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The Medical Epitome Series

CLINICAL DIAGNOSIS AND URINALYSIS

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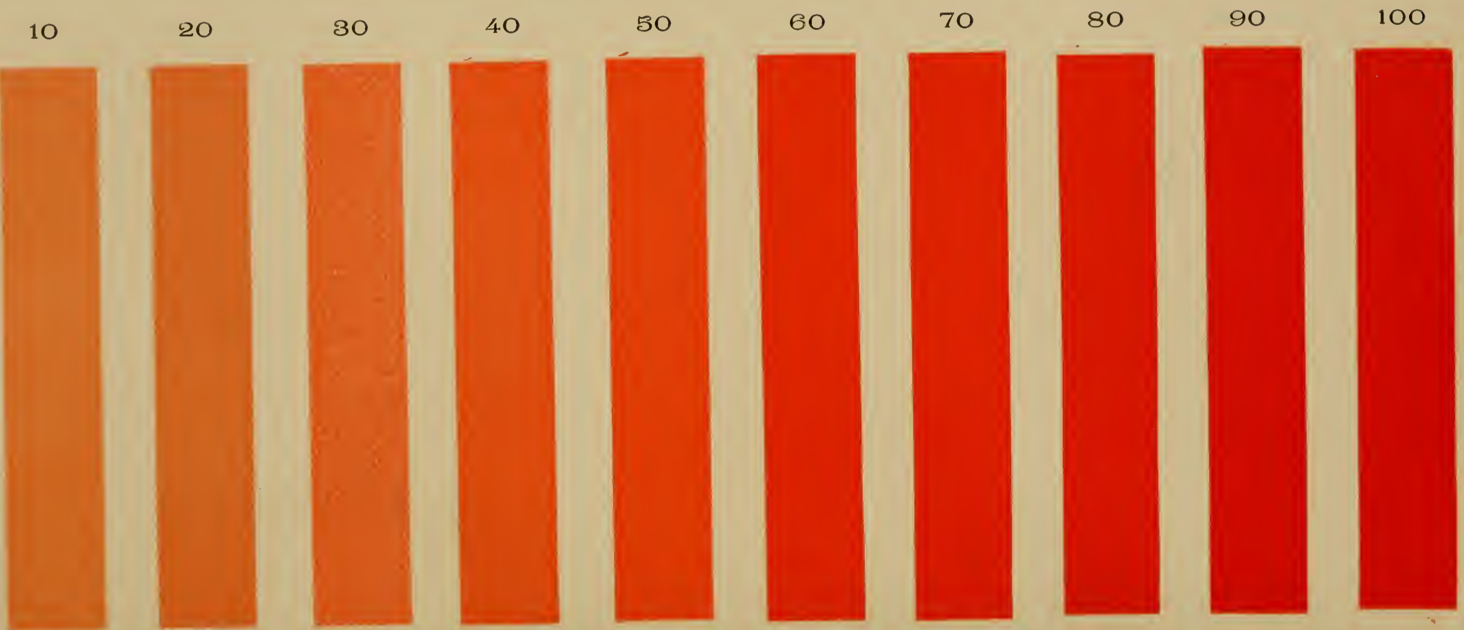

Tallquist's color scale for estimating Hemoglobin.

The Medical Epitome Series.

CLINICAL DIAGNOSIS AND URINALYSIS.

A MANUAL FOR STUDENTS AND PRACTITIONERS.

BY

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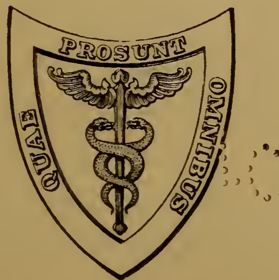
ILLUSTRATED WITH 79 ENGRAVINGS AND 1 COLORED PLATE.



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AUTHOR'S PREFACE.

THE great importance of laboratory examinations in all branches of medicine and surgery has come to be universally recognized; but the ability to put the methods into practice, and to derive the information they can give, has not yet been as widely acquired by the profession. This brief volume, covering the essentials, is intended to serve the needs of physicians and students rather than those of experts.

The practical side of laboratory work in its relation to the diagnosis of disease is emphasized. Many of the time-consuming quantitative analyses are intentionally omitted or merely mentioned. To be of value they should be made by experienced chemists. An attempt is made to explain fully the most important tests and procedures, and to anticipate many of the difficulties and mistakes of the inexperienced worker. No claim is made for originality or completeness. Many of the standard works have been freely consulted—such as Simon, Von Jaksch, Nichols, Ewing, Cabot, Musser, DaCosta, Vierordt, Purdy, Peyer, Osler, and others.

Numerous practical suggestions also have been obtained from the work in the clinical laboratory of the University of Michigan as instituted by Drs. Dock and Cowie. I am greatly indebted to Mr. Charles L. Bliss, late instructor in physiological chemistry in the University of Michigan, now government physiological chemist at Manila, for very valuable aid in the preparation of the section on Urinalysis.

J. R. A.

DENVER, COLORADO.

EDITOR'S PREFACE.

In arranging for the editorship of *The Medical Epitome Series* the publishers established a few simple conditions, namely, that the Series as a whole should embrace the entire realm of medicine; that the individual volumes should authoritatively cover their respective subjects in all essentials; and that the maximum amount of information, in letterpress and engravings, should be given for a minimum price. It was the belief of publishers and editor alike that brief works of high character would render valuable service not only to students, but also to practitioners who might wish to refresh or supplement their knowledge to date.

To the authors the editor extends his heartiest thanks for their excellent work. They have fully justified his choice in inviting them to undertake a kind of literary task which is always difficult—namely, the combination of brevity, clearness, and comprehensiveness. They have shown a consistent interest in the work and an earnest endeavor to coöperate with the editor throughout the undertaking. Joint effort of this sort ought to yield useful books, brief manuals as contradistinguished from mere compends.

In order to render the volumes suitable for quizzing, and yet preserve the continuity of the text unbroken by the interpolation of questions throughout the subject-matter, which has heretofore been the design in books of this type, all questions have been placed at the end of each chapter. This new arrangement, it is hoped, will be convenient alike to students and practitioners.

V. C. P.

NEW YORK, 1905.

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CLINICAL DIAGNOSIS AND URINALYSIS.

CHAPTER I.

GENERAL CONSIDERATIONS.

EQUIPMENT AND SCOPE OF WORK.

In purchasing laboratory supplies one should deal with an absolutely reliable firm. Because of the technical skill and experience required in the preparation of some of the solutions and stains, it is well for beginners to purchase them already prepared. This statement refers especially to the decinormal sodium hydrate solution and the like, and such stains as Wright's, Gram's, and Ehrlich's triacid stains.

Grübler's stains (powders) have the reputation of being the best on the market.

Laboratory tables should be painted black.

Laboratory diagnosis includes examination of the blood, stomach-contents, sputum, feces, urine, transudates and exudates, and of the various secretions and excretions of the body.

Special apparatus and reagents are required for some of this work, but the same apparatus may be employed in many of the foregoing subdivisions.

Among the **general utility articles** are to be mentioned the following:

Microscope (triple nose-piece, 2 eye-pieces, 3 objectives)—*i. e.*, oil-immersion and high and low dry lens.

Thermostat.

Glass rods, different sizes.

Glass tubing, different sizes.

Filter-paper, 4 inch, 6 inch, 8 inch, etc.

Funnels, different sizes (2 inch, 4 inch, etc.).
Graduates, different sizes (100 c.c., 500 c.c., 1000 c.c.).
Centrifuge.
Two-gallon syphon bottle with distilled water.
Teasing needles.
Platinum loop.
Cover-glass forceps.
File.
Canada balsam (for mounting permanent specimens; keep in wide-mouthed bottle).
Turpentine.
Cedar oil.
Xylol.
Glass slides—white, not green glass.

Cover-slips are sold in four thicknesses, Nos. 0, 1, 2, 3. No. 1 is best for general work, since it is thin enough for use with the oil-immersion lens, and is not so easily broken as No. 0. The *square* $\frac{3}{4}$-inch cover-glass should be used.

Cleaning Glassware.—In cleaning glassware that has never been used, Cabot, after a long experience with various chemicals, has given them up, and uses nothing but soap and water, with thorough polishing.

The glassware may be placed in acetic acid for twenty-four hours, more or less (if in a hurry, a minute will do), then thoroughly washed in water and transferred to a large-mouthed bottle containing alcohol. When wanted, it is dried and polished with a clean soft cloth.

The following **cleaning fluid for glassware** is recommended by Nichols as especially useful for glassware that has been soiled:

Potassium bichromate,	10 parts;
Sulphuric acid (commercial),	10 "
Water,	100 "

The glassware is left in this fluid for twenty-four hours, then thoroughly rinsed with water and transferred to alcohol, to be used as wanted.

Carbol Fuchsin.—

Fuchsin (S.),	1 part;
Absolute alcohol,	10 parts;
5 per cent. solution of carbolic acid,	100 "

Löffler's Methylene-blue.—

Concentrated alcoholic solution of methylene-blue,	30 parts;
1 : 10,000 aqueous solution of potassium hydrate,	100 "

Gram's method requires the following solutions:

1. *Aniline Water.*—Prepare by adding aniline oil to 10 c.c. of distilled water, drop by drop, shaking thoroughly after the addition of each drop, until the solution becomes opaque. Filter through moistened filter-paper.

2. *Aniline Water Gentian-violet.*—Treat the foregoing solution with 10 c.c. of absolute alcohol and 11 c.c. of a concentrated alcoholic solution of gentian-violet. This combined solution keeps only a few days, and should, therefore, be made up fresh.

3. *Lugol's Solution.*—

Iodine,	1 part;
Potassium iodide,	2 parts;
Water,	300 "

Gram's method **is employed for** the differentiation of certain bacteria, especially the *gonococcus*, and for staining the capsule of *diplococcus pneumoniæ* and other germs.

Application of Gram's Method.—1. Cover spread with aniline water gentian-violet (made within two weeks) and heat to steaming-point.

2. Wash in water.

3. Cover with Lugol's solution for one-half to two minutes to decolorize.

4. Rinse in 95 per cent. alcohol until the violet color disappears to the naked eye.

5. Wash in water and mount.

When thus treated, certain **bacteria retain the stain**, such as

diplococcus pneumoniæ, diphtheria bacillus, tubercle bacillus, anthrax bacillus, streptococci and staphylococci.

The **bacteria which become decolorized** are the following: gonococcus, typhoid bacillus, colon bacillus, influenza bacillus, and cholera spirillum.

Fat-detection by Sudan III.—*Test-solution.*—Make a saturated alcoholic solution of Sudan III. Let stand for several days; then mix 1 part of this solution with 1 part of alcohol and 1 part of water. The mixture is at once turbid, but clears on standing. Sudan III. stains fat red and leaves everything else unstained. Do not treat specimens with alcohol or ether before or after staining.

Test.—A drop or two of the solution is run under the cover-glass covering the specimen; under the microscope the neutral fat is seen to take on a red color.

Iodine Test for Starch.—Starch in granules or in solution strikes a deep-blue color in the presence of iodine. The **stock test-solution** is Lugol's. A drop or two of this solution is allowed to run under the cover-glass covering the specimen. In testing liquids for the presence of starch, dilute a few drops of this solution with water to a light-yellow color, and add a few drops of the suspected fluid. A deep blue indicates starch, a deep brown indicates **erythrodextrin.**

Glycogen granules treated by the first method turn a deep mahogany brown under the microscope.

Iodide Test for Starch.—Starch does not react to iodides in combination, but on setting free the iodine with nitric acid a deep-blue color develops.

Technic.—A strip of starch-paper is moistened with the fluid to be tested and then touched with a drop of nitric acid. The characteristic blue color develops in the presence of iodides.

Ethereal Extracts.—In certain tests the watery solution of the substance is shaken with ether or chloroform in order to extract the substance from the water. On standing the two fluids separate. Either the water or the ether may be removed by means of a pipette. Another method is to place the mixture in a filter which has been previously moistened

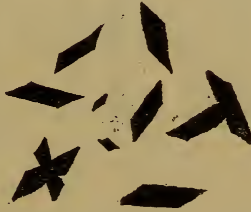
with water. The watery portion will then filter through. If ether is used for moistening the filter, the ethereal portion will filter through.

Guaiacum Test for Blood.—To 4 or 5 c.c. of tincture of guaiacum add from $\frac{1}{3}$ to $\frac{1}{2}$ as much hydrogen peroxide, or an equal amount of "ozonized" turpentine (expose turpentine to light and air for a long time). The suspected fluid—in a long glass tube placed at the bottom of a test-tube—is allowed to run slowly out and underlie the reagent. At the junction of the two liquids a robin's egg blue layer forms, either immediately or in the course of a few minutes, if hæmoglobin is present.

Iodides and iodine also give a blue color.

Hæmin Test for Blood.—A drop of a 0.6 per cent. salt solution is evaporated on a slide. A small bit of the suspected material, well teased, is placed upon the layer of crystallized salt. Place a cover-slip over it, and allow glacial acetic acid to run in under the slip, filling the spaces. Heat carefully (three-quarters to one minute) till bubbles of gas begin to form

Fig. 1.



Hæmin crystals.

beneath the cover. During evaporation glacial acetic acid is further added, drop by drop, from the edge of the slip, until a faint reddish-brown tint appears. Now hold specimen farther away from the flame, and slowly evaporate the last traces of acid. Add a drop of glycerin and examine under the microscope. If blood is present, hæmin (or Teichmann) crystals form, in the shape of light- or dark-brown rhombic plates or columns. The size of the crystals varies with the manner in which they are produced. The more slowly the acetic acid is evaporated the larger the crystals.

Weights and Measures.

1 kilogram	= exactly	15,433.6 grains. 2.679 pounds Troy. 2.205 pounds avoirdupois.	= approximately	$2\frac{2}{3}$ pounds Troy. $2\frac{1}{5}$ pounds avoirdupois.
1 gram	= exactly	15.4336 grains or approximately		$\frac{1}{4}$ drachm.
1 centigram	= "	0.1543 " "		$\frac{1}{6}$ grain.
1 milligram	= "	0.0154 " "		$\frac{1}{60}$ "
1 ounce Troy	= exactly	31.1009 grams or approximately		31 grams.
1 drachm	= "	3.8876 " "		4 "
1 grain	= "	0.0648 " "		6 centigrams.
1 litre	= exactly	33.8682 fluidounces or		2.1168 pints.
1 millilitre or 1 cubic centimetre	= "	16.2567 minims.		
1 pint	= exactly	472.4736 cubic centimetres.		
1 fluidounce	= "	29.5296 " "		
1 minim	= "	0.0615 " "		
1 metre	= exactly	39.37 inches or approximately		40 inches.
1 centimetre	= "	0.3937 " "		$\frac{2}{5}$ inch.
1 millimetre	= "	0.0394 " "		$\frac{1}{25}$ "
1 micromillimetre (μ)	= "	0.000039 " "		$\frac{1}{25000}$ inch.
1 inch	= exactly	0.0254 meter or approximately		25 millimetres.

It is often desirable to convert *Centigrade* (C.) into *Fahrenheit* (F.). The following formula will be found useful:

$$C. = \frac{5\,(F. - 32)}{9} \qquad F. = \frac{9\,C.}{5} + 32.$$

QUESTIONS.

Mention the various methods of cleansing glassware.
Describe the method of preparing carbol-fuchsin and Löffler's methylene-blue.
Describe Gram's stain, its practical application, and its special value.
Describe a method for the detection of fat.
What is the iodine test for starch?
What is meant by ethereal extracts?
Describe the guaiacum and hæmin tests for blood.
Give the formulas for converting Fahrenheit into Centigrade, and *vice versa.*

CHAPTER II.

BLOOD.

THE TECHNIC OF BLOOD-WORK—PRACTICAL SUGGESTIONS.

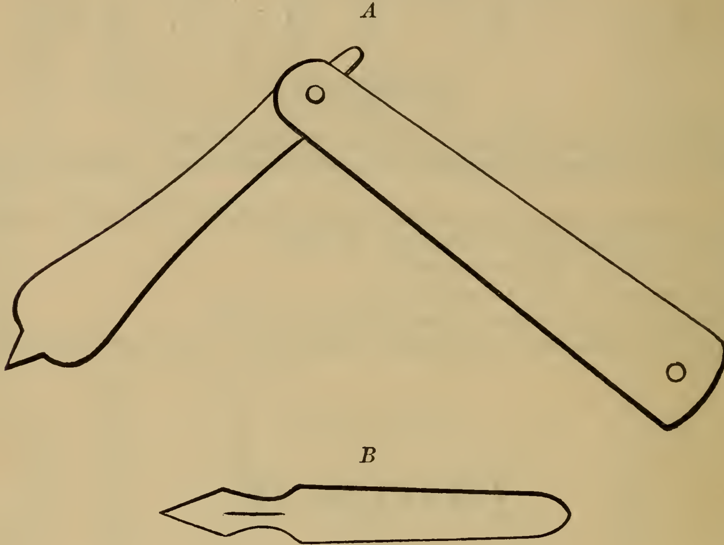
Blood-stickers.—The **German blood-sticker,** illustrated in Fig. 2, is the most satisfactory made; it costs 50 cents. The **pen-sticker** costs 5 cents and is very good. A pen with one nib broken is a useful substitute. The **Hagerdorn needle** is also used by many.

Cleaning Blood Pipettes.—In hospitals and laboratories where a great deal of blood-work is done an **aspirator** (Fig. 3) will be found extremely useful.

The cold-water faucet is threaded and the appliance screwed on. A rather large bottle with a doubly perforated rubber cork is attached, on one side by *noncollapsible* rubber tubing to the aspirator, and on the other to the blood-counter. The water is turned on, and its rush through the aspirator produces a suction which is felt in the blood-counter. This instrument is placed for a few seconds in a bottle of dilute acetic acid,

then in alcohol, and finally in ether, and these fluids sucked through it, thereby thoroughly cleaning and drying it. It is

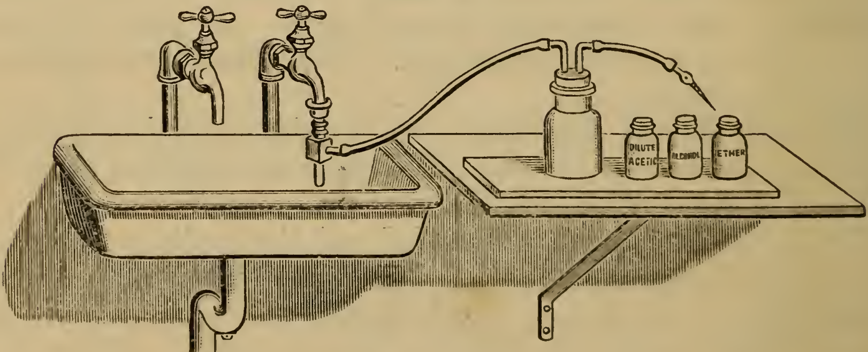
Fig. 2.



convenient to keep these four bottles grouped together in a rack.

Common Method of Cleaning a Blood Pipette.—Remove the rubber tubing attached to the blood-counter. Apply the

Fig. 3.



mouth and suck dilute acetic acid, alcohol, and ether through the pipette, thus cleaning and drying it.

The dilute acetic acid and alcohol may be forced out by blowing through the pipette, but the ether must be expelled by forcible, downward, jerking movements of the hand, since moisture would be forced into it by the mouth. If clean and thoroughly dry, the glass ball in the bulb will roll about freely on moving the pipette. Compressed air furnishes a handy means for drying the pipette.

Coagulated Blood in Pipette.—This accident occasionally happens to beginners because of slow manipulation. In order to remove the coagulum do not insert a needle or pen into the lumen, as there is danger of breaking off the tip of the instrument. It is best to place the pipette in a test-tube filled with sulphuric acid and allow it to digest the coagulum for twenty-four to forty-eight hours. Then pursue the ordinary method of cleaning. If this is not successful, as sometimes happens, break up the coagulum by the careful insertion of a fine hypodermatic wire. Even with careful routine cleansing a film of coagulated albumin is apt to adhere. This should occasionally be removed by cleansing with sulphuric acid.

Rubber Tubing for Blood-counters.—Small rubber catheters are much better than the rubber tubing supplied with the blood pipettes. When **thickening of Canada balsam** occurs, it should be thinned with xylol.

Cleansing Lenses.—Cedar oil should be removed from the oil-immersion lens immediately after using by touching it with filter-paper or rice-paper. It frequently happens that cedar oil dries on the objective, obscuring the field of vision. It may be removed by rubbing the lens with a soft cloth or absorbent cotton moistened with either alcohol or xylol. A camel's-hair brush is useful for dusting slides and cover-slips.

Diluting Fluids for Counting Red Corpuscles.—

Toisson's:

Methyl-violet, 5 B,	0.025 gm.;
Sodii chlor.,	1.000 gm.;
Sodii sulph.,	8.000 gm.;
Neutral glycerin,	30.000 cm.;
Aqua dest.,	160.000 cm.

Gowers':

Sodii sulph.,	gr. cxij;
Acid. acet.,	ʒv;
Aqua dest.,	℥iv.

Hayem's:

Hydrarg. bichlor.,	0.5 gm.;
Sodii sulph.,	5.0 gm.;
Sodii chlor.,	1.0 gm.;
Aqua dest.,	200.0 gm.

Toisson's fluid is most commonly used, but is dirty and deposits considerable sediment. *It should be filtered.* Its only advantage is that it stains the white corpuscles and makes it possible to count them along with the red cells. However, if one has a white counter, the cells are best counted separately.

Diluting Fluid for Counting Leukocytes.—0.3 to 0.5 per cent. of glacial acetic acid in distilled water.

Wright's Stain.—(1) 0.5 per cent. solution of sodium bicarbonate. Dissolve thoroughly.

(2) To (1) add 1 per cent. of methylene-blue (Grübler's B X or Ehrlich's).

(3) Steam (2) 1 hour in steam sterilizer.

(4) Cool mixture.

(5) Pour into large flask and add to it, stirring, enough 1 : 1000 watery solution of eosin (Grübler yellow, water-soluble) until the mixture becomes purple in color, and a yellow metallic scum forms on the surface and a finely granular precipitate appears in suspension. This takes about 500 c.c. of eosin solution to 100 c.c. of alkaline solution.

(6) Filter (5); do *not* wash; let filter dry and collect dry powder.

(7) With powder make a saturated solution in methyl alcohol—*i. e.*, 0.3 gram in 100 c.c. of methyl alcohol.

(8) Filter (7) and add to filtrate 25 per cent. of methyl alcohol. This is the staining fluid.

The Ehrlich Tricolor Mixture.—

Saturated watery solution of orange-green,	6 c.c.;
Saturated watery solution of acid fuchsin,	4 c.c.

Mix these and then add a few drops at a time, shaking between each addition,

Saturated watery solution of methyl-green, 6.6 c.c.;

Then add:

Glycerin,	5.0 c.c.;
Absolute alcohol,	10.0 c.c.;
Water,	15.0 c.c.

Shake well for one to two minutes. Let stand for twenty-four hours. *Do not filter.*

This formula is suggested by Cabot, though there are many other methods of making it.

Eosin.—

Eosin,	0.5 gram;
Alcohol (70 per cent.),	100.0 c.c.

Hæmatoxylon (Delafield's).—To 400 c.c. of a saturated solution of ammonium alum add 4 grammes of hæmatoxylon crystals dissolved in 25 c.c. of strong alcohol. Leave this exposed to the light and air in an unstoppered bottle for three or four days. Filter and add 100 c.c. of methyl alcohol. Allow the solution to stand until the color is sufficiently dark. Then filter and keep in tightly stoppered bottles. The stain should ripen for at least two months before using.

Eosin and Methylene-blue.—(*a*) A saturated alcoholic solution of Ehrlich's blood eosin. (*b*) A saturated watery solution (1 per cent.) of Ehrlich's rectified methylene-blue. The latter should be at least one week old.

After several weeks, methylene-blue in solution diminishes

in staining power, while the alcoholic eosin absorbs water and becomes less selective and more powerful.

Ehrlich's Dahlia Solution.—

Absolute alcohol,	50.0 c.c.;
Glacial acetic acid,	12.5 c.c.;
Distilled water,	100.0 c.c.
Add dahlia to saturation.	

Iodophilia Mixture.—

Iodi. sublim.,	1 gm.;
Pot. iodidi,	3 gm.;
Aq. dest.,	100 c.c.
Acacia ad syrupum.	

Nocht-Romanowsky Stain for Malarial and other Parasites.—
1. To 1 ounce of polychrome methylene-blue (Grübler) add 5 drops of a 3 per cent. solution of acetic acid (U. S. P., 33 per cent.).

2. Make a saturated 1 per cent. watery solution of methylene-blue, preferably Ehrlich's (Grübler) or Koch's, dissolving the dye by gentle heat. This solution improves with age, and should be at least one week old.

3. Make a 1 per cent. watery solution of Grübler's *watery* eosin.

The mixture is prepared as follows: To 10 c.c. of water add 4 drops of the eosin solution, 6 drops of neutralized polychrome blue, and 2 drops of a 1 per cent. methylene-blue solution, mixing well. The specimens may be fixed either in alcohol or by heat, and are immersed, specimen side downward, for one to three hours, and will not overstain in twenty-four hours.

THE BLOOD AS A WHOLE.

The blood as a whole **consists of** a fluid portion, the **plasma,** in which are suspended cells of three kinds: **red blood-cor-**

puscles (erythrocytes), white blood-corpuscles (leukocytes), and **blood-plates.**

Total Volume.—Most text-books give the total volume of the blood as about one-thirteenth of the body weight. If the recent researches of Haldane and Smith prove to be trustworthy, this deeply rooted idea will be modified. Smith estimates the average volume of blood in health as 3240 c.c., or 3420 grammes. In the 14 normal cases studied, the blood-mass varied from 2830 to 4550 grammes, or one-thirtieth to one-sixteenth of the body weight. A correct knowledge of the total blood volume would be of remarkable clinical value in diagnosing an anæmia and plethora. The methods employed by these observers will be referred to in another chapter. Unfortunately they are not sufficiently easy of application to be put into general use.

Color.—The red color of the blood is due to an albuminous iron-containing substance called *hœmoglobin*, which is combined in the red blood-corpuscles. It has an extremely strong affinity for oxygen, combining with it to form oxyhæmoglobin. The excess of oxyhæmoglobin in the arterial blood over that in the venous blood gives the former its bright-red color and the latter its bluish appearance. The hæmoglobin of normal blood, represented by its red color, is arbitrarily placed at 100 per cent. The varying color of blood in disease is dependent upon the amount of hæmoglobin and oxyhæmoglobin present; a great excess of the leukocytes, however, tends to give the blood a whitish or creamy appearance, such as is seen in leukæmia.

Reaction.—The reaction of the blood during life is alkaline, owing to the presence of disodium phosphate (Na_2HPO_4) and sodium carbonate. It is lower in women and children than in men, and is influenced by physiological processes, such as digestion, exercise, etc. In a large number of pathological conditions it is diminished, such as the prolonged use of acids, and in pernicious anæmia, leukæmia, nephritis, diabetes, hepatic disease, carcinoma, high fevers, and toxic conditions. An increase in the alkalinity is brought about by the prolonged use of alkalies and by cold baths.

Coagulation.—The most striking phenomenon which the blood exhibits on exposure to the air is the formation of a clot. If a bit of this clot be examined microscopically, it will be found to consist of a dense network of fibres which is filled with blood-corpuscles. These may be washed out, leaving the fibrin network intact.

Fibrin belongs to the class of the so-called coagulated albumins, and probably does not occur in the circulating blood, but is formed during the process of coagulation.

Blood-serum is the straw-colored fluid which separates from the clot during the process of coagulation. It differs from blood-plasma in the absence of fibrinogen and the presence of large quantities of fibrin-ferment and traces of fibrinoglobulin in the blood-serum.

In *the plasma* the following albumins are found: fibrinogen, serum-globulin, and serum-albumin.

Agglutinins are very important constituents of the blood-serum; they are susceptible to examination, and furnish valuable clinical information, as is seen in the Widal test in typhoid fever. They appear in the blood-serum as a result of bacterial infection, each variety of germ elaborating a special agglutinin, which has a specific agglutinating effect only upon that species of germs.

Pigment-granules are sometimes present in the plasma, as in malaria, melanosis, and sometimes in Addison's disease.

Hæmoglobin occasionally passes into solution in the blood-plasma—*i. e.*, *hæmoglobinæmia.*

The following additional substances are found in the blood: fats, soaps, cholesterin, sugar, glycogen, and at times lecithin. Numerous extractives have been found in the blood, such as urea, uric acid, kreatin, carbamic acid, sarcolactic acid, hippuric acid, and, under pathological conditions, xanthin, hypoxanthin, paraxanthin, adenin, guanin, leucin, tyrosin, lactic acid, cellulose, β-oxybutyric acid, acetone, and biliary constituents. These numerous substances are of practically no clinical importance.

Chemical analysis of the blood shows the following com-

position, calculated for 1000 parts, according to the table of C. Schmidt:

	Man.	Woman.
Corpuscles	513.01	369.20
Water	349.70	272.60
Hæmoglobin and globulins	159.60	120.10
Mineral salts	3.70	3.55
Plasma	486.90	603.80
Water	439.00	552.00
Fibrin	3.90	1.91
Albumin and **extractives**	39.90	44.79
Mineral salts	4.14	5.07

Specific gravity of normal blood varies from 1056 to 1060, being somewhat higher in men than in women. It varies greatly in disease, running practically parallel to the hæmoglobin per cent.

PHYSIOLOGY OF THE BLOOD.

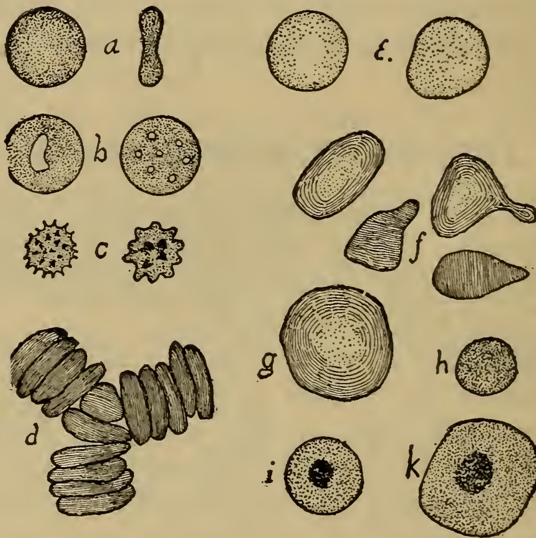
Examination of a fresh drop of normal blood under the microscope reveals chiefly two things, red corpuscles and white corpuscles; less prominently blood-plates, Müller's "blood-dust," and a fibrin network.

Red Corpuscles.—Under normal conditions the number of red corpuscles per cubic millimetre is quite constant, averaging about 5,000,000 in the male and 45,00,000 in the female. This number is greatly increased or diminished in disease. In vigorous health the number often reaches 6,000,000. High altitudes increase the number of cells up to 8,000,000 or 9,000,000. In the newborn the number of cells is high for a few days, 7,000.000 to 8,000,000. The red corpuscle is a round biconcave disk, with a yellowish or greenish-yellow appearance. It varies considerably in size under normal conditions, but the average may be taken as 7.5 μ. It seems to be larger in northern than in southern latitudes. Red blood-cells are all of the same round shape, except as they are indented, bent, and curved by striking against each other, or against white corpuscles, as they are moved in the plasma-currents. These corpuscles tend to form rouleaux, rows of cells standing on end or slightly tilted, like piles of

coins. The cells also become crenated with great facility. One should become thoroughly familiar with all forms of crenation, since the early stage of this process produces a deceptive appearance in the cell, which resembles the young form of the malarial parasite.

White corpuscles attract attention by their color, size, granules, amœboid movements, or by the fact that they are not moved by the blood-current like the red cells. In healthy adults they vary considerably in number, averaging

Fig. 4.



Normal and abnormal red blood-corpuscles: *a*, Normal corpuscles, side and edge view; *b*, vacuole formation; *c*, crenated corpuscles; *d*, rouleau formation; *e*, pale corpuscles, deficient in hæmoglobin; *f*, poikilocytes; *g*, macrocyte; *h*, microcyte; *i*, normoblast; *k*, megaloblast. (Nichols.)

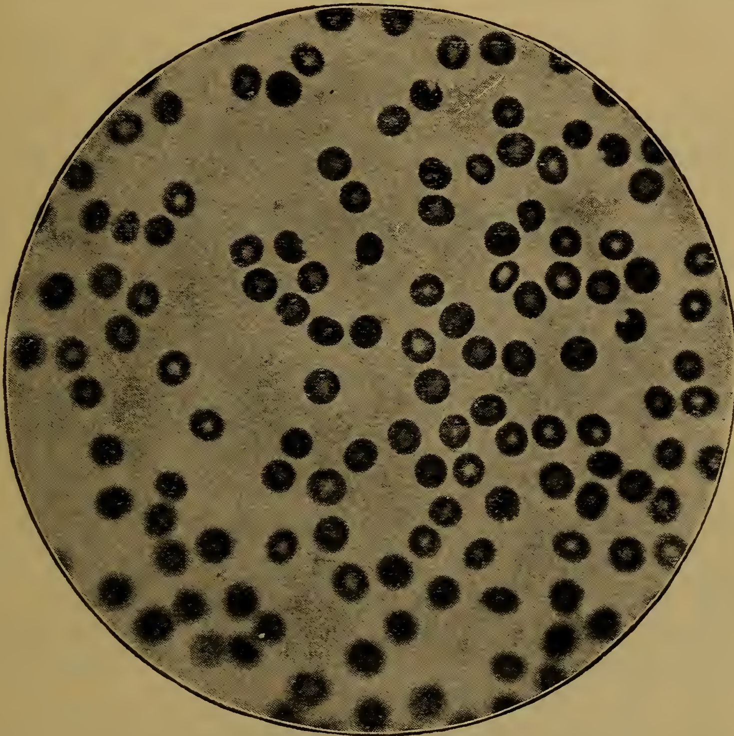
about 7500 per cubic millimetre. The normal ratio of red to white cells is about 1 : 600. They vary much in size, from 10 μ to 13.5 μ, depending on the variety.

Blood-plates are small irregularly shaped disks, about half the size of a red cell, and having a tendency to clump in bunches. They are seldom noticed in normal unstained blood, probably because of slow manipulation. Their number is variously estimated at from 180,000 to 860,000 per cubic

millimetre. They do not contain hæmoglobin, and show no signs of a nucleus.

Blood-dust.—Müller has given the name "hæmokonia" to small round colorless granules ($\frac{1}{4}$ to 1 μ in diameter) which may be found in normal and pathological blood. They are highly refractile, have a rapid dancing movement, but are without power of locomotion.

Fig. 5.



Normal blood. × 350. (Cabot.)

Fibrin Network.—If a fresh blood-drop preparation is exposed to the air for some time, a network of fine fibrin threads forms, and may be seen under the microscope, running between the corpuscles and sometimes radiating from a centre which is perhaps a mass of blood-plates. Under certain conditions this network is increased or diminished.

QUESTIONS.

Describe the method of cleansing the blood pipette.
How should coagulated blood be removed from the pipette?
Describe the method of cleansing lenses.
Mention the diluting fluids used in counting red and white blood-corpuscles.
Mention the important blood-stains.
What is the total volume of blood in man?
The colors of the blood are due to what substance?
What is the reaction of the blood and upon what does it depend?
Upon what does the coagulation of the blood depend?
What is the specific gravity of the blood?
What are agglutinins?

CHAPTER III.

CLINICAL EXAMINATION OF THE BLOOD.

PRELIMINARY REMARKS.

A PROPER appreciation of blood-examinations and the correct deductions to be drawn therefrom must take into account **certain physiological and pathological laws.**

Among these factors may be mentioned abnormal dilatation or contraction of the peripheral bloodvessels, local anæmias and congestions, concentration of the blood as in diabetes, and physiological variations for age and sex. Muscular exertion, profuse perspiration, ingestion of fluids, and temporary cyanosis alter the quality of the blood.

The blood is also strikingly influenced by the nervous system acting through cerebral (psychical) or medullary centres, or through local vasomotor nerves.

Therapeutic measures, such as the application of cold, heat, massage, electricity, aspiration of fluids, administration of purges, diaphoretics, vasodilators and vasoconstrictors, work like changes.

Clinical Examination of the Blood includes the following Procedures.—1. Examination of the fresh drop.

2. Estimation of the percentage of hæmoglobin.
3. Estimation of the specific gravity.
4. Counting the red corpuscles.

5. Counting the white corpuscles.

6. Study of the stained spread.

7. Widal serum test, and—

Less Frequently Applied Procedures.—8. Estimation of the alkalinity.

9. Estimation of the comparative volume of plasma and corpuscles.

10. Estimation of the total volume of blood.

11. Estimation of the time and completeness of coagulation.

12. Bacteriological examination of the blood.

13. Cryoscopic examination of the blood.

The physician should use his judgment in deciding upon which or how many of these procedures must be carried out. Frequently the examination of the fresh drop is sufficient. Again, only hæmoglobin estimation and red and white counts will be found necessary. Other cases require, in addition to the above examination, careful study of the stained spread.

The Widal test should be made only in suspected typhoid cases, and often is the only examination required.

Method of Securing Blood.—The finger-tip or lobe of the ear is cleansed with alcohol or ether, or simply water, care being taken not to rub too briskly, as the local blood conditions may be altered by the massage. A cold, bloodless finger should not be chosen, since it does not represent the actual blood condition of the patient. If rubbing is necessary, sufficient time should elapse to allow the circulation to equalize. A moderately deep puncture should be made, so that only slight pressure will cause the blood to flow.

Fresh Drop Examination.—1. **Macroscopical.**—Its general appearance; color, whether pale or red, streaked or creamy; its manner of flow; coagulation, rapid or slow.

2. **Microscopical.**—This procedure is very simple. A thoroughly cleansed and polished cover-slip is brought into contact at its centre with a small drop of blood directly it appears on the finger-tip. It is immediately dropped upon a clean slide. If the manœuvre be properly done, the blood will spread over the whole cover-slip, leaving a film one corpuscle deep.

Success depends largely on cleanliness and quickness. If it be seen that the blood will not run well, slight pressure with the forceps will assist.

Such preparations remain in good condition for several hours, since the periphery of the blood coagulates and seals the specimen. To preserve them longer, cover the borders with vaselin.

This fresh drop preparation reveals a great many things to the experienced observer. He studies the color of the red corpuscles, their shape and size, the relative number of cells both red and white, the kind of white cells, the peculiarity of their granules, small or large, the amœboid motion of leukocytes, the presence of rouleau formation, the approximate amount of fibrin, the relative number of blood-plates, and the presence of parasites.

Estimation of the Hæmoglobin.—Tallqvist Method.— This method is the most easily applied and gives fairly accurate results. In fact, ten Tallqvist estimations may be made in the time required for one Fleischl. It consists in the comparison of the color of a fresh drop of blood soaked up by a white filter-paper, and a scale of colors varying from 10 to 100 per cent. The comparison must be made immediately after the drop has lost its moist appearance and before it dries.

Extensive experience shows that the readings from Tallqvist and Fleischl differ very little indeed, perhaps 3 to 7 per cent.

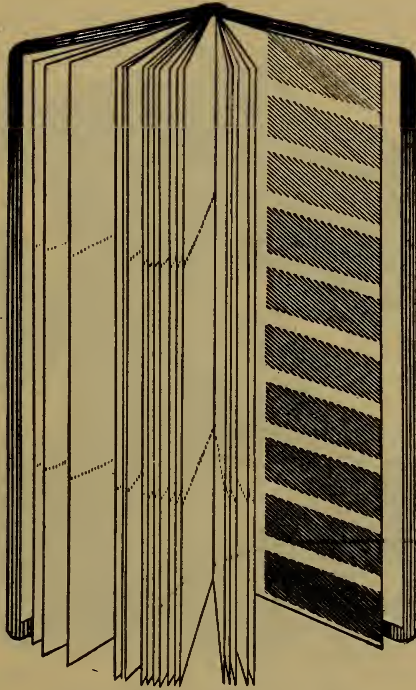
Another instructive procedure is to compare a drop of blood from the patient with that from a full-blooded, healthy individual. The contrast is often striking, and a very close estimate may be made as to the percentage of hæmoglobin.

The use of the **Fleischl hæmoglobinometer** has been the most accurate and practical method of estimating hæmoglobin until the introduction of **Miescher's modification of the Fleischl instrument**, and still later **Dare's hæmoglobinometer.**

Fleischl's Hæmoglobinometer.—(*a*) This *apparatus* (Fig. 7) consists of a metal stand with plate and plaster mirror (*S*),

which reflects diffused light through a circular opening in the plate. Beneath the plate, by means of a rack and wheel (*T*), slides a colored glass wedge fixed in a graduated frame (*P*). The glass wedge and graduated scale are so arranged as to indicate the percentage of Hb corresponding to the different portions of the wedge. In the circular opening of the plate fits a cylindrical metallic cell (*G*), with glass bottom and

Fig. 6.



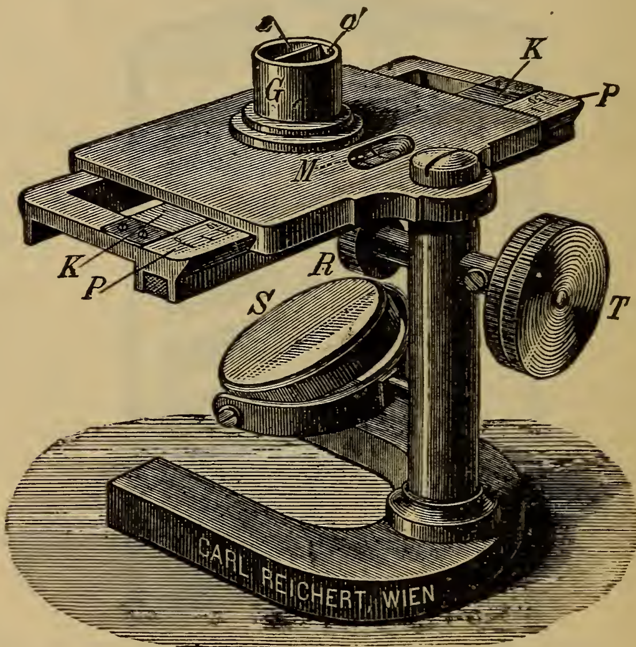
Tallqvist's hæmoglobin scale.

metal partition, one compartment of which lies directly over the glass wedge. The other compartment (*a*) being filled with diluted blood, one is enabled to make a close comparison of the color of the dissolved blood with that of the glass wedge. The blood is measured by an automatic capillary pipette, while a slowly running dropper is provided with which to add distilled water. On the handle of each pipette is stamped a number indicating its cubic contents. On the

stand of each instrument is also a number showing the capacity of the tubes with which it can be used.

(*b*) *Procedure.*—The pipette should be thoroughly clean and in working order. It is brought into contact with a drop of fresh blood expressed with very slight pressure, and should at once fill level-full. Do not immerse in the drop, or blood will adhere to the sides, thus furnishing one source of error.

Fig. 7.



Fleischl's hæmoglobinometer.

The tube of blood is immediately transferred to a compartment half-filled with distilled water and swished about till the blood is dissolved. On removal a few drops of distilled water are allowed to run through the tube in order to remove any remaining hæmoglobin. The blood is throughly mixed with the handle of the pipette. Both chambers are now filled even-full with distilled water. The thick round cover-glass is carefully adjusted, avoiding the inclosure of air and the

forcing of dissolved blood from its chamber into that containing only distilled water.

The cover-glass need not be used if the reading is promptly made. It must be done in a dark room by means of candle or gaslight. The light is placed about eighteen inches from the stand. An improvised paper tube which exactly fits the cell is used to look through. With low percentages of Hb a dim light is essential.

When the cell is in place and the light adjusted, the wedge is moved with quick turns till the colors in the two chambers exactly correspond. It is often useful to close the eyes and glance at the colors quickly, as a prolonged gaze tires the eye and makes it less accurate in the judgment of colors. When dealing with low percentages of hæmoglobin it may be advisable to use double the quantity of blood, as the matching of colors is more exact in the middle of the scale. An error of 5 per cent. must be allowed.

Miescher's Modification of Fleischl's Hæmoglobinometer.—This instrument (Fig. 8) gives more accurate results than any other. The blood is diluted with distilled water by means of a graduated pipette very similar to that of Thoma, dilutions of 1 : 200, 1 : 300, and 1 : 400 being made according as the tube is filled with blood to the mark $\frac{1}{1}$, $\frac{2}{3}$, or $\frac{1}{2}$. There are two cells, one with a depth of 15 mm., the other with a depth of 12 mm.

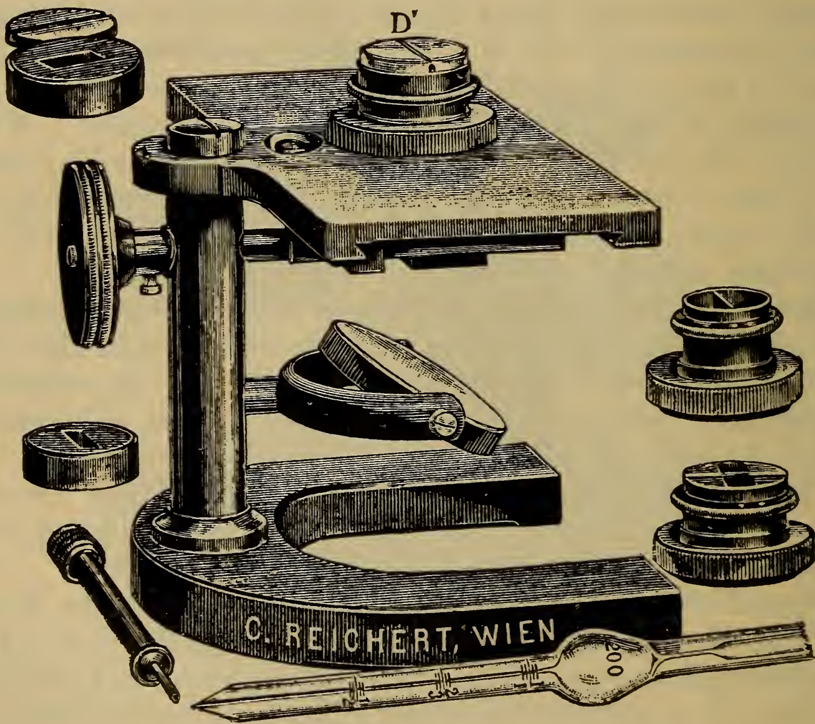
The percentage of hæmoglobin is obtained with the deeper cell, the other being used as a control and giving only $\frac{12}{15}$ of the actual percentage. These cells have a projecting partition dividing the compartments, along which a grooved cover-glass (*D*) may be slid without mixing the blood and water. The cells are covered with diaphragms, transmitting a ray of light which includes only 3 degrees on the scale, thus giving practically a single color of the wedge to compare with the blood. The pipette is agitated thoroughly, mixing the hæmoglobin, the diluting fluid blown from the tube, and one chamber in each cell is filled with the diluted blood, the other with distilled water. Cover-glasses and diaphragms are adjusted and the readings made. That of the small cell should be four-fifths that

of the larger. These may be used to correct each other. A dilution of 1 : 200 is generally used. If the dilution is 1 : 300, the reading must be multiplied by $1\frac{1}{2}$; if 1 : 400, by 2.

Oliver's Hæmoglobinometer.—For the application of this method the reader is referred to Ewing or Cabot.

Jolle's Ferrometer.—By means of this apparatus the iron in the blood is estimated, and with a formula the hæmoglobin

Fig. 8.



Miescher's hæmoglobinometer.

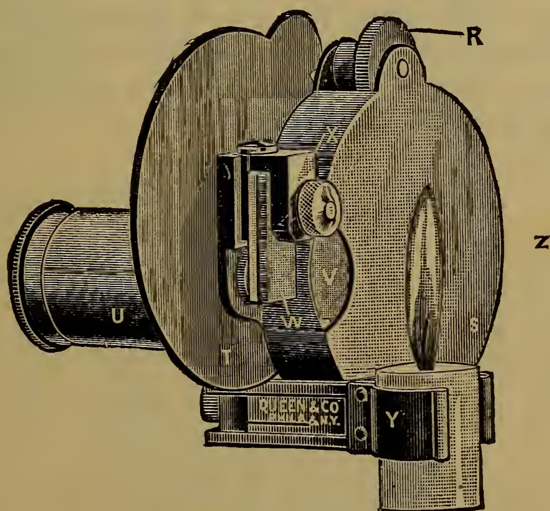
calculated. The reader is again referred to Ewing or Cabot for a description of the method.

Dare's hæmoglobinometer is highly recommended by Cabot. At Johns Hopkins it has supplanted the older instruments. In it the undiluted blood is used. Blood is drawn by capillary attraction into the slit between two slabs of glass, one transparent, the other translucent and white, so as to diffuse

the light used for illumination. Its color is compared with different portions of a circular disk of colored glass. The readings are a trifle higher than those obtained with the Fleischl instrument. Full directions accompany each apparatus. Its cost (twenty dollars), its bulk, and the time taken for cleansing, make it much less practical than Tallqvist's apparatus.

Color-index.—By this term is meant the ratio existing between the hæmoglobin per cent. and that of the red cells, on the basis of 5,000,000 red cells equalling 100 per cent. of

Fig. 9.



Dare's hæmoglobinometer.

hæmoglobin. By this rule 1,000,000 red cells should correspond to 20 per cent. of hæmoglobin.

In some diseases, such as pernicious anæmia, the hæmoglobin per cent. is higher than the red cell per cent. because of the increased size of cells. To illustrate: in a given case the hæmoglobin is 30 per cent.; the red count 1,000,000, or 20 per cent.; the color-index is $\frac{3}{2}$.

In chlorosis the color-index is low.

Estimation of the Specific Gravity.—An indirect method of calculating the per cent. of hæmoglobin is by estimating

the specific gravity of the blood. The best method is that of Hammerschlag. Such a mixture of chloroform and benzol is made in a urinometer cylinder as will give a specific gravity of 1059 (normal specific gravity of the blood), unless dealing with a very anæmic blood, when time will be saved by making a lighter mixture. The finger is freely punctured, a large drop of the blood drawn up into a capillary tube (a medicine-dropper will answer) and quickly deposited in the liquid; or the blood may be squeezed from the finger and allowed to drop into the cylinder. It should float in the middle of the liquid. If it sinks, its specific gravity is greater than that of the mixture; if it rises, it is lighter. In case it sinks, add sufficient chloroform (which is heavier than water) to cause it to remain floating in the middle of the liquid. Place the urinometer in the mixture, note the specific gravity, and compare with the appended scale.

Specific gravity.	Hæmoglobin.	Specific gravity.	Hæmoglobin.
1030.0 =	20 per cent.	1049.0 =	60 per cent.
1035.0 =	30 "	1051.0 =	65 "
1038.0 =	35 "	1052.0 =	70 "
1041.0 =	40 "	1053.5 =	75 "
1042.5 =	45 "	1056.0 =	80 "
1045.5 =	50 "	1057.5 =	90 "
1048.0 =	55 "	1059.0 =	100 "

The urinometer should be perfectly dry. It is well to have several drops of blood in the glass; add the chloroform and benzol a few drops at a time, and stir thoroughly with a glass rod after each addition.

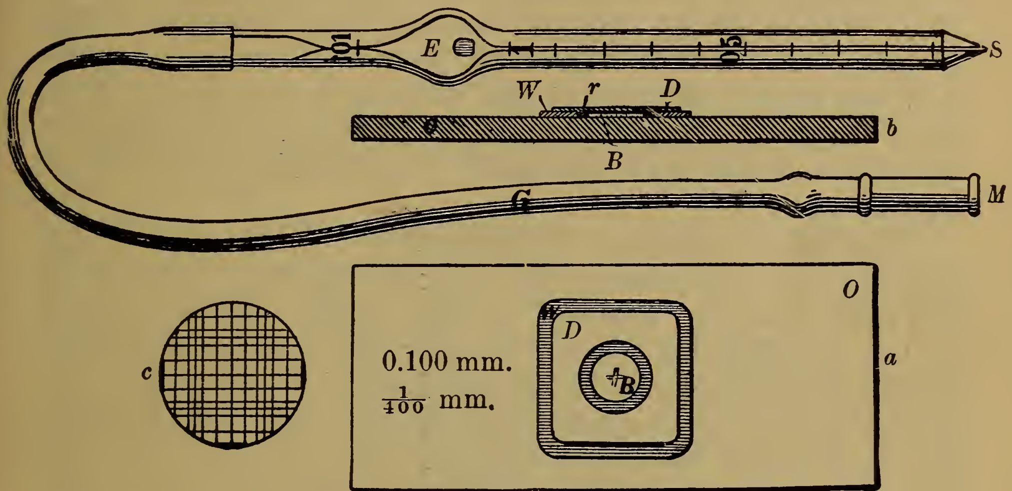
The mixture of chloroform and benzol may be filtered and kept in a special bottle, and used over and over again.

Counting the Red Corpuscles.—The finger is cleansed and punctured. As soon as the blood is flowing freely, a red-blood pipette is brought into contact with the drop, suction made, and the blood drawn up to the mark 0.5. In the case of an extremely anæmic patient draw it up to the mark 1.

Considerable experience is required to do this with exactness. If the mark is slightly overreached, touch the point of

the pipette against the towel till the column is brought back to the 0.5 mark.

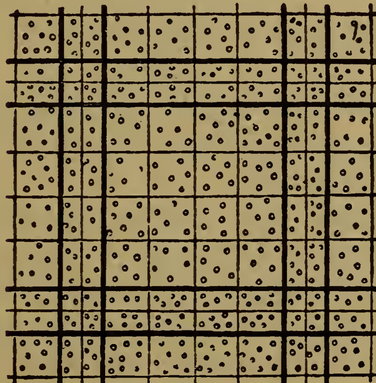
FIG. 10.



Thoma-Zeiss blood-counting apparatus.

(Beginners will find it useful to practise drawing up a colored fluid like Toisson's before experimenting with blood,

FIG. 11.



Appearance of blood in the Thoma-Zeiss cells.

which is likely to coagulate in the pipette and give much trouble.)

When the blood is drawn to the proper mark, and that on

the outside of the tube wiped off (being careful not to touch the point), the pipette is immediately plunged into the diluting solution and suction made as soon as it is below the surface. This is drawn up until the mark 101 is reached. The pipette, held in the horizontal position, is tapped rapidly with the fingers, in order to mix thoroughly the blood. Next blow out the diluting fluid and place a medium-sized drop of the diluted blood upon the small glass cylinder in the counting-chamber.

Considerable practice is required to obtain a drop of the proper size. In securing the correct dilution of the blood, the rubber tubing attached to the pipette should be long enough to allow the eyes to be brought on a level with the 0.5 mark, otherwise too much blood will be drawn up. The pipette must be thoroughly clean, dry and free from bubbles. The next step is the careful adjustment of the cover-slip over the drop of diluted blood. The drop should now nearly cover the central glass slide; there should be no air bubbles in it; it should not overrun the gutter. If the slide be held up to the light on a level with the eyes, a play of colored rainbow rings (Newton's) may be seen. This indicates that the technique has been correct. A few minutes should elapse before counting to allow the corpuscles to settle.

The counting-chamber is exactly $\frac{1}{10}$ of a millimetre deep. The ruled square used for counting reds is a square millimetre divided into 400 small squares, so that each small square is $\frac{1}{400}$ square millimetre. Use the low dry lens, with most of the light shut off.

It is well to adopt the rule of beginning with the lower right square, and counting upward. Every fifth square above and to the left is subdivided by an extra line to facilitate the counting. The corpuscles lying on the upper and left lines are counted; those on the lower and right are not. Continue this upward course till five squares are counted, then take the next square to the left and go down 5, then the next to the left and go up again 5 squares, and so on till the number of corpuscles in 200 small squares is counted. This sum is divided by 200, thus giving the number in each small square.

To calculate the number of corpuscles per cubic millimetre, multiply by 100, because the blood has been diluted 100 times; then by 400, because each small square is $\frac{1}{400}$ of a square millimetre; then by 10, as the square millimetre is only $\frac{1}{10}$ of a millimetre deep. This gives the number of corpuscles in a cubic millimetre. In short with a dilution of 1:100, the number of corpuscles in each small square is multiplied by 400,000. If the dilution has been 1 : 200, multiply by 800,000.

Counting the White Corpuscles.—A white-blood counter, with which it is possible to make dilutions of 1 : 20 and 1 : 40, is used. The diluting solution is 0.3 per cent. of glacial acetic acid, which destroys the red corpuscles.

A free puncture must be made, since a much larger drop is required than in making the red count. Because of the large bore of the pipette great care should be taken in sucking up the blood, and later in securing the proper-sized drop for counting. In depositing this drop on the counting-chamber, the pipette must be held in an almost horizontal position. The diluted blood flows out without blowing. The diaphragm of the microscope is adjusted so as to shut off considerable light, in order to make prominent the white corpuscles. They appear as small granular balls.

Several methods of calculating the number of leukocytes have been devised.

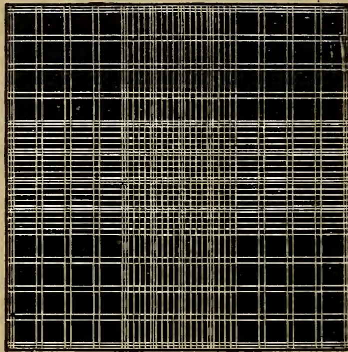
1. Turck's modification of the Thoma-Zeiss blood-counter[1] (Fig. 12) is intended for the accurate and rapid counting of leukocytes. It differs from the Thoma-Zeiss counting-chamber in having 9 square millimetres accurately ruled off, instead of 1. The central square millimetre is divided into 400 small squares, as in the original, and is used for counting red cells. By means of this counting-chamber the leukocytes in 9 square millimetres may be counted with each drop of the diluted blood, thus rendering it unnecessary to count more than 2 or 3 drops. The presence of so many lines makes it much easier to count the cells and less likely that they will be counted more than once or not at all.

[1] Wiener klinische Wochenschrift, July 10, 1902, p. 717.

Example.—In a space of 9 square millimetres 270 leukocytes were counted, an average of 30 to each square millimetre. Multiply 30 by 10, to give the number in a cubic millimetre—that is, 300. Next multiply 300 by 20, the number of times the blood was diluted, giving a total of 6000 per cubic millimetre.

2. **By Use of Thoma-Zeiss Counting-chamber and a Specially Calculated Leukocyte Table.**—This rapid method is employed almost entirely in Dock's clinic. The tube of the microscope is drawn out until the periphery of the microscopical field

Fig. 12.



Türck.

exactly cuts the corners of the large square millimetre. With some microscopes this can not be done. A piece of dark cardboard, out of which has been cut a circle of the correct size to give the above field, should be placed in the eye-piece of such instruments. The slide is moved about and all corpuscles in several of such fields are counted. Two or three drops should be treated in this way. The sum of the corpuscles is divided by the number of fields, giving the average for a field. This number is found on the table and the number of leukocytes is read off. This method, known as the calibration method, is sufficiently accurate for all practical purposes. Upon it is based a still shorter method, devised by F. T. Wright, which is fully described in another paragraph.

Leukocyte Table (dilution 1:40).

1— 254	41—10,441	81—20,626
2— 509	42—10,695	82—20,881
3— 763	43—10,950	83—21,136
4— 1,018	44—11,204	84—21,390
5— 1,274	45—11,457	85—21,645
6— 1,528	46—11,714	86—21,900
7— 1,782	47—11,938	87—22,154
8— 2,037	48—12,223	88—22,409
9— 2,292	49—12,478	89—22,664
10— 2,546	50—12,732	90—22,928
11— 2,801	51—12,987	91—23,173
12— 3,056	52—13,243	92—23,426
13— 3,310	53—13,496	93—23,682
14— 3,565	54—13,751	94—23,937
15— 3,820	55—14,006	95—24,191
16— 4,074	56—14,260	96—24,446
17— 4,329	57—14,575	97—24,701
18— 4,583	58—14,770	98—24,955
19— 4,838	59—15,024	99—25,210
20— 5,093	60—15,278	100—25,464
21— 5,348	61—15,533	101—25,719
22— 5,602	62—15,788	102—25,974
23— 5,857	63—16,043	103—26,228
24— 6,112	64—16,297	104—26,483
25— 6,366	65—16,552	105—26,737
26— 6,621	66—16,807	106—26,992
27— 6,875	67—17,061	107—27,247
28— 7,130	68—17,316	108—27,501
29— 7,385	69—17,571	109—27,756
30— 7,639	70—17,825	110—28,011
31— 7,894	71—18,080	111—28,266
32— 8,148	72—18,335	112—28,520
33— 8,403	73—18,589	113—28,775
34— 8,657	74—18,844	114—29,030
35— 8,912	75—19,099	115—29,284
36— 9,167	76—19,353	116—29,539
37— 9,421	77—19,608	117—29,793
38— 9,676	78—19,868	118—30,046
39— 9,931	79—20,117	119—30,302
40—10,186	80—20,372	120—30,557

Short Method of Calculating Leukocytes.—The following excellent method, suggested by F. J. Wright, is used in the Calumet and Hecla Hospital:

Dilute blood 1:40, count 5 fields (the periphery of the field should cut the corner of the large square), divide their

sum by 2, and add 2 ciphers. This gives the number of leukocytes with sufficient accuracy for practical purposes.

Example: 150 leukocytes are counted in 5 fields. 150 ÷ 2 = 75. Add 2 ciphers, and 7500 is obtained, the approximate number of leukocytes per cubic millimetre.

When leukocytes are very numerous, as in some cases of leukæmia, it is best to employ the red counter and make dilutions of 1 : 100.

Durham's modified hæmatocytometer combines the use of the Thoma-Zeiss counting-chamber with a self-measuring pipette, for obtaining the blood, pipettes for measuring the diluting fluid, and small test-tubes for making the dilution, giving dilutions of 1 : 200, 1 : 100, and 1 : 50. It has many advantages for the unskilled person, and is much more easily cleaned than the Thoma-Zeiss pipettes.

For a full description of this method see Cabot.

QUESTIONS.

What are the constituents of normal blood?
Mention the various factors which alter the quality of the blood?
What procedures are included in the clinical examination of the blood?
Describe the *fresh drop* examination.
Describe the various methods of estimating the hæmoglobin.
What is meant by the *color-index?*
Describe Hammerschlag's method of estimating the *specific gravity.*
Describe the best method of counting the red blood-corpuscles.
Mention the various methods of counting the white blood-corpuscles.

CHAPTER IV.

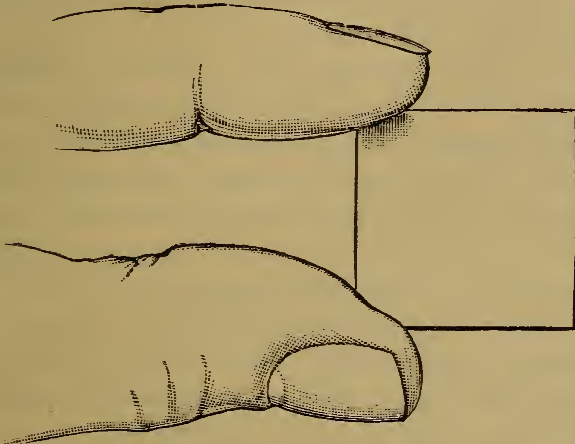
STUDY OF THE STAINED SPREAD.

MAKING SPREADS.

(1) **Cover-glass Method.**—The cover-glasses should be thoroughly cleaned and well polished. Place them upon a blood-board covered with filter-paper. Clean the finger, prick it, wipe off the first drop of blood, pick up the cover-

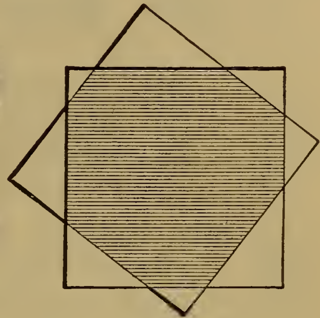
glass with forceps, touch its centre to the exuding drop before it becomes too large. Place upon another cover-glass in such

Fig. 13.



Proper method of holding a cover-glass. (Cabot.)

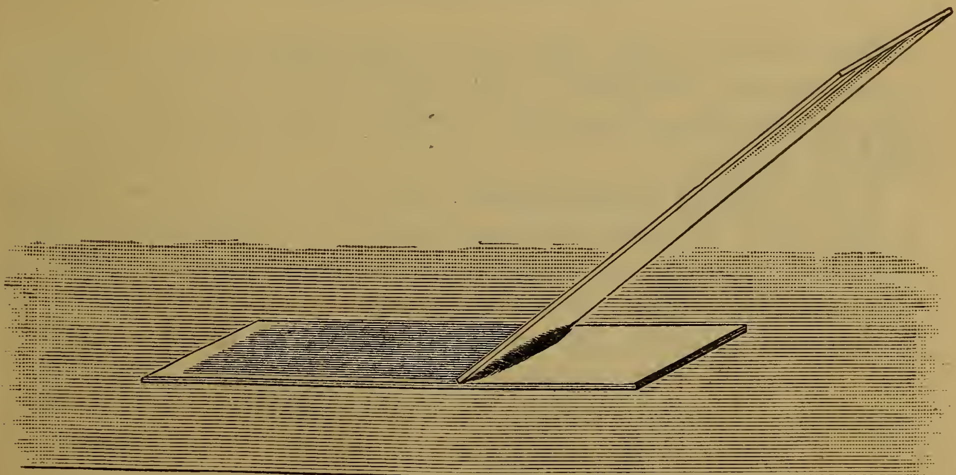
Fig. 14.



Illustrating the position of cover-glass during the spreading of blood-films. (Cabot.)

a way that all corners project. The blood should spread in a thin fim. Pick up with forceps and slide the cover-glasses

Fig. 15.



Slide method of making blood-smears. (Ewing.)

apart along the same plane, the lower cover being held by the thumb and forefinger of the left hand, the upper by the

forceps or the thumb and forefinger of the right hand. The essentials for a good spread are: first, quickness; second, clean cover-glasses; third, drop of proper size.

If the cleaned cover-slips are passed through the flame just before spreading, better films will result.

(2) **Slide Method.**—This method has become widely adopted. For the inexperienced it is the better method. It is very easy of application, and one is certain to find some part of the large field which is well spread. The polished edge of a slide held between thumb and fingers is touched to a medium-sized fresh drop of blood and pressed against another slide near its end. As soon as the blood spreads across the edge of the smearer, draw it gently and evenly along the lower slide till the drop is exhausted. The blood should be pushed before the smearer (Fig. 15). A cigarette paper may be used as a spreader in place of the upper slide.

To secure the best spreads, they should be immediately dried over a gas or alcohol flame, but for the majority of clinical work air-dried specimens are sufficiently good.

FIXING THE FILMS.

Depending on the stain to be used, films may be fixed or remain simply air-dried. The selection of the stain decides whether the film should be fixed by heat or one of several fluids or gases.

If the **Ehrlich triacid stain** is to be used, fixation by heat is essential. If eosin and hæmatoxylon or eosin and methylene-blue are to be used, fix in a solution of equal parts of absolute alcohol and ether for from five to thirty minutes, or in the same solution (30 c.c. each) to which 5 drops of a saturated alcoholic solution of corrosive sublimate are added (five minutes). Absolute alcohol alone, or 2 per cent. chromic acid, or exposure to the vapor of 45 per cent. formaldehyde may also be used. If in a hurry, the air-dried spread may be stained with eosin and hæmatoxylon, with fair results.

Fixation by Heat.—A small dry heat sterilizer with ther-

mometer registering 200° C. is heated by a Bunsen burner or alcohol lamp.

The blood-spreads are placed film downward upon the shelf of this sterilizer, and heat applied to 115° to 150° C. The degree of heat necessary must be determined for each sterilizer by heating a number of films at different temperatures and staining with triacid.

The film showing red corpuscles stained a bright yellow indicates the correct temperature for that oven. As soon as the heat reaches the proper temperature it should be turned off.

For routine work, fixation in the free flame of the Bunsen burner or alcohol lamp is very satisfactory. The cover-glass, specimen side up, is grasped with forceps and passed slowly through the flame, about twenty times, until it is too hot for the hand to bear. This means a temperature between 110° and 150° C. If overheated, the film changes color. Most mistakes are made on the side of underheating, with incomplete fixation and subsequent vacuolization of the cells. The film may also be fixed by holding it above the free flame, at such a distance that the heat is just bearable by the hand.

STAINING THE SPREAD.

Probably what will prove to be the best all-round stain in blood-work is one which has been devised by Wright, of Harvard. The somewhat troublesome fixing of films is done away with, as this stain works best with fresh unfixed spreads. Its application is extremely simple, and its results show that it is more widely applicable than any stain ever devised. It is the best malarial stain known. For the method of making the stain, see Chapter I.

Staining of Blood-films.—1. Make films of the blood, spread thinly, and allow them to dry in the air.

2. Cover the preparation with the alcoholic solution of the dye for one minute.

3. Add water to the alcoholic solution of the dye on the preparation, drop by drop, until the mixture becomes semi-

translucent and a yellowish metallic scum forms on the surface. Allow this mixture to remain on the preparation for two or three minutes.

4. Wash in water (preferably distilled) until the film has a yellowish or pinkish tint in its thinner or better spread portions.

5. Dry between filter-paper and mount in balsam.

Dried blood-films may be kept for some weeks without impairment of their staining properties; but if kept too long, will not give good results.

MICROSCOPICAL APPEARANCES IN BLOOD-FILMS STAINED BY THE METHOD OF WRIGHT.

Red cells are orange or pink in color; polychromatophilia and punctate basophilia (the granular degeneration of Grawitz) are well brought out. The **nucleated red cells** have a deep-blue nuclei, and the cytoplasm is usually of a bluish tint.

Lymphocytes have dark purplish-blue nuclei, and robin's egg blue cytoplasm in which a few dark-blue or purplish granules are sometimes present.

Polynuclear neutrophilic leukocytes have dark-blue or dark-lilac colored nucleus, and the granules are usually of a reddish-lilac color.

Eosinophilic leukocytes have blue or dark-lilac colored nuclei. The granules have the color of eosin, while the cytoplasm in which they are imbedded has a blue color.

Large mononuclear leukocytes appear in at least two forms. Each form has a blue or dark-lilac colored nucleus. The cytoplasm of one form is pale blue, and of the other form is blue with dark-lilac or deep-purple colored granules, which are usually not so numerous as are the granules in the polynuclear leukocytes.

Mast-cells appear as cells of about the size of polynuclear leukocytes with purplish or dark-blue stained, irregular-shaped nuclei, and cytoplasm, sometimes bluish, in which numerous coarse spherical granules of variable size are imbedded. These granules are of dark-blue or of a dark-purple color, and may appear almost black.

Myelocytes have dark-blue or dark-lilac colored nuclei, and blue cytoplasm in which numerous dark-lilac or reddish-lilac colored granules are imbedded.

The **blood-plates** are deeply stained, and are a prominent feature of nearly every blood preparation. They appear as blue or purplish rounded or oval bodies, usually of a diameter of a third to a half of that of a red blood-corpuscle. Sometimes they appear in groups or masses, and at first sight may be regarded as precipitates. In many instances they have the appearance of being within a red corpuscle and surrounded by an unstained zone of its cytoplasm.

Malarial Parasites.—The body of a malarial parasite stains blue, while the color of the chromatin varies from a lilac color through varying shades of red to almost black. In the young forms of the tertian and æstivo-autumnal parasite the chromatin appears as a spherical very dark-red body, while in the older forms of the tertian parasite it has a more lilac or purplish-red color, and may appear in the form of a reticulum. In the intermediate forms the color of the chromatin may present variations between these extremes. The inexperienced observer may mistake the blood-plates apparently situated within the red blood-cells for malarial parasites. This will not occur if he bear in mind that the young parasite of all the three kinds should present by this method a dark-red spherical nucleus, and a cytoplasm which is usually in the form of a definite ring. This method of staining will bring out dark-red staining granules in the red corpuscles harboring malarial parasites provided the stain after the water has been added to it is allowed to remain on the preparation for at least five minutes, and not to decolorize for so long a time as with the ordinary stain.

Following the success of the Romanowsky stain in demonstrating the chromatin material of parasites, the methylene-blue-eosin stains have acquired a deserved prominence. Among these may be mentioned Jenner's, Rosin's, Leishman's, and Wright's. Jenner and Wright use pure methyl alcohol as solvents of their stains, while Rosin and Leishman employ pure ethyl alcohol as a solvent. Wright's stain

may be selected from this list as probably the best, though the others are excellent.

As a differential stain for leukocytes, Jenner's stain has proved uncertain in staining neutrophile granules. The staining of the chromatin in the nuclei of malarial parasites is seldom accomplished satisfactorily. With Wright's stain, on the other hand, the chromatin is stained as beautifully as it is with the Nocht Romanowsky method. It must be remembered that those mixtures of methylene-blue and eosin which stain the chromatin material of the cell in varying shades of violet depend for this characteristic not upon methylene-blue, but upon its oxidation product, the methylene-azure of Bernthsen; and that the solutions in alcohol (ethyl or methyl) of neutral stain—Reuter, Leishman, Wright —which has been prepared from polychrome (alkaline) methylene-blue probably contain a large amount of methylene-azure eosin in proportion to the methylene-blue-eosin.

The **Ehrlich tricolor mixture** has for years been the most popular stain with many workers. The objections to it are (1) that it is difficult to prepare a good stain, and (2) that the films must be fixed by heat. (The first objection may be overcome by sending to Walter Dodd, apothecary to the Massachusetts General Hospital, who furnishes an absolutely reliable stain for 65 cents a small bottle.) For method of making the stain, see Chapter I.

A drop of the stain is spread over the film, allowed to remain five minutes or more, and washed off with water. (It is impossible to overstain.) The specimen should look orange-yellow; if it is brown or red, it is underheated—not overstained. If overheated, everything is blurred and dim under the microscope. Hewes improves the definition of the nuclei by pouring upon the film for a second or two a saturated aqueous solution of methylene-blue, after the triple stain has been washed off with water. This also brings out the malarial parasite.

Appearance of Films Stained with the Ehrlich Tricolor Mixture.—Blood-films, properly heated and stained with the above mixture, when normal, react as follows:

Red Cells.—The hæmoglobin of the red cells stains with the orange-G, and if the spread is properly heated takes a brilliant yellow or orange tint. If overheated, they have a feebly stained, washed-out look; while if underheated, they are brown or gray. The degree of pallor in the centres corresponds to the amount of hæmoglobin in the corpuscle. One who is accustomed to spreading blood in a uniform way can judge approximately of the number of red cells.

The fibrin and blood-plates are not seen. The plasma does not stain in normal cases. A certain amount of débris is often present, usually stained pink.

White Cells.—The chief value of the triple stain is the fact that it enables one to recognize the important varieties of leukocytes, by differentially staining them.

In normal blood the following varieties of white cells are found:

1. **Small Lymphocytes.**—These are made up chiefly of a round blue nucleus about the size of a red cell, surrounded by a thin coating of protoplasm. This is faintly stained or invisible with Ehrlich's triple stain, but takes on a dark patchy blue when Hewes' after-stain is used. With this after-stain the nucleus becomes an intense indigo color.

2. **Large Lymphocytes or Large Mononuclear Cells.**—These are simply larger and paler, and the nucleus occupies relatively less of the cell than in the small lymphocytes and stains less readily. In some bloods there are no intermediate forms. Lymphocytes are either "small" (5 to 10 μ in diameter) or "large" (13 to 15 μ in diameter).

In other cases we find every intermediate size, both of nuclei and of the cells as a whole. It is not rare to find the so-called mononuclear cells, whose nucleus has a deep cut in one side, or has divided into two parts. Cabot groups the large mononuclear leukocyte with the large lymphocyte.

3. **Transitional forms** resemble the large lymphocytes, except that they have an indentation in their nucleus, either narrow or wide. Both the nucleus and protoplasm are pale.

The cells known as polynuclear or poly(morpho)nuclear neutrophiles constitute the vast majority of those found in

ordinary pus. The nucleus stains deeply. It is very irregular in shape, being compared to the letters Z, S, E; at times it seems to have several distinct nuclei, but this is due to the fact that the nucleus dips down deep and then comes again to the surface. There are usually underground connections. The body of the cell is filled with small *neutrophilic* granules. They are much smaller than the large round eosinophile granules. They stain violet or purple, at times pink. The younger the cell the more violet the color. They are called neutrophile because they do not stain with acid stains like eosin, or with basic stains like methylene-blue. It requires a high power to see these granules; with a low power they look like a diffuse stain. They rarely occur except in cells whose nucleus has reached the polymorphous stage.

The **eosinophiles,** or coarse granular oxyphile cells, have polymorphous nuclei and granules; but the nucleus is paler and more loosely connected to the granules. The granules are spherical or oval, of nearly uniform size, and much larger than the neutrophile. They have a strong affinity for acid coloring-matter (eosin and fuchsin). They are of a copper or burnt sienna color. The eosinophiles are the most actively amœboid of all the corpuscles, and it may be for this reason that the different parts of the cell seem so loosely strung together. In cover-glass specimens we more often find a separation of the nucleus and the granules than in any other cells.

The **basophilic "mast-cell"** is a constituent of normal blood; 0.5 per cent. is the maximum number in health. The triple stain does not bring it out well.

Eosin and Hæmotoxylon.—This stain is especially useful in studying the blood of pernicious and other severe anæmias, since it brings out the finer structure of the nuclei. The spread should be fixed in ether and alcohol. Cover the spread with eosin for about one-half minute, wash in water, then examine under low-power lens to learn whether the corpuscles have the proper tint. If too deeply stained, wash still further. Cover with filtered hæmatoxylon for about one minute, wash, dry between filter-paper, and mount. The

red cells should be stained a light pink; the nuclei of white cells and nucleated red cells a dark purple. The only granules stained are the eosinophiles. The malarial parasite is not stained by this method.

Eosin and Methylene-blue.—Method.—Fixation, alcohol, or alcohol and ether. Cover the smear with eosin for one-half to one minute; wash in water. From the appearance of the film one soon learns whether the preparation is overstained or understained. To be certain, examine under a low-power lens. If overstained, wash longer in water; if understained, add more eosin. Next cover stain with methylene-blue for one minute, wash quickly, and dry.

This method may be used for ordinary blood-work. Its chief advantages are that it stains the basophilic granules and malarial parasites. It does not bring out the neutrophile granules or nuclei clearly. One must guard against overstaining with eosin, otherwise the methylene-blue does not act well.

Demonstration of Mast-cells with Ehrlich's Dahlia Solution.—The large basophilic granules of these cells retain basic dyes with greater tenacity than most other basophilic granules.

Stain several hours, wash in water, decolorize in alcohol, till the nuclei fade, and again wash in water. The nuclei of leukocytes are then very pale blue, and the mast-cell granules very dark blue or black.

Demonstration of Glycogenic Granules with Iodophilia Mixture.—For the preparation of this mixture and its application, see Chapter VII. (Diseases in which Diagnosis Depends on Blood Examination).

Origin of the Different Varieties of Physiologic Leukocytes.

I. The myelogenous group. (From the bone-marrow.)	(*a*) Poly(morpho)nuclear (*b*) Eosinophiles. (*c*) Mast-cells. (*d*) Large mononuclear cells.
II. The lymphogenous group. (From adenoid tissue.)	Lymphocytes of all sizes.

Normal Per cent. of Each Variety.

(*a*)	Small lymphocytes,	20–30	per cent.
	Large "	4–8	" "
(*b*)	Polymorphonuclear neutrophiles,	62–70	" "
(*c*)	Eosinophiles,	$\frac{1}{2}$–4	" "
(*d*)	"Mast-cells,"	$\frac{1}{40}$–$\frac{1}{2}$	" "

In infancy the percentage of lymphocytes is much larger (40 to 60), and the polymorphonuclear only 18 to 40 per cent.

Poorly nourished, debilitated people show an excess of lymphocytes and a diminution in the polynuclear cells. The opposite condition prevails in vigorous health.

Pathologic Leukocytes.

1. **Myelocyte.**—This is a mononuclear neutrophile, and has many points of resemblance to the polynuclear neutrophile; it is the same cell in an early stage of growth.

This cell makes up the larger portion of the leukocytes of the marrow, and differs from any variety found in normal blood.

It is found in the blood in various diseased conditions, and resembles very closely the large lymphocytes, differing only in possessing neutrophile granules. It differs from the polynuclear neutrophile in the shape of its nucleus, but the granules in both are alike. The nucleus is usually spherical or egg-shaped, and is in close contact with the cell-wall for a comparatively large portion of its extent.

The average	diameter	of	100 myelocytes,	15.75 μ.
"	"	"	100 polynuclear leukocytes,	13.50 μ.
"	"	"	100 large lymphocytes,	13.00 μ.
"	"	"	100 eosinophiles,	12.00 μ.
"	"	"	100 small lymphocytes,	10.00 μ.
"	"	"	100 red corpuscles normal,	7.50 μ.

Eosinophilic Myelocytes.—Myelocytes having eosinophile instead of neutrophile granules occasionally occur.

The eosinophile granules do not all take the same stain; some are darker than others.

Atypical cells:

I. Degenerated or Moribund Leukocytes.—(1) A homogeneously stained mass looking like a washed-out, structureless nucleus that has lost its protoplasm and become ragged at the edges (karyolysis).

(2) The same intensely stained.

(3) Vacuolization of the nucleus or of the protoplasm.

In the granular leukocytes the granules are scattered about the field, and the nucleus is pale, structureless, and deformed.

II. Transitional Neutrophile.—Between marrow-cell and polymorphonuclear.

III. Türck's "Stimulation Forms."—Described by Weil as nongranular myelocytes. They are associated with stimulation of the bone-marrow, grave anæmia, and all conditions in which there is a leukocytosis.

Differential Counting.—For satisfactory work a mechanical stage is essential. At least 500 leukocytes should be counted and checked off opposite their names according to the list given on pages 58 and 61. Begin in the lower right-hand corner of the film, and, by turning the thumb-screw, work upward, covering the film carefully, checking off the leukocytes as they appear and are recognized. When the top of the film is reached, move one field to the left and work downward; continue till a sufficient number of corpuscles are counted. At the same time watch for alterations in the red corpuscles, as, for example, nucleated reds.

QUESTIONS.

What are the various methods of making blood-spreads?
Mention several methods of fixing blood-spreads.
What advantage has the methylene-blue-eosin stains over the older stains?
Mention the points of distinction between the various kinds of leukocytes.
What is the normal per cent. of each variety of leukocytes?
Of what importance is differential counting?

CHAPTER V.

PATHOLOGICAL CONDITIONS OF THE BLOOD.

Classification.—The following deviations from the normal may be observed:

1. Diminution in hæmoglobin.
2. Diminution in the red count.
3. Increase or diminution in the white count.
4. Alteration in color-index.
5. Diminution in the specific gravity.
6. Alterations in the size and shape of the red cells—*i. e., poikilocytosis.*
7. Alterations in the staining properties of the red cells—*i. e., polychromatophilia.*
8. Presence of abnormal forms of red cells.
9. Presence of abnormal forms of white cells.
10. Alteration in the normal ratio of different varieties of white cells.
11. Presence of parasites in the blood.

Various combinations of these changes are shown in many of the diseases which are discussed in another chapter.

Poikilocytosis.—By this term is meant unusual alterations in the size and shape of the red corpuscles; some are very large, almost twice the size of the average red cell; some very small, being only about half the size.

In shape these cells resemble dumb-bells, Indian clubs, beets, etc. All sorts of irregular, ragged cells are to be found.

Polychromatophilia.—With special stains certain of the red cells take on a darker color than the remainder of the cells, the tint varying with the stain used. This is due to a degenerative process, which changes the staining properties of the cells, so that they take up several colors.

Endoglobular Degeneration.—This consists of the presence of clear, unstained spaces of various shapes within the corpuscles. In the fresh specimen these spaces are clear and change their shape continually.

Granular Degeneration of Red Cells (Grawitz) **or Punctate Basophilia of Red Cells.**—In certain diseases—malaria, lead-poisoning, severe anæmia, etc.—red cells often show small bluish granules. These are well brought out by Wright's stain. The weight of evidence suggests that they are remnants of nuclei. Red corpuscles harboring malarial parasites often show these granules.

Abnormal Forms of Red Cells.

Nonnucleated: (*a*) microcyte—a very small red cell; (*b*) megalocyte—a very large red cell.

Nucleated: (*a*) microblast; (*b*) normoblast; (*c*) megaloblast; (*d*) atypical forms of nucleated red cells—called metrocytes by some authors.

Microblast.—A very small red cell, made up chiefly of a nucleus similar to that of the normoblast. There is a narrow rim of protoplasm around the nucleus. It has perhaps the same significance as the megaloblast.

Normoblast.—It differs from the normal red cell in having a deeply stained, round nucleus, about one-half the diameter of the whole cell, situated somewhat excentrically. At times the nucleus is so situated that it looks as if the cell were extruding it.

Megaloblast.—This does not occur anywhere in the healthy adult body. It is found in the early fœtal marrow, and in the marrow and blood of grave forms of anæmia. According to Ehrlich, the megaloblast indicates the presence of the fœtal types of blood formation. It is a grave prognostic sign, and when present in excess of normoblasts it indicates a pernicious form of anæmia. The only exception which clinicians have found to this view is the fact that the anæmias due to intestinal parasites, and showing a pernicious blood condition, may recover under appropriate treatment.

Megaloblasts may be found in milder forms of anæmia, but the normoblast is the prevailing type. The typical megaloblast is a very large red cell, at times twice as large as the average (11 to 20 μ in diameter). Its protoplasm frequently shows marks of degeneration (polychromatophilia). The nucleus is very large and pale, filling most of the cell, thus

contrasting greatly with the normoblast. It does not stain evenly, but has a mesh-like appearance, with darker and lighter areas. The entire cell reacts differently with Wright's, Ehrlich's triple, and the eosin-hæmatoxylon stains (see description of these stains). The cell may be circular, but is more often oval or somewhat irregular.

Metrocytes.—One frequently finds in anæmic blood nucleated red cells, which can not be classed as typical normoblasts or megaloblasts. The whole cell is too small, or the nucleus is lacking in certain characteristics. These are classed as *atypical* forms of nucleated red cells, or *metrocytes.*

The real criterion of the two varieties, according to Pappenheim, is the structure of the nuclear network (pale nuclei with delicate chromatin network is indicative of a young megaloblast).

Most megaloblasts in the blood are young, while most normoblasts are old, as shown by their small, dark, coarse-skeined nuclei. For ordinary work Cabot gives the following rule: Any nucleated red cell more than 10 μ in diameter should be classed as a megaloblast, whatever the appearance of its nucleus; and any nucleated red cell less than 10 μ in diameter is probably a normoblast, whatever the appearance of its nucleus.

Karyokinesis.—Red cells with dividing nuclei are frequently found in severe anæmias—especially in leukæmia.

Degeneration of Leukocytes.—Glycogenic degeneration of leukocytes or iodophilia is demonstrated by means of a stain made up of iodine in mucilage of acacia (see Chapter I.). The mixture is painted on a slide, and the unfixed cover-glass preparation pressed down upon it. When the reaction is present, the protoplasm of the leukocytes stains brown, slight or intense, with deeply stained flakes or granules.

Acute Degeneration of Leukocytes.—Very little is known about this, but Ewing recognizes the following evidences of acute degeneration:

(*a*) Increased acidophile staining tendency in the neutrophile granules.

(*b*) Diminution in the number of neutrophile granules.

(*c*) Swelling and fragmentation of the bodies of leukocytes.

(*d*) Nuclear changes; staining less deeply with basic dyes; irregularity of outline and lobes shrunken.

Chronic Degeneration of Leukocytes.—This is best seen in myelogenous leukæmia. One finds leukocytes showing loss of granules, presence of vacuoles, granules of glycogen, faded irregular nuclei, distortion and fragmentation of cell-bodies.

Complete subdivision of nuclei of polynuclear cells into 6 to 10 hyperchromatic segments is rather characteristic of leukæmic blood.

Hydropic and **fatty degeneration** may be present.

Perinuclear Basophilia of Neusser.—By this is meant the presence of basic staining granules about the nuclei of polynuclear and other leukocytes. It is of no clinical importance.

Myelocyte.—This cell is a mononuclear neutrophile, and has many points of resemblance to the polynuclear neutrophile—in fact, it is the young form of that cell. It differs from the large lymphocyte chiefly in being larger and having neutrophile granules. Its nucleus is usually spherical or egg-shaped, and is in close contact with the cell-wall for a comparatively large portion of its circumference. Its average diameter is 15.75 μ.

Eosinophilic Myelocytes.—The myelocyte rarely shows eosinophile granules.

The Ehrlich triacid stain alone differentiates the myelocyte from the large lymphocyte—by means of its granules.

QUESTIONS.

What is meant by poikilocytosis, polychromatophilia, endoglobular degeneration, and punctate basophilia?

Describe microcyte, megalocyte, microblast, normoblast, megaloblast, and metrocyte.

CHAPTER VI.

THE WIDAL REACTION.

The Widal clump reaction depends on the presence of a substance called **agglutinin** in the blood of typhoid fever patients. It has the peculiar power of causing the typhoid bacilli to lose their motion and to clump.

The blood from other diseases possesses this power, but not to so great a degree.

In a limited time high dilutions of typhoid blood show this clumping effect, while similar dilutions of other bloods do not.

This **agglutinating substance is present in:**

(*a*) The whole blood—fluid or dried.

(*b*) The plasma and serum—fluid or dried.

(*c*) Blister fluid, fluid contents of normal serous cavities, tears, pus, breast-milk, etc.

The whole blood or its serum—fluid or dried—is usually employed.

Method of Obtaining Body Fluids.—(1) **Serum.**—In hospital work, where a centrifugal machine is at hand, it is best to use blood-serum. It is obtained as follows: A piece of glass tubing (small bore) is drawn out to a fine point, and rubber tubing attached to the large end. One or two large punctures are made in the finger (which has been well massaged previously), the fine point of the tube placed in the drop and suction made with the mouth till considerable blood is drawn into the pipette. This is sealed by holding the pointed end in the flame of a match, and immediately centrifugated. The clot should be loosened around the edge to allow the escape of serum. A file-mark is made at the junction of clot and supernatant serum. With the finger over its top, break the tube and blow out the serum from the upper piece into a receptacle. If desired, dilutions without limit can be made with this serum as follows: One drop is placed in a small glass dish and 10 drops of distilled water added,

giving a dilution of 1 : 10. With the typhoid culture, the cover-glass preparation is made with a drop of this dilution, then 10 drops more of distilled water added, giving a dilution of 1 : 20, the cover-glass preparation again made, then 10 drops more added, and so on. The same pipette must be used for measuring the drop of serum and the water in order to secure accuracy.

Whole Blood Method.—In private practice this is more easily carried out than the above :

(1) An accurate dilution of 1 : 10, 1 : 20, or 1 : 40 can be made with a white-blood counter, using distilled water as a diluting fluid.

(2) A convenient and easily applied method (especially if the preparation is to be sent by mail) is to allow a good-sized drop to dry on a cover-slip or glazed paper. If paper has been used, cut out the blood-drop and place in a test-tube containing 2 drops of water; by agitating it the blood will dissolve. To obtain a dilution of 1 : 10, 8 drops of water or bouillon are added.

The Culture of the Typhoid Bacillus.—A young—eighteen to twenty hours old—typhoid culture is required; it must be actively motile. It may be grown on agar or in peptone bouillon, in the thermostat at 37° C. The disadvantage of the bouillon is that the growth is often very small. Stock cultures are grown on agar at room temperature, and are transplanted about once a month.

Actively motile cultures show the clumping best.

Preparation of Specimen for Microscopical Examination.—A drop of the blood or serum diluted as desired, 1 : 10, 1 : 40, etc., is placed upon a cover-glass. By means of a platinum wire a bit of the typhoid culture is transferred from the agar tube to this drop and well stirred in order to separate thoroughly the bacilli. A concave slide, rung with vaselin, is placed upon the cover-glass, thus giving a hanging drop, and the preparation immediately examined under the microscope. A control slide should be made with distilled water for comparison. The preparation should now be carefully examined every few minutes for loss of motility and

signs of clumping. The length of time for the development of this phenomenon should be noted.

If a bouillon culture is used, a drop of the serum or whole blood is added to 10 or 40 drops of the bouillon in a small test-tube, depending on whether a dilution of 1 : 10 or 1 : 40 is desired. A drop of this mixture is then transferred to a cover-slip and a hanging drop made as before.

Ficker's Typhoid Diagnosticum furnishes the general practitioner, with an easily applied and trustworthy substitute for the Widal test. The fluid is manufactured by Merck, of Darmstadt. The entire apparatus costs $1.85. von Tiling carries out the test as follows: Prick the finger, catch a few drops of blood on a slide or a piece of filter paper, let it dry and later dissolve it with normal salt solution in the proportion of 1 : 10. This diluted blood is mixed with the diagnostic fluid in the proportion of 1 : 5 and 1 : 10 in two of the little test-tubes, which, therefore, contain a serum dilution of 1 : 50 in one glass and 1 : 100 in the second. Then a third glass is filled with the diagnostic fluid alone. The reaction is positive if after from ten to twelve hours the fluid in the second or third glass begins to get clear, for in this case the bacilli clot together and sink to the bottom. Sometimes the reaction proves positive in a shorter time, sometimes it may take twenty hours, but if no clearing of the fluid occurs in this time, the test is considered negative.

Dilution and Time Limit.—Although many able men believe that a dilution of 1 : 10, with a time limit of half an hour, is sufficiently accurate, most authorities favor a high dilution with a special time limit; for example, 1 : 40 with a limit of two hours.

Value in Diagnosis.—From statistics we learn that about 95 per cent. of typhoid fever patients show the Widal reaction at some time during its course.

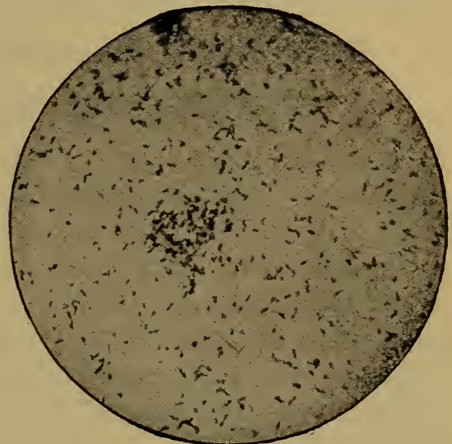
A negative result does not prove the absence of typhoid, however; only a positive result is of value. The negative results may sometimes be explained by the fact that the disease, though clinically resembling typhoid fever, is due to infection with the paracolon or colon group.

Time and Appearance of Widal's Reaction.—Most observers give the sixth or the eighth day as about the earliest

Fig. 16.

Pure culture. (Cabot.)

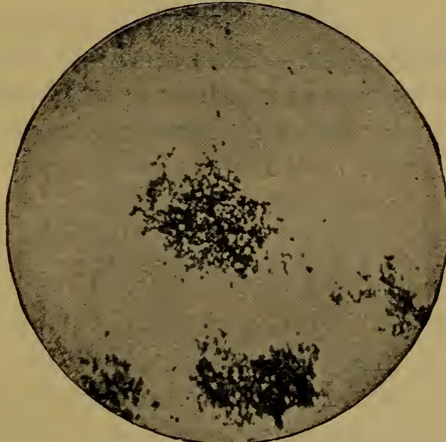
Fig. 17.



Partial reaction. (Cabot.)

for the reaction to make its appearance. In mild cases the reaction may disappear before the end of the fever, but it

Fig. 18.



Typical clumping. (Cabot.)

usually persists several months, and has been known to continue for years.

QUESTION.

Describe in detail the Widal reaction.

CHAPTER VII.

LESS FREQUENTLY APPLIED PROCEDURES.

ESTIMATION OF ALKALINITY OF THE BLOOD.

The alkaline reaction of the blood may be demonstrated by repeatedly drawing a strip of red litmus-paper, thoroughly moistened with a concentrated solution of common salt, through the blood, and rapidly washing off the corpuscles with the same solution. The estimation of the degree of alkalinity has so far proved to be a complicated, unsatisfactory procedure, and is of little practical importance to the clinician. The reader is referred to larger works on clinical diagnosis, where a description of von Jaksch's and Löwy's methods may be found.

Engel's alkalimeter furnishes the most useful clinical method of estimating the alkalinity.

INDIRECT METHODS OF ESTIMATING THE NUMBER OF RED CELLS.

Oliver's Hæmocytometer.—This method estimates the number of corpuscles by means of their optical effect. It is very useful in many cases, but can never serve as a substitute for counting. The reader is referred to Ewing, p. 35, for details.

ESTIMATION OF COMPARATIVE VOLUMES OF PLASMA AND CORPUSCLES.

The **hæmatocrit of Hedin**, as modified by Judson Daland, is another indirect method of estimating the number of red and white corpuscles. It can not be recommended as a satisfactory substitute for counting. It simply ascertains the relative volume of corpuscles and plasma in a drop of blood. A glance at a stained specimen of anæmic blood soon convinces one that corpuscles vary much in size in different diseases. In such cases inaccurate results are obtained with the hæmatocrit.

Method of Using the Hæmatocrit.—A graduated capillary tube is filled with blood. By means of a centrifugal machine the corpuscles are thrown to the bottom. The height of the blood column is noted on the scale, and the number of red and white cells calculated.

ESTIMATION OF TOTAL BLOOD VOLUME.

Blood examination, until recent years, has been limited to samples of blood from the peripheral circulation, and deductions concerning the rest of the blood drawn therefrom. This, however, did not give a correct idea of the total blood volume. The following method has been suggested by Haldane and Smith: The patient inhales a measured volume of CO; after two or three minutes a few drops of blood are taken for analysis, and the percentage to which the hæmoglobin has become saturated with carbonic oxide is estimated by the carmine method.[1]

ESTIMATION OF THE TIME AND COMPLETENESS OF COAGULATION.

Normally, clotting occurs in about three minutes, but in the exanthemata, in the various forms of the hemorrhagic diathesis, in obstruction of the biliary tract with or without jaundice, and in the various anæmias it may be very much delayed.

WRIGHT'S METHOD.

This method consists in the use of a set of from six to twelve capillary tubes (0.01 to 0.0125 inch in diameter), into which a column of blood is aspirated. The tubes are placed perpendicularly in a rack, and at regular short intervals the blood is blown from each one of them. When it becomes impossible to blow it out, coagulation has set in, and the time is noted.

Hayden thinks he can distinguish between secondary and pernicious anæmia by the incomplete formation of serum in the latter.

[1] See *Journal of Physiology*, xxii., p. 232; *Ibid.*, xxv., p. 331.

CRYOSCOPY.[1]

The Determination of the Freezing-point.—*The Molecular Concentration of the Blood.*—An important peculiarity of the blood, first established by Korányi, is the fact that its *molecular concentration* is constant under normal conditions, but is changed in certain diseases, and almost exclusively in affections of the kidneys. These organs serve as regulators of the molecular composition of the blood, and this equalization is explained upon the theory of *osmotic pressure.* Since the osmotic pressure of a solution is proportional to the number of molecules, determination of the former would give the molecular weight. In practice this is accomplished in an indirect manner. Upon the basis of the fact that the lowering of the *freezing-point* of a solvent (*e. g.*, water) by addition of foreign substances is in proportion to the osmotic pressure of the solution, a determination of the molecular weight is directly obtained by measurement of this lowering.

Determination of the freezing-point is done in Beckmann's apparatus. This consists essentially of a very delicate thermometer graduated in 100 parts, in which each Celsius degree is again divided into 100 degrees. The thermometer is placed in a glass cylinder in which the fluid to be examined is kept constantly agitated by means of a platinum stirrer. Glass cylinder, thermometer, and fluid are then placed in a freezing mixture at —4° C., and the fluid cooled under constant stirring. A moment then arrives when the fluid suddenly congeals. At the point of change from the fluid to the solid state heat is liberated, which causes the mercury column rapidly to rise to a certain point, where it remains stationary for some time—the *physical* freezing-point. On long standing it again sinks and acquires gradually the temperature of the surrounding freezing mixture. If the freezing-point of distilled water is now determined in the same manner (the scale of Beckmann's thermometer is an arbitrary one and the zero point is not specifically designated), and the freezing-point of the solution is subtracted from that of the water, the

[1] From *κρύος* = ice.

figure indicating how much lower the solution freezes than the water is obtained. In blood this difference is —0.56° C. We say, therefore, in brief: The freezing-point of the blood amounts to 0.56, and an especial designation, therefore, has been selected: "δ," while "Δ" indicates the freezing-point of the urine. The procedure in blood examination is as follows: From a constricted vein of the arm (of course, under aseptic precautions) 15 to 20 cubic centimetres of blood are taken by means of puncture with a sharp cannula. The blood is then placed in the glass cylinder used for freezing and defibrinated by shaking with the platinum ring, when the freezing is at once begun. In a second glass cylinder the freezing-point of distilled water is determined each time. The latter procedure is considered necessary because the position of the mercury in the U-shaped Beckmann thermometer is easily subject to variations and because the thermometers with fixed 0 point appear not to be reliable. After some practice the whole procedure, including venous puncture, requires about thirty minutes. The molecular concentration of the blood in health is shown by a lowering of the freezing-point of —0.55° to —0.57° C. On the other hand, this value undergoes an essential change—*i. e.*, the freezing-point is lower when an affection of *both* kidneys exists. The degree of difference in comparison to the normal freezing-point of the blood is in proportion to the severity of the renal affection. While in mild degrees of nephritis normal values are still found, the freezing-point becomes lower and lower with increased insufficiency of the kidneys. Lowerings to below 0.70° C. have been observed. On the other hand, the differential diagnostic and prognostic fact is to be emphasized that in unilateral renal affection, even when the latter has advanced to complete destruction of the organ, the freezing-point shows the normal height, provided the other kidney is healthy. From this we obtain the important law that a lowering of the freezing-point of the blood below —0.58° C. almost invariably indicates a bilateral affection of the kidney (Lenhartz).

BACTERIOLOGICAL EXAMINATION OF THE BLOOD.

For this examination a moderately large quantity of blood must be obtained under strictly aseptic conditions. Prepare the arm as for venesection, and when the veins are full insert a sterilized needle to which a sterilized syringe is attached. Suction is made, and as much blood as desired withdrawn and immediately expelled into the culture-medium before coagulation sets in.

Of late, important work has been done in demonstrating the presence of the Bacillus typhosus in the blood, even before the Widal reaction can be obtained. As a result of improved technique the various bacteria, such as the streptococcus, staphylococcus, gonococcus, pneumococcus, etc., are much more frequently demonstrated in the blood than formerly. Rosenow[1] found pneumococci in 77 of 80 cases, and Kinsey[2] demonstrated pneumococci in 19 of 25 cases examined.

Technique.—It is the opinion of all that have met with success that comparatively large amounts of blood and bouillon should be employed. Kinsey's results are particularly instructive. He reports two series of cases of 25 each. In the first series from 8 to 9 cubic centimetres of blood were added to 50 cubic centimetres of bouillon, making a dilution of 1 : 6. The results were 3 positive and 22 negative. In the second series 1 part of blood (3 to 6 cubic centimetres) to 15 or 20 parts of bouillon were employed, with the result that 19 cases were positive and 6 negative, most of the 6 negative cases being unfavorable cases. Cole employed both bouillon and litmus-milk. Kinsey and others prefer the bouillon medium.

DISEASES IN WHICH DIAGNOSIS DEPENDS ON OR IS FACILITATED BY BLOOD EXAMINATION.

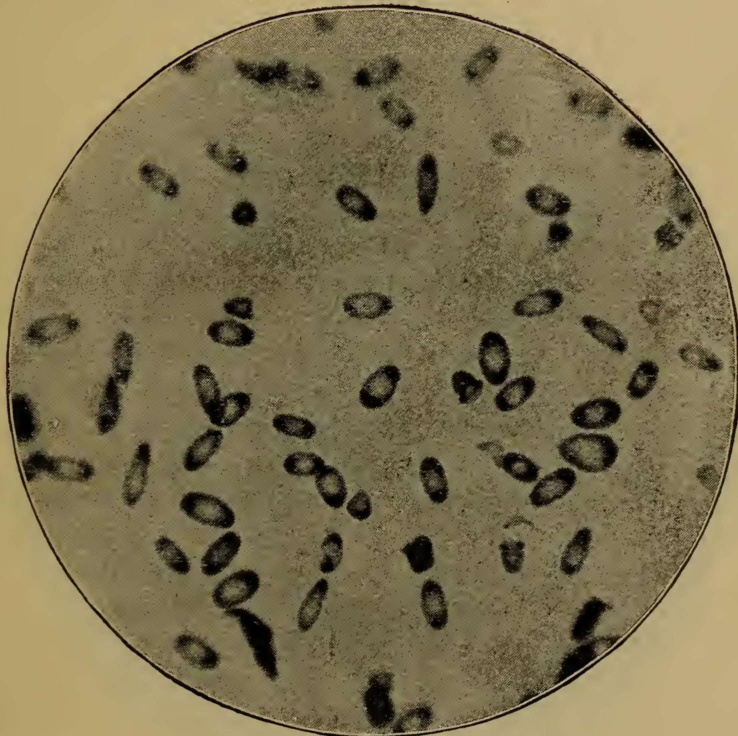
1. **Primary pernicious anæmia** is characterized by the following deviations from the normal blood condition (Figs. 19, 20, 21).

[1] *Transactions of the Chicago Pathological Society*, 1903, p. 265.
[2] *Journal of the American Medical Association*, March 19, 1904.

(*a*) The **hæmoglobin** is greatly reduced, frequently to even 20 per cent. or less.

(*b*) The **color-index** is usually high, owing to the increase in the size of many of the red cells and the excess of hæmoglobin which they contain.

Fig. 19.



Elongated or oval corpuscles in a case of pernicious anæmia. (Cabot.)

(*c*) The number of **red corpuscles** is greatly reduced. The average in Cabot's cases was about 1,200,000 per cubic millimetre. The lowest reported count is that by Quincke, of 148,000 per cubic millimetre.

(*d*) The presence of **unusual forms of red corpuscles**, marked poikilocytosis, polychromatophilia, and nucleated red cells in which *megaloblasts* exceed normoblasts.

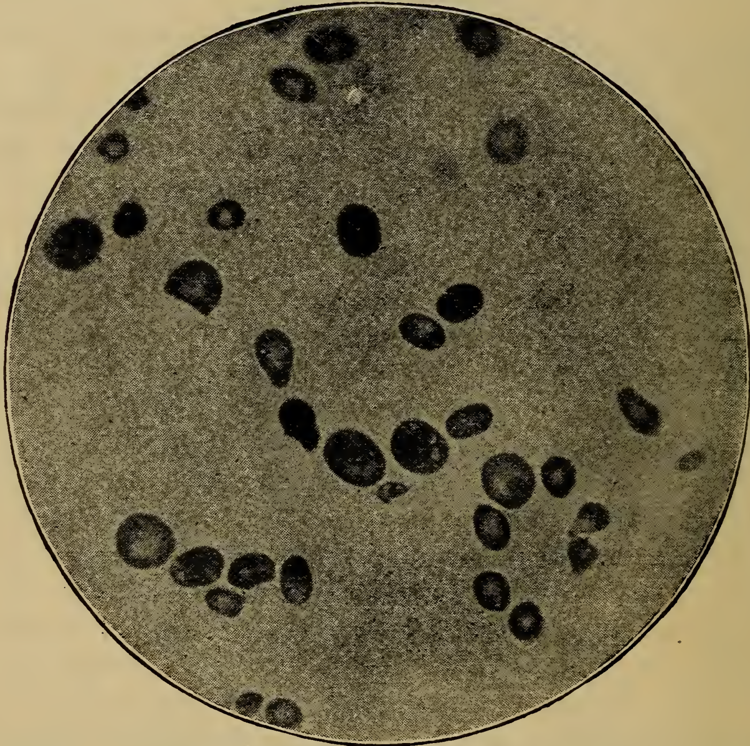
(*e*) **Oxygen capacity** decreased—volume variable.

(*f*) **Blood-drop** pale—may be streaked; is remarkably fluid; very slow in coagulating; absence of rouleau formation.

Rarity of blood-plates and loss of retraction on the part of the clot (Hayem).

Considerable diminution in the number of leukocytes. Cabot's 110 cases averaged 3800.

Fig. 20.

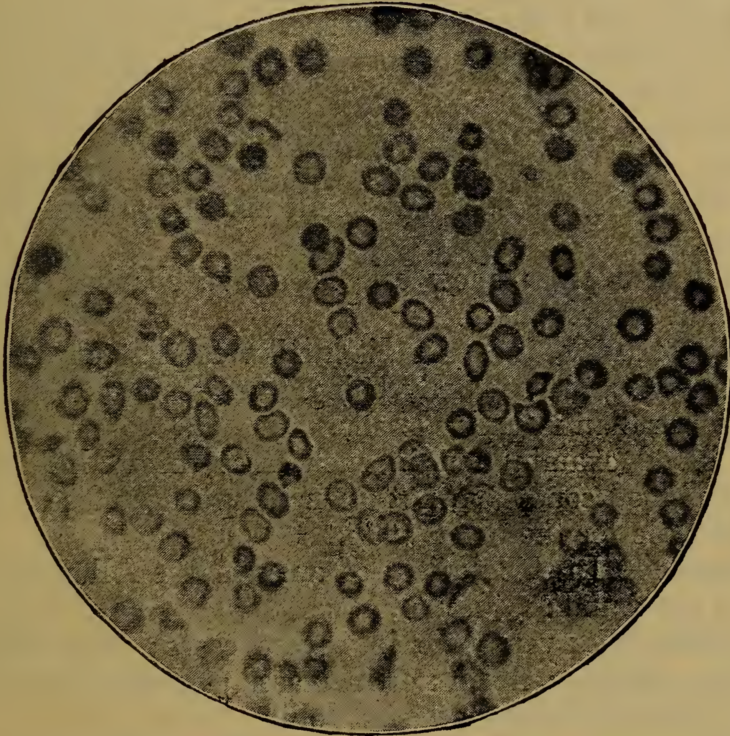


Pernicious anæmia. × 350. Note the relatively large size and well-stained centres of the cells. (Cabot.)

2. **Secondary Pernicious Anæmia.**—The blood condition is practically the same as in the primary form, differing only in its etiology. In the latter no definite cause can be found, but the former may be the result of parasites in the intestinal tract, frequent hemorrhages, etc. In spite of the pernicious blood condition, this variety of anæmia may recover under appropriate treatment.

Experience has shown that when the blood condition of pernicious anæmia is present (with the exception of the anæmia due to the ankylostoma, etc.) the prognosis is always grave, and a fatal issue may be expected within a few years, more or less. The most important diagnostic point is the preponderance of megaloblasts over normoblasts or the presence of a single gigantoblast.

FIG. 21.



Chlorosis. × 350. Note small size and pale centres. (Cabot.)

Fatal Anæmia with Hypoplastic Marrow (Aplastic Anæmia).—This is a very fatal anæmia of which only a few cases have been reported. Marked reduction in the red and white cells and hæmoglobin, with absence of nucleated red cells, great diminution in the percentage of polynuclear neutrophiles, and hypoplastic bone-marrow, are the chief blood signs.

3. **The Secondary Anæmias.**—These are very numerous, and vary in degree from the mildest to the most severe, the latter approaching the pernicious anæmias in blood changes.

The *mild forms* are characterized by: (*a*) lack of hæmoglobin; (*b*) lowered specific gravity, with perhaps only slight reduction in the red count. (We see such cases in poorly nourished women and in those living in unhygienic surroundings.)

Moderate cases show a more marked diminution in the hæmoglobin and reduction in the specific gravity, endoglobular changes, poikilocytosis, changes in staining properties, diminution in the average diameter of the corpuscles, with loss of power to form rouleaux, and slight leukocytosis.

Severe cases show all of the above changes, together with reduction in the number of red cells and the presence of normoblasts.

Very severe cases show all the above changes, with the addition of megaloblasts.

The **color-index**, in contradistinction to that of pernicious anæmia, is low, there usually being a greater reduction in the hæmoglobin than in the red cells.

The blood examination in such cases throws light simply upon the blood condition, not upon the primary disease which is responsible for the blood state.

Chlorosis.—In this variety of anæmia the following blood changes are found:

(*a*) **Blood color** very pale in severe cases; very fluid, but coagulates rapidly; fibrin not increased; specific gravity low, running parallel with the hæmoglobin.

(*b*) **Red cells** small, pale, and often deformed. The average count is in the neighborhood of 4,000,000, and seldom goes below 1,000,000. Nucleated red corpuscles (normoblasts) are rare.

(*c*) **White cells** not increased, but there is lymphocytosis and occasionally eosinophilia.

(*d*) **Blood-plates** increased.

(*e*) **Hæmoglobin-index** low, in contrast to that of pernicious anæmia, viz., a blood count of 4,000,000 red cells may furnish

only 40 to 50 per cent. of hæmoglobin. It is most difficult to distinguish chlorosis from secondary anæmia. This is due chiefly to the presence of leukocytosis in the latter. It is true, however, that the complications of chlorosis usually produce leukocytosis.

According to Smith, the blood volume is greater than normal by more than one-half. He concludes that there is really a large absolute increase in the number of red and white cells disguised by an excess of plasma. Treatment diminishes the amount of plasma, but does not increase the oxygen capacity. Very often the blood examination only allows us to diagnose "chlorotic condition of blood," and is of value from the therapeutical standpoint, but does not throw light upon the nature of the primary disease.

Leukæmia.—Blood examination is absolutely essential for the diagnosis of this disease, and enables us to distinguish its different forms. Leukæmia is of two kinds: myelogenous and lymphatic.

Myelogenous Leukæmia.—Fresh drop—opaque in color, sluggish in flow, difficult to spread, coagulation normal. The red-cell count varies with the stage of the disease; on an average it is a trifle over 3,000,000; hæmoglobin is usually diminished; color-index 0.6 in Cabot's cases. Nucleated red cells, chiefly normoblasts, are very numerous, even though the patient may not be anæmic. Variations in size and shape correspond with the degree of anæmia.

White Cells.—The average number per cubic millimetre in Cabot's 44 cases was 438,000; the highest being 1,072,222, and the lowest 98,000. The ratio between the red and the white cells in this disease may be 1 : 2, and even 1 : 1. During the stage of improvement the white cells may number as low as 10,000, and still show the characteristic type.

In contrast to the polynuclear cells of leukocytosis, the myelocytes are only slightly amœboid. The chief characteristic of leukæmia is the presence of enormous numbers of myelocytes, often averaging as high as 35 per cent. of the white cells.

The poly(morpho)nuclear cells are relatively much dimin-

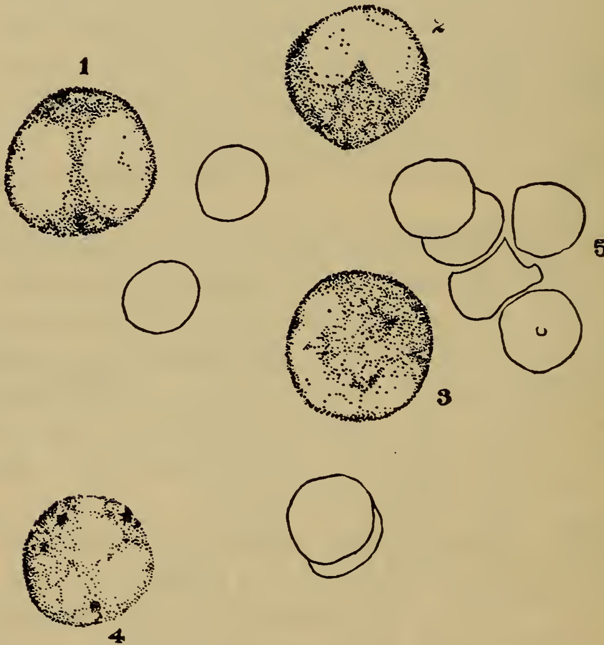
ished, but absolutely much increased. They show greater variation in size, staining properties, and size and shape of nucleus, than in any other disease.

The **lymphocytes** are reduced in percentage from 20 to 30 to 10.6 per cent.

Eosinophiles are absolutely much increased; relatively they may or may not be.

Polynuclear eosinophiles, dwarf and giant eosinophiles, and eosinophilic myelocytes may also be present.

Fig. 22.



Atypical leukocytes, seen in leukocytosis: 1, leukocytes with polar arrangement of nuclei (mitosis?); 2, 3, leukocytes with nuclei resembling those of myelocytes; 4, leukocytes containing two kinds of granules. (Cabot.)

The **condition of leukæmic blood is polymorphous.** There is no fixed type of cell; each variety shades through intermediate forms into some other variety; no two cells are alike.

Lymphatic Leukæmia.—This is divided into two varieties—chronic and acute.

Chronic:

1. Red cells about 3,000,000 or lower; nucleated forms rare.
2. White cells about 300,000.
3. Small lymphocytes usually over 90 per cent.
4. Myelocytes and eosinophiles very scanty.

Acute:

1. Red cells much diminished; nucleated forms infrequent.
2. Large forms of lymphocytes usually predominate. Many of them often show signs of degeneration.
3. Neutrophiles and eosinophiles very scanty.

DIFFERENTIAL DIAGNOSIS THROUGH BLOOD EXAMINATION.

Blood examination, including staining and differential counting, makes it possible to distinguish leukæmia from—

(1) Hodgkin's disease;
(2) Tumors of spleen and vicinity;
(3) Enlargements of lymphatic glands from tuberculosis, syphilis, and malignant diseases;
(4) Hydronephrosis;
(5) Large leukocytosis from any cause;
(6) Chronic malaria;
(7) Amyloid disease.

Hodgkin's Disease.—Blood examination furnishes the only clinical means of distinguishing Hodgkin's disease from lymphatic leukæmia. The physical signs of enlarged glands are the same, but the negative character of the blood excludes lymphatic leukæmia. It can not, however, help us to exclude syphilis or tuberculosis (many believe Hodgkin's disease and tuberculosis of the glands to be identical) or malignant disease, as the blood conditions may be the same in all these diseases.

Splenomegaly.—In patients with anæmia and an enlarged spleen blood examination makes it possible to exclude leukæmia of either variety. The majority of cases of splenomegaly show an enlarged spleen together with a moderate

anæmia and a leukopænia. Blood examination, however, does not enable us to exclude a chronically enlarged spleen from other causes; for example, an ague cake.

In **purulent affections** the leukocytes, when treated with a preparation of iodine, give a definite reaction, which is termed *iodophilia*.

Solution.—

Iodii sublimat.,	1 part;
Potassii iodidi,	3 parts;
Aqua dest.,	100 "
Acacia ad syrupum.	

This is painted on a slide and the unfixed cover-glass preparation pressed down upon it. In nonsuppurative diseases the red cells stain dark yellow; white cells light yellow, with very refractile citron-colored nuclei.

In purulent affections the protoplasm of the leukocytes stains brown, slight or intense, with deeply tinted flakes or granules.

After an abscess is opened the color soon disappears. Cold abscesses do not produce this reaction. It may occur in pneumonia, in puerperal sepsis without localization, and in nonsuppurative terminal infections.

Malaria.—The diagnosis can be made with absolute certainty by finding Plasmodium malariæ in the blood.

There are three varieties of the malarial parasite: (1) tertian, (2) quartan, and (3) æstivo-autumnal. It is very seldom that the quartan or æstivo-autumnal parasite is found farther north than Baltimore, most of the cases in this latitude being of the tertian variety. There are certain differences in the morphology of these parasites which the beginner will find difficult to recognize. This is especially true of the young forms.

There are two methods of examining the blood for malarial parasites: (1) fresh blood; (2) stained specimen. The fresh drop method is extremely useful for diagnosis, the study of the vibratory motion of the pigment, amœboid motion of the

parasite, exflagellation, escape of the parasite from the cell, and occasionally the formation of vacuoles. Slide and cover-slips should be thoroughly cleaned and polished. The slip is touched to a small drop of blood exuding from the finger and immediately placed upon the slide. The blood should spread in a thin layer one cell deep, in order that the flat surface of the cells can be seen. It is best examined with a $\frac{1}{12}$ oil immersion. The beginner should secure the blood when the parasites are old and well developed, a few hours before the expected chill, as they are easy to recognize at this stage. Even an expert finds it impossible to be certain of his diagnosis of the younger forms of malarial parasites in the fresh drop. It is very difficult to distinguish between the young hyaline forms and the presence of vacuoles, or crenation changes in the red cells. A mechanical stage is almost a necessity for accurate work. A negative fresh drop examination should always be controlled by examination of the stained specimen.

The **flagellate bodies** may be studied in the fresh drop (on a warm stage if possible). They usually appear in from ten to twenty minutes. They form chiefly from the larger tertian and æstivo-autumnal parasites; less often from the quartan. They represent the male element in the sexual reproduction of the malarial parasite. They may be stained by keeping the films moist for ten to twenty minutes in a Petri dish containing wet blotting paper and sealed with vaselin. (The moisture favors the process of flagellation.) The preparation is then dried and stained.

Staining Dry Specimens.—Spreads are made in the ordinary way, care being taken to secure a thin film. It is unnecessary to fix them if Wright's stain is to be used. For staining by other methods, they should be fixed in 95–97 per cent. alcohol, or in equal parts of alcohol and ether for fifteen to thirty minutes (though five minutes will suffice).

By far the simplest and best method of staining the malarial organism is with *Wright's stain.* Most beautifully stained specimens, which even rival those done by the Nocht-Romanowsky method, are obtained in a few minutes. The staining

reaction is very similar to that of the Nocht-Romanowsky. Excellent colored plates can be found in Ewing, which illus-

FIG. 23.

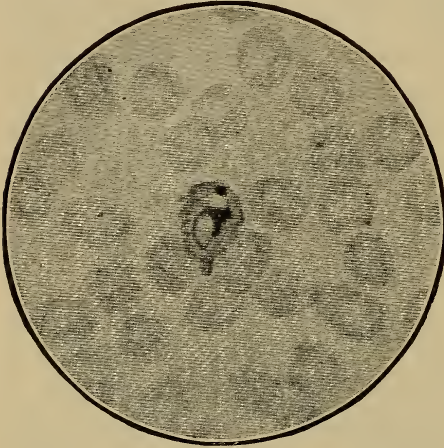


FIG. 24.

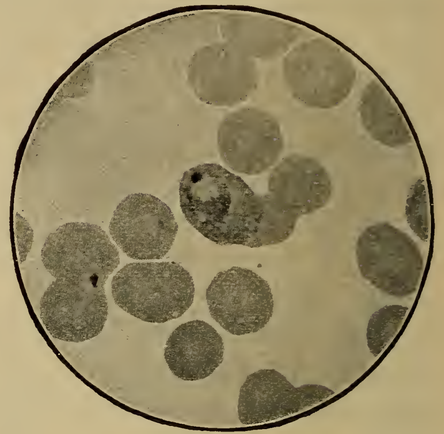


FIG. 25.

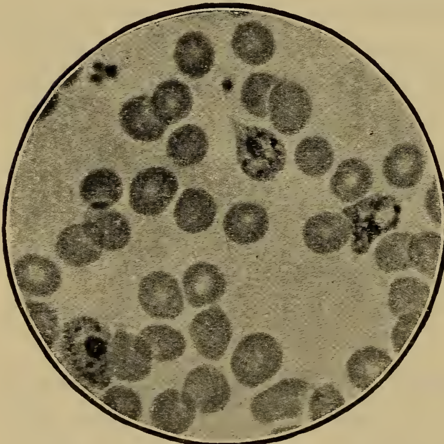


FIG. 26.

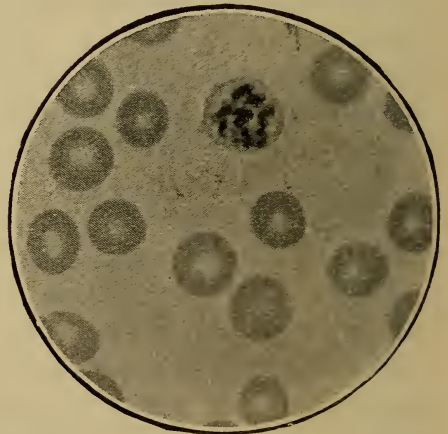


FIG. 23.—Tertian fever. (Cabot.)
FIG. 24.—Tertian fever. Marked stippling of infected corpuscle. Single chromatin body. Achromatic zone very distinct. (Cabot.)
FIG. 25.—Tertian. (Cabot.)
FIG. 26.—Intracorpuscular. Segmenting body in tertian fever. Stippling of corpuscle. (Cabot.)

trate the working of this stain, and will help the reader in the study of the development and morphology of the parasite.

The body of the malarial parasite stains blue, while the

Fig. 27.

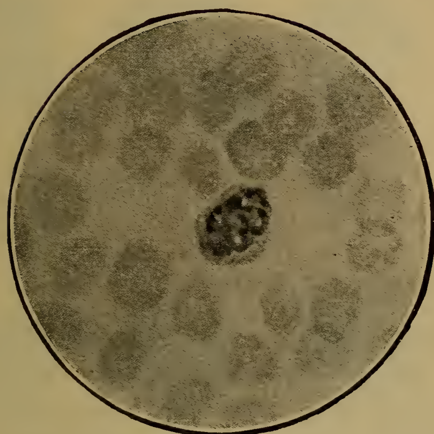


Fig. 28.

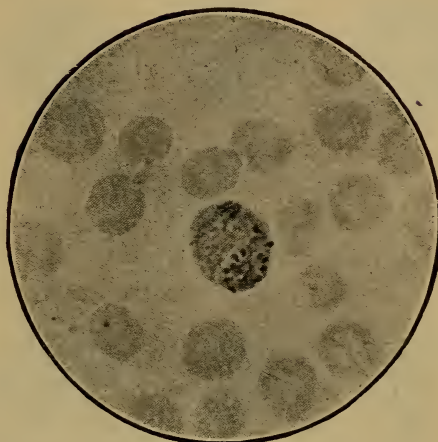


Fig. 29.

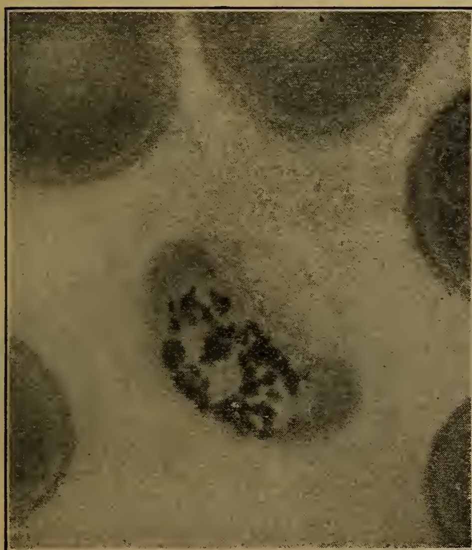


Fig. 30.

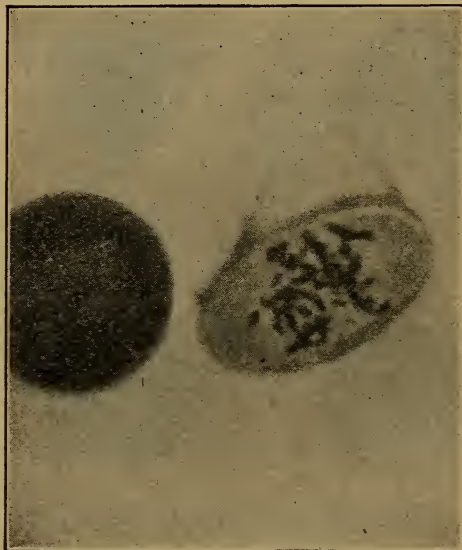


Fig. 27.—Segmentation of malarial organism in tertian fever. Stippling of corpuscle. (Cabot.)

Fig. 28.—Tertian fever. Double infection of corpuscle, one organism segmenting. (Cabot.)

Fig. 29.—Crescentic parasite distending a red blood-corpuscle. (Cabot.)

Fig. 30.—Ovoid form of the æstivo-autumnal parasite distending a red blood-corpuscle. A portion of the corpuscle projects above the parasite and is much distorted. The dark line around the parasite also represents the remnants of the corpuscle. (Cabot.)

Fig. 31.

Flagellate malarial organism. (Thayer.)

color of the chromatin varies from a lilac color, through different shades of red, to almost black. In the young forms of the tertian and æstivo-autumnal parasites the chromatin appears as a very dark-red spherical body, while in the older

Fig. 32.

The first twelve figures show the malarial plasmodium. It is a pale amœboid body inside the red corpuscle. It increases in size at the expense of the corpuscles. In the last four of the twelve it is enlarged, and contains pigment-granules derived from the hæmoglobin. The figures of the fourth row show progressive stages in the process of cleavage of the plasmodium, and shifting of the pigment-granules. In the fifth row the process of cleavage is seen to be completed, and final isolation of the spores has taken place. The dark granules are pigment-granules. The last row shows oval parasites—Laveran's corpuscles observed in atypical cases of malaria. (From Golgi, "Studien über Malaria" *Fortschritte der Medicin*, Bd. iv., Talfel III.)

forms it has a more lilac or purplish-red color, and may appear in the form of a reticulum.

In the intermediate forms the color of the chromatin may present variations between these extremes. If examined in water instead of Canada balsam, the distinct red color of the chromatin is more apparent.

The blood-plates situated seemingly within the red corpuscle must not be mistaken for the young form of the parasite.

The young parasite of all three kinds presents by this

method a dark-red spherical nucleus, and a cytoplasm which is usually in the form of a definite ring.

Some of the red cells harboring parasites show by this method dark-red staining granules.

The *Nocht-Romanowsky* is an excellent stain, but is more complicated and requires several hours for its completion. For its application, see page 28. The remarks on the Wright stain apply very well to this stain.

Eosin and methylene-blue is the method commonly used, but is much inferior to Wright's. The solutions mentioned on page 27 are used. This eosin staining should be light.

Thionin Method.—Fix specimens five minutes in 95 per cent. alcohol, to 100 c.c. of which has been added 1 c.c. of formalin. Stain one to three minutes in the following mixture: saturated alcoholic solution of thionin, 20 c.c.; 20 per cent. carbolic acid, 100 c.c. The fixing solution must be fresh; the staining fluid at least one week old. The rings are deeply stained, and the specimens do not fade.

TERTIAN PARASITE.

Morphology.—The malarial parasite is introduced into the circulation by means of a certain species of mosquito (*Anopheles*). The tertian variety goes through a life-cycle in the human blood in forty-eight hours. The youngest form appears in the red corpuscle during or soon after the chill, as an oval body 2 μ in diameter.

It is identical with the spore of the parent rosette. Stained according to the *Wright method,* it shows an outer rim of bluish protoplasm, enclosing a single large nuclear body, which takes a dark-red stain, which is usually enclosed or accompanied by a clear achromatic substance—"the milk zone" of Gautier.

In the fresh specimen this young form is refractive, but does not show the complete lack of color or the sharp outlines that the vacuoles of the red corpuscles do. They change their position, but not their shape, and are never pigmented. The red corpuscle is often swollen. The parasite grows,

assumes a ring shape, and usually preserves this up to the presegmenting stage. A few grains of pigment may be found in the rings.

Large Ring Forms.—In from six to eight hours the ring has enlarged and developed an outgrowth which is actively amœboid in the fresh condition, throwing out numerous threads. The chromatin divides into several large granules.

Spheroidal Bodies.—The body and nucleus develop rapidly in size, and toward the end of twenty-four hours the parasite occupies three-fourths of the swollen cell in the form of a spheroidal body. The pigment is abundant—mostly found in the outer zone. There is a gradual subdivision of the chromatin granules.

Third Quarter.—The full-grown parasite is homogeneous, richly pigmented, and occupies at least four-fifths of the swollen cell. Ewing thinks that between the twenty-fourth and fortieth hours of the cycle very little change takes place in the structure of the parasite. The nucleus occupies the entire ring, and undergoes changes associated with reproduction.

Presegmenting bodies begin to appear in the blood eight to ten hours before the chill. The body of the parasite becomes reticulated.

The completed process of segmentation shows itself in the *tertian rosettes*, which appear in the blood three to four hours before the chill, but are most abundant *just* before it. These rosettes are of large size, and have from ten to twenty spores. These spores separate, and appear in the plasma as small free hyaline bodies. They soon enter the red cells and begin a new cycle of intracorpuscular existence.

Instead of segmenting, the parasite sometimes escapes from the red cell and appears as an *extracorpuscular* form. This is about the size of a red blood-cell, and does not exhibit amœboid movements. They sometimes slowly disintegrate and disappear; at other times they show fragmentation, vacuolation, or flagellation.

Fragmentation is a division by the process of budding. Occasionally *vacuoles* form in the large free parasite.

Flagellation.—In a small percentage of cases the extracorpuscular bodies become flagellated. Their pigment collects in the centre and becomes very active, and several thread-like processes or flagella are protruded from the periphery. These are several times longer than the diameter of the corpuscle and have slight bulbous enlargements. These flagella may whip about vigorously, causing great disturbance among the red cells. They sometimes break off. The significance of flagellation is now understood, the flagella being a form of spermatozoon and associated with the reproductive process (MacCallum).

Double Infection.—The blood may be infected with two sets of the tertian parasite (double tertian), causing a paroxysm daily. This fact is recognized by finding in the blood one set of full-grown parasites and another of half-grown parasites. Each set requires the full forty-eight hours for the completion of its cycle.

The **quartan parasite** requires seventy-two hours for the completion of its cycle. The youngest form in the stained preparation can not be distinguished from the tertian organism, but it may be suspected from the smaller red cell. In the fresh specimen the organism is more highly refractive than the tertian. It becomes more easy of identification after a few hours' development, because it takes the form of a ring which is smaller, more compact, and more richly and coarsely pigmented. In the fresh specimen at this stage the amœboid motion is slower and the organism is more refractive.

It is easier to distinguish these two parasites during the presegmenting stage. Quartan presegmenting bodies are much more numerous. They are more coarsely reticulated, relatively smaller and more richly pigmented, and lie in shrunken cells.

Blood may be infected with one, two, or three sets of quartan parasites, each ripening on a certain day, and forming a single, double, or triple quartan infection, with one chill every three days, or two chills every three days, or a chill daily.

The **æstivo-autumnal parasite** in its youngest form resembles the tertian and quartan; it is smaller and less refractile than either.

Signet-ring.—At an early period it assumes a ring shape; many of these rings develop a thickening of one segment, giving them the appearance of a signet-ring. Multiple infection with the young form is common, three rings often being found in a single cell. Ewing reports having found seven rings in one red cell in smears from the marrow of a fatal case.

In the majority of cases the ring forms seen in the peripheral blood fail to show any pigment. The segmenting forms resemble those of the tertian, but are smaller; the cell is shrunken and the spores are smaller, but not less numerous. Segmentation takes place in the spleen or in other internal organs. Before and during the paroxysms no parasites at all may be found in the peripheral blood.

Crescentic bodies are very striking objects, and are found in cases of æstivo-autumnal fever. The adult form does not appear until the fifth to seventh day after the paroxysm. Their length is slightly greater than the diameter of the red corpuscle. They are crescentic or elliptical masses of protoplasm, containing near their centre a collection of coarse dark pigment-granules. A dim curved line can be seen on the concave side of the crescents, joining the two ends. In stained specimens this faintly stained line is seen to be the remnant of a red corpuscle. Little is known of the nature and significance of crescents. They are persistent and resistant to treatment.

The transverse segmentation, lateral budding, and vacuolation are probably degenerative changes. Crescents frequently, on being exposed to the air for a few moments, assume the spheroidal form, and soon from one or more points pseudopodia (so-called flagella) shoot out with active lashing movements.

The æstivo-autumnal parasite is associated with the irregular and protracted, continued, and remittent, chronic and cachectic, or the more malignant forms of malarial disease.

It is seldom seen in temperate regions, but is abundant in the tropics.

Melanæmia.—By this is meant the pigment left after the breaking up of the segmenting forms; it is free in the plasma.

Melaniferous Leukocytes.—Leukocytes which have taken up these pigment-granules, or the granules remaining from disintegrated parasites, are termed melaniferous.

Filariasis.—The filariæ found in the blood of man are embryos, the adult form being lodged in the lymphatics. There are four varieties: Filaria nocturna, Filaria diurna, Filaria perstans, and Filaria Demarquaii.

The only variety which is found at all frequently in the United States is Filaria nocturna. It is common in the tropics, and many cases have been reported in the southern part of the United States. Its adult form is the nematode, Filaria Bancrofti. The embryo is found in the blood only during sleep or at night. It is about 0.3 mm. long and 0.0075 mm. in diameter, small enough to pass through the capillaries. It is present in small numbers. The live worm exhibits active wriggling motions, knocking the blood-cells about. It has a slender, worm-like shape; the posterior end is tapering and pointed, the anterior rounded and blunt. It is enclosed in a delicate membrane, which does not interfere with its movements.

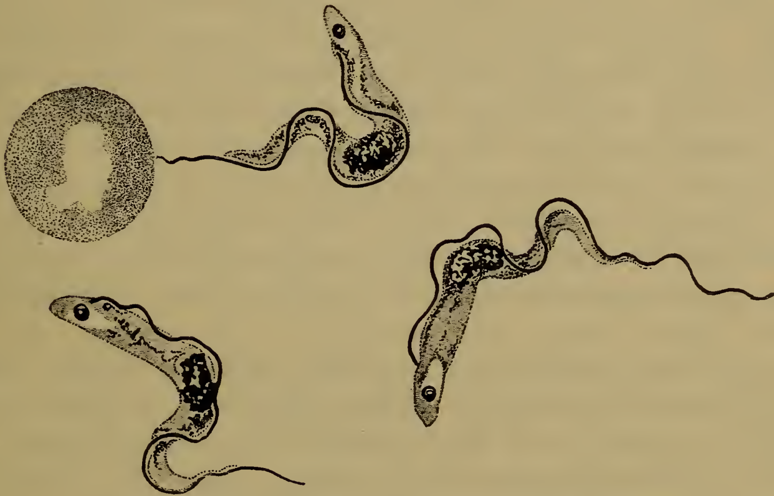
Filaria diurna is found in the peripheral blood in the daytime; *perstans* continuously. Both are found in Western Africa, and may be present in the blood for years without producing symptoms.

Relapsing Fever.—The **spirillum of relapsing fever** (Spirochæta Obermeieri) is a rare parasite, easily found in the fresh blood or stained preparation. Sometimes it is present in large numbers during and for a day or two before the paroxysms of this disease. They are slender, wavy or spiral filaments, 30 to 40 μ long, and actively motile.

TRYPANOSOMIASIS.

The trypanosome has of late acquired great importance because of its discovery in man in cases of sleeping sickness, and because of its possible etiological association with several other tropical diseases. Bruce demonstrated the organism in the blood in 13, and in the cerebrospinal fluid obtained by lumbar puncture, in all of 38 cases of sleeping sickness. Castellani had previously found the parasite in the cerebrospinal fluid in 20 of 34 cases. Dutton, in 1902, was the first to demonstrate the occurrence of trypanosomes in man. In

FIG. 33.



Trypanosoma gambiense in human blood. (Dutton.)

animals, such as frogs, dogs, rats, ground-hogs, and horses, their occasional presence had long been known. Novy and McNeal succeeded in cultivating the Surra Trypanosome of the Philippines.

Trypanosoma gambiense (Dutton) is from 8 to 25 μ long, and from 2 to 2.8 μ broad. It is provided with an undulating membrane and a flagellum, which starts from a centrosome or micronucleus, lying in the posterior end of the animal, and projects somewhat beyond the anterior end (Fig. 33). There is an oval nucleus which is centrally located and

is made up of chromatin granules. In the wet preparation the organism exhibits slow spiral movements. It is found free in the blood-plasma, but may also be seen in the interior of leukocytes. They stain with Wright's stain similar to the malarial organism. Their number in a blood preparation is not large. During apyrexia they are not found. Infection probably occurs through mosquitoes.

The Leishman-Donovan Parasites.—Leishman and Donovan have recently described a new parasite found in the spleen, pulp and blood, intra vitam, in cases of Dumdum fever. (Dumdum is a station seven miles from Calcutta.) This is a small round or oval body, 2 or 3 μ in diameter, occurring in enormous numbers among the spleen cells and red corpuscles. Stained by Romanowsky's method they show a quantity of chromatin, of a very definite and regular shape, which clearly differentiates them from blood-plates or possible nuclear detritus. This chromatin appeared in the form of a more or less definitely circular mass or ring, applied to which, though apparently not in direct connection with it, is a much smaller chromatin mass, usually in the form of a short rod set perpendicularly or at a tangent to the circumference of the larger mass. The outlines of the sphere or oval enclosing these masses of chromatin are only faintly visible by this method of staining. These bodies are scattered freely among the cells, as a rule, isolated one from another, but here and there aggregated into clumps composed of 20 to 50 members (Leishman).

SPOTTED FEVER.

Wilson, Chowning, and Anderson have described a small organism called the Pyroplasma hominis, which is found in the blood of patients suffering with the so-called spotted fever of Montana, Oregon, and Nevada. It is described as an intracorpuscular, amœboid, non-pigmented organism. It sometimes has a terminal dark spot, and sometimes occurs in pairs when it is not amœboid. It is transmitted by the Rocky Mountain tick (Derma-centor reticulatus).

Craig (*American Medicine*, December 10, 1904) has made

special investigations of the blood in various diseases, and is convinced that these supposed parasites are nothing but areas in the blood-corpuscles devoid of hæmoglobin—vacuoles probably. The imperfect and difficult staining is simply due to the fact that some of the stain extended into the areas. Craig finds similar appearances in other diseases. He also points out the improbability that the disease is due to animal parasites. Neither the blood nor the urine shows such changes as are observed in Texas fever; the blood is rather thicker than normal. Stiles has also been unable to confirm the earlier reports, and in general shares Craig's views.

Bacteræmia.—Bacteriological examination of the blood, though a difficult procedure, is of great value in recognizing the different forms of bacteræmia. Typhoid fever may be recognized in this way before the development of the Widal reaction by the detection of Bacillus typhosus in the blood. The gonococcus, Diplococcus lanceolatus of pneumonia, and the different forms of pus organisms have been demonstrated in the blood by special cultural methods, thus establishing the diagnosis of different forms of bacteræmia. In malignant endocarditis this examination is of great value.

The blood is secured with a syringe from one of the veins at the bend of the elbow, under strict aseptic precautions. Because of the bactericidal power of the blood, it is difficult to grow the bacteria; consequently large quantities of fluid media, broth, etc., should be used—a proportion of 100 parts of the media to 1 of the blood. A fairly large quantity of blood is required—0.5 c.c.—because in most cases bacteria are not numerous in the peripheral circulation. For details of the method, readers should consult text-books on bacteriology.

Leukopænia.—An absolute reduction in the number of leukocytes below the lowest normal limit in the circulating blood is termed leukopænia or hypoleukocytosis. It is the opposite of leukocytosis or hyperleukocytosis. The lowest normal limit is usually given as 5000 per cubic millimetre.

It is rather seldom in any condition that the leukocytes fall much below 3000. Koblanck reports a remarkable

case in an epileptic man twenty-five years of age. In a careful examination of 20 stained cover-glass preparations he found only 1 leukocyte. Cabot refers to an unusual case of lymphatic leukæmia in which the white count fell from 40,000 to 419 per cubic millimetre in the course of three weeks as the result of the development of an acute septicæmia. There are two classes of leukopænia: (1) physiological; (2) pathological.

Physiological leukopænia may occur after prolonged cold baths, short hot baths, and stimulation of sensory nerves. A change in the distribution of the leukocytes in the vessels takes place as a result of vasomotor influences. Malnutrition and starvation are prominent factors in causing a reduction in the number of leukocytes. The faster Succi showed a decrease in his leukocytes from 14,530 to 861 per cubic millimetre after a seven-day fast. This number increased to 1530 on the eighth day, and remained at about this figure during the remaining twenty-two days of the fast. If disease is excluded, the number of leukocytes, especially the polynuclear, may be taken as an index of the patient's nutrition, a low count indicating poor nutrition.

Pathological Leukopænia.—It is rather difficult to separate leukopænias and a simple absence of leukocytosis. The fact that some of the most important diseases (when devoid of complications) show an absence of leukocytosis is a valuable aid in diagnosis. The following diseases are included in this list: **typhoid fever**; **tuberculosis**—including incipient phthisis, miliary tuberculosis, tuberculous peritonitis, tuberculous ostitis and periostitis, tuberculous pleurisy, tuberculous pericarditis.

If during the course of these diseases a leukocytosis develops, it points to the presence of a new factor; in typhoid fever, for instance, one of its numerous complications should be suspected and looked for, such as phlebitis, perforation, hemorrhage, peritonitis, abscess, bronchitis, etc.

Pulmonary tuberculosis inevitably becomes a mixed infection as the lesions increase, with a resulting leukocytosis.

DaCosta states that leukopænia, or at least an absence of leukocytosis, may occur during the course of the following

additional infectious diseases: measles, influenza, malarial fevers, Malta fever, and leprosy.

A combination of an intense infection and feeble resisting power may result in a very low white count, as in certain cases of pneumonia and appendicitis.

A well-marked leukopænia may be expected in about one-fourth of the cases of chlorosis and about three-fourths of the cases of pernicious anæmia. It is also found in some severe cases of secondary anæmia and in splenomegaly. Chronic gastro-enteritis in infancy reduces the white count below the normal.

An intercurrent infection may produce a leukopænia, as in Cabot's case referred to above.

Various investigators have produced a decrease in the number of leukocytes by the administration of different substances hypodermatically. Bohland found that it followed the injection of ergot, sulphonal, tannic acid, camphoric acid, atropine, agaracine, and picrotoxine. Delezene injected various anticoagulant substances—peptone, diastase, and eel-serum—with a resulting marked leukopænia. The leukopænic phase which precedes the development of leukocytosis has been referred to in the chapter on Leukocytosis.

In typhoid fever there is a gradual decrease in the number of leukocytes after the first week, the lowest counts being found during the fifth and sixth weeks. This rule, according to Winter, does not hold good in all cases, but it is so constant that if in a given case of noneruptive fever the number of leukocytes is normal or subnormal, it is a strong point in favor of the diagnosis of typhoid fever. There is a progressive diminution in the percentage of polymorphonuclear cells, which continues into the stage of early convalescence. The percentage of lymphocytes is increased throughout the fever, the increase being most marked in the stage of convalescence. The degree of the leukopænia corresponds in a general way to the severity of the disease (not taking into account the effect of complications). Counts as low as 9000 and 3000 are not rare. In pernicious anæmia the white-cell count runs parallel with the red-cell count and the hæmoglobin per cent. In some cases it is very low, falling to 1000

per cubic millimetre. As stated above, leukopænia is found in about three-fourths of the cases of pernicious anæmia, which is in marked contrast to the tendency toward leukocytosis in secondary anæmias.

Ehrlich believes that in these conditions there is a lessened proliferative function of the bone-marrow, which results in a diminution in the output of leukocytes by this organ.

Leukocytosis.—By leukocytosis is meant an increase of the number of leukocytes in the circulating blood above that which is normal for the individual. This increase must affect chiefly the polynuclear leukocytes or each variety in such a way that the relative proportions of the different leukocytes remain the same as in health.

Normal Percentage of Each Variety in the Adult.—

(*a*)	Small lymphocytes,	20–30	per cent.
	Large lymphocytes,	4–8	"
(*b*)	Polymorphonuclear neutrophiles,	62–70	"
(*c*)	Eosinophiles,	0.5–4	"
(*d*)	Mast-cells,	0.1–0.5	"

Myelocytes represent a pathological variety of leukocytes; hence, an increase of leukocytes involving especially the myelocytes is not considered a leukocytosis, but represents a special blood disease, which is considered under the heading Leukæmia.

Again, an increase of leukocytes involving especially the lymphocytes is not a leukocytosis, but is termed a lymphocytosis or a lymphatic leukæmia.

Some authorities prefer the terms hyperleukocytosis and hypoleukocytosis to indicate an increase and a decrease in the number of leukocytes, using the word leukocytosis to mean the normal number of leukocytes.

The normal number of leukocytes varies within quite a wide range in healthy adults (5000 to 10,500). People in a poor condition of nutrition, but with no special disease, have a low leukocyte count with a reduced percentage of polynuclear cells; while those in vigorous health have a high leukocyte count, even approaching a slight leukocytosis, with an increased percentage of polynuclear cells.

The estimation of the number of leukocytes is of great

value in the diagnosis and prognosis of disease, and aids materially in operative decisions, when considered in connection with other diagnostic and prognostic data. Considered by itself it is useless.

It is highly important to keep in mind the fact that a leukocytosis may be *physiological.*

Varieties of Physiological Leukocytosis.—(*a*) Newborn; (*b*) digestion; (*c*) pregnancy; (*d*) post-partum; (*e*) after violent exercise, massage, and cold baths; (*f*) moribund state.

In the *newborn* there is a leukocytosis varying from 17,000 to 21,000—greatly increased by digestion. This gradually decreases as the child grows older, till about the sixth year, when it approaches the normal adult standard. It must be kept in mind that the leukocyte count of a child is greatly influenced by the backwardness or forwardness of its development.

Attention to *digestion leukocytosis* is often overlooked in the estimation of leukocytes and the deductions drawn therefrom. After a meal rich in proteids the leukocyte count may increase in health about 33 per cent. A vigorous person whose fasting leukocyte count is 9000 may have a count of 12,000 three to four hours after a meal. The best time to make a fasting leukocyte count is before breakfast, since during the day there is more or less digestion leukocytosis most of the time. In certain diseases—other than those of the digestive tract—there may be quite a marked digestion leukocytosis. Cabot gives the following examples:

In a case of pneumonia the count before food was 10,400, after food 21,700; neurasthenia, before food 7500, after food 13,500.

Any disease of the gastro-intestinal tract, whether functional or organic, may prevent the appearance of the digestive leukocytosis.

In chronic gastritis there may be an absence of digestion leukocytosis, or it may be slight and late in appearing. In dilated stomach it may be absent. In the majority of cases of cancer of the stomach it does not occur.

Pregnancy.—Most primiparæ show a moderate degree of leukocytosis during the later months of pregnancy, averaging

about 13,000. It is not so common in multiparæ. The fact that in this condition there is no digestive leukocytosis suggests that the pregnancy leukocytosis may be simply a prolonged digestive leukocytosis.

The fact that there is normally a moderate leukocytosis during the post-partum period is of value because it might be taken as an evidence of *sepsis.*

Violent exercise, massage, and cold baths such as the typhoid bath, cause a moderate temporary leukocytosis—comparable to the *digestion* leukocytosis.

Moribund, or *terminal, leukocytosis* occurs during the terminal stages of different diseases, and in most cases is due to peripheral stasis. In some cases it is thought that the terminal infections may be responsible. The increase in white cells is moderate, seldom exceeding 20,000 or 30,000, and is usually in the polymorphonuclear cells. Occasionally, as in the case of pernicious anæmia reported by Cabot, the increase in the lymphocytes is so marked as to resemble lymphatic leukæmia.

Pathological leukocytosis.—Cabot makes the following classification :

(1) Posthæmorrhagic.
(2) Inflammatory.
(3) Toxic.
(4) Malignant disease.
(5) Therapeutical and experimental.

THEORY EXPLAINING PATHOLOGICAL LEUKOCYTOSIS.

Present evidence tends to show that this process is a general one involving the entire circulatory system—that a drop of blood from the finger or the ear may be taken as an index of the blood condition in the deeper vessels of the body. Leukocytosis is symptomatic of an excessive output and rapid development of leukocytes by the bone-marrow, due to the influence of *chemotaxis.*

The **chemotactic theory** may be stated as follows: The presence in the blood of certain chemical substances, produced by infective agents, is capable of exerting both an

attractive and a repellant influence upon the amœboid leukocytes. If cells are attracted by such substances, the phenomenon is known as a positive chemotaxis; if they are repelled, it is called negative chemotaxis. This effect upon the cells of the blood may be produced by bacteria or their products—necrotic tissue—which have gained entrance to the circulation, and thermal and mechanical irritants. It would seem that different varieties of leukocytes—polynuclear neutrophiles, eosinophiles, lymphocytes—respond to different stimuli; in one instance we have an ordinary leukocytosis, as in pneumonia, in which the polymorphonuclear cells are chiefly increased; in another, as in trichiniasis, an eosinophilia; in a third a lymphocytosis.

It seems reasonable to conclude that leukocytosis is a conservative process on the part of nature, and represents an attempt to destroy the infectious agent or its product by mechanical means—*i. e.*, phagocytosis; or by chemical means—the production of chemical substances (alexins) which act as bactericidal or antitoxic agents. Grabit-Schewsky states that these processes are most active at the period of maximum leukocytosis.

Just previous to the development of leukocytosis there is usually a stage in which the leukocyte count is low. This is called by Löwit the *leukopœnic phase.* Goldscheider and Jacob have proved that this is dependent purely upon an altered distribution of the cells in favor of the deeper vessels.

Pathological leukocytosis differs from the physiological in being usually of larger extent and of greater duration, and in being almost always accompanied by a relative and absolute increase in the polymorphonuclear leukocytes. There is also a change in the cell-structure in certain cells. A small percentage of the polymorphonuclear cells resemble myelocytes, having a nucleus which is on the border-line between the two cells: 1 to 3 per cent. of the cells have become so altered that they can not be distinguished from myelocytes.

Posthæmorrhagic Leukocytosis.—Following a large hæmorrhage there is usually within an hour a considerable leukocytosis, from 16,000 to 18,000. In hæmorrhage from the stomach this disappears again in a day or two, while in ordinary traumatic hæmorrhage it persists longer.

L. of C.

Inflammatory and Infectious Leukocytosis.—To the clinician the determination of leukocytosis in the numerous infectious and inflammatory conditions is of more practical value, from the standpoint of diagnosis and prognosis, than the leukocytosis in all other conditions.

In the consideration of this variety of leukocytosis and the deductions to be drawn from it, it is well to keep in mind the following facts:

There is no direct connection between leukocytosis and **fever**, since many febrile processes—typhoid fever, for instance—run their entire course, if uncomplicated, without leukocytosis, even showing a hypoleukocytosis.

Purulent and **gangrenous processes** usually cause a higher leukocytosis than serous processes (compare Empyema and Pleurisy); the amount of leukocytosis depends on the severity of the infection and the resisting power of the patient.

A leukocytosis which increases from hour to hour suggests an acute spreading inflammatory process, and its detection is of great value, in cases of acute appendicitis, in influencing the surgeon regarding operation and prognosis. Wright and Joy (*Medical News*, April 5, 1902) come to the following conclusions from a study of 124 cases of **appendicitis** in which they have blood records, and about as many more in which they have no records:

1. The leukocyte count is a valuable aid to prognosis in appendicitis.
2. This aid is distinct from its diagnostic value.
3. A high stationary or an increasing count indicates a morbid condition of increasing severity which demands operation, no matter what the clinical symptoms may be.
4. A low stationary or a decreasing count indicates that the severity of the case is abating, and that an operation may be safely postponed. Cases in which a falling count is accompanied by unmistakable signs of a generally bad condition form rare exceptions to this general principle, and in them there is no chance of error.
5. No arbitrary set of prognostic values to be assigned to various degrees of leukocytosis can be constructed. The important point is to follow any scheme in which one learns

to have confidence, provided the essential principle be preserved.

6. The count indicates when operation should be performed for the best interests of the patient.

7. Circumstances often render it desirable to postpone operation in appendicitis. Study of the blood enables it to be determined whether this may be done with safety, and often renders such postponement permissible.

When appendical abscess is walled off and stationary, leukocytosis is less than in the advancing process, and does not increase from hour to hour.

Leukocytosis is present in the following **inflammatory diseases** (Cabot):

Asiatic cholera, relapsing fever, typhus fever (according to the majority of observers), scarlet fever, diphtheria and follicular tonsillitis, syphilis (secondary stage), erysipelas, bubonic plague, yellow fever (some cases).

Pneumonia, smallpox (suppurative stage), malignant endocarditis, puerperal septicæmia, and all pyæmic and septicæmic conditions, actinomycosis, trichinosis, glanders, acute multiple neuritis (febrile stage), acute articular rheumatism, septic meningitis and cerebrospinal meningitis, cholangitis, cholecystitis, empyema of gall-bladder, acute pancreatitis, endometritis, cystitis (some cases), gonorrhœa.

Abscesses of all kinds and situations—felon, carbuncle, furunculosis, tonsillar and retropharyngeal abscess, appendicitis, phlebitis (some cases), pyonephrosis, perinephritic abscess, pyelonephritis, osteomyelitis, empyema, psoas and hip abscesses when not simply tuberculous, abscess of lung, liver, spleen, ovary, prostate; salpingitis, pelvic peritonitis, epididymitis.

Pericarditis, peritonitis, arthritis (serous or purulent, nontuberculous), conjunctivitis.

Gangrenous inflammations of the appendix, lung, bowel, mouth (noma).

Many inflammatory diseases of the skin, such as dermatitis, pemphigus, pellagra, herpes zoster, prurigo, some cases of universal eczema.

A **miscellaneous class producing leukocytosis** (toxic under Cabot's classification) includes that of illuminating-gas-

poisoning, quinine-poisoning, rickets, uric acid diathesis, gout, acute yellow atrophy of the liver, advanced cirrhosis of the liver (some cases), especially with jaundice, acute gastro-intestinal disorders (ptomains?), chronic nephritis, usually in uræmic cases, after injections of tuberculin and thyroid extract, normal salt solution (intravenous), after ingestion of salicylates, potassium chlorate, or phenacetin, during or after prolonged chloroform narcosis, ether narcosis (according to some observers).

Malignant Diseases and Leukocytosis.—The position of the tumor, its size, rapidity of growth, the number, size, and position of its metastases, and the resisting power of the patient—all have a marked effect upon the number of leukocytes in malignant disease.

There may be a leukopænia in cancer of the œsophagus, due to the starvation which a new growth in that location causes. If the cancer is small and without metastases, as in the early epithelioma of the lip, the leukocyte count is normal. Excessively high counts are never found. In rapidly growing and extensive neoplasms of the lung, liver, and kidneys, counts of 50,000, 40,000, and 28,000 have been made. Sarcoma usually produces a more frequent and larger leukocytosis than carcinoma. When all cases are considered, absence of leukocytosis is perhaps more common in malignant disease than its presence.

Therapeutical and Experimental.—Pohl found that most of the so-called tonics and stomachics produce a slight leukocytosis in animals. Winternitz injected a large variety of substances subcutaneously, and found that the degree of leukocytosis was parallel to the degree of local reaction excited.

Lymphocytosis is an absolute and relative increase in the circulating lymphocytes. The ordinary white count can not, of course, determine this fact, but resort must be had to the differential count of stained films. A moderate white count might show a lymphocytosis. If lymphocytosis is associated with an increase in the total white count, it can not be distinguished from lymphatic leukæmia except by the history and physical signs. Taking the adult blood as our standard, lymphocytosis is normal for healthy infants. Certain of the

diseases of infancy increase the lymphocytes remarkably, such as cholera infantum, rickets, various intestinal troubles, scurvy, hereditary syphilis, and especially pertussis, which disease, according to Meunier, may quadruple the lymphocytes. There is no rule governing the size of the lymphocytes; sometimes it is the larger, sometimes the smaller, and often no division can be made between the two.

In many debilitated conditions in the adult the percentage of lymphocytes is increased, due simply to a diminution in the number of polynuclear neutrophiles; and this must not be called a lymphocytosis.

Diagnostic value of lymphocytosis is seen chiefly in the diagnosis of lymphatic leukæmia when associated with the presence of glandular tumors. Whooping-cough must first be proved absent. If associated with eosinophilia, it may suggest obscure syphilitic disease.

Eosinophilia is an absolute increase in the number of eosinophiles in the circulating blood. There is a variation from 25 to 500 per cubic millimetre in the healthy adult blood. Physiologically, eosinophilia occurs in young infants, in women during the menstrual period, and after coitus.

Pathologically, it has been reported in a large number of diseases, but from the standpoint of practical diagnosis is of more value in trichiniasis than in any other disease. The following list of diseases in which eosinophilia is found with the greatest regularity is taken from Da Costa:

Diseases of the Skin.

Dermatitis herpetiformis.
Eczema.
Leprosy.
Lupus.
Pellagra.
Pemphigus.
Prurigo.
Psoriasis.
Scleroderma.
Urticaria.

Helminthiasis.

Ankylostomiasis.
Ascaris lumbricoides infection.
Oxyuris vermicularis infection.
Tænia mediocanellata infection.
Trichiniasis.

Miscellaneous Conditions.

Postfebrile malarial fever.
Pneumonia.
Rheumatic fever.
Scarlet fever.
Septicæmia.
Bronchial asthma.
Splenomedullary leukæmia.

Diseases of the Bones.

Hypertrophy.
Osteomalacia.
Malignant neoplasms.

QUESTIONS.

In what diseases is the coagulation time of the blood altered?
Describe Wright's method of estimating the coagulation time of the blood.
What is meant by cryoscopy? Describe the technique and philosophy of the procedure.
What bacteria are found in the blood?
Describe the technique of blood culture.
Distinguish between secondary anæmia and pernicious anæmia.
Distinguish between chlorosis and pernicious anæmia.
Differentiate myelogenous from lymphatic leukæmia.
In what diseases is a differential diagnosis dependent upon blood examination?
What is meant by iodophilia?
Mention the different methods of examining the blood for the malarial parasite.
Name the varieties of malarial organisms.
What are the points of distinction between these different plasmodia?
Describe the appearance of the stained tertian parasite in its various stages of development.
What is the significance of segmentation and flagellation?
Describe the parasites of filariasis, relapsing fever, dumdum fever, spotted fever, and trypanosomiasis.
What is leukopænia, leukocytosis? Explain the philosophy of each.
In what diseases are they of diagnostic value?
What is digestive leukocytosis?
What is lymphocytosis?
Of what diagnostic value is eosinophilia?

CHAPTER VIII.

THE STOMACH.

APPARATUS REQUIRED FOR GASTRIC WORK.

STOMACH-TUBE with bulb.
Politzer bag for aspiration.
Atomizer bulb for inflation.

Rubber apron—Turck's if desired.
Graduates, 100 c.c., 200 c.c.
Urine jars, 1000 c.c.
Small beakers or whiskey glasses.
Graduated volume pipettes, 2 c.c., 5 c.c., 10 c.c.
Glass funnels 3 and 6 inches in diameter.
Burette graduated to 0.1 c.c., with Shellbach's band, or a float, fitted either with a pinchcock or stopcock.
Burette stand, either tripod or retort stand with double burette clamp.
Test-tube stand.
Small test-tubes.
Cork-puncher.
Glass rods, glass tubing.
Filter-paper.
Slides and cover-slips.
Porcelain capsules, 2½ inches, 3 inches.
Thermostat.
Microscope.
Special 1 ounce drop reagent bottles with vitrified labels.
Glass irrigator, 3000 c.c. capacity, with irrigator spout, cap, and irrigator frame.
Glass funnel for syphon stomach-tube (Kny-Scheerer Co. catalogue, No. 18,055).
Suction flask, with single perforated rubber cork.
Aspirator, same as in blood work, for rapid filtration (Fig. 1).

REAGENTS REQUIRED FOR STOMACH WORK.

Decinormal sodium hydrate solution (prepared by chemist).
Phenolphthalein solution (1 per cent., alcoholic).
Sodium alizarin sulphonate solution, 1 per cent., aqueous.
Dimethyl-amido-azo-benzol solution, 0.5 per cent., alcoholic.

Gunzburg reagent:

Phloroglucin,	2 grammes;
Vanillin,	1 gramme;
Absolute alcohol,	30 c.c.

Dilute hydrochloric acid, U. S. P. official.

Carbolic acid solution for Uffelmann's test: Put a few c.c. of carbolic acid in a 4-ounce bottle of distilled water; shake, let stand, and use supernatant liquid.

Ferric chloride solution, officinal.

Lugol's solution.

Sodium chloride.

Glacial acetic acid.

Nitric acid.

Ether.

Pepsin in 0.5-gramme powders or tablets.

Bottle of Mett albumin tubes or simple disks.

Litmus-paper.

Congo-red paper—soak filter-paper in solution of dye, dry and cut into strips.

Dimethyl paper—soak filter-paper in solution of dye, dry and cut into strips.

Methylene-blue stain.

OBJECTS OF STOMACH EXAMINATIONS.

1. To show the condition of the stomach secretions.
2. To show the condition of the motor power of the stomach.
3. To show the condition of the absorptive power of the stomach.
4. To show the presence of abnormal organic constituents, such as yeast, sarcines, bacteria, blood, pus, tissue bits from stomach-wall or new growth.
5. To show the size, position, and shape of the stomach.

GENERAL REMARKS.

The student must bear in mind the fact that the normal stomach reacts differently to varying kinds of stimuli. *Food* is the natural stimulant, and useful conclusions concerning gastric digestion can only be drawn following the administration of test-meals. The stomach also reacts differently to different foods, both as regards the quality and quantity of

food; for instance, a test-meal containing meat stimulates the secretion of more hydrochloric acid than a test-meal of carbohydrates. A large meal stimulates the secretion of more of the stomach juices than a small meal, and remains in the stomach longer.

Following the administration of test-meals, free hydrochloric acid does not appear in the contents until the proteids of the food are saturated with this acid, thus forming the combined hydrochloric acid.

The analysis of gastric juice obtained by mechanical and electrical stimulation differs materially from that obtained after the ingestion of food; consequently its analysis is of no special clinical value.

The reader is cautioned against placing too much reliance for diagnosis and therapeutics on the chemical analysis of the stomach contents. Even more important is it to direct attention to the motor power, size of the stomach, evidences of fermentation, ulceration, or new growths.

The symptoms in diseases of the stomach are due to fermentation and its products, excess of hydrochloric acid, and hyperæsthesia of the gastric mucous membrane (nervous influences).

Our knowledge of the process of digestion comes from analyses of the stomach contents removed after the taking of food at different periods during the process of digestion.

The gastric juice differs rather widely in health, depending on the individual, the character and quantity of food, and the period of digestion at which it is removed. Even with the same sort of test-meal, removed after the same length of time, it will vary from day to day; consequently it is well to give a series of three test-meals either of the same kind or, better, an Ewald-Boas breakfast, a Riegel dinner, and an ordinary meal. The average test-meal of bread and water does not furnish a fair test of the stomach's secreting capacity. It does not appeal to the appetite and by the stimulation of the senses of sight, taste, and smell stimulate the production of the psychical gastric juice, which Pawlaw has proved to be an important factor.

Analysis of Gastric Juice, Obtained during Fasting, containing Saliva, per Mille:

Specific gravity,	10020.000
Water,	994.400
Organic material,	3.190 [1]
Free hydrochloric acid,	0.200
Chloride of sodium,	1.460
Chloride of potassium,	0.550
Chloride of calcium,	0.060
Phosphate of calcium, magnesium, and iron,	0.125

TEST-MEALS.

The test-meals most commonly employed are the following:

Ewald and Boas Test-breakfast.—One roll or two slices of white bread with crust removed (35 to 70 grammes); 350 c.c. of water or weak tea. The dry bread is chewed thoroughly, then washed down with the liquid. Remove one hour after the beginning of the breakfast. Under normal conditions the following should be found (Van Valzah and Nizbet):

Amount	30 to 50 c.c.	yellowish mixture easily filtered.	
	Thirty minutes.	Sixty minutes.	Ninety minutes.
Total acidity	20 to 30	50 to 60	30 to 40
Combined acids	20 to 30	40 to 50	25 to 35
Free HCl	0	10 to 15	5 to 10

Digestive power of filtrate (Hammerschlag's test, about 90 per cent.). Filtrate in dilution of 1 : 3000 with normal HCl solution digests disk of albumin after remaining in thermostat at 37° C. for twenty-four hours.

Rennet ferment coagulates milk in dilution of 1 : 40.

Rennet zymogen coagulates milk in dilution of 1 : 160.

Free HCl appears in thirty minutes, reaches its height in about one hour, and, diminishing, continues to the end of digestion.

Acetic acid and *potassium ferrocyanide* give a slight cloudi-

[1] Pepsin, 3.

ness after the first half-hour, up to early digestion. The *biuret* reaction (rose) runs the same course. *Fehling's* solution is reduced during the first hour. Lugol's solution gives a brownish-purple color during the first one and a half hours.

There should be no blood, a small amount of mucus, perhaps a little bile, and a small number of bacteria; no organic acids; no signs of fermentation, such as long bacilli, sarcines, or yeast.

The stomach should be empty in two to two and a half hours after the beginning of the meal.

Boas Test-breakfast.—A tablespoonful of rolled oats is added to 1000 c.c. of water; this is boiled down to 500 c.c. A little salt may be added. Remove *one* hour later.

Indications.—This meal is employed in suspected cases of cancer of the stomach, where it is important to determine the presence of lactic acid, since it contains none of this acid, while test-meals containing bread do. The stomach should be thoroughly washed out the night before.

The chemical findings are about the same as those for the Ewald-Boas test-breakfast.

Riegel Test-dinner.—Soup, 400 c.c.; finely chopped or scraped beef, 200 grammes; a slice or two of wheat bread (50 grammes); and a glassful of water. Remove four hours later.

Amount . . . . 40 to 80 c.c.

	Two hours.	Three hours.	Four hours.
Total acidity	40 to 50	45 to 70	60 to 80
Combined HCl	40 to 50	45 to 60	50 to 60
Free HCl	0	0 to 5	10 to 20

Free HCl appears in about two and a half hours, continues about two hours, and disappears about twenty minutes before the stomach becomes empty.

The cloudiness with acetic acid and potassium ferrocyanide and the biuret reaction (rose) begin near the end of the first hour, and disappear during the last fourth of the period of digestion. Very few striated muscle-fibres can be found. The stomach should be empty in five hours.

Hammerschlag, about 90 per cent.

Rennet ferment and zymogen are the same as in the test-breakfast.

Lugol's solution produces a brownish-violet coloration.

Ordinary Meal.—It is a good custom to examine occasionally the stomach contents removed four hours after the patient's average dinner, and make the analysis as after the regular test-meals.

Contraindications to the Use of the Stomach-tube.—Chief among these are aneurysm of the aorta and inflammatory conditions of the œsophagus. Advanced arteriosclerosis, especially with involvement of the coronary arteries, with a history of attacks of angina pectoris, forbid the passage of the stomach-tube except in those accustomed to its use. Contrary to common opinion, it is frequently used as a therapeutical measure in cases of valvular heart lesions, even during the stage of incompensation. Cancer and ulcer of the stomach, instead of always contraindicating its use, are at times strong indications for its use unless there is a history of recent hemorrhage. Good judgment should govern one in the selection of cases.

Stomach-tube.—The tube most commonly employed is manufactured by the Goodrich Rubber Company. The most satisfactory tube is made of soft rubber and has a *bulb* attachment; sizes 21 E, 22 A, 33 F. Its outside diameter is $\frac{7}{16}$ inch, lumen $\frac{4}{16}$ inch. A smaller tube is used for children. The entire length of the tube, including the bulb, is about 65 inches; length to bulb is about 43 inches. A white ring indicates the distance to which the tube should be introduced in the average case. One soon learns the correct distance by experience. The tube most often employed has an opening at the end and one on the side, a short distance above. Some prefer a tube with a blind end and lateral openings, because there is less danger of damaging the stomach mucous membrane by suction into the terminal opening. The tube should not be too flexible. It is well to cut it in two about 12 inches from the funnel end and insert a piece of glass tubing, in order the better to inspect the character of the washings as they pass through.

A rubber or glass funnel can be used. A special glass funnel for this purpose is manufactured by the Kny-Scheerer Co.

Patients with syphilis, tuberculosis, and cancer should have tubes for their exclusive use.

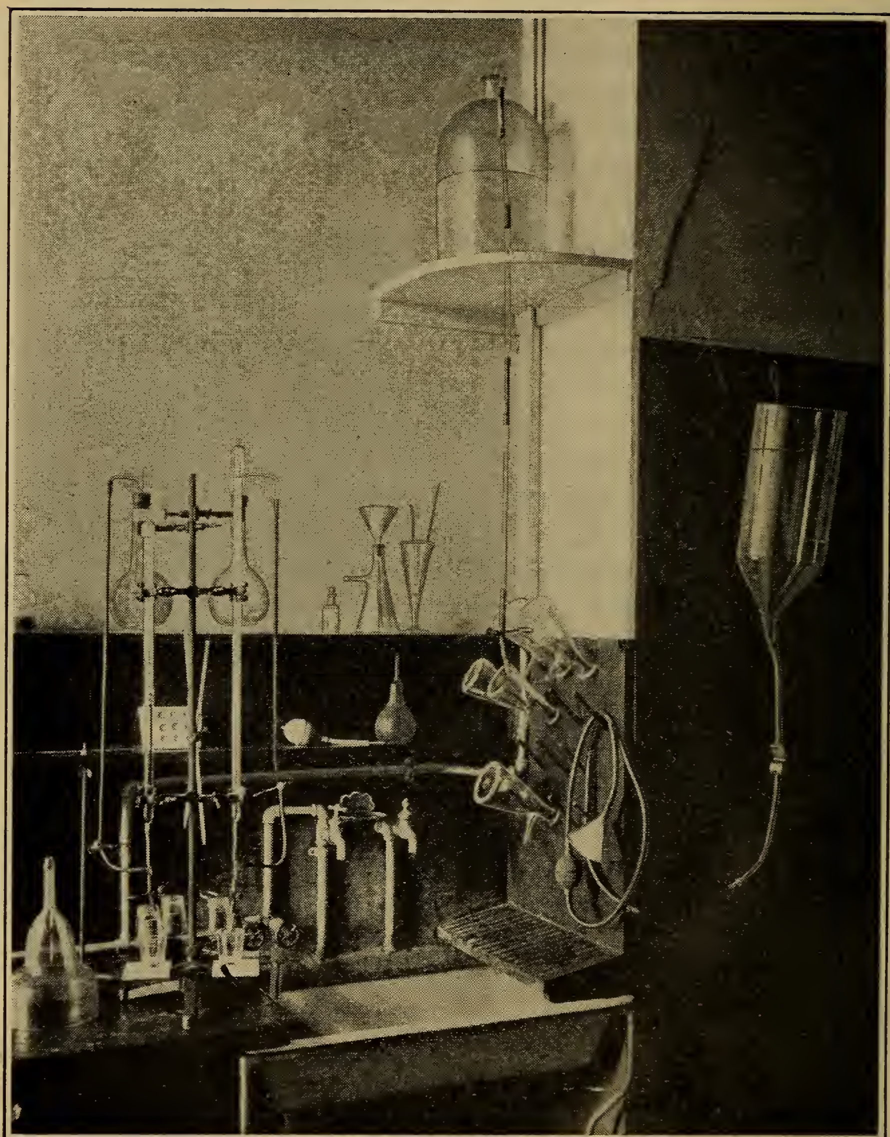
Passing the Tube.—The patient should be told that it is not a serious operation, and that it will not interfere with his breathing. There is usually no need of introducing the fingers of the left hand into the mouth to guide the tube. Moisten the tube in water or glycerine, hold it in the fingers of the right hand, as one would a pen, four to five inches from the end, aim directly at the middle of the posterior pharyngeal wall, cautioning the patient not to bend his head backward too far, tell him to swallow, and push the tube forward without hesitancy. In most cases it will enter the canal readily. With the left arm thrown around the patient's head from behind, the tube is held in place between the index and middle fingers, and rapidly pushed into the stomach with the right hand. Occasionally a spasmodic contraction of the œsophageal muscles at the isthmus of the fauces or lower down will obstruct the passage. Steady pressure will overcome this resistance in a few seconds. If the tube is quite flexible, it may not enter the isthmus at all, but bend to the side and pass around the patient's mouth. An extremely irritable pharynx may be sprayed with cocaine, or a pledget of cotton may be soaked in a 5 per cent. solution of cocaine and sucked for five minutes, care being taken not to swallow the saliva. Such procedures are almost never necessary. The tube may meet an organic stricture, due to cancer, ulcer, or tumor pressing from the outside.

REMOVAL OF THE STOMACH CONTENTS.

The contents may be obtained in three ways:

(1) **Self-expression.**—By bearing down, as at stool, or by coughing, the abdominal muscles and stomach are made to contract and thus force out the contents. Slight vomiting movements may be produced by moving the tube back and forth, thus facilitating the expulsion.

FIG. 34.



Showing on upper shelf drop reagent bottle, suction flask, and platinum wire and loop; on lower shelf, Politzer bag and atomizer bulb; on table, beginning at the left, funnel for syphon stomach-tube, Cowie burette stand, reagent bottle, rack for suction flask, etc., bulbed stomach-tube, irrigator with spout cap.

(2) **Suction.**—The modified Politzer bag of Ewald or the aspirator of Boas can be used. (See Fig. 34.) Tubes are

now manufactured with the Boas aspirator attached. With the fingers of the left hand the tube is compressed between the bulb and the stomach. The *bulb* is now compressed, the first pressure relaxed, and the tube *beyond* the bulb then compressed, and the pressure on the bulb relaxed. The bulb sucks the stomach contents into itself. They are now expelled by again compressing the tube toward the stomach, and then compressing the bulb. The *Politzer bag* when used is first compressed and then attached to the stomach-tube, and on expanding draws the contents into itself. The aspirator used in cleansing the blood pipettes will be found very convenient for this purpose.

(3) **Position and Gravity.**—With the patient in the horizontal or knee-elbow position, the contents can sometimes be more easily removed.

The tube should be just through the cardia and held in the mouth to prevent it dragging on the larynx.

Failure to obtain contents may be due to several causes:

(1) The stomach may be empty. Cases are met with in which the test-breakfast is forced into the bowel in less than half an hour.

(2) The tube may be plugged with food or mucus. Compression of the Boas bulb or the Politzer bag may free the tube. An extra effort on the part of the patient may dislodge the obstruction. The tube may be introduced too far or not far enough.

It may be necessary to remove the tube, cleanse it, and introduce it again.

The introduction of water for syphon purposes renders the analysis unsatisfactory, and is not to be recommended. If unsuccessful in obtaining the stomach contents by the above methods, very often enough can be obtained for analysis by firmly compressing the tube with the fingers and withdrawing, the tube being in many instances partially filled with stomach contents.

EXAMINATION OF THE STOMACH CONTENTS.

Macroscopical Examination.—(*a*) **Quantity.**—The quantity varies much with the size of the stomach, condition of the motor power, presence of obstruction, size of meal, and period at which it is removed.

(*b*) **Odor.**—The contents in health have a characteristic stomach odor. In fermentative conditions there is a disagreeable, rancid odor.

In *health*, following a test-meal, the contents should be moderately fluid, not thick and tenacious; the food is fairly well digested and broken into small pieces, yellowish in color, and on standing separates into two layers—lower solid, upper liquid.

A small amount of mucus is present; there should be no macroscopical blood unless it comes from irritation of the œsophagus by the tube. A small amount of bile is often found when the tube is passed on those unaccustomed to its use. Pus should not be present.

In *disease* the quantity may be diminished or very much increased. There may be great excess of mucus, which is easily recognized by its appearance. Food eaten many hours, even days, before may be present. Fresh red blood, or dark altered blood, excess of bile, a brownish scum in which sarcines are often found, pieces of gastric mucous membrane, or bits of tissue from new growths or ulcers, all should be looked for. The naked eye usually detects the presence of bile. This examination is most satisfactorily made by pouring the residue after filtration upon a plate and examining with the aid of teasing-needles.

Microscopical Examination of Normal Contents.—The findings vary with the meals. Vegetable cells, starch-granules, altered meat-fibres, a few bacteria, a few red and white cells (result of tube irritating throat and œsophagus), fat-globules, possibly leptothrix, may be found.

Microscopical Examination of Abnormal Contents.—This shows in different cases undigested meat-fibres, vegetable cells, numerous red cells, pus-cells, sarcines, yeast-cells,

numerous bacteria, most important of which are the lactic acid bacilli. (Plate X.) These are better recognized by a study of the figures than by description. Sarcines, yeast-cells, and lactic acid bacilli are all signs of fermentation. Whenever a bit of the stomach mucous membrane is obtained, it should be sectioned and examined by a pathologist. It is recognized under the microscope by the special arrangements of cells. In cases of suspected carcinoma or atrophic gastritis such examination is extremely valuable.

It has been claimed that the presence of lactic acid bacilli (Oppler-Boas) is quite positive evidence of cancer of the stomach, but too much stress must not be placed on them as evidence, as observers of late years have found them rather frequently in non-malignant conditions.

Pus and **blood** are recognized by their appearance under the microscope, as described in other chapters.

If necessary, the hæmin crystal test or the guaiacum test may be applied for the demonstration of blood.

Sarcinæ occur in characteristic squares, resembling cotton bales.

Yeast-cells appear as oval bodies 3 to 10 μ long, showing the budding formation. There may be chains of three or four, or more commonly one larger body with a small one springing from it.

Oppler-Boas Bacillus.—This is a long, large, non-motile bacillus, usually growing in chains which take a zigzag course. Frequently pairs of bacilli are seen joined together at an angle.

Ralston Williams has succeeded in growing these bacilli on glucose-agar.

They may be stained with methylene-blue. Under the oil immersion they appear made up of short rods.

Filtration of Stomach Contents.—For rapid work a suction flask is attached to the aspirator and the contents filtered. An ordinary funnel and filter-paper can be used, but the process is slow if much mucus is present.

CHEMICAL ANALYSIS OF GASTRIC JUICE.

Qualitative Tests.—Free acid of any kind turns Congo-red solution or paper a bright blue. Free organic acids can not be distinguished from free mineral acids by this test. Acid salts do not give the reaction.

Test.—A strip of Congo-red paper is dipped into the stomach juice, or to a few cubic centimetres of Congo-red solution (made by dissolving a bit of the powder in water) a few drops of stomach filtrate are added.

Free Hydrochloric Acid.—This acid is recognized very well by *Günzberg's test.* Two or three drops of phloroglucin vanillin solution and an equal amount of filtrate are placed in an evaporating-dish and slowly heated over a flame, care being taken not to burn the contents. If free hydrochloric acid is present a beautiful rose-red color appears, especially at the periphery of the drop. This is due to the formation of minute red crystals.

Dimethyl-amido-azo-benzol paper is a yellow bibulous paper which strikes a red color in the presence of free hydrochloric acid.

Free Organic Acids.—Uffelmann's test is most commonly employed.

Application.—To a small amount of carbolic acid solution of any strength a drop of ferric chloride solution is added; a very dark-blue results; *distilled* water is added till this solution becomes an amethyst-blue color. A few drops of the filtrate are added. If lactic acid is present in moderate amount, it becomes a canary yellow; if in small amount, a greenish yellow.

The reaction is unsatisfactory. Free HCl clears up the blue solution, acetic and combined HCl give it a yellowish-brown color; and butyric acid a grayish opalescent appearance.

Kelling's Test.—Kelling's is a more reliable test for lactic acid. It is applied as follows: 5 c.c. of gastric juice are diluted with 10 volumes of water and treated with 1 or 2 drops of a 5 per cent. aqueous solution of ferric chloride. In the presence of lactic acid a distinct greenish-yellow color is seen if the tube is held to the light. A positive reaction is obtained only in the presence of lactic acid.

Strauss' Test.—Strauss' apparatus is filled with gastric juice to the mark 5 c.c., then ether is added to the 25 c.c. line. After shaking thoroughly the separated liquids are allowed to escape by opening the stopcock until the 5 c.c. mark is reached. Distilled water is then added to the 25 mark, and the mixture treated with 2 drops of the official tincture of the sesquichloride of iron, diluted in the proportion of 1 : 10. On shaking, an intensely green color appears if more than 1 pro mille of lactic acid is present, while a pale green is obtained in the presence of 0.5 to 1 pro mille. Small amounts of lactic acid do not show with this test.

The exact quantitative estimation of lactic acid is not of clinical value. If so desired, Boas' method may be used, for a description of which the reader is referred to Simon or v. Jaksch.

The recognition of butyric, acetic, and fatty acids is not important. The first two are usually recognized by their odor, especially if they are heated.

Quantitative Determination of the Acidity of the Gastric Juice.—The best results are obtained by selecting ONE good method for constant use. Such a one is

TOPFER'S METHOD.—This requires a burette, graduated pipettes, 3 small beakers, decinormal sodium hydrate solution, and 3 color reagents, solutions of phenolphthalein, sodium alizarin sulphonate, and dimethyl-amido-azo-benzol.

Solutions containing phenolphthalein turn pink in the presence of an alkali, becoming permanently pink when the mixture becomes even faintly alkaline.

Sodium alizarin sulphonate is stated to be unaffected by hydrochloric acid in combination with proteid.

Dimethyl-amido-azo-benzol in alcoholic solution becomes red in the presence of free hydrochloric acid.

The following determinations are made by means of color reactions:

1. Total acidity.
2. Combined hydrochloric acid.
3. Free hydrochloric acid.
4. Organic acids and acid salts.

Procedure.—The stomach contents are filtered :

1. *Total acidity :*

a. By means of a graduated volume pipette place 10 c.c. of filtrate in a beaker or whiskey glass.

b. Add 2 drops of phenolphthalein (indicator).

c. Titrate with decinormal sodium hydrate solution, adding it drop by drop, stirring after the addition of each drop, until the first permanent pink is detected.

(This color reaction indicates that the acid filtrate has been made slightly alkaline by the addition of sodium hydrate, since phenolphthalein solutions turn pink in the presence of an alkali.)

Read off the number of cubic centimetres of sodium hydrate required to bring about this end-reaction ; for example, 10 c.c.

2. *Combined hydrochloric acid :*

a. Place 10 c.c. of filtrate in a beaker.

b. Add 2 drops of sodium alizarin (indicator).

c. Titrate with sodium hydrate solution as in (1) until a permanent pure violet color is obtained.

Read off the number of cubic centimetres of sodium hydrate required to bring about this end-reaction ; for example, 6 c.c. This figure does not indicate the combined hydrochloric acid, but instead the entire acidity minus the combined hydrochloric, since alizarin does not react to hydrochloric acid combined with proteids. Hence the combined hydrochloric is calculated by subtracting 6 c.c. from 10 c.c. = 4 c.c.

3. *Free hydrochloric acid :*

a. Place 10 c.c. of filtrate in a beaker.

b. Add 2 drops of dimethyl-amido-azo-benzol (indicator). (The mixture will turn red in the presence of free HCl.)

c. Titrate with sodium hydrate until the mixture turns a lemon yellow.

Read off the number of cubic centimetres required ; for example, 2 c.c.

The indicator (dimethyl) reacts to free mineral acids, but is not affected by organic acids unless present in more than 0.5 per cent.

4. *Organic Acids and Acid Salts.*—Subtract the acidity due to combined HCl and free HCl from the total acidity

and the result is the organic acids and acid salts; for example, 10 c.c.—(4 c.c. + 2 c.c.) = 4 c.c.

To illustrate the estimation of per cent. of *acid in the above calculations.*

One cubic centimetre of the decinormal sodium hydrate solution represents 0.00365 gramme of hydrochloric acid.

Total acidity = 10 c.c. × 0.00365, or 0.0365 gramme for every 10 c.c. of filtrate used; or for 100 c.c. of filtrate 0.3650 gramme or per cent.

Combined HCl = 4 c.c. × 0.00365, or 0.01460 gramme for every 10 c.c. of filtrate used; or for 100 c.c. of filtrate 0.1460 gramme or per cent.

Free HCl = 2 c.c. × 0.00365, or 0.00730 gramme for every 10 c.c. of filtrate used; or for 100 c.c. of filtrate 0.0730 gramme or per cent.

Organic acids and acid salts = 0.3650 — (0.1460 + 0.0730) or 0.1460 gramme or per cent.

Combined HCl	= 0.1460	per cent.
Free HCl	= 0.0730	"
Organic acid and acid salts	= 0.1460	"
Total acidity	= 0.3650	"

SCHEME ILLUSTRATING THE ABOVE PROCEDURES.

1. **Phenolphthalein indicates**	Total acidity	Free HCl, combined HCl, organic acids and acid salts.
2. **Sodium alizarin sulphonate indicates**	The entire acidity except combined HCl	Free HCl, organic acids and acid salts.
3. **Dimethyl-amido-azo-benzol indicates**	Free HCl	Free HCl.
4. **Organic acids and acid salts**	1 — (2 + 3).	

a. Total acidity, 10 c.c.
b. Combined HCl, 4 c.c.
c. Free HCl, 2 c.c.
d. Organic acids and acid salts, 4 c.c.

Substitute for Percentage Calculation.—The number of cubic centimetres of decinormal sodium hydrate solution required to bring about the end color reactions in 100 c.c. of stomach filtrate in each of these calculations is used to indicate the degree of acidity.

For example: If in estimating the total acidity 10 c.c. of filtrate were used, and it required 10 c.c. of sodium hydrate solution to bring about the end-reaction, it would require 100 c.c. of sodium hydrate to bring about this reaction if 100 c.c. of filtrate were used. Consequently, total acidity is represented by 100. If only 1 c.c. of filtrate is used, the number of cubic centimetres of sodium hydrate is multiplied by 100.

Example:

10 c.c. of filtrate.	Sodium hydrate.	Figure.	Per cent.
Total acidity	10 c.c.	100	0.3650
Combined HCl	4 c.c.	40	0.1460
Free HCl	2 c.c.	20	0.0730
Organic acids and acid salts.	4 c.c.	40	0.1460

It is helpful to notice that each 25 of acidity corresponds approximately to $\frac{1}{10}$ per cent. by weight.

SHORT METHOD.

In many stomach analyses it is necessary to determine only the total acidity and free hydrochloric acid.

A unique method for determining these figures is the following:

a. Place 10 c.c. of filtrate in a beaker.

b. Add 2 drops of dimethyl (indicator) and determine the amount of free HCl present by titration with sodium hydrate.

c. Add to this same filtrate 2 drops of phenolphthalein, and continue the titration with sodium hydrate till the first permanent pink results. The *total acidity* is represented by the entire number of cubic centimetres of sodium hydrate used.

It frequently happens that not sufficient stomach contents are obtained to furnish 10 c.c. of filtrate for each of the three

titrations, and the few cubic centimetres required for the digestion test. Under these circumstances 2 c.c. or 5 c.c. may be used and calculations made accordingly.

If the normal pipette is used for measuring the filtrate, the last drop should not be blown out. It is recognized by the presence of a white ring near its tip.

Digestion Test.—Following the determination of the acidity of the gastric juice, the next test is for the presence of *pepsin.*

Common Method.—If free hydrochloric acid has been demonstrated to be present, a small test-tube is partially filled with the filtrate and a small disk of coagulated egg-albumin added to it. This is placed in the thermostat for twenty-four hours and examined from time to time. If free HCl has been proved absent or in very small quantity, another test-tube should be prepared in the same way and a couple of cubic centimetres of a 0.4 per cent. solution of HCl added. Two or three drops of official dilute HCL may be used instead. This second test-tube will furnish evidence of pepsinogen, since free HCl changes it into pepsin.

Digestion is shown by the periphery of the disk becoming translucent; and a rough idea of the amount of pepsin can be gained by noting the depth to which this translucent area extends.

The egg-albumin disks are prepared as follows: An egg is boiled hard, shell removed, and the white carefully separated from the yolk. With a cork-borer numerous cylinders of the white are removed and cut into disks of the same size and preserved in glycerine. Before use the glycerine should be washed off with water.

Mett Method of Determining Peptic Digestion.—Ralston Williams describes this method as follows:

"Briefly described, glass tubes filled with coagulated egg-albumin are placed in 2 to 4 c.c. of gastric juice and kept in the incubator for ten hours. At the end of this time the tubes are removed and the number of millimetres of albumin digested is estimated. This is ascertained by measuring the portions of the ends of the tubes that are clear, including also the clouded

or opalescent zone, if one be present. For this purpose a scale graduated to 0.5 mm., a small lens magnifying 2 to 3 diameters, and a small black glass plate are employed. With very little practice one can correctly read fifths of a millimetre. The digestion which goes on within the first ten hours, according to Ssamojloff, corresponds to the normal period of stomach digestion. Franz Jung states that from 5.5 to 5.9 mm. should be digested in ten hours; however, the results obtained in the clinical laboratory of the University Hospital at Ann Arbor would indicate that these figures are too high, and the 3.5 to 4.5 mm. would be more nearly correct.

It has been established by Borrissow and Schutz that the amount of pepsin in one juice, as compared with another, is as the squares of the number of millimetres digested are to each other. The foregoing fundamental statements are gathered from Jung's abstracted translation of Pawlow's book, "The Work of the Digestive Glands" (*Jour. A. M. A.*, May 10, 1902), and is the basis of the experiments that follow.

There are several ways of preparing and preserving the Mett tubes, the one employed by the writer is as follows: Thin-walled glass tubing having a calibre of 1.5 mm. is cleansed in distilled water and dried. It is then cut into lengths of about 10 cm., convenient for storage for use. These then are filled by suction with the white of a fresh egg. The egg-albumin must be free from air bubbles, one of the chief sources of annoyance and error being the presence of these air bubbles. In filling the tubes the endeavor is made to secure only the more fluid portion of the egg. This can be done readily by care and manipulation. As each tube is filled its ends are passed slowly through a white gas flame, thus forming small coagulated plugs, which serve temporarily to prevent the escape of albumin. When a sufficient number of these tubes are thus prepared they are placed in a basin of distilled water, supported on glass rods. The water is then heated to a temperature between 90° and 95° C., which is maintained for five minutes. Care is taken to keep an equable temperature throughout the basin. From here the tubes are transferred to a 66 per cent. watery solution of glyc-

erine, in which they are preserved. When it is wished to make a test a tube is selected, the glycerine washed therefrom, and about 10 mm. of one is snipped off, a sharp Stubb's file being employed for this purpose. This piece is thrown away and another about 18 mm. long is taken. In this way a portion is secured free from the action of glycerine, which has an inhibiting effect on the digestion of the egg. This small tube, placed in 3 or 4 c.c. of gastric juice is put into the incubator, and left for either ten or twenty-four hours. The digestion in twenty-four hours is proportionately more. For the reasons before stated ten hours is preferred, but usually the twenty-four-hour period is much more convenient and is therefore used. Frequently in the digested tubes two zones will be seen, one perfectly clear, from which the albumin has entirely disappeared, the other hazy or cloudy. In other tubes cone-shaped translucent plugs are formed. The explanation of this is not quite clear, since in the more perfect tubes the digestion is sharply defined, there being only the clear digested and solid or undigested zones. The results may be recorded as the number of millimetres, but for purposes of comparison these figures should be squared, this being the true index to the amount of pepsin in the sample.

Nirenstein and Schiff have shown that the strength of one gastric juice, as compared to another, is as the squares of the number of millimetres digested, only in juices up to a certain strength. Beyond a certain point the rule does not hold good. Furthermore, they have shown that the soluble chlorides and carbohydrates interfere. They have obviated these difficulties by diluting the gastric juice fifteen times, 16 : 1, under which conditions the laws are correct. For practical purposes, however, the old way is sufficiently accurate. Jung[1] draws the following conclusions from his work with this method:

(1) The normal values for pepsin digestion are, according to Mett's method, 5.5 to 5.9 mm.

(2) Subacidity and anacidity have lower values than normal or superacidity—*i. e.*, 1.9 mm. average.

[1] *Jour. Am. Med. Assoc.*, May 10, 1902.

(3) Superacidity, generally speaking, has high and highest values of pepsin, yet there are cases of unusually high HCl figures with disproportionately low pepsin values.

(4) The diminution of pepsinogen does not run proportional with that of HCl. Even with a deficiency of HCl the value of pepsin can be higher than that of mild subacidity. Comparison average of Hammerschlag and Mett methods:

Mett	5.5 mm. superacidity;	1.9 mm. subacidity.
Hammerschlag . .	6.0 " "	4.5 " "

Hammerschlag's Method of Quantitative Estimation of Pepsin.—Three Esbach's tubes are employed (albuminimeters). Tube A is filled to the mark U with a mixture of 10 c.c. of a 1 per cent. solution of serum-albumin in 0.4 per cent. hydrochloric acid, and 5 c.c. of filtered gastric juice. The second tube (B), which is the standard, is also filled to the mark U, but 0.5 gramme of pepsin is added to the serum solution instead of the gastric juice. The third tube (C) simply contains a mixture of the serum solution and 5 c.c. of water. Place in thermostat for one hour at temperature of 37° C. Esbach's reagent is added to each tube to the mark R. After standing for twenty-four hours the amount of precipitated albumin is read off, and the difference between that in A and C compared with that in B.

Rennet and its Zymogen.—A few drops of the stomach filtrate are added to 10 or 15 c.c. of milk in a test-tube, and placed in a thermostat at a temperature of 37° C. If the rennet is normal, the milk will be coagulated solid or with the separation of a small amount of whey in ten to fifteen minutes. If the curdling takes place more slowly, rennet is deficient.

If the filtrate does not show hydrochloric acid, add a small amount of calcium chloride solution to the mixture. Rennet zymogen, if present, is then converted into active rennet and coagulates the milk.

Tests for Proteids.—Examine for acid albumin or syntonin, albumin, albumose, and peptone. The customary tests for proteids are used.

Acid albumin, if present, is precipitated by carefully neutralizing the filtrate with the decinormal solution. An excess of either alkali or acid redissolves the precipitate.

Albumin.—After removing syntonin by neutralizing and filtering, the filtrate may be tested for albumin by the various well-known tests, such as Heller's ring test, acetic acid and potassium ferrocyanide, etc.

Albumose.—Precipitate the syntonin and albumin by boiling the unneutralized filtrate. Filter, and test filtrate for albumose as follows:

Mix equal quantities of the cooled filtrate and a saturated solution of sodium chloride; add a drop or two of acetic acid. A turbidity or precipitate in the cold, disappearing on heating and reappearing on cooling, indicates presence of albumose. The filtrate should also give the biuret reaction.

Peptone.—Remove albumin, syntonin, and albumose as above indicated. The filtrate should be negative to the albumin tests. If peptone be present, it should give the biuret reaction—a violet-red or purplish color on the addition of a drop or two of dilute copper sulphate solution. Tannin and some other substances also give this reaction.

Carbohydrates.—Starch, erythrodextrin, sugar, and achroödextrin, may be present in the filtrate.

Starch.—Dilute a small amount of Lugol's solution to a light-yellow color; add a few drops of gastric filtrate. If unchanged starch is present, a blue color results; if ertyhrodextrin a deep brown or mahogany.

For detecting sugar the customary tests are used, such as Fehling's, etc.

EXAMINATION OF STOMACH CONTENTS DURING FASTING.

This procedure is of value in demonstrating dilatation and diminished motor power of the stomach, or the presence of an obstruction at the pylorus. If food is found in the stomach which has fasted overnight (eight to twelve hours), it gives evidence of one or all of the above conditions.

The fasting stomach is also examined to learn whether there is an excessive secretion of gastric juice. It should be washed out the night before. Normally the fasting stomach contains from a few cubic centimetres up to 50 or 60 c.c. of juice. Above this amount indicates hypersecretion, so-called Reichman's disease. This juice should be subjected to the same tests as the juice obtained after a test-meal.

FIG. 35.

Collective view of vomited matter. (Eye-piece III., objective 8 A, Reichert.) *a*, muscle-fibres; *b*, white blood-corpuscles; *c*, *c′*, squamous epithelium; *c″*, columnar epithelium; *d*, starch-grains, mostly changed by the action of the digestive juices; *e*, fat-globules; *f*, sarcinæ ventriculi; *g*, yeast-fungi; *h*, forms resembling the comma bacillus found by v. Jaksch once in the vomit of intestinal obstruction; *i*, various micro-organisms, such as bacilli and micrococci; *k*, fat-needles, between them connective tissue derived from the food; *l*, vegetable cells. (v. Jaksch.)

Ordinary Meal.—In patients complaining of indigestion much can be learned by removing the ordinary meal and subjecting it to all of the above routine examinations. In fact, the writer believes that in many instances such a meal may prove much more valuable than the test-meals.

The *vomitus* should also be subjected to the above examinations—macroscopical, microscopical, and chemical.

In suspected cancer of the œsophagus with regurgitation

chemical examination often throws much light on the case. If the various acids (free HCl and combined HCl) and ferments are present, it indicates that the material vomited is from the stomach. If absent, it indicates that the material had not reached the stomach (stricture of œsophagus with dilatation) or that a condition of achylia gastrica is present.

The reaction of the vomitus to litmus-paper is of no value.

EXAMINATION OF MOTOR POWER OF THE STOMACH.

This is one of the most important of stomach examinations, and the *one most often neglected.*

The best method is by the use of the stomach-tube. The average meal should be passed on into the intestine in six or seven hours. At the expiration of this time after an ordinary meal the tube should be passed and the stomach washed out. Presence of food indicates deficiency in motor power. The usual method is to wash out the stomach in the morning, before breakfast, nothing having been eaten since 6 o'clock the evening before.

In mild cases of diminished motor power food may be retained in the stomach from meal to meal during the day, being finally passed into the intestine during the long fast of the night.

Another but much less satisfactory method consists in administering a gramme of salol in three or four capsules immediately after a meal. Salol is unchanged in the acid contents of the stomach, but as soon as it mixes with the alkaline contents of the intestine it is decomposed into phenol and salicylic acid. The latter is absorbed and excreted in the urine, where it may be recognized. The addition of a few drops of ferric chloride solution, in the presence of salicylic acid or its derivatives, gives a brown or violet color. The urine should be tested every half to one hour for this reaction, and again after twenty-four hours. Normally it appears in sixty to seventy-five minutes. The salol should be entirely excreted in twenty-four hours. A delayed appearance of the reaction indicates diminished motor power. Nega-

tive examinations during the first twenty-four hours indicate pyloric stenosis. Too many factors are involved to make this a satisfactory test. Stomach contents must be acid, intestinal contents alkaline, and the kidneys should be normal.

Diminished motor power is present in many gastric conditions, such as cancer of the stomach, with stricture of the pylorus, benign stricture of the pylorus, dilatation, gastric atony, gastroptosis, chronic gastritis, etc.

ABSORPTIVE POWER OF THE STOMACH.

Test.—A capsule containing 0.2 gramme of potassium iodide (all traces being carefully removed from outside of the capsule) is given shortly before a meal. The saliva is now examined every two or three minutes with starch-paper for a trace of potassium iodide. Normally a violet color is obtained in six and a half to eleven minutes, a bluish tinge in from seven and a half to fifteen minutes.

A delayed appearance of the reaction is observed in most diseases of the stomach, especially in dilatation and carcinoma, less so in chronic gastritis, variable in ulcer ; but absolute dependence can not be placed on this reaction, since it has been obtained in dilatation and chronic gastritis within the normal time limit.

SIZE, SHAPE, AND POSITION OF THE STOMACH.

A fairly accurate idea of these three points may be gained by distending the stomach with air by means of the stomach-tube and atomizer bulb. This is a satisfactory method in those who take the tube well. In others it is well to distend the stomach with carbonic acid gas generated in the following way : 6 grammes of tartaric acid dissolved in half a glassful of water are swallowed, and immediately followed by a solution of 7 grammes of sodium bicarbonate in half a glassful of water. The stomach is immediately distended, revealing its position, shape, and in a general way its size. It should never be distended in those giving a history of ulcer or recent hemorrhage.

The **capacity** of the stomach is determined by measuring the quantity of water which can be poured into it through

the tube without causing special distress. That of the **normal stomach** varies with the individual, measuring on an average from 1000 to 1500 c.c. Ewald considered 1700 c.c. abnormally large. The extremes may be placed at 800 c.c. and 2000 c.c. for the adult.

Classification of Digestive Conditions.

Normal HCl acidity = Euchlorhydria.
Increased HCl acidity = Hyperchlorhydria.
Diminished HCl acidity = Hypochlorhydria.
Absent HCl acidity = Achlorhydria.

Normal pepsin = Eupepsia.
Increased pepsin = Hyperpepsia.
Diminished pepsin = Hypopepsia.
Absent pepsin = Apepsia.

Organic Acid Fermentation.

Duration of Digestion.

1. Normal length of time.
2. Increased length of time.
3. Diminished length of time.

QUESTIONS.

What are the objects of stomach examinations?
Mention the reagents required in this work.
Describe the test-meals commonly employed.
What are the contraindications to the use of the stomach-tube?
Describe the correct method of passing the tube.
What means are employed in the removal of the stomach contents?
What points are considered in the macroscopical examination of the stomach contents?
What are the microscopical findings in normal and abnormal stomach contents?
What is Günzberg's test for *free hydrochloric acid?*
Describe Kelling's test for lactic acid.
Describe in detail Töpfer's method for the quantitative determination of the acidity of the gastric juice.
Describe Mett's method of determining peptic digestion.
What facts are learned by the examination of the stomach contents during fasting?
What methods are used in determining the motor power of the stomach?
Describe the test for determining the absorptive power of the stomach.
How are the size, shape, and position of the stomach determined?
Classify the digestive conditions.

CHAPTER IX.

THE FÆCES.

The **fæces consist** of undigested particles of food, intestinal mucus, unabsorbed intestinal secretions, epithelial cells, and bacteria.

The **number of stools per day** varies with the individual. The average person has one formed stool a day, usually at a regular time.

There are numerous exceptions to this rule, as many healthy persons have normally only one stool in two or three days, while others have two or three daily. So in deciding whether there is constipation or diarrhœa, it is necessary to learn the "stool habit" of the individual

The **amount of fæces** varies with the diet. It is much larger with a carbohydrate than with a proteid diet, 60 to 250 grammes being the extremes in health.

The **consistence of the fæces** depends on the character of the food in health. With a vegetable diet (containing 80 to 85 per cent. of water) it is much softer than with a proteid diet (containing 60 to 65 per cent. of water). Normal stools are usually cylindrical and firm. A mushy stool, however, may be normal for some persons. When fæces remain long in the intestine the moisture is absorbed, and they are passed as round, hard, scybalous masses.

Odor of Fæces.—The presence of skatol and indol, products of albuminous decomposition, is largely responsible for the obnoxious odor of fæces.

Sulphuretted hydrogen, methane, and traces of phosphin add to the odor.

Color of Fæces.—The color varies according to the food, pathological products present, and medicine ingested. The ordinary color varies from light to a blackish brown. Exclusive milk diet produces a light-yellow stool. Under normal conditions the color is never due to native biliary coloring-matter, but chiefly to the presence of hydrobilirubin.

Starches tend to produce a yellow, chlorophyll a greenish color. In obstructive jaundice the stool is of an ash or light-gray color.

Blood in the stool (unless fresh) always gives it a dark appearance, the so-called tarry stool; this is due to the formation of hæmatin. Iron, manganese, and bismuth produce a dark-brown or black color, owing to the formation of the sulphides of those metals. The green color of calomel stools is probably due to the presence of biliverdin. Santonin, rhubarb, and senna produce a yellow color; hæmatoxylin a red color resembling that of blood.

The **chemical reaction of the fæces** varies much, depending on the kind of fermentation present. In intestinal catarrh with acid fermentation it is acid; with alkaline fermentation it is alkaline.

Macroscopical Examination of Normal Fæces.—Undigested particles of food, skins of various animal and vegetable foods, berries, seeds and stones, woody vegetable fibres, large pieces of connective tissue, undigested pieces of fruits, grains of corn, flakes of casein, etc., are all frequently seen.

Foreign bodies of various sorts are sometimes swallowed, and passed in the stools. It is necessary to keep this in mind when dealing with children, the hysterical, and the insane.

Small quantities of mucus may be found in health, and particles resembling sago grains may be present as a result of overindulgence in starchy food.

Stools of Vegetarians.—The color of the stools of vegetarians is light brown, rarely becoming black as in the case of meat eaters. If eggs are largely used, however, and taken without proper mastication, the stools may become dark from the decomposition of the albumin. The consistence depends on the quantity of fluids or fruit that is eaten. When fruit, fruit juices or sugars are eaten in large quantity the stools are soft.

When, on the other hand, the diet consists largely of grains the stools are apt to be hard; and if the diet consists chiefly of bread with a small quantity of fruit, and when the food is masticated with very great thoroughness, the stools are likely

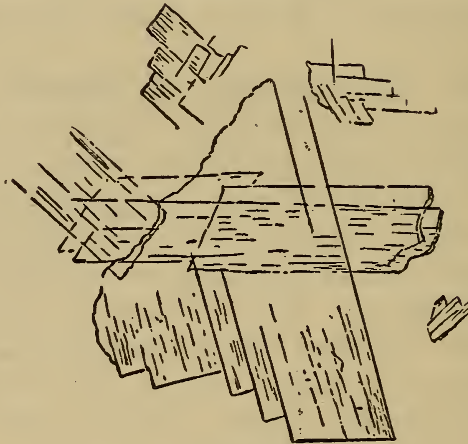
to be small and very dry. When fats are taken freely the stools are light in color (Kellogg).

Macroscopical Examination of Pathological Fæces.— **Mucus**, when present in large quantities, indicates a catarrh of the mucous membrane of the intestine.

In **mucous colitis** large strips of mucus (sometimes in the form of molds of the intestine, resembling sausage skins) are passed in the stools.

Usually, when the mucus is mixed with the fæces and appears in small bits, it comes from the small intestine; but in dysentery it may be mixed with the thin stool and come

FIG. 36.



Cholesterin crystals. (Simon.)

from the large intestine. When the hard fæces are covered with mucus, or when it appears in shreds, it is derived from the large intestine. The "rice-water" stool of cholera is so named because of the presence of bits of mucus resembling grains of rice.

Gall-stones should be sought for in all suspected cases of cholelithiasis. The *Boas sieve* will be found very useful for this purpose. These stones vary from the size of a pea to that of a hen's egg. They may be soft and crumbling masses (intrahepatic calculi, made up chiefly of cholesterin, Fig. 36), or hard and many-faceted.

They are usually light in weight, and vary in color from pale yellow to brown and green, or have a mottled appearance, and change color on exposure to air.

Following the administration of olive oil, lumps of soap may form in the intestine and be mistaken for gall-stones.

Blood.—When bright red in color, it usually comes from the rectum (hæmorrhoids, fissures, etc.) or some part of the large intestine. However, if a large hæmorrhage takes place in the small intestine (as in typhoid fever), the blood may pass quickly through the bowel and appear unaltered in the stool. Blood coming from the stomach and small intestine is usually altered by the juices of these organs and has a black color, the so-called "tarry" stool.

Pus may appear in the stool in an unmixed state when abscess cavities rupture into and discharge through the bowels. It is often mixed with mucus or blood when derived from ulcerative conditions, as in dysentery, tuberculosis, etc. It may coat the stool or be mixed with it.

Fat.—A small amount of fat is present in the normal stool. In diseases of the pancreas, jaundice, and diarrhœa it is present in unusually large amounts, giving the stool a greasy or clay-like appearance.

Microscopical Examination of Normal Fæces.—Vegetable cells, starch-granules, muscle-fibres, elastic tissue, connective tissue of white fibrous variety, fat-globules, and flakes of casein are to be found.

Under normal conditions muscle-fibres are not numerous unless unusually large quantities of meat have been eaten. Starch-granules if in excess—except in young children—indicate a pathological condition of the gastro-intestinal tract. They are easily recognized by the blue color they assume after running a few drops of Lugol's solution under the cover-glass preparation.

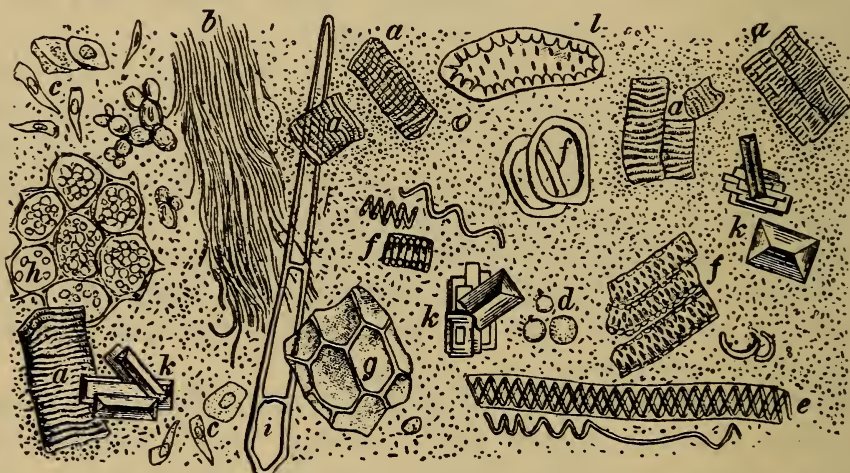
Fat may occur in droplets or in the form of needle-like crystals or highly refractive polygonal masses of a yellowish or reddish-yellow color.

Numerous bacteria are present in normal fæces (Fig. 37).

Method of Obtaining Specimen.—Probably the most im-

portant practical point connected with the microscopical examination of the fæces is the proper method of obtaining the stool. It should be passed into a warm receptacle, and the examination made as soon afterward as possible. This is essential when examining for Amœba coli or trichomonades, since the diagnosis is rendered certain only by the detection of the characteristic movements of these organisms, which cease as soon as the fæces become cold. The amœba may be kept active by the use of a warm stage. A good method of obtaining a specimen for examination at one's own conve-

Fig. 37.



Collective view of the fæces. (Eye-piece III., objective 8 A, Reichert): *a*, muscle-fibres; *b*, connective tissue; *c*, epithelium; *d*, white blood-corpuscles; *e*, spiral cells; *f*, *i*, various vegetable cells; *k*, triple phosphate crystals in a mass of various micro-organisms; *l*, diatoms. (v. Jaksch.)

nience is by the introduction of a rectal tube. In the various diarrhœas a small amount of fæces will be brought away by the tube. In using the rectal speculum a quantity of the desired material can usually be obtained. As a routine measure, in order to secure a satisfactory stool for examination, an ounce of Carlsbad salt should be given before breakfast. Several liquid stools usually result. If the material is tenacious, such as the bloody mucus in cases of amœbic dysentery or the muco-pus of tuberculosis, it can be transferred to a glass slide with the teasing-needle; if watery, it can be

drawn up into a small glass pipette by capillary attraction and a drop or two transferred to the slide and a cover-glass placed over it. This is examined with a high, dry objective.

For staining, spreads should be made and fixed as with sputum preparations.

Morphological Elements derived from the Alimentary Canal.

1. **Epithelial Cells.**—The cylindrical and goblet cells are almost always so altered in the normal fæces that it is difficult to recognize them.

Pavement epithelial cells, when present, come from the anal orifice.

2. **Leukocytes** are almost never found in normal stools.

3. **Red blood-corpuscles** may occasionally be present in very small numbers.

4. **Structureless granules** in large numbers may be seen in every stool.

5. **Necrotic tissue** and pieces of new growth may be present in the fæces.

6. **Crystals.**—A large number of crystals—of almost no diagnostic importance—are found; these are needle-like crystals of free fatty acids (Fig. 38). Calcium and magnesium salts, neutral calcium phosphate and ammonio-magnesium phosphate. Calcium oxalate crystals occur in abundance following ingestion of certain vegetables, as sorrel and spinach. Calcium carbonate and sulphate, and (in children) lactate of calcium are rare. Hæmatoidin crystals are never found in normal stools. Charcot-Leyden crystals are found under certain pathological conditions, and are supposed to indicate the presence of intestinal parasites.

Vegetable and Animal Parasites.—Vegetable parasites are always present in enormous numbers. It is not yet known what relation they bear to the process of digestion. It has been proved that they are not entirely essential. Fungi are rarely found. Schizomycetes belong to the normal constituents.

About 97 per cent. of bacteria are derived from the ingested food and 3 per cent. from the saliva. Most of these are non-pathogenic, but under suitable conditions a small per-

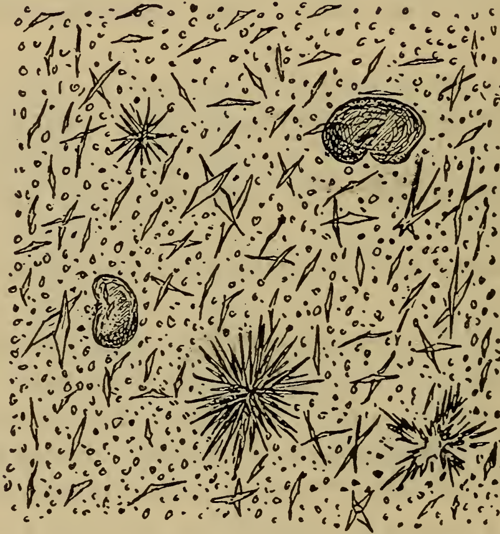
centage develop pathogenic properties. There are two large classes found in normal stools:

Class I. are stained a yellow or yellowish-brown with iodo-potassic iodide.

Class II. are colored blue or violet.

Routine bacteriological examinations of the fæces are of little practical value because of the enormous number of bacteria of all kinds that occur in the fæces and the almost

FIG. 38.



Fatty crystals obtained from the feces. (Simon.)

insurmountable difficulty of isolating them, and because, as a rule, there are easier methods of recognizing disease.

In acute infectious tropical dysentery a specific bacillus, that of Shiga and Flexner, or that of Strong, is found in the intestinal discharges.

In typhoid fever the Eberth bacillus is present in the stools, but it is difficult to isolate because of its close resemblance to numerous members of the colon group. The specific organism of Asiatic cholera is present in the fæces in that disease.

Tubercle Bacillus.—The examination of the stools for

tubercle bacilli, or of discharges for pus obtained during rectal examination, is a very important procedure in establishing or excluding the diagnosis of intestinal tuberculosis. It must be remembered that the stools may be contaminated by swallowed sputum.

Method of Search for the Tubercle Bacilli.—If pus is found, cover-glass preparations should be made, fixed, and stained, as in the sputum examination. To find tubercle bacilli in fæces, dilute the stool with 10 volumes of water in a wide-mouthed bottle of 200 c.c. capacity. Mix thoroughly and let stand for twenty-four hours. The tubercle bacilli will be found in the narrow layer between the thin liquid and the more solid sediment. With a pipette, some of this material is drawn off and spreads made from it.

The fæces may be carefully inspected in the receptacle, and suspected bits of mucus or muco-pus removed with teasing-needles, and spread upon cover-slips and examined in the routine way.

For method of differentiating from smegma bacilli, see chapter on Urine.

Animal Parasites.—The adult form and the ova are found in the human intestine and fæces.

They belong to the classes of:

I. Protozoa.
II. Vermes or worms.
III. Insects.

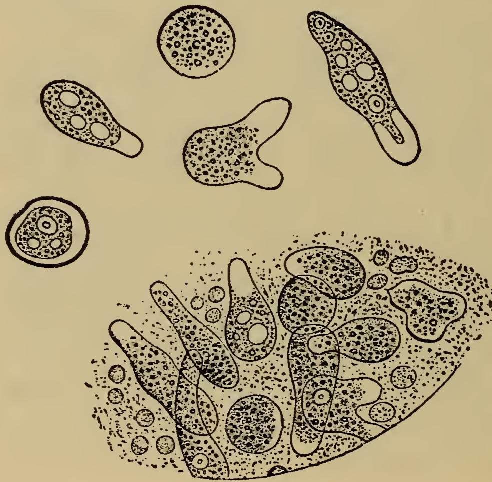
Only a few are common to man in the United States. Special mention is made of these alone. The possibility of contamination of the stools must always be kept in mind.

I. *Protozoa.*—*Amœba coli* is the only protozoon of pathological importance. Its etiological relation to amœbic dysentery has been proved by many observers. It is, of course, rarely found in the temperate zone, and is of more interest to tropical medicine. However, it has been found in the northern part of the United States, and every case of dysentery should be examined for this parasite. It is found in abscesses of the liver complicating tropical dysentery.

The *Amœba coli* is found especially in the mucopurulent or

gelatinous masses of the fæces. These organisms may be so numerous as to fill completely the field under the microscope. They vary in size from 12 to 35 μ in diameter. They consist of a clear outer zone (the ectosarc) and a granular inner zone (endosarc), a nucleus, and one or two vacuoles. They are easily recognized during their active stage by their peculiar amœboid movements, which greatly alter their shape. Pseudopodia are thrust out from the periphery and the remainder of the cell flows into it. When cold, it is difficult to recognize them, and they are likely to be mistaken for swollen, altered,

Fig. 39.



Amœba coli. (Hallopeau.)

granular epithelial cells (Fig. 39). They are well stained by Wright's method.

Trichomonas and *Cercomonas intestinalis* are frequently found in diarrhœal stools, but seem to have no causal relation. They are pear- or oval-shaped bodies possessing flagella. The trichomonas is the larger, and, in addition to the flagella, shows an undulating membrane (Fig. 40).

Other protozoa found in the intestine are coccidia, cercomonas, Megastoma entericum, and Balantidium coli.

II. (*Vermes Worms*).—*Cestodes* (*Tapeworms*).—These are recognized in the macroscopical examination of the fæces by

discovering the segments (proglottides), either singly or a number of them joined together. The important part, and that most difficult of recognition, is the head. It is joined to the slender neck—and its variety can be learned by microscopical examination under a low power.

Tænia saginata is the most common tapeworm in Europe

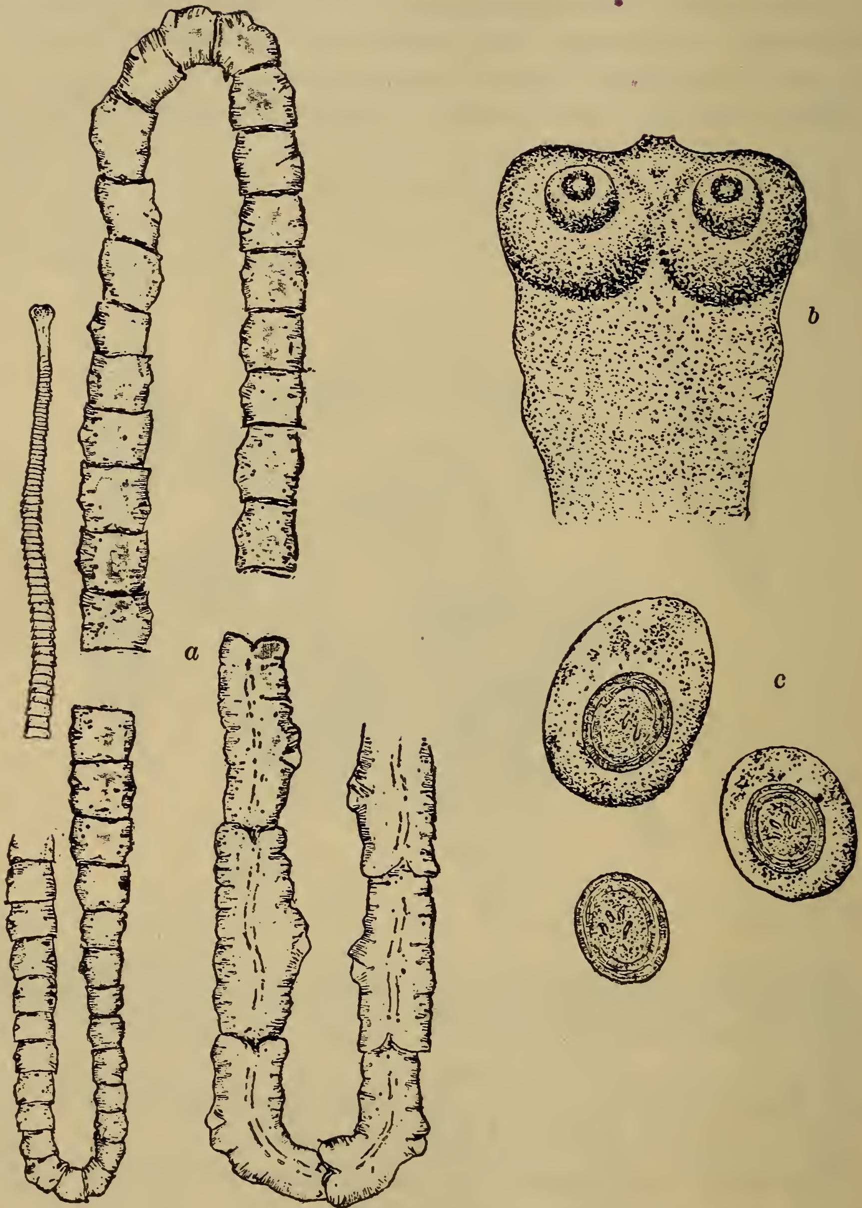
Fig. 40.

Trichomonas intestinalis: *a*, *a'*, *c*, trichomonas of the urine, after Marchand; *b*. Trichomonas vaginalis, after Donné; *b'*, same, after Scanzoni and Kölliker; *d*, Trichomonas intestinalis, after Piccardi; *e*, *e'*, *e''*, same, amœboid forms; *f*, *f'*, trichomonas of the urine, after Dock.

and North America. It is the beef tapeworm, and is recognized by the fact that its head is unarmed.

The head is surrounded by four pigmented suckers, each of which is encircled by a dark ring. Each segment contains male and female generative organs. The uterus occupies the centre, and has numerous dichotomous branches, about twenty

FIG. 41.

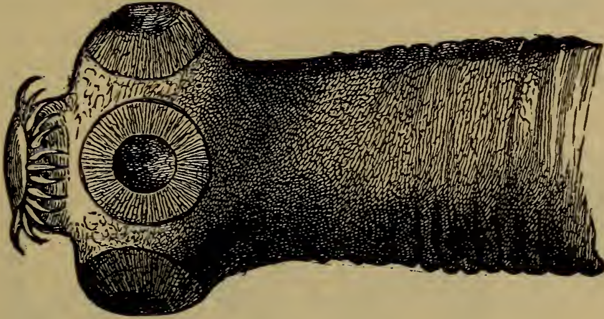


Tænia saginata: *a*, natural size; *b*, head much enlarged; *c*, ova much enlarged. (Simon.)

on a side (Fig. 41). The ova are elliptical, brown, and usually enclosed in a vitelline membrane.

Tœnia Solium.—A pork tapeworm, rarely found in the United States, but common in Asia and Africa. It is usually

Fig. 42.

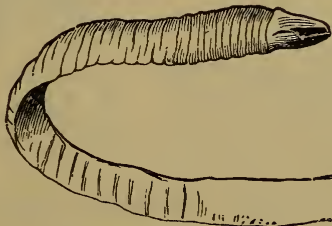


Head of Tænia solium ; × 45. (Leuckart.)

much shorter than the saginata, and its distinguishing characteristic is its armed head. In addition to the four pigmented suckers there is a rostellum at the tip, furnished with twenty-four to twenty-six hooklets arranged in a double row. The mature segments differ from those of the saginata in having a uterus with only five to seven branches (Fig. 42).

The ova are round, of a brownish color, and surrounded

Fig. 43.



Bothriocephalus latus.

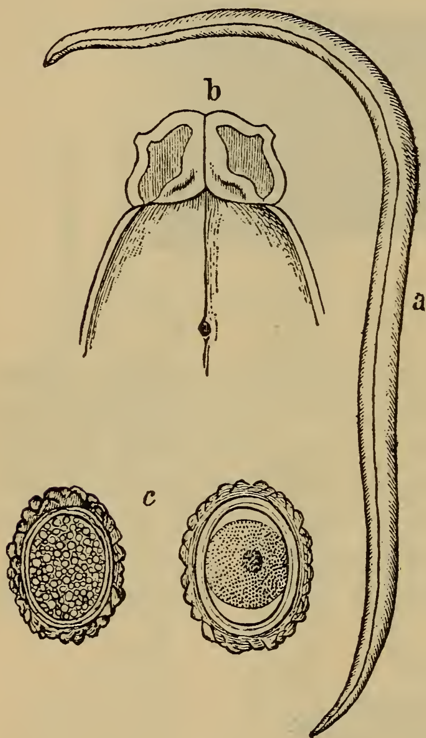
with a thick, radially striated membrane. The hooklets of the embryos can usually be found in their interiors.

Bothriocephalus Latus.—This worm is found much more rarely than the other two in this country. It is very large,

being 5 to 9 m. in length. Its head is shaped like a bean, and on its flat surface are two distinct grooves which probably act as suckers.

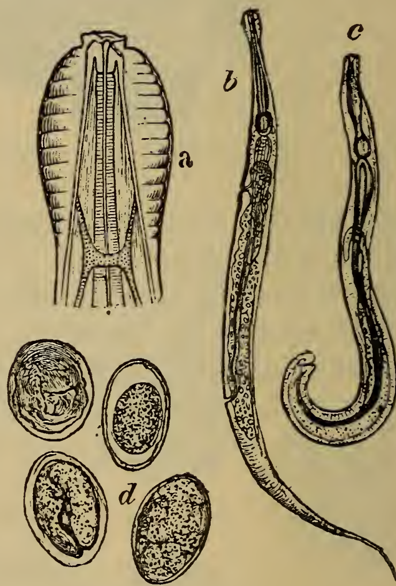
The segments are almost square. The genital apparatus opens in the median line. The uterus presents four to six

Fig. 44.



Ascaris lumbricoides. (Eye-piece I., objective 8 A, Reichert): *a*, worm, half natural size; *b*, head slightly magnified; *c*, eggs. (v. Jaksch.)

Fig. 45.



Oxyuris vermicularis: *a*, head; *b*, male; *c*, female; *d*, eggs. (v. Jaksch.)

convolutions on each side. In water they have a rosette-like appearance (Fig. 43).

The *ova* are oval, enclosed in brown envelopes, at the anterior ends of which a lid can be recognized. It is found in Europe and Japan, and is of marked pathological interest because it produces a severe form of *anœmia*.

Nematodes (filiform, resembling a thread) differ from ces-

todes in having the sex distinct. The female is always larger than the male. The most important of these are:

Ascaris lumbricoides, Oxyuris vermicularis, Uncinaria duodenalis (very important variety) (Anchylostoma duodenale), and Trichinella spiralis.

Ascaris lumbricoides is the most common human parasite, and is found chiefly in children. The female is 7 to 12 inches long, the male from 4 to 8 inches. It is cylindrical, pointed at both ends; four longitudinal bands can be seen,

Fig. 46.

Anchylostomum duodenale: *a,* male, natural size; *b,* female, natural size; *c,* male, magnified; *d,* female, magnified; *e,* head (eye-piece II., objective C, Zeiss); *f,* eggs. (v. Jaksch.)

and it is striated transversely. It may be reddish in color or yellowish brown. Its head is trilobed (Fig. 44).

The ova may be found in large numbers in the fæces. They are small, oval, 60 to 75 mm. in size, brownish red, with a thick covering.

Oxyuris Vermicularis.—This is a small round worm; the female measures 10 mm., the male 4 mm. in length. They may be present in the lower bowel and fæces, and look like bits of thread (Fig. 45).

The ova are about 50 by 24 μ in size, coarsely granular, and surrounded by a double-contoured envelope.

Uncinaria Duodenalis: (syn., Anchylostoma duodenale (Fig. 46). The great importance of this parasite as a cause of certain forms of anæmia has been properly emphasized during the past few years. It is a blood-sucking parasite, and is one of the most dangerous met with in the human being. It has a wide distribution, being found in Italy, Germany,

Fig. 47.

Eggs of Uncinaria americana in different stages of development. (Personal observation.) Magnified about 300. (Simon.)

Switzerland, Belgium, and Egypt. C. N. Stiles has recently shown that the hook-worm, found in the United States and West Indies, is a distinct species. He has denominated it the Uncinaria americana. This investigator has demonstrated that it is a very common parasite in the sandy regions of the South. Infection with it is very common among the poorer classes of this region, probably as a result of the habit of dirt-eating. The stools of all cases of severe anæmia

should be carefully examined for the eggs of this parasite. The adult worm is almost never found in the fæces (Fig. 46).

The parasite is fairly common in dogs, cattle, and sheep.

FIG. 48.

Trichina spiralis in muscle. (Simon.)

The female is 10 to 18 mm. long; male 6 to 12 mm., with an expanded copulatory pouch and slender penile organ at its posterior extremity. The head is turned dorsally and has a

hollowed mouth armed with six hooklets. The ova are abundant in the fæces, are elliptical (30 by 50 μ), and have a thin, colorless, vitelline envelope, enclosing varying numbers of rapidly dividing cells. These segmenting bodies rapidly develop outside of the human body, so that after twenty-four to forty-eight hours embryos may be found in the same fæces in which the eggs were observed, or fully developed ova may be found after allowing the fæces to stand for only a few hours. (Fig. 47.)

Examination of the Fæces in Uncinariasis.—There are two methods, the microscopical and the gross.

Microscopical Examination.—Take a small amount of the fæces, preferably from near the surface, spread this out in a drop of water on a microscopic slide and cover the preparation with a cover-slip. Examine under the low dry objective, with not too strong illumination, or for closer study with the high dry objective. Do not mistake the egg of the uncinaria for that of the Ascaris lumbricoides, which has a thick, gelatinous, often mammillated, covering, and an unsegmented protoplasm, or the egg of the Oxyuris vermicularis, which has a thin, asymmetrical shell (one side being almost straight) and containing an embryo, or for the egg of the whip-worm (Trichuris trichiura, more commonly known as Trichocephalus dispar), which possesses a smooth, thick shell, apparently perforated at each pole, and an unsegmented protoplasm (Stiles).

Gross Examination.—Give a small dose of thymol, 10 to 15 grains, followed in two hours by oil, and collect all of the stools passed. Wash the stools thoroughly several times in a bucket, and examine the sediment for worms about half an inch long, about as thick as a hair-pin, and with one end curved back to form a hook (Stiles).

Trichinella Spiralis.—The adult forms of the trichina occur in the intestine. They are 1.5 to 4 mm. long, and may be found in the stools in trichiniasis. The larval forms can be demonstrated by pressing a small piece of favorable muscle-fibre between slides, and examining under the microscope with a low power (Fig. 48).

Chemistry of the Fæces.—The clinician seldom finds the

chemical analysis of the fæces of any practical value; hence the reader is referred to the larger text-books for its consideration.

QUESTIONS.

What factors are responsible for the consistence, odor, and color of fæces?
What are the macroscopical findings in normal and abnormal fæces?
What are the microscopical findings in normal fæces?
Name the varieties of parasites found in fæces.
Mention the important vegetable parasites found in fæces.
Classify the animal parasites found in fæces.
Describe the *Amœba coli.*
What is the difference between a cestode and a nematode?
Mention the important cestodes and nematodes.
Describe the Uncinaria duodenalis and its ova.
Describe the methods of examining the fæces in uncinariasis.
How are the larval forms of Trichinella spiralis demonstrated?

CHAPTER X.

SPUTUM.

GENERAL CONSIDERATIONS.

Apparatus for examination of sputum includes—
Glass teasing-needles made from glass rods.
Platinum loop.
Glass plate, 5 inches square.
Krönig's sputum plate or a soup plate with bowl painted black.
Slides.
Cover-slips.
Stains and **reagents** for examination of sputum—
Carbol-fuchsin.
Löffler's methylene-blue.
Gabbets stain.
Reagents for Gram's stain.
Eosin, same as in blood work.
Nitric acid, about 30 per cent.

The **term sputum means** that material which is brought up from the pharynx and respiratory tract by the acts of cough-

ing, hawking, and at times vomiting. It is usually associated with diseases of the pharyngo-respiratory mucous membranes, and its examination is often of great value in throwing light upon diseases of the respiratory and neighboring organs.

General Remarks.—Sputum examination has become one of the most important and useful of laboratory diagnostic measures. To be of the largest value, it must be combined with a proper physical examination. In most instances the examination of the sputum furnishes only an etiological, not a pathological, diagnosis. At times it is possible to make a diagnosis of beginning tuberculosis from the physical signs when tubercle bacilli can not be found in the sputum. On the other hand, it frequently happens that the physical signs are very indefinite, possibly indicating a slight bronchitis; in such cases tubercle bacilli are often demonstrated and the diagnosis clinched.

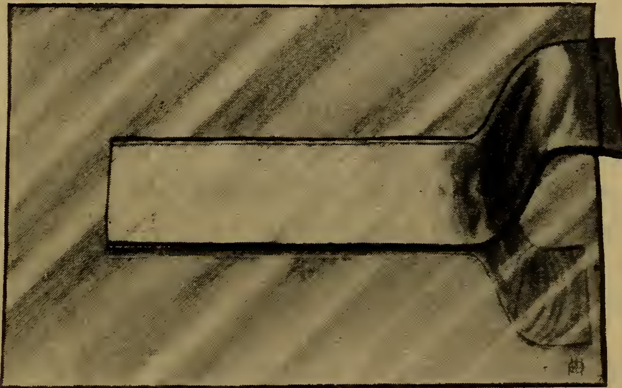
Failure to find the bacilli may be due to the fact that the sputum is chiefly from the throat and contains none of the suspected bacteria. In a great many cases, especially when the bacilli are scarce, it is due to faulty technique. One frequently sees sputum examined in the following imperfect manner: A platinum loop is dipped at random into the sputum cup and a small amount of sputum withdrawn, placed upon a cover-slip, spread, fixed, and stained, with negative results. Such a method is very faulty, and can not be depended on except in advanced cases of tuberculosis, when the entire sputum is filled with bacilli.

Correct Method of Obtaining Sputum.—The patient should be told to clean the mouth thoroughly and expectorate into a clean sputum cup or wide-mouthed glass bottle, in order to avoid contamination with food, since food particles roughly resemble the opaque bits which are rich in tubercle bacilli. Throat sputum, except in cases of laryngeal tuberculosis, seldom shows anything of importance. To secure sputum from those with suspicious signs but scant expectoration, it is sometimes useful to administer potassium iodide in doses of 3 grains every three or four hours.

Correct Method of Examining Sputum.—The sputum should be poured upon a glass plate. This may be an ordinary window pane or a cleaned photograph plate. By means of two sharp-pointed glass teasing-needles the sputum is torn apart and carefully inspected. A second method consists in the use of a glass slide (Fig. 49). The sputum is pressed out between plate and slide; about an inch of the slide extends beyond the plate and is used as a handle. If present, the suspicious opaque bits can be readily seen. The slide is moved about so that fresh portions of sputum are examined successively.

When suspicious particles are found by these macroscopical

Fig. 49.



Author's sputum slide.

methods, they are either transferred immediately to cover-slips and spreads made or, better, the plate is transferred to the microscope, and the particles examined *in situ* with a low-power dry lens. By this means it can be immediately determined whether it is a bit of food, elastic tissue, or a collection of pus. The slide can now be slipped off and the bit transferred to a cover-slip if staining procedures are indicated.

Methods of Spreading Sputum.—1. Transfer the suspected bit of sputum to a cover-glass, place upon it another cover-glass, press down with forceps until the material is spread out in a thin layer, and slide them apart,

2. The material may be spread in a thin layer with the teasing-needle or the platinum loop.

The cover-slips are dried in the air or held in the fingers above a flame, then fixed by passing them several times slowly through the flame of a Bunsen burner or alcohol lamp.

Sputum Examination.—The examinations of the sputum which are of value to the clinician are macroscopical and microscopical.

Macroscopically the following points should be noted:

(1) Quantity in twenty-four hours or during a certain stated period.

(2) Character { (*a*) consistence. (*b*) color. (*c*) odor.

The **amount of sputum** varies much with the disease. In some conditions only a few cubic centimetres are raised in twenty-four hours, in others 1000 c.c. and more. In incipient tuberculosis, early stages of acute bronchitis, and dry pleurisy, and some cases of croupous pneumonia the cough may be frequent, with little or no expectoration. On the other hand, in some cases of chronic bronchitis, advanced tuberculosis with cavities, hemorrhage from the lungs, pulmonary œdema, bronchiectasis, and perforations into the lungs of pus from the thorax or abdomen, there is a large amount of sputum.

In bronchiectasis large quantities of mucopurulent sputum may be raised in a short time, with change of position, especially on rising in the morning.

Consistence of Sputum.—The consistence corresponds in a general way to the amount; it may vary from a watery to an extremely tenacious sputum. Whether mucin or nuclein derivatives are the cause of the tenacity is not thoroughly understood.

In œdema of the lungs the sputum is liquid and resembles blood-serum, and is covered by a frothy surface layer. When the sputum is mostly pus, as in pulmonary gangrene, pulmonary abscess, putrid bronchitis, and following the perforation of an empyema or subdiaphragmatic abscess into the lungs, it

is quite liquid. In croupous pneumonia the sputum is so tenacious that the cup containing it may be inverted without losing a drop, provided there is not an associated bronchitis.

This is also the case following an attack of bronchial asthma and in the beginning stages of acute bronchitis.

Color of Sputum.—The color may vary from colorless mucoid to the dark-brown sputum containing altered blood. It may be gray, yellow, green, red, or brown. Admixture with fresh blood gives the red color, while admixture with pus (depending on the number of leukocytes) gives a color varying from gray to green. Green sputum may be due to admixture with bile, as in the perforation of a liver abscess into the lung, jaundice, and pneumonia accompanied by jaundice.

The sputum in amœbic abscess of the liver which has perforated the lung has a color resembling anchovy sauce—reddish-brown, of a brick-dust color—due to blood-pigments and corpuscles.

Red sputum, varying in intensity with the amount of blood present, is found in pneumonia, tuberculosis, heart disease, gangrene and abscess of the lungs. In pneumonia the sputum gradually changes from a bright-red color, due to unchanged blood, to a rusty or orange shade. In low types of the disease it resembles prune juice.

Prune-juice sputum (dark mucoid) is present in a large percentage of cases of cancer of the lungs.

Greenish-yellow sputum in coin-like lumps is found in influenza (Pfeiffer).

In anthracosis the sputum may be very dark in color, so-called "black spit."

Odor of Sputum.—Sputum is usually odorless, but at times, as in fœtid bronchitis and pulmonary gangrene, the stench is most obnoxious.

In bronchiectasis, perforating empyema, and ulcerative processes the odor is sweetish.

In perforating empyema an odor resembling old cheese is sometimes present.

Varieties of Sputum.—

Homogeneous	Mucoid. Purulent. Serous. Sanguineous.
Heterogeneous	Mucopurulent. Mucoserous. Serosanguineous. Sanguinomucopurulent.

Sputum crudum is an example of pure mucoid sputum, and is seen in the first stages of bronchitis.

Nummular sputum is made up of roundish, coin-like disks, which sink in water. It is found in the second and third stages of phthisis.

Sputum globosum consists of fairly dense, round, grayish-white masses, secreted in old cavities.

Cheesy particles, varying in size from that of a millet-seed to that of a pea, are seen in cases of tuberculosis. They are usually rich in tubercle bacilli and elastic tissue. It is important to search for such particles in the macroscopical examination of sputum.

Caseous masses which form in the crypts of tonsillar tissue are very common and frequently cause much unnecessary worry. They are coughed up or brought up by clearing the throat. Their microscopical examination is negative. They have a very foul stench, due to fatty acids.

Macroscopical examination of sputum by means of **teasing-needles, glass plate and slide** is of great value in facilitating the microscopical examination.

Opaque particles may contain elastic tissue or tubercle bacilli.

Fibrinous casts may appear as masses of a white color; they are often yellowish brown or reddish yellow, owing to the presence of blood coloring-matter. They are found in fibrinous bronchitis, pneumonia (either before or after resolution has taken place), and in cases of diphtheria where the disease has extended into the finer ramifications of the bronchi. The suspected bits should be transferred from the glass plate or Krönig's sputum plate, and shaken out in water in order to

unravel them. These casts may vary from 12 cm. in length by several millimetres in thickness, to very small fragments 0.5 to 3 cm. in length. Those found in pneumonia are small; those in fibrinous bronchitis come from the smaller and medium-sized bronchi.

These casts branch dichotomously and contain a cavity in

Fig. 50.

Fibrinous coagulum from a case of croupous pneumonia. (Bizzozero.)

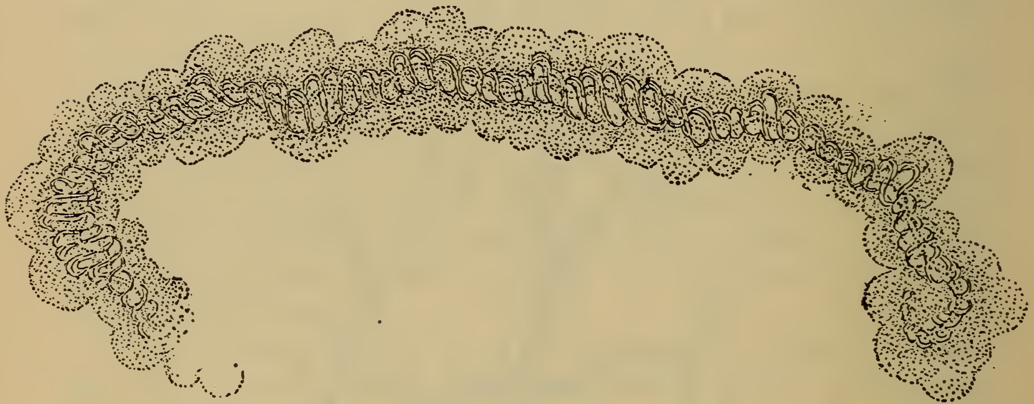
their larger portion, while the finer branches appear to be solid (Fig. 50).

Curschmann's spirals occur in bronchial asthma, also in chronic bronchitis and even in pneumonia. Macroscopically they appear as thick, yellowish-white masses, which show a spirally twisted appearance,

Microscopically with the low power they are seen to consist of a spirally twisted network of extremely delicate fibrils, which is wound around a clear, colorless central thread. In this mass are found epithelial cells and leukocytes which are mainly of the eosinophile type. Not all spirals are perfect; the central thread may be absent or the spiral arrangement may be imperfect. They vary in length from 1 to 1.5 cm. Their presence usually indicates a desquamative catarrh of the bronchi and alveoli (Fig. 51).

Echinococcus membranes, concretions, and foreign bodies are rarely found.

FIG. 51.



A Curschmann spiral from a case of true bronchial asthma. (Simon.)

Microscopical examination of sputum consists in the use of a low dry lens with the glass plate and slide, and of a high dry lens with cover-glass and slide, and of an oil immersion with stained preparations.

The **objects of chief interest and diagnostic value** are:

(1) Elastic tissue.
(2) Parasites.
(3) Red blood-cells.

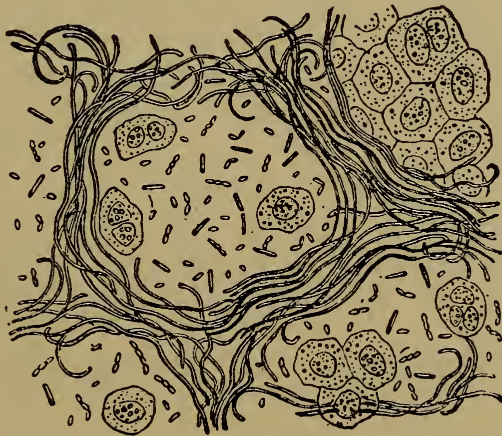
Of less value are:

(1) Leukocytes.
(2) Epithelial cells.
(3) Crystals.
(4) Food particles.

The examination should begin with the employment of the glass plate under a low-power lens. Attention is directed especially to the small opaque bits.

Examination for Elastic Tissue.—A portion of sputum is placed upon a glass plate about 5 inches square, a size convenient for handling on the stage of the microscope. With a slide for a spatula the sputum is pressed out into thin layers, and all suspicious bits examined. Elastic tissue-fibres have the following appearance under the low power of the microscope; they are very slender threads of varying lengths, curl-

FIG. 52.



Elastic fibres in the sputum. (Eye-piece III., objective 8 A, Reichert.) (v. Jaksch.)

ing and branching somewhat at their ends. They refract light in such a way as to give them the appearance of having a double wavy contour with a colorless centre. Occasionally they show an alveolar arrangement, making plain their origin (Fig. 52).

Macroscopically, particles of food, masses of epithelium, and débris are most often mistaken for these suspicious bits which contain elastic fibres.

Microscopically, the beginner frequently mistakes vegetable fibres, such as lint from a towel, etc., and masses of leptothrix for elastic tissue.

The more slender fibre with its double contour and curling, branching ends distinguishes the elastic fibre (Fig. 52).

Whenever elastic tissue is found, it is certain that a destructive process is going on in the respiratory tract. It is especially important when associated with tubercle bacilli. It is most frequently found in tuberculosis, but may also be present in abscess of the lung, bronchiectasis, and occasionally in pneumonia. In gangrene of the lung, elastic tissue usually is not found (perhaps it is destroyed by a ferment).

Vegetable parasites found in sputum are: tubercle bacilli; Diplococcus pneumoniæ; bacillus of influenza; streptococci and staphylococci; Leptothrix buccalis; Oïdium albicans; Aspergillus fumigatus; Mucor corymbifer, etc.

Tubercle Bacillus.—By far the most important sputum examination is that for tubercle bacilli. After selecting the suspicious bits by macroscopical examination they are transferred to cover-slips or slides, and spreads made as directed. These are fixed by passing through a flame several times, specimen side up, and are then ready to be stained. If the specimen is spread upon a slide, forceps are unnecessary, and cover-slips may be dispensed with, unless permanent specimens are desired.

Methods of Staining.—The following solutions are required:

(1) Carbol-fuchsin.
(2) 25 per cent. nitric acid.
(3) Löffler's methylene-blue.

The cover-glass, held with forceps, is covered with a few drops of carbol-fuchsin, placed over a flame till it steams, then moved aside, and returned to the flame several times.

The stain is washed off in water, and the specimen dipped into the nitric acid solution for a few seconds till pretty thoroughly decolorized, only a faint pink tint remaining.

The specimen is again washed in water till it is certain that all traces of nitric acid are removed. It is then covered with a few drops of the methylene-blue solution for one-half to one minute. This is washed off with water and the specimen dried between filter-paper and mounted in Canada

balsam, and examined with the oil immersion; or it may be examined in water instead of Canada balsam.

The bacilli appear as red rods on a blue background, measuring from 3 to 4 μ in length by 0.3 to 0.5 μ in breadth. They may appear homogeneous, or as made up of small red beads with unstained spaces between; they may be straight or curved, and occasionally branched.

The beginner often mistakes the edges of cells or particles of dirt, which retain the red color, for these bacilli.

Very little dependence can be placed upon the relation between the number of bacilli and the severity of the disease. In some incipient cases many bacilli are found in the mucoid sputum; while, on the other hand, in some advanced cases and in acute miliary tuberculosis the bacilli are scarce.

As a rule, however, the severity of the disease corresponds in a general way with the number of bacilli.

Gabbet's Method.—The fixed spreads are stained with carbol-fuchsin as in the above; washed in water, and covered with Gabbet's solution, which consists of 75 parts of water, 25 of sulphuric acid, and 2 of methylene-blue. This is allowed to remain for from fifteen to sixty seconds, until by washing in water the red color is seen to have disappeared and been replaced by the blue. The preparation is dried between filter-paper and mounted in Canada balsam.

If the physical signs are suspicious and the tubercle bacilli have not been found, it is wise to resort to the following devices to increase the chance of finding them:

(1) Nuttal has demonstrated that these bacilli will multiply in the sputum itself at a certain temperature. It is well then to place the specimen of sputum in the incubator for twenty-four to forty-eight hours and then examine it.

(2) The use of the centrifugal machine also increases the chance of finding the bacilli. The following procedure may be employed: About 100 c.c. of sputum are boiled with double the amount of water, to which from 6 to 8 drops of a 10 per cent. solution of sodium hydrate have been added, until a homogeneous solution has been attained, water being added from time to time to allow for evaporation. This is

then centrifugated or set aside for twenty-four to forty-eight hours and the bottom portions examined for tubercle bacilli and elastic tissue.

The only organisms likely to be mistaken for the tubercle bacillus are the bacillus of leprosy and the smegma bacillus. All three organisms are characterized by the great tenacity with which they retain acid stains and the great difficulty with which they take up basic dyes. The latter two organisms are, however, almost never found in the sputum.

In the examination of urine and fæces, however, it is necessary to exclude smegma bacilli by the use of Pappenheim's stain or decolorization in alcohol.

Diplococcus Pneumoniæ.—In the great majority of cases of lobar pneumonia this organism is the etiological agent, and can be found in the sputum. It is a rod-shaped diplococcus surrounded by a characteristic capsule, which serves to distinguish it from other cocci.

Welsh's Method of Demonstrating the Capsule.—Spread and dried cover-glass preparations are treated first with glacial acetic acid, which is allowed to drain off, and is replaced (without washing in water) with anilin gentian-violet solution. The stain is repeatedly added until all the acid is displaced. The specimen is now washed in a weak salt solution (about 2 per cent.) and examined in this—not in balsam.

Gram's method stains the diplococcus and its capsule blue-black.

Loffler's methylene-blue stains the diplococcus well, but does not stain the capsule.

Specific stains for the capsule are often unsatisfactory, and have little advantage over Löffler's methylene-blue.

Bacillus Pneumoniæ of Friedlander.—This is a larger organism than the pneumococcus, and appears in the form of plump, short rods surrounded by a capsule. It occurs occasionally in pneumonia, but is probably not a cause of the genuine lobar variety.

Bacillus of Influenza (Pfeiffer).—This germ is found in the sputum of this disease, and its recognition is of diagnostic importance. Cover-glass preparations are made in the usual

way, and stained with Löffler's methylene-blue for from five to ten minutes, washed in water, and mounted in balsam or water.

The organisms appear as extremely small blue rods. The end stains more deeply than the middle portion.

Streptococci and Staphylococci.—These organisms are extremely common in sputum, but are not specific in any pulmonary disease. They are found especially in tuberculosis, bronchitis (acute and chronic), pneumonia, pulmonary gangrene and abscess, etc.

Actinomycosis.—Actinomycosis has been positively shown to be a variety of infections due to different species of *streptothrix*, instead of being, as was formerly believed, a single disease entity, caused by a single micro-organism (Actinomyces bovis). Because of this fact it seems advisable to substitute the name streptothricosis for actinomycosis.

A number of cases of pulmonary streptothricosis have been reported in which the physical findings could not be distinguished from those of tuberculosis. The sputum and pus from cavities in the lungs contained branched threads which were "as acid-fast as tubercle bacilli." Various investigators have described several varieties of streptothrix as the cause of pulmonary infections. At the present time it is impossible to classify them definitely.

In the pus derived from ulcerating actinomycotic tumors, in the sputum of cases of pulmonary actinomycosis, and in the fæces of intestinal cases, characteristic yellow granules, measuring from 0.5 to 2 mm. in diameter, may be found.

These granules, when pressed out under a cover-slip and examined with the microscope, will be seen to consist of numerous threads, which radiate from a centre in a fan-like manner and show club-shaped extremities.

Leptothrix buccalis is a common non-pathogenic mouth micro-organism of the fission fungi type. It is frequently present in the sputum, and in the unstained specimen has been mistaken by beginners for elastic tissue. In pulmonary gangrene it is known as Leptothrix pulmonalis. It takes on a violet or bluish color when treated with Lugol's solution.

Oidium albicans, or **Saccharomyces albicans**, produces a stomatitis called *thrush*, and is seen most often in children and tuberculous adults. It belongs to the order of yeast-fungi, and consists of branching filaments, from the ends of which ovoid torula cells develop (Fig. 53).

Aspergillus fumigatus, **Mucor corymbifer**, and **Sarcina pulmonalis** may occasionally be found in sputum, but are of little importance.

An important practical point is the fact that a number of pathogenic micro-organisms can be found in the sputum or mouth in health. Such are Diplococcus pneumoniæ, strepto-

FIG. 53.

Oïdium albicans, the vegetable parasite of muguet or thrush. (Reduced from Ch. Robin.)

cocci and staphylococci, Bacillus pneumoniæ of Friedländer, Bacillus coli communis, and even the bacillus of diphtheria.

Animal Parasites.—Portions of echinococcus cysts—*i. e.*, pieces of membrane and hooklets—are occasionally found in the sputum when the parasite has entered the lungs or neighboring organs.

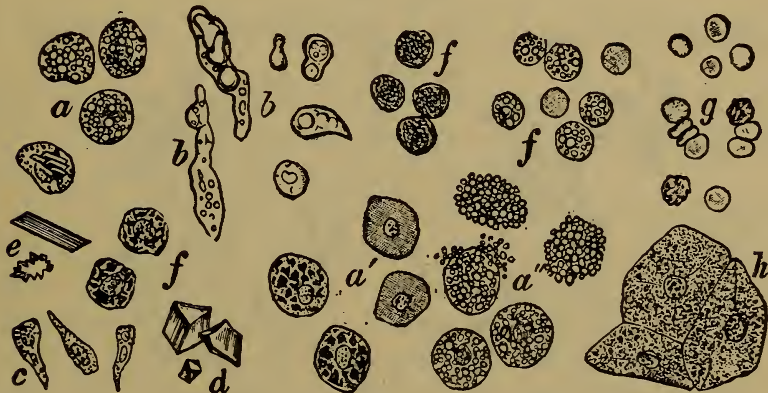
Trichomonades, similar to Trichomonas vaginalis, have rarely been observed in cases of gangrene of the lungs and in the pus removed from lung *cavities* post mortem.

Amœba coli, when found in sputum, make it certain that an hepatic abscess has perforated into the lungs.

Distoma pulmonale, a lung parasite, is the cause of a disease resembling phthisis, and occurs frequently in Japan. The worm and ova are found in the sputum.

Red blood-cells (Fig. 54) in small numbers are found in almost every sputum, being derived from inflamed mucous membranes. When present in large numbers, they are of importance, especially in phthisis. They occur in almost all pulmonary diseases. The appearance of the red cell depends upon the length of time it has been in the lung, and will vary from a typical cell to its shadow or a mere fragment. The

FIG. 54.



Epithelium, leukocytes, and crystals of sputum. (Eye-piece III., objective 8 A, Reichert): *a*, *a'*, *a''*, alveolar epithelium; *b*, myelin forms; *c*, ciliated epithelium; *d*, crystals of calcium carbonate; *e*, hæmatoidin crystals and masses; *f*, *f*, *f*, white blood-corpuscles; *g*, red blood-corpuscles; *h*, squamous epithelium. (v. Jaksch.)

presence of blood-pigment is not always indicated by a red color. It may show a golden-yellow or greenish tinge, having undergone certain chemical changes.

Leukocytes, usually polynuclear, are seen in every sputum. In bronchial asthma a large number of eosinophile and even basophilic leukocytes are found. The sputum of pulmonary abscess, perforating empyema, and putrid bronchitis is made up chiefly of degenerating leukocytes.

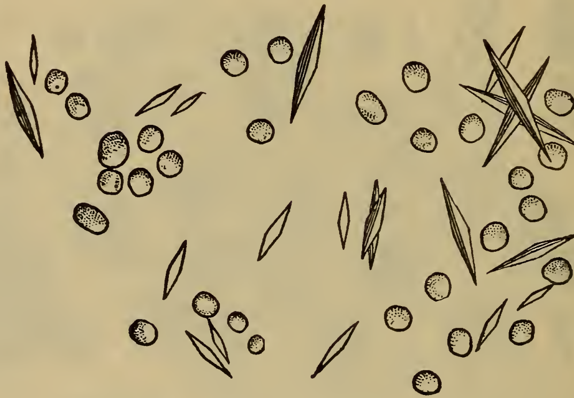
Epithelial cells are present in the sputum, but are of little importance in determining the location of the pulmonary disease.

The cylindrical epithelial cells are usually so much altered that they resemble leukocytes. It has been proved that alveolar epithelial cells occur in almost every known pulmonary disease, and also in normal expectoration after a very forcible expiration.

Heart-disease cells are alveolar epithelial cells, containing numerous hæmatoidin granules. They are found in the sputa of bronchitis associated with chronic heart disease (see Fig. 4).

Crystals.—The following crystals have been found in sputum: Charcot-Leyden, hæmatoidin, chloesterin, margarin, tyrosin, calcium oxalate, uric acid, and triple phosphates.

Fig. 55.



Charcot-Leyden crystals. (Scheube.)

None of these is of diagnostic importance, although at one time Charcot-Leyden crystals were supposed to have an etiological relation to asthma. They are more often present in this disease than in any other pulmonary affection, but they have also been found in acute and chronic bronchitis and in phthisis (Fig. 55).

QUESTIONS.

Describe the correct methods of obtaining and examining sputum.

Mention the different varieties of sputum.

Upon what factors do the varying colors of sputum depend?

What points are to be considered in the macroscopical examination of sputum?

What are the chief objects of interest and diagnostic value in the microscopical examination?

Describe elastic tissue and the best method for its examination.
What is a Curschmann spiral?
Name the vegetable parasites found in the sputum.
Describe Gabbet's method of staining tubercle bacilli.
What bacilli are most likely to be mistaken for tubercle bacilli?
Describe Welsh's method of capsule staining.
Of what importance is the streptothrix in pulmonary infections?
Of what significance are Charcot-Leyden crystals and eosinophile leukocytes?

CHAPTER XI.

MISCELLANEOUS EXAMINATIONS.

1. CLINICAL BACTERIOLOGICAL EXAMINATIONS.

Most of this work consumes very little time when carried out by microscopical examination of stained cover-slip preparations, as described in preceding chapters.

In case of a mixed infection or difficulty in recognizing bacteria by the fresh cover-glass method, it is occasionally necessary to resort to more time-consuming examinations, such as cultural methods and animal inoculation.

For this purpose a sterilizing oven, Petri plates, and culture-media are needed in addition to apparatus before mentioned.

Reliable culture-media may be purchased from the leading manufacturing pharmacists. The following will serve ordinary purposes:

(1) Bouillon, 2 per cent. glucose-bouillon if desired.
(2) Agar, 2 per cent. glucose-agar if desired.
(3) Löffler's blood-serum.

Bacteria are isolated by means of (1) plate cultures or (2) the streak method.

Plate Cultures.—For this method three sterilized Petri plates and three tubes of agar are employed.

The culture-medium is melted in the tubes by heating over a Bunsen flame, and then placed in a hot water-bath. Cool melted material below 50° C. and inoculate before solidifica-

tion commences. With a sterilized platinum loop the first tube is inoculated with two or three loopfuls of the material and stirred thoroughly. The platinum loop is sterilized again, and the second tube inoculated with two or three loopfuls of material from tube No. 1, and well mixed.

The loop is sterilized once more, and then tube No. 3 is inoculated with several loopfuls of material from tube No. 2. The cotton plugs are again removed and the upper portions of the tube heated in the flame and the contents poured into Petri dishes. They are now placed in the thermostat or set aside at room temperature. In the growth which follows the colonies of bacteria are separated. They may now be transplanted to different tubes, thus rendering it possible to isolate and study them.

The **streak method** is much more simple. The platinum loop is charged with the material and drawn several times over the surface of the culture-medium. In this way the bacteria may be separated and grown in isolated colonies. The different bacteria may be recognized by their cultural characteristics, or may be transferred to cover-slips and studied microscopically.

Animal inoculation is especially practical clinically for the diagnosis of tuberculosis and rabies. Suspected sediments are injected into the peritoneal cavities of guinea-pigs or rabbits. Solid material is introduced through a small abdominal incision under aseptic precautions. The symptoms are carefully observed, and later a postmortem is performed and the necessary bacteriological and histological examinations made.

Bacteriological examination of the following bacteria is practical, and may be of great value:

Diphtheria Bacillus.—Spreads may be made directly from the throat, stained, and examined microscopically.

A better method is to inoculate a tube of Löffler's blood-serum with the material from the diseased throat. Two tubes are required: (1) Löffler's blood-serum; (2) a tube containing a swab, made by winding absorbent cotton around a wire. Both tubes are plugged with cotton and sterilized.

The sterilized swab is rubbed against the membrane and then smeared over the surface of the blood-serum. The swab is burned or returned to its tube. The culture-tube is placed in the thermostat at 37° C. If diphtheria bacilli are present, characteristic colonies should grow in from eighteen to twenty-four hours. They are large, elevated, rounded, grayish or creamy-white, moist, discrete or confluent, with a centre denser than the periphery.

Spreads are made from these colonies and stained with Löffler's methylene-blue or by Gram's method. The bacillus is nonmotile, 2.5 to 3 μ in length, 0.5 to 0.8 μ in thickness. It is a straight or slightly bent rod, with rounded ends. Irregular forms are common, such as rods with one or both ends swollen. They stain unevenly—some areas deeply, others slightly—giving a beaded or segmented appearance. The most characteristic thing about this bacillus, and one which greatly facilitates its recognition, is the presence of small granules near the poles of the bacillus, which stain blue with Neisser's method, while the body of the organism is colored brown. *Streptococci* and *staphylococci* frequently produce exudates in the throat which resemble the diphtheritic membrane.

Tubercle Bacillus.—Because of their small numbers, it is often impossible to demonstrate tubercle bacilli in urine, exudates, transudates, etc., by staining methods. Animal inoculation should then be resorted to. The fluid is centrifugated and the sediment injected.

In examining urine, fæces, and genito-urinary discharges for the tubercle bacilli, the spread should always be decolorized in alcohol, in addition to nitric acid, in order to exclude the smegma bacilli. Pappenheim's stain may be used for this purpose.

Diplococcus Intracellularis Meningitidis.—This resembles the gonococcus in its morphology and staining properties. It is associated with infectious cerebrospinal meningitis, and is obtained from the cerebrospinal fluid and meningeal exudates.

Micrococcus lanceolatus of pneumonia is recognized by staining methods. (See Sputum.)

Cerebrospinal Fluid.—Examination of this fluid may be of great diagnostic aid in diseases of the cerebrospinal tissues, especially in meningitis.

There may be inflammatory, suppurative, hæmorrhagic, and dropsical processes; one looks for alterations in the amount, appearance, specific gravity, quantity of albumin, and the presence of pus, blood, and bacteria. The presence of a turbid fluid is important. The chief examinations are macroscopical, microscopical, and bacteriological.

The fluid should be centrifugated, spreads made from the sediment, stained, and examined for the various bacteria with the oil immersion.

The tubercle bacillus, Diplococcus intracellularis meningitidis, Micrococcus lanceolatus, staphylococcus, streptococcus, and typhoid bacillus are the chief causes of meningitis.

The spreads should be stained by appropriate methods. If the microscopical examination is unsatisfactory, cultural methods or animal inoculation should be resorted to.

Lumbar Puncture.—Cerebrospinal Fluid as Obtained by Lumbar Puncture.—This simple operation should be performed in all obscure conditions suggesting cerebrospinal involvement. The patient should lie on the side, with the back bent forward as far as possible, in order to separate the vertebræ and make the lumbar region prominent.

The sitting posture may also be assumed with the back bent forward. A local anæsthetic is usually all that is necessary. Very often the patient is comatose and requires none. A long, strong, aspirating-needle is introduced midway between the second and third or third and fourth lumbar vertebræ, about an inch to the side, and directed a trifle inward. It should be inserted from 2 to 3 cm. in a child and from 7 to 8 cm. in the adult. As soon as the subarachnoid space is entered, the fluid makes its appearance, usually drop by drop; but when large in amount and under great pressure, in a stream.

Normal cerebrospinal fluid is a clear, limpid, noncoagulating liquid, with a specific gravity of 1005 to 1007. Microscopically a few endothelial cells, leukocytes, and fibrin-filaments

may be seen. A few cubic centimetres of fluid can nearly always be obtained. It is increased in hydrocephalus and meningitis; in some cases 100 c.c. have been withdrawn.

In fibrinous and purulent conditions it is decreased, or can not be obtained. The same is true in adhesions or when tumors press upon the canal.

Articular Fluid.—Examination of synovial fluid—obtained by aspiration or incision—may throw considerable light upon the nature of the disease. The same methods of examination should be followed as in the above.

Transudates and **exudates** of the various cavities of the body, pleura, pericardium, and peritoneum, should be examined in the same way. Frequently by microscopical and cultural means, or animal inoculation, the etiological factor can be discovered.

The examination of the sediment of the various exudates by staining with hæmatoxylin may be of value in the diagnosis of malignant growths of the serous membranes. Dock has found that in cancerous effusions there are more cells showing mitoses than in simple or tuberculous inflammations.

Transudates are found in noninflammatory conditions, and are usually clear and light yellow in color, while **exudates** are found in inflammatory conditions and are darker in color and turbid.

The specific gravity of transudates is usually below 1018; while in exudates it is usually above this figure, and may reach 1030.

Exudates contain much more albumin.

Microscopically the transudates are free from microörganisms, and show only a few isolated leukocytes and endothelial cells.

Exudates contain many more formed elements, and may be serous, serofibrinous, seropurulent, purulent, putrid, hæmorrhagic, chylous or chyloid. They may be free from microorganisms, but more often they are not.

In empyemas, various streptococci and staphylococci, the pneumococcus, and Fränkel's diplococcus may be found. If entirely free from microörganisms, tuberculosis should be

suspected. *Pus* made up of a large percentage of mononuclear leukocytes instead of *poly*nuclear strongly suggests a tuberculous origin.

Discharges from the various mucous membranes of the body, such as the urethral, vaginal, nasal, conjunctival, etc., should be examined microscopically by means of spreads and appropriate stains.

Average Human Milk.—Analysis taken from Rotch's *Pediatrics:*

Reaction, amphoteric or slightly alkaline.
Specific gravity, 1028 to 1034.

Water	87 to 88	per cent.
Total solids	12 to 13	"
Fats	3 to 4	"
Milk-sugar	6 to 7	"
Proteids	1 to 2	"
Total mineral matter	0.1 to 0.2	"

Average Cows' Milk.—

Reaction, slightly acid.
Specific gravity, 1029 to 1033.

Water	86 to 87	per cent.
Total solids	13 to 14	"
Fats	4	"
Sugar	4.5	"
Proteids	4	"
Total mineral matter	0.1	"

Laboratory examination of milk may be of value from the standpoint of infant feeding.

The specific gravity and the determination of the per cent. of fat are the two chief practical tests, since the other solid ingredients vary proportionately with the fat. From these a fairly accurate idea of the nutritive strength of the milk can be gained. The *total solids* can be approximately calculated (for cows' milk) by adding 1.2 times the per cent. of fat to one-fourth of the specific gravity at 15° C.

Fat Estimation.—This is most easily made by means of the *lactoscope of Feser.* Draw milk into the pipette up to the mark M; empty into cylinder C. Rinse pipette with

water and add washings to the milk. Shake and add water until the black lines upon the milk-colored glass plug A can just be discerned. The height to which the mixture reaches is noted on the scales. The figure on the right indicates the percentage amount of fat, while that on the left indicates the number of cubic centimetres of water which have been added.

The *simplest method of determination of fat* is by the cream gauge (Holt) (Fig. 50). Although its results are only approximate, they are in most cases sufficiently accurate for clinical purposes.

The tube is filled to the zero mark with freshly drawn milk, which stands at a room temperature for twenty-four hours, when the percentage of cream is read off. The ratio of this to the fat is approximately 5 : 3 ; thus 5 per cent. cream indicates 3 per cent. fat, etc. For a more accurate determination the best ready method is probably the modification by Lewi of the Leffmann and Beam test for cows' milk. This is a centrifugal test requiring special tubes (made by Richard & Co., New York) used in the ordinary centrifuge for urine. Only 6 c.c. of milk are necessary ; and if carefully made the results are almost as accurate as by a chemical analysis.

The *Babcook fat-tester* is frequently employed. Equal volumes of milk and commercial sulphuric acid are mixed in a test-bottle which has a long, graduated neck. This is centrifugated at once—while still hot. After whirling, the bottle is filled to the neck with hot water, returned to the machine, and whirled again for one or two minutes, after which it is filled with hot water to about the 7 per cent. mark. It is again whirled for a short time. The fat separates and its percentage is noted on the scale.

Specific Gravity.—This is best determined with the lactodensimeter of Quevenne. The instrument is graduated for a temperature of 60° F., so it is necessary to correct the specific gravity for different temperatures. The error, however, is very small, and if taken at room temperature need not be considered.

QUESTIONS.

Mention several important culture-media.
Describe the *plate culture* method of isolating bacteria.
Describe the *streak method.*
What is meant by *animal inoculation,* and when is it indicated?
Describe in detail the correct method of making a bacteriological examination in a case of diphtheria.
Of what importance is examination of cerebrospinal fluid?
Describe the method of making lumbar puncture.
What is the difference between a transudate and an exudate?
What are the two chief practical tests in the examination of milk?
Mention several methods for determining fat per cent.

CHAPTER XII.

URINALYSIS.

APPARATUS AND REAGENTS USED IN URINALYSIS.

1. Test-tube, 4 and 6 inches.
2. Test-tube stand and brush.
3. Urinometer (preferably Squibb's).
4. Doremus ureometer.
5. Esbach's albuminimeter.
6. Burette graduated in 0.2 or 0.1 c.c.
7. Graduated cylinders, 100 c.c. and 500 c.c. or 1000 c.c.
8. Flasks of several sizes.
9. Conical glasses.
10. Nest of beakers.
11. Set of porcelain evaporating dishes.
12. Glass funnels, 2-inch and 5-inch.
13. Nipple pipettes, 1 c.c.
14. Glass tubing of different sizes.
15. Glass rods of different sizes.
16. Bunsen burner or alcohol lamp.
17. Centrifuge, centrifuge tubes.
18. Litmus-paper, blue and red.
19. Filter-paper, to fit funnels.

20. Small file.
21. Slides and cover-slips.
22. Stains—carbol-fuchsin, methylene-blue, Gram's stain.
23. Microscope.

Several test-tubes should be file-marked in order that the same quantities of reagents and urine may always be used in making such tests as the diazo, indican, etc.

Esbach's Reagent.—10 grammes of picric and 20 grammes of citric acid, dissolved in 1000 c.c. of distilled water.

Fehling's Solutions.—Two solutions, kept in separate rubber-stoppered bottles.

Solution I.: 34.64 grammes of pure crystallized copper sulphate, dissolved in distilled water and diluted to 500 c.c.

Solution II.: 173 grammes of tartrate of potassium and and sodium, and 60 grammes of sodium hydrate dissolved in distilled water, and diluted to 500 c.c.

Purdy's Solution for Sugar Determination.—

Pure cupric sulphate, 4.752 grammes;
Potassium hydroxide, 23.50 grammes;
Strong ammonia (U. S. P., specific gravity 0.90), 350 c.c.;
Glycerin, 38 c.c.;
Distilled water, to 1000 c.c.

Prepare by dissolving the cupric sulphate and glycerin in 200 c.c. of distilled water with the aid of gentle heat. In another 200 c.c. of distilled water dissolve the potassium hydroxide. Mix the two solutions, and when cooled add the ammonia. Finally with distilled water bring the volume of the whole up to exactly 1000 c.c.

Diazo Solutions.—

Solution I. { Sulphanilic acid, 1 gramme;
Hydrochloric acid (concentrated), 50 c.c.;
Distilled water, ad., 1000 c.c.

Solution II. { Sodium nitrite, 0.5 gramme;
Distilled water, ad., 100 c.c.

The nitrite solution readily oxidizes to nitrate; it is therefore necessary to keep it in a well-stoppered bottle. It should be fairly fresh.

Hypobromite solution, for Doremus ureometer.

Solution 1.	Caustic soda,	100 grammes;
	Distilled water,	250 c.c.
Solution 2.	Bromine.	

Sudan III. Test Solution.—Saturate a certain amount of alcohol with Sudan III. After standing several days, 1 part of this solution is mixed with 1 part of alcohol and 1 part of water. It is turbid at first, but clears on standing.

Lugol's Solution.—

Iodine,	1 gramme;
Potassii iod.,	2 grammes;
Water,	300 c.c.

Silver Nitrate Solution.—Dissolve 5 grammes of silver nitrate in distilled water and dilute to 100 c.c. Keep in dark-colored bottles.

Ferrocyanide of Potassium Solution.—Dissolve 10 grammes of potassium ferrocyanide in water enough to make 100 c.c.

Ferric Chloride Solution.—Dissolve 10 grammes of ferric chloride in enough water to make 100 c.c.

Potassium or Sodium Hydrate Solution.—Dissolve 10 grammes in 100 c.c. of water.

Barium Chloride Solution.—Dissolve 10 grammes of barium chloride in enough water to make 100 c.c.

Nitric acid (HNO_3), sp. gr. 1.42.

Hydrochloric acid (HCl), sp. gr. 1.20.

Sulphuric acid (H_2SO_4), sp. gr. 1.84.

Ammonium hydrate (NH_4OH), sp. gr. 0.96.

Glacial acetic acid ($HC_2H_3O_2$).

Ether.

Chloroform.

Distilled water.

Sodium nitroprusside powder.

Phenylhydrazin hydrochloride.

Sodium acetate.

Sodium chloride.

Value of Urinalysis.—A properly made analysis of urine may furnish much valuable information concerning body metabolism, the diagnosis and prognosis of both renal and other diseases. On the other hand, an enormous amount of valuable time and labor may be wasted by ill-judged resort to quantitative analyses, such as estimations of urea, uric acid, phosphates, sulphates, etc. These estimations are on occasions of value, but as ordinarily made by anyone except an expert are worse than useless. For instance, urea is often estimated for a single day or in a separate portion of urine; the nitrogen intake through the food is not regarded, and the nitrogen passed in the fæces is not taken into consideration in the calculation. No diagnostic, prognostic, or therapeutical deductions can be drawn from such examinations; in fact, they may be very misleading. To be of value, quantitative estimations must be made for a number of days in succession, under known conditions of diet, rest, etc. In most cases information of much greater value can be obtained by the more simple and easily applied qualitative tests and the microscopical examination of the sediment.

Characteristics of Normal Urine.—Recently passed normal urine is clear, with no visible cloud or sediment; turbidity in freshly passed urine in the majority of cases indicates an abnormal condition. However, at certain times during the day, especially two to three hours after a heavy meal, the freshly passed urine in health may be distinctly turbid from the presence of amorphous phosphates. After standing for some time a normal urine develops a light cloud which settles to the bottom. This cloud consists of mucus, containing a few round granular cells, somewhat larger than normal leukocytes, so-called *mucous* corpuscles, and a few pavement epithelial cells derived from the genito-urinary organs.

In normal urine which is allowed to stand for some time at ordinary temperature, and more quickly at a somewhat elevated temperature, certain important changes take place as the result of the development of ammoniacal fermentation. The reaction gradually changes from acid to alkaline; a bulky sediment forms, made up of triple phosphates, earthy phos-

phates, and ammonium urate, in addition to enormous numbers of bacteria. The supernatant liquid is permanently cloudy even after filtration, owing to the presence of numerous bacteria. Freshly voided urine exhibiting such a sediment indicates the presence of germs in the genito-urinary tract. If urine be kept in a cool place, this decomposition may be delayed for days, and instead a deposit of amorphous urates or uric acid may appear.

A recognition of the fact that such marked changes in urine can take place on standing is of great practical importance. It emphasizes the necessity of examining *fresh* samples of urine, especially in warm weather. The tests for albumin and sugar, and the microscopical examination of the sediment in particular, are very unsatisfactory in decomposing urines; not only this, but they may also lead to a wrong diagnosis.

The **amount of urine** varies much in health, depending on many factors, such as the quantity of liquid taken, variety of food ingested, amount of water lost through the skin, bowels, and lungs; age, sex, and season also cause variation in the amount. The limit may be placed at 600 to 1800 c.c.; and yet even these limits may be exceeded in some instances. The average for the adult in the United States, according to Simon, is 1000 to 1200 c.c. for twenty-four hours for men and a trifle less for women. Children pass relatively more than adults. The amount of solid matter in the urine is also higher for men than for women.

In disease the amount may vary from none at all, as in complete suppression (anuria), or a few ounces as in acute Bright's disease (oliguria), to several litres (polyuria), as in diabetes mellitus or insipidus and chronic interstitial nephritis.

The quantity is measured with graduated cylinders.

The **specific gravity of urine** is as variable as the quantity, ranging ordinarily from 1015 to 1025; these limits, however, can be extended considerably in health. It depends upon the quantity of solids in solution. In disease the specific gravity may be high with a large volume, as in diabetes mellitus, or low with a small volume, as in many chronic wasting diseases

and in the later stages of acute disease, indicating defective elimination of solids.

In estimating the specific gravity, the Squibb urinometer is used for routine work. For very accurate estimations the Lohnstein urinometer is best, especially in making the fermentation test for sugar. The following precautions should be observed: the urinometer should not adhere to the side of the cylinder; all foam should be removed from the surface of the urine with filter-paper; the reading should be taken at the lower meniscus. If great accuracy is essential, the temperature of the urine must be taken into consideration, since the specific gravity increases or decreases 1 degree for every 3 degrees Centigrade above or below 15° C., the temperature at which the instrument is standardized. For most clinical work the temperature is disregarded provided the urine be at the room temperature.

Reaction of Urine.—Normally the reaction of the twenty-four hours' urine, and also of separate portions, is acid; but in many healthy people, at different periods of the day, it is faintly acid, neutral, or even faintly alkaline, especially after hearty meals. The urine of those living on strictly vegetable diet will usually be very faintly acid or alkaline. The acidity is due to diacid phosphate, chiefly of sodium, while alkalinity is due to the monoacid or primary phosphates and to carbonates of sodium and potassium.

Litmus-paper is used to test the reaction; acid urine turns blue litmus red, alkaline urine turns red litmus blue. Neutral urine does not change the color of either.

Color.—This varies from a very light yellow or even watery, to a very dark brownish yellow. The depth of color is proportional to the amount of water present, and thus to the dilution of urinary pigments.

Pathologically the color of urine varies a great deal. It may be the color of water in the polyuria of nervous conditions and chronic interstitial nephritis. In febrile conditions it is usually very highly colored. The red tints of urine are usually due to the presence of blood; the dark-brown tints may be due to methæmoglobin. The urine may be almost

black in the presence of melanotic cancer, especially after standing for some time. Bile gives a brownish or greenish-brown color. Blue urine may be present in cholera and typhus, owing to the presence of indigo. Certain drugs alter the color of urine. Turbidity in urine may be due to pus, urates, phosphates, bacteria, and epithelium.

Acid urines, as a rule, are darker in color than alkaline.

The **odor of normal urine** is characteristic; after eating certain substances or administration of certain drugs more or less peculiar odors are imparted. If ammoniacal fermentation has taken place, the urine smells of ammonia; if the urine is decomposed, a strong, putrid, ammoniacal odor is noticed. Acetone is easily recognized if present in any quantity by a fruity odor. Rarely, however, is the odor of clinical value, though it may give a clue.

Constituents of Normal Urine.—Normal urine is a watery solution of the waste products of metabolism and the decomposition products of excess of ingested food. It is evident that the composition will vary from hour to hour during the day, being a very dilute solution after eating or drinking, and very concentrated in the early morning hours. The total amount of urine passed by an individual in one day will, however, be very nearly the same in composition as that of another day, other things being equal. The constituents of urine may be divided into two groups, **organic** and **inorganic**, or mineral. Of these, the most important are:

Organic.—Urea, uric acid, and alloxuric or xanthin bases; creatinin; hippuric acid; indoxyl, phenol, and cresol as conjugate sulphates; other sulphur compounds (neutral, or unoxidized sulphur); pigments and chromogens.

Inorganic.—Chlorides, sulphates, phosphates of sodium, potassium, calcium, magnesium, and ammonium. Oxalates and carbonates may be present also.

CONSIDERATION OF THE IMPORTANT NORMAL CONSTITUENTS OF THE URINE, VARIATIONS IN THE QUANTITY OF WHICH MAY INDICATE DISEASE.

Water.—Upon this constituent depend the concentration and dilution, and consequently the quantity of urine excreted. It varies much in amount, depending upon many factors such as are mentioned under *Amount.*

Solids.—The total daily amount of solids excreted in the urine is more constant than that of the water. If estimated for a number days in succession under known conditions, it may be of clinical value. One estimation is a waste of time.

Composition of Normal Urine (Bliss):

Organic matter, about 35 grammes.

Urea, about 30 grammes.

The remaining organic matter consists principally of uric acid, hippuric acid, creatinin, extractives, and coloring-matter.

Mineral matter, about 25 grammes. Half to two-thirds of this consists of sodium chloride; the remainder is made up of sulphates, chlorides, and phosphates of the alkalies and alkaline earths.

Urea, $CO\langle^{NH_2}_{NH_2}$, is the most important nitrogenous constituent of the urine. It is of clinical significance, since it to a large extent represents the nitrogenous katabolism of the body. Under normal conditions it represents about 85 per cent. of the total nitrogen eliminated by the kidneys. The normal daily amount of urea excreted in the urine varies from 25 to 35 grammes. The liver is held to be the seat of the formation of urea.

Simon says: "It might be supposed that an accurate idea of tissue destruction could be formed from a quantitative estimation of the urea. This is not the case, since, in pathological conditions especially, the quantitative relations existing between the excretion of urea and the remaining nitrogenous constituents is subject to wide variations. The urea may disappear entirely from the urine, the nitrogen being eliminated in the form of other compounds. So it is

necessary to determine the total nitrogen excreted by the kidneys when one wishes to gain an accurate insight into the nitrogenous metabolism.

The elimination of urea, and of nitrogen in general, is subject to great variations, depending on the amount of nitrogen ingested, and that resulting from tissue destruction, which in turn is largely influenced by the body weight. The elimination of nitrogen should always be compared with the amount of food ingested. A variable amount (in disease it may be considerable) of nitrogen is eliminated in the fæces; consequently this factor must be reckoned with if accurate conclusions are to be drawn.

The tediousness and the difficulty of such estimations render them entirely impractical. Approximate results can be obtained by parallel estimation of chlorides. In health the amount of the chlorides is equal to about one-half that of the urea. Whenever the amount of urea is greatly in excess of the normal amount of chlorides, an increased tissue destruction may be inferred, and *vice versâ*.

Urea is increased in amount in fevers, diabetes, excessive bodily exercise, and with a meat diet. An important clinical point is the fact that its total excretion is diminished in uræmia, kidney diseases with impaired excretory power, in liver diseases such as cirrhosis, in which the urea-forming function of the organ is impaired. Little is definitely known about the relation of urea, uric acid, etc., to gout and lithæmia. In certain diseases of the kidney the amount of urea formed may be decreased, but the kidneys are unable to excrete all; and in certain liver diseases, while the amount of urea formed is diminished, the nitrogenous substances which go to urea formation are eliminated and the total nitrogen excreted remains the same.

Characteristics of Urea.—Owing to its ready solubility, it appears in the urine in solution. In concentrated solution it is precipitated in the form of crystals of nitrate of urea on the addition of nitric acid. This is shown in the application of Heller's test, urea nitrate crystals forming just above the junction of the two liquids.

Urea is converted into ammonium carbonate by certain bacteria ($CON_2H_4 + 2H_2O = (NH_4)_2CO_3$). This is the commonly observed ammoniacal decomposition which is seen whenever urine stands for a length of time at ordinary temperature, and in pathological conditions inside the bladder, as in cystitis, etc.

Xanthin bases enter into the formation of the nucleins, the essential chemical constituents of the nuclei of body-cells. Those in the urine are derived from food and nuclear katabolism. They have practically the same origin, significance and fluctuations as uric acid. The quantity is ordinarily about one-tenth that of uric acid. Clinically they have the same significance.

Uric acid is an oxidation product of the xanthin bases, and the chief form in which they are excreted. It represents the katabolic breaking down of the cell-nuclei.

The amount of uric acid excreted daily in the urine varies from 0.4 to 0.8 gramme, having a proportion to the amount of urea excreted of 1 : 40 to 1 : 60. Physiologically it is increased by the ingestion of food rich in cells, such as liver, kidney, etc.; pathologically, by increased breaking down of tissues.

Uric acid is increased in conditions attended with leukocytosis, such as leukæmia (may be 5 grammes in twenty-four hours), pneumonia, etc.; and in febrile conditions, acute rheumatism, and in so-called uric acid diathesis. It is diminished in leukopænic and anæmic conditions, and especially in kidney diseases associated with uræmia.

Characteristics.—Uric acid has a strong tendency to crystallize upon contact with any solid substance. This fact explains the large percentage of uric acid calculi. The deposit of uric acid is pathological if it occurs in urine passed within four to six hours. Normal urine deposits uric acid after standing ten hours or more. If uric acid is deposited soon after being voided, it suggests the possibility of its being deposited in the urinary tract, with the formation of gravel and uric acid calculi.

The chief form in which uric acid occurs in the urine is

as the urates of sodium, potassium, calcium, and magnesium. High acidity forces the uric acid from these combinations. They are more soluble than uric acid, and more so in warm than in cold liquid. The mixed urate deposit is a reddish, granular-looking sediment, the so-called "brick-dust deposit." It may vary from a pink to a brick-red. This deposit dissolves on heating the urine. It gives the murexid reaction, similar to uric acid. It dissolves in solutions of the caustic alkalies, and is decomposed by mineral acids with precipitation of uric acid crystals.

Hippuric acid, benzoic acid, and creatinin are found in very small amounts in normal urine. Time spent in their examination is wasted from the clinical standpoint.

Ammonium compounds, similar to the corresponding sodium and potassium salts, are normally excreted in small amounts.

Total nitrogen in the urine is represented by the above substances. It gives a more exact idea of nitrogenous metabolism than urea alone.

Sulphates.—The sulphur of the urine is excreted chiefly in the form of mineral sulphates and the conjugate or ethereal sulphates. They resemble urea in being produced chiefly by the decomposition of albuminous material, either taken in with the food or that broken down in katabolic processes. The quantity varies from 1.5 to 3 grammes daily, calculated as SO_3, fluctuating parallel to urea.

Mineral sulphates make up the bulk (nine-tenths) of the urinary sulphates. Sodium sulphate is most abundant, with a small proportion of potassium, ammonium, and perhaps calcium and magnesium sulphates. These salts all appear in solution. Calcium sulphate very rarely appears in the sediments.

Conjugate Sulphates.—Indol, phenol, and similar substances generated in the course of albuminous decomposition become oxidized and combine with potassium or sodium sulphate to form the conjugate or ethereal sulphates. They make up about one-tenth of the excreted sulphates. They are formed in the intestine, absorbed, and then excreted by

the kidneys, chiefly as indoxyl potassium sulphate—*i. e.* (indican), phenol potassium sulphate, potassium sulphates of cresol and traces of catechol, and skatoxyl.

Indican is the most conspicuous, and may be taken as representative of this class. Increase of indican indicates an increase of the total ethereal sulphates, and thus an increase in bacterial putrefaction in the intestine.

Unoxidized Sulphur Compounds.—Only small amounts of sulphur other than sulphates occur in the urine normally. Pathologically cystin and other substances are found; hydrogen sulphide may be absorbed from the intestine in putrefactive processes or from foul abscesses, and be excreted by the kidneys. It is usually associated with indican.

Sulphates are increased by meat diet, excessive exercise, acute fevers, especially acute rheumatism and meningitis, leukæmia, etc.

Conjugate sulphates are small or large, depending on the extent of intestinal decomposition. They are increased in the various gastric and intestinal diseases (in practice this fact is chiefly noted by the test for indican).

Phosphates.—The quantity excreted varies between 2.5 and 3 grammes, depending largely on the amount ingested, increasing with animal, decreasing with vegetable diet. A certain amount is derived from the katabolism of body-tissue, muscle-cell, bone, nerve-cell, blood-corpuscles. Some tissues (nerve) contain more phosphorus than others. The normal proportion between the excretion of phosphoric acid and nitrogen is 1 : 7.

In urine phosphates occur as sodium, potassium, calcium, and magnesium salts of the tribasic acid, H_3PO_4. Most important of these is diacid sodium phosphate, NaH_2PO_4, to which the acidity of the urine is principally due. The phosphates vary with the reaction of the urine. In acid urine there are diacid sodium phosphate and diacid calcium phosphate; in amphoteric urine, besides these are found disodium phosphate, monocalcium phosphate, and monomagnesium phosphate; in alkaline urines, trisodic phosphate, neutral calcium phosphate, and neutral magnesium phosphate may be

present. The alkaline phosphates exceed the earthy phosphates by about one-third. Sodium is combined with more of the phosphoric acid than potassium. Alkaline phosphates of sodium and a small amount of potassium are freely soluble in fluids of any reaction, and only appear in the urine in solution. The normal excretion amounts to 1.5 to 3 grammes daily, calculated as P_2O_5.

Earthy Phosphates are those of calcium and magnesium. They are practically insoluble in pure water, very readily soluble in acid, insoluble in alkaline fluids. In alkaline urine they occur as a white precipitate. Earthy phosphates may be precipitated by heating urine, but the urine clears up on adding a drop or so of acid.

Phosphates are decreased in most acute fevers, the degree corresponding to the severity of the disease. This is due to failure of excretion. They are also decreased in acute and chronic nephritis, anæmia, hysteria, chronic lead-poisoning, acute yellow atrophy of the liver, cirrhosis of the liver, etc. An *increase* occurs in the so-called "phosphatic diabetes," some nervous diseases, some bone diseases, starvation, and convalescence.

Triple phosphate, ammoniomagnesium phosphate, indicates ammoniacal fermentation, occurring either within the bladder (fresh urine) or outside the bladder.

Chlorides.—These are excreted almost entirely as sodium chloride, with a small amount of potassium, ammonium, calcium, and magnesium chlorides. From 10 to 16 grammes are excreted in twenty-four hours. They are derived from the food (surplus over body need), not from body metabolism, and thus vary in health with the habits of the individual. Their estimation is at times of clinical importance. They are diminished in most acute fevers (the degree depending on the severity of the disease), excessive secretion of gastric juice, exudations and transudations, these withdrawing the chlorides that would otherwise appear in the urine. They are increased during the absorption of exudates and transudates, in convalescence, and in polyuria.

Oxalic acid is present in normal urine in very small

amounts. It is derived chiefly from vegetable foods. It may be derived in part from uric acid through a process of oxidation, or from carbohydrates from incomplete oxidation. The *quantity* is small, varying from a faint trace to 20 milligrammes in twenty-four hours. The recognition of calcium oxalate crystals in the sediment is ordinarily taken as an index of the excretion, but is misleading, since with a quantitative diminution in the excretion there may be numerous crystals of calcium oxalate, and *vice versâ.*

Oxalic acid is increased in gastro-intestinal disturbances, diabetes, and in some cases of albuminuria, and in the so-called oxalic acid diathesis. This fact is determined in practice chiefly by recognition of calcium oxalate crystals in the sediment, but the method is untrustworthy. The frequent presence of calcium oxalate crystals in the urine of an individual should lead to suspicion that it may be deposited within the bladder and thus cause the formation of calculus.

Pathological Substances.—As a result of disease of the genito-urinary system, or of local or general disease in other parts of the body, or of altered metabolism, any of the above-mentioned constituents of normal urine may be either increased or diminished. More important than this, however, is the occurrence of *additional* substances. These are as follows:

1. *Proteids:* serum-albumin, serum-globulin, and albumose. Of very rare occurrence are nucleo-albumin, fibrin, and mucin.

2. *Carbohydrates:* glucose; less frequently lævulose, lactose, sucrose, maltose. Very rarely are pentoses, glycogen, dextrin, animal gum, and inosite found. Normal urine contains a trace of glucose, barely enough to respond to the most delicate tests, and not enough to interfere with any clinical tests.

3. Acetone, aceto-acetic or diacetic acid, β-oxybutyric acid.

4. Diazo substances.

5. Ammonia.

6. Bile, bilirubin, bile salts.

7. Blood and its constituents: albumin, blood-corpuscles (red and white), hæmoglobin and its derivatives.

8. Pus-cells—when few in number called leukocytes.
9. Casts; cylindroids.
10. Spermatozoa.
11. Epithelium.
12. Tissue débris, floating particles, etc.
13. Clap-threads, mucous threads.
14. Crystals of various normal and abnormal substances, amorphous débris, and calculi.
15. Parasites, vegetable and animal.

Less important constituents are fat, volatile and nonvolatile fatty acids, lactic acid, alcohol; glycuronates, glycerin-phosphoric acid, alkapton; cystin, xanthin, cholesterin, leucin, tyrosin, ferments, and toxins.

Albumins.—Of the several albumins mentioned on another page, *serum-albumin* is of the greatest clinical importance. When speaking of urinary albumin this variety is always indicated.

The presence of albumin in the urine must always be considered *abnormal*, though the clinician must keep in mind that it may be transient and unimportant, as in the so-called "physiological albuminuria." Even in this variety there is probably disturbance in the nutrition of the epithelium of the capillaries of the tufts, or of the cells surrounding the glomerulus.

Albumin is present in the urine in the following conditions, varying greatly in amount:

(*a*) Functional albuminuria; (*b*) febrile albuminuria; (*c*) hæmic changes—*i. e.*, purpura, scurvy, chronic poisoning by lead or mercury, syphilis, leukæmia, severe anæmia, bile and sugar in the blood; (*d*) certain nervous diseases (neurotic albuminuria), after epileptic attacks, apoplexy, tetanus, blow on the head, etc.; (*e*) congestion of the kidney, active or passive; (*f*) organic diseases of the kidney—acute and chronic, Bright's disease, amyloid and fatty degeneration, suppurative nephritis, and tumors; (*g*) all affections of the pelvis, ureter, bladder, and urethra associated with the formation of pus; (*h*) hæmorrhage along the urinary tract.

Serum-globulin is always found together with serum-albu-

min; in amyloid degeneration the proportion is unusually high. It responds to the same tests and has the same significance.

Albumose (*Peptone*).—Traces of albumose are found in many acute diseases, and in chronic suppuration. Albumose often accompanies serum-albumin. Urine containing albumin may after decomposition fail to respond to the tests; in such cases positive tests for albumose may be obtained.

Hæmoglobin occurs in the urine (hæmoglobinuria) whenever there is such a destruction of red blood-corpuscles that the liver can not transform into bilirubin all the blood-containing matter set free, as in poisoning by potassium chlorate, in extensive burns, certain infectious diseases, malaria, hæmoglobinuria of the newborn, etc. Whenever blood occurs in urine, hæmoglobin is present within the corpuscles. After these disintegrate it will be in solution in the urine, although likely in modified form.

Nucleo-albumin is inconstant in disease of the kidneys, but has been found frequently in cases of acute nephritis, and sometimes in febrile and functional albuminuria. It may be still present when serum-albumin and serum-globulin can no longer be demonstrated.

Mixed albuminuria, in which several of the above substances are present at the same time, is not very infrequent.

Carbohydrates.—Glucose (dextrose, grape-sugar), $C_6H_{12}O_6$, is the only important member of the group, from the clinical standpoint. Its presence, when persistent, indicates diabetes mellitus. It is frequently present in small amounts for short periods, as in febrile affections, alimentary disturbances, and dietetic errors, and is then not of serious import.

The specific gravity of the urine is increased even higher, up to 1040, or in proportion to the amount of sugar present in spite of the large amount of water. But it is sometimes present even with low specific gravity if the amount of water is very large or the other constituents small. Other carbohydrates may appear along with glucose. Sugar may be present up to 500 grammes or even more in twenty-four hours.

During the late stages of pregnancy and during lactation, lactose in very small amount is frequently present in urine, but is not likely to respond to the ordinary sugar-tests.

Clinically the remaining carbohydrates are of very minor importance; the reader interested in their chemistry is referred to larger text-books.

Acetone; Diacetic Acid; β-oxybutyric Acid.—These three substances are closely related, and are of great pathological importance, as their presence in diabetic urine indicates the possible approach of diabetic coma. Acetone appears in very small quantities in normal urine, the amount being so small, however, that special procedure is necessary to demonstrate it; diacetic and oxybutyric acids never occur normally. Acetone is most abundant when little albuminous food and no carbohydrates are eaten. It is derived from proteid material. Diacetic acid has practically the same significance as acetone, and is met with in diabetes, digestive disturbances, and fevers. It is now generally believed that β-oxybutyric acid is the cause of diabetic coma. The amount of this substance excreted in twenty-four hours may be enormous. Sülz found in 3 cases, 65, 100, and 226 grammes respectively. Acetone and diacetic acid are derivative products of β-oxybutyric acid.

Diazo Substances.—Of great practical importance is the detection in the urine of substances which give the Ehrlich diazo reaction. This test is of great clinical value in the diagnosis and prognosis of typhoid fever; likewise in prognosis in pulmonary tuberculosis, since a persistent reaction indicates a severe case. It occurs sometimes in acute tuberculosis, measles, smallpox, and scarlet fever, but seldom in other diseases. It occurs in a large per cent. of all typhoid cases, but almost never in the other diseases for which typhoid may be mistaken (with the exception of acute miliary tuberculosis). It may appear as early as the fifth day, and its disappearance usually indicates subsidence of the disease. A reappearance suggests a relapse.

Bile appears in the urine in jaundice, both of the hæma-

togenous and hepatogenous varieties. Bilirubin and bile salts are present, the former alone being of importance.

Blood in the urine (hæmaturia) results from hæmorrhage at some point along the genito-urinary tract. Examination of the urine during the menstrual period is very likely to show blood and its various constituents unless the patient is catheterized. Whenever blood-corpuscles are found in urine, the other constituents may be assumed to be present. A faint albumin reaction should therefore be ascribed to this cause rather than to a kidney lesion. Hæmatin, hæmatoidin, and hæmatoporphyrin are rarely present in the urine.

Pus is found in the urine in many conditions involving the genito-urinary tract, such as pyelitis, pyelonephritis, renal and vesical stone, genito-urinary tuberculosis, cystitis, urethritis, rupture of an abscess into the urinary passages, leucorrhœa, etc. A small amount of albumin accompanies pus, fat, and lymph.

Leukocytes are often present in small numbers in normal urine. In catarrhal and inflammatory conditions they are more numerous. They may be derived from the vaginal discharges. They are mostly polynuclear, and become altered and disintegrated by ammoniacal urine.

Casts of the uriniferous tubules are of remarkable diagnostic importance, nearly always indicating disease of the kidneys, though a few hyaline casts in middle life are of no great significance. They are cylindrical bodies, 20 to 50 μ in diameter, having the shape and size of small sections of the uriniferous tubules; they are unbranched, usually with one or both ends rounded, though one end may be broken off sharply. Various substances enter into their composition, giving the following classification: (*a*) hyaline, (*b*) epithelial, (*c*) granular, (*d*) fatty, (*e*) waxy, (*f*) leukocyte, (*g*) blood, (*h*) pseudocasts made up of bacteria or urates. They may be of considerable aid in the prognosis and the diagnosis of the variety of Bright's disease. Hyaline and granular casts are common to all varieties. Blood and epithelial, and especially leukocyte casts are most commonly seen in acute Bright's disease. Dock believes that the

presence of enormous numbers of dark, coarsely granular casts is a bad prognostic sign.

Cylindroids are hyaline bodies, longer and more slender than casts, and tapering toward a wavy point, giving them a whip-like appearance, and at times showing a coating of granules. They are of very common occurrence, and at most indicate only a slight degree of renal irritation. It is believed that they are formed in the uriniferous tubules.

Spermatozoa are found in the urine after sexual intercourse, masturbation, or emissions, in spermatorrhœa, and sometimes following epileptic convulsions. They may be found in the urine of women after intercourse.

Epithelium.—Very little concerning their site of origin can be learned from the epithelium, since the same kind lines the renal pelvis, ureters, and bladder. In normal urine varying numbers of epithelial cells appear, shed from the mucous membrane of the urinary tract. The urine from the female nearly always contains numerous cells, many of them coming from the vagina. The cells may appear singly or in patches. The urine is often distinctly cloudy.

Squamous cells—i. e., pavement cells—are shed from the most superficial layers of the renal pelvis, ureters, bladder, and vagina.

Spherical cells are much smaller than the above. They resemble a leukocyte somewhat, but are larger and have a single round nucleus. Some of them may originate from the renal tubules, but most of them are derived from the deeper layers of the renal sinus, ureters, and bladder. In large numbers they may indicate a catarrhal condition.

Elongated caudate cells of varying shapes may appear in the urine. Their origin and significance are the same as those of the *spherical* cells.

Tissue débris when formed in the urine may afford valuable information in regard to new growths.

Clap-threads are found floating in recently passed urine, even many years after a supposed cure of the gonorrhœa.

Mucous threads are frequently found in the urine in catar-

rhal conditions, especially of the prostate, and may be entirely independent of a gonorrhœa.

Crystals are of extremely common occurrence in normal, and especially in abnormal, urine. They are not, however, of much pathological significance, except as they indicate ammoniacal decomposition and excess of uric acid, when they suggest the possible presence of calculi. They will be considered in detail under microscopical examination of urine.

Amorphous débris is usually present in all urine, especially after standing. It is derived from broken-down cellular material or insoluble salts.

Calculi.—Uric acid and urates, oxalate of lime, and earthy phosphates are the most common constituents of urinary calculi. Very rarely cystin, xanthin, carbonate of lime, indigo, and urostealith form calculi. Renal sand, uric acid, or urates may be passed in the urine.

Parasites.—Vegetable and animal parasites are at times found in the urine.

Vegetable parasites are much the most common, and consist of bacteria and rarely of fungi.

In normal urine, freshly passed, bacteria are very seldom found except in small numbers. After being voided, they develop, as a result of contamination from outside, in enormous numbers, causing ammoniacal fermentation.

Pathogenic bacteria are derived mostly from local infections in the course of the urinary tract. Of great importance are gonococci in gonorrhœa, tubercle bacilli in genito-urinary tuberculosis, streptococci, staphylococci, colon bacilli, and other germs associated with the causation of cystitis and pyelitis.

In some of the acute infectious diseases, such as typhoid fever (in particular), scarlet fever, croupous pneumonia, erysipelas, the causative germ is excreted in the urine.

Sometimes enormous numbers of bacteria are voided in the urine without demonstrable cause (idiopathic bacteriuria).

Fungi.—If found in the urine, they are nearly always due to contamination after the urine is voided. Saccharomycetes, or yeast-fungi, may be found in old urine in diabetes mellitus.

In the United States **animal parasites** are almost never found in urine. Trichomonas, protozoa, and amœba rarely appear. Trichomonas vaginalis is the most common.

Worms are rarely found. Blood-moulds resembling earth worms are occasionally reported as examples of Eustrongylus gigas. In Egypt Schistosoma hæmatobium is common. Larval filaria and echinococcus may be found in the urine. Intestinal parasites are almost never found in the urine.

Lymph occurs in the urine very rarely except in connection with filariasis, when there is rupture of lymph-vessels into the urinary tract (lymphuria, chyluria).

Fat does not occur in the normal urine. When it occurs in large amounts, recognizable with the naked eye, it is termed *lipuria*. Small amounts, recognized with the microscope, occur whenever there is fatty degeneration of the renal epithelium, pus-corpuscles, or tumor particles in the urinary tract. In chyluria, a tropical affection usually associated with filariasis, there is a large amount of fat in the urine, due to leakage of chyle into some part of the urinary tract.

Toxins are excreted in the urine in health and disease, but very little is known about them.

Foreign Matter.—Vegetable fibres, such as threads from towels, starch-granules from toilet powders used on the genitals, oily globules, and various forms of dirt from unclean receptacles, find their way into urine, and give the beginner trouble in microscopical examinations.

QUESTIONS.

What are the characteristics of normal urine?
Upon what factors does the specific gravity of urine depend?
What is the reaction of normal urine and upon what does it depend?
What are the various colors of urine in disease? Upon what factors does the color depend?
Mention the constituents of normal urine.
Under what conditions is a quantitative estimation of urea of value?
What is uric acid?
In what condition is uric acid increased? In what decreased?
In what forms is the sulphur of the urine excreted?
Of what significance is *indican?*
Name the different phosphates of the urine.

In what conditions are the chlorides diminished and increased?

Of what significance is the frequent presence of calcium oxalate crystals in freshly voided urine?

Mention the pathological substances found in the urine.

In what conditions is albumin found in the urine?

Of what significance is the presence of acetone, diacetic acid, and β-oxybutyric acid?

In what diseases are diazo substances found in the urine?

Under what conditions are blood and pus found in the urine?

What are casts? Of what significance are they?

What are cylindroids, and what is their significance?

Mention the various cells found in urine.

Name the pathological bacteria found in urine.

In what conditions are lymph and fat found in the urine?

CHAPTER XIII.

EXAMINATION OF THE URINE.

It is self-evident that it is impossible for the physician to make complete urinary examinations according to text-book directions. Not only that, but it would be a great waste of time in the majority of cases, so far as useful, accurate clinical knowledge is concerned. This statement applies especially to the quantitative analyses of the various solids, such as urea, uric acid, chlorides, sulphates, phosphates, etc. In the vast majority of cases such time-consuming analyses have thrown very little practical light on the diagnosis and treatment of cases which could not have been more easily and satisfactorily gained by other methods of examination.

On the other hand, too much emphasis can not be laid upon the necessity of a routine examination for the chief abnormal constituents in every important case.

As an example of a very sensible and useful guide in urine examination the following scheme, used by Professor Dock in his clinic, is *appended.* In it the essential methods for clinical diagnosis are considered. They may be modified in private practice as the physician sees fit.

Scheme for Recording Urinary Examinations.—A complete examination of a fresh sample, catheterized if necessary, should be made as soon as possible after the patient is seen; also of the first twenty-four hours' urine.

In this examination note the following points:

Physical and Chemical Examination.—*Name, Date, Quantity, Specific gravity, Reaction, Color, Odor, General Appearance* (clear or turbid; floating particles, clap-threads).

Albumin.—Boiling and nitric acid; ferrocyanide and acetic acid, Heller test if in doubt. Test for other albuminous bodies if indicated.

Sugar.—Fehling. If positive, make fermentation test.

Acetone.—Diacetic acid, β-oxybutyric acid.

Bile.—Foam, Gmelin's test.

Indican.—Amount.

Microscopical Examination.—*Centrifuge* a specimen for sediment; note amount and appearance. Record the following:

Crystals.—Kind and amount.

Casts.—Full description.

Blood-corpuscles.—Condition.

Leukocytes.—Kind.

Pus, spermatozoa, epithelium, bacteria, protozoa, fat-globules or crystals.

Test for such other substances as may be suspected or indicated.

In all cases of *pyuria* stain for tubercle bacilli and gonococci.

In all cases of *fever* and *new growths* make the *diazo test.*

In *diabetes* keep careful note of acetone, diacetic acid, and β-oxybutyric acid.

In *albuminuria* examine day and night urine separately.

If the first examination is negative, note daily the quantity, specific gravity, and reaction, and make a careful examination at once if any alteration is noted. Make a careful examination once a week, anyway.

If the first examination is positive, repeat daily the complete examinations, with full notes, especially of any variation.

Determination of Total Solids.—(1) **Specific Gravity Method.**—From the specific gravity the total amount of solid material in the urine can be calculated with sufficient accuracy for all practical purposes. Assuming the volume to be constant, the increase or decrease in the specific gravity depends entirely upon the increase or decrease in solids excreted ; the substances which will cause these variations are, of course, those which are given off in fairly large amounts—that is, the urea, and chlorides, sulphates, and phosphates ; and in pathological urine, sugar and albumin. An increase in uric acid, for instance, can not be great enough to affect the specific gravity noticeably.

It has been found by experiment that if the last two figures of the specific gravity at 15° C.—that is, the figures in the second and third decimal places—be multiplied by 2.33 (Trapp's or Haeser's coefficient), the product will represent approximately the amount of solid matter in 1 litre of urine. If this figure is multiplied by the number of litres in the twenty-four hours' urine, the total amount of solid matter excreted in twenty-four hours is obtained. Example :

24 hours, urine	= 2000 c.c.
Sp. gr.,	= 1.020.
20×2.33	= 46.6.
46.6×2	= 93.2 grammes.

Results by this method agree very closely with those obtained by the long and tedious chemical methods. This method can not be used with diabetic urine.

It is evident that such a procedure as this would not give a correct result unless the total twenty-four hours' urine were collected, measured, and the specific gravity taken, since the urine varies greatly in its composition at different times during the day. In the morning, upon rising, it has a high specific gravity, while after meals or after drinking much liquid the gravity will be very low. Absurd results will be obtained from the estimation of solids based upon separate portions.

(2) **By Evaporation and Weighing.**—5 c.c. of urine, accurately measured, are placed in a watch-glass containing a little

dry sand (sand and crystal having been previously weighed): this is placed over a dish containing concentrated sulphuric acid, and under the receiver of an air-pump which has been made perfectly air-tight with vaselin. The receiver is exhausted; at the end of twenty-four hours it is exhausted again, and the urine allowed to remain another twenty-four hours; at the end of this time the crystal is weighed, the difference between the two weights obtained indicating the amount of solids in 5 c.c. of urine, from which the percentage and total amount are readily calculated.

Urea.—The quantitative estimation of urea is based upon the decomposition of urea into carbon dioxide and nitrogen by means of sodium hypobromite:

$$CON_2H_4 + 3NaOBr = 3NaBr + CO_2 + 2H_2O + 2N.$$

The carbon dioxide formed is absorbed by an excess of the sodium hydrate added to the hypobromite solution, while the nitrogen is set free and can be measured.

Doremus Ureometer (Fig. 56).—A small amount of urine is poured into the smaller tube *B*, while the stopcock *C* is closed. This is then opened for a moment to allow its lumen to become filled, then closed. Tube *A* is now washed out with water and filled with solution of caustic soda. By means of a curved 1 c.c. nipple pipette add 1 c.c. of bromine, and a sufficient amount of water to fill the bend of the tube. (By this means a fresh solution of sodium hypobromite is formed.) Now allow 1 c.c. of urine (less if concentrated) to flow into the longer tube and mix with the hypobromite solution, when brisk effervescence occurs, and the nitrogen rises to the top of the tube. After a short time the volume of gas present is read from the scale.

The degrees marked upon the tube show the number of grammes or grains of urea contained in the amount of urine employed. This being 1 c.c., the total amount of urea is obtained by simply multiplying the reading of the instrument by the volume of the urine in cubic centimetres.

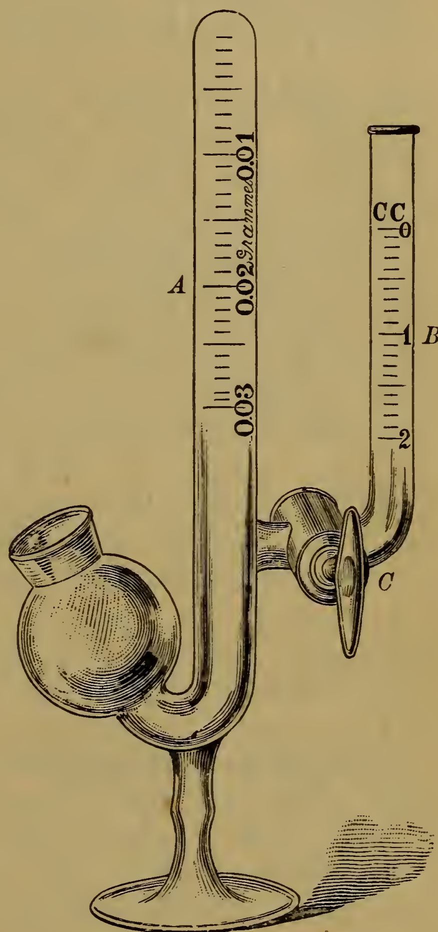
This method gives fairly accurate results provided proper care is exercised. About a quarter of an hour should be

allowed for the gas to collect; after that time the gas which still continues to be given off will not affect the reading much. Since gas expands with rise in temperature, the instrument should not be held in the hand or placed in a warm place.

Uric Acid.—Qualitative Test. — *Murexid.* — A small portion of the sediment or of suspected particles is treated with a few drops of nitric acid in a porcelain dish; this is then carefully evaporated to dryness, best on a water-bath; a yellowish spot remains if uric acid or xanthin is present. On exposing this spot to the vapor of ammonia it will assume a purplish color. This is due to the formation of ammonium purpurate (murexid). If it is now moistened with a drop of potassium hydroxide, it will become violet if due to uric acid.

For the quantitative estimation of uric acid, the reader is referred to the larger text-books. The Folin modification of the Hopkins' method is recommended as being the simplest and the most practicable.

Fig. 56.



Doremus ureometer.

Chlorides.—Qualitative Test.—Remove albumin from a few c.c. of urine (see p. 198). Acidify with several drops of nitric acid, and add a few drops of a 1 : 20 solution of silver nitrate. In the presence of chlorides a white precipitate of insoluble silver chloride forms ($AgNO_3 + NaCl = AgCl + NaNO_3$). A general idea of the

quantity present may also be gained, a heavy caseous precipitate pointing to a large amount.

For accurate quantitative tests, which the clinician will find of little value, the reader is referred to the larger text-books.

Sulphates. — (1) **Preformed Sulphates.**—*Test.* — Strongly acidify a few cubic centimetres of urine with acetic acid and treat with a few drops of barium chloride. A cloud or white precipitate of barium sulphate forms and indicates their presence. Conjugate sulphates give no precipitate, it being necessary first to split these into their component parts; this is done by boiling with a mineral acid.

(2) **Conjugate Sulphates.**—Filter the precipitate obtained above, after allowing it to stand for some time in order to have complete precipitation of the simple sulphates; add several drops of hydrochloric acid and boil for some minutes, with the addition of a few drops more of barium chloride if necessary. Any precipitate or cloudiness now is caused by conjugate sulphates. It is well to test the filtrate with a little barium chloride before adding the hydrochloric acid, in order to be sure the simple sulphates were all removed.

For quantitative analysis consult the larger text-books. The method used is exactly the same as the foregoing qualitative test, carried out in a quantitative manner.

For clinical purposes the indican test is taken as an index of the quantity of conjugate sulphates present.

Phosphates.—**Qualitative Test.**—Ferric chloride precipitates phosphoric acid as ferric phosphate, which is insoluble in acetic acid. Acidify a few cubic centimetres of urine with a few drops of acetic acid, then add a few drops of 10 per cent. ferric chloride solution. A yellowish-white precipitate occurs in the presence of phosphates.

Test for Earthy and Alkaline Phosphates.—Render 10 c.c. of urine alkaline with ammonia; a flocculent precipitate is due to the earthy phosphates. After precipitating the earthy phosphates as above, filter, acidify the filtrate with acetic acid, and test with ferric chloride as described above.

For quantitative analysis, see larger text-books.

Under certain conditions the earthy phosphates are precipitated from the urine on heating, and may at first sight be mistaken for albumin. On the addition of a drop of nitric acid they are dissolved and the urine clears.

The xanthin bases, hippuric acid, creatinin, oxalic acid, benzoic acid, leucin, and tyrosin are of practically no clinical importance, so methods for their detection and estimation are intentionally omitted.

Tests for Albumin.—The urine should be perfectly clear in order to test for small amounts of albumin. Turbidity from phosphates can be removed by adding a few drops of nitric acid; from urates by heating. If the turbidity is due to the presence of bacteria or very fine particles, it can not be removed by ordinary filtration through filter-paper, which serves the purpose in most cases. Under these circumstances the addition of insoluble substances, like chalk, magnesia, etc., to precipitate the phosphates may clarify the urine, which may then be filtered. The use of the Chamberland filter will completely clarify the urine.

Tests for albumin are far too numerous; they vary much in delicacy, some being too delicate for practical purposes, others not delicate enough. Only a few of those which are most useful will be mentioned.

Heat and Nitric Acid.—If more than a trace of albumin is present, this is an extremely useful test, both qualitatively and quantitatively.

A test-tube partially filled with perfectly clear urine is heated to boiling over a flame. A cloudiness may result, due to albumin or earthy phosphates. A little nitric acid is then added; if the cloudiness is due to the phosphates, the urine immediately becomes perfectly clear, while if due to albumin it remains or may even be increased.

It is necessary to bear in mind the fact that earthy phosphates are liable to be precipitated by heat, this being due to a change in the conditions. Also that very slight amounts of albumin may not be precipitated by heat alone, but the addition of nitric acid will cause all of it to be thrown out of solution in the form of very fine or coarse floccules. It may

be difficult to notice any change on simple observation; but by holding a tube of the urine beside the tested portion, and observing against a dark background for comparison, even an extremely faint turbidity will be easily seen; after standing for a few moments these floccules collect into larger ones and settle. Such comparison tests are often very helpful in other cases.

It is necessary to have the amounts of acid and urine in proper proportion, since too little acid may fail to cause precipitation, an acid albumin forming, and too much strong acid may redissolve the precipitate if the boiling be continued. Best results are obtained by adding about one-tenth to one-twentieth as much acid as there is urine. An idea of the amount of albumin may be obtained by observing the bulk of the precipitate; and by always using the same amounts of urine and reagent, data will be given which are very useful for comparison.

Acetic Acid and Heat.—Albumin may be precipitated by faintly acidifying the urine with acetic acid and heating. Unlike nitric acid, acetic acid must be used in very small amount, barely enough to acidify; if more is used, an acid albumin forms which is not precipitated even by continued boiling. A single drop of glacial acetic acid may be too much. The addition of a cubic centimetre or so of a saturated solution of sodium chloride (common salt) will aid in the completeness of the reaction, and may be necessary with dilute urines. This is the best method for removing albumin preparatory to testing for sugar.

If a large amount of albumin is present, it will coagulate and form a solid mass in the test-tube. The character of the precipitate should be carefully noted after the addition of nitric acid. According to Dock, the presence of coarse granules indicates primary kidney disease, while that of fine granules indicates some cause outside of the kidneys, such as heart disease.

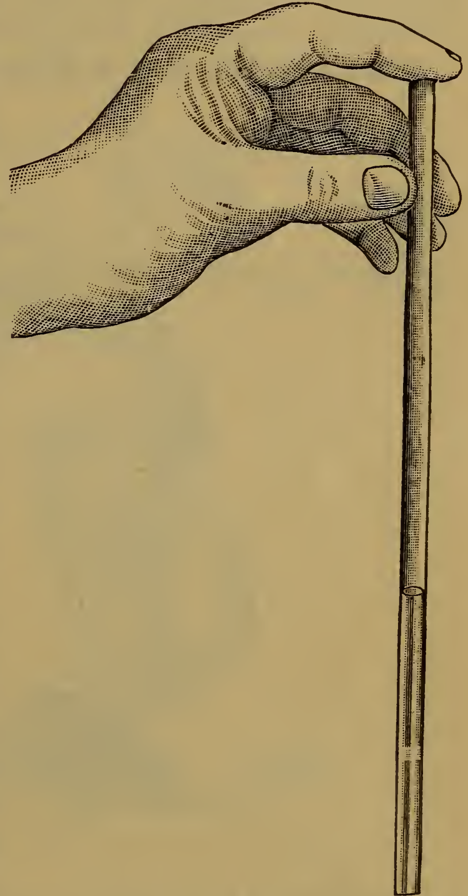
Heller's Ring Test.—If properly applied, this is a very satisfactory and delicate test. The ordinary method of over-

laying nitric acid with urine poured from a bottle is extremely unsatisfactory, as the fluids are usually mixed.

The following are excellent modifications of this method :

Boston's Pipette Method.—A piece of glass tubing about eight or ten inches long and about one-fourth to one-fifth inch in diameter is needed. With the index finger firmly pressed over the top of this tube, it is introduced into the urine, and by relaxing the pressure of the finger a *small amount* of urine is drawn up to the distance of about one inch. The tube is then placed under the tap and washed, and its *outer surface* is wiped dry, and introduced into a test-tube containing pure nitric acid, and the pressure of the finger again relaxed, allowing about as much acid to run in. Pressure is again applied, the tube removed and examined. There is a very sharp line of demarcation between the two liquids. If albumin is present, a distinct white ring forms at the junction of the two liquids, increasing with the percentage of albumin present (Fig. 57).

Fig. 57.



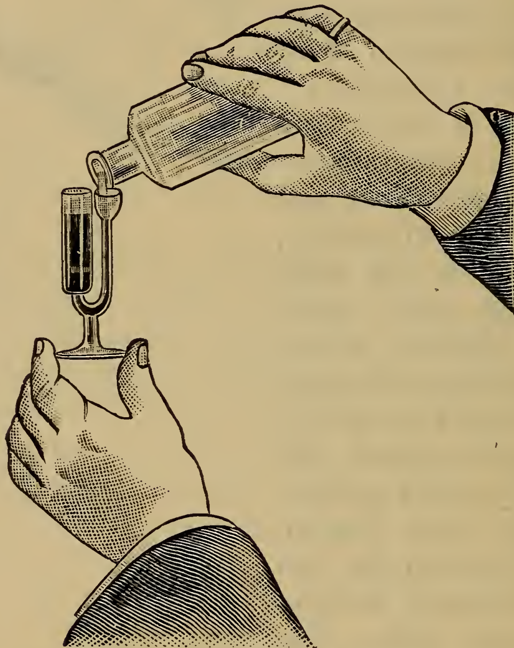
Boston's pipette method.

The **horismoscope**, manufactured by Nelson, Baker & Co., of Detroit, is useful in making all ring tests, but is easily broken. In testing urine for albumin, fill the large tube of the instrument two-thirds full of clear urine. Then pour into the funnel tube 25 or 30 drops of nitric acid ; this will pass into the capillary tube and form a layer be-

neath the urine. If albumin is present, a distinct white zone will presently appear at the point of contact, sharply defined against the black background, the amount of albumin being indicated by the density of the opaque ring. The tube should be free from air-bubbles before the addition of the acid. They can be driven out by merely tilting the instrument. A descriptive pamphlet accompanies the apparatus (Fig. 58).

Simon's Modification of Heller's Test.—This is a highly use-

Fig. 58.

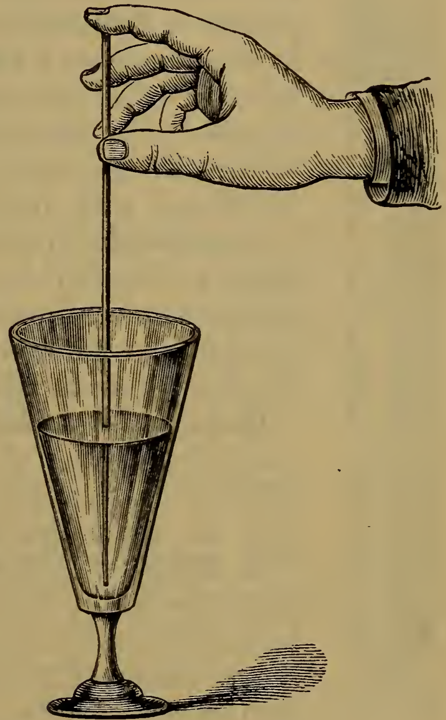


Horismoscope.

ful qualitative test, and furnishes valuable quantitative suggestions. About 20 c.c. of urine are placed in a conical glass, and by means of a pipette a few cubic centimetres of nitric acid are carried to the bottom of the glass and allowed slowly to run out, by gradually lessening the pressure of the finger on the pipette. The last portion of acid should be retained in the pipette, since if allowed to dribble as the pipette is removed, it will cause more or less mixing (Fig. 59).

By carefully following these directions, the nitric acid forms a distinct layer beneath the urine. If albumin is present, a distinct white cloud will form as a ring *at* the junction of the two liquids. The extent and intensity of this ring vary with the amount of albumin. The glass should be allowed to stand for some time, when a number of important points may be brought out. The same quantities of urine and reagent should always be used in each test, in order to draw satisfactory comparisons.

FIG. 59.



Nitric acid test. (Simon.)

Uric acid in excess is indicated by the appearance, within five to ten minutes, of a distinct ring in the clear urine a little *above* the zone of contact, similar in appearance to the albumin ring. If this ring does not appear in five to ten minutes, uric acid is probably present in diminished amount or the urine is quite dilute. The size of the ring indicates the extent of increase of the uric acid. In a urine containing more than 25 grammes of urea to the litre, an appearance like hoar frost will be noted on the sides of the vessel, due to the formation of urea nitrate. With 50 grammes and over per litre a dense mass of urea nitrate separates out.

Biliary urine treated in this way, if the nitric acid contains a little nitrous acid, shows the typical color-play of bilirubin.

Indican is shown by the appearance of a ring which is more or less violet blue, and situated *above* that referable to

the normal urinary pigment. It varies from a light blue to a deep indigo-blue, depending upon the amount present.

Potassium Ferrocyanide Test for Albumin.—This test is sufficiently delicate for all clinical purposes. To a few cubic centimetres of clear urine in a test-tube several drops of acetic acid are added, then a few drops of a 10 per cent. solution of potassium ferrocyanide are allowed to fall into the mixture; if even a trace of albumin is present a distinct cloudiness ensues, and if present in large amount a flaky precipitate. It is best seen by comparing the tube, as the ferrocyanide is being dropped in, with another tube containing clear urine. If the urine is very concentrated, it should be diluted with distilled water.

FIG. 60.

Esbach's albuminimeter.

Occasionally the addition of acetic acid alone produces a cloud; this may be due to urates or urinary mucin (nucleoalbumin). If this happens, the urine should be refiltered, diluted with water, and then the ferrocyanide added.

Quantitative Estimation of Albumin.—1. As mentioned under the heat and nitric acid test, the bulk of the precipitated albumin, $\frac{1}{10}$, $\frac{1}{4}$, $\frac{1}{2}$, etc., will give an **approximation of the quantity.**

2. **Esbach's Method** (Fig. 60).—A special, graduated test-tube, called the albuminimeter, and Esbach's reagents are required. The tube is filled to the *U* mark with the filtered urine, and then with the reagent to the point *R*. The tube is closed with a rubber stopper, inverted twelve times, and set aside for twenty-four hours. At the end of this time serum-albumin, serum-globulin, the albumoses, uric acid, and creatinin will have settled down and the amount per thousand, in grammes, can be read off from the scale. The reaction of the urine should be acid, a few drops of acetic acid being added if necessary, and the specific gravity should not exceed 1.006 or 1.008. If higher, a definite amount of distilled water may be added and the proper calculations made.

Tests for Albumoses.—(1) **Biuret Test.**—Render the cold

filtrate alkaline with a solution of sodium hydrate; a pink or rose color develops on the addition of a very dilute solution of copper sulphate, added drop by drop.

(2) To a test-tube partially filled with cold filtered urine, add a few cubic centimetres of **nitric acid.** If a cloud appears, which disappears on warming and reappears on cooling, it indicates the presence of albumose.

(3) Strongly acidify the urine with **acetic acid** and add an equal volume of a saturated solution of common salt. A precipitate occurring which dissolves on heating and reappears on cooling indicates albumose. To remove albumin, which is usually present with albumose, filter the liquid while hot. The albumoses are in solution in the hot filtrate and reappear on cooling.

The separate recognition of *serum-globulin, peptone,* etc., is of no special clinical value. Globulin responds to the albumin tests exactly as albumin does; is associated with it, and has the same significance. Peptone will give the biuret test just as albumose will. Albumin and globulin will also give this test, though heat is required, and the color is deeper, more of a violet.

Tests for Carbohydrates.—Albumin if present should be removed.

Glucose Qualitative Tests.—1. Fehling's Test.—Equal parts of the copper sulphate and the alkaline tartrate solutions are placed in a test-tube, diluted with 3 or 4 volumes of water, and boiled. The urine is then added, a little at first, and more if necessary; not more than an equal volume should be used. Sugar produces a precipitate of yellow hydroxide of copper or red cuprous oxide. The solution should not be boiled after the addition of the urine, only warmed for a moment. It must be kept in mind that other substances present in the urine may reduce cupric oxide, such as uric acid and creatinin, also though rarely milk-sugar, pyrocatechin, hydrochinon, and bile-pigment. Following the ingestion of some medicines reducing substances also appear. These may be disregarded if care is taken not to boil after the addition of the urine, since the

precipitation of cuprous oxide in the presence of sugar takes place before the boiling-point is reached. Sometimes a slight reduction of Fehling's solution is caused by the uric acid; the cuprous oxide is, however, held in solution by creatinin, and instead of a red precipitate there is a reddish or brownish solution.

2. **Haines's Test.**—Haines's test is the best of the copper tests.

Formula.—Take pure copper sulphate, 30 grains; distilled water, $\frac{1}{2}$ ounce; make a perfect solution and add pure glycerin, $\frac{1}{2}$ ounce; mix thoroughly and add 5 ounces of liquor potassæ.

Test.—Take about 1 drachm of the solution and gently boil it in an ordinary test-tube. Next add from 6 to 8 drops —not more—of the suspected urine, and again gently boil. If sugar be present, a copious yellow or yellowish-red precipitate is thrown down. This test solution is stable and can be kept indefinitely.

3. **Phenylhydrazin Test.**—This is an extremely delicate test, perhaps five or six times more so than Fehling's, if made as follows: Place in a test-tube about 0.5 gramme each of phenylhydrazin hydrochloride and sodium acetate (about as much as can be placed on a 5-cent piece), fill tube one-third to one-half full of urine, and heat over a flame till the solution is clear. Remove from the flame for a moment, then bring to a boil again, and repeat three times, removing the tube from the flame between times. Set aside to cool, being careful not to disturb the formation of crystals by shaking. (With sugar phenylhydrazin forms an insoluble crystalline compound, phenylglucosazon.) Examine in about fifteen minutes. With a pipette transfer some of the crystals to a slide, cover with a cover-glass, and examine under the microscope. If present, these crystals appear as delicate bright-yellow needles arranged in bundles and sheaves.

To illustrate the delicacy of this test, a 4 per cent. sugar urine was diluted 120 times, and gave the test. The same urine diluted 20 times failed to give the Fehling test.

Quantitative Sugar Tests.—(1) **Fermentation Test.**—

With yeast, sugar undergoes fermentation, with the formation of alcohol and carbonic acid. The specific gravity of the urine is lowered by this process, and upon this fact is based the quantitative estimation. Two 6-ounce bottles are filled two-thirds with urine; to one a fourth of a cake of fresh Fleischmann's yeast is added, in small pieces. Nothing is added to the other. Both are corked to prevent contamination, a slit being cut in the cork of bottle No. 1 to allow the gas to escape. Great care must be taken not to contaminate No. 2. The bottles are set aside in a warm room for twenty-four hours; at the end of this time No. 1 is tested for sugar with Fehling's solution; if there is no reaction, the urine is filtered and the specific gravity very carefully taken. The specific gravity of No. 2 is also taken at the same time, and the specific gravity of No. 1 subtracted from it. The figure obtained is multiplied by Robert's factor, 0.230, the product resulting representing the percentage of sugar.

Example.—Specific gravity before fermentation, 1040
Specific gravity after fermentation, 1020

20

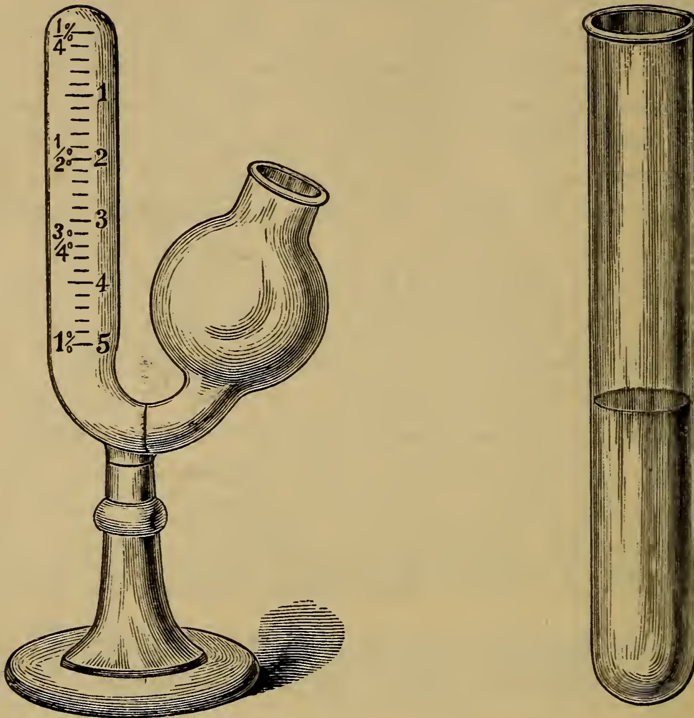
$$20 \times 0.230 = 4.60 \text{ per cent.}$$

For a very accurate estimation of the specific gravity, Lohnstein's urinometer should be used, reading to the fourth place.

The **saccharimeter of Einhorn** or **Lohnstein** may be employed. Either is extremely handy and fairly accurate. The percentage of sugar corresponding to the displacement of urine by gas can be read off the instrument directly. The objection to the fermentation test is that it requires a wait of twenty-four hours or longer for its completion (Fig. 61).

(2) **Purdy's Modification of the Pavy Method.**—This is by all means the most practical method of estimating the percentage of sugar. The quantitative estimation of sugar by the Fehling method is so time-consuming and the end-reaction so uncertain that in the hands of most physicians it is extremely unsatisfactory.

Fig. 61.



Einhorn's saccharimeter. (Simon.)

To demonstrate the simplicity and accuracy of Purdy's method, a series of sugar estimations, by all three methods—*i. e.*, Fehling, Purdy, and fermentation—was carried out in the clinical laboratory of the University of Michigan on a diabetic urine, by Dr. Cleaves, with the following results:

July.	Fehling.		Purdy.		Fermentation.		Quantity.
	Per cent.	Grammes	Per cent.	Grammes	Per cent.	Grammes	c.c.
12	1.50	13.90	1.43	13.23	. .	. .	925
13	1.40	14.97	1.30	13.32	1.38	14.83	1075
14	0.89	5.78	0.87	5.56	0.92	5.98	650
15	1.47	14.41	1.48	14.43	1.51	14.72	975
16	1.13	8.27	1.11	8.32	1.15	8.62	750
17	1.16	10.15	1.18	10.32	1.15	10.06	875

From the above figures it can be seen that the results with Purdy's method approach very closely those obtained by the

Fehling and the fermentation methods. Purdy's method is based on the fact that a definite amount of sugar will cause the complete reduction of a definite amount of the reagent, the latter being of a certain prescribed strength. The cupric oxide is reduced to cuprous, and this, instead of being precipitated as a red granular precipitate, is held in solution by the ammonia of the reagent, giving at the end a perfectly clear, colorless liquid. *The end-point is thus very easily* determined. The reagent is of such strength that exactly 35 c.c. will be just reduced completely by 0.020 gramme of sugar.

Technic of Purdy's Method.—Place 35 c.c. of the reagent in a flask of 150–200 c.c. capacity, add about 2 volumes of water, and bring the contents to the boiling-point. Run the urine from a burette into the boiling copper solution, drop by drop, until the blue color begins to fade; then still more slowly, three to five seconds elapsing after each drop, until the blue color just completely disappears, and leaves the solution perfectly transparent and colorless. The number of cubic centimetres of urine required to decolorize the 35 c.c. of the reagent contain exactly 20 milligrammes (0.02 gramme) of sugar.

Example.—If it require 2 c.c. of urine to reduce 35 c.c. of the test-solution, there is present 1 per cent. of sugar; if it require 4 c.c., there is present 0.5 per cent. of sugar. The total quantity of sugar excreted in twenty-four hours can easily be calculated. Suppose 2000 c.c. of urine are excreted, and 2 c.c. reduce the copper, 2 c.c. = 0.020. 2000 c.c. = 1000×0.020, or 20 grammes in twenty-four hours.

Precautions for Purdy's Technic.—It will be noticed after the determination that upon standing for some time the contents of the flask slowly assume the blue color again. This is due to reoxidation, which is somewhat rapid, and should not be mistaken for imperfect reduction or defect in manipulation.

The best results are obtained by first diluting the urine before the titration if the amount of sugar is large. The diluted urine should contain about 1 per cent. of sugar, and the calculation is made according to the dilution.

Polariscopic Methods.—By means of the polariscope the percentage of sugar can be estimated; and also the kind of

sugar can be determined. For the use of the instrument the reader is referred to special books.

Tests for Acetone in the Urine.—**Legal's Test.**—To a few cubic centimetres of urine in a test-tube add a crystal of sodium nitroprusside, render alkaline with sodium or potassium hydroxide, and shake till it is dissolved. The dark-red color produced may be due to creatinin or to acetone. The addition of a few drops of acetic acid changes this to a wine-red color if acetone is present; if absent, the color changes to a yellow. This test may also be used for creatinin, the change from red to yellow on the addition of acetic acid being characteristic.

Lieben's Test.—Phosphoric acid is added to the urine, about 1 gramme to the litre; 500 to 1000 c.c. of this are distilled, only 10 to 15 c.c. being distilled over. To this add a few drops of potassium or sodium hydroxide and enough of dilute Lugol's solution to give a yellowish color, then sodium hydroxide till the color just fades. If acetone is present, a yellow precipitate of iodoform appears, which is recognizable by its odor on heating, and by the form of the crystals, very small six-sided plates or stars.

Diacetic or Acetoacetic Acid in the Urine.—**Gerhardt's Test.**—A few cubic centimetres of urine are treated with a strong solution of ferric chloride, added drop by drop. If a precipitate of phosphates occurs, this is filtered off and more iron added to the filtrate. A wine-red color indicates the presence of diacetic acid. A second portion of the urine is boiled and similarly treated. If the reaction occurs after the urine has been boiled, it is not due to diacetic acid.

If in the second urine no reaction is obtained, a third portion is acidulated with sulphuric acid and extracted with ether. The ethereal extract when treated with ferric chloride solution gives a wine-red color, which disappears on standing for twenty-four to forty-eight hours, if diacetic acid is present, especially if acetone is abundant.

Salicylates in the urine give a similar reaction on the addition of ferric chloride, but the color is permanent, while that due to diacetic acid gradually fades. Diacetic acid is readily changed to acetone by heating, or even by standing.

β-oxybutyric Acid.—If after fermentation of the sugar the urine rotates the plane of polarized light to the left, β-oxybutyric acid is present. Such rotation, before fermentation, may be caused by lævulose.

Tests for Bile in the Urine.—Bilirubin is the only bile-pigment met with in fresh urine. It is never found in normal urine, so is an infallible sign of disease.

Foam Test.—This is a very satisfactory method of demonstrating the presence of bile. A few cubic centimetres of urine in a test-tube are shaken vigorously ; if bile is present, the foam takes on a greenish or greenish-yellow iridescence, and is quite permanent.

Morphological elements, casts, etc., are stained by the bilirubin, and appear yellow under the microscope.

Gmelin's Test.—Make a ring-test just as in making the Heller test for albumin, using concentrated nitric acid which contains some nitrous acid fumes. If bilirubin is present a band of colored rings appears just above the point of contact of the two liquids, exhibiting the colors of the rainbow. The green is the most characteristic and important, especially when taken in conjunction with the pink. These colors are caused by the oxidation of bilirubin through a series of products; the green is due to biliverdin, and represents the first product, it is therefore nearest the oxidizing agent. The nitric acid can be prepared by simply allowing it to stand exposed to sunlight until it becomes colored yellow.

Rosenbach's Modification of Gmelin's Test.—Filter the urine through thick filter-paper ; unfold the filter and place a drop or so of concentrated nitric acid, which contains some nitrous, upon its inner surface. If bilirubin is present, the typical rings are produced, the green being the most typical and important.

Iodine Test.—Pour 1 c.c. of tincture of iodine, diluted with 8 parts of alcohol, on the surface of the urine in a test-tube. A green ring at the point of contact of the two liquids shows the presence of bile.

Tests for Indican in the Urine.—**Jaffé's Test.**—Bliss has modified this test by using the official solution of ferric

chloride, instead of the solution of calcium hypochlorite, which spoils very quickly. To a few cubic centimetres of urine in a test-tube add an equal volume of concentrated hydrochloric acid, 2 or 3 drops of ferric chloride or hypochlorite solution, and 3 or 4 c.c. of chloroform. Shake thoroughly but not vigorously, and set aside. Indoxyl is set free, and is oxidized to indigo-blue, which is taken up by the chloroform; this settles to the bottom of the tube. The intensity of the blue color depends on the amount of indican present. Albumin does not interfere with the reaction. Bile-pigments interfere with the reaction, and must be removed by careful addition of a solution of lead subacetate. Potassium iodide, because of the liberation of iodine, colors the chloroform more or less violet. It is well to have marked test-tubes, and to use the same amounts of urine and reagents each time, and thus become familiar with the comparative colors in different urines.

This test can be made quantitative by comparing with a set of tubes of a chloroform solution of indigo-blue of known strengths; the volumes of solution must, of course, be the same in all.

Diazo Reaction of Ehrlich.—For method of preparing the reagent (see page 186). To 5 c.c. of sulphanilic acid solution add 2 drops of sodium nitrite solution; this gives a mixture of about 40 : 1. To this mixture add an equal volume of urine, and mix carefully by reversing the test-tube several times. Now add about 2 c.c. of ammonia, letting it run down the side of the test-tube. At the point of contact of the ammonia and the mixture rings of various tints form, ranging from light yellow, through dark yellow, orange and brown, to eosin or garnet, depending upon the urine. The formation of a *red ring* is an indispensable part of the true Ehrlich diazo reaction. It is also essential that, on shaking, the foam takes on a pink color. This color varies considerably in intensity, depending on the strength of the reaction, from the palest rose to the deepest pink, but must not be any other color, such as salmon, orange, etc. The foam is also persistent.

Tests for Blood in the Urine.—The color of the urine suggests its presence, and may vary from "smoky" when little blood is present, to red or brown. The sediment may be reddish if the corpuscles settle. The **hæmin crystal** and **guaiacum tests** may be applied (see page 21). The presence of red blood-corpuscles under the microscope is pathognomonic. Albumin, white cells, and hæmoglobin can always be demonstrated in the presence of blood. For recognition of red cells (see page 31). In alkaline urine the corpuscles disintegrate rapidly, and may not be demonstrable after ammoniacal fermentation has begun.

Fat in the Urine is most readily detected by the microscope (see page 190). If necessary, the specimen may be treated with Sudan III. This solution has an affinity for fat only, staining it red, and leaving everything else unstained. Specimens must not be treated with alcohol or ether, since the fat would be dissolved out. A drop of the solution is allowed to run under the cover-glass preparation; under the microscope the neutral fat-particles are seen to take on a red color.

Fat may be removed from urine by shaking with ether, removing the ethereal layer after it has risen, and evaporating. The fat may then be subjected to chemical tests.

Pancreatic Urine Test.—The diagnosis of diseases of the pancreas has assumed such importance in medicine and surgery of late years, and it is such a difficult field of diagnosis, that a trustworthy urinary test would be welcomed with open arms. Cammidge, working in conjunction with Mayo Robson, has introduced the following test: Sufficient work has not as yet been done with the reaction to prove its value in pancreatic diagnosis. However, it is to be hoped that it will prove to be all that its author claims for it.

Pancreatic Reaction.—Cammidge describes the procedure in which he calls reaction "A" as follows:

The specimen of urine to be examined is filtered, and 10 c.c. of the filtrate are poured into a small flask. One cubic centimetre of strong hydrochloric acid is added and, a funnel having been placed in the neck to act as a condenser, the flask

is placed on a sand bath and gently boiled for ten minutes after the first sign of ebullition is detected. A mixture of 5 c.c. of the filtered urine and 5 c.c. of distilled water is then poured into the flask, which is afterward cooled in running water. The excess of acid is now neutralized by slowly adding 4 grammes of lead carbonate and, after standing for a few minutes to allow of the completion of the reaction, the urine is filtered through a moistened filter-paper and the flask is washed out with 5 c.c. of distilled water on to the filter. To the clear filtrate are now added 2 grammes of powdered sodium acetate and 0.75 gramme of phenylhydrazin hydrochlorate, and the mixture is boiled for from three to four minutes on the sand bath. The hot fluid is then poured into a test-tube and allowed to cool undisturbed. After the lapse of a period, varying with the severity of the case from one to twenty-four hours, a more or less abundant flocculent yellow deposit is found at the bottom of the tube, and this when examined under the microscope with a $\frac{1}{6}$-inch objective is seen to consist of sheaves and rosettes of golden-yellow crystals. As the presence of sugar in the urine would obviously vitiate the results thus obtained, it is necessary before proceeding to the test to make sure that the untreated urine does not give a reaction with phenylhydrazin. This may roughly be done with Fehling's solution, or some similar test, but it is better to carry out a control experiment with phenylhydrazin hydrochlorate and sodium acetate in the manner I have just described, omitting the preliminary boiling with hydrochloric acid. Should the control experiment reveal even a trace of sugar, it must be removed by fermentation, with subsequent boiling to expel the alcohol formed, before the investigation is proceeded with. The presence of albumin in the urine is also liable to cause trouble, and it is best got rid of either by treatment with ammonium sulphate or by acidifying with acetic acid, heating and filtering. The results obtained by this method were found not to be absolutely trustworthy, although a useful aid in diagnosis, since a positive reaction was also obtained in patients suffering from certain other diseases where active tissue-change was taking place, such as cancer, adenitis, pneumonia, etc.

For a long time the positive reaction given by these non-pancreatic cases diminished the practical usefulness of the test, but eventually a means by which the cases of pancreatitis might be distinguished was devised, and we now believe that, by combining the results of the two, it is possible in the large majority of cases to make a trustworthy diagnosis from the examination of the urine alone. The differentiating test depends on the fact that the formation of the crystals described in reaction "A" is interfered with in inflammation of the pancreas by a preliminary treatment of the urine with perchloride of mercury, while such treatment does not affect the appearance of crystals in cases of cancer of the pancreas and the other conditions which give rise to a positive reaction. The procedure, which I shall refer to as the "B" reaction, is as follows: Twenty cubic centimetres of the filtered urine are thoroughly mixed with half its bulk of a saturated solution of perchloride of mercury. After standing for a few minutes it is carefully filtered, and to 10 c.c. of the filtrate 1 c.c. of strong hydrochloric acid is added. The mixture is then boiled for ten minutes on a sand bath and subsequently diluted with 5 c.c. of filtrate from the mixed urine and perchloride of mercury solution and 10 c.c. of distilled water. After cooling it is neutralized with 4 grammes of lead carbonate and the succeeding stages of the operation are carried out as in reaction "A."

Experiments were then set on foot to still further differentiate the forms of disease, and a careful observation of the crystals isolated from the urines of various types of pancreatic disease showed that while there is a general resemblance, certain variations occurred and the differing rate of solubility of crystals in dilute sulphuric acid was remarked and found to be of still greater value. If the crystals obtained in reaction "A" are observed under the microscope while a 1 : 3 dilution of sulphuric acid is being irrigated under the cover-glass, they will be seen to turn brown when the acid reaches them and dissolve. In acute pancreatitis the interval that elapses between the first appearance of the brown color and complete solution varies from a few seconds to one-half or

three-quarters of a minute. In chronic pancreatitis it extends from half to one and a half or, rarely, to two minutes, while in malignant disease the crystals do not completely disappear until three to six minutes. The practical results of the examination of the urine he summarizes as follows:

1. If no crystals are found by either the "A" or "B" method the pancreas is not at fault, and some other explanation of the symptoms ought to be sought. 2. If crystals are obtained by the "A" method, but not by the "B" reaction, active inflammation of the pancreas is present, and surgical interference is generally indicated. (*a*) The crystals obtained by the "A" method will in acute inflammation dissolve in 33 per cent. sulphuric acid in about half a minute. (*b*) In chronic inflammation the crystals obtained by the "A" method will take one or two minutes to disappear. 3. If crystals are found in preparations made by both the "A" and the "B" methods there may be (*a*) malignant disease of the pancreas, when the crystals will, as a rule, take from three to five minutes to dissolve, and operation is inadvisable; (*b*) a damaged pancreas, due to past pancreatitis, when the crystals will dissolve in from one to two minutes; (*c*) some disease not connected with the pancreas, when the crystals dissolve in about one minute. In the latter, two (*b*) and (*c*), the urgency of the symptoms and the condition of the patient must decide the need for an exploratory incision, but there is generally not much difficulty in referring the case to one or other of the groups when the clinical history is considered in conjunction with the result of the examination of the urine.

QUESTIONS.

Give scheme for urinary examinations.
What is Trapp's method for the determination of *total solids?*
Describe a quick method for the quantitative determination of urea.
What is the *murexid* test for uric acid?
Describe a qualitative test for *chlorides.*
What is meant by *conjugate sulphates?*
Describe a qualitative test for phosphates.
Describe the Boston pipette method of testing for albumin.
Describe Simon's modification of Heller's test.
Describe the potassium ferrocyanide test for albumin.

Describe the volume method of quantitatively estimating albumin.
Describe Esbach's method.
Mention several tests for *albumose.*
What is Fehling's test for sugar? Haines' test?
Describe Purdy's method of quantitatively estimating sugar.
Describe Legal's test. Lieben's test.
Describe Gerhardt's test.
How are salicylates in the urine distinguished from diacetic acid in the urine?
Describe Gmelin's test. Also the foam test for bile.
Describe Jaffe's test for indican.
Describe the diazo-reaction of Ehrlich.
Of what value is cryoscopy in urinalysis?

CHAPTER XIV.

URINARY SEDIMENTS.

Macroscopical Examination.—Normal urine on standing for some time gradually deposits a faint white cloud occupying the lower part of the vessel, and made up of mucus, a few epithelial cells and mucous corpuscles. A dense sediment of amorphous urates is common, especially in cold weather, and varies in color from almost white to pink or brown; this is the so-called "brick-dust" deposit; it disappears on warming the urine or on the addition of caustic alkali. Uric acid is often deposited from very acid, highly colored urines, in the form of small grains looking like red pepper; it can be demonstrated by the murexid test or its solubility in caustic alkali. A voluminous white sediment usually means earthy phosphates, though it may have ammonium urate, triple phosphate, or pus with it, or it may be pure pus. This, however, is usually shiny or sticky, while the phosphate sediment is light and fluffy; such urines are usually alkaline.

A yellowish-white sediment may consist of pus; if the urine is acid, it is separated into small freely moving particles; but if alkaline, it consists of a viscid, stringy, coherent mass.

A chocolate-brown sediment usually indicates the presence of blood in the urine; clots of blood may come from the kidney, ureter, bladder, or urethra, and may at times resemble worms.

Microscopical Examination.—The microscopical examination of urinary sediments is a very important part of urine analysis, and very frequently throws much light on both diagnosis and prognosis.

The freshly passed urine is sedimented by means of the centrifuge or by standing for several hours in a conical vessel, covered to keep out dust. The first method is preferable, as it requires only two or three minutes; if the latter method be used, certain changes may take place in the urine during the long wait, modifying the sediment, and thus incorrect conclusions may be drawn regarding it.

In the freshly passed urine small floating particles or mucous threads, technically termed "floaters," may be seen. These may be fished out by means of a glass tube and examined separately. Special attention should be given to these mucous threads, the so-called "clap-threads," and to the minute opaque cheesy bits; the former should be stained and examined for gonococci, the latter for tubercle bacilli. These particles can be obtained by the following method: With the index-finger pressed firmly over the top, a pipette or glass tube is brought near the particles, pressure is then slightly relaxed and the particles drawn up into the tube; pressure is increased again, the tube withdrawn and held for a moment to allow settling, and then they are deposited on a slide or cover-slip; excess of urine is removed with a strip of filter-paper. The specimen is then dried, stained, and examined. After the urine is sedimented specimens are transferred to a slide in exactly the same way as just described for floating particles, and examined with the microscope. The specimens should not be covered with a cover-slip, and a low power should be used; furthermore, the light should be fairly well shut off. When in doubt as to the recognition of certain of the smaller elements, as blood-corpuscles, etc., or when desiring to study their finer structure, a cover-slip

Fig. 62.

Extraneous matters found in urine: *a*, cotton-fibres; *b*, flax-fibres; *c*, hairs; *d*, air-bubbles; *e*, oil-globules; *f*, wheat-starch; *g*, potato-starch; *h*, rice-starch granules; *i*, *i*, *i*, vegetable tissue; *k*, muscular tissue; *l*, feathers.

should be applied and a higher power used. For the recognition of bacteria it may be necessary to prepare the specimen, dry, fix, and stain it, as with sputum.

Specimens of sediment must always be examined while

still wet, since as the water evaporates all the solid constituents of the urine will be left in crystalline or amorphous form covering everything else. For this reason it is well to prepare one at a time; and if the examination is to be prolonged, hanging-drop preparations, as made in bacteriological examinations, will be found very convenient. (See under Widal Reaction.) On the other hand, too much fluid will be liable to run off the slide on to the stage of the instrument. Excess of urine can easily be removed with a strip of filter-paper.

It is frequently desirable to treat a specimen of sediment with a reagent and notice the effect under the microscope. The common way of adding the reagent to the edge of the cover-slip and allowing it to flow under will usually result unsatisfactorily; some of the reagent is almost certain to get on top of the slip, and may get on the objective. A much better plan is to place a small drop of the concentrated sediment and a drop of the reagent side by side on the slide, allow them to run together, and cover if necessary. Such a preparation is much neater and there is no danger of injuring the microscope.

Classification of Urinary Sediments.—

<table>
<tr><td rowspan="2">Chemical, or Unorganized</td><td>Crystalline.</td></tr>
<tr><td>Amorphous.</td></tr>
<tr><td rowspan="3">Anatomical, or Organized</td><td>Formed elements, as blood-corpuscles; pus-cells; epithelium; casts; cylindroids; spermatozoa.</td></tr>
<tr><td>Parasites, animal and vegetable.</td></tr>
<tr><td>Foreign bodies; fragments of tissue; clap-threads; blood-clots, etc.</td></tr>
</table>

Chemical Sediments.—These, as a rule, are of little diagnostic or prognostic value. Most of the substances entering into their formation occur in solution in normal urine; but as a result of changes from normal conditions to abnormal, or in some cases to slightly different but still normal conditions, they are rendered insoluble, and so appear as a sedi-

ment. Such changes, which may at times be considered as pathological, and at other times as simply due to alteration in diet, habits, etc., are hyperacidity of the urine; decrease in acidity or even change to alkaline; increase in the amount of a normal constituent above the usual amount; decrease in the volume of the urine, rendering it more concentrated, and thus relatively increasing the amounts of the substances in solution; the occurrence of certain substances as a result of eating certain articles of food; development of ammonia. This last is always pathological if occurring before the urine is passed, and is always to be expected in urine which is kept for some time.

Some of the substances found in chemical sediments always occur in crystalline form; some always are amorphous; a few may take either form, but usually the crystalline. A few are rare, and need practically no consideration; these are cystin, xanthin, cholesterin, leucin, and tyrosin. These always occur as crystals when found in the sediment; but they may remain in solution. The first three are of interest, since they have been found in calculi. If there is reason to suspect their presence, the reader should consult large books for methods of identification and tests.

The commonly occurring sediments are composed of uric acid and urates; the various phosphates; oxalate and carbonate of lime. Sulphate of lime and hippuric acid are rare, their solubility precluding their deposition except in very concentrated urine. Fat or oil in the form of globules, or crystals, of fatty acids and soaps, are not common except as accidental constituents. Certain coloring-matters, such as hæmatoidin, bilirubin, indigo, etc., are also very rare, though indigo is frequently seen in the sediment of old decomposed urines.

If the following facts be borne in mind, the identification of a sediment will not be a difficult matter. A sediment is composed only of such substances as are insoluble in the urine under the existing conditions of concentration, reaction, etc.; none of the substances is absolutely insoluble, though some are practically so; a sediment therefore represents the excess

over the amount that can be held dissolved. Whenever, by any change in the conditions or by any combination of circumstances, an insoluble compound can form, the tendency will be for it to form; the equilibrium existing between the substances in the solution is thus destroyed and the reaction will continue until equilibrium is again restored. Certain new chemical compounds may thus be formed.

Substances which crystallize will always take a certain definite form when possible; but various causes may interfere, and instead of well-developed, perfect crystals, there will be more or less imperfect forms, or even an amorphous deposit; often the crystals will show the *tendency* toward the normal form. When crystals are being deposited slowly they will grow; triple phosphate, for instance, will appear as very small well-formed crystals at first, but if examined a few days later, after the ammoniacal fermentation has progressed further, the crystals will be much larger, and may even be large enough to be recognized with the naked eye. The influences which modify crystal formation are especially the rate and the presence of other substances. As a rule, the slower the formation, the more perfect the form; and the rate may be retarded by slow cooling, slow evaporation, etc. Triple phosphate may be used again as an illustration: if ammonia is produced in the urine by bacteria, and thus slowly, the triple phosphate will appear in almost perfect form; if, however, ammonia be added to the urine directly, the triple phosphate is formed at once and precipitated; no time is given for the characteristic form to be taken, and fern-leaf-shaped or star-shaped crystals will be seen. Ammonium urate, if made by adding ammonia to a solution of uric acid, nothing else being present, will consist of needles; but as found in a urinary sediment, it is almost always in the form of small more or less highly colored balls; the other constituents of the urine prevent it from taking the needle-shape; the small spicules so frequently seen show the tendency toward needles; and sometimes a burr-like mass of crystals can be observed.

Some substances on being deposited from urine, or from

other solutions also, will enclose and drag down suspended particles or coloring-matter; for example, uric acid deposited from urine is almost invariably colored, the more deeply the more intense the color of the urine; on the other hand, triple phosphate and calcium oxalate are never colored, but are bright and shining. A familiar illustration of an analogous process is the clarification of water by means of alum, the bulky, voluminous precipitate of aluminum hydrate, in settling, acting as a drag-net, will carry down suspended particles, bacteria, etc. This process may be used in clarifying urine which will not filter clear.

FIG. 63.

Uric-acid crystals of various shapes.

Uric Acid.—Uric acid itself has a very slight solubility; it forms two kinds of salts, acid and normal. Sodium acid urate, $C_5H_3NaN_4O_3$, may be taken as the type of the former; and $C_5H_2Na_2N_4O_3$ the latter. The constituents of normal urine increase the solubility of these difficultly soluble forms, holding them as the normal urates, which are easily soluble, and they do not therefore appear as sediment in fresh urine. The acid salts also have very slight solubility. It is as normal salts that uric acid exists in urine; by a change in the conditions, excess in the amount, the degree of acidity, lowering of the temperature, etc., there is a change to the more

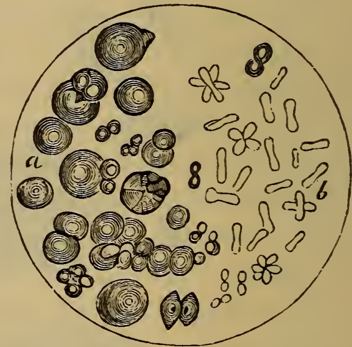
insoluble form, either the acid urate or uric acid, and the appearance of a sediment. This frequently occurs in cold weather as a result of simple cooling. Uric acid crystals are almost always colored either a deep yellow or brownish red, or even brown, rarely a pale yellow. The rhombic prism is the essential form of the crystal, but it may present all sorts of modifications of this form, star-shaped and fan-shaped clusters. Colored crystals occurring in acid urine are in all probability uric acid.

Urates.—As explained above, urates are very frequently deposited as a result of cooling of the urine; an interchange

Fig. 64.

Sodium urate: *a*, *a*, from a gouty concretion; *b*, *b*, artificially prepared by adding liquor sodæ to the amorphous urate deposit. (Roberts.)

Fig. 65.



Ammonium urate spontaneously deposited: *a*, spheres and globular masses; *b*, dumb-bells, crosses, rosettes, (Roberts.)

takes place between the normal urate and the acid phosphate, with formation of the acid urate and alkaline or secondary phosphate. The acid urate is thus precipitated. The same reaction may go farther and form uric acid. The urates are chiefly those of sodium and potassium, sometimes also of calcium and magnesium; they are spoken of as the amorphous, or acid, or mixed urates, or brick-dust deposit. Ammonium urate, as stated, will appear only when there is ammonia. The urates rarely appear in crystalline form, though sometimes small needles are found. The usual form is a rather dense, sandy deposit, cream-colored, yellow, pink

or rose-colored, or even brown, depending upon the color of the urine. Under the microscope small sandy particles are seen, usually yellowish or brownish, with now and then a crystal of uric acid. If there should be any doubt, the following very simple characteristic tests can be applied:

(*a*) A drop of caustic soda or potash added to the sediment on the slide will cause it to dissolve immediately. (*b*) A drop of hydrochloric acid added to the sediment will in two or three minutes produce numerous very small colorless

Fig. 66.

Ammonium urate. (Musser.)

crystals of uric acid. (*c*) Simply warming some of the urine in a test-tube will cause a solution of the deposit and clearing-up of the urine (Figs. 64 and 65).

Calcium Oxalate.—Calcium oxalate is usually found in acid urine, frequently associated with uric acid or urates; but occasionally in alkaline urine, and at times associated with triple phosphate. It is almost always in the form of small colorless shining octahedra, the so-called envelope crystals; these have the appearance of small squares with lines con-

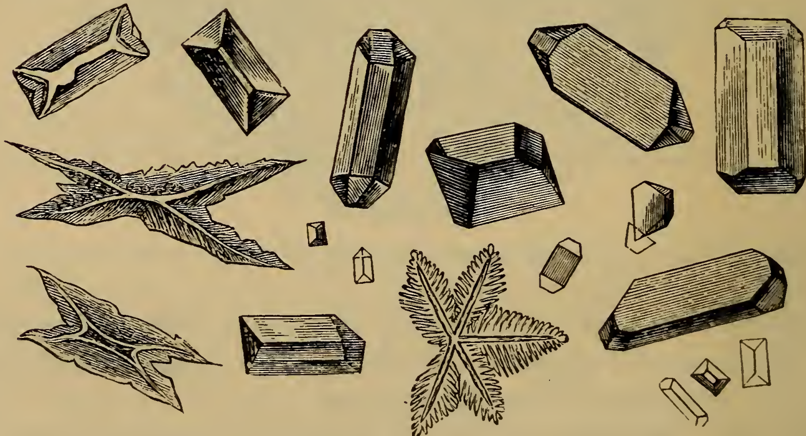
necting the corners and crossing at the middle. It rarely occurs as small dumb-bells, ovals, or amorphous (Fig. 67).

Fig. 67.

Calcium oxalate crystals.

The solubility in hydrochloric acid and insolubility in acetic acid or caustic alkali may aid in identifying it.

Fig. 68.



Various forms of triple phosphates. (Finlayson.)

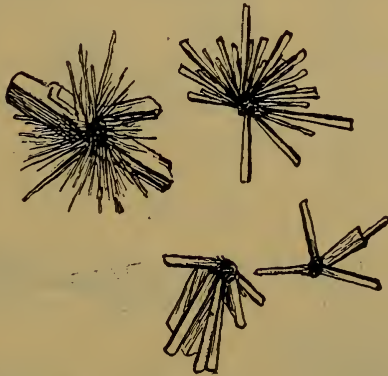
Phosphates.—Whenever the urine becomes alkaline from any cause, the phosphates of calcium and magnesium, $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$, may be expected; if ammonia is

present, ammonium magnesium phosphate, NH_4MgPO_4, or triple phosphate will also appear. In urine which is very faintly acid these may be found, as explained, or rarely the acid phosphate of calcium, $CaHPO_4$.

The first named, earthy or amorphous phosphates, occur as a very voluminous, colorless, fluffy deposit, hardly visible under the microscope.

Triple phosphate occurs almost invariably as the so-called "coffin-lid" crystals, colorless prisms; rarely as fern-leaf-shaped or in stars of leaf-shaped crystals, and these only when ammonia has been added to the urine or has developed very rapidly (Fig. 68). The acid phosphate of calcium forms colorless wedge-shaped crystals, often in bundles (Fig. 69).

Fig. 69.



Monocalcium phosphate crystals.

The reaction of the urine and the solubility of the deposit in acids will distinguish phosphates from anything else.

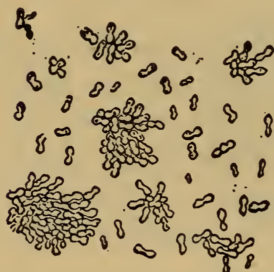
Calcium Carbonate.—This is frequently found in ammoniacal urine in the form of very small colorless granules or dumb-bells. The effervescence with a drop of acid will identify it (Fig. 70).

Cystin.—This is a very rare urinary deposit. It occurs most often in small six-sided plates with a "mother-of-pearl" appearance, or in the form of four-sided square prisms (Fig. 71).

Xanthin.—This is found very infrequently, and occurs in the form of small fusiform crystals.

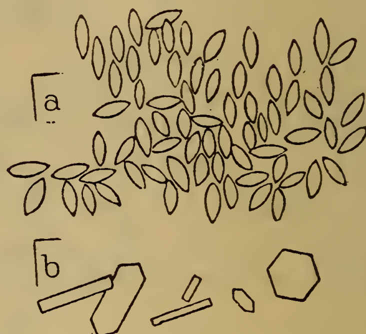
Leucin and Tyrosin.—These are very rare, being found

FIG. 70.



Calcium carbonate crystals.

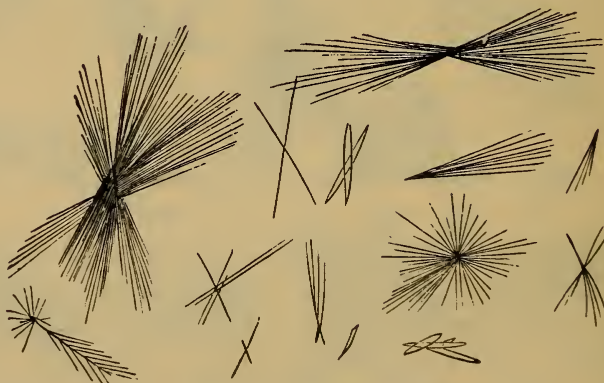
FIG. 71.



a, Crystals of xanthin (Salkowski; *b*, crystals of cystin (Robin).

only in severe diseases of the liver. Leucin occurs in mulberry-like masses. Tyrosin appears as very fine needles arranged in the form of sheafs. Both are insoluble in alco-

FIG. 72.



Tyrosin crystals. (Charles.)

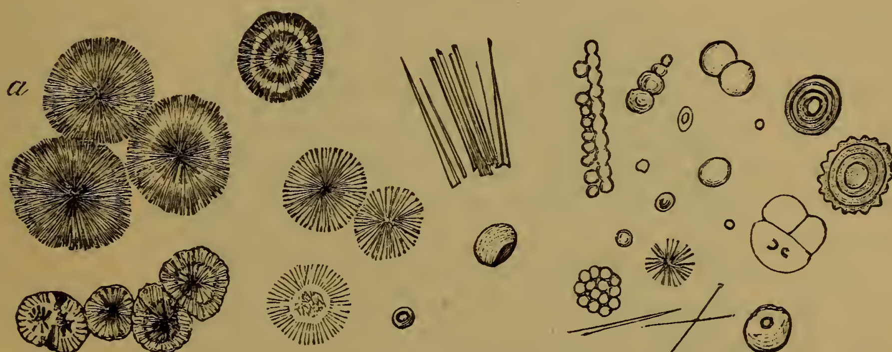
hol or ether, readily soluble in acids, alkalies, or warm water (Figs. 72 and 73)

Organized Sediment.—**Red blood-corpuscles** can usually be recognized under the microscope with the low power.

If there is any doubt, the specimen should be covered with a cover-slip and a high dry power used. In freshly passed urine they usually show a light-yellow color and the typical biconcave appearance. If they have become shrunken, crenated, or the hæmoglobin has become dissolved out, they may be recognized with difficulty. Only rarely are chemical tests required.

If a hemorrhage has occurred within the urinary tract, blood-clots may be found; these may be moulded into more or less definite-shaped masses if formed within the tubules

FIG. 73.



Crystals of leucin (different forms): (Crystals of kreatinin-zinc chloride resemble the leucin crystals depicted at *a*.) The crystals figured to the right consist of comparatively impure leucin. (Charles.)

(blood-casts) or in the ureters; but they are usually simply masses of clotted blood, especially if formed in the bladder.

Leukocytes and Pus-cells.—A few leukocytes may be present in normal urine. An excess of leukocytes or *pus* always indicates disease in some part of the urinary tract, except in the female, when the cells may be numerous as a result of contamination with vaginal discharge. In such cases they are associated with a corresponding increase in the vaginal epithelium. A catheterized specimen of urine should then be examined.

The appearance of pus-corpuscles depends on the reaction of the urine. In acid and neutral urines they are usually well preserved; but in alkaline urine they swell up, become

opaque, and the nucleus is recognized with difficulty, except after the addition of acetic acid.

In pyogenic conditions the leukocytes are all polynuclear.

The presence of a good many mononuclear leukocytes makes the presence of genito-urinary tuberculosis extremely suspicious.

Pus can be most easily demonstrated by pouring off the greater part of the urine and adding a small piece of caustic soda or potash, when a viscid, sticky, slimy mass is formed.

Epithelial cells occur in small numbers in the sediments of normal urine. An increased number of cells indicates an inflammatory condition in some part of the urinary tract. Unfortunately it is impossible to be absolutely certain as to which part of the urinary tract certain cells come from, since the cells from the bladder, ureters, and the pelvis of the kidneys are practically alike. Hence definite conclusions can very seldom be drawn from the microscopical examination alone.

Cells are of three kinds:

(1) Round cells;

(2) Conical and caudate cells;

(3) Squamous, or pavement cells.

Round cells are a trifle larger than leukocytes and have a more distinct nucleus. They may be confused with pus-cells, but in the latter it requires the addition of acetic acid to bring out the nucleus, which is furthermore polynuclear. In fatty degeneration of the kidney, fat can be demonstrated in these cells by means of Sudan III.

Conical and caudate cells have their origin in the superficial layers of the pelvis of the kidneys and the neck of the bladder; the latter have the longer processes.

Squamous or **flat cells** may come from the ureters, the bladder, the vulva and vagina of the female, and the prepuce of the male. They are large polygonal cells with a distinct nucleus and slightly granular protoplasm (Fig. 74).

Tube-casts.—There are three main classes:

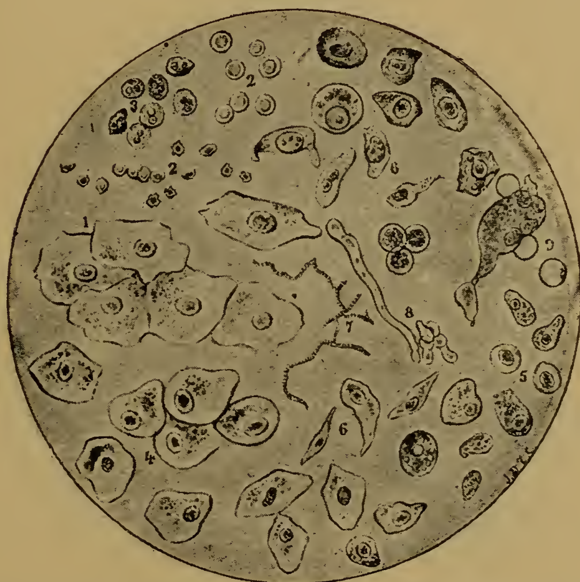
(1) Hyaline and waxy casts. These are clear, almost

transparent, homogeneous bodies; they may be narrow or broad. There are also composite casts, chiefly hyaline, but more or less covered with granules and organized elements.

(2) Those consisting of organized elements embedded in a hyaline matrix; blood-casts, leukocyte casts, epithelial casts, bacterial casts.

(3) Those consisting of the débris of organized bodies; granular casts, fatty casts.

FIG. 74.



Cellular elements from the urine: 1, squamous epithelium; 2, red blood-corpuscles; 3, polynuclear leukocytes; 4, transitional cells; 5, epithelium from the kidneys; 6, epithelium from the bladder; 7, Micrococcus ureæ; 8, yeast-fungi. (Musser.)

The inexperienced worker best recognizes and studies casts by comparing his microscopical preparation with good cuts.

Casts usually indicate nephritis, acute or chronic; but may be found in cases of renal calculi, icterus, diabetes, and sometimes in secondary congestion of the kidney, and in fevers.

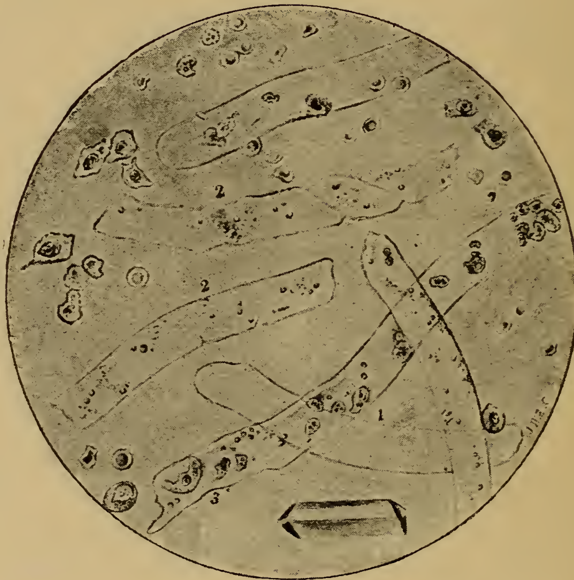
Hyaline casts are clear, translucent cylinders, which are easily overlooked on account of their slight refraction of light. Their borders are clearly marked and their ends may

be rounded or have a broken-off appearance; they are seldom long, and are either straight or slightly curved. In diameter they vary from that of a white blood-cell (narrow) to five or six times this diameter (broad). It is seldom that they do not show some granulation.

It is essential to have most of the light shut off when searching for these casts.

Waxy casts are somewhat similar to hyaline, but have a

FIG. 75.



Hyaline casts from a case of acute nephritis: 1, plain hyaline cast; 2 granular deposit on hyaline cast; 3, cellular deposit (blood and epithelium). (Musser.)

light-yellow color, refract the light better, and are usually larger and seem more cylindrical. They have a waxy appearance (Fig. 75).

Blood-casts consist most often of hyaline casts whose surface is covered with blood-cells, or of masses of blood-corpuscles pressed into cylindrical shape. The same is true of *epithelial* and *leukocyte* casts (Fig. 76).

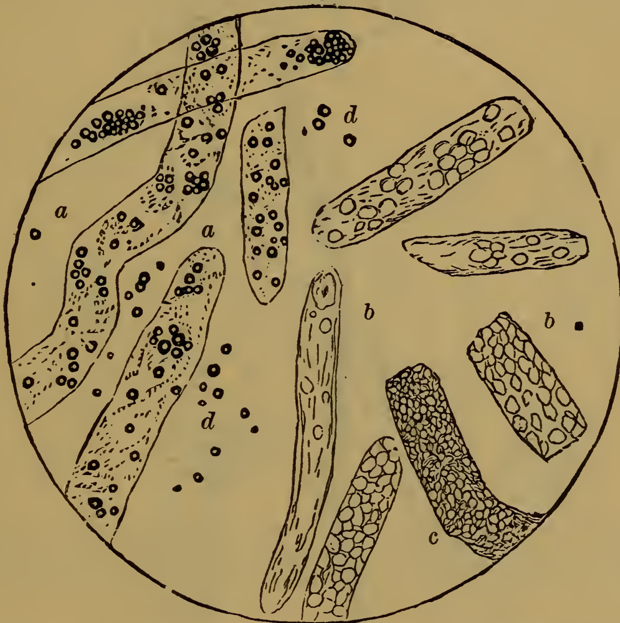
Granular casts have as their basis the hyaline cast; this is covered with fine or coarse granules, few or many, or

is granular from a disintegration of the cast itself. The granules may be so coarse and numerous as to give the cast a dark appearance. Enormous numbers of coarse dark granular casts have, according to Dock, a grave prognostic indication.

Fatty casts are simply hyaline or granular casts on which are deposited minute oil drops, or sometimes crystals of fat.

Cylindroids resemble hyaline casts, except in shape and

FIG. 76.



a, Fatty casts ; *b* and *c*, blood-casts ; *d*, free fatty molecules. (Roberts.)

size. They are much larger, and usually taper from a thick end to a slender wavy or twisted point, or they may show several constrictions in their course. They may have a striated or ribbed appearance (Fig. 77). They have practically no pathological significance.

False casts have been described. They consist of urinary crystals or amorphous salts moulded into cylindrical forms.

Spermatozoa are easily recognized by their tadpole appearance (Fig. 78).

Fig. 77.

a and *b*, cylindroids from the urine in congested kidney. (v. Jaksch.)

Parasites—Vegetable and Animal.—Vegetable.—The bacteria of most importance in urinary examination are the gonococcus, the tubercle bacillus, and the colon bacillus and typhoid bacillus.

Clap-threads may be fished out of freshly passed urine or the urine may be centrifugated, and spreads made of the sediment, dried in the air, and fixed in a flame. These are now stained with methylene-blue solution or by Gram's method or with neutral red or Kresylechtviolett.

Tubercle Bacillus.—Any opaque particles are fished out of the suspected urine, or it is centrifugated and spreads made, dried, and fixed as above. The specimen is then double-stained with carbol-fuchsin and methylene-blue, as in sputum examination. Tubercle bacilli in the urine are often

FIG. 78.

Human semen : *a*, spermatozoa ; *b*, cylindrical epithelium ; *c*, bodies enclosing lecithin-granules ; *d*, squamous epithelium from the urethra ; *d'*, testicle-cells ; *e*, amyloid corpuscles ; *f*, spermatic crystals ; *g*, hyaline globules. (v. Jaksch.)

arranged in thick masses, having an S-shaped form. It may be difficult to demonstrate the germs by staining methods. The urine is then sedimented, and 1 or 2 c.c. of the sediment injected into the peritoneal cavity of a guinea-pig. In the course of six weeks the pig should develop tuberculosis if the urine contains tubercle bacilli. The pig is posted, and tubercles found in the various organs of the abdomen and perhaps chest.

Smegma Bacillus.—This is important because it resembles the tubercle bacillus very closely in its morphology and staining characteristics. It is found on the external genitals of

both sexes, and at times in the urethra. It may find its way into the urine and fæces, and thus be mistaken for the tubercle bacillus. It does not cause disease; hence animal inoculation is a certain means of differentiating the two germs.

They can also be differentiated by *Pappenheim's method of differentiating Bacillus tuberculosis from the smegma bacillus.* The specimen is stained with carbol-fuchsin solution: then after draining off the excess of stain, it is dipped from three to five times into Pappenheim's solution (1 part of corallin (rosolic acid) in 100 parts of absolute alcohol, to which methylene-blue is added to saturation. This mixture is treated further with 20 parts of glycerin). The specimens are washed in water, dried between filter-paper, and mounted in Canada balsam. The tubercle bacilli are stained red, and all other germs, including smegma bacilli, are stained blue.

A *simpler method of differentiation* is to stain with carbol-fuchsin, decolorize in 33 per cent. nitric acid, then wash in 95 per cent. alcohol for thirty seconds, and counterstain with methylene-blue. Smegma bacilli hold the red color in the presence of acids, but not in the presence of alcohol.

Gonococci.—(1) *Stain by Gram's method* (see page 19), then counterstain with dilute carbol-fuchsin 1 : 8, without heat, or with saturated aqueous solution of Bismarck-brown with heat to the steaming-point. Wash in water and mount. Diplococci within leukocytes, which have been decolorized by Gram's stain, and have taken the counterstain of red or brown, are to be considered as gonococci.

(2) *Neutral Red Stain.*—This is an excellent stain. In a dilution of about 1 : 15,000 it shows a selective action for gonococci, staining them a red color, while other organisms, with the exception of the Diplococcus urethræ communis, which takes a somewhat lighter red, remain unstained. Morse gives the following directions:

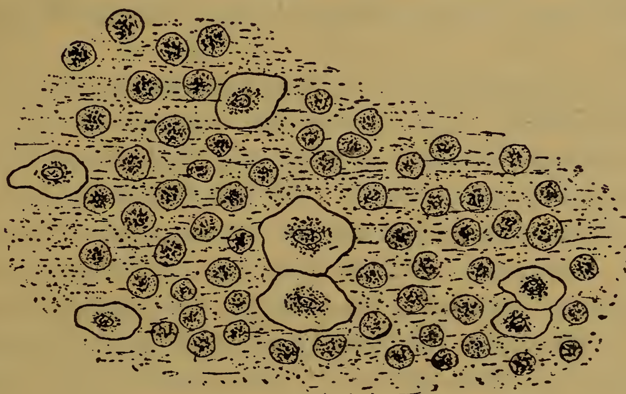
1. Make a stock 1 per cent. aqueous solution of neutral red.
2. To a beaker of distilled water add sufficient of this stock solution to give a sherry-wine color (about 1 : 15,000).
3. Float fixed spread, specimen side down, upon this solution for about five minutes.

4. Wash quickly in distilled water and dry with filter-paper.

(3) *Methylene-blue.*—With this stain the diplococci within the leukocytes are usually considered as gonococci. The stain is easily applied, but is not specific, as all cocci in the preparation take the same blue stain.

Gram and Neutral Red Stain for Gonococci (Morse): 10 c.c. water; 2 c.c. anilin oil; shake; filter clear. Add 1 c.c. alcohol and 1 c.c. saturated gentian-violet solution.

Fig. 79.



A gonorrhœal thread. (Simon.)

1. Stain in this cold for two minutes.
2. Leave in Gram's (I in KI) solution for three minutes.
3. Wash in alcohol till decolorized.
4. Wash in water.
5. Float in neutral red solution 1 : 5000 about five minutes.
6. Dip once in distilled water and dry with filter-paper.

As is well known, certain bacteria are stained by Gram's stain, while others are not (see Gram's stain). The Diplococcus urethræ communis resembles the gonococcus very closely in its morphology; it is a little larger and longer. The average observer is likely to mistake it for the gonococcus. It is stained by the Gram method, while the gonococcus is not. The above stain will differentiate these two organisms.

Bacteriuria is the name given to a condition in which

micro-organisms are present in considerable numbers in urine at the time of voiding. These can be demonstrated by preparing the sediment, fixing, and staining with methylene-blue.

The bacteria of the infectious diseases can sometimes be demonstrated by appropriate methods of culture and staining.

The trichomonas is rarely found in the urine.

Sarcines, yeasts, and moulds are rarely found.

Animal parasites are rarely found, and then chiefly in residents of the tropics.

Among them are the echinococcus, Filaria sanguinis hominis, Bilharzia hæmatobia, Distoma hæmatobium, Strongylus gigas.

Calculi.—Examination of the freshly passed urine may throw considerable light upon the nature of the calculus. Small crystalline masses, gravel, or deposits of uric acid or calcium oxalate may be seen. If the urine is distinctly acid at the time the gravel was passed or the attack of renal colic occurred, it is almost certain that the calculus is either uric acid or calcium oxalate; in a large percentage of the cases the former, especially if directly on cooling the urine precipitates crystals of uric acid. If the fresh urine shows continually the presence of crystals of calcium oxalate, it is most likely a calcium oxalate calculus.

In alkaline urines the calculus is usually of the mixed phosphate variety, or has a nucleus composed of uric acid or calcium oxalate and coated with phosphates.

Accidental Substances.—Extraneous matter found in the urine may consist of fibres of various kinds, such as cotton, linen, or woollen; starch-granules or oil drops, or particles of dust. These may appear in the urine as a result of contamination with towels, clothing, toilet preparations, dust, etc.

CRYOSCOPY.

Molecular Concentration of the Urine.—The method of determining the freezing-point of the urine is the same as that for the blood.

Lenhartz gives the following statement of the value of cryoscopy in the examination of urine: "In defective renal function and consequent retention in the blood of substances which should be excreted in the urine, a diminution of the molecules in the urine must occur in the same ratio. In other words, *in disturbed renal function the freezing-point of the urine (Δ) rises above the normal.*

"It has been shown that in healthy kidneys this varies between 0.87° and 2.42° C., according to the conditions of metabolism. In order to obtain an approximate idea of the actual amount of molecules excreted, the daily amount of urine must, of course, be considered. In order to obtain standard values, the product of Δ and the amount of urine = V (valence number) has been calculated. The figures given by different authorities vary between 766 and 3770. While the practical value of the molecular determination of the urine in internal medicine is limited (because the limits of the normal value, which among other things are decidedly influenced by solid and liquid foods, are very variable) the researches of Kummel and Rumpel have shown that cryoscopy is of the greatest advantage in the diagnosis of unilateral renal affections. For this purpose it is necessary to collect the urine of both kidneys separately by ureteral catheterism. The urine of each kidney is then examined in regard to its freezing-point and also its urea and sodium chloride content. If Δ of one kidney shows a normal value, while Δ of the other kidney is under 0.87° C., this indicates an affection of the latter."

The foregoing remarks indicate that the value of cryoscopy is extremely limited, as the members of the profession who are thoroughly familiar with ureteral catheterism in the male, and the technique of cryoscopy will always be very few and confined to the larger medical centres.

QUESTIONS.

What points are to be noted in the naked-eye examination of urine?

Describe the correct method of making a microscopical examination of urine.

Classify urinary sediments.

What substances are most commonly found in chemical sediments?

What substances are rarely found?
Mention the important points in the law of crystalization.
Describe the various forms of uric acid crystals.
Describe the various forms of urates.
Mention several simple tests for the detection of urates.
Describe the common form of calcium oxalate crystals.
Describe the various forms of phosphates.
Describe calcium carbonate, cystin, xanthin, leucin, and tyrosin crystals.
Describe the red blood-corpuscles in varying conditions of the urine.
Give several methods for recognizing pus in the urine.
Of what significance is an excess of mononuclear leukocytes in the urine?
Name the different areas of the urinary tract in which similar cells are found.
Mention the chief classes of tube casts.
In what conditions are tube casts found, and what is their significance?
Describe cylindroids. False casts.
Describe the various methods of demonstrating tubercle bacilli in the urine.
Describe a differential stain for the gonococcus.
What is meant by bacteriuria?
Of what value is sediment examination in determining the nature of calculi?
Mention accidental substances found in urinary sediments.

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