person. They are quite constantly one-third larger than the pus-cells.

The following description from Heitzmann sets forth clearly the method of looking for renal cells:

"In every case examined, the first step is to look for pus-corpuscles, which are known to be small in some people and are usually the smallest granular corpuscle seen. As soon as these are decided upon, the next step is to determine whether bodies distinctly larger than these are

present. If such bodies, one-third larger than pus-corpuscles, are found in at least moderate numbers, we can be certain that they are epithelia from the convoluted narrow tubules of the kidney. The presence or absence of nuclei has no significance whatever, although such a nucleus is usually found in the kidney epithelia, but may be invisible in the pus-corpuscles. The relation between the size of the pus-corpuscles and that of the epithelia from the convoluted tubule is always the same—that is, the latter are one-third larger than the former. If the pus-corpuscle happens to be small in the case examined, the kidney epithelia will be small, but if large, the epithelia will be larger. The comparative sizes of the different smaller formations

Fig. 60.—Corpuscles and epithelia, showing their comparative sizes.

found in the urine are illustated in Fig. 60. The smallest corpuscles with double contour, and which are not granular, are the red corpuscles; the next in size, being the smallest granular corpuscles, are the pus-corpuscles; then follow the smallest epithelia found in urine, one-third larger than the pus-corpuscles—the epithelia from the convoluted tubules of the kidney. Finally, the next larger epithelia are shown, always twice the size of the pus-corpuscles, which are those from the ureter or from the prostate gland, between which no difference can be noted. If this relationship is kept in mind, no mistake can be made, though it must be remembered that when an individual small epithelial cell is found, the diagnosis cannot be positively made until they are compared with the pus-corpuscles."

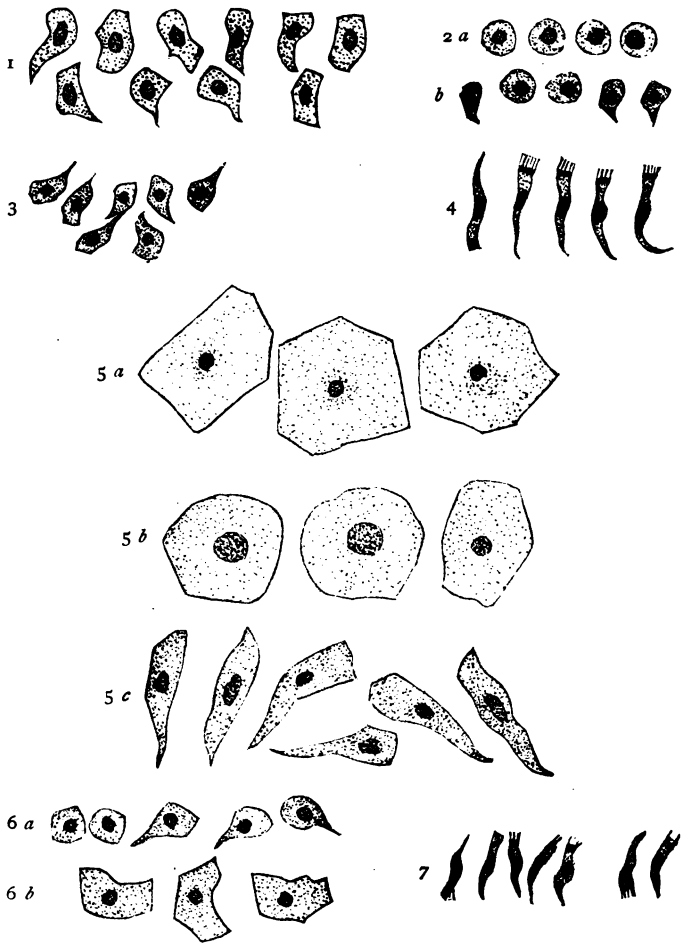
The epithelia from the straight collecting tubules are less frequently seen, and occur in larger numbers only in the severe forms of nephritis. They are about the same size as the cuboidal, but narrower. Kidney epithelia may occur in clusters or in cast-like groups (epithelial casts, *q. v.*).

The clinical significance of the presence of renal epithelia is not always appreciated. They are indicative of renal inflammation, and when casts are absent, as is often the case, they assist in making the diagnosis of nephritis. Renal epithelia are also present in suppurative conditions of the kidney, and, in combination with pus and cells from the pelvis, assist in making the diagnosis of pyelonephritis. It is very important, therefore, to look for renal epithelia whenever a renal affection is suspected.

Epithelia from the Urethra.—The *male urethra* is lined with stratified epithelia which vary in different portions of the canal. In the fossa navicularis there is a stratified squamous lining. Cells from this portion are seen in the early stages of acute urethral inflammation, after instrumentation, etc. They are flat, smaller than bladder cells, averaging five pus-cell diameters, with large nuclei (Fig. 61). The anterior urethra is lined with columnar stratified epithelium. The most common urethral cells come from this portion, and the majority of urethral cells in urethritis (both in urine, shreds, and discharges) are of this type. They are oval, irregular, elongated, but maintain a fairly wide transverse diameter. Occasionally they are caudate and narrower (deeper cells). Their longest diameter is about four times that of a pus-cell (see Plate 10 and Fig. 80, A).

In the membranous urethra the cells are practically

PLATE 10



EPITHELIA IN THE URINE FROM THE URETHRA AND GENITAL ORGANS. × about 500.

1. *Urethra.* 2. *Prostate:* (*a*) Acini, (*b*) ducts. 3. *Vesicles.* 4. *Ejaculatory ducts.* 5. *Vagina:* (*a*) Superficial, (*b*) middle, (*c*) deep layer. 6. *Cervix:* (*a*) Cuboidal, (*b*) columnar. 7. *Uterine mucosa.*

identical with those of the anterior urethra. In the prostatic portion there is a gradual transition toward the flat stratified epithelium of the bladder. The cuboid and columnar epithelia from this portion come from the numerous glands opening into the prostatic cavity.

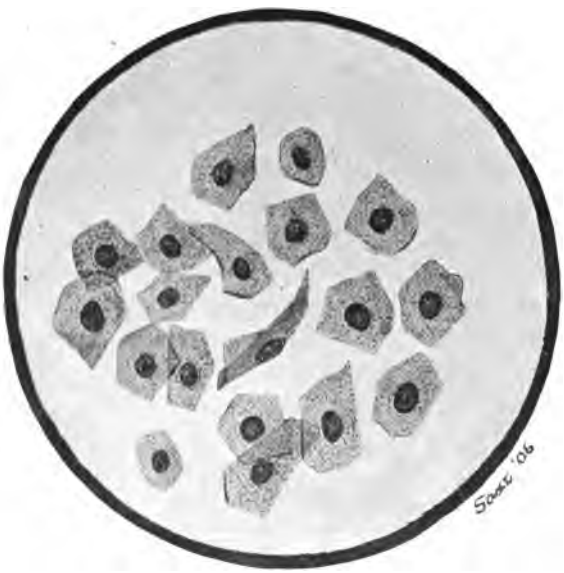


Fig. 61.—Epithelia from the fossa navicularis.

In chronic urethritis, large flat, horny epithelia, looking like vaginal, and practically of the same size as the latter, but with very small, round nuclei, appear in the urine, as a result of the keratification of the urethral mucosa, which is transformed in places frankly into the stratified squamous type. These cells, when numerous and when occurring in shred-like masses with pus, indicate the probability of stricture formation (see Fig. 80, B).

In the *female* the urethra is lined with a very variable type of cells. In some women the squamous, in others the columnar, type predominates. The squamous cells are most numerous toward both ends of the canal. As a rule, squamous cells are most commonly found in the female urethra, while in the male, cylindric cells predominate. The size of urethral cells in both male and female is about three to five "pus-cell diameters" (in the longest direction), though the squamous cells may reach much larger sizes.

Epithelia from the Prostate.—These are cuboid or columnar, the latter derived from the duct of the gland. The cuboid epithelia are twice the size of pus-cells, and identical in appearance with those from the ureter, but larger than those from the kidney. If these epithelia are present in large number, while renal and pelvic epithelia are absent or few in number, the cells of ambiguous size are undoubtedly prostatic and not ureteral. The accompanying presence of other prostatic elements (mucus-plugs, spermatozoa, etc.) also assists in the diagnosis. Prostatic epithelia may be found in the urine after coitus, after massage of the prostate, and in the sediment of urine in cases of prostatic inflammation and prostatic abscess.

Epithelia from the ejaculatory ducts and seminal vesicles may also be found in the urine, and are of the columnar ciliated variety. The cilia may become broken, but the character of the cell can be made out from the delicate striation of rods along the upper surface. These cells are very narrow and long, about five pus-cell diameters in length. Cells from the *vesicles* are of medium size, elongated or irregular, highly granular, about two and one-half pus-cell diameters in size, and often adherent

to spermatozoa. These epithelia may be found in the urine in cases of vesiculitis, tuberculosis of the vesicles, etc., and after massage of the vesicles.

Epithelia from the Vagina.—These are the largest epithelia found in urine. Those from the upper layers are flat; from the middle layers, cuboid; from the deepest layers, columnar. The flat variety is present in most urines in women in health. They are increased in leukorrhea, and may be present in masses, accompanied by bacteria, mucus, and granular matter. They may be shrunken and folded, and may contain fat-globules, which have no significance. They have very pale, fine granulations. The cuboid epithelia in the middle layer are seen in vaginitis in considerable numbers, and in chronic cases contain fat-granules. The columnar epithelia are seen in ulcerations and deep-seated inflammations. All these vaginal cells are larger than those of the corresponding layers in the bladder. They average seven or eight pus-cell diameters in size (the columnar, in their longest dimension), but may reach even larger sizes.

Epithelia from the Uterus.—These are not so frequently found in the urine, but should be remembered, as they may give rise to confusion. Epithelia from the *cervix* are flat, cuboid, or columnar, usually irregular in shape, and smaller than those in the vagina. They are practically identical with the irregular epithelia from the urethra, and can hardly be differentiated. Epithelia from the *mucosa of the uterus* are tall, columnar, ciliated, of delicate structure. They are about one-fourth to one-fifth shorter than those of the ejaculatory duct, and less irregular, but if the patient's sex is known there is no need for this differentiation. Their presence indicates endometritis.

Extraneous Epithelia.—In both normal and pathologic urines scales of epidermis in the form of flat, horny cells from the genitals (prepuce, clitoris, and labia) are often present. They are recognized by their zigzag contours, by the absence of nuclei, the presence of fat-globules and dust particles, their pallor, and their shrivelled condition.

CASTS

True casts of the kidney were first described by Henle in 1842, but they had been seen by Vigla, Quevenne, and Rayer in the years 1837 to 1840 in France, and by Simon and Nasse in 1842 in Germany. The first detailed description was given in 1867 by Rovida. Casts may be defined as cylindric bodies which represent the molds of the uriniferous tubules of the kidney. The origin of renal casts is still unsettled. At one time they were considered as the product of secretion of the epithelia of the tubules, or as masses of transformed or disintegrated epithelia (Rovida). Later on their production was ascribed (Ribbert) to the transudation of coagular elements of the blood, which pass into the tubules as a result of lesions in the walls of the latter, solidify in the lumen, and are voided with the urine. This last view is probably more close to the correct solution of this question than the others, and casts are probably the products of an exudation from the blood-vessels with the admixture of fragments of epithelia.

One serious defect in this theory which has not yet been remedied or explained is the fact that undoubtedly there are cases in which casts are present in the urine without any albumin being detected by chemic means. The occurrence of casts without any renal disease is now a well-

established fact (see below), and recent experimental and clinical research seems to point to the probability that casts may be regarded as evidences of an effort of nature to keep the renal tubules open and free from accumulations of dead epithelia. The epithelium shed is transformed into a plastic permeable mass and the latter is driven out with the stream of urine as soon as it becomes sufficiently loosened.

Clinical Significance.—In speaking of albuminuria we have tried to emphasize the fact that the presence of albumin does not necessarily mean the presence of nephritis. The same is true of casts. At least some varieties of casts occur without any renal lesions, indeed, may occur under certain conditions in health. That casts may occur without renal disease is now well established by the researches of Nothnagel, Senator, and others. The occurrence of casts in the urine after muscular exercise (Penzoldt, 1893, von Noorden, 1904), after drinking alcoholic beverages (Luthje), and after taking large doses of irritating substances (Filosofoff, 1905), such as pepper, mustard, onions, alcohol, turpentine, have also been well demonstrated.

Filosofoff[1] examined 50 healthy young men whose history was free from alcoholism and acute nephritis, and whom he had watched for a long period on a known diet. These men were kept constantly at rest during the observation. In 17 of these men he found hyaline casts; in 12 granular casts; and in 13 cylindroids. In other words, 52 per cent. of these young men showed some form of cylindric elements in the urine.

It seems settled that casts may occur without the presence of albumin. In the writer's experience, which em-

[1] Roussky, Vratch, 1905, No. 50, p. 1561.

braces an examination, up to the time of writing, of over 10,000 specimens of urine, at least 10 per cent. of all specimens in which casts were found contained no albumin that was apparent upon clinical tests. Actual figures are not available, but this is a conservative estimate. The casts found in these cases were usually hyaline, and sometimes faintly granular. Hyaline casts are found much more frequently in healthy urines than is generally supposed, provided the examiner uses proper care in his work.

Although the presence of a few casts without albumin in an otherwise normal urine is still looked upon as evidence of an impaired risk by insurance companies, and although unfortunately some physicians continue to make the diagnosis of Bright's disease upon this basis, the writer is convinced from his experience that the clinical significance of casts in these circumstances has been very greatly exaggerated. In order to make a diagnosis of renal disease it is always important to weigh separately each microscopic and each chemic finding as a part of the evidence, and to base our judgment upon the particular combination found in each case. When thus considered the presence of casts is a very useful factor in localizing and differentiating renal disease.

In the following pages a detailed description of the various casts will be given. Pure hyaline and faintly granular casts are the most common forms, and are not necessarily nephritic in origin. Coarse granular, epithelial, fatty, and other forms are always pathologic.

Classification of Casts.—The following table (Ogden) shows a useful classification of tube-casts:

I. Hyaline (transparent) casts.... { (1) Pure hyaline. (2) Fibrinous. (3) Waxy.

II. Granular casts ..... (1) Fine. (2) Coarse. (3) Pigmented.
III. Epithelial casts.
IV. Fatty casts.
V. Blood-casts.
VI. Pus-casts.
VII. Crystalline casts ..... (1) Urate. (2) Oxalate. (3) Cystin.
VIII. Bacterial casts.

Hyaline Casts.—There are three varieties of hyaline casts: (1) Pure hyaline; (2) fibrinous; (3) waxy.

Pure Hyaline Casts.—These are pale, delicate, transparent, homogeneous cylinders, varying in diameter and in length, usually with rounded ends and parallel and straight sides, but sometimes indented, twisted, serpentine in shape, with ragged ends. It is very difficult to detect pure hyaline casts, except by constant focusing with the fine adjustment of the microscope and by diminishing the amount of light in the field. Pure hyaline casts occasionally contain some very fine pale granules, or here and there show upon their outlines a blood-cell, a leukocyte, a droplet of oil, etc. Such casts are still in the hyaline class. The source of the hyaline casts may be to a certain extent deduced from their diameter. The smaller, narrower casts arise from the convoluted tubules, while the larger ones come from the straight or collecting tubules.

The mode of origin of hyaline casts is still unsettled. According to Rovida, they are the products of secretion by the tubular epithelium, but experiments on animals made by Ribbert show that they are probably due to the exudation of albumin within the tubules. Whatever may be their origin, the mere presence of pure hyaline casts must not lead to the hasty conclusion of the existence of renal disease.

Hyaline casts are commonly found, however, in a great many diseases and disturbances of the kidney, especially in chronic interstitial nephritis, in amyloid kidney, and in passive congestion. In acute nephritis and in active congestion they are most numerous. They may be the only indications of a severe nephritis, and, on the other hand, these casts are met with in the urine of persons with

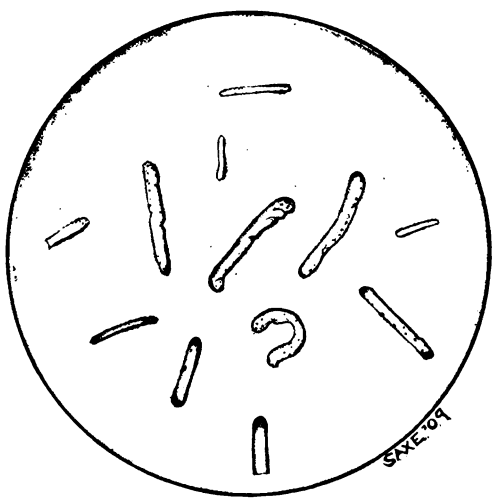
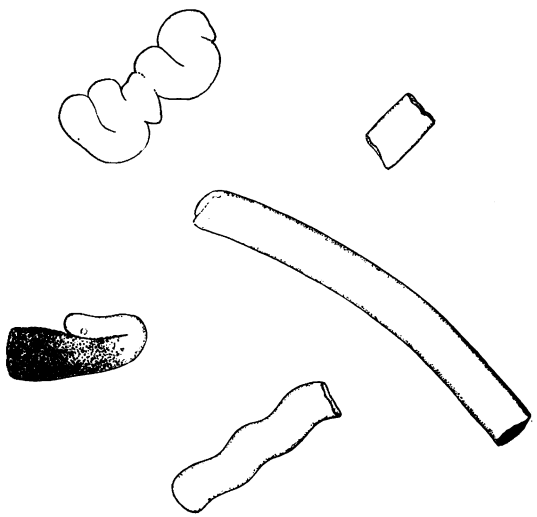


Fig. 62.—Hyaline casts viewed in a darkened field of the microscope.

healthy kidneys in whom no albuminuria or any other sign of renal inflammation had ever been observed. They may be evidences of a renal irritation caused by a strain upon elimination due to overabundant food, excess of meat in the diet, or intestinal intoxication. In such cases albumin is often absent, and the casts disappear after the cause is removed. To find hyaline casts we use, in preference, fresh urine, and sediment it, instead of centrifuging.

PLATE II



FIBRINOUS CASTS (*Ogden*).

Fibrinous casts are dense, highly refractive, transparent, and of a yellowish color, which ranges between pale yellow and deep brown. Like pure hyaline casts, they vary in shape and size, but are, as a rule, larger than the average hyaline cast. They should not be confounded with *waxy* casts, which are always perfectly colorless. The term fibrinous casts is inappropriate, as they have nothing to do with fibrin and are suggestive only by their yellow color. Like pure hyaline casts, they may be studded with fine granules and may have adhering blood or fat-droplets. Their presence indicates an acute disease or disturbance in the kidneys, and fibrinous casts are, as a rule, seen only temporarily during the acute stage of these conditions. They are not unfavorable signs in prognosis.

Waxy Casts.—These casts are very highly refractive and always perfectly colorless and transparent. They are usually large in diameter, and vary in shape like the pure hyaline. They may be studded with coarse granules, and may have adhering the same elements as the pure hyaline casts. They are so dense that they may look cracked or broken into segments, with irregular ends. They are never present in active congestion and in acute nephritis, but are found in advanced stages of chronic nephritis, both interstitial and parenchymatous. They are, as a rule, of bad prognostic significance. They are often seen in amyloid infiltration of the kidneys, and in this condition are of some diagnostic value, although not pathognomonic, as they occur in other chronic diseases of the kidney. It must be remembered, however, that the term "waxy" is misapplied to these casts, as they show the "amyloid reaction" with methyl-violet and potassium iodid and iodin solution, in the absence of amyloid de-

generation, and, on the other hand, this reaction is absent in some cases when this degeneration exists.

All the other casts to be described are practically modifications of the hyaline cast, which forms the groundwork upon which various elements adhere or into which they become imbedded. A hyaline cast covered with granules is a *granular* cast; one covered with epithelia is an *epithelial* cast, etc.

Granular casts consist of a hyaline basis in which fine, coarse, or pigmented granules are imbedded. These

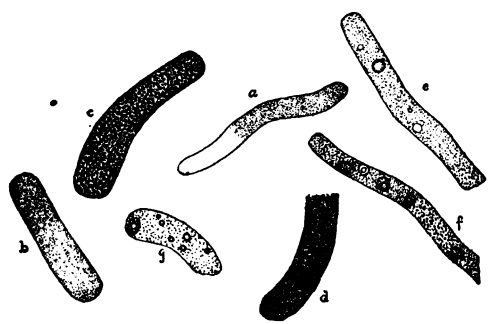


Fig. 63.—*a*, Hyaline and finely granular cast; *b*, finely granular cast; *c*, coarsely granular cast; *d*, brown granular cast; *e*, granular cast with normal and abnormal blood adherent; *f*, granular cast with renal cells adherent; *g*, granular cast with fat and a fatty renal cell adherent (Ogden).

granules probably come from the disintegration of renal epithelia, or partly from the destruction of blood-cells. The pigmented granules, also called "brown granules," probably derive their color from blood-pigment. Granular casts may be stained yellow or brown by bile.

These casts vary considerably in dimension, and are often broken, the end often being concave or zigag. Some-

times they show fragments of epithelial cells and transition forms which prove that the granules are derived from the epithelia, and that granular casts are degenerated forms of epithelial casts. They may be found in any inflammatory condition of the kidney, and do not indicate any particular disease of this organ. Their presence is about equally significant with that of renal epithelia as evidence of nephritis.

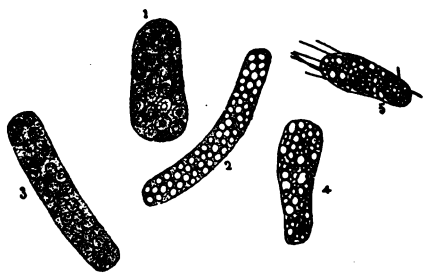


Fig. 64.—1, Epithelial cast; 2, blood-cast; 3, pus-cast; 4, fatty cast; 5, fatty cast with a compound granule and fatty renal cell adherent (crystals of the fatty acids protruding) (Ogden).

Epithelial Casts.—When a cast is entirely covered with renal epithelium, it is called an epithelial cast. The basis of this formation may be either hyaline or granular, and the cells may be imbedded or only adherent. When only one or two cells adhere to a hyaline cast, the term "hyaline with adherent epithelium" should be used. The outlines of the cells are not always distinguishable, but their nuclei usually stand out prominently. The cells may contain fat-globules, and leukocytes may adhere to these casts along with the epithelium.

Perfectly formed epithelial casts appear in urine under such conditions only as cause the separation of renal epi-

thelium in the entire circumference of a renal tubule. They always indicate an active inflammatory process in the parenchyma of the kidney, and "their presence alone suffices to establish the existence of acute nephritis or the supervention of a fresh paroxysm in that disease" (von Jaksch). The intensity of the inflammatory process is in proportion to the number of these casts. They are rarely found in chronic and interstitial nephritis and in amyloid kidney. Epithelial cylinders with a lumen may be seen in

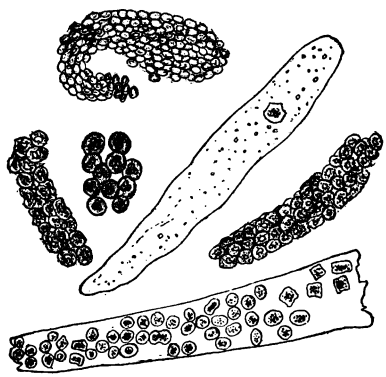


Fig. 65.—Blood-casts, composed wholly of red or white corpuscles, or hyaline substance covered with blood-corpuscle.

cases of great congestion in which the lining of the tubules is thrown off unchanged.

Blood-casts.—These casts consist of a hyaline or granular base, covered with red blood-cells or of cylinders of coagulated blood (fibers) with imbedded blood-cells. The occurrence of blood-casts indicates a hemorrhage into the tubules. According to the length of time which these casts have remained in the tubules, the blood-cells on them may be normal or abnormal (see p. 275).

Blood-casts are found in the renal hematuria due to tuberculosis, stone, tumor, etc.; in acute nephritis, acute congestion, and hemorrhagic infarcts of the kidney. Their presence simply shows the existence of renal hemorrhage, and not necessarily of renal disease.

Fatty casts are cylinders which are thickly studded with fat-droplets and granules. The term does not apply

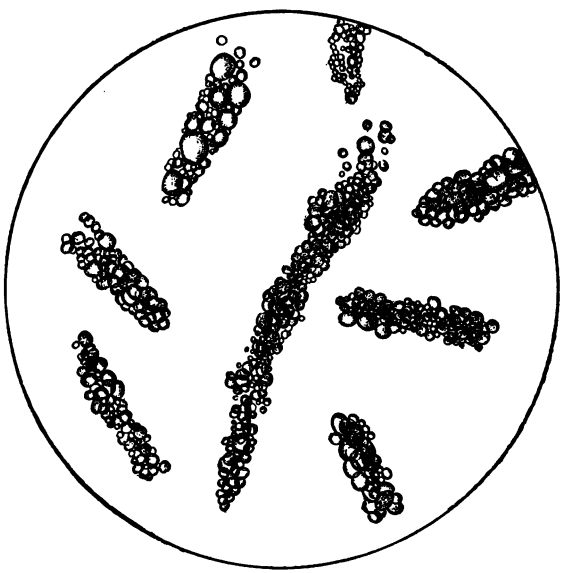


Fig. 66.—Fatty casts (after Peyer).

to hyaline or granular casts which show one or two oil-globules. They occur in short, highly refracting cylinders, and may show bristling, needle-like hair or crystals of fatty acids. The minute fat-drops are highly glistening, and should not be confounded with granules, which have a duller luster.

These casts were first discovered by Knoll, and are usual in protracted cases of chronic nephritis with a tendency to fatty degeneration of the kidney. Fatty degeneration, it must be remembered, shows an advanced stage of alteration in the renal cell. Fatty casts are also found in the fatty stage of acute nephritis, and occasionally in severe renal congestions.

Pus-casts are hyaline or granular cylinders covered with pus-cells or leukocytes. They are often confounded with epithelial casts because the pus-cells are often so

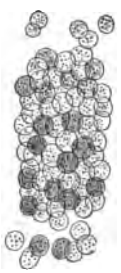


Fig. 67.—Casts formed of leukocytes from a case of acute nephritis (von Jaksch).

Fig. 68.—Casts of urates from a case of emphysema (von Jaksch).

granular that the nuclei are obscured. The addition of dilute acetic acid dissolves the granular matter and shows the nuclei of the leukocytes.

Pus-casts are not met with, except in a chronic suppurative process in the kidney, such as an abscess, tuberculosis, and chronic pyelonephritis. In cases of chronic pyelitis or pyelonephritis there may be casts from the pelvis, calices, or from the straight tubules, consisting of large and irregularly shaped plugs of coagulated material covered with pus-cells. In acute non-suppurative nephritis and in acute

exacerbations of chronic nephritis there may be hyaline or granular casts, with a few adherent pus-cells, but these do not constitute true pus-casts.

Crystalline Casts.—These are simply hyaline or granular casts to which crystals or amorphous granules of a chemic sediment are adherent or in which these are imbedded. A *urate* cast is one covered with crystals of ammonium urate, usually of the characteristic spheric shape. The *cystin* cast is rarely seen in cystinuria, and is covered with cystin crystals. The *calcium oxalate* casts are covered with the crystals of this substance. *Amorphous urates*, when present in large amounts, may coat hyaline or other casts and obscure their true morphology. In such cases it is important to dissolve the urates in warm water, as has been described on page 258.

Bacterial casts are cylinders covered with bacteria, but masses of germs that resemble these casts, and that sometimes are seen in urine undergoing decomposition, do not constitute bacterial casts. The latter are sometimes confounded with the pigmented or brown granular casts, but resist the action of acetic acid and strong alkalis. Bacterial casts are rare, and usually indicate renal suppuration or renal embolism.

FALSE CASTS OR CYLINDROIDS

False casts, mucous casts, or cylindroids are occasionally found in urine, and seem to be pure mucous molds of tubules. Their true chemic nature is not exactly known, but they have little clinical significance and are frequently present without any albumin. They are smooth, long, flat, transparent, finely fibrillated, with indistinct, wavy, longitudinal striations, and *have tapering ends*. They are

characterized especially by their great length, and are often twisted or folded. The chief interest in false casts is their resemblance to true casts, and they must be carefully distinguished by means of the peculiarities just described. Occasionally, epithelia or other elements may adhere to them and increase their resemblance to true casts.

False casts are found usually wherever there is irritation of the bladder and lower urinary passages which has extended up to the kidneys. They are also found in urine of very high specific gravity, but it must be remembered that they are not identical with mucus threads, although chemically false casts and mucous threads are probably the same. Mucus in the urine may often be found moulded into false casts upon prolonged and rapid centrifuging.

MUCOUS THREADS

Mucous threads appear in normal urine in the form of more or less opaque, ropy masses of irregular shape, but sometimes in the form of long and transparent shreds, the ends of which taper or split in fine divisions, which fade away imperceptibly. They are often covered with amorphous urates, and may be mistaken for casts. They are, however, flat and not cylindric; generally very much narrower than casts, and their granules can be dissolved with a little heat on the addition of acids or alkalis. The presence of these threads has no very marked clinical significance unless there is an excessive amount of mucus, which indicates irritation or inflammation in some part of the genito-urinary tract. Mucin threads may be demonstrated by the addition of a little acetic acid and a little solution of iodin, potassium iodid, tartaric acid, or dilute

mineral acids, but an excess of the latter will redissolve the mucin. Mucin threads may be found in urine without addition of acids, being probably precipitated by the action of acids formed in acid fermentation. The greater the

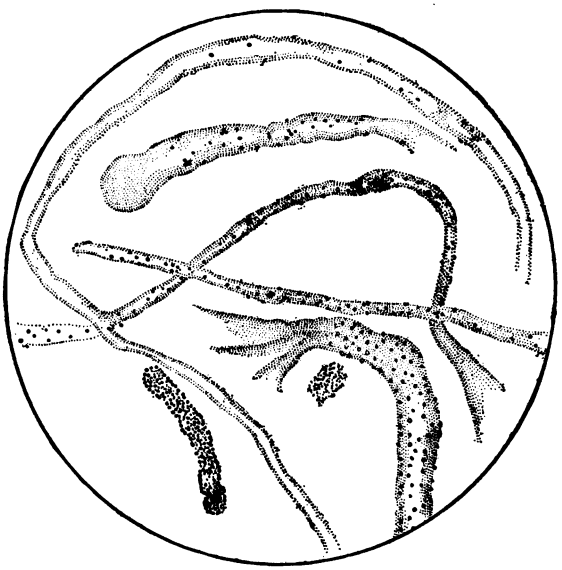


Fig. 69.—False casts (after Peyer).

irritation in the genito-urinary tract, the more opaque, the thicker, and the more ropy becomes the mucus, and appears then in the form of clouds mixed with epithelial cells from the seat of the trouble.

PROSTATIC PLUGS

Prostatic plugs are large, colorless, or yellow molds of the prostatic ducts (Fig. 70), cylindric, with rounded ends, or of irregular shape. Their outlines are not sharply

defined, as they generally are found in clouds of mucus, but their chief characteristic is the fact that they have imbedded in them or adherent to them spermatozoa and epithelial cells from the prostatic ducts. Their presence indicates an inflammation of the prostate involving the ducts, and they are said to occur in urines after massage

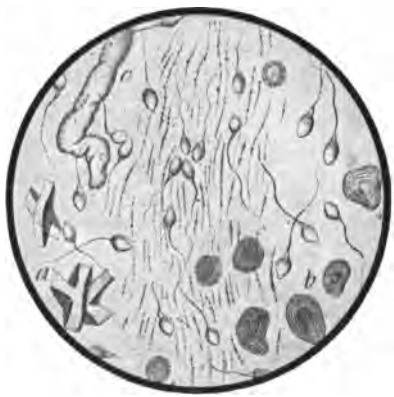


Fig. 70.—Spermatozoa: At *a* are so-called Böttcher crystals; at *b*, amyloid bodies; at *c*, a prostatic plug (Jakob).

of the prostate, but the examination of a very large number of such specimens by the writer showed that they are very rare in massage-urine. They are interesting because they are apt to be mistaken for casts. Their occurrence is, in fact, a matter of some doubt.

AMYLOID BODIES

Amyloid bodies, or *corpora amylacea*, are minute bodies which are found in the acini of the prostate, and occasionally occur in the urine,[1] especially after massage of a

[1] Amyloid bodies are also found in urethral shreds, and are said to be present normally in the urine in very small numbers.

prostate in the state of chronic follicular prostatitis. The word "amyloid" is a misnomer, as these bodies have nothing to do with starch chemically. They are more or less opaque, rounded bodies, of either homogeneous or lamellated structure. Their central portions consist of a darker or more densely lamellated core. They are not affected by hot water nor dissolved by strong nitric acid; they are colored red with methyl-violet, while starch takes a blue color. Amyloid bodies, when treated with iodin, often show a violet color which becomes blue on the addition of sulphuric acid. Their clinical significance is doubtful. In old men larger masses of these bodies may occur, visible to the naked eye, and surrounded with a mixture of phosphates to form the so-called prostatic concretions which form in the follicles of the prostate.

SPERMATOZOA

Spermatozoa are bodies about 50 μ in length, consisting of an oval head, a minute body, and a long, delicate, whip-like tail (Fig. 70). In fresh semen they show active eel-like movements, but in urine they usually become motionless in a short time. They require the high power for their detection and fine focusing for their demonstration. In the urine they may be accompanied by mucus, prostatic plugs, amyloid bodies, and by epithelia from the prostate, the vesicles, and the neck of the bladder. The so-called spermin crystals (Böttcher's crystals) which are present in prostatic secretion never occur in the urine, as they do not form in acid media.

Clinical Significance.—Spermatozoa are found frequently in the urine of healthy men, and of both sexes after coitus. They are found also in men after nocturnal

emissions, and sometimes in the urine in acute fevers, sepsis, and convulsions. Their most important clinical bearing is in cases of *spermatorrhea*, when they are constantly found in the urine, and also in cases of acute and chronic prostatitis and seminal vesiculitis, or of congestion or irritation of the prostatic region. The presence of an inflammatory condition in cases in which spermatozoa are found may be inferred from the coexistence of mucus, pus, epithelia from the region concerned, and from the broken tails and immovable condition of the spermatozoa.

Dead spermatozoa frequently are found curled up into various grotesque shapes.

The detection of spermatozoa is important from the medicolegal aspect in cases of *suspected rape*. It is easy to show their presence in vaginal secretion, and stained linen may be soaked in either water or salt solution and the sediment examined. A chemic reaction which may aid in their recognition was described in 1897 by Florence, of Lyons. A few drops of the suspected fluid are treated with a drop of Florence's reagent, which consists of:

Potassium iodid	1.65 gm.
Iodin	2.64 "
Distilled water	30.00 "

This reaction is probably dependent on the presence of cholin. If semen is present, small, dark, rhombic crystals appear, resembling closely the hemin crystals described in Teichmann's test for blood.

SAGO BODIES AND OTHER VESICULAR ELEMENTS IN MASSAGE-URINE

By massage-urine is meant the urine voided by the patient after the contents of the prostate and seminal

vesicles have been expressed into the prostatic urethra by kneading and stripping these organs through the rectum. In such urines a variety of semisolid elements occur; the microscopic structure and clinical significance of which were made a subject of research by the writer.[1]

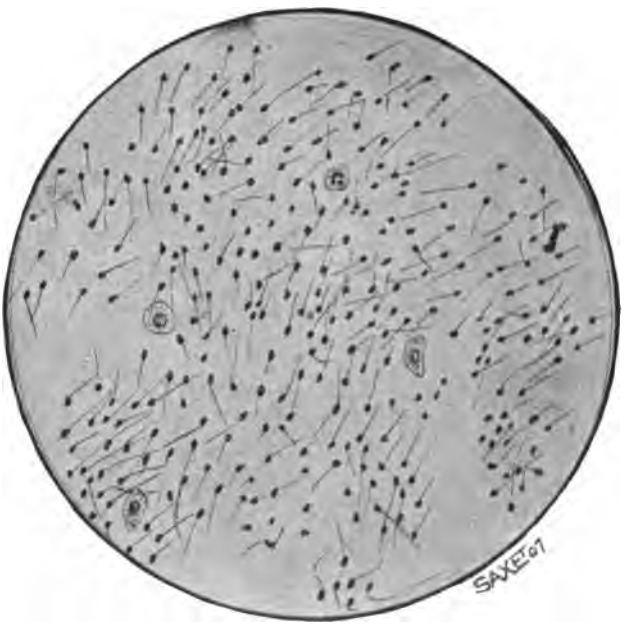


Fig. 71.—Stained smear taken from a sago body (×257. Eosin methylene-blue).

One or more of the following classes of elements may be found present in these urines: (1) "sago bodies"; (2) "sugar granules"; (3) vesicular "skins"; (4) vesicular casts and vesicular shreds.

[1] Saxe, A Study of Sago Bodies and other Vesicular Elements in Massage-urine, etc., New York Med. Jour., Nov. 23, 1907.

Sago bodies were originally described by Lallemand and Trousseau in the urines of patients with spermatorrhea. They consist of sago-like masses of colloid material of the size of a small pea or lentil in which are embedded numerous motionless spermatozoa. The latter are often packed

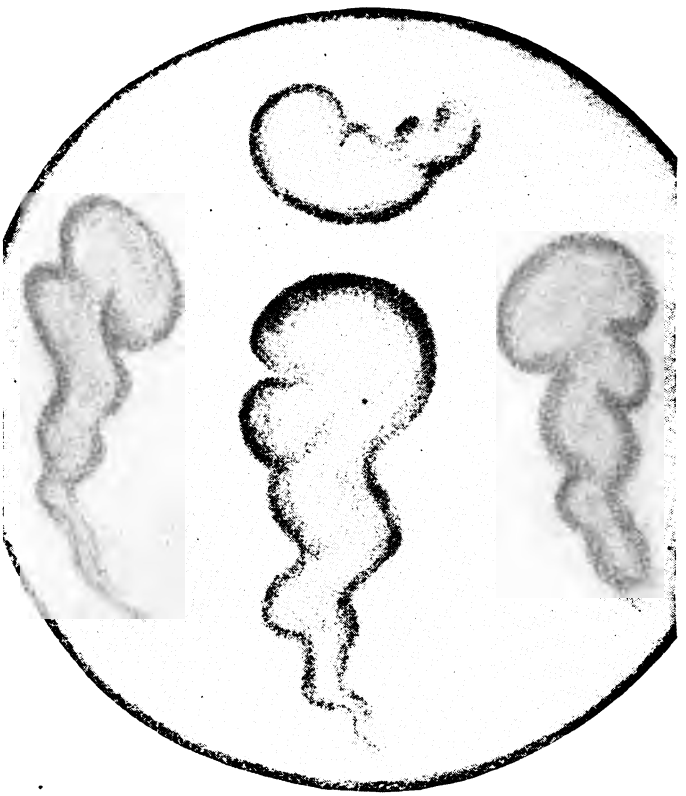


Fig. 72.—Casts from the vesicles and the ampulla of the vasa deferentia in a case of atonic vesiculitis. (×5. In dish with salt solution.)

closely at the edges, in several layers. These bodies contain epithelia from the vesicles and occasionally a leukocyte (Fig. 71). The sago bodies are derived from the recesses of the seminal vesicles which offer molds for their formation.

Sugar granules are much smaller, of the size of a pinhead or a little larger, and are glassy, translucent, having the appearance of melting sugar. They settle at the bottom of the vessel and dissolve rapidly. Their structure is practically the same as that of the sago bodies, save that they contain fewer spermatozoa. They may consist merely of a translucent colloid matrix, highly refractive. The writer found them to be

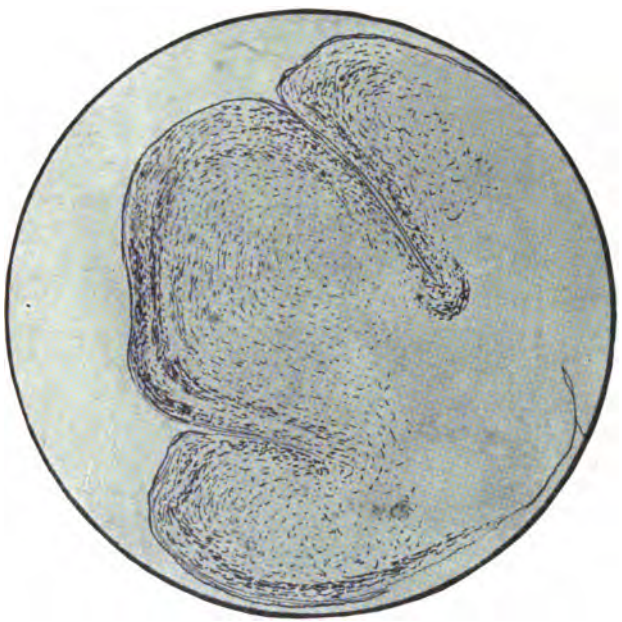


Fig. 73.—Vesicular cast, showing the characteristic concentric arrangement of the spermatozoa and the lobulation. (⅔ obj. Eosin-methylene-blue.)

fragmented masses of secretion from parts of the vesicle where there were few or no spermatozoa—*i. e.*, probably from the fundus of the organ.

The **vesicular skins** are fine, translucent or slightly opaque, whitish pellicles, often presenting an appearance of hollow shells, like the thin shells of lemon seeds, grouped in masses. When removed from the urine they collapse into viscid shreds. The skins are probably composed of inspissated or thickened vesicular secretions which have lain for a consid-

erable time in the vesicles, and which have been stripped by massage. Microscopically they contain the same elements as the sago bodies, but densely packed and mixed with ropy mucoid material.

Vesicular casts are moulded forms of vesicular contents (see Figs. 72–74) which may be grouped in grape-like masses or may occur

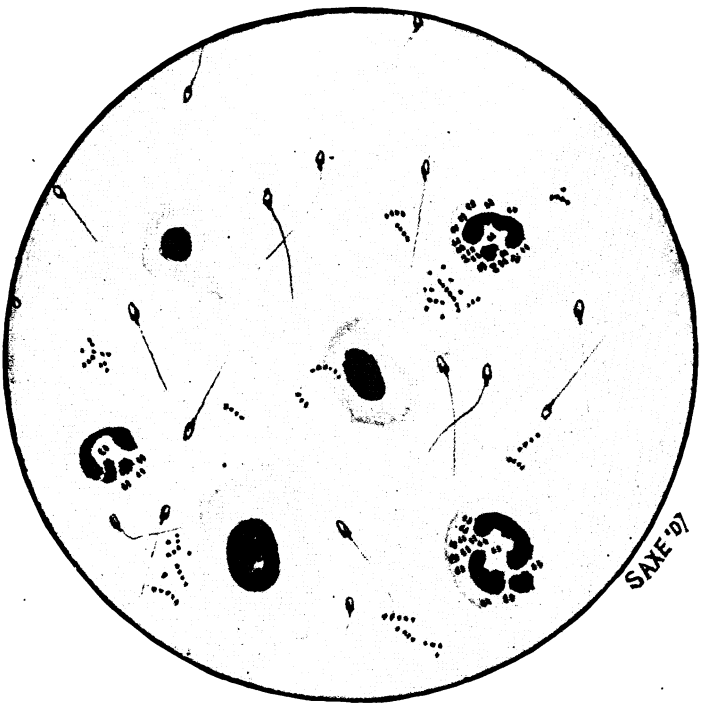


Fig. 74.—Gonococci and streptococci, epithelia and pus-cells, in stained smear from a vesicular cast (Gram's stain: ×960).

in elongated, sausage-like forms. They are semi-opaque, whitish, and are easily seen with the naked eye. Microscopically, they present a lobulated border, with concentric layers of spermatozoa in dense masses at the edges and numerous spermatozoa in the central portions (Fig. 73). They may contain gonococci, staphylococci, streptococci, etc., in vesiculitis due to these germs (Fig. 74).

Vesicular shreds are often of large size and look like pieces of egg-membrane. They consist of a mucoid matrix in which are imbedded many spermatozoa, vesicular epithelia, and pus-cells. They often contain bacteria.

Clinical Significance.—A careful study of the clinical significance of these bodies showed that the sago bodies, the sugar granules, and the "skins" may occur without any vesicular inflammation. They may be present in normal individuals who are relatively sexually continent, but they are increased as the result of a stagnation of secretion in the vesicles in that type of vesicular atony which the writer has termed "spermatostasis." Vesicular casts and vesicular shreds, on the other hand, especially when they contain pus-cells and bacteria, are evidences of vesiculitis.

Smears from any of these structures may be made by washing the bodies in normal salt solution, spreading thinly on a slide, fixing with equal parts of alcohol and ether for fifteen minutes, and staining with eosin and methylene-blue according to the method described for urine sediments on p. 244. Gram's stain may be used to show gonococci, which, however, are rarely found. An examination of stained smears of the massaged material is essential before making a diagnosis of vesiculitis, as the mere presence of even large amounts of semisolid massaged bodies is not conclusive evidence of vesicular inflammation. The finding of pus and of bacteria is necessary before the diagnosis of vesiculitis can be made.

URETHRAL SHREDS

Urethral shreds occur in the urine in chronic inflammations of the urethra, particularly in chronic gonorrhea and

stricture. Very little information concerning shreds appears in the text-books and the writer herewith presents a summary of his own studies on these elements, based on extensive clinical material.[1]

The Author's Technic.—Shreds may be obtained directly from the urine or by washing the different portions of the urethra, as described in text-books on urology. They should be fished out with sterile platinum wires and spread on slides. They should then be fixed with equal parts of alcohol and ether for ten minutes, stained for from one to two minutes in Unna's polychrome methylene-blue, washed thoroughly in distilled water, and dried. They may then be immediately examined after mounting in balsam, or if still better pictures are desired, the dried film should be dehydrated for a few seconds in alcohol, dried with filter-paper, and cleared in xylol or oil of cloves. On drying, the film may be mounted in balsam and examined. For the detection of gonococci in shreds, Gram's stain (see p. 331) may be used instead of the polychrome blue, the other steps in the technic being the same.

Urethral shreds proper may be divided into four varieties: pus shreds, mucopus shreds, mucous shreds, and epithelial shreds, each of which have special naked-eye and microscopic characteristics.

Pus Shreds (Fig. 75).—These are dense, heavy opaque, yellowish-white, often thick and shaggy, usually short and friable. They tend to sink readily to the bottom of the vessel. Microscopically, they consist of innumerable pus-cells, closely packed in a scarcely visible matrix of homogeneous character, and contain singly or in small groups epithelia from the urethra. The latter may be normal or they may be in a state of

[1] See Saxe, A Study of Shreds in the Urine in their Relation to Diagnosis and Prognosis, New York Med. Jour., March 2, 1907.

hyalin degeneration. In this case, the writer has found them to stain quite differently from normal epithelia, with polychrome blue (Fig. 76). They stain diffusely without chromatic distinction between nucleus and cell body, and are markedly more reddish than normally staining urethral cells, which show deep purple nuclei and pale bluish-violet cell bodies. For a fuller description of urethral epithelia, see page 294.

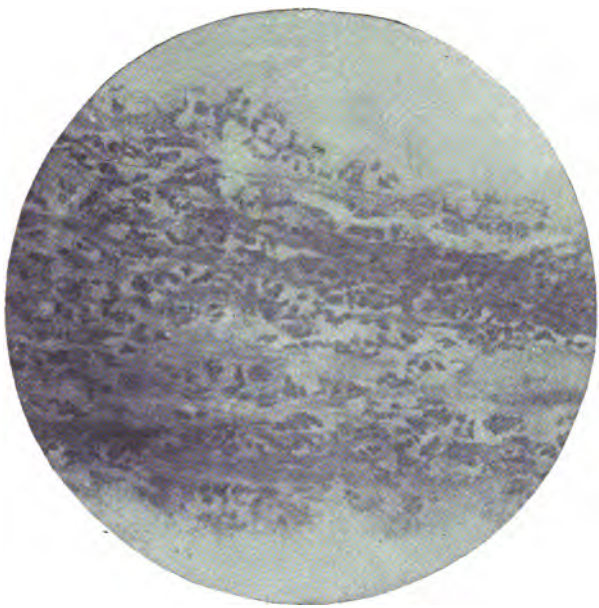


Fig. 75.—Pus shred, showing pus, epithelia, and strands of matrix (× 333).

Mucopus Shreds.—These are longer, more wavy or twisted, very irregular in shape, thinner, more translucent, grayish white, and show faint whitish longitudinal streaks and occasional large opaque nodes. One of their ends may curl up into a knob, causing them to resemble commas. Microscopically (Fig. 76), they consist of the same elements as the pus shreds, save that the homogeneous mucoid matrix is much more abundant, while the pus-cells are fewer in number and appear in scattered groups. Some of the pus-cells are fragmented, showing the beginning of disintegration. The epithelia vary in number and may be so numerous

as to justify the name epitheliomucous shreds. They show the same changes as those found in pus shreds.

Mucous Shreds.—These are the lightest of all urinary shreds and persistently float at or near the surface of the fluid. They are long, thin, almost transparent, with faint gray striations. When removed from the urine they appear as translucent, viscid streaks of mucus. Microscopically (Fig. 77), they consist of a mucoid matrix arranged in fibrillated layers and staining a deep purple with polychrome blue in contrast to the

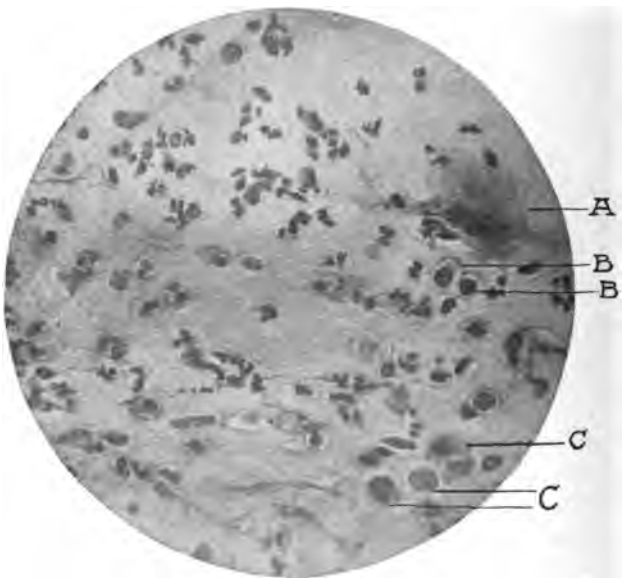


Fig. 76.—Mucopus shred, showing amyloid body (A) and contrast between normal (B) and chromatically degenerated epithelia (C) (× 249).

lighter shade seen in the matrix of pus shreds and mucopus shreds. Within the strands of mucus there are usually a few epithelia which show distinctly by contrast, and occasionally there are the remains of a few pus-cells.

Epithelial Shreds.—Of these there are two varieties: The first consist of large masses of epithelia with a moderate proportion of pus-cells, and without any distinctly visible matrix. These shreds are found in the urine as short, flaky, or scaly masses, usually rather loosely knit and

shaggy. Their microscopic appearance is shown in Fig. 78. They are characteristic of very chronic urethral inflammations, specially of stricture formation.

The second variety of epithelial shreds are purely epithelial and are comparatively rare. They are very thin, semitransparent, sink quite rapidly, wrinkle, and look like bits of desquamated epidermis. They are composed simply of a pavement of large pale, flat cells, which cling

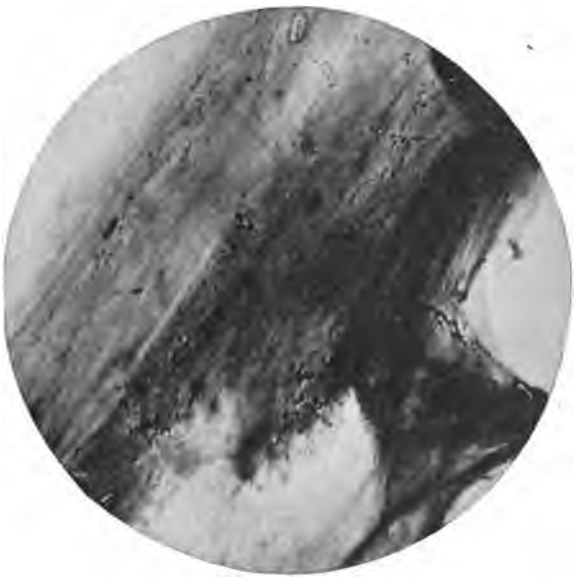


Fig. 77.—Mucous shred in a case of chronic urethritis with glandular lesions, showing stratification of matrix and remains of pus-cells (× 147.5).

to one another, occasionally overlapping (Fig. 79). The squamous cells found in the epithelial shreds of both varieties are altered urethral cells which are characteristic of chronic urethritis with hard infiltration (Fig. 80). In this condition the cells of the superficial layers of the urethral mucosa are transformed into squamous cells, contrasting with normal urethral epithelia, which are commonly of the stratified cylindric type. The pure epithelial shreds are seen sometimes after the passage of instruments or after applying solutions of silver nitrate through the ureth-

roscope. They may appear, however, spontaneously in patients with hard infiltrates in the urethra.

Comma Shreds.—These are of two varieties in the writer's experience: The false comma shreds (already mentioned), which may come from any part of the urethra and which are nothing but mucopus shreds curled up at one end. They may come from any part of the urethra and appear in the urine as short comma-like structures which have been erroneously supposed to come from the prostatic ducts. The true comma shreds are

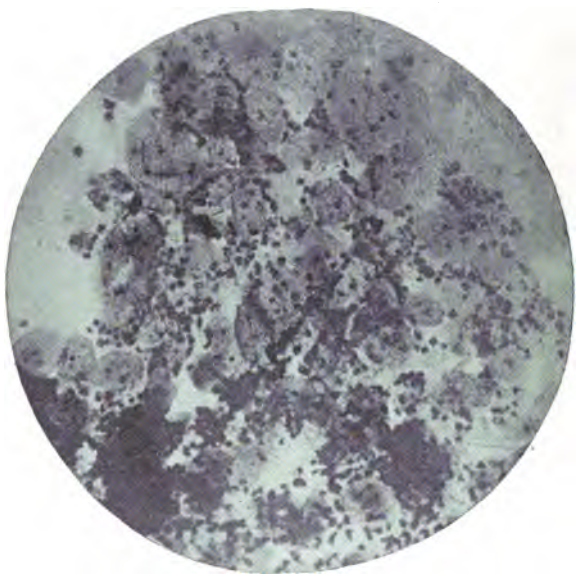


Fig. 78.—Epithelial shred from a case of stricture 12 F. at P. S. junction, showing flat epithelia and pus-cells (× 167.4).

rarely found in the urine, except in the last glass in a test involving a sequence of glasses into which the patient is asked to urinate. (The Kollmann five-glass test.) They are sometimes found in urine after prostatic massage. These true comma shreds come from the prostatic ducts and outwardly resemble the false comma shreds, but microscopically they consist of epithelial masses from the prostatic ducts, usually arranged in two layers, one of which is composed of cylindric, the other of smaller rounded, cells.

Clinical Significance of Shreds.—The examination of shreds in the urine is of great importance in diagnosticating the stage of a chronic urethritis and in determining the character of the lesions in the urethra, and is of value to some extent in the prognosis of chronic urethritis.

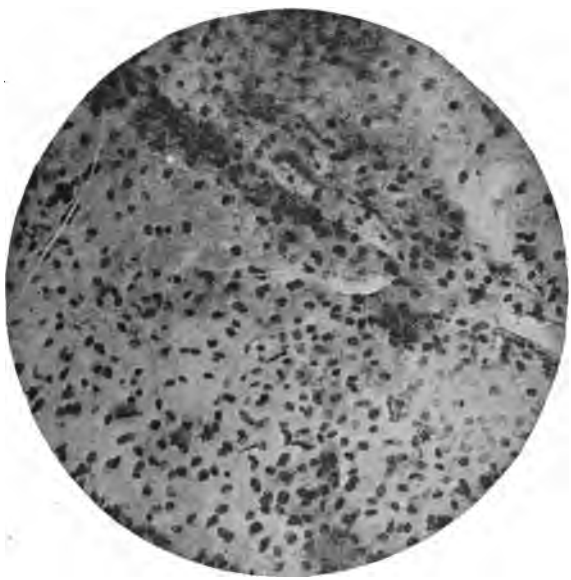


Fig. 79.—Pure epithelial shred in a case of chronic urethritis with hard infiltration in the bulbous urethra, showing pavement of cells and no pus (×147.5).

The examination of shreds is not of much value in localizing the affection in the anterior or posterior urethra. The presence of prostatic or vesicular epithelia and of spermatozoa in the shreds may point to the involvement of the prostatic urethra, but the localization of a urethritis must be carried out by other methods which do not belong to urine analysis.

Urethral shreds appear with a fair degree of regularity with each stage of urethritis, the order usually observed beginning with a subacute case and ending with a pronouncedly chronic condition with induration and stricture formation: (1) Pus shreds; (2) mucopus shreds; (3)

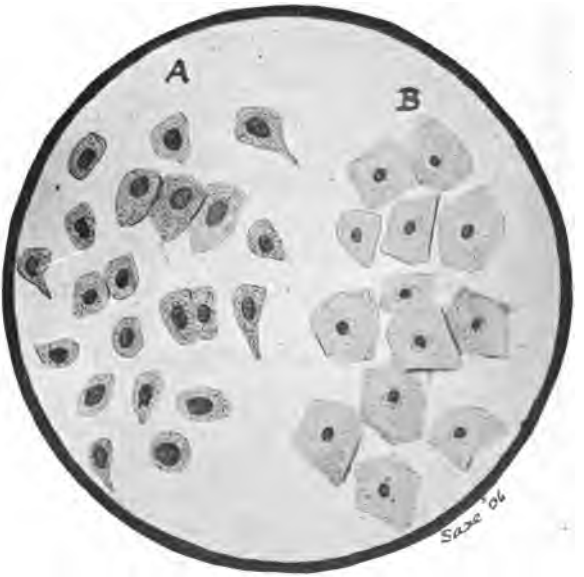


Fig. 80.—Epithelia found in shreds: A, Normal urethral epithelia; B, modified flat cells from the superficial layers in the stage of hard infiltration.

mucous shreds, and (4), epithelial shreds. While this is the typic evolution of shreds in chronic urethritis, this rule is subject to many exceptions. For example, if an acute exacerbation occurs in the course of a chronic case, some of the types of shreds which had been present earlier will reappear. Moreover, the same urine may contain

several varieties of shreds, as different parts of the urethra may not keep equal pace in the progress of the disease.

Each of the varieties of shreds described, however, has a distinct significance as regards the pathologic process in the urethra which each type represents. Pus shreds indicate a subacute or chronic exudative process. They are the first signals of chronicity and appear in the chronic stage whenever an active inflammatory process is going on. As the urethritis becomes more chronic the pus-cells gradually disappear and the epithelia and mucoid matrix increase, bringing the shreds closer to the mucopus variety. The latter are first found when the chronic process begins to assume the catarrhal type and when the glandular involvement first comes to the fore. The mucopus shreds are the most frequent and the most persistent type of shred in the urine. As the exudative inflammation gradually disappears and the catarrhal phase frankly sets in, the next variety, the mucous shreds, appear. The mucous shreds signalize chronic soft infiltration with predominant glandular involvement. There may be single mucous shreds persisting for a long time. If the process now heals, this last shred disappears. But if hard infiltration develops, as is usually the case, shreds of flat epithelia, with or without pus, are seen. The epithelial shreds with pus are evidences of ulcerative conditions or of strictured areas around which there are ulcerating or granulating lesions.

MICRO-ORGANISMS

The urine may contain bacteria, molds, or yeasts. Fresh urine is said to be sterile in normal persons if obtained directly from the bladder, but is contaminated in transit by the bacteria always present in the urethra.

If urine is allowed to stand in the air for some time, it becomes turbid from *masses of bacteria* of the non-pathogenic variety. The conversion of urea into ammonium carbonate is probably produced by several bacteria, of which the **Micrococcus ureæ** (Fig. 81) is the most important. This germ is found very frequently on the surface of decomposing urine, and occurs in chains of round, highly refracting dots. It is contantly present in the air. Large varieties of other bacteria which take part in the decomposition of the urine have been described.

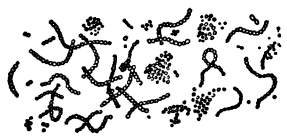


Fig. 81.—Micrococcus ureæ (after von Jaksch).

Molds are not seen normally in decomposing urine, but often occur in diabetic urines floating on the surface.

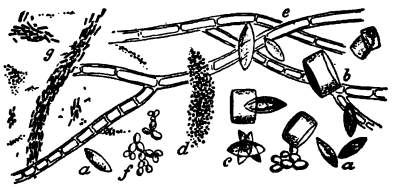


Fig. 82.—Sediment from fermenting diabetic urine with casts of micrococci: *a*, *b*, *c*, Various forms of uric acid; *d*, micrococci in form of casts; *e*, molds; *f*, yeast fungi; *g*, bacilli and micrococci (after von Jaksch).

A variety of molds may be found in urines which have been allowed to stand in uncovered vessels.

Yeasts.—The beer-yeast fungus is found in diabetic urine or in urine contaminated accidentally with sugar. It occurs in oval, transparent cells, free or in groups of

three or more, and multiplies rapidly in acid urine, but ceases to grow when it becomes alkaline. They should not be mistaken for red blood-cells, and are distinguished by their oval shape and the presence of a cell body without a focal point.

The **Sarcina urinæ** is found occasionally in urine, resembles the sarcina of the lung, and occurs in cubes of eight cells, each arranged like a bale of goods. In some cases very many sarcinæ are present. Their clinical significance, if any, is not established.

Pathogenic Bacteria.—Of these, a large number have been found in urine. In a great variety of the suppurative conditions of the urinary tract the bacteria of pus-formation are found—*Streptococcus pyogenes; Staphylococcus pyogenes albus, citreus,* and *aureus.* In many specific diseases, as in typhoid fever, anthrax, glanders, erysipelas, and tuberculosis, the germs of these affections are found in the urine.

Technic of Preparing Bacterial Smears from Urine.—Much difficulty is often encountered in making good stained smears from urine for bacterial study, and this is especially the case when the urine is clear, contains no pus, and little epithelial or mucoid material. The urinary salts, especially urea, interfere materially with staining (see Methods of Staining Urinary Sediments, p. 244).

The best results are obtained by thoroughly centrifuging the sediments. If any visible sediment can be obtained the centrifuge tube should be quickly drained and the sediment adhering to the bottom scraped off with a platinum loop, the tube being held bottom up during this procedure. In this way smears with a minimum of urinary salts will be obtained. If very scanty sediments are present, especially if there is no pus, the urine should be diluted with from one to two volumes of alcohol, allowed to settle, and the sediment centrifuged thoroughly. Smears made from such sediments are quite free from urinary salts.

If the smear thus obtained will not adhere to the slide, which is the case when there are no pus-cells and few epithelia, the addition of a little

egg-albumen and glycerin to the sediment on the slide will often help to secure better films. The mixture is thinly spread upon the slide with the platinum loop.

After the film is spread the slide is fixed by being passed *thrice* through the flame of a Bunsen burner. A better way is to cover the film with equal parts of absolute alcohol and ether and allow the fluid to evaporate slowly till the film is dry. The film is then ready to be stained. The methods of staining will be described in dealing with the different germs.

The most important pathogenic germs in the urine are the *Bacillus coli*, the gonococcus, and the tubercle bacillus.

The **Bacillus coli** (colon bacillus) is not a single germ, but is a name for a large group of germs. It varies in length, but usually is a short, thick bacillus with rounded ends (1 to 2.5 μ long and 0.5 μ thick), sometimes occurring in pairs and sometimes so short as to look like a coccus. It is very common in urinary infections, and is derived usually from the intestinal tract, where it is constantly met with. It is feebly motile in the urine and decolorizes by Gram's stain (see below). It can be stained with any of the ordinary anilin dyes. When a bacillus of the type described is found in urine the practitioner must content himself with the statement that a bacillus resembling morphologically the colon bacillus is present, unless he wishes to make further studies with the aid of cultures. As these are not ordinarily used in practice, the methods of cultivating the *Bacillus coli* are here omitted. (Compare text-books on Bacteriology.) As it is very important often to determine the exact germ contained in a urinary infection, the urine, drawn by a sterile catheter into a sterile bottle, should be sent promptly to a competent bacteriologist for such examinations. A pure culture of the germ in question can serve for the preparation of a vaccine for the treatment of the infection by Wright's method.

The *Bacillus coli* is one of the most frequent causes of cystitis. Melchior found it in 37 out of 72 cases of cystitis—29 times in pure culture. He found that the *Bacillus coli* is not pathogenic when injected into normal bladders, but when there is obstruction to the flow of urine, or an injury, or a previous irritation this germ may produce cystitis, pyelitis, pyelonephritis, etc.

Gonococcus.—The gonococcus of Neisser is the specific agent of gonorrhea, and is a diplococcus, consisting of groups of kidney-shaped cocci with flattened surfaces facing each other. They are found in the discharge from the urethra in gonorrheal urethritis, and it is characteristic of them that they occur within the bodies of the pus-cells. It is often very difficult to demonstrate gonococci in urine. For this purpose the specimen must be chosen from the portion of urine which contains most pus and threads, preferably after massage of the prostate and vesicles, and must then be centrifuged, the thickest part of the sediment spread on several slides, and allowed to dry in the air. It is then fixed by heat or, better, with alcohol and ether (see p. 330), and is then stained as hereafter described. A thin part of the specimen where there are pus-cells must be sought, and the slides carefully gone over to find the characteristic diplococcus within the pus-cells. Inasmuch as there are germs in the urine which resemble the gonococcus very closely, the true germ of gonorrhea may be distinguished by *Gram's method* as follows:

This method is of no value unless correctly applied, and, unfortunately, its proper application is not always taught in text-books. Its principle is as follows: All germs stain readily with a solution of gentian violet. Some of them

form a purple compound of gentian violet and iodin when an iodin solution (Gram's solution) is added to the first stain. This compound is insoluble in alcohol, and the germs forming this compound are *Gram-positive—i. e.*, they stain purple with this method. On the other hand, other germs do not form the iodin-gentian-violet compound. When alcohol is added to the film after the iodin is poured off, the former reagent does not alter the Gram-positive bacteria, but removes all the violet color from the others. The latter are known as *Gram-negative* germs. When a fainter contrast stain is added which does not interfere with the purple color of the Gram-positive germs, the bacteria which had been decolorized by the addition of alcohol take up the contrast stain and are readily distinguished from the purple-colored Gram-positives.

The gonococcus is Gram-negative—*i. e.*, it does not stain purple with Gram's method. The pseudogonococcus is, on the other hand, Gram-positive, and takes the purple color with this method. Other germs in the Gram-negative class are the *Bacillus coli* and *Proteus vulgaris*. On the other hand, the *Streptococcus pyogenes* and the *Staphylococcus aureus* are Gram-positive.

In using Gram's stain the writer has found that the original Gram's method is far more satisfactory and more trustworthy than any of the modifications more recently introduced. Students are warned to adhere to it strictly and not to use the shorter but less reliable methods, such as that of Nicolle (decolorization with acetone-alcohol). The best results are obtained with absolute alcohol, as was pointed out by Weinrich, and as the writer has been able to prove in a study of many hundreds of specimens. The directions given should be followed minutely. The

following *technic of Gram's stain* is, therefore, recommended to the student and practitioner:

I. The fixed film is stained for one and one-half minutes in carbol-gentian violet (the original method prescribes anilin-gentian violet, but the carbolic solution keeps well, while the anilin solution must be made freshly):

℞ Saturated alcoholic solution gentian-violet.. . 1 part
Carbolic acid in water (5 per cent.) . . . . . 9 parts

II. *Without washing*, but after simply pouring off the violet, the slide is covered with Gram's iodin solution:

℞ Iodin. . . . . 1 part
Potassium iodid. . . . . 2 parts
Water. . . . . 300 "

This is poured off after a few seconds, and some fresh Gram's solution is poured upon the film. After repeating this application of the iodin solution two or three times the solution is allowed to remain on the slide for two minutes. It is then poured off.

III. Without washing in water, the film is washed with *absolute alcohol*, gently rocking the slide to and fro, and adding fresh alcohol. Not more than thirty seconds must be used for this decolorization. If the alcohol is used longer, there is danger of decolorizing the Gram-positive germs. The alcohol is drained off and the slide quickly washed for a few seconds in distilled water.

IV. The slide may then be dried by waving through the air a few times and the counter-stain should be applied. Bismark brown makes the best contrast stain. It is prepared as follows:

Bismark brown. 3.0
Alcohol (90 per cent.). 30.0
Mix and add
Hot distilled water. 70.0

Mix and cool. Stain, when cold, for thirty seconds. Great care should be taken not to overstain with the brown color, as the purple bacteria may take on a brownish hue if too much brown is used. The gonococci appear brown, likewise the pus-cells and the epithelia. The pseudo-gonococci and other Gram-positive germs appear purple.

Instead of the Bismark brown a weak aqueous solution of fuchsin or of eosin may be used, staining the gonococci red, while the Gram-positive germs stain purple. With the red stains there is rather more danger of overstaining the contrast color than with the brown, but in either case great care should be taken to guard against this error.

V. The film is now washed for a few seconds in water and is dried and mounted in balsam. An oil-immersion lens $\frac{1}{12}$-inch focus is necessary for the accurate study of gonococci.

There is a diplococcus closely resembling the gonococcus, and decolorizing with Gram's method, which can only be

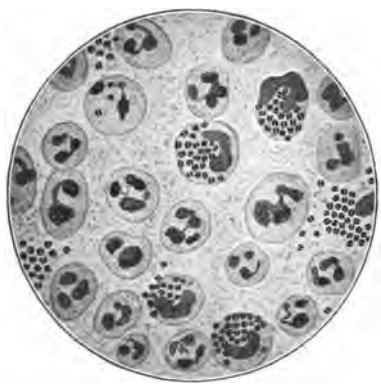


Fig. 83.—Pus from gonorrhea, showing gonococci (Jakob).

distinguished by cultural methods. In men this diplococcus occurs in but 4 to 5 per cent. of chronic urethral infections. Hence in 96 per cent. of cases Gram's stain is accurate in revealing the gonococcus in the male.

When diplococci of the characteristic kidney shape occur within the bodies of pus-cells and do not stain purple with this method, one may be quite satisfied, if the patient is of the male sex, that the gonococcus of Neisser is present. In women, however, no method short of a culture gives

positive assurance that we are dealing with the true gonococcus, as the female genitals often harbor diplococci negative to Gram and resembling gonococci in other respects, which prove to be non-gonorrheal germs on culture.

It is in chronic gonorrhea, with scarcely any discharge excepting a morning drop and a few shreds, that gonococci are looked for in the urine. Wherever there is a sufficient discharge the germs should be looked for in the pus. The absence of the gonococcus from the urine is of no conclusive negative value, as the germ may be deeply lodged in the follicles of the prostate or the urethra.

The Tubercle Bacillus.—It is still more difficult to discover the tubercle bacillus in the urine. For this purpose it is important that the urine should be fresh and, preferably, it should be obtained by catheter after thoroughly cleansing the external genitals, and examined at once. In this manner we prevent its destruction by decomposition, and also avoid the admixture of the smegma bacillus, which is found on the prepuce and glans, and which resembles the tubercle bacillus very closely.

Technic of Staining.—The sediment obtained by the method decribed on p. 329 is smeared on several clean slides. The smears are fixed with heat in the usual way. The slides should be immersed in carbol-fuchsin solution in a tray or dish and the latter kept over a Bunsen flame till the dye begins to steam. The writer uses an aluminum box provided with a rack for six slides, which is filled with carbol fuchsin and placed on a piece of asbestos over a tripod under which is a burner. The films are well stained in five minutes or even less. They are removed from the tray; the excess of dye is allowed to drip off and the decolorizing solution is at once applied. The best decolorizer is a 3 per cent. solution of pure HCl in 95 per cent. alcohol. This is poured on the slide repeatedly until no more red color comes off, and the film appears grayish. After washing off the acid with water the films are counter-stained with aqueous methylene-blue for about two minutes, thoroughly washed in water, dried, and mounted in balsam. They are examined with a $\frac{1}{12}$ oil-immersion lens.

The acid and alcohol decolorize smegma bacilli, but do not affect the tubercle bacilli, which remain stained by the fuchsin, while the other germs, pus-cells, etc., are stained blue. The tubercle bacillus is recognized by its characteristic form and grouping. It occurs in long, thin rods, slightly curved or bent, and often crossed and arranged in parallel formation. Its ends are slightly clubbed and it presents a series of unstained vacuoles.

Finally, injections of portions of the sediment of a urine suspected of containing tubercle bacilli into guinea-pigs offer a useful means of diagnosis. The lesions obtained in these animals, however, take some time to develop, and a negative result, even in these animal experiments, is not always conclusive.

Technic of Animal Inoculation.—The sediment is thoroughly centrifuged in a sterile tube and the urine is decanted. Sterile normal salt solution is substituted for the urine and the sediment is again centrifuged. This is repeated several times until a suspension of the sediment in salt solution is obtained fairly clear of urinary salts. The suspension should measure 1 or 2 cc. in bulk, and should be drawn into a sterile hypodermic syringe. The guinea-pig is held belly-side up by an assistant. The groin is rendered aseptic and shaved as for a surgical operation. The glands in the groin are felt as very minute prominences, or if they are not felt, their site is easily guessed at. The suspension is injected slowly into the subcutaneous tissue of the groin of the animal as nearly as possible into the region of the glands. The region is then massaged and the glands, if felt, are squeezed for a minute or two. The guinea-pig should be kept under observation (with good air, food, and shelter) for at least six weeks. The animal is then killed by means of chloroform and an autopsy is performed. The presence of tuberculosis in the lungs, peritoneum, glands, and other organs should be looked for.

During the period of observation the animal's inguinal glands may be examined from time to time. If they swell, the autopsy should be performed sooner, within twenty-one days. Bloch has recently excised the smaller inguinal glands after from nine to eleven days and found tuberculosis in microscopic sections of the lymph-nodes of guinea-pigs.

PLATE 12

TUBERCLE BACILLI IN URINARY SEDIMENT; × 800. (*Ogden.*)

The absence of tubercle bacilli in an ordinary microscopic examination of the sediment is by no means decisive in diagnosis, and tuberculous lesions may exist in the kidneys or elsewhere in the genito-urinary tract without any discharge of tubercle bacilli into the urine. (See Tuberculosis of the Kidneys, etc., Part IV, p. 358.)

PARASITES

It is very rare to find parasites in the urine, and a detailed description of these must be sought in works on pathology. The following parasites have been found in urine on rare occasions:

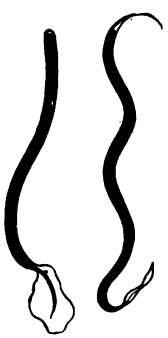


Fig. 84.—Embryos of Filaria sanguinis: Length, 0.0075 to 0.21 mm.; thickness, 0.004 to 0.36 mm. (after Scheube).

Filaria sanguinis hominis, the parasite of chyluria, discovered by Lewis, of Calcutta, and found in the blood and urine of persons passing a milky urine.

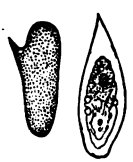


Fig. 85.—Eggs of Distomum hæmatobium (Bilharzia hæmatobia): Length, 0.12 mm.; breadth, 0.05 mm. (after Bilharz).

Distoma hæmatobium, a form found in eastern South Africa, whose eggs may infect the urinary passages, the veins, etc. They produce a sharp pain when they are passed along the urethra, and the urine contains blood, pus, fat, and the eggs of distoma.

Echinococcus.—The hooklets of *Tænia echinococcus* are found in urine in cases of parasitic cysts in the kidney, or in cases of rupture of such cysts into the urinary tract from some neighboring organ.

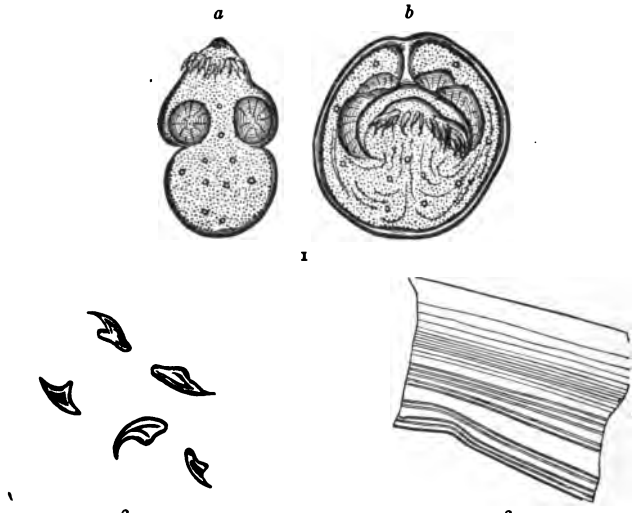


Fig. 86.—Echinococcus elements: 1, Free scolices; *a*, rostellum projected; *b*, rostellum withdrawn; 2, hooklets; 3, membrane (× cross-section) (after Heller).

Ascarides.—The presence of these worms in the urine may be due to the accidental washing away of one of these parasites from the region of the anus, or to an abnormal communication between the intestine and the urinary tract.

QUESTIONS ON CHAPTER XX

What does the term organized sediments include? What is the relative importance of organized and unorganized sediments?

Blood.—What influences the appearances of red cells in urine?

What are "normal" blood-cells? Describe them.

What is the first change blood undergoes when it enters the urine?

What are "abnormal blood-cells"?

How is normal blood distinguished from abnormal on gross inspection?

Describe hemin crystals.

How may blood be removed from a sediment?

What is the clinical significance of blood in the urine?

What are the characteristics of blood from the kidney? What are the causes of blood from the kidney?

What are the characteristics of bleeding from the bladder?

Pus.—Describe a typic pus-cell. What occurs on the addition of acetic acid? Iodin?

What occurs in the pus-cells in ammoniacal urine?

What do fat-globulins in the pus-cells show?

What is the significance of pus in the urine?

In what conditions of the kidney are pus-cells found in the urine? Pus from the bladder? The prostate? The urethra?

What precaution should be taken in women in regard to pus in the urine?

Epithelia.—What epithelia occur in normal urines?

What difficulties present themselves in distinguishing epithelia from one part of the tract from those derived from another part?

What are the three great classes of epithelia? Which of these is sometimes ciliated?

What is the appearance of epidermal scales?

Describe epithelia from the *vagina;* from the *uterus;* from the *bladder.*

What does the presence of bladder epithelia indicate?

How can the intensity of a cystitis be judged by the character of these cells? What is meant by an endogenous pus-corpuscle?

Describe epithelia lining the *pelvis.* What does their presence indicate?

Describe the two types of cells from the *ureter.* When are they present in sediments?

Describe a typic *renal epithelial cell.* What does their presence indicate? What two forms are distinguished and where do they come from?

How are renal epithelia distinguished from other cells of similar appearance?

What do epithelia from the straight collecting tubules indicate?

What is the clinical significance of renal epithelia?

What is the appearance of *urethral epithelia?* Of those from the deeper layers? From the prostatic portion? From the *prostate?* The ejaculatory duct? The vesicles?

Casts.—Define casts. What are the theories of their origin?

What is the relation of casts to albumin?

What is the clinical significance of casts and when have they been seen without renal disease?

Name the principal classes of casts.

Describe a *pure hyaline cast.* What may be concluded from the size of these casts? What is their mode of origin? What do they indicate?

Describe *fibrinous casts.* What do they indicate and what is their prognostic value?

Describe a *waxy cast.* In what conditions are they found? What is their prognostic meaning?

Define a *granular cast.* Where do the granules come from? What do they indicate?

Define an *epithelial cast.*

What term is used when only a few epithelia adhere to a cast?

What does the presence of epithelial casts show?

What are epithelial cylinders with a lumen?

Define a *blood-cast.* What does their presence indicate? When are they found in the urine?

Define *fatty casts.*

What variety of casts may be mistaken for fatty casts? When are these casts found?

Define *pus-casts.* What do they indicate?

What varieties are found in pyelitis?

Define *crystalline casts.*

Define *bacterial casts.* What do they indicate? How are they distinguished from brown granular casts?

Describe *false casts.* What are they made of? Why are they interesting? Where are they found?

Describe *mucous threads.* How do they differ from casts?

What does an excess of mucus show?

How are mucin threads demonstrated?

Descibe *connective-tissue shreds.* What do they indicate and in what diseases are they found in urinary sediments?

What are *prostatic plugs?* Describe them. What does their presence indicate?

Describe *amyloid bodies.* What is their significance?

Describe *spermatozoa* and their appearance in the urine. What does

their presence indicate? How are they shown in medicolegal investigations? Describe Florence's reaction.

What elements occur in the urine after the vesicles have been massaged? How may these be examined microscopically? What is their clinical significance?

Give the classes of urethral shreds, their clinical significance, and the method of staining them.

Describe *micrococcus ureæ*. Describe *yeast fungi*.

What impedes the growth of yeast in urine?

Describe *sarcina urinæ*.

What *pathogenic bacteria* may occur in urine? What three germs are important diagnostically?

Describe the *gonococcus*. May they be isolated in urine?

What is Gram's method and what is its value?

How do the gonococci stain by this method?

Describe the method of staining for *tubercle bacilli* in the urine. What methods may be used to facilitate the precipitation and isolation of tubercle bacilli in the urine? What method may be used when the urine sediment shows no tubercle bacilli?

Describe the appearance of the bacillus in stained specimens.

What *parasites* are sometimes found in the urine?

PART IV

URINARY DIAGNOSIS

CHAPTER XXI

CHARACTERS OF THE URINE IN DISEASES OF THE KIDNEY AND RENAL PELVIS

In the foregoing pages we have discussed the methods of examination and the clinical significance of the various normal and abnormal constituents of the urine. In applying this knowledge to diagnosis the student's chief concern will be the recognition and differentiation of the various diseases of the kidney. In doing so he will meet the difficulty of deciding as to the *particular lesion* which is present in the kidney, and as to *what stage* the process has reached. Unfortunately, each renal disease, though clearly defined pathologically, *does not present a separate and distinct type of urine.* Not only this, but two or more types of lesions may be present in the same kidney at the same time, and may obscure each other to a certain extent, so that only repeated examinations, the careful elimination of all external factors influencing the constitution of the urine, and the scrupulous weighing of each element in the findings, will lead to a correct diagnosis. In the following summary, which is necessarily brief, are given the characteristics of

the urine in the various diseases of the kidney, and with restrictions and limitations just mentioned borne in mind, these data may be used in the diagnosis of renal disease.

ACUTE CONGESTION OF THE KIDNEY

Acute or *active* congestion of the kidney is always a secondary condition, due to disturbances of circulation or to the action of toxic or irritant substances. The most frequent cause is exposure to cold and wet, which chills the surface of the body and induces a congestion of the internal organs. In some diseases, such as delirium tremens and acute mania, sudden changes in the circulation may also cause renal congestion. Local irritation through the presence of gravel or calculi (oxalic, uric acid, phosphatic, etc.), and general irritation, induced by drugs or by the poisons of infectious diseases, may also cause acute congestion in the kidney. Of the drugs, we may mention arsenic, lead, mercury, cantharides, turpentine, cubebs, carbolic acid, salicylic acid, and the essential oils. Of the toxins concerned in renal congestion, we may mention those of pneumonia, typhoid, erysipelas, measles, scarlet fever, diphtheria, acute rheumatism, acute tuberculosis, cerebrospinal meningitis, malaria, intestinal intoxication, and enterocolitis in children. In chronic disases, such as tuberculosis and rheumatism, there may be transient congestions due to the temporary accumulation of toxins. In local suppuration in any part of the body there may also be absorption of toxin and renal congestion. Large amounts of bile irritate the kidney in their transit and considerable amounts of sugar often cause congestion. This condition is also observed in strictures, enlarged prostate, and other obstructive diseases of the uri-

nary tract; after operations on the urethra, bladder, etc. In itself acute congestion is a transient condition, but is chiefly interesting because it often precedes acute nephritis.

The **urine** varies according to the degree and duration of the congestion. In mild cases it may be normal, except a few red cells, a few hyaline casts, a few renal epithelia, and a small amount of albumin. The quantity is usually increased at first; the acidity may be normal; the specific gravity may be somewhat lowered, and the total solids somewhat reduced.

In severe cases the urine changes completely. It is usually high colored, reddish or smoky, and contains visible amounts of blood. The quantity is small—sometimes less than 300 cc. The acidity is increased; the specific gravity is usually higher than normal. The total solids are increased. Albumin is always present—sometimes in considerable amounts for a day or two. A few hyaline and finely granular casts, blood-casts, red cells, renal epithelia, and crystals (these causing irritation) are found in the sediment. In acute congestion that has lasted some time we find fatty renal epithelia; occasionally small fatty casts and fat-globules; some pus-cells, pelvic epithelia, etc., showing the extent and severity of the process. Acute congestion is differentiated from nephritis chiefly by the transitory nature of the signs in the urine. Albumin is usually present in small amounts, and the casts are few in number, appear suddenly, and diminish rapidly. The urine just before death in chronic interstitial nephritis resembles that of acute congestion, except that its specific gravity is low and the solids reduced.

CHRONIC CONGESTION

Chronic or passive congestion is always secondary to a disturbance of circulation due to disease of the heart, arteries, or the liver, to tumors of the abdomen, or to pregnancy.

The **urine** is diminished in quantity in uncomplicated cases. Its color is usually high, brownish red, but if diuretics have been given, or if the patient is in a bad state of nutrition, it may be pale. It is generally somewhat cloudy, owing to increase of urates and mucus, and, as a rule, strongly acid in reaction. The specific gravity is typically high, but may be normal or low in pale urines. The absolute amount of solids is diminished, especially if there is dropsy. The relative amount of solids is increased, except in extreme cases of dropsy. There is usually a slight trace or a trace of albumin; occasionally albumin is absent and the degree of congestion does not correspond with albuminuria. The sediment contains a few small hyaline casts, with occasional adherent red and white blood-cells, and a few free red cells, although blood is often absent. In pregnancy the urine is lighter, of a lower specific gravity, and occasionally contains much albumin, without any complication in the shape of nephritis. In such cases we must look out for convulsions. The urine of chronic congestion is diagnosed from that of chronic interstitial nephritis by the low specific gravity, the small amount of urea, and the predominance of urine passed at night in interstitial nephritis. The two urines are difficult to distinguish from each other in interstitial nephritis near death, when the quantity is practically normal. This is particularly true when blood is absent in chronic congestion, as is the case in patients with compensation which is beginning to fail.

ACUTE DIFFUSE NEPHRITIS

Acute nephritis, acute diffuse nephritis, and acute Bright's disease are the names applied to an acute degenerative or inflammatory process in the kidneys. The causes of this condition include those mentioned as producing acute congestion, and acute nephritis may be the sequel of an acute hyperemia. The most frequent cause is some acute infectious fever, such as scarlatina, diphtheria, pneumonia, typhoid fever, and influenza. Sudden and violent exposure to cold sometimes operates as a cause, and occasionally the acute inflammation follows extensive burns, erysipelas, and other local affections of the skin. Acute nephritis may also occur in women during pregnancy.

According to Councilman, acute diffuse nephritis includes a number of pathologic processes, any one of which may predominate, or be exclusively present, or be combined with other lesions in the list: (1) Acute degeneration of the kidney; (2) acute glomerular inflammation; (3) acute hemorrhagic inflammation; (4) acute interstitial inflammation.

Characters of the Urine.—The *quantity* is diminished at times to an extreme degree, and at the height of the disease it may be reduced to a few ounces. If the disease loses its acute character, the quantity increases, but during the acme of the disease there may even be suppression, which, if it lasts long, may be considered fatal.

The *specific gravity* varies with the quantity and increases to 1025, 1030, or even higher during the acute stage of the disease, while during convalescence it becomes lower as the volume increases.

The *color* also varies with the quantity voided and with the amount of blood, but the typic urine of this disease

is dark brown, approaching chocolate in color, smoky, opaque, and dull in lustre. During convalescence the urine gradually clears up and becomes lighter. The *reaction* is always highly acid, except when large amounts of alkaline salts are given in the treatment. Of the normal constituents, the *urea* and the *chlorids* are diminished in quantity, and the *total solids* (actual) are correspondingly reduced. The relative amount of solids, however, varies with the volume excreted, and in the early stage, when the urine is very scanty and concentrated, this amount may be normal or high. During convalescence the amount of urea and chlorids increases, but during the acme of the disease the urea is often reduced to 100 grains (6.0 gm.), or even less, for twenty-four hours (Purdy).

There is always a large amount of *albumin*, usually from 0.5 to 1 per cent. by Esbach's method. It is in these cases that the urine coagulates on heating, so that it appears almost solid, whence the erroneous expression of "100 per cent. of albumin" has arisen. In rare cases albumin is absent throughout the disease. In a few cases it is present intermittently, and in some cases it appears suddenly toward the acme of the disease. The amount of albumin decreases toward convalescence.

The amount of *blood* in the urine in acute diffuse nephritis varies considerably, both in different individuals and in different periods of the disease. Usually it appears early and disappears before the albuminuria vanishes. The amount of blood and the amount of albumin are valuable prognostic indications, and the sudden increase of albuminuria and hematuria shows a relapse of the disease.

The *sediment* is usually copious and brownish or reddish, owing to the presence of blood, urates, and a large amount

of coloring-matter. The predominant element under the microscope is the red blood-cell, which is usually present in considerable numbers, and appears in the abnormal form (see p. 275), unless the hemorrhage is very marked. There are always some pus-corpuscles in moderate numbers; epithelium from the renal tubules is always in evidence, and may be present in large numbers. There may be a few caudate cells from the pelvis or some cells from the calices.

Hyaline and finely granular casts, coarsely granular, epithelial blood, and fibrinous casts are usually present in considerable numbers, together with masses of granular débris from broken-down cells.

If the disease goes on after the acute stage subsides, it enters the so-called *fatty stage*, which is characterized by fatty renal cells and fatty casts, the number of which depends upon the severity of the preceding acute stage. During the convalescent stage the character of the urine becomes gradually normal, the sediments show fewer casts, fewer renal cells, and fewer blood-cells, until there may be only a trace of albumin, a few hyaline and granular casts, and an occasional blood-cell or renal cell. This urine is identical in microscopic features with that of acute congestion. Acute nephritis very often becomes chronic, and during the convalescence there may be relapses or exacerbations in which the urine again shows the features of the acute stage.

Differentiation.—In mild acute nephritis it is not always easy to differentiate from severe acute congestion by examining the urine alone. We must rely chiefly on the persistence of marked albuminuria, of a considerable amount of blood, and of many casts.

The second stage (fatty stage of acute nephritis) may be mistaken for subacute parenchymatous nephritis complicated by an acute process. In the latter the acute complication will gradually subside, leaving the disease in its original form. In the subacute condition the amount of albumin is usually larger, and the total amount of urea is much lower than in the acute cases.

CHRONIC DIFFUSE NEPHRITIS

(Chronic Nephritis with Exudation; Chronic Glomerular Nephritis; Chronic Arteriosclerotic Nephritis; Chronic Parenchymatous and Interstitial Nephritis.)

This disease either follows acute nephritis or develops as a chronic condition from the start. In this condition the lesions are both parenchymatous and interstitial. It is well for the student, therefore, to understand the changes in the urine which are supposed to characterize each of these distinct sets of lesions. As has already been said in the introductory paragraphs of this section (p. 342) the urine does not always show the precise lesion existing in the kidney, and a diagnosis of the exact lesion is not always possible *intra vitam*.

Interstitial lesions give rise to an increased quantity, a pale color with a low specific gravity, and a diminution of the relative and absolute solids, especially of urea. The parenchymatous lesions are represented by a comparatively large amount of albumin and the presence of renal casts and of renal cells in various stages of fatty degeneration. The rule is that in chronic diffuse nephritis the interstitial changes predominate, but this is by no means always so.

The causes of chronic diffuse nephritis are the same as those of the acute form, and the chronic type is often the sequel of acute nephritis; but it is well to remember that

chronic diffuse nephritis is more apt to develop from acute nephritis due to scarlet fever, diphtheria, pneumonia, or other infectious diseases.

Characters of the Urine.—The *quantity* is diminished, as a rule, but not so markedly as in acute nephritis, and the daily amount fluctuates much more markedly than in the acute form. In the late stages, when the interstitial changes gain the ascendency, the volume increases. The *specific gravity* varies with the quantity, but is usually low—at least below 1020, and in the later stages it is often below 1010. The *color* is variable, but generally light, the urine being often cloudy, owing to the presence of a large amount of sediment.

There is always some *albumin*—sometimes a large amount, up to 1 per cent.; but the average is between $\frac{1}{8}$ and $\frac{1}{4}$ per cent. The amount of albumin fluctuates, and seems to be in fairly constant relation to the specific gravity. It generally increases for a time, but in the later stages, tending toward the interstital type, the amount of albumin often becomes reduced. The *total solids* are more or less diminished, although there may be a temporary increase of solids when dropsy subsides under diaphoretics. *Urea* and *chlorids* are below the normal, and *indican* is either normal or increased. The most important features of the urine are found in the *sediment*. The latter is abundant, and contains numerous casts, of practically all types. The characteristic casts are, however, the so-called fatty casts, which indicate an advanced chronic parenchymatous lesion. In the earlier stages these fatty casts are few, and the hyaline, faintly granular, and epithelial casts predominate. In the more advanced cases the fatty casts become more numerous, and they are accompanied by casts with

dark and prominent granules and by broad casts from the large straight tubules. In addition to casts, the sediment contains many renal cells, some of which are fatty, but usually no blood is seen unless there is an acute exacerbation. A few pus-cells are usually seen. Connective-tissue shreds are always present, and a fair number of large shreds may be seen in the sediment. Pelvic epithelia, ureteral and bladder epithelia may also be present, but are not characteristic. In advanced cases waxy casts may be found, sometimes in large numbers, and as the interstitial lesions increase the sediment becomes more and more scanty, the casts and renal epithelia fewer, and the fatty elements less numerous.

CHRONIC PARENCHYMATOUS NEPHRITIS

(Subacute or Chronic Glomerular Nephritis; Fatty Degeneration of the Kidneys; Chronic Diffuse Nephritis of the Parenchymatous Type; Chronic Degeneration of the Kidneys.)

This is a chronic disease of the parenchyma of the kidney, characterized by marked changes in the glomeruli and the epithelial lining of the renal tubules. The vascular tufts of the glomeruli are swollen, the number of cell nuclei in them is increased, and the glomerular blood-vessels are degenerated and become obliterated. The capsule of the glomerulus shows proliferation of epithelium and new connective-tissue growth. The tubular epithelium is extensively diseased, necrosed, and becomes detached. There are edema and proliferation of connective tissue to a minor extent in the intertubular tissue.

This condition often accompanies chronic diseases—*e. g.*, syphilis, tuberculosis, malaria, etc.—but it may be a sequel of acute nephritis—*e. g.*, after scarlet fever. In such cases it is at first subacute and later becomes chronic.

Chronic parenchymatous nephritis may occur in old persons, and in some cases without discoverable cause. It is one of the possible sequels of heart disease and of other disturbances of circulation (emphysema, pleuritic effusions, etc.) such as cause chronic congestion.

Characters of the Urine.—The urine varies at different stages of the disease. During the *active stage*, or during acute exacerbations, when there is marked dropsy, the *amount* of urine is scanty, the *color* high or bloody, the *reaction* markedly acid, the *specific gravity* high—up to 1035. The *solids*, especially urea and chlorids, are low, on account of dropsy, but relatively there is an increase of urea unless there are interstitial changes setting in strongly. A very large amount of *albumin* is present—up to 3, 4, or even 5 per cent.—but the average is 0.75 per cent. The reddish or dark *sediment* is abundant, contains urates, hyaline, granular, and fatty casts, and fatty epithelia from the kidney. In advanced cases one finds waxy casts. Blood-cells and leukocytes are also usually present.

During the *inactive, chronic stage* the dropsy and edema are diminished or disappear, and the *amount* of urine increases, being about normal. The *specific gravity* is slightly lowered, the *solids* are diminished, though the chlorids may be higher than in the active stage (absorption of dropsy). Less *albumin* is present, but there is still a considerable amount. An abundant *sediment* is found to contain the same elements, but fewer casts and fewer renal epithelia and blood-cells are found.

During the final stage the *kidneys become atrophic*, and the urine, while about normal in *amount*, is very low in *specific gravity*, contains little albumin, and a scanty sediment consisting largely of fatty elements and waxy casts.

Differentiation.—*Subacute cases* (subacute glomerular nephritis) are noteworthy for the great frequency with which acute exacerbations occur in the inactive stage. In these exacerbations the urine resembles that of acute diffuse nephritis, with the fatty elements in prominence and with a marked proportion of blood-cells and blood-casts. Such cases cannot be distinguished from the second (fatty) stage of acute nephritis except by the clinical history. The symptoms and urinary changes of an acute diffuse nephritis are transient, those of a subacute or chronic parenchymatous nephritis, more or less permanent.

From *chronic diffuse nephritis* subacute and chronic glomerular nephritis may be distinguished not only by the clinical history, but by watching the quantity of urine voided. If this be permanently increased, the condition is chronic diffuse nephritis. The two exceptions to this rule are: (1) Cases of chronic diffuse nephritis during acute exacerbations, when the amount is often reduced. (2) Cases of chronic diffuse nephritis near death, in which the amount is normal or low. In both these cases there is no way of distinguishing the two conditions under discussion.

CHRONIC INTERSTITIAL NEPHRITIS

(Chronic Bright's Disease; Renal Cirrhosis; Sclerotic Kidney; Chronic Nephritis without Exudation; Chronic Nephritis; Chronic Diffuse Nephritis of the Interstitial Type; Chronic Catarrhal Nephritis.)

This disease may be interstitial from the beginning, or it may be an outgrowth of chronic diffuse nephritis.[1] The

[1] Delafield and Prudden ("Handbook of Pathological Anatomy and Histology," New York, 1896) do not use the classification of chronic renal inflammation which is currently employed—*i. e.*, the distinction between chronic parenchymatous and chronic interstitial nephritis and

causes of this condition are probably toxic in their ultimate analysis. Chronic poisoning with lead, arsenic, and alcohol are prominent causes. Persons with gout are subject to chronic interstitial nephritis, although just how gout produces this disease is not exactly known. Syphilis and chronic malaria are known to be accompanied often by chronic interstitial nephritis. Arterial disease (arteriosclerosis) produces changes in the arteries of the whole body which are identical with those found in the arteries of the kidney, and there is a distinct causal relation between arteriosclerosis and interstitial nephritis. In connection with urinalysis, the chief thing to be remembered about the lesions of chronic interstitial nephritis is that they are often insidious and take years to develop, and that in the early stages a diagnosis is often impossible unless the urine be carefully watched from day to day and repeatedly examined. The lesions of interstitial nephritis consist chiefly in the growth and increase of connective tissue in the stroma of the kidney, and secondarily in changes in the glomeruli and tubules. The study of the urine must be pursued bearing these facts in mind.

the designation of a combined parenchymatous and interstitial inflammation as "diffuse." These authors believe that both parenchymatous and interstitial changes are present in all chronic nephritides almost from the first, and divide chronic nephritis into two classes—chronic nephritis without exudation (corresponding to the interstitial type of other authors) and chronic nephritis with exudation (corresponding to the parenchymatous and diffuse types of other observers). In this book I have followed the classification usually employed, as it is the most convenient for urine analysis and is still adhered to by most physicians. Those who wish to use Delafield's classification will find that the urine which I describe as typic of chronic diffuse nephritis may be fairly said to be that found in "chronic nephritis with exudation," as described by Delafield. The urine herein described for interstitial nephritis practically corresponds to that found in "chronic nephritis without exudation."

Characters of the Urine.—The typic urine of chronic interstitial nephritis is increased in quantity, usually perfectly transparent, and paler than normal in color, with a markedly acid reaction and a rather low specific gravity. Albumin is present in small quantities, or it may be absent. Very few casts are seen in the sediment, chiefly of the small hyaline variety. The chlorids are not markedly changed in amount, the phosphates are reduced, and the urea is more or less diminished.

The *quantity* is usually increased until the last stage, when heart-failure sets in, and the urine is secreted in smaller amounts. A diminution of the quantity is, therefore, a bad sign in chronic interstitial nephritis. The *specific gravity* is lowered in proportion to the extent of the interstitial changes, but it is never so low as in chronic diffuse nephritis or in amyloid kidney. Usually it ranges between 1010 and 1015. In the last stage, after heart-failure, the specific gravity rises somewhat. There is some difference of opinion as to the frequency of *albuminuria* in this condition. According to Purdy, the reason why albumin is so often found absent in this disease is that the albuminuria is intermittent. It is probable that very slight traces are always present, even in the early stage. The amount of albumin is always small, unless the interstitial nephritis becomes complicated by transient acute conditions. In the last stages—in heart-failure—the albumin increases, but it never reaches the extreme limit which is seen in chronic diffuse and chronic parenchymatous nephritis.

The amount of *urea* diminishes from the first, and this reduction is proportionate to the extent of the interstitial lesions. The *phosphates* are also very constantly reduced,

but the *chlorids* do not suffer reduction to any extent. The *total solids* are greatly diminished, both absolutely and relatively.

The *sediment* contains few or no red *blood-cells*, but a moderate number of pus-cells, some containing fat-globules and granules; *epithelia* from the renal tubules, some of which are fatty; and, in severe cases, columnar epithelia from the straight collecting tubules are present in small numbers (Heitzmann). *Crystals of hematoidin* in the form of rust-brown needles and plates, either free or within the pus-cells or epithelia, may be found, indicating the chronic nature of the disease and denoting a previously existing hemorrhage. *Connective-tissue shreds* of various sizes are often noted. *Casts* from the renal tubules in this disease are rarely found in large numbers, and often they are difficult to detect. They are of the hyaline or of the finely granular type, and come usually from the narrow tubules. *Crystals* of uric acid and of calcium oxalate may be seen, especially in the early stages. The sediment, as a whole, is quite scanty, and even after centrifuging, very little is obtained.

When acute exacerbations of nephritis occur, the urine presents the altered characters which would be expected in an acute process. The quantity of albumin becomes increased and the sediment resembles that of acute nephritis.

AMYLOID KIDNEY

(Waxy Degeneration; Lardaceous Kidney.)

This is a chronic condition of the kidney which is usually the expression of amyloid degeneration in various organs of the body. It may be met with in syphilis, tuberculosis, chronic bone disease, and chronic wasting diseases. It is

frequently mistaken for chronic diffuse nephritis, although it is very important to distinguish these conditions, as the emaciated and cachectic persons who have amyloid degeneration require treatment entirely different from that applicable in chronic diffuse inflammation of the kidney.

Characters of the Urine.—The amount is increased, the color lighter than normal, but the transparency remains unaltered. The specific gravity is low, and there is a well-marked reaction for albumin in the typic cases. Besides serum-albumin there is usually a considerable amount of globulin. The sediment is usually scanty, and contains but few cells and a moderate number of casts, of the hyaline, granular, or, occasionally, the waxy variety.

It is still a matter of discussion whether *waxy casts* are characteristic of amyloid disease and consist of amyloid material. Amyloid material in tissues is distinguished by its reactions to certain stains, but it is very difficult to stain casts with these dyes. The sediment is washed repeatedly by decantation with diluted glycerin and a little methyl-violet solution is added. Both waxy and hyaline casts will assume a light reddish tint (see Waxy Casts, p. 303).

Amyloid degeneration may be complicated by parenchymatous degeneration as the result of interference with the renal nutrition. When this takes place, the urine will resemble that of diffuse nephritis so closely that it cannot be differentiated. The above description applies to typic urine of amyloid. There are certain variations, according to the stage of the process and the severity of the lesions. The *amount* of urine continues increased, as a rule, but there are often periods of temporary reduction in volume, accompanied by attacks of diarrhea. When the *specific*

gravity is comparatively high (1016 to 1118), the prognosis is more favorable, but in the average case it is low, and may even reach 1004 in the late stages. In the early stage of the disease the amount of *albumin* is usually small, then it increases, and toward the end it may again decrease, with a marked polyuria. The *solids*, especially the urea, decrease in proportion to the lesions and to the low state of nutrition. In the late stages the *casts* are very few, hyaline, and usually of comparatively large size.

Differentiation.—It is almost impossible to differentiate amyloid kidney from chronic interstitial nephritis, unless we admit that waxy casts are characteristic of the former, and this has not yet been settled. An unusually large proportion of globulin may lead to the suspicion of amyloid kidney.

TUBERCULOSIS OF THE KIDNEY

This may be primary, but more frequently it is an accompaniment of tuberculosis in other organs. The kidney is often the first organ of the urinary tract to be affected (blood infection). In other cases the infection apparently results from the extension of tuberculosis from the lower part of the urinary tract.

In order to understand the various features that are found in the urine of tuberculous kidneys it must be remembered that, in addition to the formation of tubercles, there is usually a certain amount of chronic interstitial nephritis, and that in the later stages there is also a breaking down or caseation of the tuberculous nodules in different portions of the kidneys. Besides, there are often secondary infections with pus-germs, producing suppurative pyelonephritis. All these factors play a rôle in the appear-

ances of the urine. The latter also shows marked variations, according to the position of the lesions and the extent of the process. If the lesions are mainly cortical, and so long as they are productive in character, as is the case in the early stages, there will be but slight changes in the urine. When the lesions affect the central portions of the kidney, and when caseation and ulceration occur, when small abscesses form, and when the pelvis is involved, the features of the urine are very characteristic.

Characters of the Urine.—In the *early stages* the urine becomes increased in quantity, and the desire to pass urine is more frequent than normally. The color of the urine is pale; it is cloudy, with a slightly lowered specific gravity and an acid reaction, and contains traces of albumin, a few pus-cells, and a few renal epithelia. Tubercle bacilli are not usually found in the urine in this stage.

In the *ulcerative stage* the urine is pale, cloudy, with a lower specific gravity, and ordinarily an alkaline reaction. On standing, it slowly deposits a thick sediment of pus, and from time to time there is found an appreciable amount of blood or a very marked hemorrhage. In the advanced cases the urine becomes very offensive, of ammoniacal odor, and contains small cheesy lumps. Microscopically, the chief features are pus, with usually smaller amounts of blood; numerous renal cells in various stages of degeneration and fatty change, occasionally large hyaline and granular casts, which may be covered with blood-cells when there is an acute exacerbation of the process, or an intermittent hematuria. If the pelvis and calices are involved, there appear large clumps of pus, mucus, and débris, which may be recognized as casts of the calices.

The chief diagnostic feature of the urine in these cases is

the presence of the tubercle bacillus in the sediment. The detection of this germ has already been considered on page 335. When the tubercle bacillus is absent, we cannot exclude tuberculosis of the kidney because this germ may not appear in the urine until late in the disease. In cases of doubt we must look for some other focus of tuberculosis, and we may use tuberculin injections for diagnostic purposes or inject portions of the sediment into the peritoneal cavity of a guinea-pig.

STONE IN THE KIDNEY

The effect of stone in the kidney upon the urine is threefold: (1) In cases of stone uncomplicated by inflammatory conditions there is an increase in the chemic elements which constitute the stone—*e. g.*, uric acid, calcium oxalate, etc., both in the urine itself and in the sediment. (2) When inflammation ensues due to the irritation produced by the stone or to secondary infection, either ascending or hematogenous, then there are, in addition, the signs of suppurative nephritis, and, if the pelvis is involved, of suppurative pyelonephritis. (3) When the process is complicated by chronic interstitial changes or by parenchymatous nephritis, the features of these also appear in the urine.

Characters of the Urine.—The urine is usually highly colored, concentrated, of high specific gravity, and of markedly acid reaction. It may be dark and smoky, owing to the presence of altered blood-pigment. Blood is present during and just after the attacks of colic, and appears fresh and of considerable quantity during these paroxysms. In the intervals between the paroxysms the blood may be found intimately mingled with the urine,

profoundly changed, and brownish in color. The solids are usually increased relatively, but normal absolutely. There is always some albumin when there is any blood or when there is an irritation of the kidney, but albumin may be absent in the earlier stages.

In the sediment we find the signs of acute congestion (which see), and very often an abundance of crystals, singly or in masses (small concretions; gravel; sand), of the same substance as the stone. In the earlier stages it is usual to find a few blood-cells and some scattered leukocytes, some renal epithelia, and some epithelial cells from the pelvis and the ureter, as well as some bladder-cells, showing irritation all along the tract, due to the passage of small concretions.

When inflammatory and suppurative changes are established, the urine shows the features of pyelonephritis. There is an abundant sediment of pus, more or less blood, and crystalline elements or concretions, but the latter may not be present. The urine is very turbid, and is either faintly acid or alkaline. Its specific gravity is low; the normal solids are diminished, owing to the presence of pus and of a complicating nephritis. The sediment contains chiefly pus-cells, often in clumps; shreds of connective tissue; renal epithelia, also in clumps, mixed with pus and mucus, and red blood-cells in moderate numbers. Casts may be present, but may be obscured by pus.

Differentiation.—It will be seen that the urine in the earlier stages is rather characteristic, and whenever we find the signs of congestion, hemorrhage, and an unusually abundant sediment of crystals in urine immediately on voiding, the presence of stone in the kidney may be thought of seriously. In the later stages, with suppura-

tion setting in, the differentiation of stone from tuberculosis, from tumor of the kidney, or from pyelonephritis presents serious difficulties. The finding of tubercle bacilli, on the one hand, and of tumor elements, on the other, differentiates from tuberculosis and from tumor. The passage of small concretions of masses of crystals, when seen soon after voiding, are significant of stone also in this stage, but there are undoubtedly cases in which the examiner must content himself in the suppurative stage of stone with a diagnosis of pyelonephritis "possibly due to stone."

It must be remembered that in very many cases stones may exist in the kidney, in the pelvis, or even in the ureter, without giving rise to any clinical symptoms and without showing any changes in the urine. In such cases the *x*-ray alone can help the diagnostician.

TUMORS OF THE KIDNEY

Of these, the malignant growths, hypernephroma, sarcomata, and carcinomata, are interesting in their relations to urine. Sarcoma is met with in young persons. Hypernephroma, a tumor developing from inclusions of suprarenal tissue remaining in the kidney since fetal life, has come to be more and more frequently found on operation and at autopsy. There is no way of differentiating this new growth by means of urinary analysis. Malignant tumors of the kidney are usually slow to develop, but when they have reached a certain stage they grow rapidly and lead to death in a year or two. The disease may be unilateral, especially in primary cases.

The Urine.—In malignant tumors of the kidney more or less blood is always found—occasionally large amounts of fresh blood, when there is a paroxysm of hematuria.

Albumin is present in the urine in large or smaller quantities, according to the amount of blood and the state of the renal parenchyma. Acetone is frequently found in the urine in cancer of the kidney. The amount of urine is usually increased unless the ureter is blocked. The specific gravity and the solids are altered, according to the extent of the renal destruction, the involvement of the opposite organ, and the presence of congestion or inflammation of the kidneys. As in stone of the kidney, the urine is changed, not only owing to the presence of the disease itself, but also owing to the accompaniment of nephritis, and, in the later stages, the breaking down of the tumor, the ulceration and the suppuration which ensue, especially when there is secondary infection and when the malignant disease invades the renal pelvis. The sediment of a case of malignant tumor presents the features, in the early stages, of simple acute congestion of the kidney. Later, when the ulcerative changes take place, the urine shows the features of chronic pyelitis already mentioned under the heading of Stone.

Differentiation.—It is very difficult to make a diagnosis of renal malignant tumor from the urine alone, as the only real characteristic features of such urines is the presence of portions of the tumor with their distinct alveolar structure. These are found very rarely. The presence of single tumor cells, even in considerable quantities, is not of much value, as they may not appear till the pelvis is involved, and as the cells themselves do not present any definite diagnostic features.

Whenever grotesquely shaped or pigmented epithelioid cells are found, especially in groups or nests, the suspicion of malignant tumor is reasonable.

CYSTS AND CYSTIC DEGENERATION OF THE KIDNEY

Cystic degeneration of the kidney is a congenital condition characterized by a conglomeration of cysts of various sizes occupying the renal tissue. The entire kidney may be destroyed. The affection is almost always bilateral.

Solitary cysts of various sizes may occur in the kidney as the result of the blocking of a tubule or of the capsule of Bowman. In chronic interstitial nephritis it is common to see small single cysts of this kind.

The diagnosis of congenital cystic disease is rarely made during life. Greatly enlarged kidneys; a hypertrophy of the left cardiac ventricle, and increased arterial tension are the chief diagnostic guides. Large single cysts may give rise to palpable tumors.

The **urine** is not changed in any way in some cases, when the renal tissue is comparatively unaffected. When much of this tissue is destroyed, the urine shows the signs of chronic interstitial nephritis.

ABSCESS OF THE KIDNEY

This is a circumscribed suppuration found in the kidney as the result of secondary infection in cases of injuries, stone, tuberculosis, etc. When such an abscess ruptures into the pelvis, the urine will of a sudden become markedly purulent.

The **urine** of renal abscess, aside from this sudden pyuria, shows nothing absolutely characteristic. There may be a considerable number of connective-tissue shreds from the breaking down of renal tissue. While the abscess is developing the urine is that of an acute congestion. When the abscess ruptures, there is often an abundant hematuria due to the breaking of blood-vessels.

The diagnosis is made from the history of the case and the appearances following rupture.

EMBOLISM OF THE KIDNEY

Renal embolism occurs when a thrombus formed in some blood-vessel or in the heart is carried into the renal vessels. The condition is rarely diagnosticated during life, and but little help may be obtained from the urine for its diagnosis.

The **urine** suddenly gives the characters of acute congestion—*i. e.*, it is greatly diminished in amount, with a high specific gravity, increased solids, albumins, and the characteristic bloody sediment of acute hyperemia.

ACUTE PYELITIS AND PYELONEPHRITIS

Pyelitis is an inflammation of the pelvis of the kidney. Pyelonephritis is a combined inflammation of the kidney and the renal pelvis. Acute pyelitis often complicates acute nephritis. It may be due to an extension of the disease from the kidney; to infection from below, from the bladder, or to irritation due to stones or concretions. Pyelitis alone, without nephritis, is usually due to the last-named cause.

The **urine** in acute pyelitis resembles that of acute nephritis in all respects, save that it contains more pus-cells and a large number of epithelia from the pelvic lining. When acute nephritis is associated with pyelitis, renal epithelia and casts are prominent in the sediment, and the relative importance of the coincident nephritis may be judged from this. When pus is present in considerable amounts, the term "acute suppurative pyelitis" may be used.

CHRONIC PYELITIS AND PYELONEPHRITIS

The same remarks apply to the relation of chronic pyelitis and chronic nephritis. In chronic (non-suppurative) nephritis there may be a certain amount of chronic pyelitis, shown by a few pelvic cells in the sediment, but it is the suppurative forms of chronic pyelitis and pyelonephritis that are clinically interesting. Chronic suppurative pyelitis may be due to the irritation of stones, to tuberculosis, or to tumors of the kidney or pelvis, or both; to infection through the blood in fevers; to obstruction of the ureter due to stones, stricture, etc.; to ascending infection from below (bladder); to strictures of the urethra; tumors of the bladder; movable kidney, or enlarged prostate.

The **urine** of chronic pyelitis is characterized by a diminished quantity; a foul odor, sometimes both of sulphur and of ammonia; a pale, turbid appearance, due to pus; an acid reaction on voiding, and a low specific gravity (1012 to 1015). The normal solids, especially the urea, are diminished. There is albumin present, according to the amount of blood and pus. The sediment consists of pus, in clumps or free, of blood-cells, and of numerous pelvic epithelia. If the kidney is involved, there are, in addition, renal epithelia, casts, and shreds of connective tissue. Pus-casts may be present, and large clumps of hyaline matter coated with pus from pelvic calices are seen. Crystals of various kinds may be found and may indicate the presence of stone (see Differentiation from Cystitis, p. 370).

HYDRONEPHROSIS

Hydronephrosis is an accumulation of non-purulent urine in the renal pelvis and in a sac produced by the gradual dilatation of the renal cavity, due to an obstruction

of the ureter by a stone, by pressure from without (tumors, etc.), by kinking of the ureter, etc. The kidney gradually is destroyed, and may be dilated into a thin sac forming a single cavity with the pelvis.

In cases of movable kidney the kinking of the ureter may produce hydronephrosis, which disappears when the kidney is replaced (intermittent hydronephrosis).

The **urine** presents at first signs of acute congestion, as the opposite kidney takes up the work of both. The quantity is markedly diminished, the solids somewhat lowered, and there may be some albumin. Hyaline casts, a few blood-cells, and a few renal epithelia may be seen in the sediment.

When hydronephrosis intermits—*i. e.*, is relieved—there is a sudden polyuria, with sometimes an increase in the amount of albumin and blood. The contents of the hydronephrotic sac are practically water with a slight amount of urinary salts, a trace of albumin, and a few red and white blood-cells and epithelia from the kidney and pelvis.

PYONEPHROSIS

Pyonephrosis is an accumulation of purulent urine in the renal pelvis and renal cavity, due to an obstruction of the ureter, together with infection. It may follow a hydronephrosis when infection supervenes, or it may develop from an acute or chronic pyelitis when the ureter becomes obstructed. The causes of ureteral obstruction are the same as in hydronephrosis. The kidney is gradually destroyed and atrophies, forming finally a large abscess cavity.

The **urine** shows the signs of an acute or active congestion, due to the overwork of the opposite kidney when the

obstruction is absolute. In such a case there is no pus, and the diagnosis cannot be made from the urine alone.

When the obstruction is relative, or if it is removed suddenly, there is a considerable flow of pus, and the urine assumes the characters described under Chronic Pyelonephritis. It is turbid, with an abundant greenish-white sediment, consisting of pus, epithelia from the kidney and pelvis, connective-tissue shreds, and blood (slight amounts usually), together with fragments of stone (when stone is present) and larger débris of kidney tissue. There is usually a considerable amount of albumin and a marked proportion of globulin.

URETERITIS

Inflammation of the ureter alone is rare, and is scarcely ever diagnosticated from the urine alone. Catheterization of the ureter may, however, reveal ureteritis, stricture of the ureter, calculi impacted in this canal, etc.

Ureteritis may form part of pyelitis or cystitis by extension, or may result from the irritation due to the passage of a calculus with sharp points. It may follow compression of the ureter by tumors from without, strictures, or kinks of the ureter. Tuberculous ureteritis may occur when the kidney or the bladder is similarly diseased.

The **urine** in a simple ureteritis is that of acute renal congestion, with high color, high specific gravity, and small amount of albumin and blood. The sediment shows blood, pus (few cells), and the characteristic cells of the ureteral lining.

CHAPTER XXII

CHARACTERS OF THE URINE IN DISEASES OF THE LOWER URINARY TRACT AND OF THE GENITAL ORGANS

CYSTITIS

Acute Cystitis.—This is an acute inflammation of the bladder, caused by infection with the gonococcus or with some pus-producing germ, usually by an extension of infection from elsewhere in the tract or by the use of dirty instruments. Acute cystitis also results from exposure to cold and wet; sexual excesses; acute urinary retention; the use of irritant drugs (copaiba, cantharides), or injury to the bladder. It may also occur as a complication of infectious diseases.

The urine, as a rule, is diminished in amount. It is bloody or smoky, strongly acid, with a high specific gravity and relatively high solids. The amount of albumin varies with that of blood and pus. The sediment contains fresh blood, pus, mucus, mucous threads, and many epithelia from the superficial layers of the bladder.

Chronic Cystitis.—This may follow an acute cystitis and be due to the same causes. A common cause of chronic cystitis is an enlarged prostate or some other form of urethral obstruction. In these cases the urine cannot be voided completely, and stagnates in the bladder, inducing cystitis. The same takes place in paralysis of the bladder. In tumors, stone, or tuberculosis of this

organ there is usually a chronic cystitis. Between the vagina and the bladder there may be fistulas communicating which complicate the cystitis.

The urine is not markedly affected in quantity; usually pale and cloudy or bloody, with an ammoniacal or putrid odor, and thickened in consistence (mucus). In the early stages it may be highly acid, but later becomes alkaline. The albumin depends on pus and blood. An abundant thick and heavy sediment is present, chiefly of pus; of cells from all the layers of the bladder epithelium; of mucus, amorphous phosphates, triple phosphates, and ammonium urate. A number of bacteria may be found in the urine in acute, and especially in chronic cystitis. Besides the gonococcus there may be staphylococci, various bacilli, especially of the "colon group," the *proteus vulgaris*, various diplococci, etc. Cystitis may be due to one or more varieties of these germs.

Differentiation of Pyelitis and Cystitis.—The detection of numerous epithelia from the pelvis, on the one hand, and from the bladder, on the other, does not always suffice to make a distinction between pyelitis and cystitis. In fact, very well-trained observers have been misled into regarding a specimen of urine in a case of pyelitis for one due to cystitis alone. In view of the great importance of this differentiation the recent researches of Rosenfeld, extending through a number of years, are of interest. Rosenfeld found that the urine of a pyelitis differs from that of a cystitis in several important respects, which may be briefly summed up as follows:

1. The *reaction* of the urine in pyelitis is almost always acid. An alkaline reaction is never found in uncomplicated pyelitis. An alkaline reaction, therefore, speaks for

cystitis, while an acid reaction does not exclude cystitis. The reaction must be observed in freshly voided urine.

2. In pyelitis the pus-cells are characteristically ragged and distorted in contour. In cystitis they are characteristically round and uniform in shape. The distinction should be drawn only when large numbers of pus-cells show either of these types. The finding of round forms does not exclude pyelitis necessarily, but testifies to the presence of cystitis.

3. The *red blood-cells* from a pyelitis are "abnormal," *i. e.*, washed out, altered in form, while in cystitis, except when due to tumors, they are fresh and "normal." This does not apply to hemorrhages other than microscopic.

4. The *ratio of albumin to the amount of pus* is the most important point. In cystitis there may be great amounts of pus, but the albumin never exceeds from 0.1 to 1.5 per cent. In pyelitis, however, the amount of pus may be very small, yet from 0.1 to 0.15 per cent. of albumin and over will be found, and when the pus is abundant, the albumin reaches higher amounts—up to 0.3 per cent.

TUBERCULOUS CYSTITIS

Tuberculosis of the bladder is usually secondary to that of other organs of the genito-urinary tract. The first stage—of productive lesions (tubercles) in the bladder—does not give marked changes in the urine, while the second, marked by ulceration and by complicating chronic cystitis, gives a rather characteristic urine.

The **urine** is diminished in amount, pale or bloody, and turbid, varies in reaction, with a low specific gravity and diminished normal solids. The albumin varies with the amount of blood and pus present, and may be

abundant during attacks of hematuria. The sediments consist of large amounts of pus, bladder epithelia, and blood-cells. The presence of tubercle bacilli with a large number of bladder epithelia, especially from the

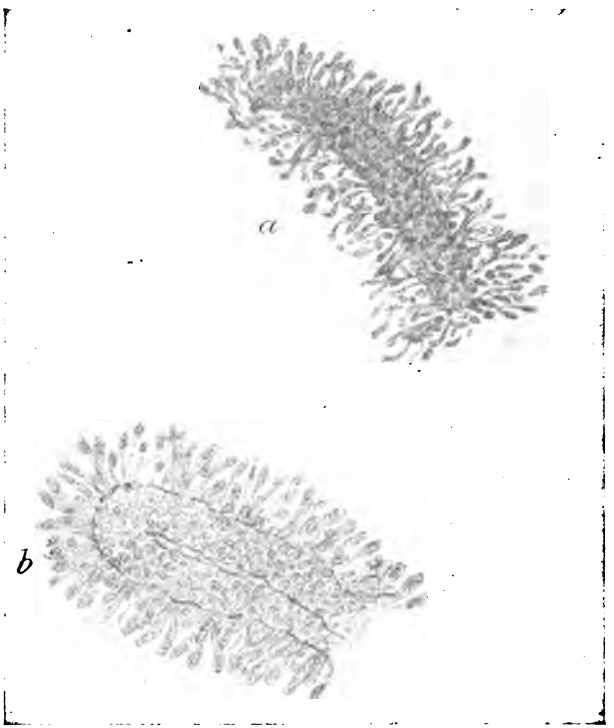


Fig. 87.—Portions of a villous growth of the bladder: *a*, Magnified 190 diameters; *b*, magnified 370 diameters (Ogden).

middle and deep layers are conclusive evidences of tuberculous cystitis. The absence of tubercle bacilli from the urine does not, however, necessarily exclude the presence of this disease.

TUMORS OF THE BLADDER

Of the benign tumors in the bladder the most common are papillomata, but fibromata and fibromyxomata also occur. Papillomata are diagnosticated in the urine from the presence of masses of villous growths of microscopic size in the sediment. In malignant growths of the bladder the urine presents the same features as in chronic cystitis, but there is apt to be more blood and even large blood-clots. Shreds of tissue and portions of the tumor may be found in it. Cells resembling those of the new growths are also sometimes seen in the sediments, but no positive diagnosis can be made from their presence. The cystoscope and exploratory operations are often required for diagnosis, but the urine alone often points to the presence of bladder growths.

STONE IN THE BLADDER

The diagnosis of stone in the bladder is not usually made from the urine alone. Sounds, and the stone-searcher are used as aids, as well as the *x*-rays and the cystoscope.

The **urine** presents the characters of acute or chronic cystitis, according to the length of time the stone has been in the bladder and the shape and size of the stone. Small concretions and an unusually abundant sediment of a crystalline or amorphous deposit, such as uric acid, calcium oxalate, etc., are sometimes clues to the diagnosis.

PROSTATITIS

Acute prostatitis is an acute parenchymatous inflammation of the prostate gland. It is caused by infection aided by congestion (exposure to cold and wet, sexual

excitement, urethral stricture, traumatism, passage of calculi). The chief infectious agent is the gonococcus, which enters the prostatic ducts from the posterior urethra in cases of gonorrheal urethritis. During an attack of gonorrhea acute prostatitis may be excited by a variety of causes, chiefly by sexual intercourse, alcoholic indulgence, instrumentation, etc.

Characters of the Urine.—Acute prostatitis is often accompanied by a congestion of the kidneys, and in such cases the urine gives the signs of renal hyperemia, with a high or bloody color, high specific gravity, scanty amount, and a trace of albumin. The urine may, however, show none of these signs except a diminished amount and a little blood. Microscopic examination reveals many red blood-cells, pus-cells, epithelia from the prostate and its ducts and from the seminal vesicles; spermatozoa, most of which are dead and have broken tails; and plugs from the prostatic ducts. The pus-cells are not greatly altered, as a rule, and the epithelium is fairly fresh.

Chronic prostatitis may follow the acute form or may be chronic from the start, due to gonorrheal infection, or sexual overindulgence, masturbation, or coitus interruptus. It may also result from chronic urethral obstruction—*e. g.*, by strictures, narrow meatus, phimosis, etc.—or from irritation or injury to the prostate by passing stones.

Characters of the Urine.—Unless kidney lesions be present at the same time, the urine is not very markedly changed in chronic prostatitis. It is usually pale, acid, turbid, with slightly lowered specific gravity, and an amount of albumin varying with the amount of pus. In the sediment we find large numbers of pus-cells, chiefly in masses,

mixed with a mucous substance; many epithelia in various states of disintegration and fatty change from seminal vesicles, the prostate, its ducts, and the neck of the bladder. There are rarely many blood-cells, but spermatozoa are in evidence, free and embedded in masses of pus, epithelia, and mucus. These masses often assume the shape of casts and plugs, already described under Acute Prostatitis. Shreds of connective tissue, mucus, and round cells, massed together with adherent spermatozoa and pus-cells, are also often found in this condition. Amyloid bodies (see p. 312) are sometimes found in considerable numbers, but usually only a few are present. They assist in the localization of the trouble in the prostate. The presence of an accompanying cystitis will give its signs already described.

Chronic prostatitis occurred in 60 per cent. of cases of gonorrheal infection in a series of 180 cases observed by the writer. In the sediment from the massage-urine in these cases the gonococcus may frequently be found after centrifuging and staining the deposit (thinly spread on a slide). The method of staining these sediments is the same as that used for smears from sago bodies, etc. (see p. 244). Very frequently other bacteria, *e. g.*, especially the staphylococcus, the streptococcus, and bacilli resembling the colon bacillus, or sometimes the pseudodiphtheria bacillus are found in these massage-urines.

TUBERCULOSIS AND CANCER OF THE PROSTATE

Tuberculous prostatitis is almost always a part of a tuberculous process in some other genito-urinary organ. The urine does not differ essentially from that which characterizes chronic prostatitis, except that its sediment on centrifuging (or after massage, on standing) contains

tubercle bacilli. Several examinations are often needed to find these germs (see Tubercle Bacillus, p. 335).

Cancer of the prostate is rare, especially the primary form. The urine is practically that of chronic prostatitis, with an occasional hemorrhage. The presence of cells with large round nuclei from the tumor cannot serve for a positive diagnosis, but in conjunction with clinical signs may hint at cancer.

SEMINAL VESICULITIS

Inflammation of the seminal vesicles occurs as the result of the same causes as prostatitis. Acute seminal vesiculitis is a complication of gonorrheal urethritis. The chronic variety occurs with or without prostatitis as a sequel to gonorrhea, stricture, etc., and from such causes as congestion, overindulgence in sexual intercourse, masturbation, etc.

The diagnosis of seminal vesiculitis is usually made by palpating the vesicles. The **urine** in the acute form shows pus-cells, blood, epithelia from the vesicles, numerous spermatozoa, shreds of vesicular lining, and plugs of coagulated matter from the vesicles. The signs of renal congestion may also be present. In the chronic form the urine shows, in addition to the signs of chronic prostatitis and chronic urethritis, masses of epithelia from the vesicles in a state of fatty change; spermatozoa more or less broken and distorted; plugs of coagulum and shreds consisting of connective tissue; numerous round cells, masses of mucus, and pus-cells.

Smears prepared with material from massage-urine and properly stained as described, show pus, epithelia, and bacteria from the vesicles (see p. 314).

SPERMATORRHEA

In the functional disturbance known as spermatorrhea semen is found in the urine, especially after defecation. The discharge of thin, mucoid matter which follows or accompanies defecation contains many spermatozoa, some of which are alive. The structures may be stained with anilin dyes or may be tested with Florence's reaction (see p. 314). In addition, spermin crystals, fat-droplets, prostatic and vesicular cells, granular matter, and mucus are found in this discharge.

URETHRITIS

In acute gonorrheal urethritis the urine is cloudy, acid, and deposits a sediment of mucopus, epithelia, and shreds from the urethra. With ensuing posterior urethritis there comes blood, in addition to these elements, in the shape of a moderate number of red cells. The amount of albumin depends upon the pus, and in other respects the urine is unchanged unless the kidney is affected.

In *chronic urethritis* the urine shows a much more scanty deposit of mucus and pus and usually a number of *shreds* from the urethra. The latter have been fully dealt with on p. 319.

The diagnosis of specific urethritis is based upon the finding of the gonococcus (see p. 331). It is always best to seek this germ in the pus pressed out of the urethra or in the "morning drop" of chronic cases. In the urine the gonococcus must be looked for in the centrifuged pus or in shreds picked out with a pipet or a platinum loop. The urine voided after prostatic (or vesicular) massage may contain gonococci. In these cases the centrifuged

sediment or the solid elements (p. 314) should be smeared on slides and stained according to the methods already described.

QUESTIONS ON CHAPTERS XXI AND XXII

1. *Acute Congestion of the Kidneys.*—What are its causes? What is the most frequent cause? Why is it chiefly interesting? What is the character of the urine? How is it distinguished from acute nephritis? From chronic interstitial nephritis just before death?

2. *Chronic Congestion of the Kidneys.*—What are its causes? Describe the urine. How is it distinguished from that of chronic interstitial nephritis?

3. *Acute Diffuse Nephritis.*—What are its causes? What pathologic processes may be present? Describe the characters of the urine. What do the amounts of blood and of urine indicate? What is the fatty stage? What other renal disease does it resemble?

4. *Chronic Parenchymatous Nephritis.*—What are its causes? Its lesions? Describe the characters of the urine in this disease. How is it differentiated from other forms of chronic nephritis?

5. *Chronic Diffuse Nephritis.*—What are its causes and lesions? Describe the urine of this disease. What are the characteristic casts in this form of nephritis? What changes in the urine indicate interstitial lesions, and what changes stand for parenchymatous lesions?

6. *Chronic Interstitial Nephritis.*—What are its causes? Its lesions? Describe the characters of the urine in this condition.

7. *Amyloid Kidney.*—What are its causes? Describe the chief characters of the urine. What is noteworthy of the quantity, the specific gravity, the solids, and the varieties of casts found?

8. *Tuberculosis of the Kidney.*—What elements in the lesions influence the characters of the urine in this disease? What is noted in the urine in early stages? In advanced stages?

9. *Stone in the Kidney.*—What three elements are at work in this disease in determining the characters of the urine? Describe the urine in the early stages; in the late stages, accompanied by infection.

10. *Tumors of the Kidney.*—What malignant growths occur in the kidney? Which is more common in children? What are the characters of the urine? On what should the diagnosis be based? What is the value of "cancer cells" in the sediment?

11. *Cysts of the Kidney.*—In what forms may cysts occur in the kidney?

What symptoms lead to a diagnosis of congenital cystic degeneration? What does the urine show in cysts of the kidney?

12. *Abscess of the Kidney.*—Define renal abscess. What are its causes? What does the urine show before rupture? After rupture?

13. *Embolism of the Kidney.*—Define it. When does it occur? What changes in the urine are found in such cases?

14. *Acute Pyelitis and Pyelonephritis.*—Define each. What are the causes of acute pyelitis? What are the characters of the urine? How is acute pyelitis distinguished from acute pyelonephritis? What is suppurative pyelitis?

15. *Chronic Pyelitis and Pyelonephritis.*—Define each. To what is chronic suppurative pyelitis due? What are the characteristics of the urine?

16. *Cystitis.*—(*a*) *Acute.*—What are the causes and the characters of the urine?

(*b*) *Chronic.*—What are its causes? What are the features of the urine? How is pyelitis differentiated from cystitis? What is peculiar of the ratio of albumin to pus in cystitis? In pyelitis?

17. *Tuberculous Cystitis.*—What are the two stages of this disease and how do they show themselves in the urine? What does the absence of tubercle bacilli show?

18. *Tumors of the Bladder.*—What benign tumors occur in the bladder? Which is the most common? How are the papillomata diagnosticated from the urine? What is the character of the urine in malignant growths of the bladder?

19. *Stone in the Bladder.*—How is it diagnosticated? What, in general, are the characters of the urine?

20. Describe the characters of the urine in *acute prostatitis;* in *chronic prostatitis;* in *tuberculosis of the prostate;* in *cancer of the prostate.*

21. What characteristics are observed in the urine of *seminal vesiculitis?* Of *spermatorrhea?*

22. Describe the sediment in the urine of *urethritis*—(*a*) acute, (*b*) chronic.

CHAPTER XXIII

THE URINE IN ABNORMAL STATES OF METABOLISM

I. DIABETES MELLITUS

THE *amount* of urine excreted is usually increased in diabetes mellitus. If there is over 3 per cent. of sugar, the increase becomes more and more marked as the amount of sugar rises. Very large amounts, even 10 liters daily, have been seen. From 2000 to 3000 cc. are commonly excreted. In moderately severe cases the amount of urine frequently reaches 5 or 6 liters daily, but diminishes when the diet is regulated. The proportion of sugar is ordinarily increased with the increase of quantity, and, what is most important, the specific gravity is not decreased in diabetes with an increased excretion of urine. Von Noorden gives the following table which demonstrates this:

Quantity.	Specific Gravity.	Sugar.
1500– 2,500 cc.	1025–1030	2–3 per cent.
2500– 4,000 "	1030–1036	3–5 " "
4000– 6,000 "	1032–1040	4–7 " "
6000–10,000 "	1036–1046	6–9 " "

The diet has a great deal to do with the quantity of urine as well as of sugar. When carbohydrates are excluded, the amount of urine diminishes, as well as the amount of glucose, and when the sugar disappears under the influence of diet, the quantity becomes almost normal, although the specific gravity remains high.

The *specific gravity* of diabetic urine, as has been hinted, is characteristically high, usually from 1030 to 1040. The *color* of the urine is usually pale yellow, sometimes greenish yellow. Sometimes the urine will ferment in the bladder with a development of gases (pneumaturia). The fermentation may give rise to cystitis.

The *form of sugar* almost invariably present in the urine of diabetes is glucose; sometimes levulose is present, but only in small quantities, not exceeding 1 per cent. When levulose is taken by mouth by diabetics, it is usually excreted as dextrose. Inosite and pentose have been found associated with glucose.

In *testing for sugar*, especially in the mild cases, the urine voided from four to six hours after a breakfast at which starch and sugar had been taken is to be preferred for examination. The largest amount is said to be excreted in the late forenoon, and another considerable rise in the sugar excretion takes place toward the evening, about 6 P. M. The morning urine and the urine passed during the night contains the smallest amount of sugar. In order to determine the amount of sugar excreted the total twenty-four-hour quantity should be collected, thoroughly mixed, accurately measured, and a sample examined. If a distinction is desired between the day and the night urine, one portion should be taken, beginning after breakfast and ending late in the evening (day urine), and a second portion, beginning at night until before breakfast of the following morning.

The severity of diabetes cannot be measured by the *quantity of sugar excreted in* twenty-four hours. This is a common practice, but very often leads to errors in prognosis. The percentage of glucose excreted rarely exceeds

8 per cent., although cases have been reported in which as much as 20 per cent. has been excreted. Von Noorden divides these cases into three groups: The mild cases, the severe cases, and the moderately severe cases. A mild case is one in which the urine becomes free of sugar a few days after the exclusion of carbohydrates from the diet. This does not mean that the patient no longer manufactures sugar in his tissues, but that the process goes on so gradually when no carbohydrates are taken in, that the organism is able to cope with the glucose manufactured, and none is excreted in the urine.

The severe cases are those in which in spite of the removal of carbohydrates from the food for days or even weeks, there is no complete disappearance of glucose from the urine. A case of moderate severity, according to Naunyn, is one in which the sugar cannot be removed from the urine by simply excluding carbohydrates from the diet, but in which the glycosuria can be checked by regulating the amount of proteids ingested. There are many gradations between the types just defined. A diabetes of moderate severity is usually a transition form between the mild and the severe types. The only way to test the severity of a case of diabetes is by a carefully regulated diet, the rules for which will be found in von Noorden's and other text-books.

The amount of sugar is increased by carbohydrates in the diet, but some carbohydrates, as potato and oatmeal, have less influence than others. Lactose, levulose, and compounds derived from these sugars have less influence than dextrose. An increased amount of proteids may also increase the sugar output, but fats do not have any influence on the glycosuria. Sugar excretion is markedly

influenced by mental strain, excitement, worry, and other psychical influences. Glycosuria is decreased by muscular work.

During fevers and acute infectious diseases there is said to be a marked diminution in the sugar excretion; although von Noorden has not found this diminution to occur as was supposed. The sugar output is diminished in certain chronic wasting diseases, as in tuberculosis and nephritis.

Albumin is frequently found in diabetic urines, and may be present apparently without a complicating nephritis. On the other hand, when chronic nephritis develops in the course of diabetes, the glycosuria may disappear (Frerichs, von Noorden, etc.). It appears that the kidneys are unable to excrete sugar when they are affected by chronic nephritis, and in such cases enormous amounts of sugar have been found in the blood. Diabetes is closely allied to gout, and glycosuria may alternate with gouty paroxysms, the sugar disappearing during the attacks.

An increased excretion of *creatinin* is often noted in diabetes as the result of a predominating meat diet. *Uric acid* is usually present in normal or slightly increased quantities.

Acetonuria is an important symptom in diabetes. It has already been discussed on page 165, but a few additional clinical points may be mentioned here.

It should be remembered that the acetone bodies occur in other conditions than diabetes. Thus, acetone occurs in starvation and in other conditions in which the same factors obtain as in starvation, for example, in high fever, in acute gastro-intestinal diseases, in obstruction of the intestine, in puerperal toxemia, etc. Von Noorden found acetone

in the urine of patients with acute pneumonia who were fed exclusively on meat broths and eggs. The acetone bodies come from the fatty acids of the organism and appear in the urine when the body is unable to burn up carbohydrates (von Noorden).

Acetonuria is absent in mild cases of diabetes and the excretion of acetone bodies is more accurately proportionate to the severity of the disease than the excretion of glucose. Von Noorden distinguishes several degrees of acetonuria. In the first degree there is only acetone in the urine. In the second degree diacetic acid is also present, while in the third degree oxybutyric acid is present.

When large amounts of acetone or diacetic acid or, in the severest cases, oxybutyric acid appear, the system of the diabetic is said to be in a condition of "acidosis." The abnormally manufactured acids are said to produce an intoxication which may be counteracted by an increased supply of alkalis. Diabetic acidosis is said to lead to the coma which characterizes the most dangerous phase of this disease. While the presence of the acids mentioned, especally in large amounts, indicates a severe type of diabetes, their occurrence does not necessarily mean impending coma. Acidosis may exist for years without coma. When the acid bodies reach a certain amount, however, coma seems to be impending. According to Herter, when there are 25 gm. of oxybutyric acid daily coma is probably approaching. As it is difficult to estimate the oxybutyric acid, a better way is to measure the amount of ammonia which is in proportion to oxybutyric acid usually. When over 3 gm. of ammonia per day are excreted there is danger of coma (Naunyn). Alkaline treatment is indicated in such cases and the urine should

be watched for the appearance of casts of the granular type in large numbers, which precede the appearance of coma, according to Külz.

II. DIABETES INSIPIDUS

This is a rare condition (variously called hydruria, polydipsia, persistent polyuria, insipid diabetes, etc.), characterized by the excretion of large amounts of urine of a low specific gravity, containing no sugar nor any other abnormal constituent.

The etiology of true (or "idiopathic") diabetes insipidus is obscure. Lancereaux found that heredity, syphilis, gout, and lithemia were concerned in 74 cases which he collected. No cause can be discovered in most cases, and we can only say that diabetes insipidus is a "manifestation of disturbed metabolism," the mechanism of which is not known.

True diabetes insipidus must be distinguished from symptomatic polyuria, due to hysteria or other nervous affections, renal disease, injuries to the skull, etc.

The **urine** in diabetes insipidus varies in quantity, as much as 20 and even 40 liters daily having been recorded. The specific gravity varies from 1001 to 1009. The color is very pale, the reaction faintly acid. No sugar is present nor any albumin or casts (distinction from interstitial nephritis). The total urea excreted may be increased owing to the ravenous appetite shown by some patients. The patients complain of great thirst, dry skin, and frequent necessity to void the bladder. The disease is of long duration and is not directly fatal, but may last a lifetime and materially weaken the patient.

III. THE TOXEMIAS OF PREGNANCY

Nitrogen Partition.—During the past few years a considerable amount of experimental work has been published by a few observers[1] which demonstrates the clinical value of determining in pregnant women suspected of toxemic conditions leading to eclampsia or to toxic pernicious vomiting (*a*) the total nitrogen and (*b*) the separate quantities of nitrogen derived from urea, uric acid, ammonia, creatinin, and the residual or "undetermined nitrogen."

While the subject is as yet comparatively new, enough evidence is at hand now to make it necessary to acquaint students and practitioners with the great importance of an accurate study of nitrogen elimination in the diagnosis and prognosis of toxemias of pregnancy. Students are referred to the original papers for details, but a brief summary of the practical aspects of this subject is as follows:

The normal relations of urea, uric acid, ammonia, etc., to the total N. output in the urine have already been given (p. 131). In pregnancy there is normally in most cases a disturbance of metabolism *lowering* the urea and *increasing* the "undetermined nitrogen" and to a less extent (Ewing and Wolf) or to a marked extent (Williams) the ammonia. After delivery the normal relations are restored.

When a toxemia develops in the course of pregnancy it is usually characterized by one of the following groups of symptoms: (1) *Persistent vomiting;* (2) the clinical condition known as the *pre-eclamptic state,* or threatened eclampsia, in which the symptoms are practically those of impending uremia: dizziness, weakness, headache, transient visual disturbances, vomiting, increased blood-pressure, and marked edema and albuminuria; (3) *eclampsia,* with or without the previous existence of the condition described under (2).

[1] Zweifel, Arch. f. Gynäk., 1904, lxxii, 1; 1906, lxxvi, 536; Williams, Johns Hopkins Hosp. Bull., 1906, xvii, 71; and Ewing and Wolf, Am. Journ. Obst., 1905, li, p. 145, and 1907, lv, No. 3.

The work of the authors mentioned above seems to show that these three groups of conditions arise from similar causes—a general intoxication of the system in which there may or may not be (opinions differ as yet) a predominance of acidosis. The behavior of the nitrogen elimination in these conditions, according to Ewing and Wolf, is as follows:

Condition.	Urea.	Ammonia.	Undetermined Nitrogen.
I. Toxemia characterized chiefly by vomiting.	Low.	Usually high.	High.
II. Pre-eclamptic state.	Low.	Variable.	High.
III. Eclampsia.	Low in proportion to severity.	Variable.	High, but not so accurately proportionate to severity as urea.

The term "undetermined nitrogen" is used to designate that amount of nitrogen which cannot be accounted for in the form of urea, uric acid, ammonia, or creatinin—*i. e.*, the nitrogen *remaining unaccounted for* after subtracting from the total nitrogen the nitrogen determined as existing in the form of the substances mentioned (each separately tested). Detailed studies showed that this "undetermined nitrogen" was due in a large measure to amino-acids, which are decomposition products of proteids that normally are further split up into ammonia, and then into urea. The undetermined nitrogen, therefore, is also called "the amino-acid nitrogen." The failure to split up amino-acids into ammonia and urea is called by some authors (Zweifel, Ewing, and Wolf) "desamidation." It is possible, though not completely proved as yet, that the toxemias of pregnancy are dependent upon this failure to split up amino-acids.

Clinical Value of Nitrogen Partition.—From the above facts one feels now that a physician does not do his full duty to his patient (as E. B. Cragin puts it) unless the nitrogen excretion is determined in all cases of *pernicious vomiting* in pregnancy and in the cases of *threatened eclampsia* in which the symptoms are vague and the other findings in the urine uncertain.

It must be remembered, however, (1) that in normal women (without toxemia) there may be a disturbed state of metabolism during pregnancy, with a lowered urea and an increased "undetermined nitrogen," and to a less marked extent an increased NH_3 nitrogen (Ewing). This may be

due to nitrogen retention in pregnancy. (2) That urea may be distinctly low and undetermined nitrogen high *without any symptoms* in some cases. Such women should be watched for a possible predisposition to eclampsia.

When vomiting is persistent or when the toxic symptoms of impending eclampsia are present, however, with a low urea ratio and a high ratio of "amino-acid" or "undetermined" nitrogen, especially when indicanuria, acetonuria, and albuminuria exist at the same time (the last three are not constant, as will be seen by reference to the appropriate sections), the patient is in a state of toxemia and should be treated accordingly.

The quantitative determinations of nitrogen necessary for this work cannot be done save in a completely equipped laboratory and should be entrusted only to experts in these analyses. The twenty-four-hour quantity of urine should be very carefully collected and the specimens sent as quickly as possible to the laboratory. The bottle in which the urine is collected should contain half an ounce of chloroform, and should be well shaken after each addition.

PART V

FUNCTIONAL RENAL DIAGNOSIS

CHAPTER XXIV

GENERAL CONSIDERATIONS; THE SECRETION OF URINE

THE OBJECTS OF DETERMINING THE RENAL FUNCTION

WITHIN the past ten years there have arisen certain new methods of urinary diagnosis which aim to determine the functional efficiency of the kidneys, or of one of these organs as compared to the other. The terms "sufficiency" and "insufficiency," as applied to the functions of an organ of the body, were first used in relation to the stomach by O. Rosenbach in the expression "gastric insufficiency." The same idea had been suggested in the writings of Stokes concerning the functions of the heart.

The "sufficiency"—*i. e.*, capacity for work or functional efficiency of an organ—depends upon the condition of that organ and the demands upon it. Therefore, sufficiency is a relative term only, and hence, if we find that an organ at a given examination shows insufficiency, we mean that it is incapable of doing the work it needs to do in the body.

The study of the functional value of the kidney is an important supplement to the study of the anatomic features of this organ in health and disease. If we can find and exactly measure the amount of work that the kidneys are doing, and if we can compare this amount of work with that of normal kidneys in a man of the same size, weight, and age, we gain a means of determining the actual value of a diseased kidney from a functional viewpoint. It is, of course, important to know what lesions are present in the kidneys; whether they are acute or chronic, parenchymatous or interstitial, but it is even of greater value to know, both for prognosis and for the choice of treatment, the amount of functional efficiency left in a diseased kidney—*i. e.*, whether it is sufficient for the present needs of the body. The functional study of an organ, as Virchow pointed out long ago, broadens our knowledge of pathology and is just as important as the anatomic study of its lesions.

But, in addition to its general value in pathology, diagnosis, and prognosis, the functional state of the kidneys, or usually of one kidney as compared with the other, has become highly important of late in *surgical affections of these organs.* Just as one ventricle of the heart may compensate for the functional inefficiency of the other, so one kidney may compensate for the functional insufficiency of its fellow. When one kidney is perfectly healthy, the removal of the other functionally useless kidney, which may be the seat of a suppurative condition, a malignant tumor, tuberculosis, etc., is not fraught with much danger to the patient's life, as the healthy kidney will take care of the work of both. On the other hand, should the opposite kidney be the seat of chronic lesions, producing

a more or less marked state of insufficiency, then nephrectomy is contraindicated. Modern surgery regards as unjustifiable any nephrectomy which has not been preceded by an examination of the comparative functional values of each kidney, the urine being drawn directly from each ureter by means of ureteral catheters introduced through a special cystoscope, or else being collected separately by means of a "segregator."

The data of ordinary urinalysis, etc., may give a clue to the anatomic conditions present or to the extent of the anatomic lesions, but do not give a ready means of calculating the actual amount of work a kidney can do. "For the fate of a patient does not depend upon the anatomic changes in the kidneys. It depends upon the interference with the function of this organ which is produced by these lesions" (Cooper). A kidney may be diseased, yet very nearly perfect in function, as enough healthy parenchyma may be left to do its work.

THEORIES OF RENAL SECRETION

We shall assume that the reader is acquainted with the anatomy, minute structure, and physiology of the kidneys. A few words must be said, however, as regards the mode of secretion of the urine by these organs. There are two principal theories as to the mode in which the urine is excreted. The first of these is the theory of Ludwig, who regards the kidneys essentially as *filters*, through which the waste-products pass from the blood out of the body, and who considers the process as a purely physical one, depending upon the principle of diffusion or osmosis.

Ludwig assumes that the blood-pressure is relatively greatest in the glomerular tufts as a result of the resistance

to the efferent circulation; and consequently a free exudation of water takes place from the tufts, with, perhaps, some dissolved salts. This concentrates the blood that reaches the convoluted tubules through the capillaries about these canals, while within the tubules flows the thin aqueous filtrate from the tufts. These conditions—a thin, watery fluid on one side of a membrane (the tubular wall) and a thickened blood on the other side—are the ideal conditions for osmosis, and so an interchange of elements occurs in the kidney, whereby water from the urinary tubules passes into the blood, while the products of tissue waste—urea and salts—pass from the blood into the tubules, where they mix with the watery fluid and so form the urine.

The second theory is that of Bowman-Heidenhain. According to this theory, the kidney is essentially a *secreting gland* and the urine is a mixture of certain characteristic elements, such as urea, etc., which are secreted by the epithelial cells of the urinary tubules, and of water and inorganic salts, such as sodium chlorid, which are filtered through the glomeruli from the blood. According to Bowman, therefore, the *glomeruli are filters* which send water and salts into the urine, while the *tubules are true secretory structures*. This theory has been confirmed by certain interesting experiments by Heidenhain, who injected methylene-blue into the blood of an animal and found soon afterward that the blue color appeared in the urine, and that epithelium of the tubules was stained blue, while the glomeruli were free from stain, thus showing that the tubular epithelium possesses selective powers like those of a secreting gland. The most important proof of the Bowman-Heidenhain theory, however, is the fact that when the epithelium of the tubules is destroyed by disease, urea and

certain toxic products (?) are retained in the blood, producing uremia, while the urine is more watery and contains a smaller percentage of its characteristic solids.

Korányi, of Budapest, to whom we shall refer later, has combined the theories of Ludwig and of Heidenhain. He admits, with Heidenhain, that water and salts are filtered through the glomeruli, while the other constituents of urine are secreted by the tubular epithelium, but he believes that there is a *perfect balance* maintained between the number of molecules of urea and allied products secreted by the epithelium of the tubules, on the one hand, and the number of molecules of sodium chlorid and other salts filtered into the lumen of the tubules through the glomeruli, on the other. The epithelium of the tubules, therefore, acts not only as a secreting cell with special properties but also as a living membrane, through which osmosis constantly takes place from the blood into the tubules, and vice versâ, so that for every molecule of urea, etc., that passes from the blood into the tubule there is reabsorbed a molecule of sodium chlorid or other salts through this living membrane of epithelium into the blood. For this reason any changes in the epithelium wrought by disease will be reflected in changes in the proportion between the urea and the salts.[1]

QUESTIONS ON CHAPTER XXIV

How many theories of urinary secretion are there at present? By what names are they known?

What is Ludwig's theory, in your own words?

[1] The above is a very brief synopsis of the theories of the secretion of the urine; for further details the student is referred to the more advanced text-books on physiology.

On what physical principles does Ludwig's theory of secretion depend?

What is Bowman-Heidenhain's theory? How does this theory regard the kidney essentially?

What part of the urine is secreted by the tubular epithelium? What part filtered by the glomeruli?

Mention experiments to confirm this theory.

What is the most important pathologic proof of this theory?

What is Korányi's teaching as regards the number of molecules of urea, etc., and of sodium chlorid, etc., filtered?

CHAPTER XXV

METHODS OF DETERMINING THE FUNCTIONAL EFFICIENCY OF THE KIDNEY

THE special methods which have been devised for measuring the functional efficiency of the kidneys, or of each of these organs separately, are as follows:

I. By determining the molecular concentration of the urine—*i. e.*, the relative number of molecules in a given volume:
   (*a*) By measuring the freezing-point (cryoscopy).
   (*b*) By measuring the electric conductivity of the urine.

II. By measuring the rate of excretion of urine in the kidneys:
   (*a*) The methylene-blue test (Achard and Castaigne).
   (*b*) The indigo-carmin test (Voelcker and Joseph).

III. By measuring the chemic activity of the kidneys:
   The phloridzin test (Casper and Richter).

IV. By measuring the rate of excretion under abnormal conditions:
   The experimental polyuria test of Albarran.

1. DETERMINING THE FREEZING-POINT OF THE URINE

Cryoscopy is the study of dissolved substances by observing the freezing-point of their solutions. The physical laws which underlie cryoscopy were discovered by Raoult in 1882, although they had been partly forecast by DeCoppet in 1872 and were later supplemented by laws

deduced from experimental researches by Vant' Hoff in 1886. The application of cryoscopy to the study of urine, blood, and other body-fluids is due to von Korányi, of Budapest, who first introduced this method into clinical work in 1897.

It would be beyond our scope to enter into the intricacies of the physics of solutions upon which the freezing-point method is based. It will be enough if we say that *the freezing-point of a solution varies in proportion to the number of molecules of a substance in a given volume of the solvent.* The more concentrated the solution, the greater the number of molecules, the *lower* the freezing-point. This is simply a more exact statement of the well-known fact that a solution of salt in water freezes at a lower temperature than does water alone. The number of hundredths of a degree Centigrade at which freezing takes place below zero represents the *ratio* of the molecules dissolved in 1 cc. of the solution. Thus, if urine may be found to freeze at 1.30° F., it may be assumed that there are 130 molecules of solids in 1 cc. of this urine. This figure is merely a conventional way of calculating, and we do not mean to state the *actual number* of molecules, but merely to designate the ratio of molecules in this particular solution as compared to other solutions.

Cryoscopy is, therefore, based upon the principle established experimentally that *for every additional molecule of dissolved solids in a solution the freezing-point sinks to some extent, and that, therefore,* by comparing the freezing-point of different solutions we can determine the ratio of molecular constitution.[1]

[1] There is, however, a very slight error in this calculation, which depends upon the fact that there is a difference between the freezing-

The **technic of cryoscopy** is by no means simple, on account of the delicacy of the observations, the variations in the freezing-point of urine being in hundredths of 1° C. A sample of the whole amount passed in twenty-four hours must be obtained, and 10 or 15 cc. of this are sufficient for the cryoscopic test. The latter is performed in a special apparatus known as the *cryoscope*, of which there are a number of modifications, the original types being constructed by Raoult and by Beckmann-Heidenhain (Fig. 88). Essentially the apparatus consists of an extremely delicate thermometer[1] graduated

Fig. 88.—Beckmann-Heidenhain's apparatus for determining the freezing-point of a solution.

point actually observed and the calculated freezing-point, expressing the sinking of the point of congealing corresponding to the molecular constitution of a solution of known quantitative composition. This difference is very slight, does not amount to more than one-sixtieth of the figure obtained, and probably depends upon what is known as *dissociation*. In virtue of this phenomenon, which takes place to a greater or less extent in all solutions of salts, the molecules are split up or dissociated into their *ions* or radicles (such as *Na* and *Cl* in the case of *NaCl*). Each of the *ions* exerts the same influence upon the freezing-point as an *independent molecule*, and so the freezing-point is lowered more markedly than it should be from the actual number of molecules corresponding to the amount of the substance dissolved.

[1] The thermometer used in all cryoscopes was devised by Beckmann. The introduction of this instrument into chemic technic may be said to

to $\frac{1}{100}$ of a degree, the bulb of which dips into a test-tube filled with the urine. Outside of this is another test-tube containing alcohol or a solution of glycerin, so that the urine is inclosed in a double-walled chamber with one of these fluids in the interspace. The outer test-tube is surrounded by a mixture of ice and salt packed in a jar. A spiral of platinum wire or of hard rubber is arranged so that by means of it the urine can be constantly stirred around the thermometer, thus keeping the temperature of all parts of the sample uniform. The mercury begins to sink rapidly when the fluid approaches the freezing-point. It then rises and fluctuates for a time until it finally settles at a point below zero, which is read accurately as the freezing-point of the urine. Various mechanic improvements, such as automatic stirrers moved by clockwork, etc., have been devised, and in France the evaporation of ether or carbon disulphid is used instead of ice for the purpose of freezing.[1]

Clinical Applications of Cryoscopy.—The total number of molecules in a given urine is expressed by the freezing-point thereof, Δ. The normal freezing-point of urine ranges between —1.30° and —2.20° C. When there are

have marked an era in the history of physical chemistry. The Beckmann thermometer is unlike the ordinary instrument in that it has a reservoir of mercury at the top of the capillary, and by shaking the thermometer one can add to or subtract from the length of the column of mercury. In this manner the thermometer may be "set" to read at any temperature. It is graduated so as to cover only a few degrees, thus giving a long scale which is subdivided into hundredths, and these, with the aid of a magnifying glass, may be read to thousandths. Before using, the freezing-point of water should be determined with it, and any deviation from 0° C. should be noted as a correction.

[1] Not because of any advantage of this method, but because of the scarcity of ice.

lesions in the kidneys interfering with the functional activity of these organs the freezing-point rises—*i. e.*, approaches zero Centigrade—because the total number of molecules excreted is diminished. A urine freezing at above —1° C. is usually considered as abnormal. In extremely severe cases of chronic nephritis, with uremia, the freezing-point is close to or actually at zero. The symbol Δ, by common consent, stands for the number of dissolved molecules in 1 cc. of urine, as expressed by the freezing-point in tenths and hundredths of a degree Centigrade. If V stands for the amount of urine eliminated in twenty-four hours, and P for the weight of the patient in kilograms, then the formula $\Delta \times \frac{V}{P}$ represents the total number of molecules eliminated in the urine per kilogram of body-weight in twenty-four hours. The comparison of the value of $\Delta \times \frac{V}{P}$ in different persons makes the freezing-point method more exact as a measure of functional activity than the mere comparison of the cryoscopic coefficient, Δ, alone.

The foregoing is a summary of the theoretic basis of cryoscopy. Unfortunately, the beautiful structures built up by Korányi and his school, by Claude and Balthazard, and others, who vied with each other in devising complete mathematic formulæ for determining the functional condition of the kidneys, have not stood the test of time and experience. Cryoscopy is a troublesome method, is subject to many errors, and even when performed with accuracy is not clinically conclusive. A normal freezing-point has been found with anatomic lesions of the kidney, and an abnormal freezing-point may occur with healthy kidneys. Cryoscopy of the urine of each kidney separately has more value, as it may be used for comparison,

but even then the freezing-point tells very little, if anything, more than the specific gravity, which is much more easily determined.

The *relation of the specific gravity of urine to its freezing-point* has been the subject of extended studies during the past ten years. Theoretically there should be a marked difference between the specific gravity and the freezing-point. The latter indicates the *number* of molecules, the former the weight of the same molecules. Thus, if a trace of albumin be present, its heavy molecules would theoretically influence the specific gravity more than the freezing-point. Yet in practice this difference is of no importance.[1] There is, therefore, a fairly constant parallelism between specific gravity and Δ. Fuchs proposes that the last two figures of the specific gravity (carried out to the third place) be multiplied by 0.075 to determine the freezing-point of normal urine in degrees Centigrade. In practice, therefore, we can usually do without cryoscopy, but can employ advantageously an accurate determination of the specific gravity of the urine from each kidney. This may be done with the aid of a pyknometer and an accurate balance or, more easily, with the writer's urinopyknometer (see p. 44).

2. DETERMINATION OF THE ELECTRIC CONDUCTIVITY OF THE URINE

This method is a complicated one, and consists in measuring the power of the urine to conduct or carry a current. This power varies with the molecular concentration of the urine. The method has not gained headway in practice because it gives no information that may

[1] Bugarszky, Fiodoroff, Kiss, etc., Kapsammer, Casper.

not be obtained by measuring the specific gravity or by determining the freezing-point.

3. THE METHYLENE-BLUE TEST

Kutner[1] was the first to conceive the idea of giving methylene-blue internally and watching the flow of urine from the ureters through a cystoscope to determine the functional activity of the kidneys. The method, however, which is at present used was worked out by Achard and Castaigne (1900).

The test consists in injecting 0.02 gm. of methylene-blue[2] intramuscularly (Kapsammer), though some authors recommend larger doses—0.05 gm. (Albarran). Normal kidneys begin to excrete the dye within fifteen or twenty minutes and the excretion is complete within forty-eight hours. The excretion must normally begin not later than within half an hour and reach its maximum in the third or fourth hour (Kapsammer).

Clinical Value.—When the kidneys are in a state of functional inefficiency due to anatomic lesions, they are unable to excrete methylene-blue properly:

(*a*) The excretion may be *delayed* and may be prolonged when it does occur (impermeability, said to be indicative of interstitial lesions—Bard).

(*b*) The excretion may be *premature* and the total *duration short* (said to mean parenchymatous changes—Bard and others).

(*c*) The excretion may be *premature* and *prolonged*

[1] Deutsche med. Wochenschrift, 1892.

[2] Merck's or Grübler's "medicinal" chemically pure methylene-blue should be used in sterile water.

(said to mean compensatory hypertrophy of a kidney—Albarran and Bernard).

It is claimed by Achard and Castaigne that methylene-blue excretion is always delayed when there are lesions in the kidney. Chauffard, Kövesi, and Kapsammer, and with them many other observers, including the present writer, find that methylene-blue is retained only in the presence of marked renal lesions. When methylene-blue appears within half an hour the kidney is functionally capable.

Sources of Error.—The most important difficulty with the methylene-blue test is the fact that the dye is often not excreted *as such*, but as a leuko-derivative or chromogen, a colorless substance from which the blue color may be obtained only by oxidation. This oxidation frequently takes place in the kidney, but sometimes a urine may appear unchanged in color although it contains methylene-blue in the form of a chromogen. By boiling with a little acetic acid the blue color is unmasked.

A further source of error lies in administering the methylene-blue and the phloridzin test in the same patient on the same day. If the phloridzin test (see below) is used first and the methylene-blue test used afterward, while sugar is still present, the sugar excreted interferes with the blue dye and the latter is excreted as a chromogen, hence is invisible. On the other hand, if the methylene-blue test is used first and the phloridzin test is applied while the urine is still blue, the sugar decolorizes the urine and converts the blue dye into an invisible chromogen.

Retention of methylene-blue and its late excretion, months or even years after the injection of the dye, may occur. E. Beer, of New York, has suggested that such retention of the dye may be used as a diagnostic sign of pyelonephritis with abscesses in which the dye is lodged. The subsequent excretion of the dye is due to the bursting of these abscesses.

To sum up, the methylene test is not absolutely reliable for the determination of the functional value of the kidneys. Its chief value, as that of several other methods, is in contrasting the function of one kidney with that of the other (urethral catheterization or separation of

urines) when the lesions are marked on one side. The indigo-carmin test is more satisfactory in many respects.

4. THE INDIGO-CARMIN TEST

In 1903 Voelcker and Joseph showed[1] that indigo-carmin was superior to methylene-blue in testing the rate of excretion of the kidneys, because the former dye, unlike methylene-blue, was not excreted as a chromogen, but always as a blue pigment.

Normal kidneys begin to excrete a solution of indigo-carmin injected into the muscles in about five minutes. The urine turns blue and the color reaches its maximum in one-half or three-quarters of an hour. The excretion lasts about twelve hours. The dose usually given (Kapsammer) is 4 cc. of a 4 per cent. suspension in normal NaCl (=0.16 gm.). The site of injection may be into the gluteal muscles, but as the dye is excreted rapidly, there may not be time to turn the patient and put him into position for cystoscopy. Kapsammer, therefore, injects the dye into the quadriceps muscles about 4 inches above the knee-cap. (The ureteral catheters should be in their places before the injection.)

Delayed excretion and lessened excretion of indigo-carmin used in this way means a functional renal disturbance. If the color does not appear for ten or twelve minutes after injecting 0.16 gm. of the dye, and then only as a greenish tint, and if the color never becomes blue, but remains greenish, there is renal insufficiency. Kapsammer emphasizes the trustworthiness of *the time of onset of the excretion* with indigo-carmin. The longer the appearance of the blue color is delayed, the more severe the lesion.

[1] Münch. med. Wochenschrift, 1903.

The *intensity of the color* is also a criterion. The dose used by Kapsammer takes twenty-four hours to be completely eliminated, but as this time varies normally, it is not so valuable as the other criteria mentioned.

5. THE PHLORIDZIN TEST

Phloridzin is a glucosid discovered by Koninck, in 1855, in the root-bark of apple, pear, and cherry trees. Von Mering, in 1885, found that phloridzin caused glycosuria when injected subcutaneously in normal persons. The glucose excreted after injections of this drug is manufactured in the kidney itself, but just where or how is not as yet definitely known. That interstitial nephritis interferes with the excretion of sugar in cases of diabetes had been known for some time (Klemperer, Fürbringer, Senator), and we have referred to this fact in the chapter on this disease (p. 383). In 1896 Klemperer showed that patients with chronic Bright's disease did not have glycosuria after the injection of phloridzin, as did normal persons.

It remained for Achard and Delamarre, in 1899, to apply these ideas to the functional diagnosis of renal affections. These authors were the first to use the phloridzin test as such for the purpose of determining whether or not the kidneys were functionally efficient, measuring this efficiency by the capacity of the kidneys to excrete sugar after injections of phloridzin.

Preparation of the Patient.—The patient should not have received any of the following drugs within a reasonable time before the test: Antipyrin, glycerin, sodium salicylate, piperazin, or jambul. All these retard sugar excretion. Furthermore, the administration of diuretics before the test is not advisable, although practised by some in order to accelerate the collection of urine after the test. Diuretics interfere with the quantitative relations of the excretion.

The patient's kidneys should not be massaged or otherwise forcibly manipulated for at least three days before the test.

Technic of the Injections.—The phloridzin used should be of the best quality, Merck's being preferred. Cases have been reported of failure of the test due to the use of impure phloridzin.

Phloridzin is insoluble in cold water and is precipitated on cooling from its solutions in hot water. Solutions may be made in hot water and the requisite amount drawn into the syringe while the fluid is hot and before the glucosid has had time to precipitate. A better way, however, is to make a solution of phloridzin in alcohol, and to add to it the required amount of water. No precipitate occurs in such solutions. The solutions should, in all cases, be freshly prepared.

The best method of preparing the solution, therefore, is to dissolve 1 part of phloridzin in 30 parts of 95 per cent. alcohol and add 70 parts of warm water. This will make a 1 per cent. solution, and from 10 to 20 minims (usually 15 minims) is a convenient dose of this solution, which should be injected warm. The syringe should have been boiled and washed with alcohol before each injection. The solution should be injected *subcutaneously* into the arm, care being taken not to inject intradermally nor too deeply into the adipose layers, as the rate of absorption varies according to the character of the tissue into which phloridzin is injected. In metric weights the dose usually used may be expressed as 0.01 gm., although some workers use higher doses.

The sugar should appear in the urine ten or fifteen minutes after the injection of phloridzin. If the function of the kidneys is insufficient, the excretion of sugar is delayed, appearing twenty minutes or a longer time after the injection. If no sugar appears within forty-five to fifty minutes after the injection, the kidney is in a state of marked insufficiency. The sugar normally disappears in about twelve hours, after reaching a maximum excretion at the end of one and a half hours.

Method of Observing the Result.—Inasmuch as the phloridzin test is now almost always applied as a means of comparing the function of one kidney with that of the opposite organ, the urine being collected for this purpose through ureteral catheters, or with the aid of a

"separator," a few words might be said as to the manner of conducting this test under these conditions. The cystoscope is introduced before the injection, and the catheters are inserted into the ureters. The phloridzin is injected, and the time of injection noted. Ten minutes are allowed to elapse, during which the urine is allowed to drop through the catheters into graduated test-tubes. The first portion of urine is used for the purpose of a general examination. At the end of ten minutes the tubes are changed, a new tube being substituted on each side. The tubes are changed after each succeeding five minutes. It is convenient to have a series of test-tubes, properly marked in series, ready for each side. The first sugar reaction normally appears in the second tube, *i. e.*, after fifteen minutes. The third tube represents the urine up to twenty minutes, etc. In this way the exact time of appearance of the sugar can be noted, and the progress of the excretion can be accurately watched.

Interpretation of the Result.—Although it seems to be well established both clinically and experimentally that functional inefficiency interferes with the excretion of sugar, there is still a question as to what criteria are to be applied to the excretion of sugar in order to make out an insufficiency. There are practically two views at present. Casper and Richter originally held and still hold that it is necessay to determine *the percentage of sugar excreted* after phloridzin injections, and for this purpose make a quantitative determination of sugar in the urine obtained at maximum excretion. This view is now gradually becoming obsolete, owing to the many sources of error which obtain in judging the quantity of sugar under these conditions. First, the percentage of sugar is affected by the polyuria, which is ordinarily produced by the mere act of ureteral catheterization, and second, there is always some leakage alongside the catheter, which makes a difference in the quantity of sugar excreted in a given time.

The second view, brought forward some years ago by Kapsammer, seems more simple and more practical in its application. According to this author, it is merely necessary to watch for the *time of appearance* of the sugar in the manner described above. The time of appearance of sugar is a more trustworthy criterion as to the functional capacity of the kidney than the variable factor of quantity of sugar excreted. Kapsammer's method is sufficiently accurate for practical purposes, and, indeed, seems to lead to fewer errors than the method of Casper and Richter. If the appearance of sugar is delayed for forty-five minutes on the "unaffected side," Kapsammer rules out the possibility of performing nephrectomy, as the functional capacity of the remaining kidney is then not sufficient to allow the patient to live. If the appearance of sugar is delayed

for thirty minutes, marked renal lesions are present, but before final decision is made other confirmatory tests, such as the indigo-carmin test and the polyuria test of Albarran (see below), are recommended.

Value of the Method.—While the phloridzin test, on the whole, is the best and most trustworthy single method of functional diagnosis we have, it is not infallible. It is very delicate, and errs perhaps in this direction. Moreover, it is not conclusive in cases in which there is a parenchymatous nephritis with few or no interstitial changes. In these cases there is commonly a normal excretion of sugar, although there are many casts, and albumin is present in considerable amount. In interstitial nephritis or in diffuse nephritis with considerable interstitial changes the phloridzin method is of the greatest value. The reason for its failure in parenchymatous types is that in these the function of the kidney may long remain comparatively good.

The failure to excrete sugar when the kidneys are comparatively healthy has been recorded by a number of observers. Beer[1] found that this interference with sugar excretion occurred at times in the "unaffected kidney" when the other was surgically diseased, and attributes this interference to the influence of the diseased kidney upon the healthy or healthier organ. He reported several cases in which the phloridzin test showed normal function in the kidney which remained after nephrectomy of its fellow, although previous to operation the test had been negative up to fifty minutes in the unaffected organ. While more evidence is needed to confirm this, Beer's work certainly throws a shadow of doubt upon some of the negative findings with the phloridzin test, and in this sense considerably diminishes its value. When the test is positive, however, every observer agrees that its interpretation of good function can be relied upon, with the possible exception, already noted, of parenchymatous nephritis. It is because of these occasional failures of the phloridzin test that a control by means of the method about to be described may be desirable.

[1] Journal Am. Med. Assoc., 1908, II, 1876.

6. EXPERIMENTAL POLYURIA

When an increased amount of water is taken by a patient with normal kidneys, there occurs a corresponding increase in quantity and a proportionate dilution of the urine. On the other hand, when the water intake is increased in a person with parenchymatous nephritis, the quantity and concentration of the urine remain more or less unchanged. This was experimentally proved by Kövesi and Roth-Schulz, in 1900, by F. Strauss, in 1902, and by Albarran, in 1905. The last-mentioned observer has published a considerable amount of evidence as to the value of what he terms "experimental polyuria" in determining the functional capacity of the kidneys.[1]

Albarran gives the patient from 400 to 600 cc. of a mineral water known as "Evian." The urine is then collected every half-hour, and its quantity, its freezing-point, the amount of urea, and of chlorids are determined. Albarran found that, as a rule, in healthy kidneys the freezing-point, the percentage of urea, and the amount of chlorids were diminished in about the same degree as the quantity of urine was increased. This was true, however, of the *percentages* of solids, but when their *absolute quantities* were determined, it was found that normal kidneys could at times excrete during an experimental polyuria not only more water, but also a larger quantity of urinary solids. In other cases, however, there was also a reduction in the absolute quantity of the solids along with the polyuria.

In diseased kidneys the decrease in the percentages of the solids excreted was much less marked, with the same amounts of urine, and the more severe the anatomic lesion of the kidney the less markedly altered were the percentages of solids in proportion to the total amount excreted.

In addition to the factors mentioned above, the specific gravity of the urine may also be employed as a criterion in connection with this test. Grünwald used Albarran's method, substituting the specific gravity for the freezing-point, and came to the same conclusions. In healthy kidneys the polyuria was noteworthy for a marked lowering of the specific gravity, while in parenchymatous nephritis the specific gravity was lowered to a lesser extent and the change in density appeared later than in

[1] Albarran, Exploration des Fonctions Rénales, Paris, Masson, 1905.

normal cases. In interstitial nephritis no polyuria of any account was produced and the specific gravity was not affected by the increased intake of water.

The experimental polyuria test is simple in technic and may be employed as a corroborative method in connection with other means of functional renal diagnosis.

SUMMARY

To sum up what has been said in the preceding pages as to the value of the various methods of functional renal diagnosis, the following brief statement may serve as a guide to those who are at a loss to select a suitable method for this purpose: A careful estimation of the specific gravity, of the urea, and, if need be, of chlorids in each of the separated urines gives for practical purposes as trustworthy results as cryoscopy, without the sources of error to which the latter is subject.

Of the other functional test, the phloridzin method stands highest in accuracy and delicacy, but its limitations must be understood and its results must be corroborated, if necessary, by the indigo-carmin test. The latter is to be preferred in every instance to the methylene-blue test. Finally, the method of experimental polyuria may be resorted to as an additional safeguard.

CHAPTER XXVI

THE TOXICITY OF THE URINE

It has been known for a long time that suppression of urine is followed by systemic poisoning of a definite type. In 1881 Feltz and Ritter showed that normal urine was poisonous by injecting it into animals and finding that a certain dose of urine was fatal. Later, Bouchard investigated the poisonous properties of normal urine more thoroughly. In order to be able to compare the toxicity of urine under various conditions, Bouchard established what is known as the *urotoxic coefficient*, which is the weight of rabbit in kilos that is killed by the quantity of urine excreted by one kilo of the person experimented upon in twenty-four hours. According to Marfan this coefficient is from 20 to 35 in health.

The toxic symptoms produced by the intravenous injection of normal urine include contraction of the pupil, which dilates just before death, somnolence, coma, marked polyuria, frequent micturition, a lowering of the temperature, diminished reflexes of the conjunctiva and cornea, and death in coma or convulsions.

The toxicity of the urine, according to Bouchard, is greater during the day than at night. The day urine is strongly narcotic and but feebly convulsive, while the night urine is the reverse. The toxicity is diminished by active exercise in the open air. Bouchard found that in

acute uremia the urine becomes non-toxic. He concludes that the urine's toxic properties are due to a number of substances which he could not isolate completely, but is not due to urea, uric acid, creatinin, etc., since these are non-toxic in large doses when injected into the blood.

Some observers—*e. g.*, Stadthagen, Beck, and v. d. Bergh[1]—deny that any specific poisonous substance occurs in normal urine. They claim that the poisonous action of the urine is due partly to the potassium salts and partly to the other normal constituents—urea, creatinin, etc.—which have very little toxic effect individually.

In *disease* the toxic power of the urine may be either increased or diminished. It is generally increased in acute infectious diseases and fevers, provided the kidneys remain healthy. The toxic powers of the urine become more or less diminished according to the extent of the damage done to the kidneys, and in extensive renal lesions the urine may become almost non-toxic. In uremia the kidneys no longer eliminate the poisons from the system, and the urine is non-toxic. The toxicity of the urine, however, is considerably raised in most diseases—*e. g.*, in tetanus (Labbé), in cholera (Bouchard), in septicemia (Feltz), in diphtheria (Roux and Yersin), etc.

Bouchard has shown that from 30 to 60 cc. of normal urine injected intravenously will kill a rabbit weighing 1 kilogram. A man weighing 60 kilos and passing 1200 cc. of urine daily secretes enough poison to kill 24 kilos of animal, if 50 cc. are necessary to kill one kilo of living matter. This standard is used in determining the relative value of the "urotoxic coefficient" referred to above.

[1] Quoted by Hammarsten, *loc. cit.*

In connection with the subject of urinary toxicity we may briefly consider the two classes of substances that have been found in the urine, and that are responsible for this toxic action—ptomains and leukomains.

PTOMAINS

PTOMAINS are complex organic substances, basic in character, resembling alkaloids in many respects, and formed by the action of bacteria upon nitrogenous matter, which may be either animal or vegetable in origin. Some ptomains are highly poisonous and are styled toxins (Brieger), while others are harmless.

According to Bouchard, Pouchet, and others, ptomains occur in normal urine and are increased in quantity in disease (Bouchard, Lépine, and Guerin, etc.). Villiers found a constant increase of these substances in measles, diphtheria, and pneumonia. Pouchet found them in cholera; Feltz, in cancer; Lépine, in pneumonia, and Griffiths and Albu have isolated a series of ptomains in a great variety of diseases. Baumann and von Udránszky showed the presence of two ptomains, first described by Brieger —putrescin ($C_4H_{12}N_2$) and cadaverin ($C_5H_{14}N_2$)—in the urine in a case of cystinuria and cystitis.

LEUKOMAINS

LEUKOMAINS are basic substances found in living tissues, which do not result from the action of bacteria, but are the product of fermentation or of retrograde changes in the organism. While leukomains are the products of normal life-processes, ptomains are the results of putrefaction.

Leukomains in the urine are divided into two groups—the uric-acid group and the creatinin group. The uric-acid

group includes bases related to uric acid—viz., adenin. hypoxanthin, guanin, xanthin, heteroxanthin, etc., carnin, episarkin, pseudoxanthin, etc. The creatinin group, according to Gautier, includes creatinin, creatin, crusocreatin, xanthocreatin, amphicreatin, and two unnamed bases.

Brieger and von Udránszky and Baumann and Stadthagen[1] deny the occurrence of ptomains and leukomains in normal urine, and present very strong objections to Bouchard's teachings.

The clinical interest in the presence of ptomains and leukomains in the urine lies in the fact that these substances are the chemic bases of infectious diseases, and that possibly specific substances may be isolated from the urine in various maladies. The subject, however, has not yet been sufficiently well worked out to bear direct application in clinical work.[2]

[1] Quoted by Hammarsten, Physiological Chemistry (Mandel), third edition, New York, 1901, p. 463.

[2] For details regarding the chemistry of ptomains and leukomains, see Vaughan and Novy, Cellular Toxins, Philadelphia, 1902. For a bibliography of ptomains and leukomains in the urine, see Neubauer and Vogel, Analyse des Harnes (Huppert), Weisbaden 1898.

APPENDIX

ROUTINE OF EXAMINATION, REAGENTS, APPARATUS, ETC.

WHILE a complete urine examination, as may be seen from the foregoing pages, calls for a great deal of time and labor and a full mastery of technical detail, routine analyses which are sufficient for every-day purposes require but a few minutes' time and are accomplished with comparatively simple means and with the fewest possible manipulations.

In the following summary I mean to give the student, the busy physician, and the medical examiner for life insurance a few hints as to the best means of shortening the time and lessening the labor of urine analysis, without unduly interfering with the trustworthiness or accuracy of the results.

Every one who has a large number of urinalyses to perform daily, or who has but a few minutes to devote to the examination of urine, will do well if he begins by devising a certain routine of work. Such routine examinations should be as rapid as is compatible with thoroughness and accuracy; they should include everything that is necessary for a clinical diagnosis, and exclude everything that is unnecessary or of purely theoretic interest. A routine of work may, indeed, be so devised that even

unnecessary movements are saved, wherever possible, by simplifying the methods and arranging the order of the tests. Such moments, wasted in unnecessary trifling with minor details, are well saved and well invested in a longer time allotted to the study of the urine under the microscope.

Selection of Methods.—The first thing to be done when contemplating the analysis of a number of specimens is to determine, once for all, the methods that are to be used, and then to adhere to these methods unless forced to deviate from them by some special circumstance. This does not, of course, mean to exclude experimenting with various methods or modifications at our leisure.

It is the beginner who is usually undecided as to what method he shall use for a certain test, etc. The experienced man, as a rule, works with one method or, at the most, with two, for the same part of the analysis.

As I have said in speaking of albumin tests, in practical work the best way is to use one test, always holding a confirmatory test in reserve in case of doubt. This principle holds good in all parts of urine analysis. Select, therefore, the best method you know of—one that has proved efficient and practical in the hands of a large number of men—and adhere to this method in your routine work. This has the obvious advantage that the man who works with one method will in a short time know all the vagaries, irregularities, possibilities, impossibilities, and sources of error in the one method which he uses.

The next rule to be observed in routine work is: In employing a given method, invariably use the same amounts of reagents, the same dilution, the same order, heating, if needed, for the same length of time—in short,

using exactly the same manipulations for the same test in each analysis. This will enable a careful worker, after a time, to estimate with fair accuracy the quantity of a constituent from the bulk of a sediment or precipitate. Of course, such adherence to routine in details can be profitable only after one knows with perfect accuracy just how to perform a test to the best advantage with whatever materials (test-tubes, etc.) one may have on hand.

Routine of Work.—Necessarily each man must be a law unto himself in devising this routine according to his tastes, the time at his disposal, and the requirements of his cases. The following order of examination is suggested as one that is apt to meet the average requirements. The tests selected are those which have proved trustworthy in my own work and which have been found satisfactory by a large number of observers.

ROUTINE OF EXAMINATION

1. **Physical Examination.**—The urine is poured into a small cylinder and a urinometer is immersed in it, together with a strip of blue litmus-paper. While the urinometer is allowed to settle in position, the *color* of the urine is determined by a comparison with Vogel's scale, which hangs directly opposite on a wall. The same glance tells us its *transparency*, the presence of a *sediment*, and, before the specific gravity is read, the *odor* is ascertained. These characteristics are noted on the record blank (see p. 420) and then the urinometer scale is read. A portion of the urine is now poured into a small filter, the *consistence* of the fluid being noted as this is done, and the filtered urine is used for many of the subsequent tests, a constant supply of it being kept up by filling the filter from time to time.

2. **Chemic Examination.**—This should begin with the estimation of *urea*, because while the nitrogen gas is forming we can attend to the other tests and then read the scale of the apparatus.

Albumin.—While the urea test is under way we proceed to look for albumin. For this purpose I usually employ first the acetic acid and heat test, after adding saturated salt solution. Unless there is a massive coagulation, I confirm the result with Heller's ring test (cold nitric acid) and the ferrocyanid test. If hyaline casts are found in the urine on subsequent examination under the microscope and if all the above albumin tests were negative, I make sure of the absence of even the faintest traces af albumin by the use of the Roberts magnesium test with the horismascope or with Spiegler's test. If albumin is found, Esbach's or Tsuchiya's quantitative method is applied. If time is pressing, Purdy's centrifugal method will suffice ordinarily, if performed accurately.

Sugar.—While the test-tube of the albumin test is allowed to stand for a few moments to show the full development of the ring or reaction, we mix and dilute the Fehling's solution and bring it to a boil. The urine is added, and if a positive reaction is obtained, we at once set up the Einhorn saccharometer, with yeast and urine, and allow it to stand for twenty-four hours. If a polariscope is available, the urine is at once prepared for polarimetry by mixing with basic lead acetate and filtering. If albumin is present it must, of course, be removed first.

Indican is the next substance looked for. Here I am in the habit, in routine work, of employing Obermeyer's simple and efficient method. The same amount of urine and of the reagent is always used, and a fair estimate is

obtained of the amount of indican in the urine. For routine quantitative work Robin's method of estimating indican is useful.

Bile.—In testing for bile we may use the filter-paper, which has become in the meanwhile well saturated with the filtering urine. While the indican test is allowed to reach its completion, a drop of pure nitric acid is placed on the filter, producing a green spot which changes to red if an excess of bile is present. Often this test is not necessary, as in Heller's test for albumin an excess of bile-pigments will give a play of colors (green, blue, violet, red, and yellow) at the point of contact of the two fluids (see Gmelin's Test, p. 190). When small traces of bile-pigments are present, Maréchal's test (p. 191) should be used to confirm the above-mentioned method.

The remaining chemic tests are used only when special indications require.

Acetone and *diacetic acid* are tested for if there was glucose in the urine, or in cases of suspected toxemia, in pregnancy, etc. For acetone the Jackson-Taylor modification of the nitroprussid test is useful. For diacetic acid the ferric chlorid method is most convenient.

The *diazo-reaction* is next tested for if necessary.

The estimation of *uric acid* may be clinically simplified by the use of Ruhemann's method. The estimation of *chlorids*, *phosphates*, and *sulphates* may be made convenient by Purdy's centrifugal method. For accurate work, however, these methods are not sufficiently exact.

The *microscopic examination* follows: The centrifuge is set going, and in the meanwhile the amount of urea is read off, and the changes, if any, in the various test-tubes that have been allowed to stand are noted. The sediment

is then examined microscopically according to the methods already outlined.

Finally, a word of warning: Do not rely upon the findings of laboratories that do their work upon "the quick-lunch-counter plan." Such laboratories unfortunately exist in some of our large cities. Urine analysis may mean life or death, and should not be intrusted to incompetent persons. If the patient cannot pay for an examination by an expert, let the physician make the principal tests himself, rather than allow the work to be "railroaded" through by one of the concerns that apply the "factory system" to clinical pathology.

Forms for Recording Analyses.—The following blank form (p. 420) is used by the writer in reporting analyses of urine when the specimen or the patient has been referred to him by other physicians. Naturally, this blank provides merely for the essential points of an examination in an average case. Space is provided, however, for additional data, quantitative and qualitative. The tabular form of expressing the principal quantities is recommended as very convenient.

For use in his own private practice the writer has devised an index card, which is shown on p. 421. These cards are filed with the histories of the cases.

BLANK FOR REPORTING URINE ANALYSES

Report No. *Date.*

Dr. *Name.*

ANALYSIS OF URINE

Transparency Reaction

Color Specific Gravity at 15° C.

Amount voided in twenty-four hours, cc., Ounces.

	Per Cent. by Weight.	Per Mille by Weight.	Grains per Ounce.	Amount in 24 hours.
Total solids.....				
Urea				
Albumin				
Sugar.....	(Polariscope)			

Indican Acetone

Bile Diacetic acid

Diazo-reaction Other constituents

EXAMINATION OF SEDIMENT

(*Obtained by Centrifuge at 1800 Revolutions for Five Minutes*)

Amorphous sediment Mucus

Crystals Pus-cells

Cylindroids Red blood-cells

Casts Epithelia

Bacteria Other elements

Remarks:

Respectfully submitted,

———————————— M. D.

INDEX-CARD FOR RECORDING URINE ANALYSES.

Dr.....Name.....Date.....No.....

Transparency	Amorphous deposit
Color	Crystals
Amount	Cylindroids
Reaction	Casts
Specific gravity	Bacteria
Urea	Mucus
Albumin	Pus
Sugar	Blood
Indican	Epithelia
Bile	From kidney
Diazo-reaction	" pelvis
Acetone	" bladder
Diacetic acid	" vagina
	Other elements

LISTS OF REAGENTS, APPARATUS, ETC.

Reagent bottles of appropriate sizes (250 cc. for liquids and 125 cc. for solids) are made by firms supplying chemic apparatus, and should be made of glass free from lead and other impurities. The bottles should be fitted with glass stoppers, and those for solutions of salts, such as sodium hydrate, etc., should have stoppers coated with a mixture of paraffin and vaselin.

All reagents should be purchased chemically pure, unless otherwise specified. All standard solutions for chemic analysis are described by the United States Pharmacopœia, and should conform to its standard unless otherwise mentioned in the text of this book.

The following list includes the principal reagents needed in urine analysis. The list is intended chiefly for the use of teachers intending to follow the methods outlined in the present book. Practitioners will find it convenient to

select from this list the reagents they require in their routine analyses.

LIQUID REAGENTS

Distilled water (H_2O).
Acid, Acetic (c. p.), U. S. P. ($HC_2H_3O_2$).
Acid, Acetic, Glacial, U. S. P.
Acid, Boric (c. p.) (H_3BO_3), sat. sol.
Acid, Hydrochloric (c. p.) (HCl).
Acid, Hydrochloric, Commercial (for cleaning glassware).
Acid, Nitric (c. p.) (HNO_3).
Acid, Nitric (brown, fuming; nitrosonitric).
Acid, Picric ($C_6H_2(NO_2)_3OH$), 8 gr. to 1 oz. = sat. sol.
Acids, Picric and Citric (Esbach's solution, see p. 71).
Acid, Salicylsulphonic, sat. sol.
Acid, Sulphuric (c. p.) (H_2SO_4).
Ammonium Hydrate, U. S. P. (NH_4OH).
Ammonium Chlorid, sat. sol.
Ammonium Sulphate, sat. sol.
Sodium Hydrate, U. S. P. (NaOH).
Sodium Hydrate Solution for Urea (Knop's).
Sodium Hydrate Solution for Urea (Rice's).
Sodium Chlorid (NaCl), sat. sol.
Sodium Nitroprussid [$NaFe(NO)(CN)_5 + 2H_2O$].
Potassium Hydrate, U. S. P. (KOH).
Potassium Ferrocyanid ($K_4Fe(CN)_6$), 1: 20.
Potassiomercuric Iodid (Tanret's, see p. 67).
Potassium and Sodium Tartrate (Fehling's, see p. 101).
Magnesium Fluid (see p. 218).
Magnesium Sulphate and Nitric Acid (Roberts, see p. 64).
Barium Chlorid ($BaCl_2$, 4 oz.; H_2O, 16 oz.; HCl, 1 oz.).
Copper Sulphate (Fehling's, see p. 101).
Silver Nitrate ($AgNO_3$), 1: 8.
Iron Chlorid (Fe_2Cl_6), 1: 10.
Obermeyer's Solution, see p. 179.
Lead Acetate ($PbO_2[C_2H_3O]_2$), 1: 4.
Lead Acetate, Basic ($Pb_2C_2H_3O_2PbO$) 1: 4.
Mercuric Chlorid (Spiegler's, see p. 66).
Mercuric Nitrate (Millon's, see p. 80).
Hydrogen Peroxid (H_2O_2), U. S. P.
Bromin (c. p.) (Br).

Bromin (Rice's solution, see p. 139).
Tincture Iodin, U. S. P.
Tincture Guaiac, U. S. P., fresh.
Turpentine.
Alcohol.
Chloroform.
Ether.
Formalin, 40 per cent.

Also the following standard volumetric solutions:

Decinormal Potassium Hydrate.
Decinormal Sodium Hydrate.
Decinormal Silver Nitrate.
Decinormal Potassium Bichromate ($K_2Cr_2O_7$).
Standard Uranium Nitrate.
Standard Sodium Acetate.
Potassium Ferrocyanid.
Standard Barium Chlorid.
Potassium Sulphate (K_2SO_4), 20 per cent.

SOLID REAGENTS

Acid, Citric.
Acid, Picric.
Ammonium Chlorid.
Ammonium Sulphate.
Copper Sulphate.
Lead Acetate.
Litmus-paper.
Magnesium Sulphate.
Mercuric Chlorid.
Phenylhydrazin.
Potassium Chlorate.
Potassium Chromate.
Potassium Ferrocyanid.
Potassium Hydrate.
Potassium Iodid.
Sodium Acetate.
Sodium Carbonate.
Sodium Chlorid.
Sodium Hydrate.
Sodium Nitrite.
Sodium Nitroprussid.
Yeast.

BACTERIAL STAINS, ETC.

1. Methylene-blue, sat. sol. in 95 per cent. alcohol (1 part in 3 of water for staining bacteria).
2. Carbol-gentian violet, see p. 333.
3. Gram's solution (iodin, 1; potassium iodid, 2; water, 300).
4. Bismark brown, see p. 333.

5. Carbol-fuchsin (Ziehl-Neelsen's: Fuchsin, sat. alcoholic sol., 10; carbolic acid solution, 5 per cent., —90).

6. HCl 3 per cent. in 95 per cent. alcohol.

7. Alcohol, absolute.

APPARATUS

Test-tubes, 2 or 3 dozen, assorted sizes.
Pipets, plain glass, ½ dozen.
Pipets, graduated, 5, 10, 25 and 50 cc.
Glass rods, 8 inches long each.
Beakers, thin glass, with lip, nest of 6.
Florence flasks, 250 cc., 500 cc.
Distilling flask, 100 cc., see p. 166.
Watch-glasses, nest of 6.
Porcelain evaporating dishes, nest of 6.
Conic glasses, 4.
Wineglasses, 2.
Ground-glass covers, square.
Cylinders for urinometer, 4 ounces, and for urine, 12 ounces.
Wash-bottle.
Funnels, glass, 3 assorted sizes.
Balance, accurate, with weights.
Test-tube rack.
Test-tube brushes, sponge end.
Filter-paper, round, assorted sizes, white "c. p." quality.
Water-bath, copper, fitted with rings.
Filter and buret stand, metal with solid base.
Wire gauze, several squares, 4 by 4 inches each.
Droppers, nipple.
Graduates for 100, 500, and 1000 cc., marked also in ounces.
Graduate for 15 cc., marked also in minims.
Urinometer (Squibb's, etc.).
Urinopyknometer (see p. 44).
Saccharometer, Einhorn's.
Doremus' or Hinds' urea apparatus.
Esbach's albuminometer.
Horismascope (albumoscope).
Uricometer, Ruhemann's.
Purin apparatus, Camerer's.
Thermometer, Centigrade.
Burets, 50 cc. each, graduated in $\frac{1}{10}$ cc.
Tripod and triangle for heating.
Bunsen burner and tubing; or
Alcohol-lamp.
Test-tube holder, wood or wire.
Platinum foil.
Platinum wire in glass-rod handle.
Centrifuge (see p. 237).
Slides and cover-glasses.
Microscope (see p. 242).
Syracuse watch glasses, 6.
Glass tray for staining, $12'' \times 8'' \times 3''$.
Ultzmann polariscope.

THERMOMETRIC EQUIVALENTS

Cent.	Fahr.	Cent.	Fahr.	Cent.	Fahr.
110°	230°	41°	105.8°	31°	87.8°
100	212	40.5	104.9	30	86
95	203	40	104.0	25	77
90	194	39.5	103.1	20	68
85	185	39	102.2	15	59
80	176	38.5	101.3	10	50
75	167	38	100.4	+5	41
70	158	37.5	99.5	0	32
65	149	37	98.6	—5	23
60	140	36.5	97.7	—10	14
55	131	36	96.8	—15	+5
50	122	35.5	95.9	—17.8	0
45	113	35	95.0	—20	—4
44	111.2	34	93.2	0.54°	= 1°
43	109.4	33	91.4	1	= 1.8
42	107.6	32	89.6	2	= 3.6

RELATIONS OF ENGLISH TO METRIC SYSTEMS

1 grain	=	64.8	milligrams.
1 ounce	=	28.3	grams.
1 pound	=	453.6	"
1 gram	=	15.432	grains.
1 kilo	=	2	pounds, 3 ounces.
1 minim	=	0.059	cubic centimeter.
1 fluidram	=	3.5	cubic centimeters.
1 fluidounce	=	28.39	" "
1 pint	=	567.9	" "
1 cubic centimeter	=	16.9	minims.
1 liter	=	35.2	fluidounces.
1 inch	=	2.54	centimeters.
1 foot	=	30.48	"
1 yard	=	91.44	"
1 centimeter	=	0.39	inch.
1 meter	=	39.37	inches.

INDEX

Kelly and Cullen's Myomata of the Uterus

Myomata of the Uterus. By HOWARD A. KELLY, M. D., Professor of Gynecologic Surgery at Johns Hopkins University; and THOMAS S. CULLEN, M. B., Associate in Gynecology at Johns Hopkins University. Large octavo of about 700 pages, with 388 original illustrations by August Horn and Hermann Becker. Cloth, $7.50 net; Half Morocco, $9.00 net.

A MASTER WORK

ILLUSTRATED BY AUGUST HORN AND HERMANN BECKER

This monumental work, the fruit of over ten years of untiring labors, will remain for many years the last word upon the subject. Written by those men who have brought, step by step, the operative treatment of uterine myoma to such perfection that the mortality is now less than one per cent., it stands out as the record of greatest achievement of recent times.

The illustrations have been made with wonderful accuracy in detail by Mr. August Horn and Mr. Hermann Becker, whose superb work is so well known that comment is unnecessary. For painstaking accuracy, for attention to every detail, and as an example of the practical results accruing from the association of the operating amphitheater with the pathologic laboratory, this work will stand as an enduring testimonial.

Surgery, Gynecology, and Obstetrics

"It must be considered as the most comprehensive work of the kind yet published. It will always be a mine of wealth to future students."

New York Medical Journal

"Within the covers of this monograph every form, size, variety, and complication of uterine fibroids is discussed. It is a splendid example of the rapid progress of American professional thought."

Bulletin Medical and Chirurgical Faculty of Maryland

"Few medical works in recent years have come to our notice so complete in detail, so well illustrated, so practical, and so far reaching in their teaching to general practitioner, specialist, and student alike."

Cullen's Uterine Adenomyoma

Uterine Adenomyoma. By THOMAS S. CULLEN, M. D., Associate Professor of Gynecology, Johns Hopkins University. Octavo of 275 pages, with original illustrations by Hermann Becker and August Horn. Cloth, $5.00 net.

ILLUSTRATED BY BECKER AND HORN

Dr. Cullen's large clinical experience and his extensive original work along the lines of gynecologic pathology have enabled him to present his subject with originality and precision. The work gives the early literature on adenomyoma, traces the disease through its various stages, and then gives the detailed findings in a large number of cases personally examined by the author. Formerly the physician and surgeon were unable to determine the cause of uterine bleeding, but after following closely the clinical course of the disease, Dr. Cullen has found that the majority of these cases can be diagnosed clinically. The results of these observations he presents in this work.

The Lancet, London

"A good example of how such a monograph should be written. It is an excellent work, worthy of the high reputation of the author and of the school from which it emanates."

Cullen's Cancer of the Uterus

Cancer of the Uterus. By THOMAS S. CULLEN, M. B., Associate Professor of Gynecology, Johns Hopkins University. Large octavo of 693 pages, with over 300 colored and half-tone text-cuts and eleven lithographs. Cloth, $7.50 net; Half Morocco, $8.50 net.

Howard A. Kelly, M. D.

Professor of Gynecologic Surgery, Johns Hopkins University.

"Dr. Cullen's book is the standard work on the greatest problem which faces the surgical world to-day. Any one who desires to attack this great problem must have this book."

Ashton's Practice of Gynecology

The Practice of Gynecology. By W. EASTERLY ASHTON, M.D., LL.D., Professor of Gynecology in the Medico-Chirurgical College, Philadelphia. Handsome octavo volume of 1099 pages, containing 1058 original line drawings. Cloth, $6.50 net; Half Morocco, $8.00 net.

THE NEW (4th) EDITION

Four editions of this work have been demanded in as many years. Among the new additions are: Colonic lavage and flushing, Hirst's treatment for vaginismus, Dudley's treatment of cystocele, Montgomery's round ligament operation, Chorio-epithelioma of the Uterus, Passive Incontinence of the Urine, and Moynihan's methods in Intestinal Anastomosis. Nothing is left to be taken for granted, the author not only telling his readers in every instance what should be done but also precisely *how to do it.* A distinctly original feature of the book is the illustrations, numbering 1058 line drawings made especially under the author's personal supervision from actual apparatus, living models, and dissections on the cadaver.

From its first appearance Dr. Ashton's book set a standard in *practical* medical books; that he *has* produced a work of unusual value to the medical practitioner is shown by the demand for new editions.

Howard A. Kelly, M. D.,

Professor of Gynecologic Surgery, Johns Hopkins University

"It is different from anything that has as yet appeared. The illustrations are particularly clear and satisfactory. One specially good feature is the pains with which you describe so many *details* so often left to the imagination."

Charles B. Penrose, M. D.,

Formerly Professor of Gynecology, University of Pennsylvania.

"I know of no book that goes so thoroughly and satisfactorily into all the *details* of everything connected with the subject. In this respect your book differs from the others."

George M. Edebohls, M.D.

Professor of Diseases of Women, New York Post-Graduate Medical School.

"I have looked it through and must congratulate you upon having produced a text-book most admirably adapted to *teach* gynecology to those who must get their knowledge, even to the minutest and most elementary details, from books."

Bandler's Medical Gynecology

Medical Gynecology. By S. WYLLIS BANDLER, M. D., Adjunct Professor of Diseases of Women, New York Post-Graduate Medical School and Hospital. Octavo of 702 pages, with 150 original illustrations. Cloth, $5.00 net; Half Morocco, $6.50 net.

THE NEW (2d) EDITION

This new work by Dr. Bandler is just the book that the physician engaged in general practice has long needed. It is truly *the practitioner's gynecology*—planned for him, written for him, and illustrated for him. There are many gynecologic conditions that do not call for operative treatment; yet, because of lack of that special knowledge required for their diagnosis and treatment, the general practitioner has been unable to treat them intelligently. This work gives just the information the practitioner needs.

American Journal of Obstetrics

"He has shown good judgment in the selection of his data. He has placed most emphasis on diagnostic and therapeutic aspects. He has presented his facts in a manner to be readily grasped by the general practitioner."

Bandler's Vaginal Celiotomy

Vaginal Celiotomy. By. S. WYLLIS BANDLER, M.D., New York Post-Graduate Medical School and Hospital. Octavo of 450 pages, with 148 original illustrations. Cloth, $5.00 net.

RECENTLY ISSUED

The vaginal route, because of its simplicity, ease of execution, absence of shock, more certain results, and the opportunity for conservative measures, constitutes a field which should appeal to all surgeons, gynecologists, and obstetricians. Posterior vaginal celiotomy is of great importance in the removal of small tubal and ovarian tumors and cysts, and is an important step in the performance of vaginal myomectomy, hysterectomy, and hysteromyomectomy. Anterior vaginal celiotomy with thorough separation of the bladder is the only certain method of correcting cystocele. Dr. Bandler shows by original illustrations the various steps in these operations.

Kelly and Noble's Gynecology and Abdominal Surgery

Gynecology and Abdominal Surgery. Edited by HOWARD A. KELLY, M. D., Professor of Gynecology in Johns Hopkins University; and CHARLES P. NOBLE, M.D., Clinical Professor of Gynecology in the Woman's Medical College, Philadelphia. Two imperial octavo volumes of 900 pages each, containing 880 illustrations, mostly original. Per volume: Cloth, $8.00 net; Half Morocco, $9.50 net.

BOTH VOLUMES NOW READY

WITH 880 ORIGINAL ILLUSTRATIONS BY HERMANN BECKER AND MAX BRÖDEL

In view of the intimate association of gynecology with abdominal surgery the editors have combined these two important subjects in one work. For this reason the work will be doubly valuable, for not only the gynecologist and general practitioner will find it an exhaustive treatise, but the surgeon also will find here the latest technic of the various abdominal operations. It possesses a number of valuable features not to be found in any other publication covering the same fields. It contains a chapter upon the bacteriology and one upon the pathology of gynecology, dealing fully with the scientific basis of gynecology. In no other work can this information, prepared by specialists, be found as separate chapters. There is a large chapter devoted entirely to *medical gynecology*, written especially for the physician engaged in general practice. Heretofore the general practitioner was compelled to search through an entire work in order to obtain the information desired. *Abdominal surgery* proper, as distinct from gynecology, is fully treated, embracing operations upon the stomach, upon the intestines, upon the liver and bile-ducts, upon the pancreas and spleen, upon the kidney, ureter, bladder, and the peritoneum. Special attention has been given to *modern technic*. The illustrations are the work of *Mr. Hermann Becker* and *Mr. Max Brödel*.

American Journal of the Medical Sciences

"It is needless to say that the work has been thoroughly done: the names of the authors and editors would guarantee this; but much may be said in praise of the method of presentation, and attention may be called to the inclusion of matter not to be found elsewhere."

Webster's Diseases of Women

Diseases of Women. By J. CLARENCE WEBSTER, M. D. (EDIN.), F. R. C. P. E., Professor of Gynecology and Obstetrics in Rush Medical College. Octavo of 712 pages, with 372 illustrations. Cloth, $7.00 net; Half Morocco, $8.50 net.

FOR THE PRACTITIONER

Dr. Webster has written this work *especially for the general practitioner*, discussing the clinical features of the subject in their widest relations to general practice rather than from the standpoint of specialism. The magnificent illustrations, three hundred and seventy-two in number, are nearly all original. Drawn by expert anatomic artists under Dr. Webster's direct supervision, they portray the anatomy of the parts and the steps in the operations with rare clearness and exactness.

Howard A. Kelly, M.D., *Professor of Gynecologic Surgery, Johns Hopkins University.*

"It is undoubtedly one of the best works which has been put on the market within recent years, showing from start to finish Dr. Webster's well-known thoroughness. The illustrations are also of the highest order."

Webster's Obstetrics

A Text-Book of Obstetrics. By J. CLARENCE WEBSTER, M. D. (EDIN.), Professor of Obstetrics and Gynecology in Rush Medical College. Octavo of 767 pages, illustrated. Cloth, $5.00 net; Half Morocco, $6.50 net.

Medical Record, New York

"The author's remarks on asepsis and antisepsis are admirable, the chapter on eclampsia is full of good material, and . . . the book can be cordially recommended as a safe guide."

Hirst's Text-Book of Obstetrics

The New (6th) Edition

A Text-Book of Obstetrics. By BARTON COOKE HIRST, M. D., Professor of Obstetrics in the University of Pennsylvania. Handsome octavo, 992 pages, with 847 illustrations, 43 in colors. Cloth, $5.00 net; Half Morocco, $6.50 net.

INCLUDING RELATED GYNECOLOGIC OPERATIONS

Immediately on its publication this work took its place as the leading text-book on the subject. Both in this country and abroad it is recognized as the most satisfactorily written and clearly illustrated work on obstetrics in the language. The illustrations form one of the features of the book. They are numerous and the most of them are original. In this edition the book has been thoroughly revised. Recognizing the inseparable relation between obstetrics and certain gynecologic conditions, the author has included all the gynecologic operations for complications and consequences of childbirth, together with a brief account of the diagnosis and treatment of all the pathologic phenomena peculiar to women.

OPINIONS OF THE MEDICAL PRESS

British Medical Journal

"The popularity of American text-books in this country is one of the features of recent years. The popularity is probably chiefly due to the great superiority of their illustrations over those of the English text-books. The illustrations in Dr. Hirst's volume are far more numerous and far better executed, and therefore more instructive, than those commonly found in the works of writers on obstetrics in our own country."

Bulletin of Johns Hopkins Hospital

"The work is an admirable one in every sense of the word, concisely but comprehensively written."

The Medical Record, New York

"The illustrations are numerous and are works of art, many of them appearing for the first time. The author's style, though condensed, is singularly clear, so that it is never necessary to re-read a sentence in order to grasp the meaning. As a true model of what a modern text-book on obstetrics should be, we feel justified in affirming that Dr. Hirst's book is without a rival."

Hirst's Diseases of Women

A Text-Book of Diseases of Women. By BARTON COOKE HIRST, M. D., Professor of Obstetrics, University of Pennsylvania; Gynecologist to the Howard, the Orthopedic, and the Philadelphia Hospitals. Octavo of 745 pages, 701 illustrations, many in colors. Cloth, $5.00 net; Half Morocco, $6.50 net.

THE NEW (2d) EDITION
WITH 701 ORIGINAL ILLUSTRATIONS

The new edition of this work has just been issued after a careful revision. As diagnosis and treatment are of the greatest importance in considering diseases of women, particular attention has been devoted to these divisions. To this end, also, the work has been magnificently illuminated with 701 illustrations, for the most part original photographs and water-colors of actual clinical cases accumulated during the past fifteen years. The palliative treatment, as well as the radical operative, is fully described, enabling the general practitioner to treat many of his own patients without referring them to a specialist. The author's extensive experience renders this work of unusual value.

OPINIONS OF THE MEDICAL PRESS

Medical Record, New York

"Its merits can be appreciated only by a careful perusal. . . . Nearly one hundred pages are devoted to technic, this chapter being in some respects superior to the descriptions in many text-books."

Boston Medical and Surgical Journal

"The author has given special attention to diagnosis and treatment throughout the book, and has produced a practical treatise which should be of the greatest value to the student, the general practitioner, and the specialist."

Medical News, New York

"Office treatment is given a due amount of consideration, so that the work will be as useful to the non-operator as to the specialist."

GET THE BEST **American** THE NEW STANDARD

Illustrated Dictionary

Just Ready—The New (6th) Edition, Reset

The American Illustrated Medical Dictionary. A new and complete dictionary of the terms used in Medicine, Surgery, Dentistry, Pharmacy, Chemistry, Veterinary Science, Nursing, and all kindred branches; with over 100 new and elaborate tables and many handsome illustrations. By W. A. NEWMAN DORLAND, M.D., Editor of "The American Pocket Medical Dictionary." Large octavo, 975 pages, bound in full flexible leather. Price, $4.50 net; with thumb index, $5.00 net.

A KEY TO MEDICAL LITERATURE

Gives a Maximum Amount of Matter in a Minimum Space

ENTIRELY RESET—A NEW WORK, WITH ADDED FEATURES

We really believe that Dorland's Dictionary is the most useful single book that the medical practitioner can own. We are confident you will get more real use out of it than out of any one book you ever bought.

Nearly every medical paper to-day contains special words which are new to most readers. If *you* want to get the best out of your journals and text-books, Dorland's Dictionary should be on your desk for ready reference.

This new edition defines all the new words, and is a safe key to capitalization, pronunciation, and etymology.

PERSONAL OPINIONS

Howard A. Kelly, M. D.,

Professor of Gynecologic Surgery, Johns Hopkins University, Baltimore.

"Dr. Dorland's dictionary is admirable. It is so well gotten up and of such convenient size. No errors have been found in my use of it."

J. Collins Warren, M.D., LL.D., F.R.C.S. (Hon.)

Professor of Surgery, Harvard Medical School.

"I regard it as a valuable aid to my medical literary work. It is very complete and of convenient size to handle comfortably. I use it in preference to any other."

Penrose's Diseases of Women

Sixth Revised Edition

A Text-Book of Diseases of Women. By CHARLES B. PENROSE, M. D., PH. D., formerly Professor of Gynecology in the University of Pennsylvania; Surgeon to the Gynecean Hospital, Philadelphia. Octavo volume of 550 pages, with 225 fine original illustrations. Cloth $3.75 net.

UP TO DATE—ACCURATE

Regularly every year a new edition of this excellent text-book is called for, and it appears to be in as great favor with physicians as with students. Indeed, this book has taken its place as the ideal work for the general practitioner. The author presents the best teaching of modern gynecology, untrammeled by antiquated ideas and methods. In every case the most modern and progressive technique is adopted, and the main points are made clear by excellent illustrations.

Howard A. Kelly, M.D.,

Professor of Gynecologic Surgery, Johns Hopkins University, Baltimore.

"I shall value very highly the copy of Penrose's 'Diseases of Women' received. I have already recommended it to my class as THE BEST book."

Davis' Operative Obstetrics

Operative Obstetrics. By EDWARD P. DAVIS, M.D., Professor of Obstetrics at Jefferson Medical College, Philadelphia. Octavo of 463 pages, with 264 illustrations.

JUST READY

Dr. Davis' new work on Operative Obstetrics is a most practical one and no expense has been spared to make it the handsomest work on the subject, as well. Every step in every operation is described minutely, and the technic shown by beautiful new illustrations. Dr. Davis' name is sufficient guarantee for something above the ordinary.

Dorland's Modern Obstetrics

Modern Obstetrics: General and Operative. By W. A. NEWMAN DORLAND, A. M., M. D., Professor of Obstetrics at Loyola University, Chicago. Handsome octavo volume of 797 pages, with 201 illustrations. Cloth, $4.00 net.

Second Edition, Revised and Greatly Enlarged

In this edition the book has been entirely rewritten and very greatly enlarged. Among the new subjects introduced are the surgical treatment of puerperal sepsis, infant mortality, placental transmission of diseases, serum-therapy of puerperal sepsis, etc.

Journal of the American Medical Association

"This work deserves commendation, and that it has received what it deserves at the hands of the profession is attested by the fact that a second edition is called for within such a short time. Especially deserving of praise is the chapter on puerperal sepsis."

Davis' Obstetric and Gynecologic Nursing

Obstetric and Gynecologic Nursing. By EDWARD P. DAVIS, A. M., M. D., Professor of Obstetrics in the Jefferson Medical College and Philadelphia Polyclinic; Obstetrician and Gynecologist, Philadelphia Hospital. 12mo of 436 pages, illustrated. Buckram, $1.75 net.

THIRD REVISED EDITION

This volume gives a very clear and accurate idea of the manner to meet the conditions arising during obstetric and gynecologic nursing. The third edition has been thoroughly revised.

The Lancet, London

"Not only nurses, but even newly qualified medical men, would learn a great deal by a perusal of this book. It is written in a clear and pleasant style, and is a work we can recommend."

Garrigues' Diseases of Women — Third Edition

A TEXT-BOOK OF DISEASES OF WOMEN. By HENRY J. GARRIGUES, A. M., M. D., Gynecologist to St. Mark's Hospital and to the German Dispensary, New York City. Handsome octavo, 756 pages, with 367 engravings and colored plates. Cloth, $4.50 net; Half Morocco, $6.00 net.

Thad. A. Reamy, M. D., *Professor of Gynecology, Medical College of Ohio.*

"One of the best text-books for students and practitioners which has been published in the English language; it is condensed, clear, and comprehensive. The profound learning and great clinical experience of the distinguished author find expression in this book."

Macfarlane's Gynecology for Nurses

A REFERENCE HAND-BOOK OF GYNECOLOGY FOR NURSES. By CATHARINE MACFARLANE, M. D., Gynecologist to the Woman's Hospital of Philadelphia. 32mo of 150 pages, with 70 illustrations. Flexible leather, $1.25 net.

A. M. Seabrook, M. D., *Woman's Medical College of Philadelphia.*

"It is a most admirable little book, covering in a concise but attractive way the subject from the nurse's standpoint."

American Text-Book of Gynecology — Second Edition

AMERICAN TEXT-BOOK OF GYNECOLOGY. Edited by J. M. BALDY, M. D. Imperial octavo of 718 pages, with 341 text-illustrations and 38 plates. Cloth, $6.00 net.

American Text-Book of Obstetrics — Second Edition

THE AMERICAN TEXT-BOOK OF OBSTETRICS. In two volumes. Edited by RICHARD C. NORRIS, M. D.; Art Editor, Robert L. Dickinson, M. D. Two octavos of about 600 pages each; nearly 900 illustrations, including 49 colored and half-tone plates. Per volume: Cloth, $3.50 net.

Matthew D. Mann, M. D.,

Professor of Obstetrics and Gynecology, University of Buffalo.

"I like it exceedingly and have recommended the first volume as a text-book. It is certainly a most excellent work. I know of none better."

Schäffer and Webster's Operative Gynecology

Atlas and Epitome of Operative Gynecology. By DR. O. SCHÄFFER, of Heidelberg. Edited, with additions, by J. CLARENCE WEBSTER, M. D. (EDIN.), F. R. C. P. E., Professor of Obstetrics and Gynecology in Rush Medical College, in affiliation with the University of Chicago. 42 colored lithographic plates, many text-cuts, a number in colors, and 138 pages of text. *In Saunders' Hand-Atlas Series.* Cloth, $3.00 net.

Much patient endeavor has been expended by the author, the artist, and the lithographer in the preparation of the plates for this Atlas. They are based on hundreds of photographs taken from nature, and illustrate most faithfully the various surgical situations. Dr. Schäffer has made a specialty of demonstrating by illustrations.

Medical Record, New York

"The volume should prove most helpful to students and others in grasping details usually to be acquired only in the amphitheater itself."

DeLee's Obstetrics for Nurses

Obstetrics for Nurses. By JOSEPH B. DE LEE, M. D., Professor of Obstetrics in the Northwestern University Medical School, Chicago; Lecturer in the Nurses' Training Schools of Mercy, Wesley, Provident, Cook County, and Chicago Lying-In Hospitals. 12mo of 512 pages, fully illustrated.

Cloth, $2.50 net.

THE NEW (3d) EDITION

While Dr. DeLee has written his work especially for nurses, the practitioner will also find it useful and instructive, since the duties of a nurse often devolve upon him in the early years of his practice. The illustrations are nearly all original and represent photographs taken from actual scenes. The text is the result of the author's many years' experience in lecturing to the nurses of five different training schools.

J. Clifton Edgar, M. D.,

Professor of Obstetrics and Clinical Midwifery, Cornell University, New York.

"It is far and away the best that has come to my notice, and I shall take great pleasure in recommending it to my nurses, and students as well."

Schäffer and Edgar's Labor and Operative Obstetrics

Atlas and Epitome of Labor and Operative Obstetrics. By DR. O. SCHÄFFER, of Heidelberg. *From the Fifth Revised and Enlarged German Edition.* Edited, with additions, by J. CLIFTON EDGAR, M. D., Professor of Obstetrics and Clinical Midwifery, Cornell University Medical School, New York. With 14 lithographic plates in colors, 139 other illustrations, and 111 pages of text. Cloth, $2.00 net. *In Saunders' Hand-Atlas Series.*

This book presents the act of parturition and the various obstetric operations in a series of easily understood illustrations, accompanied by a text treating the subject from a practical standpoint.

American Medicine

"The method of presenting obstetric operations is admirable. The drawings, representing original work, have the commendable merit of illustrating instead of confusing."

Schäffer and Edgar's Obstetric Diagnosis and Treatment

Atlas and Epitome of Obstetric Diagnosis and Treatment. By DR. O. SCHÄFFER, of Heidelberg. *From the Second Revised German Edition.* Edited, with additions, by J. CLIFTON EDGAR, M. D., Professor of Obstetrics and Clinical Midwifery, Cornell University Medical School, N. Y. With 122 colored figures on 56 plates, 38 text-cuts, and 315 pages of text. Cloth, $3.00 net. *In Saunders' Hand-Atlas Series.*

This book treats particularly of obstetric operations, and, besides the wealth of beautiful lithographic illustrations, contains an extensive text of great value. This text deals with the practical, clinical side of the subject.

New York Medical Journal

"The illustrations are admirably executed, as they are in all of these atlases, and the text can safely be commended, not only as elucidatory of the plates, but as expounding the scientific midwifery of to-day."

American Pocket Dictionary

Just Ready
New (7th) Edition

THE AMERICAN POCKET MEDICAL DICTIONARY. Edited by W. A. NEWMAN DORLAND, A. M., M.D. With 610 pages. Full leather, limp, with gold edges, $1.00 net; with patent thumb index, $1.25 net.

James W. Holland, M. D.,
Professor of Chemistry and Toxicology, at the Jefferson Medical College, Philadelphia.

"I am struck at once with admiration at the compact size and attractive exterior I can recommend it to our students without reserve."

Cragin's Gynecology

New (7th) Ed.

ESSENTIALS OF GYNECOLOGY. By EDWIN B. CRAGIN, M. D., Professor of Obstetrics, College of Physicians and Surgeons, New York. Crown octavo, 240 pages, 62 illustrations. Cloth, $1.00 net. *In Saunders' Question-Compend Series.*

Galbraith's Four Epochs of Woman's Life

Second Edition

THE FOUR EPOCHS OF WOMAN'S LIFE: A STUDY IN HYGIENE. Maidenhood, Marriage, Maternity, Menopause. By ANNA M. GALBRAITH, M. D. With an Introductory Note by JOHN H. MUSSER, M. D., University of Pennsylvania. 12mo of 247 pages. Cloth, $1.50 net.

Schaffer and Norris' Gynecology

Saunders' Atlases

ATLAS AND EPITOME OF GYNECOLOGY. By DR. O. SCHAFFER, of Heidelberg. Edited, with additions, by RICHARD C. NORRIS, A. M., M. D., Assistant Professor of Obstetrics, University of Pennsylvania. 207 colored illustrations on 90 plates, 65 text-cuts, and 272 pages of text. Cloth, $3.50 net.

Ashton's Obstetrics

New (7th) Ed.

ESSENTIALS OF OBSTETRICS. By W. EASTERLY ASHTON, M. D., Professor of Gynecology in the Medico-Chirurgical College, Philadelphia. Crown octavo, 252 pages, 109 illustrations. Cloth, $1.00 net. *In Saunders' Question-Compend Series.*

Southern Practitioner

"An excellent little volume, containing correct and practical knowledge. An admirable compend, and the best condensation we have seen."

Barton and Wells' Medical Thesaurus

A THESAURUS OF MEDICAL WORDS AND PHRASES. By WILFRED M. BARTON, M. D., Assistant to Professor of Materia Medica and Therapeutics, Georgetown University, Washington, D. C.; and WALTER A. WELLS, M. D., Demonstrator of Laryngology, Georgetown University, Washington, D. C. 12mo of 534 pages. Flexible leather, $2.50 net; with thumb index, $3.00 net.