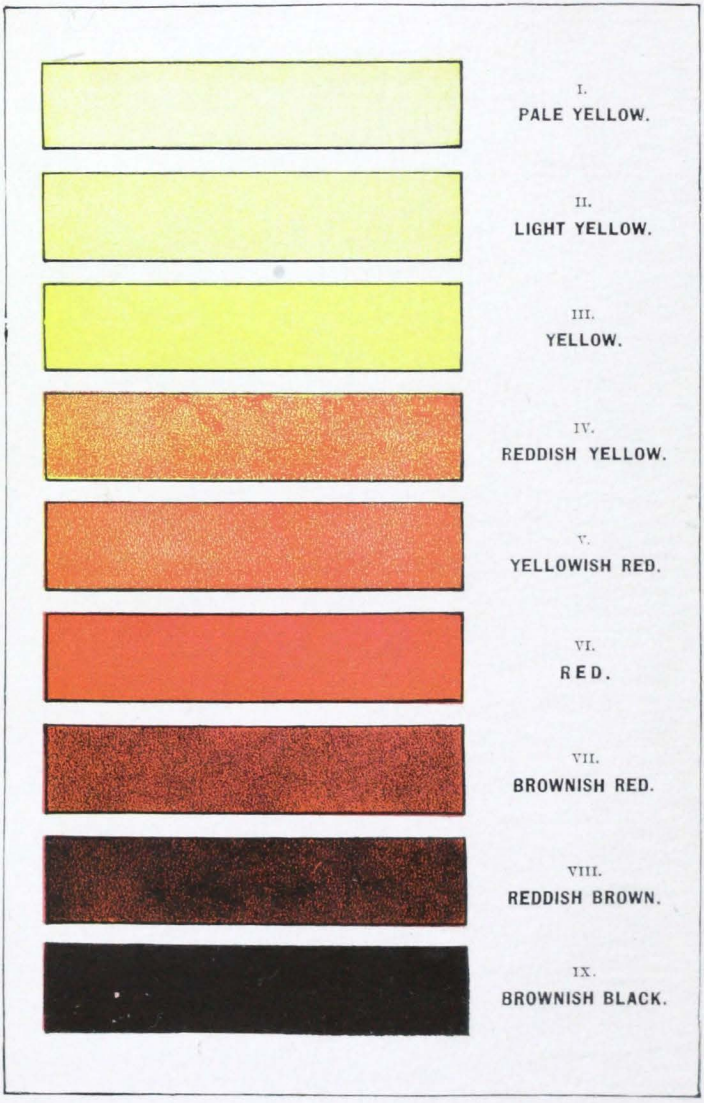
PLATE I



Scale of urinary colors, according to Vogel.

Clinical Diagnosis

A MANUAL OF LABORATORY METHODS

BY

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Illustrated

Second Edition, Revised and Enlarged

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TO

MY FATHER

Joe H. Todd, M. D.

THESE PAGES ARE

AFFECTIONATELY DEDICATED

PREFACE TO THE SECOND EDITION

WHILE the original purpose of this book—to present clearly and concisely the various laboratory methods which are of use in clinical medicine—has not been lost sight of, its scope has been somewhat enlarged in the present edition.

Each section has been carefully revised and much new material has been added to every chapter. Among the many additions may be mentioned: the use of artificial light and the importance of numerical aperture in microscopic work; photomicrography with simple apparatus; the antiformin method for tubercle bacilli; detection and significance of albumin in the sputum; Tsuchiya's modification of Esbach's test; the formalin test for ammonia and Benedict's methods for sugar in urine; volume index of red blood-corpuscles; Wright and Kinnicutt's method of counting blood-platelets; Harlow's blood-stain; a simple technic for the diagnosis of typhoid fever by blood-cultures; the Wassermann reaction, and Frothingham's impression method in the diagnosis of rabies.

Because of the growing importance of animal parasites, this chapter has been entirely rewritten and more than doubled in extent. Two new chapters have been added: one upon Bacteriologic Methods, which supplements the methods given in other portions of the book, and one upon Preparation and Use of Vaccines, including therapeutic and diagnostic use of tuberculin.

Some of the illustrations have been replaced with better ones and many new pictures have been added, including eight photomicrographs by Dr. W. P. Harlow, and a considerable number by the author. Through courtesy of Dr. Langdon Frothingham, of Harvard University, a colored plate showing Negri bodies as seen in his impression method has been included.

The author wishes to express his indebtedness to Francis Ramaley, Ph.D., Professor of Biology in the University of Colorado, and T. D. A. Cockerell, Professor of Systematic Zoology, for suggestions as to the nomenclature of animal parasites; and to Dr. A. R. Peebles, Professor of Medicine, for suggestions and aid throughout the revision.

J. C. T.

BOULDER, COLORADO.

PREFACE

THIS book aims to present a clear and concise statement of the more important laboratory methods which have clinical value, and a brief guide to interpretation of results. It is designed for the student and practitioner, not for the trained laboratory worker. It had its origin some years ago in a short set of notes which the author dictated to his classes, and has gradually grown by the addition each year of such matter as the year's teaching suggested. The eagerness and care with which the students and some practitioners took these notes and used them convinced the writer of the need of a volume of this scope.

The methods offered are practical; and as far as possible are those which require the least complicated apparatus and the least expenditure of time. Simplicity has been considered to be more essential than absolute accuracy. Although in many places the reader is given the choice of several methods to the same end, the author believes it better to learn one method well than to learn several only partially.

More can be learned from a good picture than from any description, hence especial attention has been given to the illustrations, and it is hoped that they will serve truly to *illustrate*. Practically all the microscopic struc-

tures mentioned, all apparatus not in general use, and many of the color reactions are shown in the pictures.

Although no credit is given in the text, the recent medical periodicals and the various standard works have been freely consulted. Among authors whose writings have been especially helpful may be mentioned v. Jaksch, Boston, Simon, Wood, Emerson, Purdy, Ogden, Ewald, Ehrlich and Lazarus, Da Costa, Cabot, Osler, Stengel, and McFarland.

The author wishes hereby to express his indebtedness to Dr. J. A. Wilder, Professor of Pathology in the Denver and Gross College of Medicine, for aid in the final revision of the manuscript; and to W. D. Engel, Ph.D., Professor of Chemistry, for suggestions in regard to detection of drugs in the urine. He desires to acknowledge the care with which Mr. Ira D. Cassidy has made the original drawings, and also the uniform courtesy of W. B. Saunders Company during the preparation of the book.

J. C. T.

DENVER, COLORADO.

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INTRODUCTION

USE OF THE MICROSCOPE

THERE is probably no laboratory instrument whose usefulness depends so much upon proper manipulation as the microscope, and none is so frequently misused by beginners. Some suggestions as to its proper use are, therefore, given at this place. It is presumed that the reader is already familiar with its general construction (Fig. 1).

For those who wish to understand the principles of the microscope and its manipulation—and best results are impossible without such an understanding—a careful study of some stan-

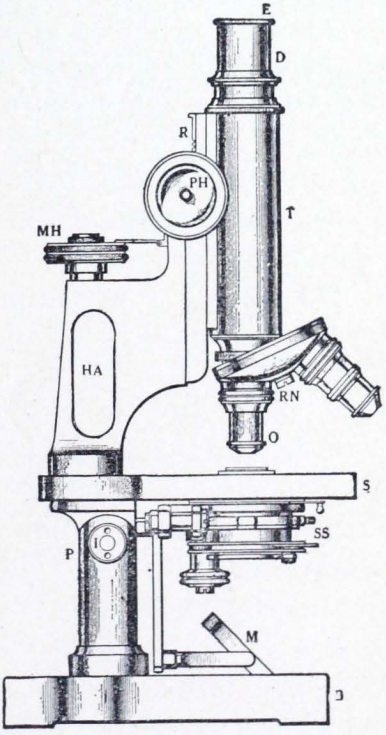


Fig. 1.—Handle-arm microscope: E, Eyepiece; D, draw-tube; T, body-tube; RN, revolving nose-piece; O, objective; PH, pinion head; MH, micrometer head; HA, handle-arm; SS, substage; S, stage; M, mirror; B, base; R, rack; P, pillar; I, inclination joint.

dard work upon microscopy, such as those of Carpenter, Spitta, and Sir A. E. Wright, is earnestly recommended. It is also recommended that the beginner provide himself with some slides of diatoms, for example, *Pleurosigma angulatum*, *Surirella gemma*, and *Amphipleura pellucida*, costing fifty cents each. Faithful practice upon such test-objects, in the light of the principles of microscopy, will enable the student to reach, intelligently, an accuracy in manipulation to which the ordinary laboratory worker attains only slowly and by rule of thumb. He will soon find that the bringing of an object into accurate focus is by no means all of microscopy.

Illumination.—Good work cannot be done without proper illumination. It is difficult to lay too much stress upon this point.

The light which is generally recommended as best is that from a white cloud, the microscope being placed by preference at a north window, to avoid direct sunlight. Such light is satisfactory for all ordinary work. Artificial light is, however, imperative for those who must work at night, and is a great convenience at all times. Properly regulated artificial light, moreover, offers decided advantages over daylight for critical work. Almost any strong light which is diffused through a frosted globe will give fair results. The inverted Welsbach light with such a globe is excellent. The following plan is much used abroad, and gives results equal to the best daylight: A Welsbach lamp or strong electric light is used, and a glass globe—a six-inch round-bottom flask answers admirably—is placed between it and the microscope, to act as a condenser (Fig. 2).

The flask should be at a distance equal to its diameter from both the light and the mirror of the microscope. In order to filter out the yellow rays the flask is filled with water to which have been added a few crystals of copper sulphate and a little ammonia.

For critical work, the method suggested by Sir A. E. Wright is to be preferred. He has shown that fog is dispelled and definition is improved if the size of the light source is so regulated that its image, thrown upon

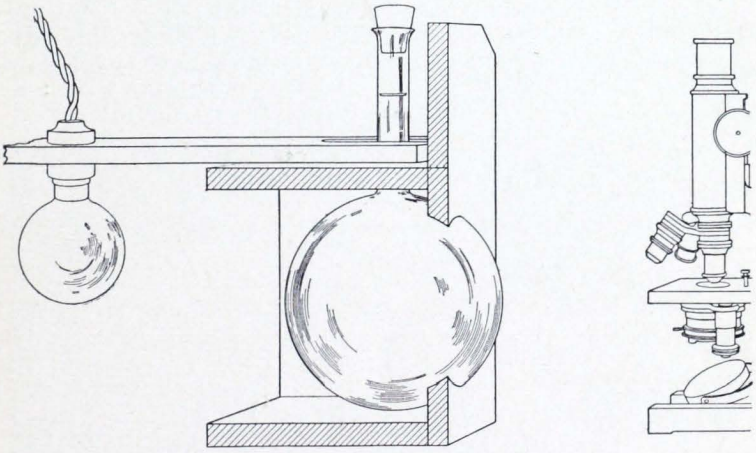


Fig. 2.—Illumination with water-bottle condenser.

the slide by the condenser, coincides with the real field of the objective. Upon this principle a very neat and satisfactory microscope lamp, shown in Fig. 3, has been designed by B. H. Matthews. It is fitted with iris-diaphragm, condensing lens, small electric light, and reflector, and has a slot in which a ray filter or ground-glass disc may be inserted.

Illumination may be either *central* or *oblique*. Central illumination is to be used for all routine work. To ob-

tain this, the mirror should be so adjusted that the light from the source selected is reflected directly up the tube of the microscope. This is easily done by removing the eye-piece and looking down the tube while adjusting the mirror. The eye-piece is then replaced, and the light reduced as much as desired by means of the diaphragm. With daylight, it is best to use the plane mirror; with artificial light, the concave mirror.

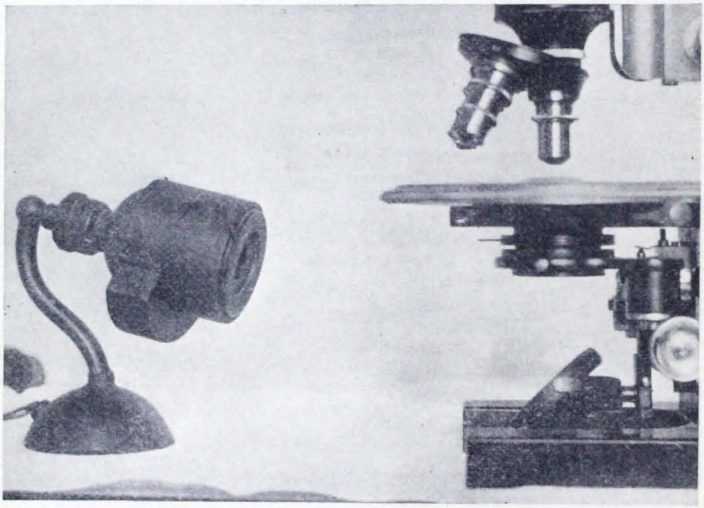
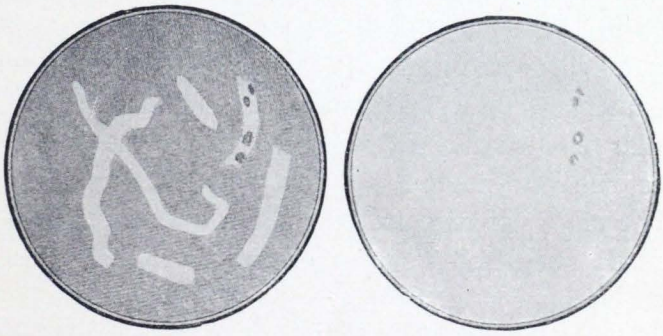


Fig. 3.—Matthews' microscope lamp with iris-diaphragm.

Oblique illumination is to be used only to bring out certain structures more clearly after viewing them by central light: as, for example, to show the edges of a hyaline cast by throwing one of its sides into shadow. Oblique illumination is obtained in the more simple instruments by swinging the mirror to one side, so that the light enters the microscope obliquely. The more complicated instruments obtain it by means of a rack

and pinion, which moves the diaphragm laterally. Beginners frequently use oblique illumination without recognizing it, and are thereby much confused. If the light be oblique, an object in the center of the field will appear to move from side to side when the fine adjustment is turned back and forth.

The amount of light is even more important than its direction. It is regulated by the diaphragm. *It is always best to use the least light that will show the object*



a *b*

Fig. 4.—*a*, Hyaline casts, one containing renal cells; properly subdued illumination; *b*, same as *a*; strong illumination. The casts are lost in the glare, and only the renal cells are seen. (From Greene's "Medical Diagnosis").

well. Unstained objects require very subdued light. Beginners constantly use it too strong. Strong light will often render semitransparent structures, as hyaline casts, entirely invisible (Fig. 4). Stained objects, especially bacteria, require much greater light.

Dark Ground Illumination.—This consists in cutting out the central rays of light and directing the peripheral rays against the object from the side. Only those rays which strike the object and are reflected pass into the objective. The object is bright upon a black back-

ground. By means of this form of illumination very minute structures can be seen, just as particles of dust in the atmosphere become visible when a ray of sunlight enters a darkened room.

Dark ground illumination for low-power work can be obtained by means of the ring stops with central discs which accompany most microscopes when purchased. The stop is placed in a special ring beneath the condenser. By varying the size of the central disc good results can be had with the lower power dry lenses.

For oil-immersion work a special condenser is necessary. With some makes it is placed upon the stage of the microscope; with others it is substituted for the regular condenser. It requires an intense light, like direct sunlight or the Liliput arc-light.

The chief use of dark ground illumination in clinical work is for demonstration of *Treponema pallidum* in fresh material (Fig. 158).

The Condenser.—For the work of the clinical laboratory a substage condenser is a necessity. Its purpose is to condense the light upon the object to be examined. For critical work the light must be focused on the object by raising or lowering the condenser by means of the screw provided for the purpose. The image of the light source will then appear in the plane of the object. This is best seen by using a low-power objective and ocular. Should the image of the window-frame or other nearby object appear in the field and prove annoying, the condenser may be raised or lowered a little. It is often advised to remove the condenser for certain kinds of work, but this is not necessary and is seldom desirable.

It is very important that the condenser be accurately

centered, and most high-grade instruments have centering screws by which it can be adjusted at any time. The simplest way to recognize whether the condenser is centered is to close the diaphragm beneath it to as small an opening as possible, then remove the eye-piece and look down the tube. If the diaphragm opening does not appear in the center of the field, the condenser is out of center.

The use of the condenser is further discussed in the following section.

Objectives and Eye-pieces.—Unfortunately, different makers use different systems of designating their lenses. The best system, and the one chiefly used in this country, is to designate objectives by their focal lengths in millimeters, and eye-pieces by their magnifying power, indicated by an " ×." Most foreign makers use this system for their high-grade lenses, but still cling to arbitrary letters or numbers for their ordinary output.

Objectives are of two classes—achromatic and apochromatic. Those in general use are of the achromatic type, and they fulfil all requirements for ordinary work. Apochromatic objectives are more highly corrected for chromatic and spheric aberration, and represent the highest type of microscope lenses produced. They are very desirable for photomicrographic and research work, but for routine laboratory work do not offer advantages commensurate with their great cost. They require the use of special " compensating " eye-pieces.

The " working distance " of an objective should not be confused with its focal distance. The former term refers to the distance between the front lens of the objective, when it is in focus, and the cover-glass. It is

always less than the focal distance, since the "focal point" lies somewhere within the objective; and it varies considerably with different makes. Long working distance is a very desirable feature.

Objectives are "corrected" for use under certain fixed conditions, and *they will give the best results only when used under the conditions for which corrected.* The most important corrections are: (*a*) For tube-length; (*b*) for thickness of cover-glass; and (*c*) for the medium between objective and cover-glass.

(*a*) The tube-length with which an objective is to be used is usually engraved upon it—in most cases it is 160 mm. The draw-tube of the microscope should be pulled out until the proper length is obtained, as indicated by the graduations on its side. When a nose-piece is used, it adds about 15 mm. to the tube-length, and the draw-tube must be pushed in for that distance.

(*b*) The average No. 2 cover-glass is about the thickness for which most objectives are corrected—usually 0.17 or 0.18 mm. Very low powers and oil-immersion objectives do not require any cover-glass. A cover should always be used with high dry lenses, but its exact thickness is more important in theory than in practice. Many immersion objectives have such short working distance that only very thin covers can be used.

(*c*) The correction for the medium between objective and cover-glass is very important. This medium may be either air or some fluid, and the objective is hence either a "dry" or an "immersion" objective. The immersion fluid generally used is cedar oil, which gives great optical advantages because its index of refraction is the same as that of crown glass. It is obvious that only objectives

with very short working distance, as the 2 mm., can be used with an immersion fluid.

To use an oil-immersion objective a drop of the cedar oil which is prepared for the purpose should be placed upon the cover, and the objective lowered into it and then brought to a focus in the usual way.

Bubbles in the oil are a frequent source of trouble, and should always be looked for when an immersion objective does poor work. They are readily seen by removing the eye-piece and looking down the tube. Immediately after use the oil should be removed with lens-paper or a soft linen handkerchief.

A useful " pointer " can be made by placing a straight piece of a hair across the opening of the diaphragm of the eye-piece, cementing one end with a tiny drop of balsam, and cutting the hair in two in the middle. When the eye-piece is in place, the hair appears as a black line extending from the periphery to the center of the microscopic field.

Numeric Aperture.—This expression, usually written N. A., indicates the amount of light which enters an objective from a point in the microscopic field. In optical language, N. A. is the sine of one-half the angle of aperture multiplied by the index of refraction of the medium between the cover and the front lens. Numeric aperture is extremely important, because upon it depends *resolving power*, which is the most important property of an objective.[1]

[1] Resolving power really depends upon two factors, the N. A. and the wave length of light, but the latter can be ignored in practice. The great resolving power of the ultra-microscope depends upon its use of light of short wave length.

Resolving power is the ability to separate minute details of structure. For example, the dark portions of a good half-tone picture appear gray or black to the unaided eye, but a lens easily resolves this apparently uniform surface into a series of separate dots. Resolving power does not depend upon magnification. The fine lines and dots upon certain diatoms may be brought out clearly and crisply (*i. e.*, they are resolved) by an objective of high numeric aperture, whereas with an objective of lower numeric aperture, but greater magnifying power, the same diatom may appear to have a smooth surface, with no markings at all, no matter how greatly it is magnified. Knowing the N. A., it is possible to calculate how closely lines and dots may lie and still be resolved by a given objective. To state the numeric aperture, therefore, is to tell what the objective can accomplish; provided, of course, that spheric and chromatic aberrations are satisfactorily corrected. An objective's N. A. is usually engraved upon the mounting.

It is an important fact, and one almost universally overlooked by practical microscopists, that the proportion of the numeric aperture of an objective which is *utilized* depends upon the aperture of the cone of light delivered by the condenser. In practice, the numeric aperture of an objective is reduced nearly to that of the condenser (which is indicated by lower-case letters, n. a.).[1] The condenser should, therefore, have a numeric aperture at least equal to that of the objective with which it is to be used. Lowering the condenser

[1] The N. A. of the objective is not reduced wholly to that of the condenser, because, owing to difraction phenomena, a small part of the unilluminated portion of the black lens is utilized.

below its focal distance and closing the diaphragm beneath it have the effect of reducing its working aperture. A condenser, whatever its numeric aperture, cannot deliver through the air a cone of light of greater n. a. than 1. It follows, therefore, that the proper adjustment of the substage condenser is a matter of great importance when using objectives of high N. A., and that, to gain the full benefit of the resolving power of such objectives, the condenser must be focused on the object under examination, it must be oiled to the under surface of the slide in the same way as the immersion objective is oiled to the cover-glass, and the substage diaphragm must be wide open. The last condition introduces a difficulty in that colorless structures will appear "fogged" in a glare of light (Fig. 4). Wright suggests that the size of the light source be so regulated by a diaphragm that its image, thrown on the slide by the condenser, coincides with the real field of the objective. and maintains that in this way it is possible to reduce the glare of light and to dispel the fog without closing the diaphragm of the condenser.

One can easily determine how much of the aperture of an objective is in use by removing the eye-piece, looking down the tube, and observing what proportion of the back lens of the objective is illuminated. The relation of the illuminated central portion to the unilluminated peripheral zone indicates the proportion of the numeric aperture in use. The effect of raising and lowering the condenser and of oiling it to the slide can thus be easily seen.

Magnification.—The degree of magnification should always be expressed in *diameters*, not *times*, which is a

misleading term. The former refers to increase of *diameter;* the latter, to increase of *area.* The comparatively low magnification of 100 diameters is the same as the apparently enormous magnification of 10,000 times.

The magnifying power of a lens is obtained by dividing 250 mm., or 10 inches (the distance of normal vision), by the focal length of the lens. The focal length of an objective is approximately twice the diameter of the front lens. Thus, the 2 mm. objective gives a magnification of 125 diameters; the 25 mm. eye-piece gives a magnification of 10 diameters, and is usually designated as a 10× eye-piece. When an objective and eye-piece are used together, the total magnification is the product of the two. In the case just cited the total magnification would be 1250 diameters. In practice, magnification can be increased in one of three ways:

(*a*) *Drawing out the tube.* Since the increased tube-length interferes with spheric correction, it should be used only with the knowledge that an imperfect image will result.

(*b*) *Using a higher power objective.* As a rule, this is the best way, because resolving power is also increased; but it is often undesirable because of the shorter working distance, and because the higher objective often gives greater magnification than is desired, or cuts down the size of the real field to too great an extent.

(*c*) *Using a shorter eye-piece.* This is the simplest method. It has, however, certain limitations. When too high an eye-piece is used, there results a hazy image in which no structural detail is seen clearly. This is called "empty magnification," and depends upon the fact that the objective has not sufficient resolving power

to support the high magnification. The extent to which magnification can be satisfactorily increased by eye-piecing depends wholly upon the resolving power of the objective, and consequently upon the N. A. The greatest total or combined magnification which will give an *absolutely* crisp picture is found by multiplying the N. A. of an objective by 400. The greatest magnification which can be used at all satisfactorily is 1000 times the N. A. For example: The ordinary 2 mm. objective has a N. A. of 1.30; the greatest magnification which will give an absolutely sharp picture is 520 diameters, which is obtained approximately by using a 4× eye-piece. Higher eye-pieces can be used, up to a total magnification of 1300 diameters (10× eye-piece), beyond which the image becomes wholly unsatisfactory.

Focusing.—It is always best to "focus up," which saves annoyance and probable damage to slides and objectives. This is accomplished by bringing the objective nearer the slide than the proper focus, and then, with the eye at the eye-piece, turning the tube up until the object is clearly seen. *The fine adjustment should be used only to get an exact focus with the higher power objectives after the instrument is in approximate focus.* It should not be turned more than one revolution.

There will be less fatigue to the eyes if both are kept open while using the microscope, and if no effort is made to see objects which are out of distinct focus. Fine focusing should be done with the fine adjustment, not with the eye. An experienced microscopist keeps his fingers almost constantly upon one or other of the focusing adjustments. Greater skill in recognizing objects will be acquired if the same eye be always used. To be

seen most clearly, an object should be brought to the center of the field.

Care of the Microscope.—The microscope is a delicate instrument and should be handled accordingly. It is so heavy that one is apt to forget that parts of it are fragile. It seems unnecessary to say that when there is unusual resistance to any manipulation, force should never be used to overcome it until its cause has first been sought; and yet it is no uncommon thing to see students, and even graduates, push a high-power objective against a microscopic preparation with such force as to break not only the cover-glass, but even a heavy slide.

It is most convenient to carry a microscope with the fingers grasping the pillar and the arm which holds the tube; but since this throws a strain upon the fine adjustment, it is safer to carry it by the base. In the more recent instruments a convenient handle-arm is provided. To bend the instrument at the joint, the force should be applied to the pillar and never to the tube or the stage.

Lens surfaces which have been exposed to dust only should be cleaned with a camel's-hair brush. Those which are exposed to finger-marks should be cleaned with lens paper, or a soft linen handkerchief wet with saliva. Particles of dirt which are seen in the field are upon the slide, the eye-piece, or the condenser. Their location can be determined by moving the slide, rotating the eye-piece, and lowering the condenser. When the image is hazy, the objective probably needs cleaning; or in case of an oil-immersion lens, there may be bubbles in the oil.

Oil and balsam which have dried upon the lenses and resist saliva may be removed with alcohol or xylol; but

these solvents must be used sparingly and carefully, as there is danger of softening the cement. Care must be taken not to get any alcohol upon the brass parts, as it will remove the lacquer. Balsam and dried oil are best removed from the brass parts with xylol.

Choice of a Microscope.—It is poor economy to buy a cheap instrument.

For the work of a clinical laboratory the microscope should preferably be of the new handle-arm type, and should have a large stage. It should be provided with a substage condenser (preferably of 1.40 n. a.), three or more objectives, and two or more eye-pieces.

The most generally useful objectives are: 16 mm., 4 mm., and 2 mm. oil immersion. The 4 mm. objective may be obtained with N. A. of 0.65 or 0.85. If it is to be used for blood-counting, the former is preferable, since its working distance is sufficient to take the thick cover of the Thoma-Zeiss instrument. For coarse objects a 32 mm. objective is very desirable. The eye-pieces most frequently used are 4× and 8×. A very low power (2×) and a very high (18×) will sometimes be found useful. The micrometer eye-piece is almost a necessity. A mechanical stage, preferably of the attachable type, is almost indispensable for blood and certain other work.

A first-class microscope, of either American or foreign make, equipped as just described, will cost in the neighborhood of a hundred dollars, exclusive of the mechanical stage.

Measurement of Microscopic Objects.—Of the several methods, the most convenient is the use of a micrometer eye-piece. In its simplest form this is similar to an ordinary eye-piece, but has within it a glass disc upon

which is ruled a graduated scale. When this eye-piece is placed in the tube of the microscope, the ruled lines appear in the microscopic field, and the size of an object is readily determined in *terms of the divisions of this scale.* The value of these divisions in inches or millimeters manifestly varies with different magnifications. Their value must, therefore, be determined separately for each objective. This is accomplished through use of a stage micrometer—a glass slide with carefully ruled scale divided into hundredths and thousandths of an inch, or into subdivisions of a millimeter. The stage micrometer is placed upon the stage of the microscope and brought into focus. From the number of divisions of the eye-piece scale corresponding to each division of the stage micrometer the value of the former in fractions of an inch or millimeter is easily calculated. The counting slide of the Thoma-Zeiss hemocytometer will answer in place of a stage micrometer, the lines which form the sides of the small squares being one-twentieth of a millimeter apart. Any eye-piece can be converted into a micrometer eye-piece by placing a micrometer disc—a small circular glass plate with ruled scale—ruled side down upon its diaphragm.

The principal microscopic objects which are measured clinically are animal parasites and their ova and abnormal blood-corpuscles. The metric system is used almost exclusively. For very small objects 0.001 mm. has been adopted as the unit of measurement, under the name *micron.* It is represented by the Greek letter μ. For larger objects, where exact measurement is not essential, the diameter of a red blood-corpuscle (7 to 8 μ) is sometimes taken as a unit.

Tuttle has suggested that in feces and other examinations a little lycopodium powder be mixed with the material. The granules are of uniform size—30 μ in diameter—and are easily recognized (Fig. 5). They furnish a useful standard with which the size of other structures can be compared.

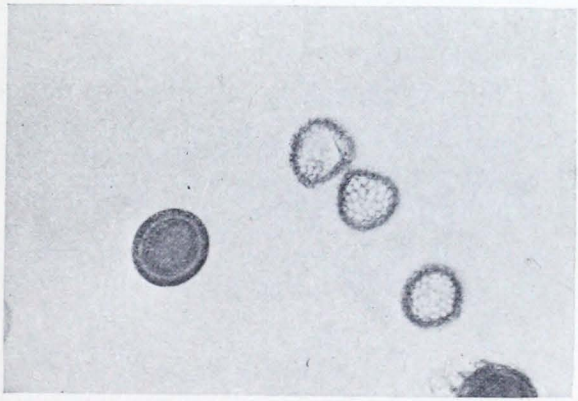


Fig. 5.—Egg of Tænia saginata. Lycopodium granules used as micrometer (× 250) (photograph by the author).

Photomicrography.—Very satisfactory pictures of microscopic structures can be made by any one with simple apparatus.

Any camera with focusing screen or a Kodak with plate attachment may be used. It is best, but not necessary, to remove the photographic lens. The camera is placed with the lens (or lens-opening, if the lens has been removed) looking into the eye-piece of the microscope, which may be in either the vertical or the horizontal position. One can easily rig up a standard to which the camera can be attached in the proper position by means of a tripod screw. A light-tight connection can be made

of a cylinder of paper or a cloth sleeve with draw-strings. The image will be thrown upon the ground-glass focusing screen, and is focused by means of the fine adjustment of the microscope. The degree of magnification is ascertained by placing the ruled plate of the blood-counting instrument upon the microscope and measuring the image on the screen. The desired magnification is obtained by changing objectives or eye-pieces or lengthening the camera-draw.

Focusing is comparatively easy with low powers, but when using an oil-immersion objective, it is a difficult problem unless the source of light be very brilliant. If one always uses the same length of camera and microscope tube, a good plan is as follows: Ascertain by trial with a strong light how far the fine adjustment screw must be turned from the correct eye focus to bring the image into sharp focus upon the ground-glass screen. At any future time one has only to focus accurately with the eye, bring the camera into position, and turn the fine adjustment the required distance to right or left.

The light should be as intense as possible, in order to shorten exposure, but any light that is satisfactory for ordinary microscopic work will answer. It is nearly always necessary to insert a color screen between the light and the microscope. Pieces of colored window-glass are useful for this purpose. The screen should have a color complementary to that which it is desired to bring out strongly in the photograph: for blue structures, a yellow screen; for red structures, a green screen. For the average stained preparation, a picric-acid yellow or a yellow green will be found satisfactory.

Very fair pictures can be made on Kodak film, but

orthochromatic plates (of which Cramer's " Iso " and Seed's " Ortho " are examples) give much better results. The length of exposure depends upon so many factors that it can be determined only by trial. It will probably vary from a few seconds to fifteen minutes. Plates are developed in the usual way,—the tank method yielding most uniform and satisfactory results,—but in order to secure all the contrast possible, they should be considerably overdeveloped.

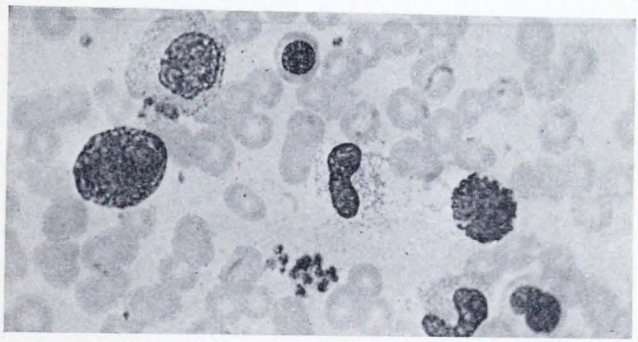


Fig. 6.—Leukemic blood (about × 650). Photograph taken with a kodak, as described in the text.

The photograph from which Fig. 6 was made was taken with a Kodak and plate attachment on an " Iso " plate, the source of light being the electric lamp and condensing lens illustrated in Fig. 2. It was focused by the method described above. The screen was a picric-acid stained photographic plate. Exposure, three and a half minutes. The picture loses considerable detail in reproduction.

CHAPTER I

THE SPUTUM

Preliminary Considerations.—Before beginning the study of the sputum, the student will do well to familiarize himself with the structures which may be present in the normal mouth, and which frequently appear in the sputum as contaminations. Nasal mucus and material obtained by scraping the tongue and about the teeth should be studied as described for unstained sputum. A drop of Lugol's solution should then be placed at the edge of the cover, and, as it runs under, the effect upon different structures noted. Another portion should be spread upon slides or covers and stained by some simple stain and by Gram's method. The structures likely to be encountered are epithelial cells of columnar and squamous types, leukocytes, food-particles, *Leptothrix buccalis*, and great numbers of saprophytic bacteria, frequently including spirochetes. These structures are described later.

The morning sputum or the whole amount for twenty-four hours should be collected for examination. In beginning tuberculosis tubercle bacilli can often be found in that first coughed up in the morning when they cannot be detected at any other time of day. Sometimes, in these early cases, there are only a few mucopurulent flakes which contain the bacilli, or only a small purulent mass every few days, and these may easily be overlooked.

Patients should be instructed to rinse the mouth well in order to avoid contamination with food-particles which may prove confusing in the examination, and to make sure that the sputum comes from the lungs or bronchi and not from the nose and nasopharynx. Many persons find it difficult to distinguish between the two. It is always desirable that the material be raised with a distinct expulsive cough, but this is not always possible. Material from the upper air-passages can usually be identified from the large proportion of mucus and the character of the epithelial cells.

As a receptacle for the sputum, a clean, wide-mouthed bottle with tightly fitting cork may be used. The patient must be particularly cautioned against smearing any of it upon the outside of the bottle. This is probably the chief source of danger to those who examine sputum. Disinfectants should not be added. They so alter the character of the sputum as to render it unfit for satisfactory examination.

When the examination is begun, the material should be spread out in a thin layer in a Petri dish, or between two small plates of glass, like photographic plates. It may then be examined with the naked eye—best over a black background—or with a low power of the microscope. The portions most suitable for further examination may thus be easily selected. *This macroscopic examination should never be omitted.*

After an examination the sputum must be destroyed by heat or chemicals, and everything which has come in contact with it must be sterilized. The utmost care must be taken not to allow any of it to dry and become disseminated through the air. It is a good plan to con-

duct the examination upon a large newspaper, which can then be burned. Contamination of the work table is thus avoided. If this is not feasible, the table should be washed off with 10 per cent. lysol solution, and allowed to dry slowly, as soon as the sputum work is finished.

Examination of the sputum is most conveniently considered under four heads: I. Physical examination. II. Microscopic examination. III. Chemic examination. IV. Characteristics of the sputum in various diseases.

I. PHYSICAL EXAMINATION

1. Quantity.—The quantity expectorated in twenty-four hours varies greatly. It may be so slight as to be overlooked entirely in beginning tuberculosis. It is usually small in acute bronchitis and lobar pneumonia. It may be very large—sometimes as much as 1000 c.c.—in advanced tuberculosis with large cavities, edema of the lung, bronchiectasis, and following rupture of an abscess or empyema. It is desirable to obtain a general idea of the quantity, but accurate measurement is unnecessary.

2. Color.—Since the sputum ordinarily consists of varying proportions of mucus and pus, it may vary from a colorless, translucent mucus to an opaque, whitish or yellow, purulent mass. A yellowish green is frequently seen in advanced phthisis and chronic bronchitis. In jaundice, in caseous pneumonia, and in slowly resolving lobar pneumonia it may assume a bright green color, due to bile or altered blood-pigment.

A red color usually indicates the presence of blood. Bright red blood, most commonly in streaks, is strongly suggestive of phthisis. It may be noted very early in the disease. A rusty red sputum is the rule in croupous

pneumonia, and was at one time considered pathognomonic of the disease. " Prune-juice " sputum is said to be characteristic of " drunkard's pneumonia." It at least indicates a dangerous type of the disease. A brown color, due to altered blood-pigment, follows hemorrhages from the lungs, and is present, to greater or less degree, in chronic passive congestion of the lung, which is most frequently due to a heart lesion.

Gray or black sputum is observed among those who work much in coal-dust, and is occasionally seen in smokers who are accustomed to " inhale."

3. Consistence.—According to their consistence, sputa are usually classified as serous, mucoid, purulent, seropurulent, mucopurulent, etc., which names explain themselves. As a rule, the more mucus and the less pus and serum a sputum contains, the more tenacious it is.

The rusty sputum of croupous pneumonia is extremely tenacious, so that the vessel in which it is contained may be inverted without spilling it. The same is true of the almost purely mucoid sputum (" sputum crudum ") of beginning acute bronchitis, and of that which follows an attack of asthma. A purely serous sputum, usually slightly blood tinged, is fairly characteristic of edema of the lungs.

4. Dittrich's Plugs.—While these bodies sometimes appear in the sputum, they are more frequently expectorated alone. They are caseous masses, usually about the size of a pin-head, but sometimes reaching that of a bean. The smaller ones are yellow, the larger ones gray. When crushed, they emit a foul odor. Microscopically, they consist of granular débris, fat-globules, fatty acid crystals, and bacteria. They are formed

in the bronchi, and are sometimes expectorated by healthy persons, but are more frequent in putrid bronchitis and bronchiectasis. The laity commonly regard them as evidence of tuberculosis. The similar caseous masses which are formed in the crypts of the tonsils are sometimes also included under this name.

II. MICROSCOPIC EXAMINATION

The portions most likely to contain structures of interest should be very carefully selected, as already described. *The few minutes spent in this preliminary examination will sometimes save hours of work later.* Opaque, white or yellow particles are most frequently bits of food, but may be cheesy masses from the tonsils; small cheesy nodules, derived from tuberculous cavities and containing many tubercle bacilli and elastic fibers; Curschmann's spirals, or small fibrinous casts, coiled into little balls; or shreds of mucus with great numbers of entangled pus-corpuscles. The food-particles most apt to cause confusion are bits of bread, which can be recognized by the blue color which they assume when touched with iodin solution.

Some structures are best identified without staining; others require that the sputum be stained.

A. Unstained Sputum

A careful study of the unstained sputum should be included in every routine examination. It best reveals certain structures which are seen imperfectly or not at all in stained preparations. It gives a general idea of the other structures which are present, such as pus-

corpuscles, eosinophiles, epithelial cells, and blood, and thus suggests appropriate stains to be used later.

The particle selected for examination should be transferred to a clean slide, covered with a clean cover-glass, and examined with the 16 mm. objective, followed by the 4 mm. It is convenient to handle the bits of sputum with a wooden tooth-pick or with a wooden cotton-applicator, which may be burned when done with. The platinum wire used in bacteriologic work is less satisfactory because not usually stiff enough.

The more important structures to be seen in unstained sputum are: elastic fibers, Curschmann's spirals, Charcot-Leyden crystals, fibrinous casts, the ray fungus of actinomycosis, and molds. Pigmented cells, especially the so-called " heart-failure cells " (p. 62), are also best studied without staining (Plate II, Fig. 1).

1. Elastic Fibers.—These are the elastic fibers of the pulmonary substance (Fig. 7). When found in the sputum, they always indicate destructive disease of the lung, provided they do not come from the food, which is a not infrequent source. They are found most commonly in phthisis; rarely in other diseases. Advanced cases of tuberculosis often show great numbers, and, rarely, they may be found in early tuberculosis when the bacilli cannot be detected. In gangrene of the lung, contrary to the older teaching, elastic tissue is probably always present in the sputum, usually in large fragments.

The fibers should be searched for with a 16 mm. objective, although a higher power is needed to identify them with certainty. Under the 4 mm. they appear as slender, highly refractive fibers with double contour, and often curled or split ends. Frequently they are found

in alveolar arrangement, retaining the original outline of the alveoli of the lung (Fig. 7, *b*). This arrangement is positive proof of their origin in the lung. *Leptothrix buccalis*, which is a normal inhabitant of the mouth, may easily be mistaken for elastic tissue. It can be distinguished by running a little iodin solution under the cover-glass (see p. 56).

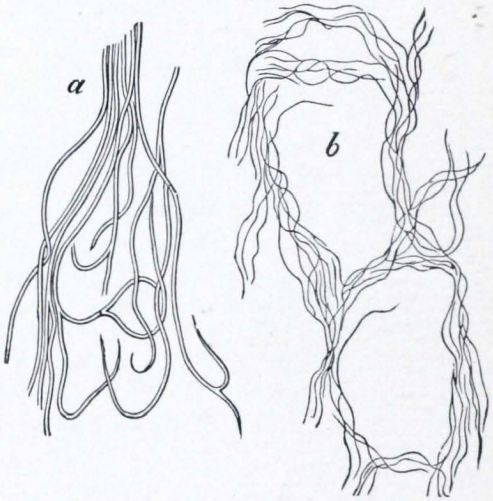


Fig. 7.—Elastic fibers from the sputum: *a*, Highly magnified; *b*, alveolar arrangement, less highly magnified (after Bizzozero).

Fatty-acid crystals, which are often present in Dittrich's plugs and in sputum which has lain in the body for some time, also simulate elastic tissue when very long, but they are more like stiff, straight or curved needles than wavy threads. They show varicosities when the cover-glass is pressed upon. The structures which most frequently confuse the student are the cotton fibrils which are present as a contamination in most

sputa. These are usually coarser than elastic fibers, and flat, with one or two twists, and often have longitudinal striations and frayed-out ends.

To find elastic fibers when not abundant, boil the sputum with a 10 per cent. solution of caustic soda until it becomes fluid; add several times its bulk of water, and centrifugalize, or allow to stand for twenty-four hours in a conical glass. Examine the sediment microscopically. The fibers will be pale and swollen and, therefore, somewhat difficult to recognize. Too long boiling will destroy them entirely.

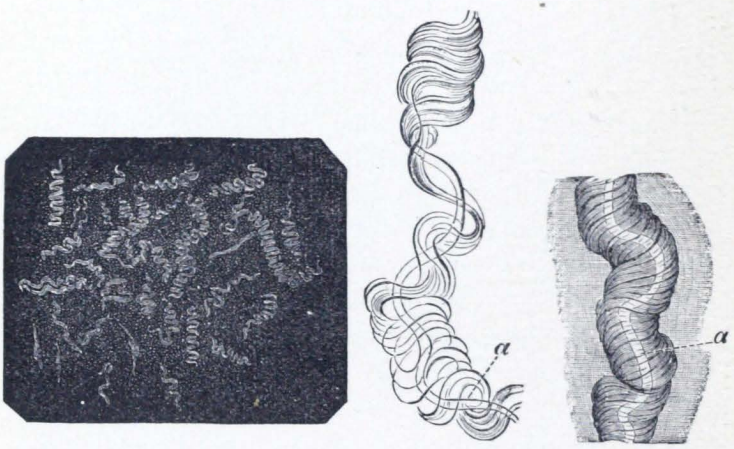
The above procedure, although widely recommended, will rarely or never be necessary if the sputum is carefully examined in a thin layer against a black background macroscopically and with a hand-lens, and if all suspicious portions are further studied with the microscope.

2. Curschmann's Spirals.—These peculiar structures are found most frequently in bronchial asthma, of which they are fairly characteristic. They may occasionally be met with in chronic bronchitis and other conditions. Their nature has not been definitely determined.

Macroscopically, they are whitish or yellow, twisted threads, frequently coiled into little balls (Fig. 8, I). Their length is rarely over half an inch, though it sometimes exceeds two inches. Under a 16 mm. objective they appear as mucous threads having a clear central fiber, about which are wound many fine fibrils (Fig. 8, II. and III.). Eosinophiles are usually present within them, and sometimes Charcot-Leyden crystals. Not infrequently the spirals are imperfectly formed, con-

sisting merely of twisted strands of mucus inclosing leukocytes. The central fiber is absent from these.

3. Charcot-Leyden Crystals.—Of the crystals which may be found in the sputum, the most interesting are the Charcot-Leyden crystals. They may be absent when the sputum is expectorated, and appear in large numbers after it has stood for some time. They are rarely found



I. II III.

Fig. 8.—Curschmann's spirals: I., Natural size; II. and III., enlarged: *a*, central fiber (after Curschmann).

except in cases of bronchial asthma, and were at one time thought to be the cause of the disease. They frequently adhere to Curschmann spirals. Their exact nature is unknown. Their formation seems to be in some way connected with the presence of eosinophilic cells. Outside of the sputum they are found in the feces in association with animal parasites, and in the coagulated blood in leukemia.

They are colorless, pointed, often needle-like, octahedral crystals (Fig. 9). Their size varies greatly, the average length being about three or four times the diameter of a red blood-corpuscle.

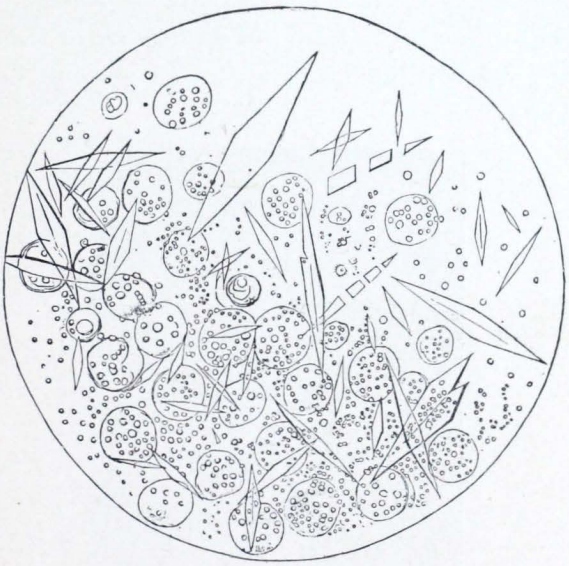


Fig. 9.—Charcot-Leyden crystals (after Riegel).

Other crystals—hematoidin, cholesterin, and, most frequently, fatty-acid needles (see Fig. 36)—are common in sputum which has remained in the body for a considerable time, as in abscess of the lung and bronchiectasis.

4. Fibrinous Casts.—These are casts of the bronchi, frequently, but not always, composed of fibrin. In color they are usually white or grayish, but may be reddish or brown, from the presence of blood-pigment. Their size varies with that of the bronchi in which they are formed. They may, rarely, be fifteen or more centimeters in length. When large, they can be recog-

nized with the naked eye by floating them out in water over a black surface; when small, a low power of the microscope must be used. Their branching, tree-like structure (Fig. 10) is usually sufficient to identify them.

Fibrinous casts are characteristic of fibrinous bronchitis, but may also be found in diphtheria of the smaller bronchi. Very small casts are often seen in croupous pneumonia.

Fig. 10.—Fibrinous bronchial cast (Sahli).

5. Actinomyces Bovis (Ray-fungus).—In the sputum of pulmonary actinomycosis and in the pus from actinomycotic lesions elsewhere small, yellowish, "sulphur" granules can be detected with the unaided eye. Without a careful macroscopic examination they are almost certain to be overlooked. The fungus can be seen by crushing one of these granules between slide and cover, and examining with a low power. It consists of a net-

work of threads having a more or less radial arrangement, those at the periphery presenting club-shaped extremities (Fig. 11). It can be brought out more clearly by running a little solution of eosin in alcohol and glycerin under the cover. This organism, also called *Streptothrix actinomyces*, apparently stands midway between the bacteria and the molds. It stains by Gram's method.

Actinomycosis of the lung is rare. The clinical picture is that of tuberculosis.

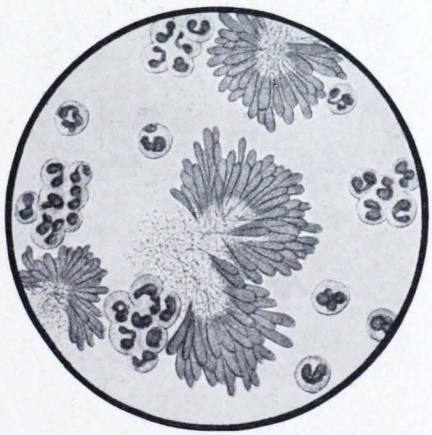


Fig. 11.—Sputum from a case of actinomycosis; stained (Jakob).

6. Molds and Yeasts.—The hyphæ and spores of various molds are occasionally met with in the sputum. They are usually the result of contamination, and have little significance. The hyphæ are rods, usually jointed or branched (Fig. 62), and often arranged in a meshwork (mycelium); the spores are highly refractive spheres. Both stain well with the ordinary stains.

In the extremely rare condition of systemic blastomycosis the specific yeasts have been found in the sputum

in large numbers. It is advisable to add a little 10 per cent. caustic soda solution and examine unstained.

7. Animal Parasites.—These are extremely rare in the sputum in this country. A trichomonad, perhaps identical with *Trichomonas vaginalis*, has been seen in the sputum of putrid bronchitis and gangrene of the lung, but its causal relationship is doubtful. In Japan, infection with the lung flukeworm, *Paragonimus westermani*, is common, and the ova are found in the sputum. The lung is not an uncommon seat for echinococcus cysts, and hooklets and scolices may appear, as may also *Amœba histolytica*, when a hepatic abscess has ruptured into the lung. Ciliated body-cells, with cilia in active motion, are not infrequently seen, and may easily be mistaken for infusoria. All the above-mentioned parasites are described in Chapter VI.

B. Stained Sputum

Structures which are best seen in stained sputum are bacteria and cells.

A number of smears should be made upon slides or covers, dried in the air, and fixed in the flame, as described on the next page. Fixation will kill the bacteria when covers are used, and the smears may be kept indefinitely; but smears on slides are often not sterile, and should be handled accordingly. One of the smears should be stained with some simple stain, like Löffler's methylene-blue, which will give a good idea of the various cells and bacteria present. Special stains may then be applied, as indicated, but a routine examination should, in all cases, include a stain by the method for the tubercle bacillus and by Gram's method.

1. Bacteria.—Saprophytic bacteria from mouth contamination are frequently present in large numbers and will prove confusing to the inexperienced. The presence of squamous cells in their neighborhood will suggest their source. Among the pathogenic organisms which have clinical importance are: tubercle bacilli; staphylococci and streptococci; pneumococci; bacilli of Friedländer; influenza bacilli, and *Micrococcus catarrhalis*.

(1) **Tubercle Bacillus.**—The presence of the tubercle bacillus may be taken as positive evidence of the existence of tuberculosis somewhere along the respiratory tract, most likely in the lung. In laryngeal tuberculosis it is not easily found in the sputum, but can frequently be detected in swabs made directly from the larynx.

Recognition of the tubercle bacillus depends upon the fact that it stains with difficulty; but that when once stained, it retains the stain tenaciously, even when treated with a mineral acid, which quickly removes the stain from other bacteria. This " acid-fast " property is due to the presence of a waxy capsule. The most convenient method for general purposes is here given in detail:

Gabbet's Method.—(1) Spread suspicious particles thinly and evenly upon a slide or a cover-glass held in the grasp of cover-glass forceps. In general, slides are more satisfactory, but cover-glasses are easier to handle while staining. Do not grasp a cover too near the edge or the stain will not stay on it well. Tenacious sputum will spread better if gently warmed while spreading.

(2) Dry the film in the air.

(3) Fix in a flame; *i. e.*, pass the cover-glass rather slowly, with film side up, three times (a slide about twelve times) through the flame of a Bunsen burner or alcohol lamp low down in the flame. Take care not to scorch. Should the film be washed off during future manipulations, fixation has been insufficient.

(4) Apply as much carbolfuchsin as will stay on, and hold over a flame so that it will steam for three minutes or longer, replacing the stain as it evaporates. If the bacilli are well stained in this step, there will be little danger of decolorizing them later. Too great heat will interfere with the staining of some of the bacilli, probably by destroying the waxy envelop upon which the acid-fast property depends. It is better to stain at room temperature for twelve to twenty-four hours.

(5) Wash the film in water.

(6) Apply Gabbet's stain to the under side of the cover-glass to remove excess of carbolfuchsin, and then to the film side. Allow this to act for one-fourth to one-half minute.

(7) Wash in water.

(8) If, now, the thinner portions of the film are blue, proceed to the next step; if they are still red, repeat steps (6) and (7) until the red has disappeared. Too long application of Gabbet's stain will decolorize the tubercle bacilli.

(9) Place the preparation between layers of filter-paper and dry by rubbing with the fingers, as one would in blotting ink.

(10) Put a drop of Canada balsam upon a clean slide, place the cover-glass film side down upon it, and examine with an immersion objective. Cedar oil or water may be used in place of balsam for temporary preparations. Smears on slides may be examined directly with an oil-immersion lens, no cover being necessary.

Carbolfuchsin is prepared by mixing 10 c.c. of a saturated alcoholic solution of fuchsin with 90 c.c. of 5 per cent. aqueous solution of phenol.

Gabbet's stain consists of methylene-blue, 2 gm.; 25 per cent. sulphuric acid, 100 c.c.

Both stains can be purchased ready prepared.

Other Methods.—The objection is often raised that decolorization is masked by the blue in Gabbet's stain, but this will not make trouble if step 8 is carefully carried out. The **Ziehl-Neelsen method** is preferred by many: After the staining with carbolfuchsin the smear is washed in 5 per cent. nitric acid until decolorized, washed in water, stained lightly with Löffler's methylene-blue, again washed, and mounted.

Pappenheim's Method.—This is the same as Gabbet's method, except that Pappenheim's methylene-blue solution is substituted for Gabbet's solution. This consists of:

Corallin (rosolic acid)	1 gm.
Absolute alcohol	100 c.c.

Saturate with methylene-blue and add 20 c.c. glycerin.

The method is very satisfactory for routine work. Decolorization of the tubercle bacillus is practically impossible: it retains its red color, even when soaked overnight in Pappenheim's solution. The stain was originally recommended as a means of differentiating the smegma bacillus, which is decolorized by it; but it is not to be absolutely relied upon for this purpose.

In films stained by these methods tubercle bacilli, if present, will be seen as slender red rods upon a blue background of mucus and cells (Plate II, Fig. 2). They average 3 to 4 μ in length—about one-half the diameter of a red blood-corpuscle. Beginners must be warned against mistaking the edges of cells, or particles which have retained the red stain, for bacilli. The appearance of the bacilli is almost always typical, and if there

seems room for doubt, the structure in question is probably not a tubercle bacillus. They may lie singly or in groups. They are very frequently bent and often have a beaded appearance. It is possible that the larger, beaded bacilli indicate a less active tuberculous process than do the smaller, uniformly stained ones. Sometimes they are present in great numbers—thousands in a field of the 2 mm. objective. Sometimes several cover-glasses must be examined to find a single bacillus. At times they are so few that none are found in stained smears, and special methods are required to detect them. The number may bear some relation to the severity of the disease, but this relation is by no means constant. The mucoid sputum from an incipient case sometimes contains great numbers, while sputum from large tuberculous cavities at times contains very few. Failure to find them is not conclusive, though their absence is much more significant when the sputum is purulent than when it is mucoid.

When they are not found in suspicious cases, one of the following methods should be tried:

(1) **Antiformin Method.**—This has lately come into use, and has superseded the older methods of concentration. The chief difficulty with the older methods, such as boiling with caustic soda, is that the bacilli are so injured in the process that they do not stain characteristically.

Antiformin is the patented name for a preparation consisting essentially of equal parts of a 15 per cent. solution of caustic soda and a 20 per cent. solution of sodium hypochlorite. It keeps fairly well. The sputum is thoroughly shaken in a corked bottle with one-fourth its volume of antiformin, and allowed to stand four to six hours in an incubator,

Fig. 1.—Heart-failure cells in sputum, containing blood-pigment, from a case of cardiac congestion of the lungs (Jakob).

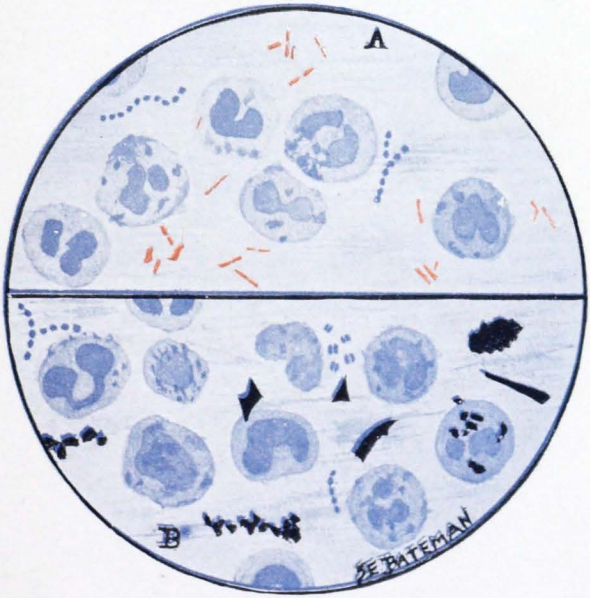


Fig. 2.—*A*, Sputum showing tubercle bacilli stained with carbolfuchsin and Gabbet's methylene-blue solution (obj. one-twelfth oil-immersion); *B*, sputum of anthracosis, showing particles of coal-dust stained with methylene-blue (obj. one-twelfth oil-immersion) (Boston).

or twenty-four hours at room-temperature. The sputum will be thoroughly liquefied. A centrifuge tubeful is thoroughly centrifugalized, the supernatant fluid is poured off and replaced with water, and centrifugalization is repeated. This washing is repeated several times. Some of the sediment is then spread upon slides (with a little egg-albumen or some of the untreated sputum to cause it to adhere), dried, fixed, and stained. The tubercle bacilli are not killed, but retain their form and staining properties unchanged. Other bacteria and cells are destroyed.

Since the bacilli remain alive, the utmost care must be used in handling, and all tubes and glassware which have come in contact with the liquefied sputum must be sterilized.

(2) **Animal Inoculation.**—Inoculation of guinea-pigs is the court of last appeal in detection of tubercle bacilli. The method is described on p. 375.

There are a number of bacilli, called *acid-fast bacilli*, which stain in the same way as the tubercle bacillus. They stain with difficulty, and when once stained, retain the color even when treated with a mineral acid; but, unlike the tubercle bacillus, most of them can be decolorized with alcohol. Of these, the smegma bacillus is the only one likely ever to cause confusion. It, or a similar bacillus, is sometimes found in the sputum of gangrene of the lung. It occurs normally about the glans penis and the clitoris, and is often present in the urine and in the wax of the ear. The method of distinguishing it from the tubercle bacillus is given later (p. 168).

Other bacteria than the acid-fast group are stained blue by Gabbet's method. Those most commonly found are staphylococci, streptococci, and pneumococci. Their

presence in company with the tubercle bacillus constitutes *mixed infection*, which is much more serious than single infection by the tubercle bacillus. It is to be remembered, however, that a few of these bacteria may reach the sputum from the upper air-passages. Clinically, mixed infection is evidenced by fever.

(2) **Staphylococcus and Streptococcus** (p. 368).—One or both of these organisms is commonly present in company with the tubercle bacillus in the sputum of advanced phthisis (Plate II, Fig. 2). They are often found in bronchitis, catarrhal pneumonia, and many other conditions.

(3) **Pneumococcus (Diplococcus of Fränkel)**.—The pneumococcus is the causative agent in nearly all cases of croupous pneumonia, and is commonly found in large numbers in the rusty sputum of this disease. It is sometimes met with in the sputum of catarrhal pneumonia, bronchitis, and tuberculosis. It is also an important factor in the causation of pleurisy, meningitis, otitis media, and other inflammations. It has been found in the saliva in health. Pneumococci are about the size of streptococci. They are ovoid in shape, and lie in pairs, end to end, often forming short chains. Each is surrounded by a gelatinous capsule, which is its distinctive feature (Fig. 12). Diplococci without capsules are common in the sputum, but have no special significance.

The pneumococcus is closely related to the streptococcus, and it is sometimes extremely difficult to differentiate them even by culture methods (for which see p. 368). The morphology of the pneumococcus, the fact that it is Gram-positive, and the presence of a capsule are, however, generally sufficient for its recognition

in smears from sputum or pus. The capsule is often seen as a halo around pairs of cocci in smears stained by the ordinary methods, particularly Gram's method, but to show it well special methods are required. There are numerous special methods of staining capsules which are applicable to other encapsulated bacteria, as well as to the pneumococcus, but few of them are satisfactory. Buerger's method can be recommended. It is especially useful with cultures upon serum media,

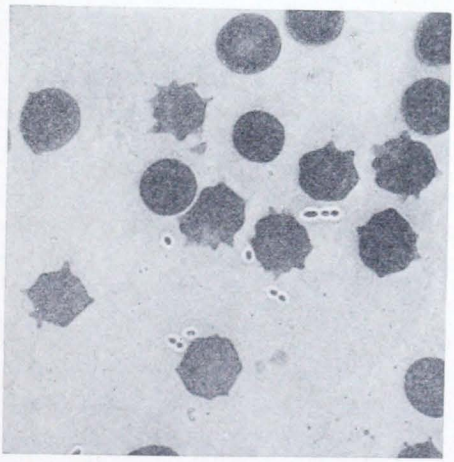


Fig. 12.—Diplococcus pneumoniæ in the blood (× 1000) (Fränkel and Pfeiffer).

but is applicable also to the sputum. Smith's new method is easier of application, and apparently gives uniformly good results. The India-ink method described for the organism of syphilis is likewise said to show capsules satisfactorily. The sputum should be fresh—not more than three or four hours old.

Buerger's Method for Capsules.—(1) Mix a few drops each of the sputum and blood-serum or egg-albumen solu-

tion (egg-albumen, distilled water, equal parts; shake and filter through cotton). Blood-serum can be obtained as described for the Widal test, p. 258. Make thin smears from the mixture, and just as the edges begin to dry, cover with Müller's fluid (potassium dichromate, 2.5 gm.; sodium sulphate, 1.0 gm.; water, 100 c.c.) saturated with mercuric chlorid (ordinarily about 5 per cent.). Gently warm over a flame for about three seconds.

(2) Rinse very quickly in water.

(3) Flush once with alcohol.

(4) Apply tincture of iodin for one to two minutes.

(5) Thoroughly wash off the iodin with alcohol and dry in the air.

(6) Stain about three seconds with weak anilin-gentian-violet freshly made up as follows: Anilin oil, 10; water, 100; shake; filter; and add 5 c.c. of a saturated alcoholic solution of gentian violet.

(7) Rinse off the stain with 2 per cent. solution of sodium chlorid, mount in this solution, and examine with a one-twelfth objective.

Buerger suggests a very useful variation as follows: After the alcohol wash and drying, the specimen is stained by Gram's method (p. 409), counterstained with aqueous solution of fuchsin, washed, and mounted in water. The pneumococcus holds the purple stain, while all capsules take on the pink counterstain.

Smith's Method.—(1) Make thin smears of the sputum or other material, which should be as fresh as possible.

(2) Fix in the flame in the usual manner.

(3) Apply a 10 per cent. aqueous solution of phosphomolybdic acid (Merck) for four to five seconds.

(4) Rinse in water.

(5) Apply anilin-gentian-violet, steaming gently for fifteen to thirty seconds.

(6) Rinse in water.

(7) Apply Gram's iodin solution, steaming gently for fifteen to thirty seconds.

(8) Wash in 95 per cent. alcohol until the purple color ceases to come off.

(9) Rinse in water.

(10) Apply a 6 per cent. aqueous solution of eosin (Grübler, w. g.), and gently warm for one-half to one minute.

(11) Rinse in water.

(12) Wash in absolute alcohol.

(13) Clear in xylol.

(14) Mount in balsam.

This is essentially Gram's method (see p. 409), preceded by treatment with phosphomolybdic acid and followed by eosin. Gram-positive bacteria like the pneumococcus are deep purple; capsules are pink and stand out clearly.

When the method is applied to Gram-negative bacteria, steps 5 to 9 inclusive are omitted; between steps 11 and 12 the preparation is counterstained with Löffler's methylene-blue, gently warming for fifteen to thirty seconds.

Anilin-gentian-violet.—Ehrlich's formula is the one generally used, but this keeps only a few weeks. Stirling's solution, which keeps much better and seems to give equal results, is as follows: gentian-violet, 5 gm.; alcohol, 10 c.c.; anilin oil, 2 c.c.; water, 88 c.c.

Formalin-gentian-violet is a satisfactory substitute for anilin-gentian-violet and is permanent. It consists of 5 per cent. solution formalin, 75 parts; saturated alcoholic solution gentian-violet, 25 parts.

Gram's Iodin Solution.—Iodin, 1 gm.; potassium iodid, 2 gm.; water, 300 c.c.

Löffler's alkaline methylene-blue is a very generally useful stain for bacteria. It is composed of 30 parts of a saturated alcoholic solution of methylene-blue and 100 parts of a 1 : 10,000 aqueous solution of caustic potash. It keeps indefinitely.

(4) **Bacillus of Friedländer (Bacillus Mucosus Capsulatus).**—In a small percentage of cases of pneumonia this organism is found alone or in company with the pneumococcus. Its pathologic significance is uncertain. It is often present in the respiratory tract under normal conditions. Friedländer's bacilli are non-motile, encapsulated rods, sometimes arranged in short chains (Fig. 13). Very short individuals in pairs closely resemble pneumococci, from which they are distinguished by the fact that they are Gram-decolorizing.

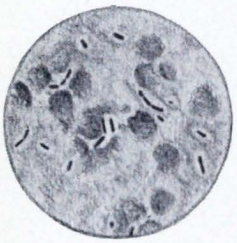


Fig. 13.—Friedländer's bacillus in pus from pulmonary abscess (obj. one-twelfth) (Boston).

(5) **Bacillus of Influenza.**—This is the etiologic factor in true influenza, although conditions which are clinically similar or identical may be caused by the pneumococcus, streptococcus, or *Micrococcus catarrhalis*. It is present, often in large numbers, in the nasal and bronchial secretions, and is also found in the local lesions following influenza. Chronic infection by influenza bacilli may be mistaken clinically for tuberculosis, and they should be searched for in all cases of obstinate chronic bronchitis.

Their recognition depends upon the facts that they are extremely small bacilli; that most of them lie within the pus-cells; that their ends stain more deeply than their centers, sometimes giving the appearance of minute diplococci; and that they are decolorized by Gram's method of staining (Figs. 14 and 149).

They are stained blue in the methods for tubercle bacilli, but are more certainly recognized by Gram's

method, followed by a counterstain. Pappenheim's pyronin-methyl-green stain is especially satisfactory.

(6) **Micrococcus Catarrhalis.**—This organism is frequently present in the sputum in inflammatory conditions of the respiratory tract resembling influenza. It is sometimes present in the nasal secretions in health. It is a Gram-negative diplococcus, frequently intracellular, and can be distinguished from the meningo-

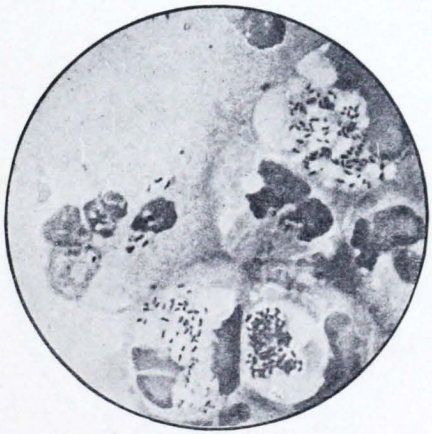


Fig. 14.—Bacillus of influenza; cover-glass preparation of sputum from a case of influenza, showing the bacilli in leukocytes; highly magnified (Pfeiffer).

coccus and gonococcus only by means of cultures. It grows readily on ordinary media.

2. Cells.—These include pus-corpuscles, epithelial cells, and red blood-corpuscles.

(1) **Pus-corpuscles** are present in every sputum, and at times the sputum may consist of little else. They are the polymorphonuclear leukocytes of the blood, and appear as rounded cells with several nuclei or one very irregular nucleus (Fig. 11 and Plate II, Fig. 2). They

are frequently filled with granules of coal-dust and are often much degenerated. Such coal-dust-laden leukocytes are especially abundant in anthracosis, where angular black particles, both intra- and extra-cellular, are often so numerous as to color the sputum (Plate II, Fig. 2, *B*). Occasionally mononuclear leukocytes are present.

Eosinophilic cells are quite constantly found in large numbers in the sputum of bronchial asthma near the

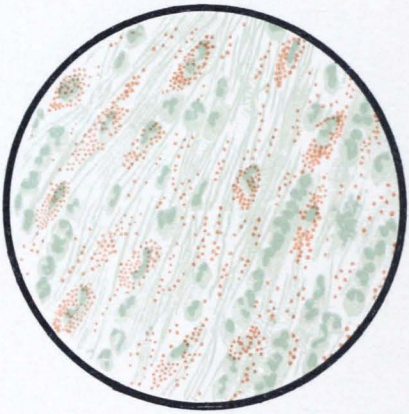


Fig. 15.—Sputum from a case of asthma showing leukocytes, some containing eosinophilic granules; free eosinophilic granules and micrococci; stained with eosin and methylene-blue (× 350) (Jakob).

time of the paroxysm, and constitute one of the most distinctive features of the sputum of this disease. They resemble ordinary pus-corpuscles, except that their cytoplasm is filled with coarse granules having a marked affinity for eosin. It is worthy of note that many of them, sometimes the majority, are mononuclear. Large numbers of free granules, derived from disintegrated cells, are also found (Fig. 15).

Ordinary pus-cells are easily recognized in sputum stained by any of the methods already given. For eosinophilic cells, some method which includes eosin must be used. A simple method is to stain the dried and fixed film two or three minutes with saturated solution of eosin, and then one-half to one minute with Löffler's methylene-blue; nuclei and bacteria will be blue, eosinophilic granules bright red.

(2) **Epithelial cells** may come from any part of the respiratory tract. A few are always present, since desquamation of cells goes on constantly. Their recognition is important chiefly as an aid in deciding upon the source of the portion of the sputum in which they are found. In suspected lung conditions it is manifestly useless to study material from the nose only, yet this is not infrequently done. They have little diagnostic value, although a considerable excess would indicate a pathologic condition at the site of their origin. Any of the stains mentioned above will show them, and they can usually be identified in unstained sputum. In general, three forms are found:

(*a*) *Squamous Cells.*—Large, flat, polygonal cells with a comparatively small nucleus (Fig. 16, *i*). They come from the upper air-passages, and are especially numerous in laryngitis and pharyngitis. They are frequently studded with bacteria—most commonly diplococci.

(*b*) *Cylindric Cells from the Nose, Trachea, and Bronchi* (Fig. 16, *f*, *h*).—These are not usually abundant, and, as a rule, they are not identified because much altered from their original form, being usually round and swollen. When very fresh, they may retain their cylindric form, sometimes bearing cilia in active motion.

(*c*) *Alveolar Cells.*—Rather large, round, or oval cells with one or two round nuclei (Fig. 16). Their source is presumably the pulmonary alveoli. Like the leukocytes, they frequently contain particles of carbon (normal lung pigment). In chronic heart disease, owing to long-continued passive congestion, they may be filled

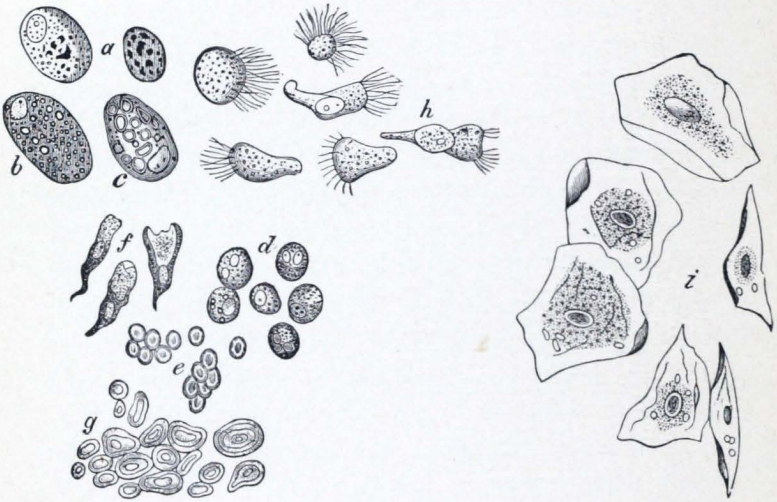


Fig. 16.—Different morphologic elements of the sputum (unstained): *a*, *b*, *c*, Pulmonary or alveolar epithelium—*a*, with normal lung pigment (carbon); *b*, with fat-droplets; *c*, with myelin globules; *d*, pus-corpuscles; *e*, red blood-corpuscles; *f*, cylindric beaker-shaped bronchial epithelial cells; *g*, free myelin globules; *h*, ciliated epithelium of different kinds from the nose, altered by coryza; *i*, squamous cells from the pharynx (after Bizzozero).

with brown granules of altered blood-pigment, and are then called "heart-failure cells." The presence of these cells in considerable numbers, by directing one's attention to the heart, will sometimes clear up the etiology of a chronic bronchitis. They are best seen in unstained sputum, appearing as grayish or colorless

balls filled with rounded granules of brown or yellow pigment. (Plate II, Fig. 1.)

Alveolar cells commonly contain fat-droplets and, less frequently, myelin globules. The latter are colorless, rounded bodies, sometimes resembling fat-droplets, but often showing concentric or irregularly spiral markings (Fig. 16, *c*, *g*). They are also found free in the sputum. They are abundant in the scanty morning sputum of apparently healthy persons, but may be present in any mucoid sputum.

(3) **Red blood-corpuscles** may be present in small numbers in almost any sputum. When fairly constantly present in considerable numbers, they are suggestive of phthisis. The corpuscles, when fresh, are shown by any of the staining methods which include eosin. They are commonly so much degenerated as to be unrecognizable, and often only altered blood-pigment is left. Ordinarily, blood in the sputum is sufficiently recognized with the naked eye.

III. CHEMIC EXAMINATION

There is little to be learned from a chemic examination, and it is rarely undertaken. Recently, however, it has been shown that the presence or absence of albumin may have clinical significance. Albumin is constantly present in the sputum in pneumonia, pulmonary edema, and tuberculosis. It is usually absent in bronchitis. A test for albumin may, therefore, be of great value in distinguishing between bronchitis and tuberculosis, a negative result practically proving the absence of tuberculosis. It is carried out as follows: The sputum is acidified with acetic acid to precipitate mucin and fil-

tered. If tenacious, it is first shaken up with water. The filtrate is then tested for albumin, as described in the chapter upon the Urine.

IV. THE SPUTUM IN DISEASE

Strictly speaking, any appreciable amount of sputum is abnormal. A great many healthy persons, however, raise a small quantity each morning, owing chiefly to the irritation of inhaled dust and smoke. Although not normal, this can hardly be spoken of as pathologic. It is particularly frequent in city dwellers and in those who smoke cigarettes to excess. In the latter, the amount is sometimes so great as to arouse suspicion of tuberculosis. Such "normal morning sputum" generally consists of small, rather dense, mucoid masses, translucent white, or, when due to inhaled smoke, gray in color. Microscopically, there are a few pus-corpuscles, and, usually, many alveolar cells, both of which may contain carbon particles. The alveolar cells commonly show myelin degeneration, and free myelin globules may be present in large numbers. Saprophytic bacteria may be present, but are not abundant.

1. Acute Bronchitis.—There is at first a small amount of tenacious, almost purely mucoid sputum, frequently blood streaked. This gradually becomes more abundant, mucopurulent in character, and yellowish or gray in color. At first the microscope shows a few leukocytes and alveolar and bronchial cells; later, the leukocytes become more numerous. Bacteria are not usually abundant.

2. Chronic Bronchitis.—The sputum is usually abundant, mucopurulent, and yellowish or yellowish-green in

color. Nummular masses—circular, " coin-like " discs which sink in water—may be seen. Microscopically, there are great numbers of leukocytes, often much degenerated. Epithelium is not abundant. Bacteria of various kinds, especially staphylococci, are usually numerous.

In fibrinous bronchitis there are found, in addition, fibrinous casts, usually of medium size.

In the chronic bronchitis accompanying long-continued passive congestion of the lungs, as in poorly compensated heart disease, the sputum may assume a rusty brown color, owing to presence of large numbers of the " heart-failure cells " previously mentioned.

3. Bronchiectasis.—When there is a single large cavity, the sputum is very abundant at intervals,—sometimes as high as a liter in twenty-four hours,—and has a very offensive odor. It is thinner than that of chronic bronchitis, and upon standing separates into three layers of pus, mucus, and frothy serum. It contains great numbers of miscellaneous bacteria.

4. Gangrene of the Lung.—The sputum is abundant, fluid, very offensive, and brownish in color. It separates into three layers upon standing—a brown deposit, a clear fluid, and a frothy layer. Microscopically, few cells of any kind are found. Bacteria are extremely numerous; among them may sometimes be found an acid-fast bacillus probably identical with the smegma bacillus. As before stated, elastic fibers are usually present in large fragments.

5. Pulmonary Edema.—Here there is an abundant, watery, frothy sputum, varying from faintly yellow or pink to dark brown in color; a few leukocytes and

epithelial cells and varying numbers of red blood-corpuscles are found with the microscope.

6. Bronchial Asthma.—The sputum during and following an attack is scanty and very tenacious. Most characteristic is the presence of Curschmann's spirals, Charcot-Leyden crystals, and eosinophilic leukocytes.

7. Croupous Pneumonia.—Characteristic of this disease is a scanty, rusty red, very tenacious sputum, containing red corpuscles or altered blood-pigment, leukocytes, epithelial cells, usually many pneumococci, and often very small fibrinous casts. This sputum is seen during the stage of red hepatization. During resolution the sputum assumes the appearance of that of chronic bronchitis. When pneumonia occurs during the course of a chronic bronchitis, the characteristic rusty red sputum may not appear.

8. Pulmonary Tuberculosis.—The sputum is variable. In the earliest stages it may be scanty and almost purely mucoid, with an occasional yellow flake, or there may be only a very small mucopurulent mass. When the quantity is very small, there may be no cough, the sputum reaching the larynx by action of the bronchial cilia. This is not well enough recognized by practitioners. A careful inspection of all the sputum brought up by the patient on several successive days, and a microscopic examination of all yellow portions, will not infrequently establish a diagnosis of tuberculosis when physical signs are negative. Tubercle bacilli will sometimes be found in large numbers at this stage. Blood-streaked sputum is strongly suggestive of tuberculosis, and is more common in the early stages than later.

The sputum of more advanced cases resembles that of

chronic bronchitis, with the addition of tubercle bacilli and elastic fibers. Caseous particles containing immense numbers of the bacilli are common. Far-advanced cases with large cavities often show rather firm, spheric or ovoid masses of thick pus in a thin fluid —the so-called "globular sputum." These globular masses usually contain many tubercle bacilli. Considerable hemorrhages are not infrequent, and for some time thereafter the sputum may contain clots of blood or be colored brown.

CHAPTER II

THE URINE

Preliminary Considerations.—The urine is an aqueous solution of various organic and inorganic substances. It is probably both a secretion and an excretion. Most of the substances in solution are either waste-products from the body metabolism or products derived directly from the foods eaten. Normally, the total amount of solid constituents carried off in twenty-four hours is about 60 gm., of which the organic substances make up about 35 gm. and the inorganic about 25 gm.

The most important organic constituents are urea, uric acid, and ammonia. Urea constitutes about one-half of all the solids, or about 30 gm. in twenty-four hours.

The chief inorganic constituents are the chlorids, phosphates, and sulphates. The chlorids, practically all in the form of sodium chlorid, make up about one-half of the inorganic substances, or about 13 gm., in twenty-four hours.

Certain substances appear in the urine only in pathologic conditions. The most important of these are proteins, sugars, acetone, and related substances, bile, hemoglobin, and the diazo substances.

In addition to the substances in solution all urines contain various microscopic structures.

While, under ordinary conditions, the composition of

urine does not vary much from day to day, it varies greatly at different hours of the same day. It is evident, therefore, that *no quantitative test can be of value unless a sample of the mixed twenty-four-hour urine be used.* The patient should be instructed to void all the urine during the twenty-four hours into a clean vessel kept in a cool place, to mix it well, to measure the whole quantity, and to bring eight or more ounces for examination. A pint fruit-jar is a convenient container. When it is desired to make only qualitative tests, as for albumin or sugar, a " sample " voided at random will answer. It should be remembered, however, that urine passed about three hours after a meal is most likely to contain pathologic substances. That voided first in the morning is least likely to contain them. To diagnose cyclic albuminuria samples obtained at various periods during the twenty-four hours must be examined.

The urine must be examined while fresh. Decomposition sets in rapidly, especially in warm weather, and greatly interferes with all the examinations. Decomposition may be delayed by adding five grains of boric acid (as much of the powder as can be heaped upon a ten-cent piece) for each four ounces of urine. Formalin, in proportion of one drop to four ounces, is also an efficient preservative, but if larger amounts be used, it may give reactions for sugar and albumin, and is likely to cause a precipitate which greatly interferes with the microscopic examination.

Normal and abnormal pigments, which interfere with certain of the tests, can be removed by filtering the urine through animal charcoal, or precipitating with a solution of acetate of lead and filtering.

Certain cloudy urines cannot be clarified by ordinary filtration through paper, particularly when the cloudiness is due to bacteria. Such urines can usually be rendered perfectly clear by adding a small amount of purified talc, shaking well, and filtering.

A suspected fluid can be identified as urine by detecting any considerable quantity of urea in it (p. 93). Traces of urea may, however, be met with in ovarian cyst fluid, while urine from very old cases of hydronephrosis may contain little or none.

The frequency of micturition is often suggestive in diagnosis. Whether it is unduly frequent can best be ascertained by asking the patient whether he has to get up at night to urinate. Increased frequency may be due to restlessness; to increased quantity of urine; to irritability of the bladder, usually an evidence of cystitis; to obstruction ("retention with overflow"); or to paralysis of the sphincter.

Clinical examination of the urine may conveniently be considered under four heads: I. Physical examination. II. Chemic examination. III. Microscopic examination. IV. The urine in disease.

I. PHYSICAL EXAMINATION

1. Quantity.—The quantity passed in twenty-four hours varies greatly with the amount of liquids ingested, perspiration, etc. The normal may be taken as 1000 to 1500 c.c., or 40 to 50 ounces.

The quantity is increased (polyuria) during absorption of large serous effusions and in many nervous conditions. It is usually much increased in chronic interstitial nephritis, diabetes insipidus, and diabetes mellitus. In these

conditions a permanent increase in amount of urine is characteristic—a fact of much value in diagnosis. In diabetes mellitus the urine may, though rarely, reach the enormous amount of 50 liters.

The quantity is decreased (oliguria) in severe diarrhea; in fevers; in all conditions which interfere with circulation in the kidney, as poorly compensated heart disease; and in the parenchymatous forms of nephritis. In uremia the urine is usually very greatly decreased and may be entirely suppressed (anuria).

2. Color.—This varies considerably in health, and depends largely upon the quantity of urine voided. The usual color is yellow or reddish-yellow, due to the presence of several pigments, chiefly urochrome. Acid urine is generally darker than alkaline. In recording the color Vogel's scale (see *Frontispiece*) is very widely used, the urine being filtered and examined by transmitted light in a glass three or four inches in diameter.

The color is sometimes greatly changed by abnormal pigments. Blood-pigment gives a red or brown, smoky color. Urine containing bile is yellowish or brown, with a yellow foam when shaken. It may assume a greenish hue after standing, owing to oxidation of bilirubin into biliverdin. Ingestion of small amounts of methylene-blue gives a pale green; large amounts give a marked blue. Santonin produces a yellow; rhubarb, senna, cascara, and some other cathartics, a brown color; these change to red upon addition of an alkali, and if the urine be alkaline when voided, may cause suspicion of hematuria. Thymol gives a yellowish-green. Following poisoning from phenol and related drugs the urine may have a normal color when voided, but becomes olive-

green to brownish-black upon standing. In susceptible individuals therapeutic doses of creosote, or absorption from carbolized dressings, may cause this change. Urine which contains melanin, as sometimes in melanotic sarcoma, and very rarely in wasting diseases, also becomes brown or black upon long standing. A similar darkening upon exposure to the air occurs in alkaptonuria (p. 126).

A pale greenish urine with high specific gravity strongly suggests diabetes mellitus.

3. Transparency.—Freshly passed normal urine is clear. Upon standing, a faint cloud of mucus, leukocytes, and epithelial cells settles to the bottom—the so-called "nubecula." Abnormal cloudiness is usually due to presence of phosphates, urates, pus, blood, or bacteria.

Amorphous phosphates are precipitated in neutral or alkaline urine. They form a white cloud and sediment, which disappear upon addition of an acid.

Amorphous urates are precipitated only in acid urine. They form a white or pink cloud and sediment ("brick-dust deposit"), which disappear upon heating.

Pus resembles amorphous phosphates to the naked eye. Its nature is easily recognized with the microscope, or by adding a strong solution of caustic soda to the sediment, which is thereby transformed into a gelatinous mass (Donné's test).

Blood gives a reddish or brown, smoky color, and may be recognized with the microscope or by tests for hemoglobin.

Bacteria, when present in great numbers, give a uniform cloud, which cannot be removed by ordinary filtration. They are detected with the microscope.

The cloudiness of decomposing urine is due mainly to precipitation of phosphates and multiplication of bacteria.

4. Odor.—The characteristic aromatic odor is due to volatile acids, and is most marked in concentrated urines. During decomposition, the odor becomes ammoniacal. A fruity odor is sometimes noted in diabetes, due probably to acetone. Urine which contains cystin may develop an odor of sulphureted hydrogen during decomposition.

Various articles of diet and drugs impart peculiar odors. Notable among these are asparagus, which gives a characteristic offensive odor, and turpentine, which imparts an odor somewhat suggesting that of violets.

5. Reaction.—Normally, the mixed twenty-four-hour urine is slightly acid in reaction. The acidity was formerly held to be due to acid phosphates, but Folin has shown that the acidity of a clear urine is ordinarily much greater than the acidity of all the phosphates, the excess being due to free organic acids. Individual samples may be slightly alkaline, especially after a full meal, or amphoteric. The reaction is determined by means of litmus-paper.

Acidity is increased after administration of certain drugs, and whenever the urine is concentrated from any cause, as in fevers. A very acid urine may cause frequent micturition because of its irritation. This is often an important factor in the troublesome enuresis of children.

The urine always becomes alkaline upon long standing, owing to decomposition of urea with formation of ammonia. If markedly alkaline when voided, it usually

indicates such "ammoniacal decomposition" in the bladder, which is the rule in chronic cystitis, especially that due to paralysis or obstruction. Alkalinity due to ammonia (*volatile alkalinity*) can be distinguished by the fact that litmus paper turned blue by the urine again becomes red upon gentle heating. *Fixed alkalinity* is due to alkaline salts, and is often observed during frequent vomiting, after the crisis of pneumonia, in various forms of anemia, after full meals, and after administration of certain drugs, especially salts of vegetable acids.

Quantitative estimation of the acidity of urine is not of much clinical value. When, however, it is desired to make it, the method of Folin will be found satisfactory. In every case the sample must be from the mixed twenty-four-hour urine and as fresh as possible.

Folin's Method.—Into a small flask measure 25 c.c. of the urine and add 1 or 2 drops 0.5 per cent. alcoholic solution of phenolphthalein and 15 or 20 gm. of neutral potassium oxalate. Shake for a minute, and immediately titrate with decinormal sodium hydroxid, shaking meanwhile, until the first permanent pink appears. Read off from the buret the amount of decinormal sodium hydroxid solution added, and calculate the number of cubic centimeters which would be required for the entire twenty-four hours' urine. Folin places the normal acidity, obtained in this way, at 617.

6. Specific Gravity.—The normal average is about 1.017 to 1.020. Samples of urine taken at random may go far above or below these figures, hence a sample of the mixed twenty-four-hour urine should always be used.

Pathologically, it may vary from 1.001 to 1.060. It is *low* in chronic interstitial nephritis, diabetes insipidus,

and many functional nervous disorders. It is *high* in fevers and in parenchymatous forms of nephritis. In any form of nephritis a sudden fall without a corresponding increase in quantity of urine may foretell approaching uremia. It is *highest* in diabetes mellitus. A high specific gravity when the urine is not highly colored should lead one to suspect this disease. A normal specific gravity does not, however, exclude it.

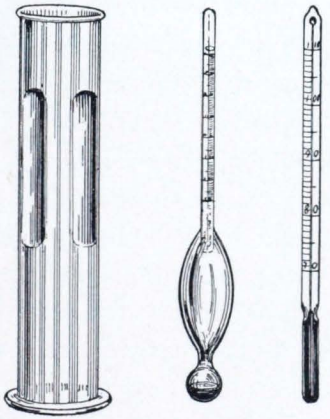


Fig. 17.—Squibb's urinometer with thermometer and cylinder.

The specific gravity is most conveniently estimated by means of the urinometer—Squibb's is preferable (Fig. 17). It is standardized for a temperature of 77° F., and the urine should be at or near that temperature. Care should be taken that the urinometer does not touch the side of the tube, and that air-bubbles are removed from the surface of the urine. With most instruments the reading is taken from the bottom of the meniscus. A long scale on the stem is desirable, because of the greater ease of accurate reading.

One frequently wishes to ascertain the specific gravity of quantities of fluid too small to float an urinometer. A simple device for this purpose, which requires only about 3 c.c. and is very satisfactory in clinical work has been designed by Saxe (Fig. 18). The urine is placed in the bulb at the bottom, the instrument is floated in dis-

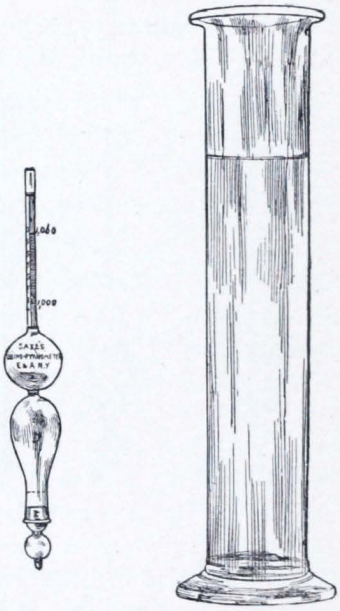


Fig. 18.—Saxe's urinopyknometer and jar for same.

tilled water, and the specific gravity is read off from the scale upon the stem.

7. Total Solids.—An estimation of the total amount of solids which pass through the kidneys in twenty-four hours is, in practice, one of the most useful of urinary examinations. The normal for a man of 150 pounds is about 60 gm., or 950 gr. The principal factors which

influence this amount are body weight (except with excessive fat), diet, exercise, and age, and these should be considered in making an estimation. After about the forty-fifth year it becomes gradually less; after seventy-five years it is about one-half the amount given.

In disease, the amount of solids depends mainly upon the activity of metabolism and the ability of the kidneys to excrete. An estimation of the solids, therefore, furnishes an important clue to the functional efficiency of the kidneys. The kidneys bear much the same relation to the organism as does the heart: they cause no direct harm so long as they are capable of performing the work required of them. When, however, through either organic disease or functional inactivity, they fail to carry off their proportion of the waste-products of the body, some of these products must either be eliminated through other organs, where they cause irritation and disease, or be retained within the body, where they act as poisons. The great importance of these poisons in production of distressing symptoms and even organic disease is not well enough recognized by most practitioners. Disappearance of unpleasant and perplexing symptoms as the urinary solids rise to the normal under proper treatment is often most surprising.

When, other factors remaining unchanged, the amount of solids eliminated is considerably above the normal, increased destructive metabolism may be inferred.

The total solids can be estimated roughly, but accurately enough for most clinical purposes, by multiplying the last two figures of the specific gravity of the mixed twenty-four-hour urine by the number of ounces

voided and to the product adding one-tenth of itself. This gives the amount in grains. If, for example, the twenty-four-hour quantity is 3 pints or 48 ounces, and the specific gravity is 1.018, the total solids would approximate 950 gr., as follows:

$$48 \times 18 = 864; 864 + 86.4 = 950.4$$

This method is especially convenient for the practitioner, because patients nearly always report the amount of urine in pints and ounces, and it avoids the necessity of converting into the metric system. Häser's method is more widely used but is less convenient. The last two figures of the specific gravity are multiplied by 2.33. The product is then multiplied by the number of cubic centimeters voided in twenty-four hours and divided by 1000. This gives the total solids in grams.

8. Functional Tests.—Within the past few years much thought has been devoted to methods of more accurately ascertaining the functional efficiency of the kidneys, especially of one kidney when removal of the other is under consideration. The most promising of the methods which have been devised are cryoscopy, electric conductivity, the methylene-blue test, and the phloridzin test. It is doubtful whether, except in experienced hands, these yield any more information than can be had from an intelligent consideration of the specific gravity and the twenty-four-hour quantity, together with a microscopic examination. They are most useful when the urines obtained from separate kidneys by segregation or ureteral catheterization are compared. The reader is referred to larger works upon urinalysis for details.

Cryoscopy, determination of the freezing-point, depends upon the principle that the freezing-point of a fluid is depressed in proportion to the number of molecules, organic and inorganic, in solution. To have any value, the freezing-point of the urine must be compared with that of the blood, since it is not so much the number of molecules contained in the urine as the number which the kidney has failed to carry off and has left in the blood, that indicates its insufficiency.

Electric conductivity refers to the power of the urine to carry an electric current. It is increased in proportion to the number of *inorganic* molecules in solution.

In the **methylene-blue test** of Achard and Castaigne a solution of methylene-blue is injected intramuscularly, and the time of its appearance in the urine is noted. Normally, it appears in about thirty minutes. When delayed, renal " permeability " is supposed to be interfered with. Since methylene-blue is sometimes excreted as a colorless derivative, indigo-carmin has been proposed as a substitute. In the absence of renal insufficiency this always gives a blue color, which begins to appear in about five minutes.

The **phloridzin test** consists in the hypodermic injection of a small quantity of phloridzin. This substance is transformed into glucose by the kidneys of healthy persons. In disease, this change is more or less interfered with, and the amount of glucose recoverable from the urine is taken as an index of the secretory power of the kidneys.

In applying these tests for " permeability," " secretory ability," etc., one must remember that the conditions are abnormal, and that there is no evidence that

the kidneys will behave with the products of metabolism as they do with the substances selected for the tests, and also that the tests throw unusual work upon the kidneys, which in some cases may be harmful.

II. CHEMIC EXAMINATION

A. Normal Constituents

Of the large number of organic and inorganic substances normally present in the urine, only a few demand any consideration from the clinician. The following table, therefore, outlines the average composition from the clinical, rather than from the chemical, standpoint. Only the twenty-four-hour quantities are given, since they alone furnish an accurate basis for comparison. *The student cannot too soon learn that percentages mean little or nothing, excepting as they furnish a means of calculating the twenty-four-hour elimination.*

COMPOSITION OF NORMAL URINE

	Grams in twenty-four hours.	Approximate average.
Total substances in solution	55–70	60
Inorganic substances	20–30	25
Chlorids (chiefly sodium chlorid)	10–15	12.5
Phosphates (estimated as phosphoric acid), total	2.5–3.5	3
Earthy $\frac{1}{3}$ of total		1
Alkaline $\frac{2}{3}$ of total		2
Sulphates (estimated as sulphuric acid), total	1.5–3.0	2.5
Mineral $\frac{9}{10}$ of total		2.25
Conjugate $\frac{1}{10}$ of total		0.25
Includes indican		Trace
Ammonia	0.5–1.0	0.7
Organic substances	30–40	35
Urea	20–35	30
Uric acid	0.4–1.0	0.7

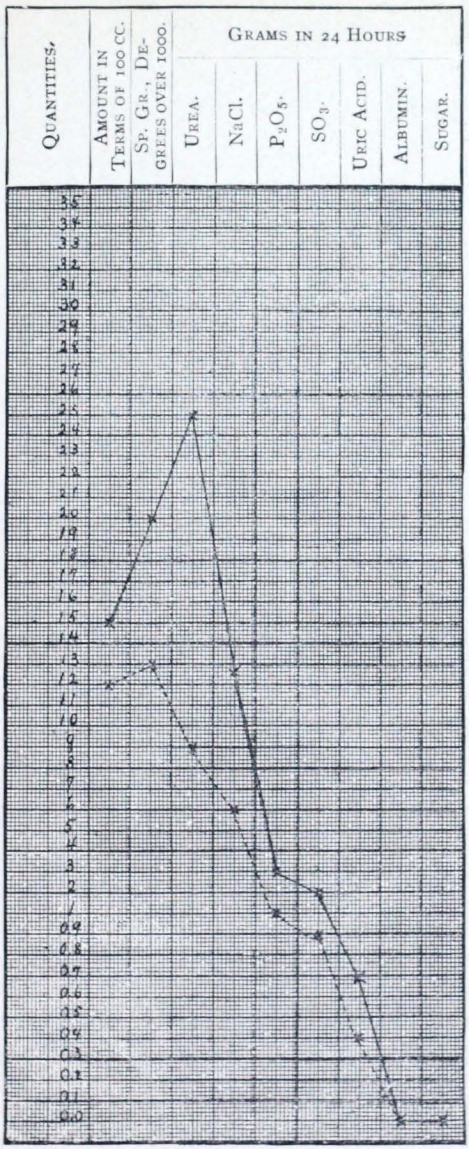


Fig. 19.—Graphic expression of quantities in the urine. Solid line, normal urine; dotted line, an example of pathologic urine in a case of cancerous cachexia (Saxe).

Although the conjugate sulphates are organic compounds, they are, for the sake of convenience, included with the inorganic sulphates in the table on p. 80.

Among constituents which are of little clinical importance, or are present only in traces, are:

Inorganic: Iron, carbonates, nitrates, silicates, and fluorids.

Organic: Creatinin, hippuric acid, purin bases, oxalic acid, benzoic acid, volatile fatty acids, pigments, and acetone.

Variations in body weight, diet, and exercise cause marked fluctuations in the total solids and in individual substances.

1. Chlorids.—These are derived from the food, and are mainly in the form of sodium chlorid. The amount excreted normally is 10 to 15 gm. in twenty-four hours. It is much affected by the diet, and is reduced to a minimum in starvation.

Excretion of chlorids is diminished in nephritis and in fevers, especially in pneumonia and inflammations leading to the formation of large exudates. In nephritis the kidneys are less permeable to the chlorids, and it is possible that the edema is due largely to an effort of the body to dilute the chlorids which have been retained. Certainly an excess of chlorids in the food will increase both the albuminuria and the edema of nephritis. In fevers the diminution is due largely to decrease of food, though probably in some measure to impaired renal function. In pneumonia chlorids are constantly very low, and in some cases are absent entirely. Following the crisis they are increased. In inflammations leading to formation of large exudates—*e. g.*, pleurisy with

effusion—chlorids are diminished, because a considerable amount becomes "locked up" in the exudate. During absorption chlorids are liberated and appear in the urine in excessive amounts.

Diminution of chlorids is also observed in severe diarrhea, anemic conditions, and carcinoma of the stomach.

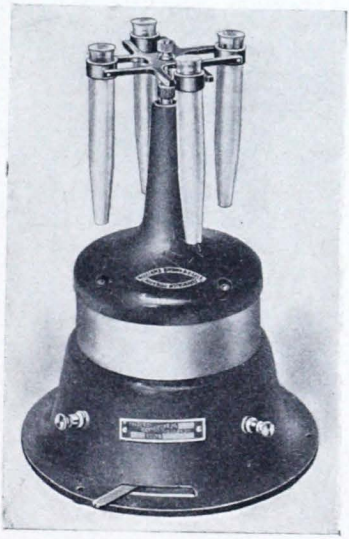


Fig. 20.—The Purdy electric centrifuge with four arms.

Quantitative Estimation.—The best method for clinical purposes is the centrifugal method.

Purdy's Centrifugal Methods.—As shown by the late Dr. Purdy, the centrifuge offers an important means of making quantitative estimations of a number of substances in the urine. Results are easily and quickly obtained, and are probably accurate enough for all clinical purposes.

In general, the methods consist in precipitating the substance to be estimated in a graduated centrifuge tube,

and applying a definite amount of centrifugal force for a definite length of time, after which the percentage of precipitate is read off upon the side of the tube. Albumin, if present, must be previously removed by boiling and filtering. Results are in terms of *bulk of precipitate*, which must not be confused with *percentage by weight*. The weight percentage can be found by referring to Purdy's tables, given later. In this, as in

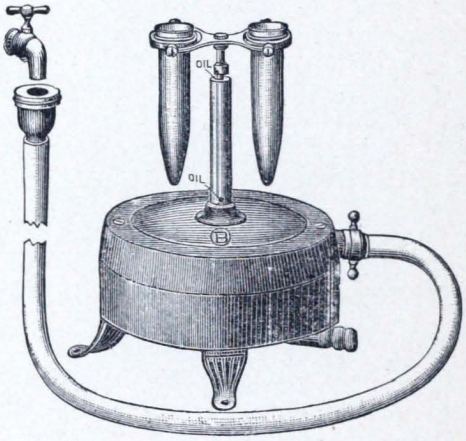


Fig. 21.—Water-motor centrifuge.

all quantitative urine work, percentages mean little in themselves; the actual amount eliminated in twenty-four hours should always be calculated.

The centrifuge should have an arm with radius of $6\frac{3}{4}$ inches when in motion, and should be capable of maintaining a speed of 1500 revolutions a minute. The electric centrifuge is to be recommended, although good work can be done with a water-power centrifuge, or, after a little practice, with the hand centrifuge. A

speed indicator is desirable with electric and water-motor machines, although one can learn to estimate the speed by the musical note.

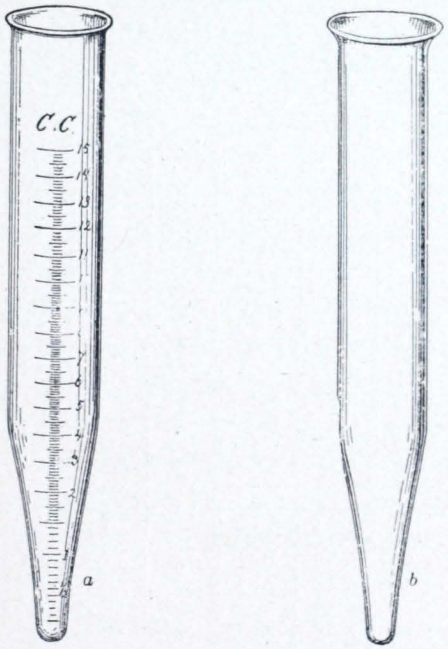


Fig. 22.—Purdy's tubes for the centrifuge: *a*, Percentage tube; *b*, sediment tube.

Estimation of Chlorids.—Fill the graduated tube to the 10 c.c. mark with urine; add 15 drops strong nitric acid and then silver nitrate solution (dram to the ounce) to the 15 c.c. mark. Mix by inverting several times. Let stand a few minutes for a precipitate to form, and then revolve in the centrifuge for three minutes at 1200 revolutions a minute. Each one-tenth cubic centimeter of precipitate equals 1 per cent. by bulk. The normal is about 10 per cent. This may be converted into terms of chlorin or sodium chlorid by means of the table upon page 86. Roughly speaking, the percent-

age of chlorin by weight is about one-twelfth the bulk-percentage.

TABLE FOR THE ESTIMATION OF CHLORIDS AFTER CENTRIFUGATION

Showing the bulk-percentage of silver chlorid (AgCl) and the corresponding gravimetric percentages and grains per fluidounce of sodium chlorid (NaCl) and chlorin (Cl).—(Purdy.)

Bulk-percentage of AgCl.	Percentage NaCl.	Gr. Per Oz. NaCl.	Percentage Cl.	Gr. Per Oz. Cl.	Bulk-percentage of AgCl.	Percentage NaCl.	Gr. Per Oz. NaCl.	Percentage Cl.	Gr. Per Oz. Cl.
¼	0.03	0.15	0.02	0.1	8	1.04	4.98	0.63	3.02
⅜	0.07	0.31	0.04	0.19	8½	1.1	5.29	0.67	3.22
½	0.1	0.47	0.06	0.28	9	1.17	5.6	0.71	3.4
1	0.13	0.62	0.08	0.38	9½	1.23	5.91	0.75	3.6
1¼	0.16	0.78	0.1	0.48	10	1.3	6.22	0.79	3.79
1½	0.19	0.93	0.12	0.57	10½	1.36	6.53	0.83	3.97
1¾	0.23	1.09	0.14	0.67	11	1.43	6.84	0.87	4.16
2	0.26	1.24	0.16	0.76	11½	1.49	7.2	0.91	4.35
2¼	0.29	1.41	0.18	0.85	12	1.56	7.46	0.95	4.54
2½	0.32	1.56	0.2	0.96	12½	1.62	7.78	0.99	4.73
2¾	0.36	1.71	0.22	1.04	13	1.69	8.09	1.02	4.92
3	0.39	1.87	0.24	1.13	13½	1.75	8.4	1.06	5.11
3¼	0.42	2.02	0.26	1.23	14	1.82	8.71	1.1	5.29
3½	0.45	2.18	0.28	1.32	14½	1.88	9.02	1.14	5.49
3¾	0.49	2.35	0.3	1.42	15	1.94	9.33	1.18	5.67
4	0.52	2.49	0.32	1.51	15½	2.01	9.65	1.22	5.86
4¼	0.55	2.64	0.34	1.61	16	2.07	9.94	1.26	6.06
4½	0.58	2.8	0.35	1.7	16½	2.14	10.27	1.3	6.24
4¾	0.62	2.96	0.37	1.8	17	2.2	10.51	1.34	6.43
5	0.65	3.11	0.39	1.89	17½	2.27	10.87	1.38	6.62
5½	0.71	3.42	0.43	2.09	18	2.33	11.2	1.42	6.81
6	0.78	3.73	0.47	2.27	18½	2.4	11.51	1.46	7.0
6½	0.84	4.05	0.51	2.46	19	2.46	11.82	1.5	7.19
7	0.91	4.35	0.55	2.62	19½	2.53	12.13	1.54	7.38
7½	0.97	4.67	0.59	2.84	20	2.59	12.44	1.58	7.56

Bulk-percentage to be read on the side of the tube.

2. Phosphates.—Phosphates are derived largely from the food, only a small proportion resulting from metab-