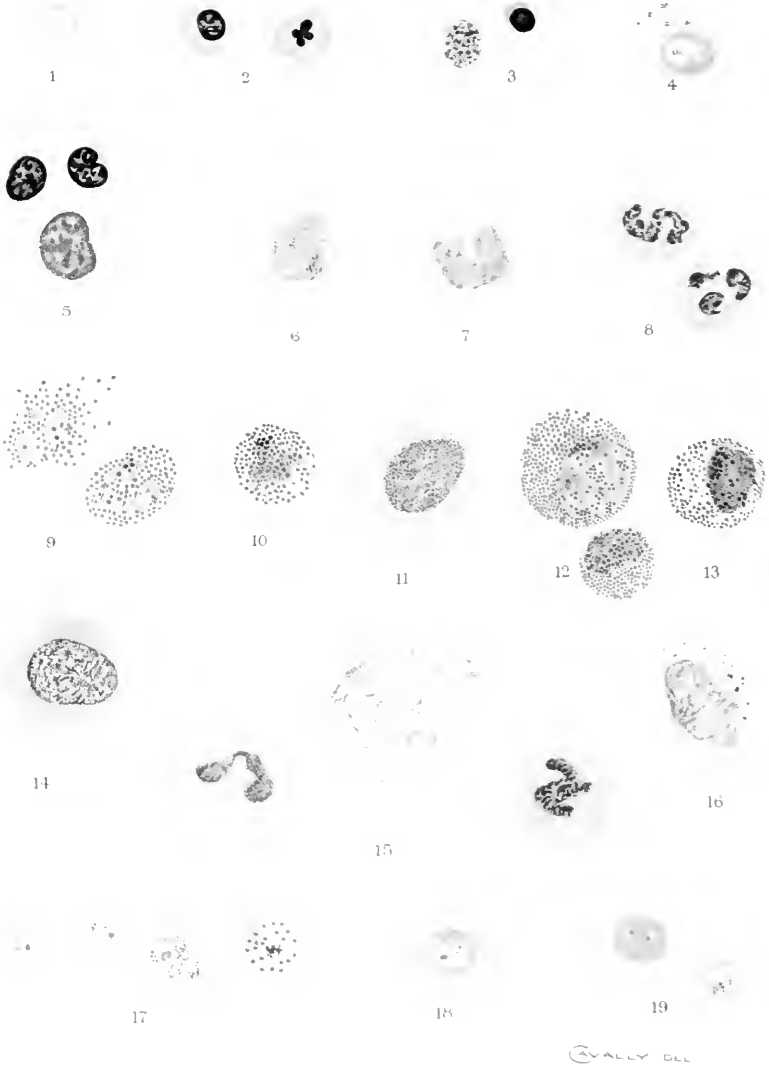
PLATE I



Clinical Diagnosis

A MANUAL OF LABORATORY METHODS

BY

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Illustrated

Third Edition, Revised and Enlarged

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TO

MY FATHER

Joe H. Todd, M. D.

THESE PAGES ARE

AFFECTIONATELY DEDICATED

PREFACE TO THE THIRD EDITION

In the present edition, as in the preceding one, the scope of this book has been somewhat enlarged and its size increased. It is believed that its value has thereby been enhanced without sacrifice of the simplicity and conciseness which were its original aim. As before, chief emphasis has been placed upon methods and microscopic morphology rather than upon the clinical significance of findings.

Much of the new material is the outgrowth of questions which have arisen in class and laboratory. To one who sees a great deal of the work of students in the clinical laboratory it soon becomes evident that errors in microscopic diagnosis spring much less frequently from ignorance of the typical appearance of microscopic structures than from imperfect preparation of the slide, faulty manipulation of the microscope, or failure to recognize extraneous matters, artefacts, and other misleading appearances. Such sources of error have been given especial attention.

Each section has been carefully revised and considerable additions have been made to every chapter, notably to those on Sputum, Urine, and Animal Parasites. The chapter upon the Use of the Microscope has been enlarged by the addition of many practical points,

including a set of practical exercises which it is hoped will prove useful to the student. The section upon Cerebrospinal Fluid has been rewritten in the light of recent advances in knowledge of this fluid. Among the newer methods which appear in this edition may be mentioned: the newly simplified Volhard method for chlorids in urine; the urease methods for urea in urine, blood, and spinal fluid; the Rimini-Burnam test for formaldehyd in urine; the Weisz permanganate (urochromogen) test; the use of edestin as a control for the glycyl-tryptophan test for gastric cancer; Huntoon's method for spores; Ponder's stain for diphtheria bacilli; and the luetin reaction.

A new chapter on Serodiagnostic Methods, including Abderhalden's test for pregnancy, the complement fixation tests for syphilis, gonorrhea, tuberculosis, and cancer, and the cobra-venom test for syphilis, has been added. This is from the pen of Ross C. Whitman, B. A., M. D., Professor of Surgical Pathology and Serology in the University of Colorado.

Thirty-five new pictures have been included. Some of these replace poorer pictures of the last edition, and in some cases from two to six have been grouped to form a single illustration, hence the total number of figures in the book has not been greatly increased. The majority of the new pictures are photomicrographs. Inadequate as is the photomicrograph in some fields, its superiority to drawings in clinical microscopy can hardly be questioned.

For aid of various sorts in the preparation of this edition the author wishes to acknowledge his indebtedness to the following members of the faculty of the University

of Colorado: Clough T. Burnett, M. D., Professor of Bacteriology; Alvin R. Peebles, M. D., Professor of Preventive and Experimental Medicine; Max M. Ellis, Ph. D., Assistant Professor of Biology; and Charles F. Poe, M. A., Ph. C., Instructor in Chemistry.

J. C. T.

Boulder, Colorado,
October, 1914.

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 stand-

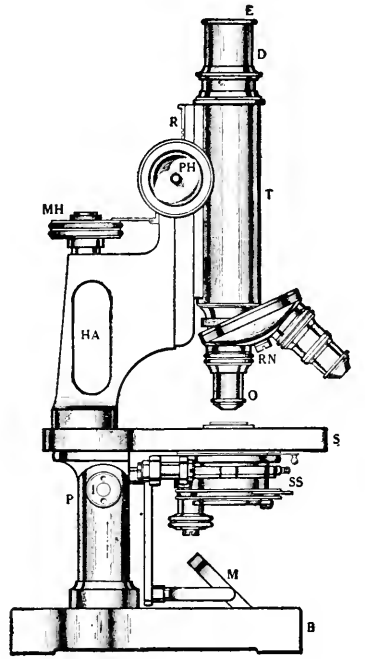


Fig. 1.—Handle-arm microscope: E, Eyepiece; D, draw-tube; T, body-tube; RN, revolving nose-piece; O, objective; PH, pinion head; MH, micrometer head; HA, handle-arm; SS, substage; S, stage; M, mirror; B, base; R, rack; P, pillar; I, inclination joint.

ard 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, and with some good preparations of stained and unstained blood. The blood slides can easily be made from one's own blood, as described in Chapter III. 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. At any other window a white window-shade is desirable. 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 elec-

tric light is used, and a spheric glass globe—a 6-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.

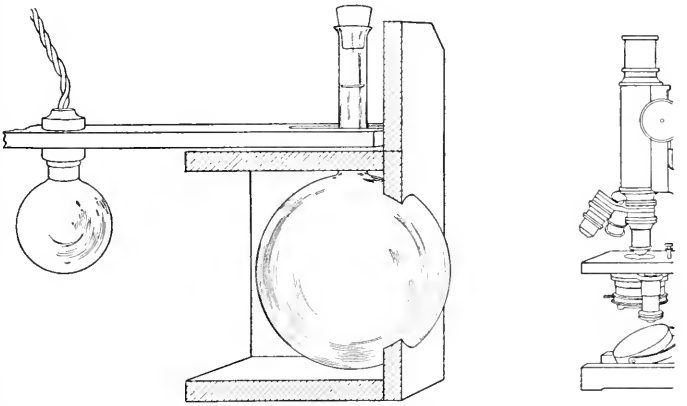


Fig. 2.—Illumination with water-bottle condenser.

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 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 disk may be inserted.

Illumination may be either *central* or *oblique*, depending upon the direction in which the light enters the microscope. To obtain **central illumination,** 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

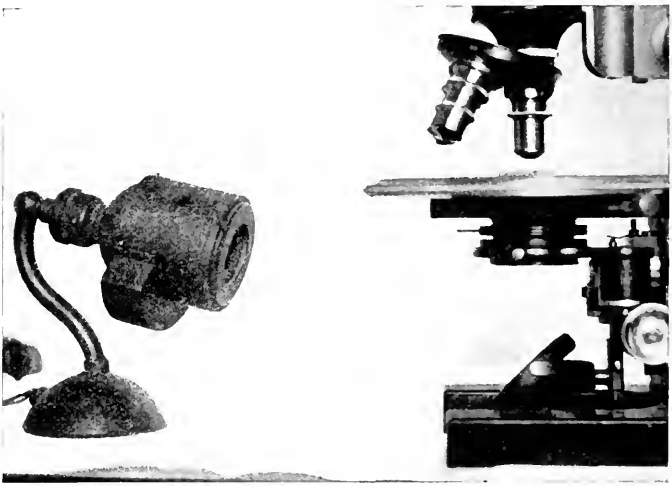


Fig. 3.—Matthews microscope lamp with iris-diaphragm.

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.

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.

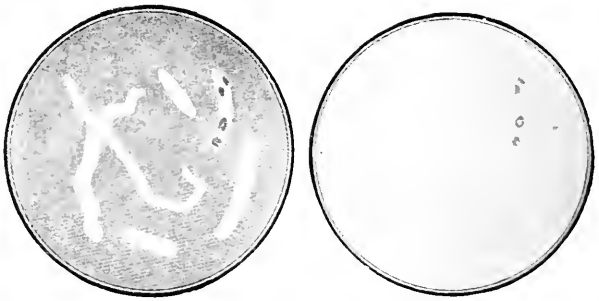
Beginners frequently use oblique illumination without recognizing it, and are thereby much confused. If the light be oblique, an object in the center of the field will appear to move from side to side when the fine adjustment is turned back and forth.

The **amount of light** admitted is also important. It is regulated by the diaphragm.

The bulk of routine work is done with central illumination, and, therefore, every examination should begin with it. Each of the forms of illumination, however—central and oblique, subdued and strong—has its special uses and demands some consideration here. The well-known rule, "Use the least light which will show the object well," is good, but it does not go far enough.

In studying any microscopic structure one considers: (1) its color, (2) its outline, and (3) its surface contour. No one form of illumination shows all of these to the best advantage. It may, therefore, be necessary to change the illumination many times during a microscopic examination. *To see color best, use central illumination with strong light.* The principle is that by which a stained glass window shows the purest color when the light is streaming through it. Strong central light is, therefore, to be used for structures such as stained bacteria, whose recognition depends chiefly upon their color, and, alternating with other forms, for stained structures in general. *To study the outline of an object use very subdued central illumination.* The diaphragm is closed to the point which trial shows to be best in each case. This illumination is required by delicate colorless objects, such as hyaline tube-casts and cholesterin crystals, which are recognized chiefly by their outline.

The usual mistake of beginners is to work with the diaphragm too wide open. Strong light will often render semitransparent structures entirely invisible (Fig. 4). *To study surface contour use oblique light of a strength suited to the color or opacity of the object.* In routine work oblique illumination is resorted to only to study more fully some object which has been found with central illumination, as, for instance, to demonstrate the cylindric shape of a hyaline tube-cast.



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").

Dark Ground Illumination.—This consists in blocking out the central rays of light and directing the peripheral rays against the microscopic object from the side. Only those rays which strike the object and are reflected upward pass into the objective. The object thus appears bright upon a black background. 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 disks which accompany most microscopes when purchased. The stop is placed in a special ring beneath the condenser. When the regular stop is not at hand, one can use an extra large round cover-glass, in the center of which is pasted a circular disk of black paper. The size of the black disk depends upon the aperture of the objective with which it is to be used, and can be ascertained by trial.

For oil-immersion work a special condenser is necessary. This is sold under the name of reflecting condenser, "dunkelfeld," dark field illuminator, etc. 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 a small arc-light.

The chief use of dark ground illumination in clinical work is for demonstration of *Treponema pallidum* in fresh material (see Fig. 166).

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 in the clinical laboratory.

The condenser is constructed for parallel rays of light. With daylight, therefore, the plane mirror should be used; while for the divergent rays of ordinary artificial light the concave mirror, which tends to bring the rays together, is best.

It is very important that the condenser be accurately centered in the optical axis of the instrument, 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 sections.

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.

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. One can get about the right thickness by buying No. 2 covers and discarding the thick ones; or by buying No. 1 covers and discarding the thinner ones. Slight differences in cover-glass thickness can be compensated by increasing the length of tube when the cover is too thin, and decreasing it when the cover is too thick. The correction necessary will depend upon the focal length and numeric aperture of the objective. With a 4-mm. objective of 0.85 numeric aperture a difference of 0.03 mm. in cover-glass thickness requires a change of 30 mm. in the tube length. Many high-grade objectives are supplied with a "correction collar," which accomplishes the same end. While for critical work, especially with

apochromatics, cover-glass thickness is very important, one pays little attention to it in the clinical laboratory. A high-power dry lens always requires a cover, but its exact thickness is unimportant in routine work. Very low-power and oil-immersion objectives may be used without any cover-glass.

(*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. A slight flash of light will be seen when the front lens touches the oil. The objective is then brought to a focus in the usual way. In order to avoid bubbles the oil must be placed upon the cover carefully and without stirring it about. Bubbles 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. If they are present, the oil must be removed and a new drop applied. Immediately after use both objective and slide should be wiped clean with lens-paper or a soft linen handkerchief. In an emergency glycerin may be used instead of cedar oil, but, of course, with inferior results.

Curvature of field, through which it is impossible to focus both center and periphery sharply at the same time, is a very noticeable defect; but it is less serious than appears at first sight, particularly for visual work. It is easily compensated by frequent use of the fine focusing adjustment. Complete flatness of field cannot be attained without sacrifice of other and more desirable properties. Some of the finest objectives made, notably the apochromatics, show decided curvature.

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. Some oil-immersion objectives have such short working distance that only very thin cover-glasses can be used.

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 with small scissors. 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.

The **formation of the microscopic image** demands brief consideration. The rays of light which are reflected upward from the mirror and which pass through the object are brought to a focus in a magnified, inverted real image. This can be focused to appear at different levels, but when the microscope is used in the ordinary

way it is formed at about the level of the diaphragm in the ocular. It can be seen by removing the ocular, placing a piece of ground glass on the top of the tube, and focusing upon it. When viewing this image a roll of paper or a cylindric mailing tube should be used to exclude extraneous light. This image, in turn, is magnified by the eye-lens of the ocular, producing a second real image, which is again inverted, and, therefore, shows the object right side up. This can be seen upon a ground glass held a few inches above the ocular, provided strong artificial light be used and the room darkened. The eye, when it looks into the microscope, sees, not this real image, but rather an inverted *virtual image* which appears about 250 mm. (10 inches) in front of the eye.

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]

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

[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 ultramicroscope depends upon its use of light of short wave length.

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 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.

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

than 1. From these considerations it follows 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 (see 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.

Another property of an objective which depends largely upon N. A. is **depth of focus,** the ability to render details in different planes clearly at the same time. The higher the N. A. and the greater the magnification, the less the depth of focus. Any two objectives of the same focal length and same N. A. will have exactly the same

depth of focus. Depth of focus can be increased by closing down the diaphragm, and thus reducing the N. A. Great depth is desirable for certain low-power work, but for high powers it does not offer advantages to balance the loss of N. A. by which it is attained. In some cases, indeed, it is a real disadvantage.

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 ascertained 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.

It is easy to find the magnifying power of any combination of objective and ocular by actual trial. Place the counting slide of the hemacytometer upon the microscope and focus the ruled lines. Now adjust a sheet of paper upon the table close to the microscope in such a position that when the left eye is in its proper place at the ocular the paper will lie in front of the right eye at the normal visual distance, *i. e.*, 250 mm. (10 inches). (The paper may be supported upon a book, if necessary.) If both eyes are kept open, the ruled lines will

appear to be projected on the paper. With a pencil mark their apparent location on the paper, and measure the distance between the marks. Divide this distance by 0.05 mm., which is the actual distance between the lines on the slide. The quotient gives the magnification. If, to take an example, the lines in the image on the paper are 5 mm. apart, the magnification is 100 diameters. The figures obtained in this way will vary somewhat as one is near or far sighted, unless the defect of vision is corrected with glasses.

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. It has been aptly compared to the enlargement, by stretching in all directions, of a picture drawn upon a sheet of rubber. No new detail is added, no matter how great the enlargement. The extent to which magnification can be satis-

factorily 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.

The Microscope in Use.—Optically, it is a matter of indifference whether the instrument be used in the vertical position or inclined. Examination of fluids requires the horizontal stage, and since much of the work of the clinical laboratory is of this nature it is well to accustom one's self to the use of the vertical microscope.

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.

Although the ability to use the eyes interchangeably is sometimes very desirable, greater skill in recognizing objects will be acquired if the same eye be always used. The left eye is the more convenient, because the right eye is thus left free to observe the drawing one may wish to do with the right hand. After a little practice one can cause the microscopic image to appear as if projected upon a sheet of paper placed close to the microscope under the free eye. This gives the effect of a camera lucida, and it becomes very easy to trace outlines. When one is accustomed to spectacles, they should not be removed.

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

One often wishes to mark a particular field upon a permanent preparation so as to refer to it again. The vernier of the mechanical stage cannot be relied upon, because it is impossible to replace the stage in exactly the same position after it has been removed and because its position is frequently changed by the slight knocks which it receives. There are on the market several "object markers" by which a desired field can be marked with ink, or by a circle scratched on the cover-glass by a minute diamond, while the slide is in place on the microscope. The circle is easily located with a low power. In the absence of these, one can, while using the low power, place minute spots with a fine pen at the edge of the field on opposite sides.

A good marking material is a cement which the author has long used for making cells, ringing cover-glasses, etc. To a few ounces of white shellac in wood alcohol add an equal volume of gasoline, shake thoroughly, and let stand for twenty-four hours, or until well separated into two layers. Pipet off the clear lower portion, add 5 to 10 drops of castor oil to each ounce, and color with any analin dye dissolved in absolute alcohol. When too thick, thin with absolute alcohol. This makes a beautiful, transparent, easy-flowing cement which does not crack and which is not attacked by xylol. Glycerin mounts which the writer ringed with it twenty years ago are still in perfect condition.

Many good workers advise against the use of spring clips to hold the slide against the stage of the microscope. Manipulation of the slide with the fingers alone certainly gives good training in delicacy of touch, and is desirable when examining infectious material which might contaminate the clips, but is most annoying in practical work. For the majority of examinations it is much more satisfactory to use a clip at one end of the slide, with just sufficient pressure to hold the slide without interfering with its freedom of movement.

Occasionally when one wishes a very low-power objective for some special work it may be desirable to unscrew the front lens of the 16-mm. objective and use the back lens only. This procedure is not recommended for critical work, and it should not be tried with high-power objectives, *which must never be taken apart.*

To attach an objective it should be supported in position against the nose-piece by means of the index-finger and middle finger, which grasp it as one would a

cigar. It is then screwed into place with the fingers of the other hand.

Care of the Microscope.—The microscope is a delicate instrument and should be handled accordingly. Even slight disturbance of its adjustments may cause serious trouble. 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.

The microscope should be kept scrupulously clean, and dust must not be allowed to settle upon it. When not in use the instrument should be kept in its case or under a cover. An expensive glass bell-jar is not needed, and, in fact, is undesirable, except for display. It is heavy and awkward to handle, and when lifted is almost certain (unless great care is exercised) to strike the microscope. It is particularly liable to strike the mechanical stage and disturb its adjustment. The simplest, cheapest, lightest, and probably the best cover for the microscope is a truncated cone or pyramid of pasteboard,

covered with creton or similar material. This is easily made at home.

Lens surfaces which have been exposed to dust only should be cleaned with a camel's-hair brush. A small brush and a booklet of lens-paper should always be at hand in the microscope case. Those surfaces which are exposed to finger-marks should be cleaned with lens-paper, or a soft linen handkerchief, moistened with water if necessary. 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. Dirt upon the objective cannot be seen as such; it causes a diffuse cloudiness. 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 may be removed with alcohol or xylol; but these solvents must be used sparingly and carefully, as there is danger of softening the cement. Some manufacturers now cement their lenses with shellac, which resists xylol. 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.

When the vulcanite stage becomes brown and discolored the black color can be restored by rubbing well with petrolatum.

Measurement of Microscopic Objects.—Of the several methods, the most convenient and accurate 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 disk 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 hemacytometer 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. When using the counting chamber with an oil-immersion lens a cover must be used; otherwise the oil will fill the ruled lines and cause them to disappear. Any eye-piece can be converted into a micrometer eye-piece by placing a micrometer disk—a small circular glass plate with ruled scale—ruled side down upon its diaphragm.

The following method of micrometry is less accurate, but is fairly satisfactory for comparatively coarse objects, such as the ova of parasites. A ruled scale corresponding to the magnified image of the hemacytometer ruling is drawn upon cardboard in the manner described for ascertaining magnifications (see p. 31). This card may be used as a micrometer, and should be marked with

the value of its graduations, and the objective, ocular, and tube length with which it is to be used. In the example cited upon p. 32 the lines are 5 mm. apart, corresponding to an actual distance of 0.05 mm. To measure an object, the cardboard is placed in the position which it occupied when made (at the right of the microscope and 250 mm.—10 inches—in front of the eye). The lines and the objects on the slide can then be seen together, and the space covered by any object indicates its size.

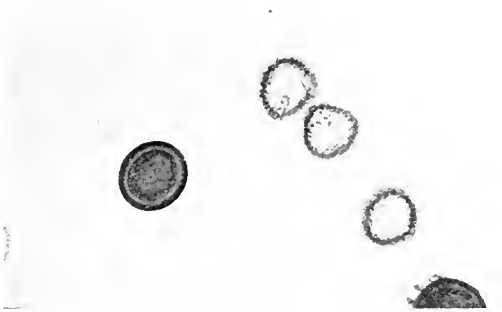


Fig. 5.—Egg of Tænia saginata. Lycopodium granules used as micrometer (× 250) (photograph by the author).

The graduations made as above indicated are too coarse for most work, and they should be subdivided. If five subdivisions are made, each will have a value of 10 μ.

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. Care must be exercised

not to use too much powder. The lycopodium is conveniently kept in a gelatin capsule, and a faint cloud can be dusted over the slide by gently scraping the edge of the lid upon the rim of the capsule.

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.

Photomicrography.—Although high-grade photomicrography requires expensive apparatus and considerable skill in its use, fairly good pictures of microscopic structures can be made by any one with simple instruments.

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 slide 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. When the camera-draw is 10 inches no change in the focusing adjustment will be necessary.

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. The light must be carefully centered. 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. Either the tray or tank method may be used, but in order to secure good contrast it is often desirable to overdevelop somewhat. Metol-hydrochinon is an excellent developer, as it gives good contrast with full detail.

The photograph from which Fig. 6 was made was taken with a Kodak and plate attachment on an "Iso"

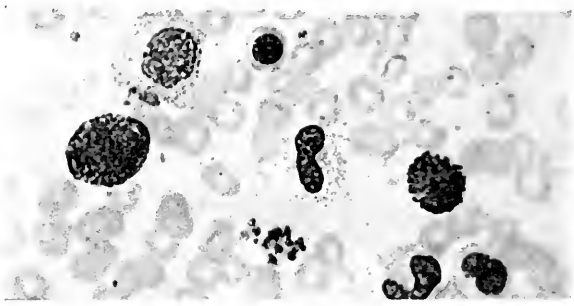


Fig. 6.—Leukemic blood (about × 650). Photograph taken with a Kodak, as described in the text.

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.

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 on a revolving nose-piece, 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 blood-counting 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 $80 to $100, exclusive of the mechanical stage.

Practical Exercises.—The following is a brief outline of certain exercises which the author has found useful in teaching microscopy. The student must learn as early as possible what can be expected of his microscope with proper manipulation. When he sits down to work his first glance should tell him whether the instrument is giving its best results. If the microscopic picture falls short of the best, he must locate the difficulty and correct it before proceeding.

1. Clean the microscope and study its parts, familiarizing yourself with the names, purposes, and movements of each (see Fig. 1).

2. Practice the manipulations necessary to locate particles of dust or dirt which appear in the microscopic field (see p. 37).

3. Try the effect of raising and lowering the condenser, and of changing from plane to concave mirror, upon the image of nearby objects which appear in the microscopic field, *e. g.*, the window-frame or trees outside the window. Note that such objects cause an unevenly illuminated or mottled field when a little out of focus.

4. Insert a "pointer" in one of the oculars (see p. 27).

5. Study illumination. Make two blood-films on slides. Stain one with Wright's or Harlow's stain. Use one unstained.

(1) Place one of these on the microscope, bring to a focus, and practice the manipulations necessary to secure (see pp. 20, 21)—

(*a*) Central illumination.

(*b*) Oblique illumination.

(*c*) Strong and subdued illumination.

The field in each case must be evenly lighted throughout, without mottling. Continue until you can adjust any desired form of illumination quickly and surely, and can recognize each by a glance into the microscope.

(2) Using the stained and unstained blood, ascertain the best form of illumination to study (see pp. 21, 22)—

(*a*) Outlines.

(*b*) Color.

(*c*) Surface contour.

Draw the corpuscles as they appear with each.

(3) Try dark ground illumination by means of the substage disk (see p. 23). Study the unstained blood-smear and draw a few corpuscles. Also examine a drop of saliva covered with a cover-glass. Note the epithelial cells, leukocytes, and miscellaneous bacteria. Use the 4-mm. objective for this.

6. With central illumination, focus upon a slide and observe how much of the numeric aperture is in use (see p. 30). Try the effect upon numeric aperture of—

(1) Opening and closing the diaphragm.

(2) Raising and lowering the condenser.

(3) Using the oil-immersion objective—

(*a*) Without oil.

(*b*) With oil between objective and cover-glass.

(*c*) With oil between slide and condenser.

7. Upon the same species of diatom compare two objectives of 3-mm. focus (therefore of same magnifying power), one of N. A. 1.4 and the other of N. A. 0.85. They will be adjusted by the instructor. Note the superior resolving power of the lens of high N. A. (see p. 29).

8. Practice using the oil-immersion objective (see p. 26). If there is difficulty in finding the specimen, move the slide about while lowering the objective to a focus. Moving objects will catch the eye as the ob ective approaches the correct focus. If a cover-glass is used, its edge can be easily found and focused upon.

Produce some bubbles in the oil by stirring it about on the slide; observe their effect on the microscopic image, and learn to detect their presence (see p. 26).

9. Image formation (see p. 27). Place any stained preparation upon the microscope. Remove the ocular and use a low-power objective. Lay a piece of ground glass across the top of the tube. This forms a screen upon which an image can be focused by means of the coarse adjustment. Try focusing it at different levels. Repeat this with the ocular in place, and with the ground glass some inches above the ocular. These exercises, especially the last, require a darkened room and strong artificial light.

10. Find by trial the magnification produced by your 16-mm. objective with the 4× ocular (see p. 31). Compare

your result with that obtained by calculation from the focal length (see p. 31).

11. Micrometry.

(1) Evaluate the scale of your micrometer eye-piece with a high-power objective, and measure accurately 10 red blood corpuscles and 10 leukocytes (see pp. 37, 38).

(2) Prepare a cardboard micrometer and measure 10 lycopodium granules (see pp. 38, 39).

12. Study the following structures, chiefly with a view to best illumination. Many of these are met as accidental contaminations in microscopic preparations and one must learn to recognize them. Make drawings of each.

Fluids are examined by placing a drop in the center of a clean slide and applying a cover-glass. The drop should be large enough to fill the space between the slide and cover, but not large enough to float the cover about. Fibers or insoluble powder may be placed in a drop of water and covered.

(1) Air bubbles produced by shaking a little diluted mucilage.

(2) Fresh milk diluted with three or four volumes of water. Prepare three slides.

(*a*) Examine one untreated.

(*b*) Treat one with solution of Sudan III. (For method see p. 183.) Note color assumed by the fat globules. This is one of the most useful tests for microscopic fat.

(*c*) Treat one with dilute acetic acid. Note clumping of globules similar to that of typhoid bacilli in the Widal test.

(3) A drop of diluted India-ink. Note the dancing motion of the particles ("Brownian motion").

(4) Starch granules. Gently scrape the freshly cut surface of a potato with a knife, place the scrapings

in a drop of water on a slide, remove coarse particles, and apply a cover-glass. Make two preparations.

(*a*) Examine one untreated. Note the variously sized starch granules, oval, colorless, concentrically striated.

(*b*) Treat one with Lugol's or Gram's iodin solution. Note change in color of granules. This is the standard test for starch.

(5) Yeast which has been growing in a dextrose solution. Make two preparations.

(*a*) Examine one unstained. Note "budding."

(*b*) Treat one with iodin solution. Compare color of yeast with that taken by starch.

(6) Mold from moldy food. Note hyphæ and spores.

(7) Various fibers and other structures mounted in a drop of water.

(*a*) Cotton.

(*b*) Wool.

(*c*) Linen.

(*d*) Silk.

(*e*) Feather tip.

(*f*) Some dust from a carpeted room.

(*g*) A hair from the head.

(8) A drop of decomposing urine. Note bacteria of various kinds, some motile, some non-motile. Make an effort to distinguish true motility from that due to currents in the fluid and to "Brownian motion."

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.

When **collecting the sample** for examination, the morning sputum, or the whole amount for twenty-four hours should be saved. 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 by the patient.

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 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.

The sputum of infants and young children is usually swallowed and therefore cannot be collected. In such cases examination of the feces for tubercle bacilli will sometimes establish a diagnosis of tuberculosis.

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. Although some of them (phenol, for example) do not interfere with detection of tubercle bacilli, they generally so alter the character of the sputum as to render it unfit for satisfactory examination.

The following outline is suggested for the **routine examination:**

1. Spread the material in a thin layer in a Petri dish or between two plates of glass. The use of glass plates is messy,

and is not to be recommended unless the top plate is much smaller than the lower one, or has some sort of handle.

2. Examine all parts carefully with the naked eye (best over a black background) or with a hand lens. The portions most suitable for further examination may thus be easily selected. *This macroscopic examination should never be omitted.*

3. Transfer various portions, including all suspicious particles, to clean slides, cover, and examine unstained with the microscope (see p. 53).

4. Slip the covers from some or all of the above unstained preparations, leaving a thin smear on both slide and cover.

5. Dry and fix the smears and stain one or more by each of the following methods:

   (*a*) For cells and bacteria in general, using pyronin-methyl green by preference (see p. 467).

   (*b*) For tubercle bacilli (see p. 63).

   (*c*) Gram's method (see p. 467).

6. When indicated, make special examinations for—

   (*a*) Capsules of bacteria (see p. 72).

   (*b*) Eosinophilic cells (see p. 77).

   (*c*) Presence of albumin (see p. 80).

After the 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. If flies are about, it must be kept covered. It is a good plan to conduct 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 or other disinfectant 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 early in the disease and generally denotes an extension of the tuberculous process. 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 lungs, 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.

5. Bronchial Casts.—Branching, tree-like casts of the larger bronchial tubes are sometimes seen. They are best examined in water. Further description is found on p. 59.

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. Unfortunately it is almost universally neglected. 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-cor-

puscles, eosinophiles, epithelial cells, and blood, and thus suggests appropriate stains to be used later. It enables one to select more intelligently the portions to be examined for tubercle bacilli.

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 unsatisfactory because not usually stiff enough. A little practice is necessary before one can handle particles of sputum readily. The bit desired should be separated from the bulk of the sputum by cutting it free with the tooth-pick. It can then be picked up by rotating the end of a fresh tooth-pick against it. *The slide must never be dipped into the sputum, nor must any of the sputum be allowed to reach its edges in spreading.*

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" (see p. 79), are also best studied without staining (Plate II, Fig. 1).

1. Elastic Fibers.—These are the elastic fibers of the pulmonary substance, where they are distributed in the walls of the alveoli, the bronchioles, and the blood-vessels (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. After the diagnosis is established they furnish a valuable clue as to the existence and rate of lung destruction. In gangrene of the lung, contrary to the older teaching, elastic tissue is probably always present in the sputum, usually in large fragments.

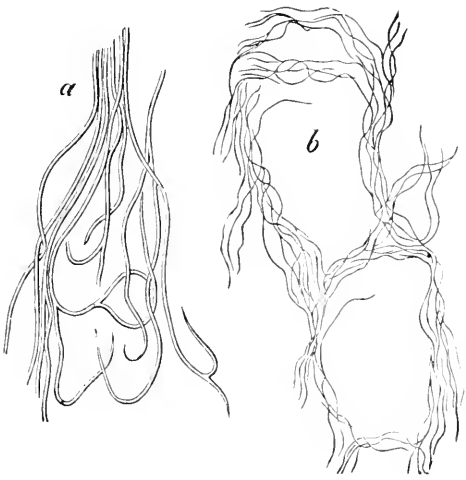


Fig. 7.—Elastic fibers from the sputum: *a*, Highly magnified; *b*, alveolar arrangement, less highly magnified (after Bizzozero).

The fibers should be searched for with a 16-mm. objective, although a higher power is needed to identify them with certainty. They may usually be more clearly seen if a drop of 10 to 20 per cent. caustic soda solution be mixed with the sputum on the slide before the cover is applied. 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. 73). 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 faily characteristic. Although not present in every attack, they probably occur at some time in every case. Sometimes they can be found only near the end of the attack. They may occasionally be met with in chronic bronchitis and other conditions. Their nature has not been definitely determined.

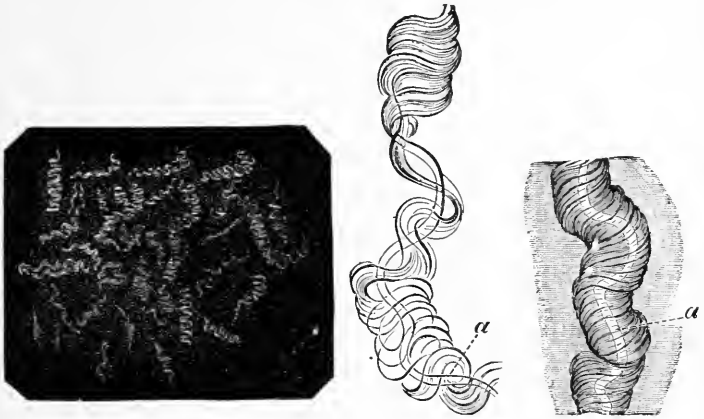


Fig. 8.—Curschmann's spirals: I Natural size; II and III, enlarged: *a*, central fiber (after Curschmann).

Macroscopically, they are whitish or yellow, twisted threads, frequently coiled into little balls (Fig. 8, I). Their length is rarely over 1.5 cm., though it sometimes exceeds 5 cm. They cannot be definitely recognized with the naked eye. 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, consisting merely of twisted strands of mucus enclosing 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

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

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 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 crystals (Fig. 9). They were formerly described as octahedral, but are now known to be hexagonal in cross-section. Their size varies greatly, the average length being about three or four times the diameter of a red blood-corpuscle.

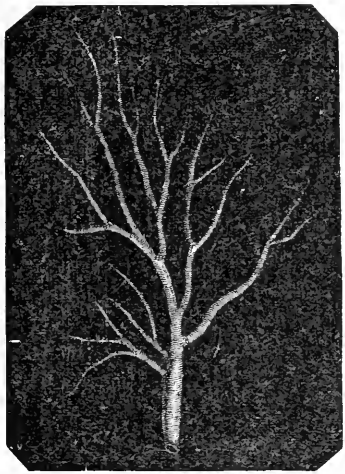


Fig. 10.—Fibrinous bronchial cast (Sahli).

Other crystals—hematoidin, cholesterin, and, most frequently, fatty-acid needles (see Fig. 35)—are common in sputum which has remained in the body for a considerable time, as in abscess of the lung and bronchiectasis.

4. Bronchial 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 recognized 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 usually indicative of fibrinous or chronic plastic bronchitis, but may also be found in

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

diphtheria of the smaller bronchi. Very small casts are often seen in croupous pneumonia.

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 network of threads having a more or less radial arrangement. In cattle, and to a less extent in man, the filaments at the periphery of the nodule present 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.

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 (see 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 westermannii*, 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. Larvæ of *Strongyloides intestinalis* and of the hook-worm have been reported. 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. These films must, of course, be thin, but it is easily possible to get them too thin. This is a common error of students who have just finished a course in bacteriology and who have there been accustomed to work with scarcely perceptible films of bacteria. It is a good plan to slide off the cover-glass from the preparation used for the unstained microscopic examinations. If this is properly done satisfactory smears will be left on both slide and cover. They are then dried in the air, and fixed in the flame, as described on page 466. 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 general stain, like Löffler's methylene blue or pyronin-methyl green (see p. 467), 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*. Their cultural characteristics are described in Chapter VIII.

(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 or lipoid substance. A number of the best staining methods are included here. Since Gabbet's method is probably the most convenient for general purposes, it is given in greater detail than the others. It is always best to select the more purulent portions of the sputum, keeping away from the mucoid parts. If bits of necrotic tissue are present they may show immense numbers of tubercle bacilli, when other portions of the specimen contain very few. One must, however, be on his guard against bits of food which resemble these "caseous particles." The specimen should be examined while fresh. It will usually liquefy upon

standing, and this, by preventing the selection of particles favorable for examination, will greatly reduce one's chances of finding bacilli.

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 Bursen burner or alcohol lamp low down in the flame. Take care not to scorch. A scorched smear has a brownish tinge, especially at the edge. The smear can likewise be fixed by flaming with alcohol as given for blood-films (see p. 265). Should the film be washed off during future manipulations, fixation has been insufficient.

(4) Apply as much carbol-fuchsin as will stay on, and hold over a flame so that it will steam for three minutes or longer, replacing the stain with a dropper as it evaporates. If the stain is allowed to evaporate completely, the preparation is ruined. 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 substance upon which the acid-fast property depends. *It is better to stain at room temperature for twelve to twenty-four hours*, although this is not often done in routine work.

(5) Wash the film in water.

(6) Apply Gabbet's stain to the under side of the cover-glass to remove excess of carbol-fuchsin, 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.

Carbol-fuchsin 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. This stain sometimes rather suddenly "goes bad," in which case it appears more transparent than normal and the bottle contains a dark red sediment.

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

Both stains can be purchased ready prepared.

Ziehl-Neelson Method.—The objection is often made to the above method that decolorization is masked by the blue in Gabbet's stain. Although this will not make trouble if step (8) is carefully carried out, most experienced workers prefer the Ziehl-Neelson method. This resembles Gabbet's method, with the following exceptions: After the staining with carbol-fuchsin the smear is washed in 5 per cent. nitric acid (or, better, a mixture of 3 c.c. concentrated hydrochloric acid and 97 c.c. 70 per cent. alcohol) until decolorized, washed in water, stained lightly with Löffler's methylene blue, again washed, and finally dried 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 stain. 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 vary considerably in size, averaging 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, even in advanced cases, several cover-glasses must be examined to find a single bacillus. At times they are

PLATE II

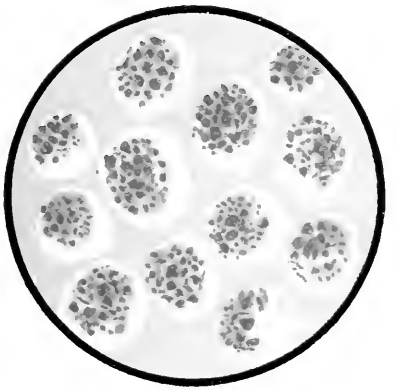


Fig. 1.—Heart-failure cells in sputum, containing blood-pigment, from a case of cardiac congestion of the lungs (Jakob).

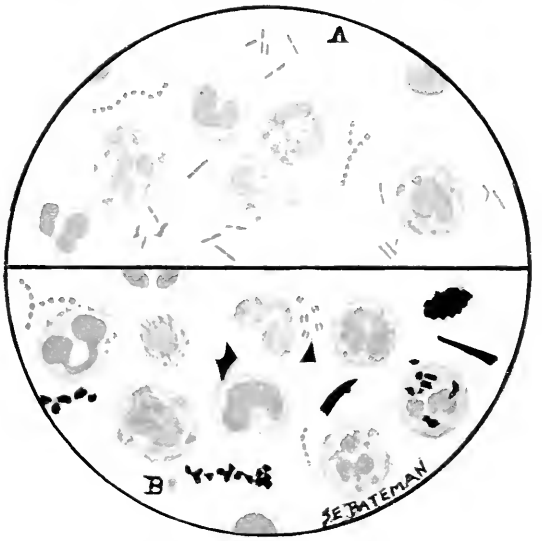


Fig. 2.—*A*, Sputum showing tubercle bacilli stained with carbolfuchsin and Gabbet's methylene-blue solution (obj. one-twelfth oil-immersion); *B*, sputum of anthracosis, showing particles of coal-dust stained with methylene-blue (obj. one-twelfth oil-immersion) (Boston).

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 it is desired to record the approximate number of bacilli present, the Gaffky table as modified by Brown may be employed, using an oil-immersion lens and 4× ocular:

I. One to four bacilli to the slide.
II. Average of one in many fields.
III. Average of one in a field.
IV. Average of two to three in a field.
V. Average of four to six in a field.
VI. Average of seven to twelve in a field.
VII. Average of thirteen to twenty-five in a field.
VIII. Average of about fifty in a field.
IX. Average of about one hundred in a field.
X. Enormous numbers in a field.

Since the sputum raised at various times in the day, and even different parts of the same sample, may vary greatly in bacillary content, such a table is of little value unless the twenty-four-hour sputum is collected and uniformly mixed before preparing the slides. This is satisfactorily accomplished by the antiformin method.

When bacilli 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 a trade 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. Substitutes appear to be less satisfactory than the original preparation.

Löffler's method is probably the best for clincial work. It kills the bacilli, so that there is no danger in handling the material. Upon this account, however, it is not applicable to isolation of tubercle bacilli for pure cultures.

Place 10 to 20 c.c. of the sputum in a small flask, with an equal amount of 50 per cent. antiformin, and heat to the boiling-point. The sputum will be thoroughly liquefied, usually within a few seconds. For each 10 c.c. of the resulting fluid add 1.5 c.c. of a mixture of 1 volume of chloroform and 9 volumes of alcohol. Shake vigorously for several minutes or until emulsification has taken place. The object is to impregnate the lipoid capsule of the bacilli with chloroform, thus increasing their specific gravity. Pour off the emulsion into centrifuge tubes and centrifugalize at high speed for about fifteen minutes. The chloroform will go to the bottom, and the sediment which collects on its surface in a thin firm layer will contain the tubercle bacilli. Pour off the supernatant liquid and transfer the sediment to glass slides, removing the excess of fluid with filter-paper. To facilitate removal of the disk of sediment *in toto* Williamson recommends the use of a centrifuge tube, the lower $\frac{1}{2}$ inch of which is of uniform caliber and the bottom of which is open and plugged with a rubber stopper. Add a little egg-albumen solution (see p. 72) or, better, some of the original sputum, to cause the film to adhere to the slide, mix well,

spread into a uniform layer, and finally dry, fix, and stain by the Ziehl-Neelson method. Löffler recommends 0.1 per cent. solution of malachite green for counterstain.

(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 (see p. 205).

Other bacteria than the acid-fast group are stained blue by Gabbet's and the Ziehl-Neelson 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.

Within the past few years much interest has centered in the so-called "Much granules." These are Gram-positive but non-acid-fast granules and rods; and are

apparently forms of the tubercle bacillus, since material containing them causes tuberculosis when injected into guinea-pigs. They may be present either alone or in company with the ordinary acid-fast form. Their recognition accounts for the well-known fact that in some cases of undoubted pulmonary tuberculosis no tubercle bacilli have been demonstrated in the sputum.

Methods for Much Granules.—(1) Stain the dried and fixed film with carbol-gentian volet, heating to steaming three times.

(2) Rinse with water and apply Gram's iodin solution for five minutes.

(3) Wash in water and apply successively 5 per cent. nitric acid one minute, 3 per cent. hydrochloric acid ten seconds, equal parts of absolute alcohol and acetone until color ceases to come off.

(4) Wash in water and counterstain with an aqueous solution of saffranin.

(5) Wash, dry, and mount.

Tubercle bacilli and Much granules will be purple; other organisms, red.

Perhaps more satisfactory is a combination of the Ziehl-Neelson and Gram's method as advocated by Fontes: After staining with carbol-fuchsin and decolorizing (see p. 65), the preparation is stained by Gram's method (see p. 467) and counterstained with Bismarck brown.

Carbol-gentian violet consists of saturated alcoholic solution of gentian-violet 10 c.c.; 2 per cent. phenol, 90 c.c.

(2) **Staphylococcus and Streptococcus** (see 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 fac-

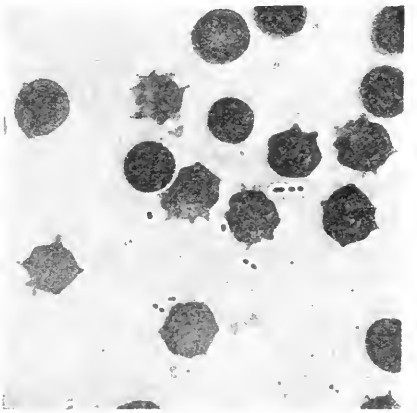


Fig. 12.—Diplococcus pneumoniæ in the blood (× 1000) (Fränkel and Pfeiffer).

tor in the causation of pleurisy, meningitis, otitis media, and other inflammations. It is frequently present 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).

The pneumococcus is closely related to the streptococcus, and it is sometimes extremely difficult to differ-

entiate them even by culture methods (for which see p. 420). 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, but is applicable also to the sputum. Smith's 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 solution (egg-albumen, distilled water, equal parts; shake, filter through cotton, and add about 0.5 per cent. phenol). Blood-serum can be obtained as described for the Widal test (see p. 498). 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 (see p. 467), counterstained with aqueous solution of fuchsin, washed, and mounted in water. The pneumococcus holds the purple stain, while all capsules take 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. 467), 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; and 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 re-

semble pneumococci, from which they are distinguished by the fact that they are Gram-decolorizing.

(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.

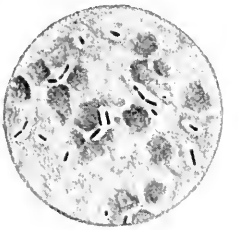


Fig. 13.—Friedländer's bacillus in pus from pulmonary abscess (one-twelfth objective) (Boston).

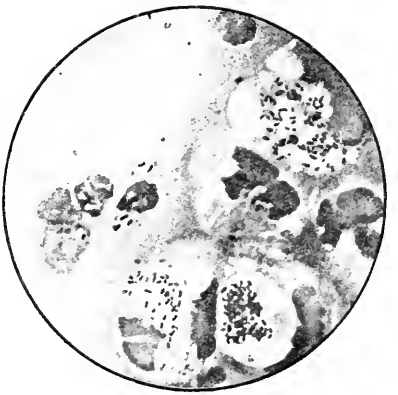


Fig. 14.—Bacillus of influenza; cover-glass preparation of sputum from a case of influenza, showing the bacilli in leukocytes; highly magnified (Pfeiffer).

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 157).

They are well stained by dilute fuchsin or by Pappenheim's pyronin-methyl green, but are more certainly recognized by Gram's method with the pyronin-methyl green for counterstain.

(6) **Bacillus pertussis.**—The whooping-cough bacillus is a minute, ovoid, Gram-negative bacillus which stains feebly with the ordinary dyes, and sometimes, though not usually, lies within pus cells. It can be demonstrated by the method given for the influenza bacillus.

(7) **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 meningococcus and gonococcus only by means of cultures. The staining method recommended for the influenza bacillus is best. 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 time of the paroxysm, and constitute one of the most distinctive features of the sputum of this disease. They

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).

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 des-

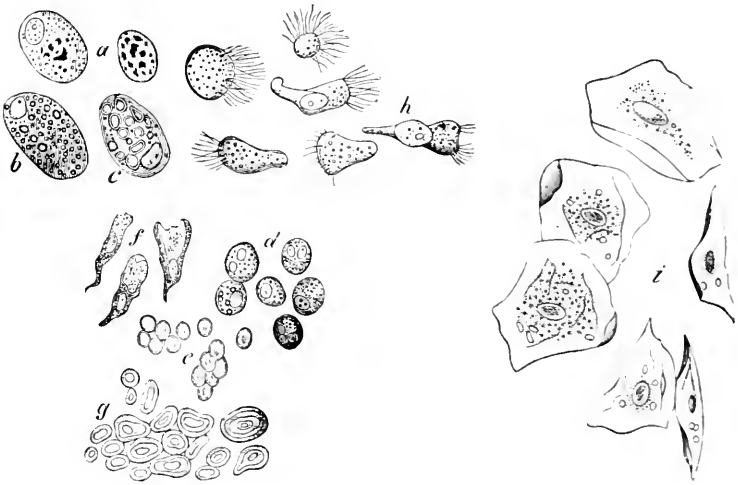


Fig. 10.—Different morphologic elements of the sputum (unstained): *a*, *b*, *c*, Pulmonary or alveolar epithelium—*a*, with normal lung pigment (carbon); *b*, with fat-droplets; *c*, with myelin globules; *d*, pus-corpuscles; *e*, red blood-corpuscles; *f*, cylindric beaker-shaped bronchial epithelial 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).

quamation 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 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. (See Plate II, Fig. 1.) Such cells are also present after pulmonary hemorrhage.

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

whether early or far advanced, generally show 0.2 per cent. or more albumin; slightly active cases, less than 0.2 per cent. The sputum must be fresh, otherwise a negative reaction may have changed to positive.

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 abun-

dant, mucopurulent, and yellowish or yellowish-green in color. Nummular masses like those of tuberculosis are sometimes 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 appear only in the morning, and is then scanty and almost purely mucoid, with an occasional yellow flake; or there may be only one very small mucopurulent mass. When the quantity is 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. Intelligent coöperation of the patient is essential in such cases. Tubercle bacilli will sometimes be found in large numbers at this stage. Blood-streaked sputum is strongly suggestive of tuberculosis, and is more com-

mon in the early stages than later. It usually indicates an advancing process.

The sputum of more advanced cases resembles that of chronic bronchitis, with the addition of tubercle bacilli and elastic fibers. Nummular masses—circular, "coin-like" disks, which sink in water—may be seen. Caseous particles containing immense numbers of the bacilli are common. Far-advanced cases with old cavities often show rather firm, spheric or ovoid grayish masses 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 extremely complex aqueous solution of various organic and inorganic substances. Most of the substances 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 the **collection of the specimen** is important and 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 fours into a clean vessel kept in a cool place, to mix it well, to measure the whole quantity, and to bring 8 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 5 grains of boric acid (as much of the powder as can be heaped upon a ten-cent piece) for each 4 ounces of urine. Formalin, in proportion of 1 drop to 4 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. Thymol, toluol, and chloroform are likewise much used. The chief objection to toluol is the fact that it floats upon the surface, and the

urine must be pipeted from beneath it. Chloroform is probably the least satisfactory. It reduces Fehling's solution; and it settles to the bottom in the form of globules which it is impossible to avoid when removing the sediment for microscopic examination. One of these preservatives may be placed in the vessel when collection of the twenty-four-hour sample is begun. Whenever possible the urine should be kept on ice.

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 or infusorial earth (Keiselguhr), shaking well, and filtering.

A suspected fluid can be **identified as urine** by detecting any considerable quantity of urea in it (see p. 114). 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. Microscipic 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 35 to 50 ounces for an adult in this country. German writers give higher figures. For children the amount is somewhat greater in proportion to body weight.

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 fairly constant—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; in the parenchymatous forms of nephritis, and during accumulation of fluid in the serous cavities. In uremia the urine is usually very greatly decreased and may be entirely suppressed (anuria).

Ordinarily, more urine is voided during the day than during the night, the normal ratio being about 100 to 50 or 60. In certain diseases, notably arteriosclerosis and cardiac and renal disease, conditions are reversed, and

the night urine (7 P. M. to 7 A. M.) equals or exceeds that passed during the day.

2. Color.—This varies considerably in health, and depends largely upon the quantity of urine voided, dilute urines being pale and concentrated urines highly colored. The usual color is yellow or reddish yellow, due to the presence of several pigments, chiefly urochrome, which is yellow. Traces of hematoporphyrin, uroerythrin, and urobilin are frequent. Uroerythrin is chiefly responsible for the deep reddish tinge of urine in acute fevers. Urobilin has clinical significance and is discussed later (see p. 158). Acid urine is generally darker than alkaline. In recording the color, Vogel's scale (see Plate III) is very widely used, the urine being filtered and examined by transmitted light in a glass 3 or 4 inches in diameter.

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 greenish 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. A bright pink or red color appearing when the urine is alkalinized may be due to phenolphthalein. 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 (see p. 157). A milky color may be due to presence of chyle, or milk may have been added by a malingering patient.

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." This is more abundant in women owing to vaginal cells and mucus. In urines of high specific gravity it may float near the middle of the fluid.

Abnormal cloudiness is usually due to presence of phosphates, urates, pus, blood, or bacteria.

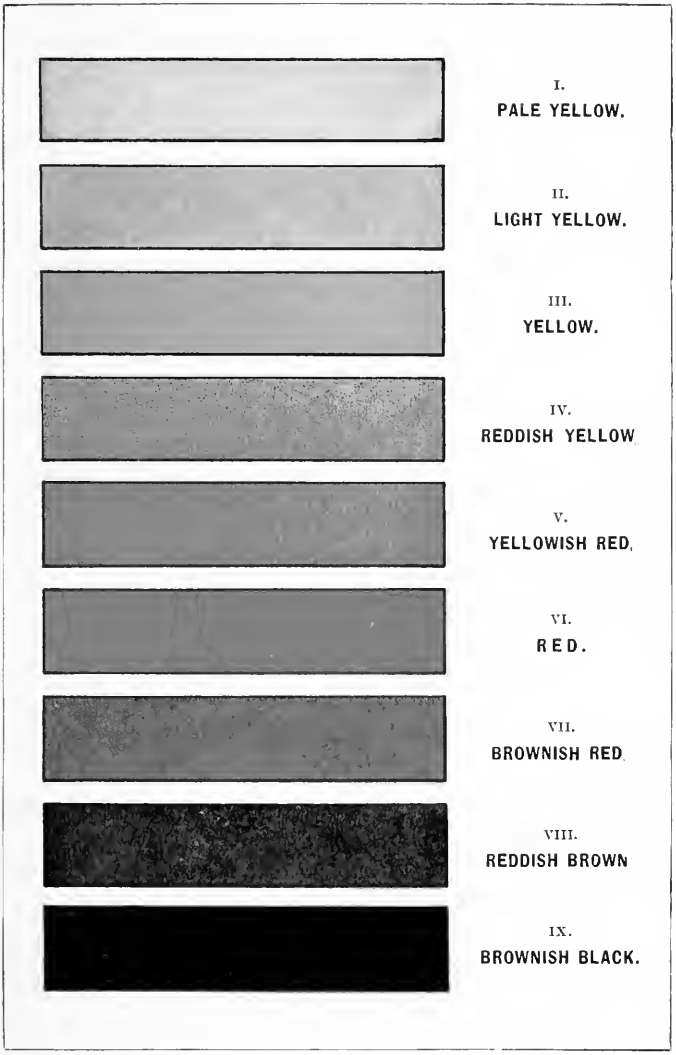
Amorphous phosphates are precipitated in neutral or alkaline urine. They form a white cloud and sediment, which disappear upon addition of an acid.

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

PLATE III



Scale of Urinary Colors, according to Vogel

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 sometimes increases for a time after the urine is voided, the so-called "acid fermentation." The acidity was formerly held to be due wholly 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 they may be amphoteric, turning red litmus-paper blue and blue paper red, owing to presence of both alkaline and acid phosphates. The reaction is determined by means of litmus-paper, which, however, is

worthless unless of good quality. That put up in vials by Squibb can be recommended.

Acidity is increased after administration of certain drugs, and whenever the urine is concentrated from any cause, as in fevers. A strongly 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, or that the paper will turn blue when held in the steam over the boiling urine. A second form of alkalinity, *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 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.

The specific gravity is most conveniently estimated by means of the urinometer (Fig. 17). Squibb's urinometer is adjusted to give accurate readings at 22.5° C.; most other instruments, at 15° C. If the urine be brought to about the right temperature, a correction for temperature will seldom be necessary in clinical work. For accuracy, however, it is necessary to add 0.001 to the urinometer reading for each 3° C. above the temperature for which the urinometer is standardized, and to subtract 0.001 for each 3° C. below that point. 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. Bubbles are easily removed with a strip of filter-paper. 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. Many of the urinometers on the market are too small to be of any real value.

One frequently wishes to ascertain the specific gravity of quantities of fluid too small to float a urinometer.

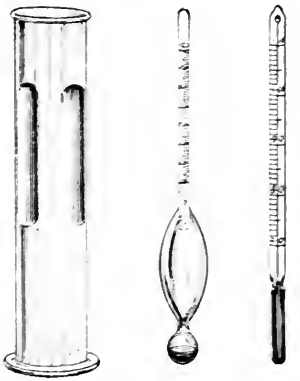


Fig. 17.—Squibb's urinometer with thermometer and cylinder.

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 distilled 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.

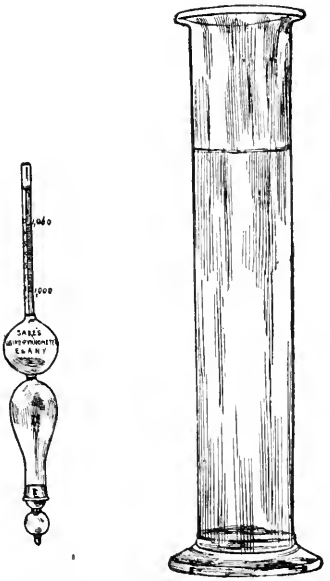


Fig. 18.—Saxe's urinopyknometer and jar for same.

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 perform-

ing 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, which uses the metric system, 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 phloridzin test, the methylene-blue test, and the phenolsulphonephthalein test. It is doubtful whether, except in the case of the last, 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. Only the phenolsulphonephthalein test will be given in detail. The reader is referred to larger works upon urinalysis for details regarding the others.

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.

Phenolsulphonephthalein Test.—This test, which was offered by Rowntree and Geraghty in 1910, consists in the intramuscular (or intravenous) injection of a solution of phenolsulphonephthalein, a drug which is eliminated only by the kidneys, and whose amount in the urine is easily estimated by colorimetric methods. The time of its first appearance in the urine and the quantity eliminated within a definite period are taken as a measure of the functional capacity of the kidneys. The test is harmless, comparatively simple, and apparently reliable. It will sometimes reveal a very serious degree of renal failure when total quantity, total solids, and urea are practically normal.

Preparation of Solution.—The solution used consists of—

Phenolsulphonephthalein..... 0.6 gm.;
$\frac{2}{N}$ Sodium hydroxid..... 0.84 c.c.;
0.75 per cent. sodium chlorid to..... 100 "

To this is added just enough of the $\frac{2}{N}$ sodium hydroxid solution to change the color to a beautiful Bordeaux red, usually 2 to 3 drops. An approximate $\frac{2}{N}$ solution of sodium hydroxid can be made by dissolving 82 gm. of the chemically pure alkali in a liter of distilled water. The phenolsulphonephthalein solution can be purchased in ampoules ready for use.

Technic.—Give the patient 300 to 400 c.c. of water to promote secretion of urine. Twenty to thirty minutes afterward empty the bladder with a catheter. Leaving the catheter in place, inject 1 c.c. of the above solution (previously sterilized by boiling) into the lumbar muscles with a hypodermic syringe. Allow the urine to drain into a test-tube in which 1 drop of 25 per cent. sodium hydroxid has been placed, and note the time of the first appearance of a faint but distinct pink color. Then, unless there be prostatic obstruction, withdraw the catheter and instruct the patient to urinate at the end of an hour from the first appearance of the color, and again at the end of the second hour, saving the urine in separate vessels. If there be objection to catheterizing, the patient may empty his bladder voluntarily when the drug is injected and then urinate every few minutes until the pink color appears.

Since the principal interest is not in the time of first appearance, but in the *quantity eliminated*, the test may be simplified by having the patient empty his bladder when the drug is injected, and again one hour and ten minutes and two hours and ten minutes afterward.

Dilute each of the two portions to exactly 1000 c.c. with

water. This is best done in 1-liter volumetric flasks. The color is yellow or orange while the urine is acid, but becomes brilliant purple red when it is alkalinized. Add sufficient 25 per cent. sodium hydroxid solution to bring out the maximum color, filter off a small quantity, and estimate the amount of phenolsulphonephthalein excreted during each of the one-hour periods, by comparing its color with that of a standard alkaline solution containing 0.006 gm. of phenolsulphonephthalein (1 c.c. of original solution) to the liter. This can be done with a fair degree of accuracy in 50-c.c. cylinders, the darker fluid being diluted until the two have exactly the same depth of color. They then contain the same percentage of the drug; and, the amount in the standard solution being known, it is easy to calculate the amount in the total quantity of urine. The cylinders may be viewed from the top, or they may be compared in a frame with a ground glass back like that of the Sahli hemoglobinometer. For greater accuracy the Rowntree and Geraghty modification of the Hellige colorimeter is recommended. When it is necessary to defer the color comparison for hours or days the urine must be kept acid, as the color generally fades after it is alkalinized.

Normal Standards.—Under normal conditions the drug first appears in the urine in five to eleven minutes after the injection. Within the first hour after its appearance 40 to 60 per cent. is eliminated; in the two hours, 60 to 85 per cent. Pathologically the elimination may be reduced to a trace or even none at all in the two hours.

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.
Water	1000–1500	1200
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	30–35	30
Uric acid	0.4–1.0	0.7

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

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

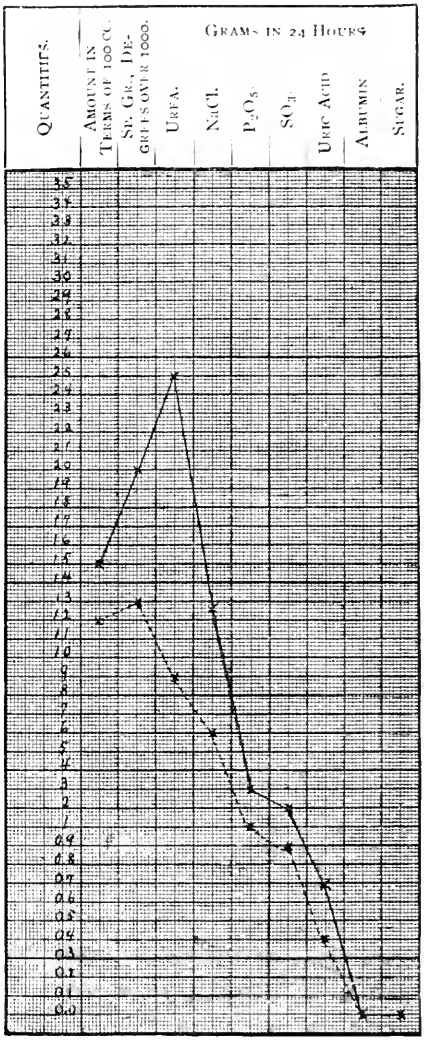


Fig. 10.—Graphic expression of quantities in the urine. Solid line, normal urine; dotted line, an example of pathologic urine in a case of cancerous cachexia (Saxe).

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.

Detection of Chlorids.—The following simple test will

show the presence of chlorids, and at the same time roughly indicate any pronounced alteration in amount:

To a few cubic centimeters of urine in a test-tube add a few drops of nitric acid to prevent precipitation of phosphates and then a few drops of silver nitrate solution of about 12 per cent. strength. A white, curdy precipitate of silver chlorid forms. If the urine merely becomes milky or opalescent, chlorids are markedly diminished.

Quantitative Estimation.—The well-known and reliable Volhard method has been simplified by Strauss, and this modification has recently been still further simplified by Bayne-Jones and by McLean and Selling, so that the method is now available for ordinary clinical work. The only difficulty is the preparation of solutions, and these can be purchased ready prepared. A less accurate, though simple and very useful, method is afforded by the centrifuge (see p. 105).

Simplified Volhard Method.—Albumin need not be removed. In an accurately graduated 50-c.c. cylinder place 5 c.c. of the urine and 10 c.c. of Solution No. 1. Mix by inverting several times. If a reddish color appears, add 3 drops of 10 per cent. potassium permanganate. After five minutes add Solution No. 2, a very little at a time, mixing after each addition, until a permanent red-brown color (best seen against a white background) appears. This is the end-point.

The solutions are so balanced that if the urine be chlorid-free the volume of fluid when the end-point is reached will be 35 c.c., and that for each gram per liter of chlorids in the urine the volume will be 1 c.c. less. Therefore, the difference between 35 c.c. and the height of the fluid at the end of the test gives directly the number of grams of chlo-

rids per liter of urine, expressed as sodium chlorid. If, for example, the fluid reaches the 28 c.c. mark, $35-28=7$ gm. of sodium chlorid per liter of urine.

A certified 50-c.c. graduated cylinder, with glass stopper, is required. The ordinary 50-c.c. graduate is inaccurate.

The **solutions** are as follows:

No. 1.—*Standard silver nitrate solution:*

Silver nitrate (C. P., anhydrous, crystallized)	29.055 gm.;
Nitric acid (25 per cent.)	900 c.c.;
Ammonioferric alum (cold saturated solution)	50 "
Distilled water to	1000 "

No. 2.—*Ammonium sulphocyanate solution:*

Ammonium sulphocyanate	7 gm.;
Distilled water	1000 c.c.

This solution is intentionally made too strong, and it must be standardized by diluting with distilled water until exactly 20 c.c. (and no less) will produce a red color when mixed with exactly 10 c.c. of Solution No. 1.

Purdy's Centrifugal Methods.—As shown by 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 most 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*

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

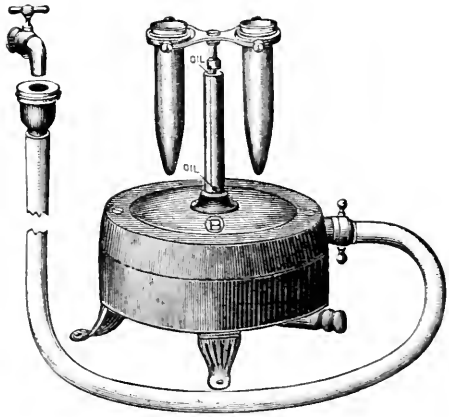


Fig. 21.—Water-motor centrifuge.

by weight. The weight percentage can be found by referring to Purdy's tables, given later. In this, as in

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 a radius of $6\frac{3}{4}$ inches when in motion, and should be capable of maintaining a speed of 1500 revolutions a minute. The

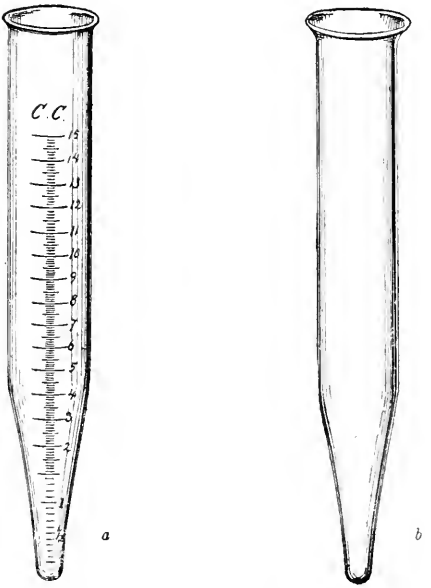


Fig. 22.—Purdy's tubes for the centrifuge: *a*, Percentage tube; *b*, sediment tube.

electric centrifuge is best, 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.

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.
[illegible]	0.03	0.15	0.02	0.1	8	1.04	4.98	0.63	3.02
	0.07	0.31	0.04	0.19	8½	1.1	5.29	0.67	3.22
	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..	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.

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 of 12 per cent. strength 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. This may be converted into percentage by weight of chlorin or sodium chlorid by means of the table upon page 108.

2. Phosphates are derived largely from the food, only a small proportion resulting from metabolism. The normal daily output of phosphoric acid is about 2.5 to 3.5 gm.

The urinary phosphates are of two kinds: *alkaline*, which make up two-thirds of the whole, and include the phosphates of sodium and potassium; and *earthy*, which constitute one-third, and include the phosphates of calcium and magnesium. Earthy phosphates are frequently thrown out of solution in neutral and alkaline urines, and as "amorphous phosphates" form a very common sediment. This sediment seldom indicates an excessive excretion of phosphoric acid. It is usually merely an evidence of diminished acidity of the urine, or of an increase in the proportion of phosphoric acid eliminated as earthy phosphates. This form of "phosphaturia" is most frequent in neurasthenia and hysteria. When the urine undergoes ammoniacal decomposition, some of the ammonia set free combines with magnesium phosphate to form ammoniomagnesium phosphate ("triple phosphate"), which is deposited in typical crystalline form (see p. 185).

Excretion of phosphates is *increased* by a diet rich in nucleins; in active metabolism; in certain nervous and mental disorders; in leukemia; and in phosphatic diabetes, an obscure disturbance of metabolism (not related to diabetes mellitus) which is associated with an increase

in the output of phosphates up to 10 gm. or more in twenty-four hours. Phosphates are *decreased* in chronic diseases with lowered metabolism; in hepatic cirrhosis and acute yellow atrophy; in pregnancy, owing to developing fetal bones; and in nephritis, owing to kidney impermeability.

Quantitative estimation does not furnish much of definite clinical value. The centrifugal method is the most convenient.

TABLE FOR THE ESTIMATION OF PHOSPHATES AFTER CENTRIFUGATION

Showing bulk-percentages of uranyl phosphate ($H[UO_2]PO_4$) and the corresponding gravimetric percentages and grains per ounce of phosphoric acid (P_2O_5).—(Purdy.)

Bulk-percentage of $H(UO_2)PO_4$.	Percentage P_2O_5.	Gr. Per Oz. P_2O_5.	Bulk-percentage of $H(UO_2)PO_4$.	Percentage P_2O_5.	Gr. Per Oz. P_2O_5.
$\frac{1}{2}$	0.02	0.1	11	0.14	0.67
1	0.04	0.19	12	0.15	0.72
$1\frac{1}{2}$	0.045	0.22	13	0.16	0.77
2	0.05	0.24	14	0.17	0.82
$2\frac{1}{2}$	0.055	0.26	15	0.18	0.86
3	0.06	0.29	16	0.19	0.91
$3\frac{1}{2}$	0.065	0.31	17	0.2	0.96
4	0.07	0.34	18	0.21	1.
$4\frac{1}{2}$	0.075	0.36	19	0.22	1.06
5	0.08	0.38	20	0.23	1.1
6	0.09	0.43	21	0.24	1.15
7	0.1	0.48	22	0.25	1.2
8	0.11	0.53	23	0.26	1.25
9	0.12	0.58	24	0.27	1.3
10	0.13	0.62	25	0.28	1.35

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

Purdy's Centrifugal Method.—Take 10 c.c. urine in the graduated tube, add 2 c.c. of 50 per cent. acetic acid, and 3 c.c. of 5 per cent. uranium nitrate solution. Mix; let stand a few minutes, and revolve for three minutes at

1200 revolutions a minute. Each 0.1 c.c. of precipitate is 1 per cent. by bulk. The percentage corresponding of phosphoric acid by weight is found by consulting the table on page 110.

3. Sulphates.—The urinary sulphates are derived partly from the food, especially meats, and partly from body metabolism. The normal output of sulphuric acid is about 1.5 to 3 gm. daily. It is increased in conditions associated with active metabolism, and in general may be taken as a rough index of protein metabolism.

Quantitative estimation of the total sulphates yields little of clinical value.

TABLE FOR THE ESTIMATION OF SULPHATES AFTER CENTRIFUGATION

Showing the bulk-percentages of barium sulphate ($BaSO_4$) and the corresponding gravimetric percentages and grains per fluidounce of sulphuric acid (SO_3).—(Purdy.)

Bulk-percentage of $BaSO_4$.	Percentage SO_3.	Gr. Per Oz. SO_3.	Bulk-percentage of $BaSO_4$.	Percentage SO_3.	Gr. Per Oz. SO_3.
$\frac{1}{8}$	0.04	0.19	$2\frac{1}{4}$	0.55	2.64
$\frac{1}{4}$	0.07	0.34	$2\frac{1}{2}$	0.61	2.93
$\frac{3}{8}$	0.1	0.48	$2\frac{3}{4}$	0.67	3.22
$\frac{1}{2}$	0.13	0.62	3	0.73	3.5
$\frac{5}{8}$	0.16	0.77	$3\frac{1}{4}$	0.79	3.79
$\frac{3}{4}$	0.19	0.91	$3\frac{1}{2}$	0.85	4.08
$\frac{7}{8}$	0.22	1.06	$3\frac{3}{4}$	0.91	4.37
1	0.25	1.1	4	0.97	4.66
$1\frac{1}{4}$	0.31	1.49	$4\frac{1}{4}$	1.03	4.94
$1\frac{1}{2}$	0.37	1.78	$4\frac{1}{2}$	1.09	5.23
$1\frac{3}{4}$	0.43	2.06	$4\frac{3}{4}$	1.15	5.52
2	0.49	2.35	5	1.21	5.81

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

Purdy's Centrifugal Method.—Take 10 c.c. urine in the graduated tube and add barium chlorid solution to the

15 c.c. mark. This consists of barium chlorid, 4 parts; strong hydrochloric acid, 1 part; and distilled water, 16 parts. Mix; let stand a few minutes, and revolve for three minutes at 1200 revolutions a minute. Each 0.1 c.c. of precipitate is 1 per cent. by bulk The percentage by weight of sulphuric acid is calculated from the table on page 111.

About nine-tenths of the sulphuric acid is in combination with various mineral substances, chiefly sodium, potassium, calcium, and magnesium (*mineral* or *preformed sulphates*). One-tenth is in combination with certain aromatic substances, which are mostly products of protein putrefaction in the intestine, but are derived in part from destructive metabolism (*conjugate* or *ethereal sulphates*). Among these aromatic substances are indol, phenol, and skatol. By far the most important of the conjugate sulphates and representative of the group is potassium indoxyl sulphate.

Potassium indoxyl sulphate, or **indican,** is derived from indol. Indol is absorbed and oxidized into indoxyl, which combines with sulphuric acid and potassium and is thus excreted. Under normal conditions the amount in the urine is small. It is increased by a meat diet.

Unlike the other ethereal sulphates, which are derived in part from metabolism, indican originates practically wholly from putrefactive processes. It alone, therefore, and not the total ethereal sulphates, can be taken as an index of such putrefaction. A pathologic increase is called indicanuria. It is noted in:

(*a*) *Diseases of the Small Intestine.*—This is by far the most common source. Intestinal obstruction gives

the largest amounts of indican. It is also much increased in intestinal indigestion—so-called "biliousness"; in inflammations, especially in cholera and typhoid fever; and in paralysis of peristalsis, such as occurs in peritonitis. Simple constipation and diseases of the *large* intestine alone do not so frequently cause indicanuria.

(*b*) *Diseases of the stomach* associated with deficient hydrochloric acid, as chronic gastritis and gastric cancer. Diminished hydrochloric acid favors intestinal putrefaction.

(*c*) *Diminished Flow of Bile.*—Since the bile serves both as a stimulant to peristalsis and an intestinal antiseptic, a diminished flow from any cause favors occurrence of indicanuria.

(*d*) *Decomposition of exudates* anywhere in the body, as in empyema, bronchiectasis, and large tuberculous cavities.

Detection of indican depends upon its decomposition and oxidation of the indoxyl set free into indigo-blue. This change sometimes takes place spontaneously in decomposing urine, causing a dirty blue color. Crystals of indigo (see Fig. 37) may be found both in the sediment and the scum.

Obermayer's Method.—Take a test-tube about one-third full of the urine and add an equal volume of Obermayer's reagent and a few cubic centimeters of chloroform. Mix by inverting a few times; avoid shaking violently. If indican be present in excess, the chloroform, which sinks to the bottom, will assume an indigo-blue color. It will take up the indigo more quickly if the urine be warm. The depth of color indicates the comparative amount of indican if the same propor-

tions of urine and reagents are always used, but one should bear in mind the total amount of urine voided. The indican in normal urine may give a faint blue by this method. Urine of patients taking iodids gives a reddish-violet color, which disappears upon addition of a few drops of strong sodium hyposulphite solution and shaking. Occasionally indigo-red will form instead of indigo-blue. This resembles the color due to iodids, but does not disappear when treated with sodium hyposulphite. Bile-pigments, which interfere with the test, must be removed if present (see p. 87).

Obermayer's reagent consists of strong hydrochloric acid (sp. gr., 1.19), 1000 parts, and ferric chlorid, 2 parts. This makes a yellow, fuming liquid which keeps well.

4. Urea.—From the standpoint of physiology urea is the most important constituent of the urine. It is the principal waste-product of metabolism, and constitutes about one-half of all the solids excreted—about 20 to 35 gm. in twenty-four hours. It represents 85 to 90 per cent. of the total nitrogen of the urine, and its quantitative estimation is a simple, though not very accurate, method of ascertaining the state of nitrogenous excretion.

This is true, however, only in normal individuals upon average mixed diet. Under pathologic conditions, the proportion of nitrogen distributed among the various nitrogen-containing substances undergoes great variation. The only accurate index of protein metabolism is, therefore, the total output of nitrogen, which can be estimated by the Kjeldahl method. The whole subject of "nitrogen partition" and "nitrogen equilibrium" (relation of excretion to intake) is an important one, but is out of the province of this book, since as yet it concerns the physiologic chemist more than the clinician.

It may be helpful to state here, however, that upon a mixed diet the nitrogen of the urine is distributed about as follows: urea nitrogen, 86.9 per cent.; ammonia nitrogen, 4.4 per cent.; creatinin nitrogen, 3.6 per cent.; uric acid nitrogen, 0.75 per cent.; "undetermined nitrogen," chiefly in amino-acids, 4.3 per cent.

Normally, the amount is greatly influenced by exercise and diet. It is increased by copious drinking of water and administration of ammonium salts of organic acids.

Pathologically, urea is increased in fevers, in diabetes when acidosis is not marked, and especially during resolution of pneumonia and absorption of large exudates. As above indicated, when other factors are equal, the amount of urea indicates the activity of metabolism. In deciding whether in a given case an increase of urea is due to increased metabolism the relation between the amounts of urea and of the chlorids is a helpful consideration. The amount of urea is normally about twice that of the chlorids. If the proportion is much increased above this, increased tissue destruction may be inferred, since other conditions which increase urea also increase chlorids.

In general, a pathologic decrease in amount of urea is due either to lessened formation within the body or to diminished excretion. *Decreased formation* of urea occurs in diseases of the liver with destruction of liver substance, such as marked cirrhosis, carcinoma, and acute yellow atrophy. The state of acidosis likewise decreases formation of urea, because nitrogen which would otherwise be built into urea is eliminated in the form of ammonia (see p. 125). *Retention* of urea occurs in most cases

of nephritis. In acute nephritis the amount of urea in the urine is markedly decreased, and a return to normal denotes improvement. In the early stages of chronic nephritis, when diagnosis is difficult, it is usually normal. In the late stages, when diagnosis is comparatively easy, it is decreased. Hence estimation of urea is of little help in the diagnosis of this disease, especially when, as is so frequently the case, a small quantity of urine taken at random is used. When, however, the diagnosis is estab-

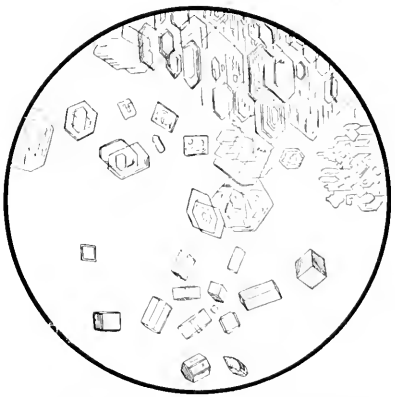


Fig. 23.—Crystals of nitrate of urea (upper half) and oxalate of urea (lower half) (after Funke).

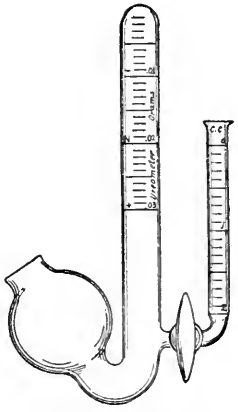


Fig. 24.—Doremus-Hinds' ureometer without foot.

lished, estimations made at frequent intervals under the same conditions of diet and exercise are of much value, *provided a sample of the mixed twenty-four-hour urine be used.* A steady decline is a very bad prognostic sign, and a sudden marked diminution is usually a forerunner of uremia.

The presence of urea can be shown by allowing a few drops of the fluid partially to evaporate upon a slide, and adding a small drop of pure, colorless nitric acid or

saturated solution of oxalic acid. Crystals of urea nitrate or oxalate (Fig. 23) will soon appear and can be recognized with the microscope.

Quantitative Estimation.—The hypobromite method, which is generally used in clinical work, is very simple, but is notoriously inaccurate. The new urease methods are much more accurate.

(1) **Hypobromite Method.**—This depends upon the fact that urea is decomposed by sodium hypobromite with liberation of nitrogen. The amount of urea is calculated from the volume of nitrogen set free. Of the many forms of apparatus devised for this purpose, that of Doremus-Hinds (Fig. 24) is probably the most convenient.

Pour some of the urine into the smaller tube of the apparatus, then open the stop-cock and quickly close it so as to fill its lumen with urine. Rinse out the larger tube with water and fill it and the bulb with 25 per cent. caustic soda solution. Add to this 1 c.c. of bromin by means of a medicine-dropper and mix well. This prepares a fresh solution of sodium hypobromite with excess of caustic soda, which serves to absorb the carbon dioxid set free in the decomposition of urea. When handling bromin, keep an open vessel of ammonia near to neutralize the irritant fumes.

Pour the urine into the smaller tube, and then turn the stop-cock so as to let as much urine as desired (usually 1 c.c.) run slowly into the hypobromite solution. When bubbles have ceased to rise, read off the height of the fluid in the large tube by the graduations upon its side. This gives the amount by weight of urea in the urine added, from which the amount excreted in twenty-four hours can easily be calculated. If the urine contains much more than the normal amount, it should be diluted.

This method has fallen into disrepute largely because of

inconstant results, and because it gives more nearly the total nitrogen than the urea. According to Robinson and Müller the discrepancies are due to insufficient mixing of urine and hypobromite and can be obviated by gentle shaking after the first vigorous reaction is over. Results are then constant, but too high, owing to decomposition of other nitrogenous constituents; and they find that with normal urine the ureometer reading multiplied by 0.917 gives the true amount of urea.

To avoid handling pure bromin, which is disagreeable, Rice's solutions may be employed:

(*a*) Bromin ..... 31 gm.;
Potassium bromid ..... 31 "
Distilled water ..... 250 c.c.;

(*b*) Sodium hydroxid ..... 100 gm.;
Distilled water ..... 250 c.c.

Equal parts of these solutions are mixed and used for the test. The bromin solution must be kept in a tightly stoppered bottle or it will rapidly lose strength.

(2) **Urease Method.**—In brief, this consists in the conversion of urea into ammonium carbonate by urease, a ferment first extracted by Takeuchi from the soy bean in 1909. The amount of urea is calculated from the amount of ammonia which is liberated from the carbonate, and transferred by an air current to a standard acid solution, and this titrated with a standard alkali solution. The method was first employed by Marshall. Van Slyke and Cullen have recently introduced certain improvements which render it more suitable for clinical laboratory use. Although the method is somewhat complicated, it requires no apparatus except that shown in Fig. 25[1], a suction filter-pump which can be attached to the water faucet, and a buret.

[1] The apparatus may be obtained complete from E. Greiner & Co., 45 Cliff Street, New York City.

Method.—Since the ammonia from the pre-existing ammonium salts of the urine as well as that derived from urea is carried over into the acid, it is necessary to carry through two tests, in one of which urease is used and in the other omitted.

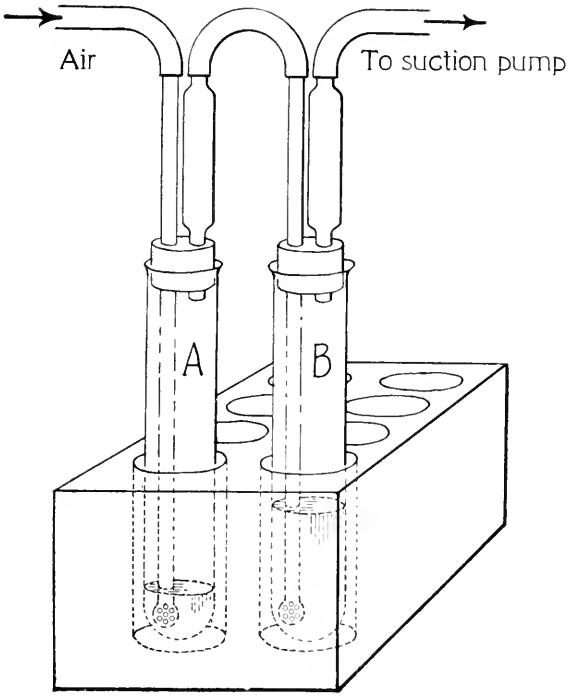


Fig. 25.—Apparatus for urease method of estimating urea.

The difference between the results obtained in the two represents the ammonia from urea alone.

(1) *For urea plus ammonia:*

(*a*) Dilute 5 c.c. of urine to 50 c.c.

(*b*) In tube A (Fig. 25) place 5 c.c. diluted urine, 1 c.c. enzyme solution, and 1 drop caprylic alcohol. (The last is

added to prevent foaming; 0.5–1 c.c. toluol, amyl alcohol, or kerosene may be substituted.)

(*c*) Close tube *A* and let stand for fifteen minutes for enzyme to act.

(*d*) In tube *B* place 5 c.c. $\frac{N}{10}$ hydrochloric or sulphuric acid, 20 c.c. distilled water, 1 drop of 1 per cent. aqueous solution of alizarin sodium sulphonate to serve as indicator, and 1 drop caprylic alcohol.

(*e*) Connect tube *A* with *B* and *B* with the filter-pump, which is attached to the water faucet.

(*f*) Pass the air current (by opening water faucet) for a half-minute. This will carry over into the acid any ammonia that may have escaped from the urine into the air space in *A*.

(*g*) Open tube *A* and pour into it 4 or 5 grams of dry potassium carbonate.

(*h*) Close *A* and pass the air current until all the ammonia has been carried over into the acid. This will require from five to fifty minutes, depending upon the rate of the current. The correct time can be determined for each pump by trial.

(*i*) Titrate the acid in *B* with $\frac{N}{10}$ sodium hydroxid from a buret. A red color indicates the end-point. The difference between the amount of $\frac{N}{10}$ acid originally taken (5 c.c.) and the amount of $\frac{N}{10}$ alkali solution used in the titration is the amount of acid which has been neutralized by the ammonia. Since each cubic centimeter of the $\frac{N}{10}$ acid solution represents 0.0017 gram ammonia, the number of cubic centimeters of the $\frac{N}{10}$ acid neutralized by the ammonia is multiplied by the factor 0.0017 to give the *number of grams of ammonia derived from the urea plus the ammonium salts in the 0.5 c.c. of urine used;* and this product by 200 to give the number of grams from 100 c.c. urine.

When extreme accuracy is essential, the following variations should be introduced: (1), the ammonia of the air should be removed by passing the air through a tube of weak sulphuric acid before it enters tube *A*; (2), a second tube of standard acid should be interposed between *B* and the suc-

tion-pump to catch any ammonia which may escape neutralization in *B*; and (3), fiftieth-normal alkali solution should be employed instead of decinormal, thus ensuring greater accuracy in titration.

Preparation of Enzyme Solution.—Dissolve 2 grams urease powder,[1] 0.6 gram dipotassium hydrogen phosphate, and 0.4 gram potassium dihydrogen phosphate in 10 c.c. water. It forms an opalescent solution, which will keep about two weeks if covered with a layer of toluol.

(2) *For ammonia alone:*

This requires a second pair of tubes which is connected in series with the first, *i. e.*, interposed between the first pair and the suction-pump in such manner that the air passes from tube *B* of the first pair to tube *A* of the second.

The technic is exactly the same as that described in the preceding paragraphs, except that 5 c.c. of *undiluted* urine are used and the enzyme solution is omitted in step (*b*). The number of cubic centimeters of the $\frac{N}{10}$ acid neutralized by the ammonia is multiplied by the factor 0.0017 to give the number of grams of ammonia in the 5 c.c. of urine used; and this product by 20 to give the number of grams of ammonia in 100 c.c. urine.

This is essentially **Folin's method for ammonia.**

(3) *Calculations:*

Find by the two methods just given:

(1) The amount of ammonia derived from the urea plus the ammonium salts of 100 c.c. urine.

(2) The amount of ammonia derived from the ammonium salts, only, in 100 c.c. urine.

The difference between (1) and (2) gives the ammonia derived from the urea alone in 100 c.c. urine. Each gram of

[1] This can be obtained from the Arlington Chemical Co., Yonkers, N. Y. It keeps indefinitely. It can also be purchased in 1-gram portions mixed with the correct amount of phosphate ready to be dissolved in the appropriate quantity of water.

ammonia represents 1.7647 gram of urea. If, for example, the difference between (1) and (2) is 1.5 grams, then $1.5 \times 1.7647 = 2.647$ grams of urea in 100 c.c. urine. From this the amount in the twenty-four-hour sample is calculated.

The above method is particularly useful in estimating urea in blood and cerebrospinal fluid. Van Slyke and Cullen use 3 c.c. of fresh blood or fluid, measured with an accurate pipet into a 100-c.c. test-tube containing 1 c.c. of 3 per cent. potassium citrate to prevent clotting. To this are added 0.5 c.c. urease solution and 2 or 3 drops caprylic alcohol, and the method carried out as above described.

5. Uric acid is the most important of a group of substances, called *purin bodies*, which are derived chiefly from the nucleins of the food, *exogenous uric acid*, and from metabolic destruction of the nuclei of the body, *endogenous uric acid*. The daily output of uric acid is about 0.4 to 1 gm. The amount of the other purin bodies together is about one-tenth that of uric acid. Excretion of these substances is greatly increased by a diet rich in nucleins, as sweetbreads and liver.

Uric acid exists in the urine in the form of urates, chiefly of sodium and potassium, which in concentrated urines are readily thrown out of solution and constitute the familiar sediment of "amorphous urates." This, together with the fact that uric acid is frequently deposited as crystals, constitutes its chief interest to the practitioner. It is a very common error to consider these deposits as evidence of excessive excretion.

Pathologically, the greatest increase of uric acid occurs in leukemia, where there is extensive destruction of leukocytes, and in diseases with active destruction of the

liver and other organs rich in nuclei. There is generally an increase during *x*-ray treatment. Uric acid is decreased before an attack of gout and increased for several days after it, but its etiologic relation is still uncertain. An increase is also noted in acute fevers.

Quantitative Estimation of Purin Bodies.—There is no accurate method which is simple enough for clinical purposes. Of clinical methods, the two given here are most satisfactory. They are based upon the same principle: precipitation and removal of phosphates, and then precipitation of purin bodies with silver nitrate which is strongly ammoniated in order to hold silver chlorid in solution. The amount of purin bodies is calculated from the bulk of the silver-purin, which in Cook's method is thrown down by the centrifuge, and in Hall's is allowed to settle for twenty-four hours. The urine must be albumin free.

(1) **Cook's Method.**—In a centrifuge tube take 10 c.c. urine and add about 1 gm. (about 1 c.c.) sodium carbonate and 1 or 2 c.c. strong ammonia. Shake until the soda is dissolved. The earthy phosphates will be precipitated. Centrifugalize thoroughly and pour off all the clear fluid into a graduated centrifuge tube. Add 2 c.c. ammonia and 2 c.c. ammoniated silver nitrate solution. Let stand a few minutes, and revolve in the centrifuge until the bulk of precipitate *remains constant.* Each $\frac{1}{10}$ c.c. of sediment represents 0.001176 gm. purin bodies.

Ammoniated silver nitrate solution is prepared by dissolving 5 gm. of silver nitrate in 100 c.c. distilled water, and adding ammonia until the solution clouds and again becomes clear.

(2) **Hall's Method.**—The instrument is shown in Fig. 26. Close the stop-cock, introduce 90 c.c. urine and 20 c.c. of

the magnesia solution, and mix by inverting a few times. Open the stop-cock and let the instrument stand for about ten minutes, or until the precipitated phosphates have settled into the lower chamber. Then close the stop-cock, and pour in ammoniated silver nitrate solution until the level of the fluid reaches the 100 c.c. mark. Mix well, and if any white precipitate of silver chlorid persists, bring it into solution by adding a few drops of ammonia. Stand the instrument in the dark for twenty-four hours and read off the bulk of the precipitate. The corresponding percentage of purin nitrogen is found by reference to a table which accompanies the instrument. Albumin must be removed before making the test.

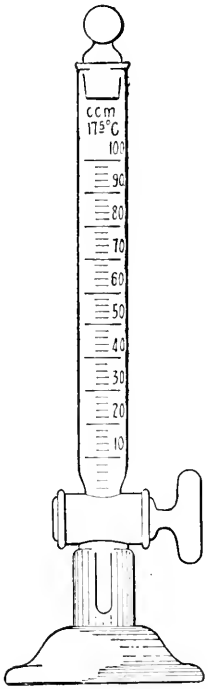


Fig. 26.—Hall's purinometer.

The *magnesia mixture* is prepared by dissoving 10 gm. of magnesium chlorid in 75 c.c. of water and adding 10 gm. of ammonium chlorid and 100 c.c. strong ammonium hydroxid. If a precipitate forms, it is dissolved by further addition of ammonia. Add water to bring the volume to 200 c.c. and finally add 10 gm. of finely powdered talcum.

The *ammoniated silver nitrate solution* used in Hall's method consists of silver nitrate, 1 gram; ammonium hydroxid, 100 c.c.; talcum, 5 grams; distilled water, 100 c.c.

Quantitative Estimation of Uric Acid.—Ruhemann's method, while not accurate, will probably answer for clinical work.

Ruhemann's Method for Uric Acid.—The urine must be slightly acid. By means of a pipet fill Ruhemann's tube (Fig. 27) to the mark *S* with the indicator, carbon disulphid, so that the lowest part of the meniscus is on a level with the mark, as indicated in Fig. 27. Next add Ruhemann's reagent until the base of the upper arch of the meniscus is level with the mark *J*. The carbon disulphid will assume a violet color. Add the urine, a small quantity at a time, closing the tube with the glass stopper and shaking vigorously after each addition, until the disulphid loses every trace of its violet color and becomes pure white. This completes the test. Toward the end the reagent should be added a very little at a time, and the shaking should be prolonged in order not to pass the end-point. The figure in the right-hand column of figures corresponding to the top of the fluid gives the amount of uric acid in parts per thousand. The presence of diacetic acid interferes with the test, as do also, to some extent, bile and albumin. Diacetic acid can be driven off by boiling; bile-pigment and albumin are removed as described elsewhere (see pp. 87 and 142).

Ruhemann's reagent consists of iodin, 0.5 gm.; potassium iodid, 1.25 gm.; absolute alcohol, 7.5 gm.; glycerin, 5 gm.; distilled water to 100 c.c.

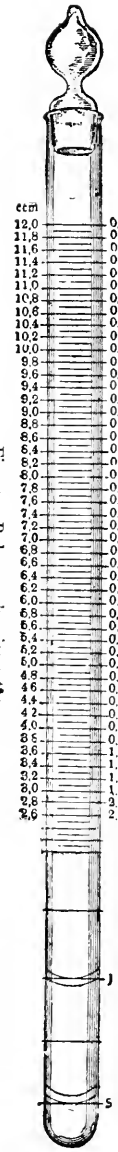


Fig. 27.—Ruhemann's uricometer.

6. Ammonia.—A small amount of ammonia, combined with hydrochloric, phosphoric, and sulphuric acids, is al-

ways present. Estimated as NH_3, the normal average is about 0.7 gm. in twenty-four hours. This represents 4 to 5 per cent. of the total nitrogen of the urine, ammonia standing next to urea in this respect.

Under ordinary conditions, most of the ammonia which results from the metabolic processes is transformed into urea. When, however, acids are present in excess, either from ingestion of mineral acids or from abnormal production of acids within the body (as in fevers, diabetes, pernicious vomiting of pregnancy, delayed chloroform-poisoning, etc.), ammonia combines with them and is so excreted, urea being correspondingly decreased. It is thus that the body protects itself against acid intoxication. A marked increase of ammonia is, therefore, important chiefly as an index of the tendency to acidosis, particularly that associated with the presence of diacetic and oxybutyric acids.

In diabetes mellitus ammonia elimination may reach 4 or 5 gm. daily. It is likewise markedly increased in pernicious vomiting of pregnancy, but *not in nervous vomiting;* and in conditions in which the power to synthesize urea is interfered with, notably cirrhosis and other destructive diseases of the liver and conditions associated with deficient oxygenation.

Quantitative Estimation.—The urine must be fresh, since decomposition increases the amount of ammonia. The following method is satisfactory for clinical purposes, though subject to some inaccuracies. It includes amino-acids with the ammonia, hence gives figures that are a little too high. Folin's method for ammonia, which is more accurate, was given on page 121.

Ronchese-Malfatti Formalin Test.—This depends upon the fact that when formalin is added to the urine the ammonia combines with it, forming hexamethylenamin. The acids with which the ammonia was combined are set free, and their quantity, ascertained by titration with sodium hydroxid, indicates the amount of ammonia.

Take 10 c.c. of the urine in a beaker or evaporating dish, add 50 c.c. water and 10 drops of 0.5 per cent. alcoholic solution of phenolphthalein. Neutralize by adding a weak caustic soda or sodium carbonate solution until a permanent pink color appears. To 5 c.c. formalin add 15 c.c. water and neutralize in the same way. Pour the formalin into the urine. The pink color at once disappears, owing to liberation of acids. Now add decinormal sodium hydroxid solution from a buret until the pink color just returns. Each cubic centimeter of the decinormal solution used in this titration corresponds to 0.0017 gm. of NH_3. This must be multiplied by 10 to obtain the percentage from which the twenty-four-hour elimination of ammonia is calculated.

The method is more complicated, but distinctly more accurate, when carried out as suggested by E. W. Brown: Treat 60 c.c. of urine with 3 gm. of basic lead acetate, stir well, let stand a few minutes, and filter. This removes certain interfering nitrogenous substances. Treat the filtrate with 2 gm. neutral potassium oxalate, stir well, and filter. Take 10 c.c. of the filtrate, add 50 c.c. water and 15 gm. neutral potassium oxalate, and proceed with the ammonia estimation as above outlined.

B. Abnormal Constituents

Those substances which appear in the urine only in pathologic conditions are of much more interest to the clinician than are those which have just been discussed. Among them are: proteins, sugars, the acetone bodies,

bile, hemoglobin, and the diazo substances. The "pancreatic reaction" and detection of drugs in the urine will also be discussed under this head.

1. Proteins.—Of the proteins which may appear in the urine, serum-albumin and serum-globulin are the most important. Mucin, proteose, and a few others are found occasionally, but are of less interest.

(1) **Serum-albumin and Serum-globulin.**—These two proteins constitute the so-called "urinary albumin." They usually occur together, have practically the same significance, and both respond to all the ordinary tests for "albumin."

Their presence, or *albuminuria*, is probably the most important pathologic condition of the urine. It is either *accidental* or *renal*. The physician can make no greater mistake than to regard all cases of albuminuria as indicating kidney disease.

Accidental or *false albuminuria* is due to admixture with the urine of albuminous fluids, such as pus, blood, and vaginal discharge. The microscope will usually reveal its nature. It occurs most frequently in pyelitis, cystitis, and chronic vaginitis.

Renal albuminuria refers to albumin which has passed from the blood into the urine through the walls of the kidney tubules or the glomeruli.

Albuminuria sufficient to be recognized by clinical methods probably never occurs as a physiologic condition, the so-called *physiologic albuminuria* appearing only under conditions which must be regarded as abnormal. Among these may be mentioned excessive muscular exertion in those unaccustomed to it; excessive ingestion of proteins; prolonged cold baths, and

childbirth. In these conditions the albuminuria is slight and transient.

There are certain other forms of albuminuria which have still less claim to be called physiologic, but which are not always regarded as pathologic. Among these are *cyclic albuminuria*, which regularly recurs at a certain period of the day, and *orthostatic* or *postural albuminuria*, which appears only when the patient is standing. They are rare and of obscure origin, and occur for the most part in neurasthenic subjects during adolescence. It is noteworthy in this connection that nephritis sometimes begins with a cyclic albuminuria.

In pathologic conditions and in most, at least, of the "functional" conditions just enumerated, renal albuminuria may be referred to one or more of the follow-lowing causes. In nearly all cases it is accompanied by tube-casts.

(*a*) *Changes in the blood* which render its albumin more diffusible, as in severe anemias, purpura, and scurvy. Here the albumin is small in amount.

(*b*) *Changes in circulation in the kidney*, either anemia or congestion, as in excessive exercise, chronic heart disease, and pressure upon the renal veins. The quantity of albumin is usually, but not always, small. Its presence is constant or temporary, according to the cause. Most of the causes, if continued, will produce organic changes in the kidney.

(*c*) *Organic Changes in the Kidney.*—These include the inflammatory and degenerative changes commonly grouped together under the name of nephritis, and also renal tuberculosis, neoplasms, and cloudy swelling due to irritation of toxins and drugs. The amount of al-

bumin eliminated in these conditions varies from minute traces to 20 gm., or even more, in the twenty-four hours, and, except in acute processes, bears little relation to the severity of the disease. In acute and chronic parenchymatous nephritis the quantity is usually very large. In chronic interstitial nephritis it is small—frequently no more than a trace. It is small in cloudy swelling from toxins and drugs, and variable in renal tuberculosis and neoplasms. In amyloid disease of the kidney the quantity is usually small, and serum-globulin may be present in especially large proportion, or even alone. Roughly distinctive of serum-globulin is the appearance of an opalescent cloud when a few drops of the urine are dropped into a glass of distilled water.

Detection of albumin depends upon its precipitation by chemicals or coagulation by heat. There are many tests, but none is entirely satisfactory, because other substances as well as albumin are precipitated. The most common source of error is mucin. When any considerable amount of mucin is present it can be removed by acidifying with acetic acid and filtering. The tests given here are widely used and can be recommended. They make no distinction between serum-albumin and serum-globulin. Usually the best time to detect albumin is in the evening or a few hours after a meal.

It is very important that urine to be tested for albumin be rendered clear by filtration or centrifugation. This is too often neglected in routine work. When ordinary methods do not suffice, it can usually be cleared by shaking up with a little purified talc or animal charcoal

and filtering. If the urine is alkaline, sufficient acetic acid should be added to make it acid to litmus.

(1) **Trichloracetic Acid Test.**—The reagent consists of a saturated aqueous solution of trichloracetic acid to which magnesium sulphate is added to saturation. A simple saturated solution of the acid may be used, but addition of magnesium sulphate favors precipitation of globulin, and, by raising the specific gravity, makes the test easier to apply.

Take a few cubic centimeters of the reagent in a test-tube or conical test glass, hold the tube or glass in an inclined position, and run the urine gently in by means of a pipet or medicine-dropper, so that it will form a layer on top of the reagent without mixing with it. If albumin be present, a white, cloudy ring will appear where the two fluids come in contact. The ring can be seen most clearly if viewed against a black background, and one side of the tube or conical glass may be painted black for this purpose.

This is an extremely sensitive test, but, unfortunately, both mucin and proteoses respond to it: urates, when abundant, may give a confusing white ring, and the reagent is comparatively expensive. It is not much used in routine work except as a control to the less sensitive tests.

A convenient and satisfactory instrument for applying this or any of the contact tests is sold under the name of "horismascope" (Fig. 28). In Boston's method of performing the ring tests the fluids are brought into contact in a glass pipet, which is immersed first in the lighter fluid and then (after wiping the outside of the pipet) in the heavier. This is widely used and is convenient for routine testing of a large number of urines, but it cannot be recommended for accuracy, owing to the small diameter of the column of fluid.

(2) **Robert's Test.**—The reagent consists of pure nitric acid, 1 part, and saturated aqueous solution of magnesium

sulphate, 5 parts. It is applied in the same way as the preceding test.

Albumin gives a white ring, which varies in density with the amount present. A similar white ring may be produced by primary proteose, thymol, and resinous drugs. White rings or cloudiness in the urine *above* the zone of contact may result from excess of urates or mucus. Colored rings near the junc-

Fig. 28.—Horismascope: adding the reagent.

tion of the fluids may be produced by iodids, urinary pigments, bile, or indican.

Robert's test is one of the best for routine work, although the various rings are apt to be confusing to the inexperienced. It is more sensitive than Heller's test, of which it is a modification, and has the additional advantage that the reagent is not so corrosive.

(3) **Ulrich's test** avoids the somewhat confusing colored rings. The reagent consists of saturated solution of common salt, 98 c.c.; glacial acetic acid, 2 c.c. It must be perfectly clear. Boil a few cubic centimeters of this fluid in a test-tube, and immediately overlay with the urine as in the preceding tests. Albumin and globulin give a white ring at the zone of contact.

(4) **Purdy's Heat Test.**—Take a test-tube two-thirds full of urine, add about one-sixth its volume of saturated solution of sodium chlorid, and 5 to 10 drops of 50 per cent. acetic acid. Mix, and boil the upper inch. A white cloud in the heated portion shows the presence of albumin.

This is a valuable test for routine work. It is simple, sufficiently accurate for clinical purposes, and has practically no fallacies. *Addition of the salt solution, by raising the specific gravity, prevents precipitation of mucin.* Proteose may produce a white cloud, which disappears upon boiling and reappears upon cooling.

(5) **Heat and Nitric Acid Test.**—This is one of the oldest of the albumin tests, and, if properly carried out, one of the best. Boil about 5 c.c. of filtered urine in a test-tube and add 1 to 3 drops of concentrated nitric acid. A white cloud or flocculent precipitate (which usually appears during the boiling, but if the quantity be very small only after addition of the acid) denotes the presence of albumin. A similar white precipitate, which disappears upon addition of the acid, is due to earthy phosphates. The acid should not be added before boiling, and the proper amount should always be used; otherwise, part of the albumin may fail to be precipitated or may be redissolved.

Quantitative Estimation.—The gravimetric, which is the most reliable method, is too elaborate for clinical work. Both Esbach's, which is very widely used, and

Purdy's centrifugal method give fair results, but Tsuchiya's modification of the Esbach method is preferable to either.

Fig. 29.—Esbach's albuminometer, improved form.

(1) **Esbach's Method.**—The urine must be clear, of acid reaction, and not concentrated. Always filter before testing, and, if necessary, add acetic acid and dilute with water. Esbach's tube (Fig. 29) is essentially a test-tube with a mark U near the middle, a mark R near the top, and graduations $\frac{1}{2}$, 1, 2, 3, etc., near the bottom. Fill the tube to the mark U with urine and to the mark R with the reagent. Close with a rubber stopper, invert slowly several times, and set aside in a cool place. At the end of twenty-four hours read off the height of the precipitate. This gives the amount of albumin in *grams per liter, and must be divided by 10 to obtain the percentage.*

Esbach's reagent consists of picric acid, 1 gm., citric acid, 2 gm., and distilled water, to make 100 c.c.

(2) **Tsuchiya's Method.**—This is carried out in the same manner as the Esbach method, using the following reagent:

Phosphotungstic acid	1.5 gm.;
Alcohol (96 per cent.)	95.0 c.c.;
Concentrated hydrochloric acid	5.0 "

The urine should be diluted to a specific gravity not exceeding 1.008. The method is said to be much more accurate than the original Esbach method, particularly with small quantities of albumin.

(3) **Purdy's Centrifugal Method.**—This is detailed in the table on opposite page. Since 10 c.c. of urine were used,

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

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

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

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

each 0.1 c.c. of precipitate is 1 per cent. by bulk. Instead of the ferrocyanid and acetic acid Tsuchiya's solution may be used.

(2) **Mucin.**—Traces of the substances (mucin, mucoid, etc.) which are loosely classed under this name are present in normal urine; increased amounts are observed in irritations and inflammations of the mucous membrane of the urinary tract. They are of interest chiefly because they may be mistaken for albumin in most of the tests. If the urine be diluted with water and acidified with acetic acid, the appearance of a white cloud indicates the presence of mucin.

True mucin is a glyco-protein, and upon boiling with an acid or alkali, as in Fehling's test, yields a carbohydrate substance which reduces copper.

(3) **Proteoses.**—These are intermediate products in the digestion of proteins and are frequently, although incorrectly, called albumoses. Two groups are generally recognized: *primary proteoses*, which are precipitated upon half-saturation of their solutions with ammonium sulphate; and *secondary proteoses*, which are precipitated only upon complete saturation.

The secondary proteoses have been observed in the urine in febrile and malignant diseases and chronic suppurations, during resolution of pneumonia, and in many other conditions, but their clinical significance is indefinite. In pregnancy, albumosuria may be due to absorption of amniotic fluid.

Primary proteoses are rarely encountered in the urine. The protein known as the "Bence-Jones body" was originally classed under this head, but its true nature is

uncertain. It is regarded as practically pathognomonic of multiple myeloma. It has recently been found in a number of cases of chronic leukemia, of both lymphatic and myelogenous types.

The proteoses are not coagulable by heat, but are precipitated by such substances as trichloracetic acid and phosphotungstic acid. The primary proteoses alone are precipitated by nitric acid.

Proteoses may be detected by acidifying the urine with acetic acid, boiling and filtering while hot to remove mucin, albumin, and globulin, and testing the filtrate by the trichloracetic acid test. As above indicated, the nitric acid test, and half and complete saturation with ammonium sulphate, will separate the two groups.

To detect Bence-Jones' protein the urine is acidified with acetic acid and gently heated in a water-bath. If this substance be present, the urine will begin to be turbid at about 40° C. and a precipitate will form at about 60° C. As the boiling-point is reached the precipitate wholly or partially dissolves. It reappears upon cooling.

2. Sugars.—Various sugars may at times be found in the urine. Dextrose is by far the most common, and is the only one of clinical importance. Levulose, lactose, and some others are occasionally met with.

(1) **Dextrose (Glucose).**—It is probable that traces of glucose, too small to respond to the ordinary tests, are present in the urine in health. Its presence in appreciable amount constitutes "glycosuria."

Transitory glycosuria is unimportant, and may occur in many conditions, as after general anesthesia and administration of certain drugs, in pregnancy, and following shock and head injuries. It may also occur

after eating excessive amounts of carbohydrates (alimentary glycosuria). The "assimilation limit" varies with different individuals and with different conditions of exercise. It also depends upon the kind of carbohydrate. The normal for glucose is about 100 to 150 gm. When more than this amount is taken at one time some of it will be excreted in the urine. Excretion lasts for a period of four or five hours.

Persistent glycosuria has been noted in brain injuries involving the floor of the fourth ventricle. As a rule, however, persistent glycosuria is diagnostic of diabetes mellitus, of which disease it is the essential symptom. The amount of glucose eliminated in diabetes is usually considerable, and is sometimes very large, reaching 500 gm., or even more, in twenty-four hours, but it does not bear any uniform relation to the severity of the disease. Glucose may, on the other hand, be almost or entirely absent temporarily.

Detection of Dextrose.—If albumin be present in more than traces, it must be removed by boiling and filtering.

(1) **Haines' Test.**—Take about 4 c.c. of Haines' solution in a test-tube, boil, examine carefully for a precipitate, and, if none is present, add 6 or 8 drops of urine. A heavy yellow or red precipitate, which settles readily to the bottom, shows the presence of sugar. Neither precipitation of phosphates, as a light, flocculent sediment, nor simple decolorization of the reagent should be mistaken for a positive reaction.

This is one of the best of the copper tests, all of which depend upon the fact that in strongly alkaline solutions glucose reduces cupric hydrate to cuprous hydrate (yellow) or cuprous oxid (red). They are somewhat inaccurate, because they make no distinction between glucose and less common

forms of sugar; because certain normal substances, when present in excess, especially mucin, uric acid, and creatinin, may reduce copper, and because many drugs—*e. g.*, chloral, chloroform, copaiba, acetanilid, benzoic acid, morphin, sulphonal, salicylates—are eliminated as copper-reducing substances. To minimize these fallacies *dilute the urine, if it be concentrated; do not add more than the specified amount of urine, and do not boil after the urine is added.* If chloroform has been used as a preservative, it should be removed by boiling the urine before making the test.

Haines' solution is prepared as follows: Completely dissolve 2 gm. pure copper sulphate in 16 c.c. distilled water, and add 16 c.c. pure glycerin; mix thoroughly, and add 156 c.c. liquor potassæ. The solution keeps well.

(2) **Fehling's Test.**—Two solutions are required—one containing 34.64 gm. pure crystalline copper sulphate in 500 c.c. distilled water; the other, 173 gm. Rochelle salt and 100 gm. potassium hydroxid in 500 c.c. distilled water. Mix equal parts of the two solutions in a test-tube, dilute with 3 or 4 volumes of water, and boil. Add the urine a little at a time, heating, but not boiling, between additions. In the presence of glucose a heavy red or yellow precipitate will appear. The quantity of urine should not exceed that of the reagent. The fallacies mentioned under Haines' Test apply equally to this.

(3) **Benedict's Test.**—This new test promises to displace all other reduction tests for glucose. The reagent is said to be ten times as sensitive as Haines' or Fehling's, and not to be reduced by uric acid, creatinin, chloroform, or the aldehyds. It consists of:

Copper sulphate (pure crystallized)..... 17.3 gm.;
Sodium or potassium citrate..... 173.0 "
Sodium carbonate (crystallized)..... 200.0 "
(or 100 gm. of the anhydrous salt).
Distilled water, to make.....1000.0 c.c.

Dissolve the citrate and carbonate in 700 c.c. of water, with the aid of heat, and filter. Dissolve the copper in 100 c.c. of water and pour slowly into the first solution, stirring constantly. Cool, and make up to one liter. The reagent keeps indefinitely.

Take about 5 c.c. of this reagent in a test-tube, and add *8 or 10 drops* (*not more*) of the urine. Heat to vigorous boiling, keep at this temperature for one or two minutes, and allow to cool slowly. In the presence of glucose the entire body of the solution will be filled with a precipitate, which may be red, yellow, or green in color. When traces only of glucose are present, the precipitate may appear only upon cooling. In the absence of glucose, the solution remains clear or shows only a faint, *bluish* precipitate, due to urates.

(4) **Phenylhydrazin Test.**—*Kowarsky's Method.*—The following directions include certain modifications which have recently been worked out by C. S. Bluemel in the writer's laboratory: In a wide test-tube take 5 drops pure phenylhydrazin, 10 drops glacial acetic acid, and 1 c.c. saturated solution of sodium chlorid. A curdy mass results. Add 3 or 4 c.c. of the urine and 4 or 5 c.c. of water. Boil vigorously for two to three minutes. The annoying bumping can be reduced or obviated by shaking continually, or, much better, by placing in the test-tube a number of pieces of glass tubing, varying in length from $1\frac{1}{2}$ to 3 inches, so as to produce an organ-pipe effect. The volume of fluid remaining should be 2 to 3 c.c. Set aside to cool, or if the glass tubes were used pour the fluid into another hot test-tube and allow to cool. Examine the sediment with the microscope, using a two-thirds objective. If glucose be present, characteristic crystals of phenylglucosazone will be seen. These are yellow, needle-like crystals arranged mostly in clusters or in sheaves (Fig. 30). When traces only of glucose are present, the crystals may not appear for one-

half hour or more. The best crystals are obtained when the fluid is cooled very slowly. It must not be agitated during cooling. The test-tubes and pieces of tubing can be cleaned when necessary by boiling in a solution of caustic soda or acetic acid.

This is an excellent test for clinical work. Bluemel finds that when applied as above directed, with the tubing to prevent bumping, it will readily detect 0.025 per cent. of glucose in urine, the crystals appearing in three to four hours. The

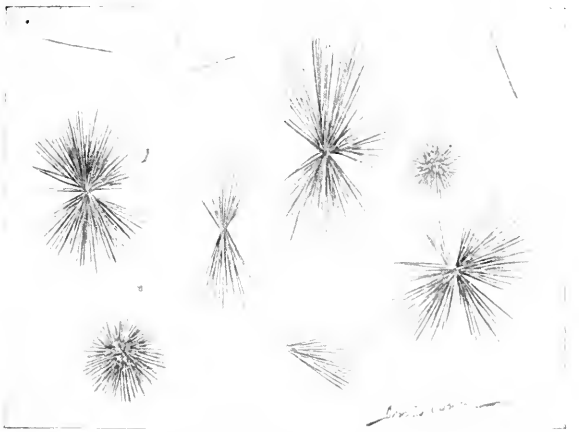


Fig. 30.—Crystals of phenylglucosazone from diabetic urine—Kowarsky's test (×500).

test has practically no fallacies excepting levulose, which is a fallacy for all the ordinary tests. Other carbohydrates which are capable of forming crystals with phenylhydrazin are extremely unlikely to do so when the test is applied directly to the urine. Even if not used routinely, this test should always be resorted to when the copper tests give a positive reaction in doubtful cases.

Quantitative Estimation.—In quantitative work Fehling's solution, for so many years the standard, has been

largely displaced by Purdy's, which avoids the heavy precipitate that so greatly obscures the end-reaction in Fehling's method. The older method is still preferred by many, and both are, therefore, given. The newer method of Benedict is likewise included, since it appears to be more exact and more satisfactory than any other titration method available for sugar work. Should the urine contain much glucose, it must be diluted before making any quantitative test, allowance being made for the dilution in the subsequent calculation. Albumin, if present, must be removed by acidifying a considerable quantity of urine with acetic acid, boiling, and filtering. The precipitate should then be washed with water and the washings added to the urine to bring it to its original volume.

(1) **Purdy's Method.**—Take exactly 35 c.c. of Purdy's solution in a flask or beaker, add twice its volume of distilled water, heat to boiling, and, still keeping the solution hot, add the urine very slowly from a buret until the blue color entirely disappears. Read off the amount of urine added; considering the strength of Purdy's solution, it is readily seen that this amount of urine contains 0.02 gm. of glucose, from which the amount in the twenty-four-hour urine, or the percentage, can easily be calculated. Example: Suppose that 2.5 c.c. of urine discharged the blue color of 35 c.c. of Purdy's solution. This amount of urine, therefore, contains exactly 0.02 gm. glucose, and the percentage is obtained from the equation: $2.5 : 100 :: 0.02 : x$, and x equals 0.8 per cent. If, then, the twenty-four-hour quantity of urine were 3000 c.c., the twenty-four-hour elimination of glucose would be found as follows: $100 : 3000 :: 0.8 : x$, and x equals 24 gm.

It will be found that after the test is completed the blue

color slowly returns. This is due to reoxidation, and should not be mistaken for incomplete reduction.

A somewhat simpler application of this method, which is accurate enough for most clinical purposes, is as follows: Take $8\frac{3}{4}$ c.c. (roughly, 9 c.c.) of Purdy's solution in a large test-tube, dilute with an equal volume of water, heat to boiling, and, while keeping the solution hot but not boiling, add the urine drop by drop from a medicine-dropper until the blue color is entirely gone. Toward the end add the drops very slowly, not more than 4 or 5 a minute. Divide 10 by the number of drops required to discharge the blue color; the quotient will be the percentage of glucose. If 20 drops were required, the percentage would be $10 \div 20 = 0.5$ per cent. It is imperative that the drops be of such size that 20 of them will make 1 c.c. Test the dropper with urine, not water. If the drops are too large, draw out the tip of the dropper; if too small, file off the tip.

Purdy's solution consists of pure crystalline copper sulphate, 4.752 gm.; potassium hydroxid, 23.5 gm.; ammonia (U. S. P.; sp. gr., 0.9), 350 c.c.; glycerin, 38 c.c.; distilled water, to make 1000 c.c. Dissolve the copper sulphate and glycerin in 200 c.c. of the water by aid of gentle heat. In another 200 c.c. of water dissolve the potassium hydroxid. Mix the two solutions and, when cool, add the ammonia. Lastly, bring the whole up to 1000 c.c. with distilled water. This solution is of such strength that the copper in 35 c.c. will be reduced by exactly 0.02 gm. of glucose.

(2) **Fehling's Method.**—Take 10 c.c. of Fehling's solution (made by mixing 5 c.c. each of the copper and alkaline solutions described on page 139) in a flask or beaker, add 3 or 4 volumes of water, boil, and add the urine very slowly from a buret until the solution is completely decolorized, heating but not boiling after each addition.

The chief objection to Fehling's method is the difficulty of determining the end-point. The use of an "outside indi-

cator," however, obviates this. When reduction is thought to be complete, a few drops of the solution are filtered through a fine-grained filter-paper on to a porcelain plate, quickly acidified with acetic acid, and mixed with a drop of 10 per cent. potassium ferrocyanid. Immediate appearance of a red-brown color shows the presence of unreduced copper.

Fehling's solution is of such strength that the copper in 10 c.c. will be reduced by exactly 0.05 gm. of glucose. Therefore, the amount of urine required to decolorize the test solution contains just 0.05 gm. glucose, and the percentage is easily calculated.

(3) **Benedict's Method.**—The following modification of his copper solution has been offered by Benedict for quantitative estimations.

The reagent consists of:

Copper sulphate (pure crystallized)	18.0 gm.;
Sodium carbonate (crystallized)	200.0 "
(or 100 gm. of the anhydrous salt).	
Sodium or potassium citrate	200.0 "
Potassium sulphocyanate,	125.0 "
Potassium ferrocyanid solution (5 per cent.)	5.0 c.c.;
Distilled water, to make	1000.0

With the aid of heat dissolve the carbonate, citrate, and sulphocyanate in about 800 c.c. of the water and filter. Dissolve the copper in 100 c.c. of water and pour slowly into the other fluid, stirring constantly. Add the ferrocyanid solution, cool, and dilute to 1000 c.c. Only the copper need be accurately weighed. This solution is of such strength that 25 c.c. are reduced by 0.05 gram glucose. It keeps well.

To make a sugar estimation, take 25 c.c. of the reagent in a porcelain evaporating dish, add 10 to 20 gm. of sodium carbonate crystals (or one-half this weight of the anhydrous salt) and a small quantity of powdered pumice-stone or tal-

cum. Heat to boiling, and add the urine rather rapidly from a buret until a chalk-white precipitate forms and the blue color of the reagent begins to fade. After this point is reached, add the urine a few drops at a time until the last trace of blue just disappears. This end-point is easily recognized. During the whole of the titration the mixture must be kept vigorously boiling. Loss by evaporation must be made up by adding water. The quantity of urine required to discharge the blue color contains exactly 0.05 gm. glucose, and the percentage contained in the original sample is easily calculated.

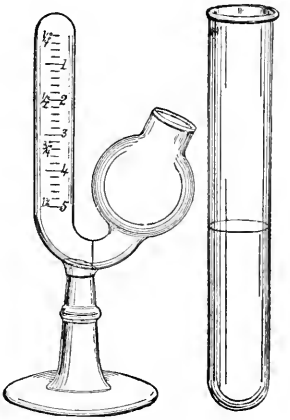


Fig. 31.—Einhorn's saccharimeter.

(4) **Fermentation Method.**—This is convenient and satisfactory, its chief disadvantage being the time required. It depends upon the fact that glucose is fermented by yeast with evolution of CO_2. The amount of gas evolved is an index of the amount of glucose. No preservative must have been added. Einhorn's saccharimeter (Fig. 31) is the simplest apparatus.

The urine must be so diluted as to contain not more than 1 per cent. of glucose. A fragment of fresh yeast-cake about

the size of a split-pea is mixed with a definite quantity of the urine measured in the tube which accompanies the apparatus. It should form an emulsion free from lumps or air-bubbles. The long arm of the apparatus is then filled with the mixture. At the end of fifteen to twenty-four hours fermentation will be complete, and the percentage of glucose can be read off upon the side of the tube. The result must then be multiplied by the degree of dilution. Since yeast itself sometimes gives off gas, a control test must be carried out with normal urine and the amount of gas evolved must be subtracted from that of the test. A control should also be made with a known glucose solution to make sure that the yeast is active. It has recently been shown that yeast can split off carbon dioxid from amino-acids, so that the results with the fermentation method are likely to be a little high.

(5) **Robert's Differential Density Method.**—While this method gives only approximate results, it is convenient, and requires no special apparatus but an accurate urinometer. Mix a quarter of an yeast-cake with about 100 c.c. of urine. Take the specific gravity and record it. Set the urine in a warm place for twenty-four hours or until fermentation is complete. Then cool to the temperature at which the specific gravity was originally taken, and take it again. The difference between the two readings gives the number of grains of sugar per ounce, and this, multiplied by 0.234, gives the *percentage* of sugar. If the original reading is 1.035, and that after fermentation is 1.020, the urine contains $1.035 - 1.020 = 15$ grains of sugar per fluidounce; and the percentage equals $15 \times 0.234 = 3.5$.

(2) **Levulose,** or **fruit sugar,** is seldom present in urine except in association with dextrose, and has about the same significance. According to von Noorden, its appearance in diabetes indicates an advanced case. Its

name is derived from the fact that it rotates polarized light to the left.

The normal assimilation limit for levulose is about 100 gm. This fact is used in the Strauss test of the functional capacity of the liver. One hundred gm. of levulose are given upon an empty stomach, and the subsequent appearance of levulose in the urine is taken as evidence of deficiency of the glycogenic function. The degree of the hepatic derangement is measured by a quantitative estimation.

Detection of Levulose.—Levulose responds to all the tests above given for dextrose. It may be distinguished from dextrose by the following:

Borchardt's Test.—Mix about 5 c.c. each of the urine and 25 per cent. hydrochloric acid (concentrated HCl, 2 parts; water, 1 part) in a test-tube and add a few crystals of resorcinol. Heat to boiling and boil for not more than one-half minute. In the presence of levulose a red color appears. Cool in running water, pour into a beaker, and render slightly alkaline with solid sodium or potassium hydroxid. Return to the test-tube, add 2 or 3 c.c. of acetic ether, and shake. If levulose be present, the ether will be colored yellow. A similar yellow color will follow administration of rhubarb and senna.

If indican be present the test must be modified as follows: Perform Obermayer's test and extract the indican with chloroform. Reduce the acidity of the indican-free urine by adding one-third its volume of water, add a few crystals of resorcinol, and proceed with Borchardt's test.

Quantitative Estimation of Levulose.—The methods are the same as for dextrose (see p. 141). It reduces copper to the same extent.

(3) **Lactose,** or **milk-sugar,** is sometimes present in the urine of nursing women and in that of women who have recently miscarried. It is of interest chiefly because it may be mistaken for glucose. *It reduces copper, but does not ferment with yeast.* In strong solution it can form crystals with phenylhydrazin, but is extremely unlikely to do so when the test is applied directly to the urine.

(4) **Maltose** and **cane-sugar** are of little or no clinical importance. Maltose has been found along with dextrose in diabetes. It reduced copper, 0.074 gm. being equivalent to 25 c.c. of Benedict's solution. Cane-sugar (sucrose) is sometimes added to the urine by malingering patients. It does not reduce copper.

(5) **Pentoses.**—These sugars are so named because they contain 5 atoms of oxygen. Vegetable gums form their chief source. They reduce copper strongly but slowly, and give crystals with phenylhydrazin, but do not ferment with yeast.

Pentosuria is uncommon. It has been noted after ingestion of large quantities of pentose-rich substances, such as cherries, plums, and fruit-juices, and is said to be fairly constant in habitual use of morphin. It sometimes accompanies glycosuria in diabetes. An obscure chronic form of pentosuria without clinical symptoms has been observed. The pentose excreted in these cases is believed to be optically inactive arabinose, although recent work indicates that ribose is present in some cases at least.

Bial's Orcinol Test.—Dextrose is first removed by fermentation. About 5 c.c. of Bial's reagent are heated in a test-

tube, and after removing from the flame the urine is added drop by drop, not exceeding 20 drops in all. The appearance of a green color denotes pentose.

The reagent consists of:

Hydrochloric acid (30 per cent.)	500 c.c.;
Ferric chlorid solution (10 per cent.)	25 drops;
Orcinol	1 gm.

3. Acetone Bodies.—This is a group of closely related substances—acetone, diacetic acid, and beta-oxybutyric acid. Acetone is derived from decomposition of diacetic acid, and this in turn from beta-oxybutyric acid by oxidation. The origin of beta-oxybutyric acid is not definitely known, but it is probable that its chief, if not its only, source is in some obscure metabolic disturbance with abnormal destruction of fats. The three substances generally appear in the urine in the order mentioned. When the disturbance is mild, acetone only appears; as it becomes more marked, diacetic acid is added, and finally beta-oxybutyric acid appears. The presence of beta-oxybutyric acid in the blood is probably the chief cause of the form of auto-intoxication known as "acid intoxication."

(1) **Acetone.**—Minute traces, too small for the ordinary tests, may be present in the urine under normal conditions. Larger amounts are not uncommon in fevers, gastro-intestinal disturbances, and certain nervous disorders. A notable degree of acetonuria has likewise been observed in pernicious vomiting of pregnancy and in eclampsia.

Acetonuria is practically always observed in acid intoxication, and, together with diaceturia, constitutes its most significant diagnostic sign. A similar or identi-

cal toxic condition, always accompanied by acetonuria and often fatal, is now recognized as a not infrequent late effect of anesthesia, particularly of chloroform anesthesia. This postanesthetic toxemia is more likely to appear, and is more severe when the urine contains any notable amount of acetone before operation, which suggests the importance of routine examination for acetone in surgical cases.

Fig. 32.—A simple distilling apparatus.

Acetone is present in considerable amounts in many cases of diabetes mellitus, and is always present in severe cases. Its amount is a better indication of the severity of the disease than is the amount of sugar. A progressive increase is a grave prognostic sign. It can be diminished temporarily by more liberal allowance of carbohydrates in the diet.

According to Folin, acetone is present in only small

amounts in these conditions, the substance shown by the usual tests, particularly after distillation of the urine, being really diacetic acid. In this connection, Frommer's test is to be recommended, since it does not require distillation, and does not react to diacetic acid unless too great heat is applied.

Detection of Acetone.—The urine may be tested directly, but it is much better to distil it after adding a little phosphoric or hydrochloric acid to prevent foaming, and to test the first few cubic centimeters of distillate. A simple distilling apparatus is shown in Fig. 32. The test-tube may be attached to the delivery tube by means of a two-hole rubber cork as shown, the second hole serving as air vent, or, what is much less satisfactory, it may be tied in place with a string. Should the vapor not condense well, the test-tube may be immersed in a glass of cold water.

When diacetic acid is present, a considerable proportion will be converted into acetone during distillation.

(1) **Gunning's Test.**—To a few cubic centimeters of urine or distillate in a test-tube add a few drops of tincture of iodin and of ammonia alternately until a heavy black cloud appears. This cloud will gradually clear up and, if acetone be present, iodoform, usually crystalline, will separate out. The iodoform can be recognized by its odor, especially upon heating (there is danger of explosion if the mixture be heated before the black cloud disappears), or by detection of the crystals microscopically. The latter only is dependable, unless one has an unusually acute sense of smell. The odor of iodin, which is also present, is confusing. Iodoform crystals are yellowish six-pointed stars or six-sided plates (Fig. 33).

This modification of Lieben's test is less sensitive than the original, but is sufficient for all clinical work; it has the advantage that alcohol does not cause confusion, and especially that the sediment of iodoform is practically always crystalline. When applied directly to the urine, phosphates are precipitated and may form large feathery, star-shaped crystals which are confusing to the inexperienced. Albumin prevents formation of the crystals, and when it is present, the urine must be distilled for the test.

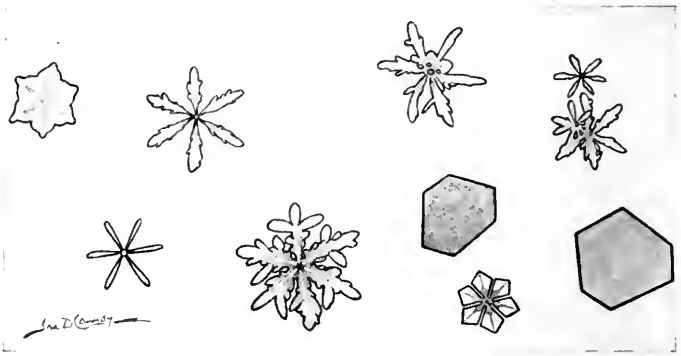


Fig. 33.—Iodoform crystals obtained in several tests for acetone by Gunning's method (×about 600).

(2) **Lange's Test.**—This is a modification of the well-known Legal test. It is more sensitive and gives a sharper end-reaction. To a small quantity of urine add about one-twentieth its volume (1 drop for each 1 c.c.) of glacial acetic acid and a few drops of fresh concentrated aqueous solution of sodium nitroprussid, and gently run a little ammonia upon its surface. If acetone be present, a purple ring will form within a few minutes at the junction of the two fluids.

(3) **Frommer's Test.**—This test has proved very satisfactory in the hands of the writer. The urine need not be distilled. Alkalinize about 10 c.c. of the urine with 2 or 3 c.c.

of 40 per cent. caustic soda solution, add 10 or 12 drops of 10 per cent. alcoholic solution of salicylous acid (salicyl aldehyd), heat the upper portion to about 70° C. (it should not reach the boiling-point), and keep at this temperature five minutes or longer. In the presence of acetone an orange color, changing to deep red, appears in the heated portion.

The test can be made more definite by adding the caustic soda in substance (about 1 gm.), and before it goes into solution adding the salicyl aldehyd and warming the lower portion.

(2) **Diacetic acid** occurs in the same conditions as acetone, but has more serious significance. In diabetes its presence is a grave symptom and often forewarns of approaching coma. It rarely or never occurs without acetone.

Detection.—The urine must be fresh.

(1) **Gerhardt's Test.**—To a few cubic centimeters of the urine add solution of ferric chlorid (about 10 per cent.) drop by drop until the phosphates are precipitated; filter and add more of the ferric chlorid. If diacetic acid be present, the urine will assume a Bordeaux-red color which disappears upon boiling. A red or violet color which does not disappear upon boiling may be produced by other substances, as phenol, salicylates, and antipyrin. Whenever the reaction is doubtful the urine should be distilled and the distillate tested for acetone.

(2) **Lindemann's Test.**—To about 10 c.c. of urine add 5 drops 30 per cent. acetic acid, 5 drops Lugol's solution, and 2 or 3 c.c. chloroform, and shake. The chloroform does not change color if diacetic acid be present, but becomes reddish violet in its absence. This test is claimed by its advocates to be more sensitive and more reliable than Gerhardt's. Uric

acid also decolorizes iodin, and if much is present double the amount of Lugol's solution should be used.

(3) **Oxybutyric acid** has much the same significance as diacetic acid, but is of more serious import.

Hart's Test.—Remove acetone and diacetic acid by diluting 20 c.c. urine with 20 c.c. water, adding a few drops of acetic acid, and boiling down to 10 c.c. To this add 10 c.c. water, mix, and divide between two test-tubes. To one tube add 1 c.c. of hydrogen peroxid, warm gently, and cool. This transforms β-oxybutyric acid to acetone. Now apply Lange's test for acetone (see p. 152) to each tube. A positive reaction in the tube to which hydrogen peroxid has been added shows the presence of β-oxybutyric acid in the original sample of urine.

4. Bile appears in the urine in all diseases which produce jaundice, often some days before the skin becomes yellow; and in many disorders of the liver not severe enough to cause jaundice. It also occurs in diseases with extensive and rapid destruction of red blood-corpuscles. Both bile-pigment and bile acids may be found. They generally occur together, but the pigment is not infrequently present alone. Bilirubin, only, occurs in freshly voided urine, the other pigments (biliverdin, bilifuscin, etc.) being produced from this by oxidation as the urine stands. The acids are almost never present without the pigments, and are, therefore, seldom tested for clinically. Crystals of bilirubin (hematoidin) (Fig. 37, 4) may be deposited in heavily bile-charged urine.

Detection of Bile-pigment.—Bile-pigment gives the urine a greenish-yellow, yellow, or brown color, which

upon shaking is imparted to the foam. Cells, casts, and other structures in the sediment may be stained brown or yellow. This, however, should not be accepted as proving the presence of bile without further tests.

(1) **Smith's Test.**—Overlay the urine with tincture of iodin diluted with nine times its volume of alcohol. An emerald-green ring at the zone of contact shows the presence of bile-pigments. It is convenient to use a conical test-glass, one side of which is painted white.

(2) **Gmelin's Test.**—This consists in bringing slightly yellow nitric acid into contact with the urine. A play of colors, of which green and violet are most distinctive, denotes the presence of bile-pigment. Colorless nitric acid will become yellow upon standing in the sunlight. The test may be applied in various ways: by overlaying the acid with the urine; by bringing a drop of each together upon a porcelain plate; by filtering the urine through thick filter-paper, and touching the paper with a drop of the acid; and, probably best of all, by precipitating with lime-water, filtering, and touching the precipitate with a drop of the acid. In the last method bilirubin is carried down as an insoluble calcium compound which concentrates the pigment and removes interfering substances such as indican.

Detection of Bile Acids.—Hay's test is simple, sensitive, and fairly reliable, and will, therefore, appeal to the practitioner. It depends upon the fact that bile acids lower surface tension. Other tests require isolation of the acids for any degree of accuracy.

Hay's Test.—Upon the surface of the urine, which must not be warm, sprinkle a little finely powdered sulphur. If it sinks at once, bile acids are present to the amount of 0.01

per cent. or more; if only after gentle shaking, 0.0025 per cent. or more. If it remains floating, even after gentle shaking, bile acids are absent. It is said that urobilin when present in large amount also reduces surface tension.

5. Hemoglobin.—The presence in the urine of hemoglobin or pigments directly derived from it, accompanied by few, if any, red corpuscles, constitutes *hemoglobinuria*. It is a comparatively rare condition, and must be distinguished from *hematuria*, or *blood* in the urine, which is common. In both conditions chemic tests will show hemoglobin, but in the latter the microscope will reveal the presence of red corpuscles. Urines which contain notable amounts of hemoglobin have a reddish or brown color, and may deposit a sediment of brown, granular pigment.

Hemoglobinuria occurs when there is such extensive destruction of red blood-cells within the body that the liver cannot transform all the hemoglobin set free into bile-pigment. The most important examples are seen in poisoning, as by mushrooms and potassium chlorate, in scurvy and purpura, in malignant malaria (blackwater fever), and in the obscure condition known as "paroxysmal hemoglobinuria." This last is characterized by the appearance of large quantities of hemoglobin at intervals, usually following exposure to cold, the urine remaining free from hemoglobin between the attacks.

Detection.—Teichmann's test may be applied to the precipitate after boiling and filtering, but the guaiac or benzidin test will be found more convenient in routine work. For further discussion of blood tests see page 306.

Guaiac Test.—Mix a few cubic centimeters each of "ozonized" turpentine and a fresh 1 : 60 alcoholic solution of guaiac. The guaiac solution may be freshly prepared by dissolving a pocket-knife-pointful of powdered guaiac in 4 or 5 c.c. of alcohol. Boil a few cubic centimeters of the urine in a test-tube, cool, and gently pour the guaiac-turpentine mixture on its surface. A bright blue ring will appear at the zone of contact within a few minutes if hemoglobin be present. The guaiac should be kept in an amber-colored bottle. Fresh turpentine can be "ozonized" by allowing it to stand a few days in an open vessel in the sunlight. Instead of turpentine, hydrogen peroxid may be used.

This test is very sensitive, and a negative result proves the absence of hemoglobin. Positive results are not conclusive, because numerous other substances—few of them likely to be found in the urine—may produce the blue color. That most likely to cause confusion is pus, but the blue color produced by it disappears upon heating. The thin film of copper often left in a test-tube after testing for sugar may give the reaction, as may also the fumes from an open bottle of bromin.

Benzidin Test.—The reagents employed are hydrogen peroxid and a saturated solution of benzidin in glacial acetic acid. The test is applied in exactly the same manner as the guaiac test just given. It has the same fallacies, but is said to be more sensitive. A green or blue color shows the presence of hemoglobin.

6. Alkapton Bodies.—The name "alkaptonuria" has been given to a condition in which the urine turns reddish brown to brownish black upon standing and strongly reduces copper (but not bismuth), owing to the presence of certain substances which result from imperfect protein metabolism. The change of color takes

place quickly when fresh urine is alkalinized, hence the name, *alkapton bodies*.

Alkaptonuria is unaccompanied by other symptoms, and has little clinical importance. Only about forty-five cases, mostly congenital, have been reported. The change in color of the urine and the reduction of copper with no reduction of bismuth nor fermentation with yeast would suggest the condition.

7. Melanin.—Urine which contains melanin likewise darkens upon exposure to the air, assuming a dark brown or black color. This is due to the fact that the substance is eliminated as a chromogen—melanogen—which is later converted into the pigment. It does not reduce copper.

Melanuria occurs in most, but not all, cases of melanotic sarcoma. Its diagnostic value is lessened by the fact that it has been observed in other wasting diseases.

Tests for Melanin.—(1) Addition of ferric chlorid gives a gray precipitate which blackens on standing.

(2) Bromin water causes a yellow precipitate which gradually turns black.

8. Urobilin.—Traces of this urinary pigment may be present under normal conditions. It is generally regarded as identical with hydrobilirubin of the feces. It is excreted as a chromogen, *urobilinogen*, which is changed into urobilin through the action of light within a few hours after the urine is voided. A great excess usually gives the urine a dark-brown color, suggesting the presence of bile. Small amounts may cause no perceptible change in color. It is derived from bilirubin, which is transformed through bacterial action in the

intestine to urobilinogen. Under normal conditions this is absorbed, carried to the liver, and there reconverted into bilirubin. When the liver cells are deranged, the transformation into bilirubin does not take place and urobilinogen is excreted by the kidneys.

The presence of any considerable quantity of the pigment or the chromogen in the urine is, therefore, a qualitative test of the functional incapacity of the liver. Clinically, urobilin is found in a great variety of diseases associated with hepatic derangement. It is especially marked in cirrhosis of the liver.

(1) **Test for Urobilinogen.**—To a few cubic centimeters of the urine in a test-tube add a few crystals of dimethyl-amino-benzaldehyd and make definitely acid with hydrochloric acid. In the presence of pathologic amounts of urobilinogen an intense red color appears. Normal amounts will cause the red color only when the urine is heated.

(2). **Schlesinger's Test for Urobilin.**—To about 5 c.c. of the urine in a test-tube add a few drops of Lugol's solution to transform the chromogen into the pigment. Now add 4 or 5 c.c. of a saturated solution of zinc chlorid in absolute alcohol and filter. A greenish fluorescence, best seen when the tube is viewed against a black background and the light is concentrated upon it with a lens, shows the presence of urobilin. Bile-pigment, if present, should be removed by adding about one-fifth volume of 10 per cent. calcium chlorid solution and filtering.

9. Diazo Substances.—Certain unknown substances sometimes present in the urine give a characteristic color reaction—the "diazo reaction" of Ehrlich—when treated with diazo-benzol-sulphonic acid and ammonia. This reaction has much clinical value, provided its lim-

itations be recognized. It is at best an empirical test and must be interpreted in the light of clinical symptoms. Although it has been met with in a considerable number of diseases, its usefulness is practically limited to typhoid fever, tuberculosis, and measles.

(1) **Typhoid Fever.**—Practically all cases give a positive reaction, which varies in intensity with the severity of the disease. It is so constantly present that it is sometimes said to be "negatively pathognomonic": if negative upon several successive days *at a stage of the disease when it should be positive*, typhoid is almost certainly absent. Upon the other hand, a reaction when the urine is highly diluted (1:50 or more) has much positive diagnostic value, since this dilution prevents the reaction in most conditions which might be mistaken for typhoid; but it should be noted that mild cases of typhoid may not give it at this dilution. Ordinarily the diazo- appears a little earlier than the Widal reaction,—about the fourth or fifth day,—but it may be delayed. In contrast to the Widal, it begins to fade about the end of the second week, and soon thereafter entirely disappears. An early disappearance is a favorable sign. It reappears during a relapse, and thus helps to distinguish between a relapse and a complication, in which it does not reappear.

(2) **Tuberculosis.**—The diazo-reaction has been obtained in many forms of the disease. It has little or no diagnostic value. Its continued presence in pulmonary tuberculosis is, however, a grave prognostic sign, even when the physical signs are slight. After it once appears it generally persists more or less intermittently until death, the average length of life after its

appearance being about six months. The reaction is often temporarily present in mild cases during febrile complications, and has then no significance.

(3) **Measles.**—A positive reaction is usually obtained in measles, and may help to distinguish this disease from German measles, in which it does not occur. It generally appears before the eruption and remains about five days.

Technic.—Although the test is really a very simple one, careful attention to technic is imperative. Many of the early workers were very lax in this regard. Faulty technic and failure to record the stage of the disease in which the tests were made have probably been responsible for the bulk of the conflicting results reported.

Certain drugs often given in tuberculosis and typhoid interfere with or prevent the reaction. The chief are creosote, tannic acid and its compounds, opium and its alkaloids, salol, phenol, and the iodids. The reagents are:

(1) Saturated solution sulphanilic acid in 5 per cent. hydrochloric acid.

(2) Aqueous solution sodium nitrite (0.5 per cent.).

(3) Strong ammonia.

Mix 100 parts of (1) and 1 part of (2).[1] In a test-tube take equal parts of this mixture and the urine, and pour 1 or 2 c.c. of the ammonia upon its surface. If the reaction be positive, a garnet ring will form at the junction of the two fluids; and, upon shaking, a distinct pink color will be imparted to the foam. The color of the foam is the essential feature. If desired, the mixture may be well shaken before the ammonia is added: the pink color will then instantly appear in that portion of the foam which the ammonia has

[1] These proportions are recommended by Greene, and are now generally used. Ehrlich used 40 parts of (1) and 1 part of (2).

reached, and can be readily seen. The color varies from eosin-pink to deep crimson, depending upon the intensity of the reaction. *It is a pure pink or red; any trace of yellow or orange denotes a negative reaction.* A doubtful reaction should be considered negative.

Substitutes for the Diazo-reaction.—Two tests, which have been offered as simple and satisfactory substitutes for the diazo, have recently found rather wide acceptance. They are supposed to be positive in the same classes of cases as the diazo and to have the same clinical significance, but are claimed to be more reliable.

(1) **Russo's Methylene-blue Test.**—To 5 c.c. of the urine in a test-tube add 5 drops of 1 : 1000 aqueous solution of methylene-blue, and mix. An emerald or mint-green color, in which there must be no trace of blue, denotes a positive reaction. There is considerable difficulty in judging the color. The correct color may be obtained for comparison by adding the methylene-blue solution to a bile-tinged urine.

Since this test was offered in 1905, it has been condemned by many workers and extolled by others. The writer's experience with it has been unfavorable.

(2) **Weisz's Permanganate Test.**—In a test-tube mix 1 c.c. of urine and 2 c.c. distilled water, and add 3 drops of 1 : 1000 aqueous solution of potassium permanganate. The appearance of a yellow color denotes a positive reaction. As suggested by Heflebower, the color is best judged by comparison with a tube of diluted urine to which no permanganate has been added.

Weisz believes the diazo-reaction to be due principally to urochromogen, which, because of the effect of certain toxins upon metabolism, fails of conversion into urochrome; and he has offered (1911) the permanganate reaction as a more satis-

factory test, both for urochromogen and for an antecedent substance which has the same significance as urochromogen, but which the diazo fails to detect. This test has been studied chiefly in its relation to prognosis in tuberculosis, in which it appears to have about the same value as the diazo, with the advantage that it is more frequently noted and is less intermittent in a given case.

10. Pancreatic Reaction.—Cammidge has shown that in cases of pancreatitis a substance capable of forming crystals with phenylhydrazin can be developed by boiling the urine with a mineral acid, and has offered the following test as an aid in diagnosis of this obscure condition. The nature both of this substance and the antecedent substance from which it is derived is not known. The difficulty and importance of diagnosis in pancreatitis warrant inclusion of the method here, even though more recent work indicates that its value is by no means so great as originally claimed.

While the test is somewhat tedious, all the manipulations are simple and require no apparatus but flasks, test-tubes, and funnels.

Technic.—Careful attention to detail is imperative. An ordinary routine examination is first made. Albumin and sugar, if present, must be removed: the former, by acidifying with acetic acid, boiling, and filtering; the latter, by fermentation with yeast after the first step of the method proper. An alkaline urine should be made slightly acid with hydrochloric acid.

(1) Forty cubic centimeters of the urine, which has been rendered perfectly clear by repeated filtration through the same filter-paper, are placed in a small flask, treated with 1 c.c. concentrated hydrochloric acid and gently boiled on a

sand-bath for ten minutes, a funnel with long stem being placed in the neck of the flask to act as a condenser (Fig. 34). After boiling, the urine is cooled in a stream of cold water and brought to its original bulk with distilled water; 8 gm. of lead carbonate are then added to neutralize the acid. The fluid is allowed to stand a few minutes and then filtered through well-moistened fine-grain filter-paper until perfectly clear.

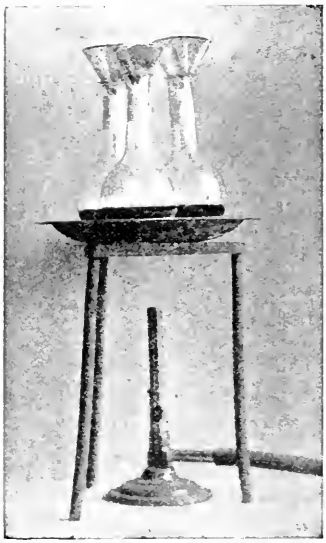


Fig. 34.—"Pancreatic reaction" flasks fitted with funnel condensers on a sand-bath (Robson and Cammidge).

(2) The filtrate is shaken up with 8 gm. powdered tribasic lead acetate and filtered. The excess of lead is then removed by passing hydrogen sulphid gas through the fluid (see page 170) or by shaking well with 4 gm. finely powdered sodium sulphate, heating to boiling, cooling to as low a temperature as possible in a stream of water, and filtering as before until perfectly clear.

(3) Ten cubic centimeters of the filtrate are then made up to 17 c.c. with distilled water, and added to a mixture of 0.8 gm. phenylhydrazin hydrochlorate, 2 gm. powdered sodium acetate, and 1 c.c. 50 per cent. acetic acid in a small flask with funnel condenser. This is boiled on a sand-bath for ten minutes, and filtered while hot through filter-paper moistened

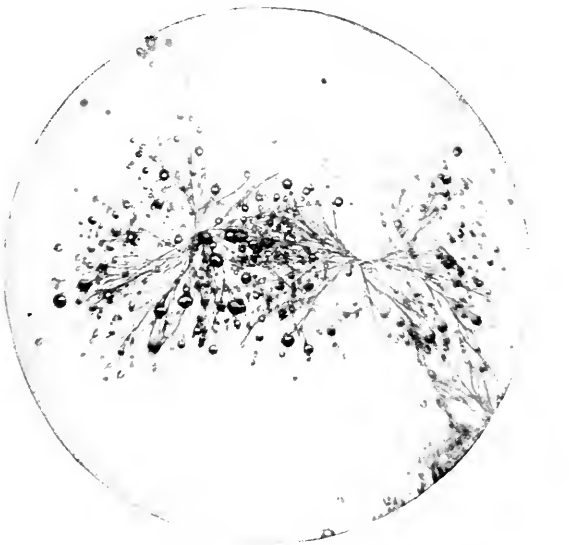


Fig. 35.—Improved "pancreatic reaction." Crystals obtained from a case of chronic pancreatitis with gall-stones in the common duct (× 200) (from a photo by P. J. Cammidge).

with hot water into a test-tube with a 15 c.c. mark. Should the filtrate not reach this mark, make up to 15 c.c. with hot distilled water. Allow to cool slowly.

(4) In well-marked cases of pancreatitis a yellow precipitate appears within a few hours; in milder cases it may not appear for twelve hours. The microscope shows this sediment to consist of "long, light yellow, flexible, hair-like

crystals arranged in sheaves, which, when irrigated with 33 per cent. sulphuric acid, melt away and disappear in ten to fifteen seconds after the acid first touches them" (Fig. 35).

(5) To exclude traces of glucose which might be overlooked in the preliminary examination a control test should be carried out in the same manner, with omission of Step (1).

11. Drugs.—The effect of various drugs upon the color of the urine has been mentioned (see p. 89). Most poisons are eliminated in the urine, but their detection is more properly discussed in works upon toxicology. A few drugs which are of interest to the practitioner, and which can be detected by comparatively simple methods, are mentioned here.

Acetanilid and Phenacetin.—The urine is evaporated by gentle heat to about half its volume, boiled for a few minutes with about one-fifth its volume of strong hydrochloric acid, and shaken out with ether. The ether is evaporated, the residue dissolved in water, and the following test applied: To about 10 c.c. are added a few cubic centimeters of 3 per cent. phenol, followed by a weak solution of chromium trioxid (chromic acid) drop by drop. The fluid assumes a red color, which changes to blue when ammonia is added. If the urine is very pale, extraction with ether may be omitted.

Antipyrin.—This drug gives a dark-red color when a few drops of 10 per cent. ferric chlorid are added to the urine. The color does not disappear upon boiling, which excludes diacetic acid.

Arsenic.—*Reinsch's Test.*—Add to the urine in a test-tube or small flask about one-seventh its volume of hydrochloric acid, introduce a piece of bright copper-foil

about $\frac{1}{8}$ inch square, and boil for several minutes. If arsenic be present, a dark-gray film is deposited upon the copper. The test is more delicate if the urine be concentrated by slow evaporation. This test is well known and is widely used, but is not so reliable as the following:

Gutzeit's Test.—In a large test-tube place a little arsenic-free zinc, and add 5 to 10 c.c. pure dilute hydrochloric acid and a few drops of iodin solution (Gram's solution will answer), then add 5 to 10 c.c. of the urine. At once cover the mouth of the tube with a filter-paper cap moistened with saturated aqueous solution of silver nitrate (1 : 1) If arsenic be present, the paper quickly becomes lemon yellow, owing to formation of a compound of silver arsenid and silver nitrate, and turns black when touched with a drop of water. To make sure that the reagents are arsenic free, the paper cap may be applied for a few minutes before the urine is added.

Atropin will cause dilatation of the pupil when a few drops of the urine are placed in the eye of a cat or rabbit.

Bromids can be detected by acidifying about 10 c.c. of the urine with dilute sulphuric acid, adding a few drops of fuming nitric acid and a few cubic centimeters of chloroform, and shaking. In the presence of bromin the chloroform, which settles to the bottom, assumes a yellow color.

Chloral hydrate appears in the urine chiefly as urochloralic acid, which reduces the copper solutions used for sugar tests. To detect it, evaporate about 500 c.c. of the urine to about one-fourth its volume, make decidedly acid with hydrochloric acid, add about 50 c.c. of ether, shake thoroughly, and separate the ether. Now evapor-

ate the ether and dissolve the residue in a little water. If urochloralic acid be present this aqueous solution will respond to Fehling's test.

Hexamethylenamin.—Interest in this drug centers chiefly in its value as a urinary antiseptic, which depends upon its decomposition with liberation of formaldehyd. According to a number of recent workers formaldehyd can be detected in the urine of only about 50 per cent. of patients who are taking hexamethylenamin. A test for formaldehyd is, therefore, necessary in order to know whether the object in administering the drug is being accomplished.

Rimini-Burnam Test for Formaldehyd.—To about 10 c.c. of the urine add successively 3 drops of 0.5 per cent. solution of phenylhydrazin hydrochlorid, 3 drops of 5 per cent. solution of sodium nitroprussid, and a few drops of a saturated solution of sodium hydroxid. The last is allowed to trickle down the inside of the tube; and if formaldehyd be present a purplish-black color, changing to green and then to yellow, will appear as it mingles with the urine.

Iodin from ingestion of iodids or absorption from iodoform dressings is tested for in the same way as the bromids, the chloroform assuming a pink to reddish-violet color; or Obermayer's reagent may be used in the same way as described for indican (see p. 113). To detect traces, a large quantity of urine should be rendered alkaline with sodium carbonate and greatly concentrated by evaporation before testing.

Lead.—No simple method is sufficiently sensitive to detect the traces of lead which occur in the urine in chronic poisoning. Of the more sensitive methods, that

of Arthur Lederer is probably best suited to the practitioner:

It is essential that all apparatus used be lead free. Five hundred cubic centimeters of the urine are acidified with 70 c.c. pure sulphuric acid, and heated in a beaker or porcelain dish. About 20 to 25 gm. of potassium persulphate are added a little at a time. This should decolorize the urine, leaving it only slightly yellow. If it darkens upon heating, a few more crystals of potassium persulphate are added, the burner being first removed to prevent boiling over; if it becomes cloudy, a small amount of sulphuric acid is added. It is then boiled until it has evaporated to 250 c.c. or less. After cooling, an equal volume of alcohol is added, and the mixture allowed to stand in a cool place for four or five hours, during which time all the lead will be precipitated as insoluble sulphate.

The mixture is then filtered through a small, close-grained filter-paper (preferably an ashless, quantitative filter-paper), and any sediment remaining in the beaker or dish is carefully washed out with alcohol and filtered. A test-tube is placed underneath the funnel; a hole is punched through the tip of the filter with a small glass rod, and all the precipitate (which may be so slight as to be scarcely visible) washed down into the test-tube with a jet of distilled water from a wash-bottle, using as little water as possible. Ten cubic centimeters will usually suffice. This fluid is then heated, adding crystals of sodium acetate until it becomes perfectly clear. It now contains all the lead of the 500 c.c. urine in the form of lead acetate. It is allowed to cool, and hydrogen sulphid gas is passed through it for about five minutes. The slightest yellowish-brown discoloration indicates the

presence of lead. A very slight discoloration can be best seen when looked at from above. For comparison, the gas may be passed through a test-tube containing an equal amount of distilled water. The quantity of lead can be determined by comparing the discoloration with that produced by passing the gas through lead acetate (sugar of lead) solutions of known strength. One gram of lead acetate crystals contains 0.54 gram of lead. Hydrogen sulphid is easily prepared in the simple

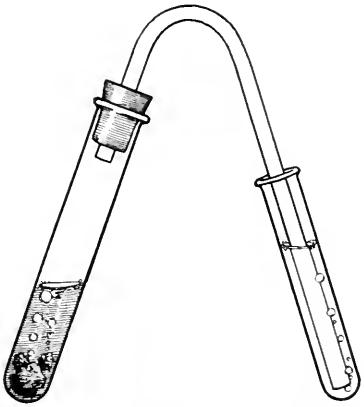


Fig. 36.—A simple hydrogen sulphid generator.

apparatus shown in Fig. 36. A small quantity of iron sulphid is placed in the test-tube; a little dilute hydrochloric acid is added; the cork is replaced; and the delivery tube is inserted to the bottom of the fluid to be tested.

Mercury.—Traces can be detected in the urine for a considerable time after the use of mercury compounds by ingestion or inunction.

About a liter of urine is acidified with 10 c.c. hydrochloric acid, and a small piece of copper-foil or gauze is

introduced. This is gently heated for an hour, and allowed to stand for twenty-four hours. The metal is then removed, and washed successively with very dilute sodium hydroxid solution, alcohol, and ether. When dry, it is placed in a long, slender test-tube, and the lower portion of the tube is heated to redness. A tube with a constriction in its upper portion is better. If mercury be present, it will volatilize and condense in the upper portion of the tube as small, shining globules which can be seen with a hand-magnifier or low power of the microscope. If, now, a crystal of iodin be dropped into the tube and gently heated, the mercury upon the side of the tube is changed first to the yellow iodid, and later to the red iodid, which are recognized by their color.

Morphin.—Add sufficient ammonia to the urine to render it distinctly ammoniacal, and shake thoroughly with a considerable quantity of pure acetic ether. Separate the ether and evaporate to dryness. To a little of the residue in a watch-glass or porcelain dish add a few drops of formaldehyd-sulphuric acid, which has been freshly prepared by adding 1 drop of formalin to 1 c.c. pure concentrated sulphuric acid. If morphin be present, this will produce a purple-red color, which changes to violet, blue violet, and finally nearly pure blue.

Phenol.—As has been stated, the urine following phenol-poisoning turns olive green and then brownish black upon standing. Tests are of value in recognizing poisoning from ingestion and in detecting absorption from carbolized dressings.

The urine is acidulated with hydrochloric acid and distilled. To the first few cubic centimeters of distillate is added 10 per cent. solution of ferric chlorid drop by

drop. The presence of phenol causes a deep amethyst-blue color, as in Uffelmann's test for lactic acid.

Phenolphthalein, which is now widely used as a cathartic, gives a bright pink color when the urine is rendered alkaline.

Quinin.—A considerable quantity of the urine is rendered alkaline with ammonia and extracted with ether; the ether is evaporated, and a portion of the residue dissolved in about 20 drops of dilute alcohol. The alcoholic solution is acidulated with dilute sulphuric acid, 1 drop of an alcoholic solution of iodin (tincture of iodin diluted about ten times) is added, and the mixture is warmed. Upon cooling, an iodin compound of quinin (herapathite) will separate out in the form of a microcrystalline sediment of green plates.

The remainder of the residue may be dissolved in a little dilute sulphuric acid. This solution will show a characteristic blue fluorescence when quinin is present.

Resinous drugs cause a white precipitate like that of albumin when strong nitric acid is added to the urine. This is dissolved by alcohol.

Salicylates, salol, aspirin, and similar drugs give a bluish-violet color, which does not disappear upon heating, upon addition of a few drops of 10 per cent. ferric chlorid solution. When the quantity of salicylates is small, the urine may be acidified with hydrochloric acid and extracted with ether, the ether evaporated, and the test applied to an aqueous solution of the residue.

Tannin and its compounds appear in the urine as gallic acid, and the urine becomes greenish black (inky, if much gallic acid be present) when treated with a solution of ferric chlorid.

III. MICROSCOPIC EXAMINATION

A careful microscopic examination will often reveal structures of great diagnostic importance in urine which seems perfectly clear, and from which only very slight sediment can be obtained with the centrifuge. Upon the other hand, cloudy urines with abundant sediment are often shown by the microscope to contain nothing of clinical significance.

Since the nature of the sediment soon changes, the urine must be examined while fresh, preferably within six hours after it is voided. When possible it should be kept on ice. The sediment is best obtained by means of the centrifuge. If a centrifuge is not available, the urine may be allowed to stand in a conical test-glass for six to twenty-four hours after adding some preservative (see p. 86).

A small amount of the sediment should be transferred to a slide by means of a pipet. It is very important to do this properly. The best pipet is a small glass tube which has been drawn out at one end to a tip with rather small opening. The tube or glass containing the sediment is held on a level with the eye, the larger end of the pipet is closed with the index-finger, which must be dry, and the tip is carried down into the sediment. By carefully loosening the finger, but not entirely removing it, a small amount of the sediment is then allowed to run slowly into the pipet. Slightly rotating the pipet will aid in accomplishing this, and at the same time will serve to loosen any structures which cling to the bottom of the tube. After wiping off the urine which adheres to the outside, a drop from the pipet is placed upon a clean slide. A hair is then placed in the drop and a large cover-glass

applied. The correct size of the drop can be learned only by experience. It should not be so large as to float the cover-glass about, nor so small as to leave unoccupied space beneath the cover. Many workers use no cover. This offers a thicker layer and larger area of urine, the chance of finding scanty structures being proportionately increased. It has the disadvantage that any jarring of the room (as by persons walking about) sets the microscopic field into vibratory motion and makes it impossible to see anything clearly; and, since it does not allow of the use of high-power objectives, one cannot examine details as one often wishes to do. It is true that a cover can be applied later, but any structure which one has found with the low power and wishes to study with the high is sure to be lost when the cover is applied. A large cover-glass (about 22 mm. square) with a hair beneath it avoids these disadvantages, and gives enough urine to find any structures which are present in sufficient number to have clinical significance, provided other points in the technic have been right. It is best, however, to examine several drops; and, when the sediment is abundant, drops from the upper and lower portions should be examined separately.

In examining urinary sediments microscopically no fault is so common, nor so fatal to good results, as improper illumination (see Fig. 4), and none is so easily corrected. The light should be central and very subdued for ordinary work, but oblique illumination, obtained by swinging the mirror a little out of the optical axis, will be found helpful in identifying certain delicate structures like hyaline casts. The 16-mm. objective should be used as a finder, while the 4-mm. is reserved

for examining details. An experienced worker will rely almost wholly upon the lower power.

It is well to emphasize that *the most common errors which result in failure to find important structures, when present, are: (a) lack of care in transferring the sediment to the slide, (b) too strong illumination, and (c) too great magnification.*

In order to distinguish between similar structures it is often necessary to watch the effect upon them of certain reagents. This is especially true of the various unorganized sediments. They very frequently cannot be identified from their form alone. With the structures still in focus, a drop of the reagent may be placed at one edge of the cover-glass and drawn underneath it by the suction of a piece of blotting-paper touched to the opposite edge; or a small drop of the reagent and of the urine may be placed close together upon a slide and a cover gently lowered over them. As the two fluids mingle, the effect upon various structures may be seen.

Urinary sediments may be studied under three heads: A. Unorganized sediments. B. Organized sediments. C. Extraneous structures.

A. Unorganized Sediments

In general, these have little diagnostic or prognostic significance. Most of them are substances normally present in solution, which have been precipitated either because present in excessive amounts, or, more frequently, because of some alteration in the urine (as in reaction, concentration, etc.) which may be purely physiologic, depending upon changes in diet or habits. Various substances are always precipitated during de-

composition, which may take place either within or without the body. Unorganized sediments may be classified according to the reaction of the urine in which they are *most likely* to be found. This classification is useful, but is not accurate, since the characteristic sediments of acid urine may remain after the urine has become alkaline, while the alkaline sediments may be precipitated in a urine which is still acid.

In acid urine: Uric acid, amorphous urates, sodium urate, calcium oxalate, leucin and tyrosin, cystin, and

Fig. 37.—Unusual urinary crystals (drawn from various authors): 1, Calcium sulphate (colorless); 2, cholesterin (colorless); 3, hippuric acid (colorless); 4, hematoidin (brown); 5, fatty acids (colorless); 6, indigo (blue); 7, sodium urate (yellowish).

fat-globules. Uric acid, the urates, and calcium oxalate are the common deposits of acid urines; the others are less frequent, and depend less upon the reaction of the urine.

In alkaline urine: Phosphates, calcium carbonate, and ammonium urate.

Other crystalline sediments (Fig. 37) which are rare and require no further mention are: Calcium sulphate, cholesterin, hippuric acid, hematoidin, fatty acids, and indigo.

The following brief table will aid the student in identifying the chemical sediments which one meets every day:

	Acid urine.	Alkaline urine.
Yellow crystals.	Uric acid—dissolve in KOH.	Ammonium urate—dissolve in HCl.
Colorless crystals.	Calcium oxalate—dissolve in HCl	Phosphate crystals—dissolve in acetic acid.
Amorphous material.	Urates—dissolve with heat.	Amorphous phosphates—dissolve in acetic acid.

1. In Acid Urine.—(1) **Uric-acid Crystals.**—These crystals are the red grains—"gravel" or "red sand"—which are often seen adhering to the sides and bottom of a vessel containing urine. Microscopically, they are yellow or reddish-brown crystals, which differ greatly in size and shape. The color is due to urinary pigments. The most characteristic forms (Plate IV and Fig. 38, 2) are "whetstones"; roset-like clusters of prisms and whetstones; and rhombic plates, which have usually a paler color than the other forms and are sometimes colorless. A very rare form is a colorless hexagonal plate resembling cystin. Recognition of the crystals depends less upon their shape than upon their color, the reaction of the urine, and the facts that they are soluble in caustic soda solution and insoluble in hydrochloric or acetic acid. When ammonia is added, they dissolve and crystals of ammonium urate appear.

A deposit of uric-acid crystals has no significance unless it occurs before or very soon after the urine is voided. Every urine, if kept acid, will in time deposit its uric acid. Factors which favor an early deposit are high acidity, diminished urinary pigments, and excessive ex-

cretion of uric acid. The chief clinical interest of the crystals lies in their tendency to form calculi, owing to the readiness with which they collect about any solid object. Their presence in the freshly voided urine in clusters of crystals suggests stone in the kidney or bladder, especially if blood is also present (see Fig. 65).

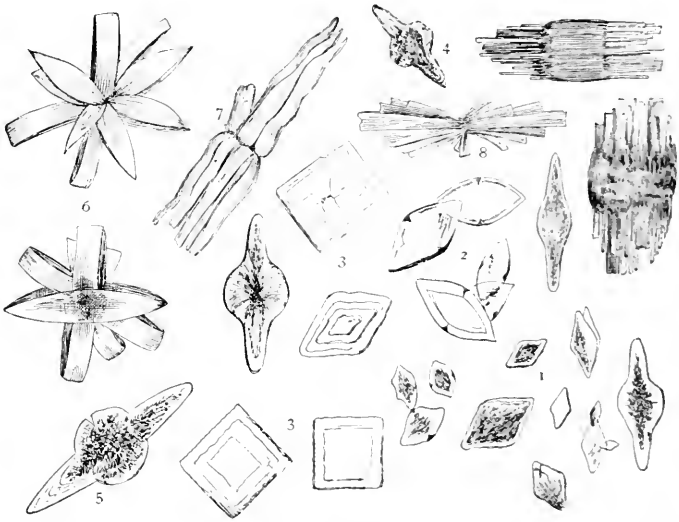
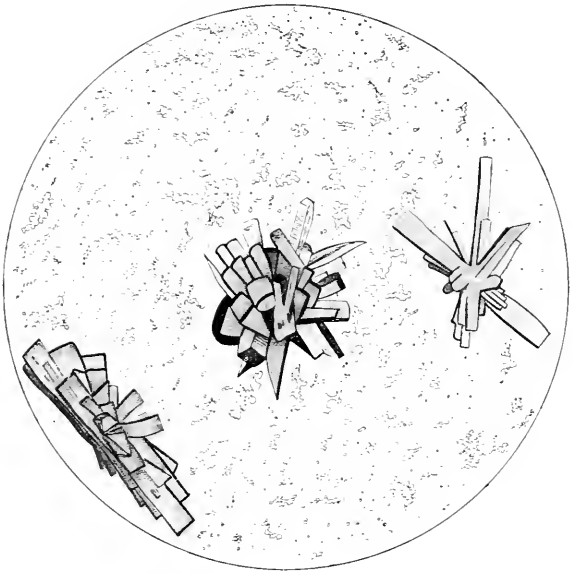


Fig. 38.—Forms of uric acid: 1. Rhombic plates; 2, whetstone forms; 3, 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosets; 7, pointed bundles; 8, barrel forms precipitated by adding hydrochloric acid to urine (Ogden).

(2) **Amorphous Urates.**—These are chiefly urates of sodium and potassium which are thrown out of solution as a yellow or red "brick-dust" deposit. In pale urines this sediment is almost white. It disappears upon heating. A deposit of amorphous urates is very common in concentrated and strongly acid urines, especially in cold weather, and has no clinical significance. Under

PLATE IV



Uric-acid crystals with amorphous urates (after Peyer).

the microscope it appears as fine yellowish granules, often so abundant as to obscure all other structures (Plate IV). In such cases the urine should be warmed before examining. Amorphous urates are readily soluble in caustic soda solutions. When treated with hydrochloric or acetic acid, they slowly dissolve and rhombic crystals of uric acid appear.

Rarely, sodium urate occurs in crystalline form—slender prisms, arranged in fan- or sheaf-like structures (see Fig. 37).

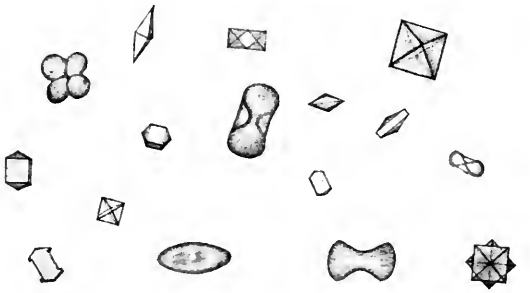


Fig. 39.—Various forms of calcium oxalate crystals (Ogden).

(3) **Calcium Oxalate.**—Characteristic of calcium oxalate are colorless, glistening, octahedral crystals, giving the appearance of small squares crossed by two intersecting diagonal lines—the so-called "envelope crystals" (see Fig. 52). They vary greatly in size, being sometimes so small as to seem mere points of light with medium-power objectives. Unusual forms, which, however, seldom occur except in conjunction with the octahedra, are colorless dumb-bells, spheres, and variations of the octahedra (Fig. 39). The spheres might be mistaken for globules of fat or red blood-corpuscles. Crystals of

calcium oxalate are insoluble in acetic acid or caustic soda. They are dissolved by strong hydrochloric acid, and recrystallize as octahedra upon addition of ammonia. They are sometimes encountered in alkaline urine.

The crystals are commonly found in the urine after ingestion of vegetables rich in oxalic acid, as tomatoes, spinach, asparagus, and rhubarb. They have no definite significance pathologically. They often appear in digestive disturbances, in neurasthenia, and when the oxidizing power of the system is diminished. When abundant, they are generally associated with a little mucus; and, in men, frequently with a few spermatozoa. Like uric acid, their chief clinical interest lies in their tendency to form calculi, and their presence in fresh urine, together with evidences of renal or cystic irritation, should be viewed with suspicion, particularly if they are clumped in small masses.

(4) **Leucin and Tyrosin.**—These substances are cleavage products of the protein molecule. They are of comparatively rare occurrence in the urine and generally appear together. In general, their presence indicates autolysis of tissue proteins. Clinically, they are seen most frequently in severe fatty destruction of the liver, such as occurs in acute yellow atrophy and phosphorus-poisoning. Crystals are deposited spontaneously only when the substances are present in large amount. Usually they will be deposited when the urine is evaporated to a small volume on a water-bath. It is best, however, to separate them from the urine as follows:

Treat 500 to 1000 c.c. of urine, which has been freed from albumin, with neutral, then with basic, lead acetate until a

precipitate no longer forms. Filter, remove excess of lead with hydrogen sulphid (see p. 170), and filter again. Concentrate to a syrup on a water-bath. Extract repeatedly with small quantities of absolute alcohol to remove urea. Treat the residue with hot dilute alcohol to which a little ammonia has been added. Filter and evaporate the filtrate to a small volume and let stand for the leucin and tyrosin to separate out. The leucin can be separated from the tyrosin by boiling with glacial acetic acid. Leucin dissolves, leaving the tyrosin, and can again be recovered by evaporating the acetic acid.

The crystals cannot be identified from their morphology alone, since other substances, notably calcium phosphate (see Fig. 43) and ammonium urate, may take similar or identical forms. It is, therefore, necessary to try out their solubility in various reagents or to apply special tests.

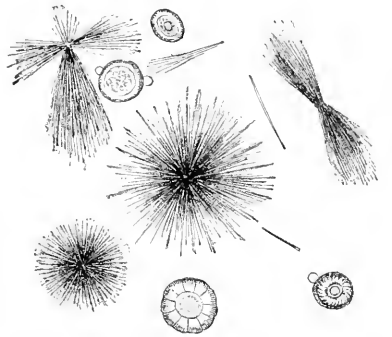


Fig. 40.—Leucin spheres and tyrosin needles (Stengel).

Leucin crystals (Fig. 40) as they appear in the urine do not represent the pure substance. They are slightly yellow, oily-looking spheres, many of them with radial and concentric striations. Some may be merged to-

gether in clusters. They are not soluble in hydrochloric acid nor in ether.

Tyrosin crystallizes in very fine needles, which may appear black and which are usually arranged in sheaves, with a marked constriction at the middle (Fig. 40). It is soluble in ammonia and hydrochloric acid, but not in acetic acid.

Mörner's Test for Tyrosin.—To a small quantity of the crystals in a test-tube add a few cubic centimeters of Mörner's reagent (formalin, 1 c.c.; distilled water, 45 c.c.; concentrated sulphuric acid, 55 c.c.). Heat gently to the boiling-point. A green color shows the presence of tyrosin.

(5) **Cystin crystals** are colorless, highly refractive, rather thick, hexagonal plates with well-defined edges. They lie either singly or superimposed to form more or less irregular clusters (Fig. 41). Uric acid sometimes takes this form and must be excluded. Cystin is soluble in hydrochloric acid, insoluble in acetic; it is readily soluble in ammonia and recrystallizes upon addition of acetic acid.

Cystin is one of the amino-acids formed in decomposition of the protein molecule, and is present in traces in normal urine. Crystals are desposited only when the substance is present in excessive amount. Their presence is known as *cystinuria*. It is a rare condition due to an obscure abnormality of protein metabolism and usually continues throughout life. There are rarely any symptoms save those referable to renal or cystic calculus, to which the condition strongly predisposes.

(6) **Fat-globules.**—Fat appears in the urine as highly refractive globules of various sizes, frequently very small.

These globules are easily recognized from the fact that they are stained black by osmic acid and orange or red by Sudan III. The stain may be applied upon the slide, as already described (see p. 175). Osmic acid should be used in 1 per cent. aqueous solution; Sudan III, in saturated solution in 70 per cent. alcohol, to which one-half volume of 10 per cent. formalin may advantageously be added.

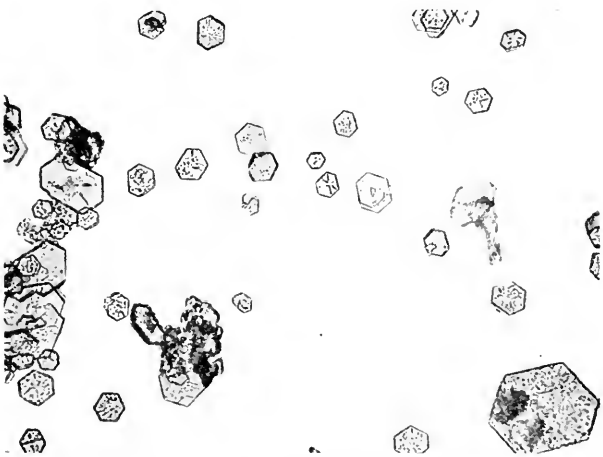


Fig. 41.—Cystin crystals from urine of patient with cystin calculus (× 200) (photograph by the author).

Fat in the urine is usually a contamination from unclean vessels, oiled catheters, etc. A very small amount may be present after ingestion of large quantities of cod-liver oil or other fats. In fatty degeneration of the kidney, as in phosphorus-poisoning and chronic parenchymatous nephritis, fat-globules are commonly seen, both free in the urine and embedded in cells and tube-

casts. Fat-droplets are common in pus-corpuscles and in degenerating cells of any kind.

In *chyluria*, or admixture of chyle with the urine as a result of rupture of a lymph-vessel, minute droplets of fat are so numerous as to give the urine a milky appearance. The droplets are smaller than those of milk, which is sometimes added by malingerers. The fluid is often blood-tinged. The condition is best recognized by shaking up with ether, which, when separated, leaves the urine

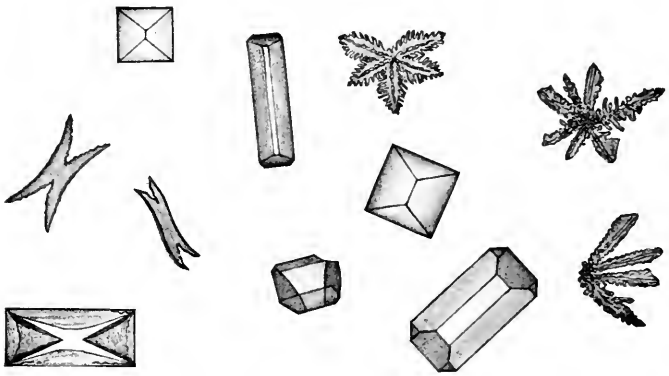


Fig. 42.—Various forms of triple phosphate crystals (Ogden).

comparatively clear. Chyluria occurs most frequently as a symptom of infection by filaria (see p. 407), the larvæ of which can usually be found in the milky urine.

2. In Alkaline Urine.—(1) **Phosphates.**—While most common in alkaline urine, phosphates are sometimes deposited in amphoteric or feebly acid urines. The usual forms are: (*a*) Ammoniomagnesium phosphate crystals; (*b*) acid calcium phosphate crystals, and (*c*) amorphous phosphates. All are readily soluble in acetic acid.

(*a*) *Ammoniomagnesium Phosphate Crystals.*—They are the common "triple phosphate" crystals, which are generally easily recognized (Figs. 42 and 66, and Plate V). They are colorless, except when bile stained. Their usual form is some modification of the prism, with oblique ends. Most typical are the well-known "coffin-lid" and "hip-roof" forms. The long axis of the hip-roof crystal is often so shortened that it resembles the envelope crystal of calcium oxalate. It does not, however, have the same luster; this, and its solubility in acetic acid, will always prevent confusion.

Fig. 43.—Crystals of calcium phosphate: 1, Common form (copied from Rieder's Atlas); 2, needles resembling tyrosin (drawn from nature); 3, large, irregular plates (from nature).

When rapidly deposited, as by artificial precipitation, triple phosphate often takes feathery, star-, or leaf-like forms. These gradually develop into the more common prisms. X-forms may be produced by partial solution of prisms.

(*b*) *Dicalcium Phosphate Crystals.*—In feebly acid, amphoteric, or feebly alkaline urines acid calcium phosphate, wrongly called "neutral calcium phosphate," is not infrequently deposited in the form of colorless

prisms arranged in stars and rosets (Fig. 43, 1). Because of the shape of the crystals it is sometimes called "stellar phosphate." The individual prisms are usually slender, with one beveled, wedge-like end, but are sometimes needle-like. They may sometimes take forms resembling tyrosin (Fig. 43, 2), calcium sulphate, or hippuric acid, but are readily distinguished by their solubility in acetic acid.

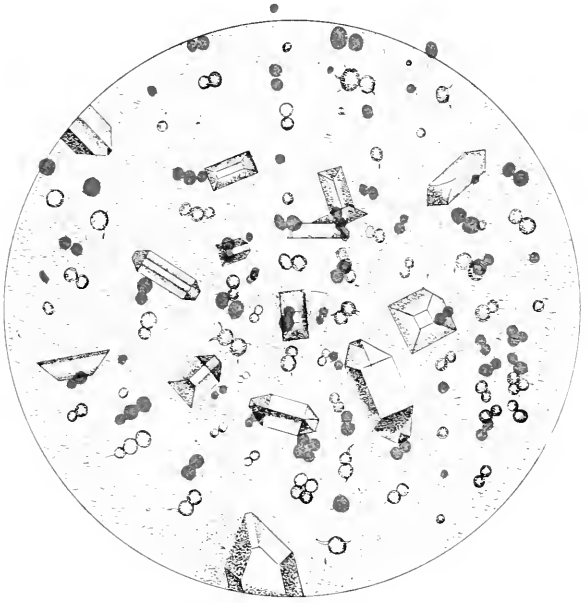
Calcium phosphate often forms large, thin, irregular, usually granular, colorless plates, which are easily recognized (Fig. 43, 3).

Fig. 44.—Indistinct crystalline sediment (dumb-bell crystals) of calcium carbonate. Similar crystals are sometimes formed by calcium oxalate and calcium sulphate (after Funke).

(*c*) *Amorphous Phosphates.*—The earthy phosphates are thrown out of solution in most alkaline and many amphoteric urines as a white, amorphous sediment, which may be mistaken for pus macroscopically. Under the microscope the sediment is seen to consist of numerous colorless granules, distinguished from amorphous urates by their color, their solubility in acetic acid, and the reaction of the urine.

The various phosphatic deposits frequently occur together. They are sometimes due to excessive excretion of phosphoric acid, but usually merely indicate that the urine has become, or is becoming, alkaline. (See Phosphates, p. 109.)

PLATE V



Sediment of alkaline fermentation (after Hofmann and Ultzmann).

(2) **Calcium carbonate** may sometimes be mingled with the phosphatic deposits, usually as amorphous granules, or, more rarely, as colorless spheres and dumb-bells (Fig. 44), which are soluble in acetic acid with gas formation.

(3) **Ammonium Urate Crystals.**—This is the only urate deposited in alkaline urine. It forms opaque yellow crystals, usually in the form of spheres (Plate V and Fig. 66), which are often covered with fine or coarse

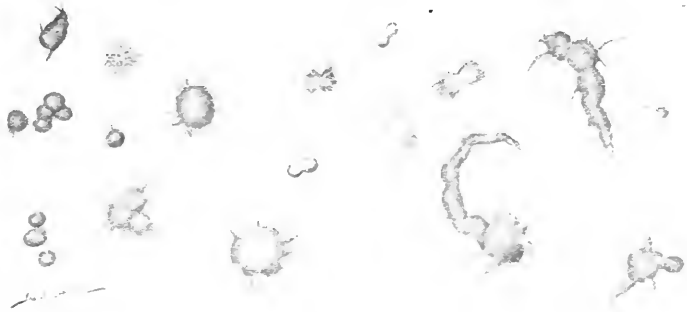


Fig. 45.—Crystals of ammonium urate (one-half of the forms copied from Rieder's Atlas; the others, from nature).

spicules—"thorn-apple crystals." Sometimes dumb-bells, compact sheaves of fine needles, and irregular rhizome forms are seen (Fig. 45). Upon addition of acetic acid they dissolve, and rhombic plates of uric acid appear.

These crystals occur only when free ammonia is present. They are generally found along with the phosphates in decomposing urine and have no clinical significance.

B. Organized Sediments

The principal organized structures in urinary sediments are: Tube-casts; epithelial cells; pus-corpuscles;

red blood-corpuscles; spermatozoa; bacteria, and animal parasites. They are much more important than the unorganized sediments just considered.

1. Tube-casts.—These interesting structures are albuminous casts of the uriniferous tubules. Their presence in the urine (known as cylindruria) probably always indicates some pathologic change in the kidney, although this change may be very slight or transitory. Large numbers may be present in temporary irritations and congestions. *They do not in themselves, therefore, imply organic disease of the kidney.* They rarely occur in urine which does not contain, or has not recently contained, albumin.

While it is not possible to draw a sharp dividing line between the different varieties, casts may be classified as follows:

(1) Hyaline casts.
    (*a*) Narrow.
    (*b*) Broad.
(2) Waxy casts.
(3) Fibrinous casts.
(4) Granular casts.
    (*a*) Finely granular.
    (*b*) Coarsely granular.
(5) Fatty casts.
(6) Casts containing organized structures.
    (*a*) Epithelial casts.
    (*b*) Blood-casts.
    (*c*) Pus-casts.
    (*d*) Bacterial casts.

As will be seen later, practically all varieties are modifications of the hyaline.

The significance of the different varieties is more readily understood if one considers their mode of formation. Albuminous material, the source and nature of which are not definitely known, but which are doubtless not the same in all cases, probably enters the lumen of a uriniferous tubule in a fluid or plastic state. The material has been variously thought to be an exudate from the blood, a pathologic secretion of the renal cells, and a product of epithelial degeneration. In the tubule it hardens into a cast which, when washed out by the urine, retains the shape of the tubule, and contains within its substance whatever structures and débris were lying free within the tubule or were loosely attached to its wall. If the tubule be small and has its usual lining of epithelium, the cast will be narrow; if it be large or entirely denuded of epithelium, the cast will be broad. *A cast, therefore, indicates the condition of the tubule in which it is formed, but does not necessarily indicate the condition of the kidney as a whole.*

The search for casts must be carefully made. The urine must be fresh, since hyaline casts soon dissolve when it becomes alkaline. It should be thoroughly centrifugalized. When the sediment is abundant, casts, being light structures, will be found near the top. In cystitis, where casts may be entirely hidden by the pus, the bladder should be irrigated to remove as much of the pus as possible and the next urine examined. In order to prevent solution of the casts the urine, if alkaline, must be rendered acid by previous administration of boric acid or other drugs. Heavy sediments of urates, blood, or vaginal cells may likewise obscure casts and other important structures. The last can be

avoided by catheterization. Urates can be dissolved by gently warming before centrifugalizing, care being taken not to heat enough to coagulate the albumin. The aluminum shield of the centrifuge tube may also be heated. Blood can be destroyed by centrifugalizing, pouring off the supernatant urine, filling the tube with water, adding a few drops of dilute acetic acid, mixing well, and again centrifugalizing; this process being repeated until the blood is completely decolorized. Too much acetic acid will dissolve hyaline casts.

Their cylindric shape can be best seen by slightly moving the cover-glass while observing them, thus causing them to roll. This little manipulation should be practised until it can be done satisfactorily. It will prove useful in many examinations.

Various methods of staining casts so as to render them more conspicuous have been proposed. They offer no special advantage to one who understands how to use the substage mechanism of his microscope. The "negative-staining" method is as good as any. It consists simply in adding a little India-ink to the drop of urine on the slide. Casts, cells, etc., will stand out as colorless structures on a dark background.

(1) **Hyaline Casts.**—Typically, these are colorless, homogeneous, semitransparant, cylindric structures, with parallel sides and usually rounded ends. Not infrequently they are more opaque or show a few granules or an occasional oil-globule or cell, either adhering to them or contained within their substance. Generally they are straight or curved; less commonly, convoluted. Their length and breadth vary greatly: they are sometimes so long as to extend across several fields of a

medium-power objective, but are usually much shorter; in breadth they vary from one to seven or eight times

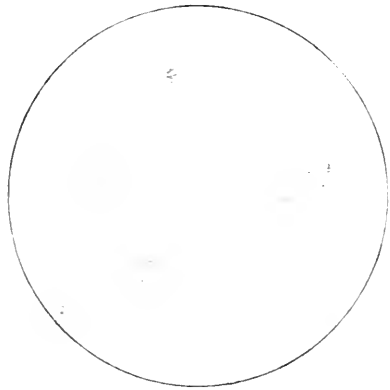


Fig. 46.—Hyaline casts showing fat-droplets and leukocytes (obj. 4 mm.) (Boston).

the diameter of a red blood-corpuscle. (See Figs. 4, 46, 47, and 51.)

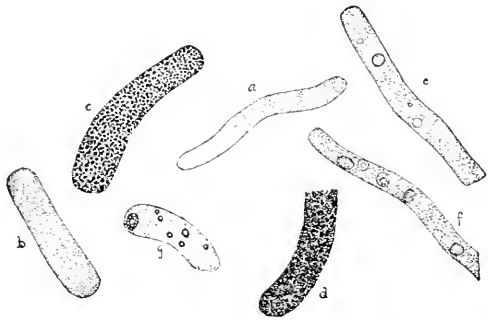


Fig. 47.—Various kinds of casts: *a*, Hyaline and finely granular cast; *b*, finely granular cast; *c*, coarsely granular cast; *d*, brown granular cast; *e*, granular cast with normal and abnormal blood adherent; *f*, granular cast with renal cells adherent; *g*, granular cast with fat and a fatty renal cell adherent (Ogden).

Hyaline casts are the least significant of all the casts, and occur in many slight and transitory conditions.

Small numbers are common following ether anesthesia, in fevers, after excessive exercise, and in congestions and irritations of the kidney. They are always present, and are usually stained yellow when the urine contains much bile. While they are found in all organic diseases of the kidney, they are most important in chronic interstitial nephritis. Here they are seldom abundant, but their constant presence is the most reliable urinary sign of the disease. Small areas of chronic interstitial change are probably responsible for the few hyaline casts so frequently found in the urine of elderly persons.

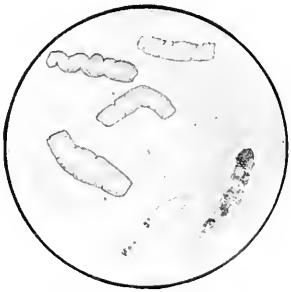


Fig. 48.—Waxy casts (upper part of figure). Fatty and fat-bearing casts (lower part of figure) (from Greene's "Medical Diagnosis").

Very broad hyaline casts commonly indicate complete desquamation of the tubular epithelium, such as occurs in the late stages of nephritis.

(2) **Waxy Casts.**—Like hyaline casts, these are homogeneous when typical, but frequently contain a few granules or an occasional cell. They are much more opaque than the hyaline variety, and are usually shorter and broader, with irregular, broken ends, and sometimes appear to be segmented. They are grayish or colorless, and have a dull, waxy look, as if cut from par-

affin (Figs. 48 and 64). They are sometimes composed of material which gives the amyloid reactions. All gradations between hyaline and waxy casts may be found. Waxy casts are found in most advanced cases of nephritis, where they are an unfavorable sign. They are perhaps most abundant in amyloid disease of the kidney, but are not distinctive of the disease, as is sometimes stated.

(3) **Fibrinous Casts.**—Casts which resemble waxy casts, but have a distinctly yellow color, as if cut from beeswax, are often seen in acute nephritis. They are called fibrinous casts, but the name is inappropriate, as they are not composed of fibrin. They are often classed with waxy casts, but should be distinguished, as their significance is much less serious.

(4) **Granular Casts.**—These are merely hyaline casts in which numerous granules are embedded (Figs. 47, 49, and 51).

Finely granular casts contain many fine granules, are usually shorter, broader, and more opaque than the hyaline variety, and are more conspicuous. Their color is grayish or pale yellow.

Coarsely granular casts contain larger granules and are darker in color than the finely granular, being often dark brown, owing to presence of altered blood-pigment. They are usually shorter and more irregular in outline, and more frequently have irregularly broken ends.

(5) **Fatty Casts.**—Small droplets of fat may at times be seen in any variety of cast. Those in which the droplets are numerous are called fatty casts (Figs. 48 and 49). The fat-globules are not difficult to recognize. Staining with osmic acid or Sudan (see p. 183) will remove any doubt as to their nature.

The granules and fat-droplets seen in casts are products of epithelial degeneration. Granular and fatty casts, therefore, always indicate partial or complete disintegration of the renal epithelium. The finely granular variety is the least significant, and is found when the epithelium is only moderately affected. Coarsely granular, and especially fatty casts, if present in considerable numbers, indicate a serious parenchymatous nephritis.

(6) **Casts Containing Organized Structures.**—Cells and other structures are frequently seen adherent to a

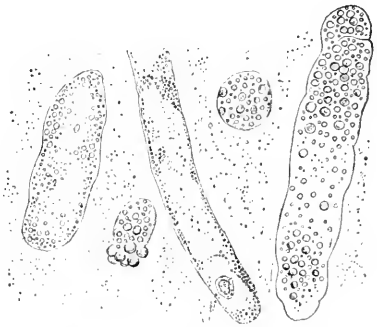


Fig. 49.—Granular and fatty casts and two compound granule cells (Stengel).

cast or embedded within it. (See Figs. 46 and 47.) When numerous, they give name to the cast.

(*a*) *Epithelial casts* contain epithelial cells from the renal tubules. The cells vary in size and are often flattened, oval, or elongated. They may be recognized as epithelial cells by irrigating with dilute acetic acid, which brings out the nucleus clearly. Epithelial casts always imply desquamation of epithelium, which rarely occurs except in parenchymatous inflammations (see

Figs. 63 and 64). When the cells are well preserved they point to acute nephritis.

(*b*) *Blood-casts* contain red blood-corpuscles, usually much degenerated (Figs. 50 and 63). They always indicate hemorrhage into the tubules, which is most common in acute nephritis or an acute exacerbation of a chronic nephritis.

(*c*) *Pus-casts* (see Fig. 65), composed almost wholly of pus-corpuscles, are uncommon, and point to a chronic suppurative process in the kidney.

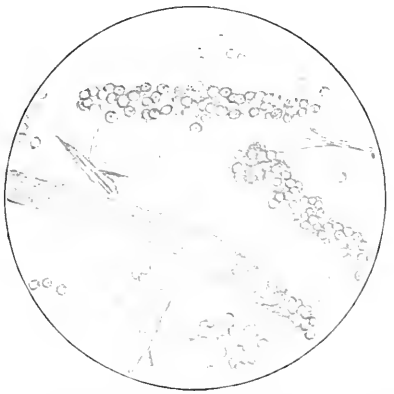


Fig. 50.—Red blood-corpuscles and blood-casts (courtesy of Dr. A. Scott [illegible] (obj. 4 mm. (Boston).

(*d*) True *bacterial casts* are rare. They indicate a septic condition in the kidney. Bacteria may permeate a cast after the urine is voided.

Structures Likely to Be Mistaken for Casts.—(1) **Mucous Threads.**—Mucus frequently appears in the form of long strands which slightly resemble hyaline casts (Fig. 51). They are, however, more ribbon-like, have less well-defined edges, and usually show faint

longitudinal striations. Their ends taper to a point or are split or curled upon themselves, and are never evenly rounded, as is commonly the case with hyaline casts.

Such threads form a part of the nubecula of normal urine, and are especially abundant when calcium oxalate crystals are present. When there is an excess of mucus, as in irritations of the urinary tract, every field may be filled with an interlacing meshwork.

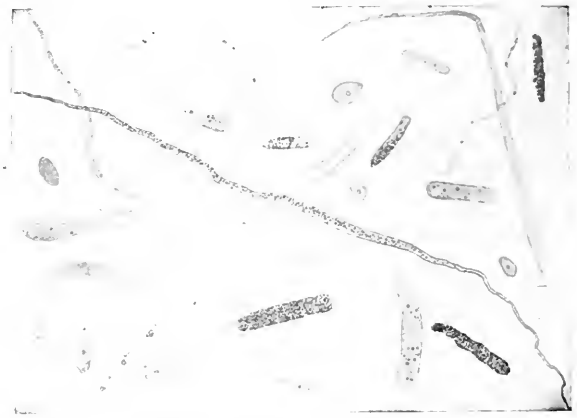


Fig. 51.—Hyaline and granular casts, mucous threads, and cylindroids. There are also a few epithelial cells from the bladder (Wood).

Mucous threads are microscopic and should not be confused with urethral shreds or "gonorrheal threads," which are macroscopic, 0.5 to 1 cm. long, and consist of a matrix of mucus in which many epithelial and pus-cells are embedded.

(2) **Cylindroids.**—This name is sometimes given to the mucous threads just described, but is more properly applied to certain peculiar structures more nearly allied to casts. They resemble hyaline casts in structure, but

differ in being broader at one end and tapering to a slender tail, which is often twisted or curled upon itself (Fig. 51). They frequently occur in the urine along with hyaline casts, especially in irritations of the kidney, and have practically the same significance.

(3) **Masses of amorphous urates,** or **phosphates,** or **very small crystals** (Fig. 52), which accidentally take a cylindric form, or shreds of mucus covered with granules, closely resemble granular casts. Application of gentle

Fig. 52.—Calcium oxalate crystals, showing a pseudocast of small crystals (Jakob).

heat or appropriate chemicals will serve to differentiate them. When urine contains both mucus and granules, large numbers of these "pseudocasts," all lying in the same direction, can be produced by slightly moving the cover-glass from side to side. It is possible—as in urate infarcts of infants—for urates to be molded into cylindric bodies within the renal tubules.

(4) **Hairs** and **fibers** of wool, cotton, etc. These could be mistaken for casts only by beginners. One

can easily become familiar with their appearance by suspending them in water and examining with the microscope (see Fig. 61).

(5) **Hyphæ** of **molds** are not infrequently mistaken for hyaline casts. Their higher degree of refraction, their jointed or branching structure, and the accompanying spores will differentiate them (see Fig. 62).

2. Epithelial Cells.—A few cells from various parts of the urinary tract occur in every urine. A marked increase indicates some pathologic condition at the site of their origin. It is sometimes, but by no means always, possible to locate their source from their form. One should, however, be extremely cautious about making any definite statement as to the origin of any individual cell. Most cells are much altered from their original shape. Any epithelial cell may be so granular from degenerative changes that the nucleus is obscured. Most of them contain fat-globules. They are usually divided into three groups:

(1) **Small, round** or **polyhedral cells** are about the size of pus-corpuscles, or a little larger, with a single round nucleus. Such cells may come from the deeper layers of any part of the urinary tract. They are uncommon in normal urine. When they are dark in color, very granular, and contain a comparatively large nucleus, they probably come from the renal tubules, but their origin in the kidney is not proved unless they are found embedded in casts. In chronic passive congestion of the kidney some of these cells may contain yellow granules of altered blood-pigment. They are analogous to the "heart-failure cells" of the sputum (see p. 79). Renal cells are abundant in parenchymatous nephritis,

especially the acute form. They are nearly always fatty —most markedly so in chronic parenchymatous nephritis, where their substance is sometimes wholly replaced by fat-droplets ("compound granule cells") (see Figs. 49, 53, and 63).

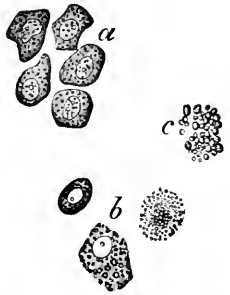


Fig. 53.—Renal epithelium from nephritic urine: *a*, Polyhedral epithelium in nephritis of scarlet fever; *b* and *c*, different grades of fatty degeneration in renal epithelium in chronic nephritis (× 400) (after Bizzozero).

(2) **Irregular cells** are considerably larger than the preceding. They are round, pear shaped, or spindle shaped, or may have tail-like processes, and are hence named large round, pyriform, spindle, or caudate cells respectively. Each contains a round or oval distinct nucleus. Their usual source is the deeper layers of the urinary tract, especially of the bladder. Caudate forms come most commonly from the pelvis of the kidney (see Figs. 54, *b*, 55, 65, and 66).

(3) **Squamous** or **pavement cells** are large flat cells, each with a small, distinct round or oval nucleus (Fig. 54, *a*). They are derived from the superficial layers of the ureters, bladder, urethra, or vagina, and when desquamation is active, appear in stratified masses. Squamous cells from the bladder are generally rounded, while those from the vagina are larger, thinner, and more angular. Great numbers of these vaginal cells, together with pus-corpuscles, may be present when leukorrhea exists.

3. Pus-corpuscles.—A very few leukocytes are present in normal urine. They are more abundant when

mucus is present. An excess of leukocytes, mainly of the polymorphonuclear neutrophilic variety, with albumin, constitutes *pyuria*—pus in the urine.

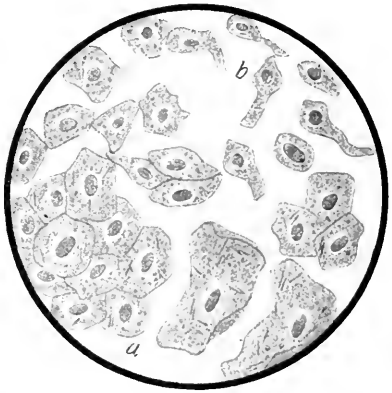


Fig. 54.—Epithelial cells from urethra and bladder: *a*, Squamous cells from superficial layers; *b*, irregular cells from deeper layers (Jakob).

Fig. 55.—Caudate epithelial cells from pelvis of kidney (Jakob).

When at all abundant, pus forms a white sediment resembling amorphous phosphates macroscopically. Un-

der the microscope the corpuscles appear as very granular cells, about twice the diameter of a red blood-corpuscle (Figs. 56 and 66). The granules are partly the normal neutrophilic granules, partly granular products of degeneration. In freshly voided urine many exhibit ameboid motion, assuming irregular outlines. Each contains one irregular nucleus or several small, rounded nuclei. The nuclei are obscured or entirely hidden by the granules, but may be brought clearly into view by running a little acetic acid under the cover-glass. This enables one to easily distinguish pus-corpuscles from

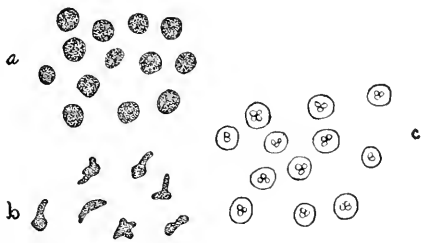


Fig. 56.—Pus-corpuscles: *a*, As ordinarily seen; *b*, ameboid corpuscles; *c*, showing the action of acetic acid (Ogden).

small round epithelial cells, which resemble them in size, but have a single, rather large, round nucleus. In decomposing urine pus is often converted into a gelatinous mass which gives the urine a ropy consistence.

Pyuria indicates suppuration in some part of the urinary tract—urethritis, cystitis, pyelitis, etc.—or may be due to contamination from the vagina, in which case many vaginal epithelial cells will also be present. In general, the source of the pus can be determined only by the accompanying structures (epithelia, casts) or by the clinical signs.

A fairly accurate idea of the quantity of pus from day to day may be had by shaking the urine thoroughly and counting the number of corpuscles per cubic millimeter upon the blood-counting slide. A drop of the urine is placed directly upon the slide. Dilution is seldom necessary. The urine must not be alkaline or the corpuscles will adhere in clumps.

Pus always adds a certain amount of albumin to the urine, and it is often desirable to know whether the albumin present in a given specimen is due solely to pus. It has been estimated that 80,000 to 100,000 pus-corpuscles per cubic millimeter add about 0.1 per cent. of albumin. If albumin is present in much greater proportion than this, the excess is probably derived from the kidney.

4. Red Blood-corpuscles.—Urine which contains blood is always albuminous. Very small amounts do not alter its macroscopic appearance. Larger amounts alter it considerably. Blood from the kidneys is generally intimately mixed with the urine and gives it a hazy reddish or brown, "smoky" color. When from the lower urinary tract, it is not so intimately mixed and settles more quickly to the bottom, the color is brighter, and small clots are often present.

Red blood-corpuscles are not usually difficult to recognize with the microscope. When very fresh, they have a normal appearance, being yellowish disks of uniform size (normal blood). When they have been in the urine any considerable time, their hemoglobin may be dissolved out, and they then appear as faint colorless circles or "shadow cells" (abnormal blood), and are more difficult to see (Fig. 57; see also Figs. 50 and 63). They

are apt to be swollen in dilute and crenated in concentrated urines. The microscopic findings may be corroborated by chemic tests for hemoglobin, although the microscope may show a few red corpuscles when the chemic tests are negative.

When not due to contamination from menstrual discharge, blood in the urine, or *hematuria*, is always pathologic. Blood comes from the *kidney tubules* in severe hyperemia, in acute nephritis and acute exacerbations of chronic nephritis, and in renal tuberculosis and malignant disease. An "idiopathic hematuria," probably of nervous origin, has been observed. The finding of blood-

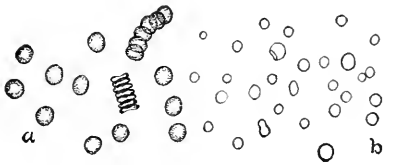


Fig. 57.—Blood-corpuscles: *a*, Normal; *b*, abnormal (Ogden).

casts is the only certain means of diagnosing the kidney as its source. Blood comes from the *pelvis of the kidney* in renal calculus (see Fig. 65), and is then usually intermittent, small in amount, and accompanied by a little pus and perhaps crystals of the substance forming the stone. Considerable hemorrhages from the *bladder* may occur in vesical calculus, tuberculosis, and new growths. Small amounts of blood generally accompany acute cystitis. In Africa the presence of *Schistosomum hæmatobium* in the veins of the bladder is a common cause of hemorrhage (Egyptian hematuria).

It is sometimes possible to gain a clue to the site of the bleeding by having the patient pass his urine in

three separate portions. If the blood be chiefly in the first portion, the bleeding point is probably in the urethra; if in the last, it is probably in the bladder. If the blood is uniformly mixed in all three portions, it probably comes from the kidney or ureter.

5. Spermatozoa are generally present in the urine of men after nocturnal emissions, after epileptic convulsions, and in spermatorrhea. They may be found in the

Fig. 58.—Spermatozoa in urine (Ogden).

urine of both sexes following coitus. They are easily recognized from their characteristic structure (Fig. 58). The 4-mm. objective should be used, with subdued light and careful focusing.

6. Bacteria.—Normal urine is free from bacteria in the bladder, but becomes contaminated in passing through the urethra. Various non-pathogenic bacteria are always present in decomposing urine. In suppura-

tions of the urinary tract pus-producing organisms may be found. In many infectious diseases the specific bacteria may be eliminated in the urine without producing any local lesion. Typhoid bacilli have been known to persist for months and even years after the attack.

Bacteria produce a cloudiness which will not clear upon filtration. They are easily seen with the 4-mm. objective in the routine microscopic examination. Ordinarily, no attempt is made to identify any but the tubercle bacillus and the gonococcus.

Tubercle bacilli are nearly always present in the urine when tuberculosis exists in any part of the urinary tract, but are often difficult to find, especially when the urine contains little or no pus.

Detection of Tubercle Bacilli in Urine.—The urine should be obtained by catheter after careful cleansing of the parts.

(1) Centrifugalize thoroughly, after dissolving any sediment of urates or phosphates by gentle heat or acetic acid. Pour off the supernatant fluid, add water, and centrifugalize again. Addition of 1 or 2 volumes of alcohol will favor centrifugalization by lowering the specific gravity.

(2) Make thin smears of the sediment, adding a little egg-albumen if necessary to make the smear adhere to the glass; dry, and fix in the usual way.

(3) Stain with carbolfuchsin, steaming for at least three minutes, or at room temperature for six to twelve hours.

(4) Wash in water, and then in 20 per cent. nitric acid, until only a faint pink color remains.

(5) Wash in water.

(6) Soak in alcohol fifteen minutes or longer. This decolorizes the smegma bacillus (see p. 69), which is often present in

the urine, and might easily be mistaken for the tubercle bacillus. It is unlikely, however, to be present in catheterized specimens. It is always safest to soak the smear in alcohol for several hours or over night, since some strains of the smegma bacillus are very resistant.

(7) Wash in water.

(8) Apply Löffler's methylene blue solution for one-half minute.

(9) Rinse in water, dry between filter-papers, and examine with the oil-emersion objective.

When the bacilli are scarce, the following method may be tried. It is applicable also to other fluids. If the fluid is not albuminous, add a little egg-albumen. Coagulate the albumen by gentle heat and centrifugalize. The bacilli will be carried down with the albumen. The sediment is then treated by the antiformin method (see p. 68).

A careful search of many smears may be necessary to find the bacilli. They usually lie in clusters (see Plate VI). Failure to find them in suspicious cases should be followed by inoculation of guinea-pigs; this is the court of last appeal, and must also be sometimes resorted to in order to exclude the smegma bacillus.

In gonorrhea, **gonococci** are sometimes found within pus-cells in the sediment, but more commonly in the "gonorrheal threads" or "floaters." In themselves, these threads are by no means diagnostic of gonorrhea. They are most common in the morning or after massage of the prostate. Detection of the gonococcus is described later (see p. 422).

7. Animal parasites are rare in the urine. Hooklets and scolices of *Tænia echinococcus* (Fig. 59) and larvæ of filariæ have been met. In Africa the ova, and even adults, of *Schistosomum hæmatobium* are common.

PLATE VI

Tubercle bacilli in urinary sediment; × 800 (Ogden).

accompanying "Egyptian hematuria." *Trichomonas vaginalis* is a not uncommon contamination. This and

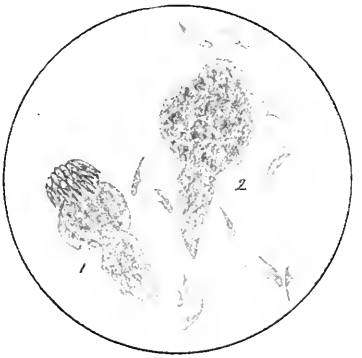


Fig. 59.—1, Scolex of Tænia echinococcus, showing crown of hooklets; 2, scolex and detached hooklets (obj. 4 mm.) (Boston).

other protozoa may be mistaken for spermatozoa by the inexperienced.

Fig. 60.—Embryo of "vinegar eel" in urine, from contamination; length, 340 μ; width, 15 μ. An epithelial cell from bladder and three leukocytes are also shown (studied with Dr. J. A. Wilder).

A worm which is especially interesting is *Anguillula aceti*, the "vinegar eel." This is generally present in

the sediment of table vinegar, and may reach the urine through use of vinegar in vaginal douches, or through contamination of the bottle in which the urine is contained. It has been mistaken for *Strongyloides intestinalis* and for the larval filaria. It closely resembles the former in both adult and embryo stages. The young embryos have about the same length as the larvæ of *Filaria bancrofti*, but are nearly twice as broad, and the intestinal canal is easily seen (compare Figs. 60 and 134).

For fuller descriptions of these parasites the reader is referred to Chapter VI.

C. Extraneous Structures

The laboratory worker must familiarize himself with the microscopic appearance of the more common of the numerous structures which may be present from accidental contamination (Fig. 61).

Yeast-cells are smooth, colorless, highly refractive, spheric or ovoid cells. They sometimes reach the size of a leukocyte, but are generally smaller (see Fig. 106, *l*). They might be mistaken by the inexperienced for red blood-corpuscles, fat-droplets, or the spheric crystals of calcium oxalate, but are distinguished by the facts that they are not of uniform size; that they tend to adhere in short chains; that small buds may often be seen adhering to the larger cells; and that they do not give the hemoglobin test, are not stained by osmic acid or Sudan, but are colored brown by Lugol's solution, and are insoluble in acids and alkalis. Yeast-cells multiply rapidly in diabetic urine, and may reach the bladder and multiply there.

Mold fungi (Fig. 62) are characterized by refractive, jointed, or branched rods (hyphæ), often arranged in a network, and by highly refractive spheric or ovoid spores. They are common in urine which has stood exposed to the air.

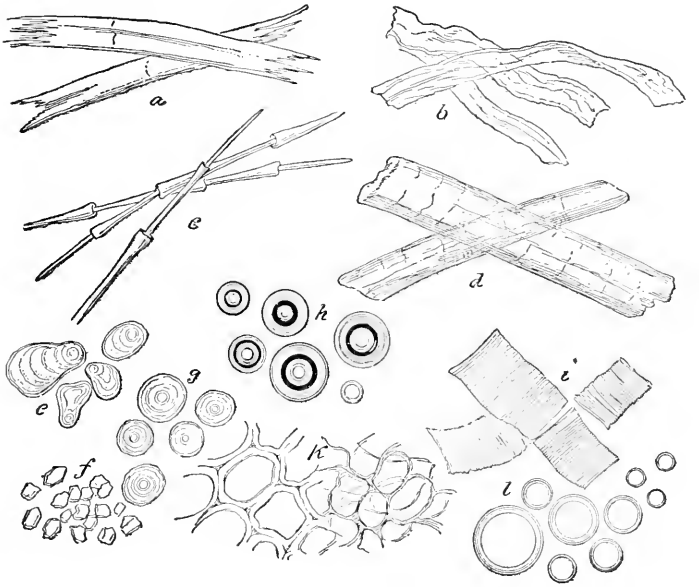


Fig. 61.—Extraneous matters found in urine: *a*, Flax-fibers; *b*, cotton-fibers; *c*, feathers; *d*, hairs; *e*, potato-starch; *f*, rice-starch granules; *g*, wheat-starch; *h*, air-bubbles; *i*, muscular tissue; *k*, vegetable tissue; *l*, oil-globules.

Fibers of wool, cotton, linen, or silk, derived from towels, the clothing of the patient, or the dust in the air, are present in almost every urine. **Fat-droplets** are most frequently derived from unclean bottles or oiled catheters. **Starch-granules** may reach the urine from towels, the clothing, or dusting-powders. They are recognized by their concentric striations and their blue

color with iodin solution. **Lycopodium granules** (see Fig. 5) may also reach the urine from dusting-powders. They might be mistaken for the ova of parasites. **Bubbles of air** (see Fig. 61, *h*) are often confusing to beginners, but are easily recognized after once being seen.

Scratches and **flaws** in the glass of slide or cover are often most assiduously studied by beginners, and are not infrequently reported as rare crystals, tube-casts, or even worms. **Dirt** upon the cover (especially when this is taken directly from the original box without cleaning) is like-

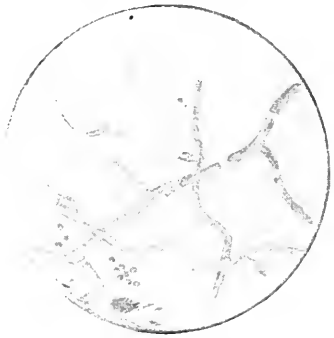


Fig. 62.—Aspergillus from urine (Boston).

wise a common source of confusion. Fibers of **muscle** (Figs. 61, *i*, and 111) and other particles which are evidently of fecal origin are usually the result of contamination, but may rarely be present in catheterized specimens. They then indicate rectovesical fistula.

IV. THE URINE IN DISEASE

In this section the characteristics of the urine in those diseases which produce distinctive urinary changes will be briefly reviewed.

1. **Renal Hyperemia.**—*Active hyperemia* is usually an early stage of acute nephritis, but may occur independ-

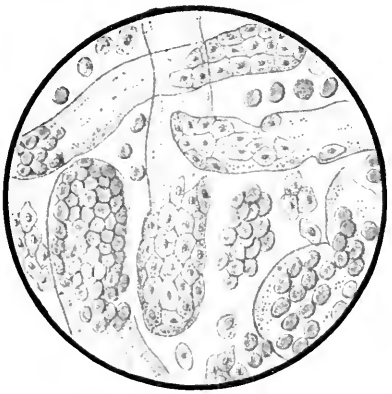


Fig. 63.—Sediment from acute hemorrhagic nephritis: Red blood-corpuscles; leukocytes; renal cells not fattily degenerated; epithelial and blood casts (Jakob).

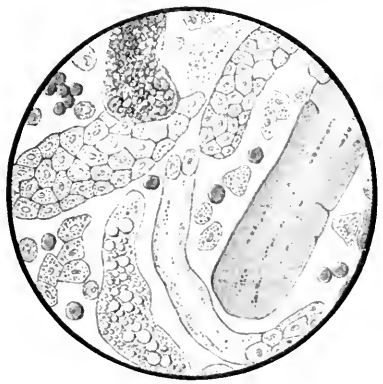


Fig. 64.—Sediment from chronic parenchymatous nephritis: Hyaline (with cells attached), waxy, brown granular, fatty, and epithelial casts; fattily degenerated renal cells, and a few white and red blood-corpuscles (Jakob).

ently as a result of temporary irritation. The urine is generally decreased in quantity, highly colored, and strongly acid. Albumin is always present—usually in

traces only, but sometimes in considerable amount for a day or two. The sediment contains a few hyaline and finely granular casts and an occasional red blood-cell. In very severe hyperemia the urine approaches that of acute nephritis.

Passive hyperemia occurs most commonly in diseases of the heart and in pregnancy. The quantity of urine is somewhat low and the color high, except in pregnancy. Albumin is present in small amount only. As the liver is usually deranged in these cases, small or moderate amounts of urobilin may be found. The sediment contains a very few hyaline or finely granular casts. In pregnancy the amount of albumin should be carefully watched, as any considerable quantity, and especially a rapid increase, strongly suggests approaching eclampsia.

2. **Nephritis.**—The various degenerative and inflammatory conditions grouped under the name of nephritis have certain features in common. The urine in all cases contains albumin and tube-casts, and in all well-marked cases shows a decrease of normal solids, especially of urea and the chlorids. In chronic nephritis, especially of the interstitial type, there may be remissions during which the urine is practically normal. The degree of functional derangement is probably best ascertained by the phenol-sulphonephthalein test (see p. 98). The characteristics of the different forms are well shown in the table on page 213, modified from Hill.

3. **Renal Tuberculosis.**—The urine is pale, usually cloudy. The quantity may not be affected, but is apt to be increased. In early cases the reaction is faintly acid and there are traces of albumin and a few renal cells.

THE URINE IN NEPHRITIS

	Physical.	Chemic.	Microscopic.
Acute nephritis.	Quantity diminished, often very greatly. Color dark; may be red or smoky. Specific gravity, 1.020 to 1.030.	Urea and chlorids low. Much albumin: up to 1.5 per cent. Reaction acid.	Sediment abundant, red or brown. Many casts, chiefly granular, blood and epithelial varieties. Red blood-cells abundant. Numerous renal epithelial cells and leukocytes.
Chronic parenchymatous nephritis. (Large white kidney.)	Quantity usually diminished. Color variable, often pale and hazy. Specific gravity, 1.010 to 1.020.	Urea and chlorids low. Largest amounts of albumin: up to 3 per cent. Reaction acid.	Sediment rather abundant. Many casts of all varieties: fatty casts and casts of degenerated epithelium most characteristic. Blood present in traces: abundant only in acute exacerbations. Numerous fattily degenerated renal epithelial cells, often free globules of fat, and a few leukocytes.
Chronic interstitial nephritis. (Contracted kidney.)	Quantity markedly increased, especially at night. Color pale, clear. Specific gravity, 1.005 to 1.015.	Urea and chlorids low in well-marked cases. Albumin present in traces (often overlooked), increasing in late stages. Reaction acid.	Sediment very slight. Few narrow hyaline and finely granular casts. No blood except in acute exacerbations. Very few renal cells. Uric acid and calcium-oxalate crystals common.
Amyloid degeneration of kidney.	Quantity moderately increased. Color pale, clear. Specific gravity, 1.012 to 1.018.	Slight decrease of urea and chlorids. Variable amounts of albumin and globulin.	Sediment slight. Moderate number of hyaline, finely granular, and sometimes waxy casts.

In advanced cases the urine is alkaline, has an offensive odor, and is irritating to the bladder. Albumin in varying amounts is always present. Pus is nearly always present, though frequently not abundant. It is generally intimately mixed with the urine, and does not settle so quickly as the pus of cystitis. Casts, though present, are rarely abundant, and are obscured by the pus. Small amounts of blood are common. Tubercle bacilli are

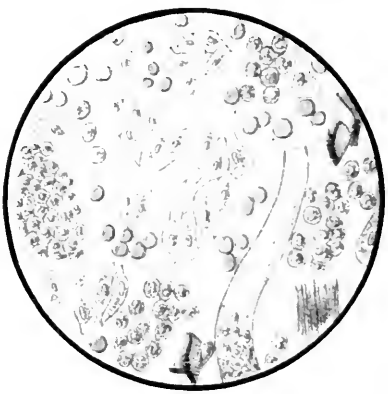


Fig. 65.—Sediment from calculous pyelitis: Numerous pus-corpuscles, red blood-corpuscles, and caudate and irregular epithelial cells; a combination of hyaline and pus casts, and a few uric-acid crystals (Jacob).

nearly always present, although animal inoculation may be necessary to detect them.

4. **Renal Calculus.**—The urine is usually somewhat concentrated, with high color and strongly acid reaction. Small amounts of albumin and a few casts may be present as a result of kidney irritation. Blood is frequently present, especially in the daytime and after severe exercise. Crystals of the substance composing the calculus—uric acid, calcium oxalate, cystin—may often

be found. The presence of a calculus generally produces pyelitis, and variable amounts of pus then appear, the urine remaining acid in reaction.

5. **Pyelitis.**—In pyelitis the urine is slightly acid, and contains a small or moderate amount of pus, together with many spindle and caudate epithelial cells. Pus-casts may appear if the process extends up into the kidney tubules (Fig. 65). Albumin is always present, and its amount, in proportion to the amount of pus, is decidedly greater than is found in cystitis. This fact is of much value in differential diagnosis. Even when pus is scanty, albumin is rarely under 0.15 per cent., which is the maximum amount found in cystitis with abundant pus.

6. **Cystitis.**—In *acute* and *subacute* cases the urine is acid and contains a variable amount of pus, with many epithelial cells from the bladder—chiefly large round, pyriform, and rounded squamous cells. Red blood-corpuscles are often numerous.

In *chronic* cases the urine is generally alkaline. It is pale and cloudy from the presence of pus, which is abundant and settles readily into a viscid sediment. The sediment usually contains abundant amorphous phosphates and crystals of triple phosphate and ammonium urate. Vesical epithelium is common. Numerous bacteria are always present (Fig. 66).

7. **Vesical Calculus, Tumors, and Tuberculosis.**—These conditions produce a chronic cystitis, with its characteristic urine. Blood, however, is more frequently present and more abundant than in ordinary cystitis. With neoplasms, especially, considerable hemorrhages are apt to occur. Particles of the tumor are sometimes

passed with the urine. No diagnosis can be made from the presence of isolated tumor cells. In tuberculosis tubercle bacilli can generally be detected.

8. **Diabetes Insipidus.**—Characteristic of this disease is the continued excretion of very large quantities of pale, watery urine, containing neither albumin nor sugar. The specific gravity varies between 1.001 and 1.005. The daily output of solids, especially urea, is increased.

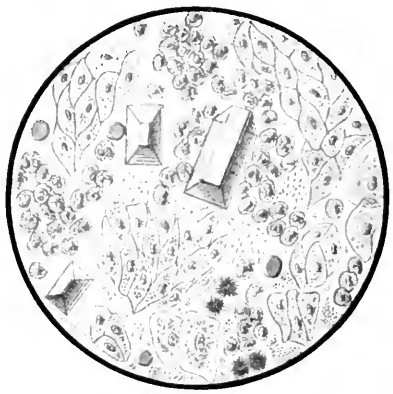


Fig. 66.—Sediment from cystitis (chronic): Numerous pus-corpuscles, epithelial cells from the bladder, and bacteria; a few red blood-corpuscles and triple phosphate and ammonium urate crystals (Jakob).

9. **Diabetes Mellitus.**—The quantity of urine is very large. The color is generally pale, while the specific gravity is nearly always high—1.030 to 1.050, very rarely below 1.020. Sometimes in mild or early cases the urine varies little from the normal in quantity, color, and specific gravity. The persistent presence of glucose is the essential feature of the disease. The amount of glucose may be small, but is often very great, sometimes exceeding 8 per cent., while the total elimination may

exceed 500 gm. in twenty-four hours. It may be absent temporarily. Acetone is generally present in advanced cases. Diacetic and oxybutyric acids may be present, and usually warrant an unfavorable prognosis. Accompanying the acidosis there is a corresponding increase in amount of ammonia.

CHAPTER III

THE BLOOD

Preliminary Considerations.—The blood consists of a fluid of complicated and variable composition, the plasma, in which are suspended great numbers of microscopic structures: viz., red corpuscles, white corpuscles, blood-platelets, and blood-dust.

Red corpuscles, or **erythrocytes,** appear as biconcave disks, red when viewed by reflected light or in thick layer, and straw colored when viewed by transmitted light or in thin layer. They give the blood its red color. They are cells which have been highly differentiated for the purpose of carrying oxygen from the lungs to the tissues. This is accomplished by means of an iron-bearing protein, hemoglobin, which they contain. In the lungs hemoglobin forms a loose combination with oxygen, which it readily gives up when it reaches the tissues. Normal erythrocytes do not contain nuclei. They are formed from preëxisting nucleated cells in the bone-marrow.

White corpuscles, or **leukocytes,** are less highly differentiated cells. There are several varieties. They all contain nuclei, and most of them contain granules which vary in size and staining properties. They are formed chiefly in the bone-marrow and lymphoid tissues.

Blood-platelets, or *blood-plaques*, are colorless or slightly bluish, spheric or ovoid bodies, usually about one-third or

one-half the diameter of an erythrocyte. Their structure, nature, and origin have not been definitely determined.

The **blood-dust of Müller** consists of fine granules which have vibratory motion. Little is known of them. It has been suggested that they are granules from disintegrated leukocytes.

The **total amount** of blood is usually given as one-thirteenth of the body weight, but more recent investigations indicate that it averages about one-twentieth.

The **reaction** is alkaline to litmus.

The **color** is due to the presence of hemoglobin in the red corpuscles, the difference between the bright red of arterial blood and the purplish red of venous blood depending upon the relative proportions of oxygen and carbon dioxid. The depth of color depends upon the amount of hemoglobin. In very severe anemias the blood may be so pale as to be designated as "watery." The formation of carbon-monoxid-hemoglobin in coal-gas-poisoning gives the blood a bright cherry-red color; while formation of methemoglobin in poisoning with potassium chlorate and certain other substances gives a chocolate color.

The clear, pale, straw-colored fluid which remains after coagulation (see p. 221) and separation of the clot is called **serum.** In the serum are found the numerous substances which the tissues elaborate for protection against bacterial and other harmful agents. In most cases these substances, or "antibodies," are elaborated only when the harmful agent is present in the body, and they are "specific," that is, they are effective only against the one disease which has called them forth. A test for the presence of the antibody is, therefore, a

test for the existence of the particular disease. The various tests based upon these principles have within recent years become a very important part of clinical laboratory work. They are discussed in the chapter upon Serodiagnostic Methods.

The **viscosity** of the blood has received considerable attention within recent years. It is evident that variations must markedly influence the load carried by the heart, but estimations of viscosity have so far proved of comparatively little clinical importance. Compared with distilled water, the normal viscosity is 4.5. It is reduced in primary and secondary anemia (roughly proportional to the grade of anemia), nephritis, cardiac

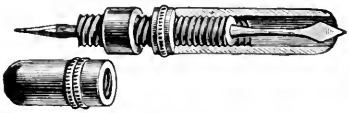


Fig. 67.—Daland's blood-lancet.

lesions with edema, and usually in leukemia and malaria. It is increased in polycythemia, diabetes mellitus, icterus, and usually in pneumonia. Measurement of viscosity is simple. The Hess instrument is one of the best. It is accompanied by directions for use.

Obtaining the Blood.— For most clinical examinations only one drop of blood is required. This may be obtained from the lobe of the ear, the palmar surface of the tip of the finger, or, in the case of infants, the plantar surface of the great toe. In general, the finger will be found most convenient. With nervous children the lobe of the ear is preferable, as it is less sensitive and its situation prevents their seeing what is being done. An edematous or congested part should be avoided; also a cold, appar-

ently bloodless one. The site should be well rubbed with alcohol to remove dirt and epithelial débris and to increase the amount of blood in the part. After allowing sufficient time for the circulation to equalize, the skin is punctured with a blood-lancet (of which there are several patterns upon the market) or some substitute, as a Hagedorn needle, aspirating needle, trocar, a spicule of glass, or a pen with one of its nibs broken off. Nothing is more unsatisfactory than an ordinary round sewing-needle. The lancet should be cleaned with alcohol before and after using, but need not be sterilized. It must be very sharp. If the puncture be made with a *firm, quick, rebounding stroke*, at not too great a distance, it is practically painless. The first drop of blood which appears should be wiped away, and the second used for examination. The blood should not be pressed out, since this dilutes it with serum from the tissues; but moderate pressure some distance above the puncture is allowable.

When a larger amount of blood is required, it may be obtained with a sterile hypodermic syringe from one of the veins at the elbow, as described on p. 293.

Clinical study of the blood may be discussed under the following heads: I. Coagulation. II. Hemoglobin. III. Enumeration of erythrocytes. IV. Color index. V. Volume index. VI. Enumeration of leukocytes. VII. Enumeration of plaques. VIII. Study of stained blood. IX. Blood parasites. X. Tests for recognition of blood. XI. Special blood pathology.

I. COAGULATION

Coagulation consists essentially in the transformation of fibrinogen, one of the proteins of the blood-plasma,

into fibrin by means of a ferment called thrombin. The presence of calcium salts is necessary. The exact nature of the process is still undetermined. The resulting coagulum is made up of a meshwork of fibrin fibrils with entangled corpuscles and platelets. The clear, straw-colored fluid which is left after separation of the coagulum is called *blood-serum*. Normally, coagulation takes place in two to eight minutes after the blood leaves the vessels. The time is affected by the temperature, the size of the drop, cleanliness of the instruments, and other factors. Pathologically, it is delayed in hemophilia, purpura, scurvy, and icterus. In treatment, calcium salts, especially the lactate and acetate, are used to hasten coagulation; citric acid, to retard it.

Fig. 68.—Showing difference in shape of blood-drops before and after coagulation (Duke's method).

For certain purposes, notably in bacteriologic and opsonic work, it is desirable to prevent coagulation of blood which has been withdrawn. This may be accomplished by receiving it directly into a solution of 1 per cent. sodium citrate (or ammonium oxalate) in normal salt solution. This precipitates the calcium salts which are necessary to coagulation.

The simplest method of ascertaining the coagulation time is to receive several drops of blood (well rounded drops 4 to 5 mm. in diameter) on a clean slide and to draw a needle through one or another of them at one-minute intervals. When the clot is dragged along by the needle, coagulation has taken place. Duke uses a glass

slide to which two glass disks 5 mm. in diameter are cemented. Well-rounded drops of blood are received on the disks and the slide is inverted across the top of a glass or beaker containing water at 40° C. and covered with a towel. Coagulation is judged by the shape of the drop when the slide is held in a vertical position (Fig. 68).

For more accurate work the method of Russell and Brodie as modified by Boggs is now generally used.

Boggs' Method.—The instrument is shown in Fig. 69. The bottom of the box (*A*) and the cone (*B*) are of glass.

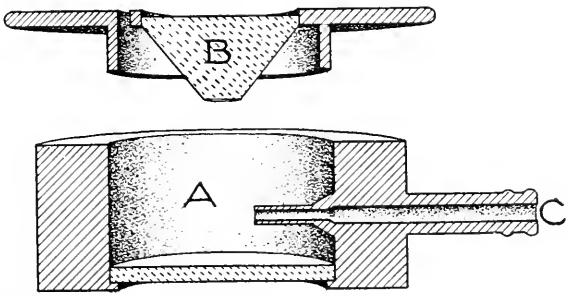


Fig. 69.—Boggs' coagulation instrument: A, moist chamber; B, glass cone; C, tube through which air is blown.

The instrument must be absolutely clean. Obtain the blood from a freely flowing puncture. When a large drop has formed, touch the small end of the cone to its surface. Quickly invert the cone into the box. Place the instrument on the microscope and blow puffs of air against the drop of blood at intervals by means of a rubber bulb attached to *C*, meanwhile watching the motion of the corpuscles with a low power of the microscope. Coagulation has occurred when

the corpuscles move *en masse* in a radial direction and spring back to their original position (Fig. 70, *D*). The time is

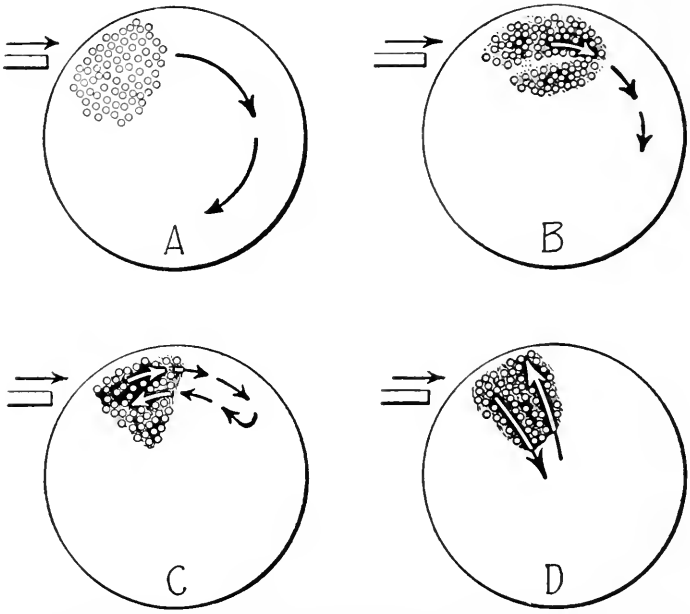


Fig. 70.—Diagram showing the direction taken by red corpuscles in Boggs' method for coagulation time: Radial movement of the corpuscles, *D*, indicates the end-point (after Boggs).

counted from the first appearance of the blood from the puncture to the end-point.

II. HEMOGLOBIN

Hemoglobin is an iron-bearing protein. It makes up about 14 per cent. of the total weight of the blood. Normally, it is found only within the red corpuscles. When it is dissolved out of these cells and appears in the plasma, the condition is known as hemoglobinemia.

The actual amount of hemoglobin is never estimated clinically: it is the relation which the amount present bears to the normal which is determined. Thus the expression, "50 per cent. hemoglobin," when used clinically, means that the blood contains 50 per cent. of the normal. Theoretically, the normal would be 100 per cent., but with the methods of estimation in general use the blood of healthy persons ranges from 85 to 105 per cent.; these figures may, therefore, be taken as normal.

Increase of hemoglobin, or *hyperchromemia*, is uncommon, and is probably more apparent than real. It accompanies an increase in number of erythrocytes, and may be noted in change of residence from a lower to a higher altitude; in poorly compensated heart disease with cyanosis; in concentration of the blood from any cause, as the severe diarrhea of cholera, and in "idiopathic polycythemia."

Decrease of hemoglobin, or *oligochromemia*, is very common and important. It is the distinctive and most striking feature of the anemias (see p. 309). In secondary anemia the hemoglobin loss may be slight or very great. In mild cases a slight decrease of hemoglobin is the only blood change noted. In very severe cases, especially in repeated hemorrhages, malignant disease, and infection by the hookworm and *Dibothriocephalus latus*, hemoglobin may fall to 15 per cent. Hemoglobin is always diminished, and usually very greatly, in chlorosis (average about 40 to 45 per cent.), pernicious anemia (average about 20 to 25 per cent.), and leukemia (usually about 40 to 50 per cent.).

Estimation of hemoglobin is less tedious and usually more helpful than a red corpuscle count. It offers

the simplest and most certain means of detecting the existence and degree of anemia, and of judging the effect of treatment in anemic conditions. Pallor, observed clinically, does not always denote anemia.

There are many methods, but none is entirely satisfactory. Those which are most widely used are here described:

(1) **Von Fleischl Method.**—The apparatus consists of a stand somewhat like the base and stage of a microscope

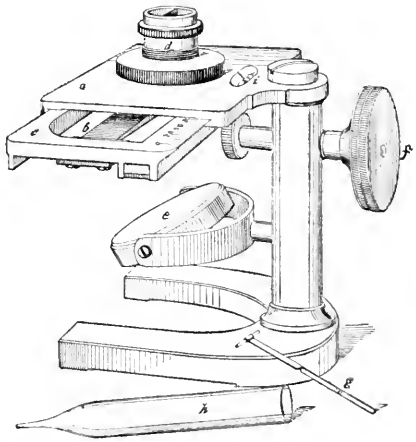


Fig. 71.—Von Fleischl's hemoglobinometer: *a*, Stand; *b*, narrow wedge-shaped piece of colored glass fitted into a frame (*c*), which passes under the chamber; *d*, hollow metal cylinder, divided into two compartments, which holds the blood and water; *e*, plaster-of-Paris plate from which the light is reflected through the chamber; *f*, screw by which the frame containing the graduated colored glass is moved; *g*, capillary tube to collect the blood; *h*, pipet for adding the water; *i*, opening through which may be seen the scale indicating percentage of hemoglobin.

(Fig. 71). Under the stage is a movable bar of colored glass, shading from pale pink at one end to deep red at the other. The frame in which this bar is held is marked with a scale of

hemoglobin percentages corresponding to the different shades of red. By means of a rack and pinion the color-bar can be moved from end to end beneath a round opening in the center of the stage. A small metal cylinder, which has a glass bottom and which is divided vertically into two equal compartments, can be placed over the opening in the stage so that one of its compartments lies directly over the color-bar. Accompanying the instrument are a number of short capillary tubes in metal handles.

Having punctured the finger-tip or lobe of the ear, as already described, wipe off the first drop of blood, and from the second fill one of the capillary tubes. Hold the tube horizontally, and touch its tip to the drop of blood, which will readily flow into it if it be clean and dry. Avoid getting any blood upon its outer surface. With a medicine-dropper rinse the blood from the tube into one of the compartments of the cylinder, using distilled water, and mix well. Fill both compartments level full with distilled water, and place the cylinder over the opening in the stage, so that the compartment which contains only water lies directly over the bar of colored glass. If there are any clots in the hemoglobin compartment, clean the instrument and begin again.

In a dark room, with the light from a candle reflected up through the cylinder, move the color-bar along with a jerking motion until both compartments have the same depth of color. The number upon the scale corresponding to the portion of the color-bar which is now under the cylinder gives the percentage of hemoglobin. While comparing the two colors, place the instrument so that they will fall upon the right and left halves of the retina, rather than upon the upper and lower halves; and protect the eye from the light with a cylinder of paper or pasteboard. After use, clean the metal cylinder with water, and wash the capillary tube with water, alcohol, and ether, successively. Results with this instrument are accurate to within about 5 per cent.

(2) The **Fleischl-Miescher** instrument, a modification of the preceding, is generally considered the most accurate hemoglobinometer available. It is, however, better adapted to laboratory use than to the needs of the clinician. Detailed instructions accompany each instrument. The chief differences from the von Fleischl are: (1) The blood is more accurately measured and diluted, a pipet like that accompanying the hemocytometer being used; (2) 0.1 per cent. solution of sodium carbonate is used instead of water for diluting; (3) the glass bar is more accurately colored; (4) there are two cylindric cells, one four-fifths the depth of the other; and (5) the cell is covered with a glass disk and a metal cap with a slit through which the reading is made.

(3) The **Sahli hemoglobinometer** (Fig. 72) is an improved form of the well-known Gowers instrument. It consists of a hermetically sealed comparison tube containing a suspension of acid hematin, a graduated test-tube of the same diameter, and a pipet of 20-c.mm. capacity. The two tubes are held in a black frame with a white ground-glass back.

Place decinormal hydrochloric acid solution in the graduated tube to the mark 10. Obtain a drop of blood and draw it into the pipet to the 20 c.mm. mark. Wipe off the tip of the pipet, blow its contents into the hydrochloric acid solution in the tube, and rinse well. The hemoglobin is changed to acid hematin. Place the two tubes in the compartments of the frame; let stand one minute; and dilute the fluid with water drop by drop, mixing after each addition, until it has exactly the same color as the comparison tube. The graduation corresponding to the surface of the fluid then indicates the percentage of hemoglobin. Mixing may be done by closing the tube with the finger and inverting, but care should be exercised to see that none of the fluid is removed by adhering to the finger. Decinormal hydrochloric acid solution may be prepared with sufficient accuracy for this purpose by adding

15 c.c. of the concentrated acid to 985 c.c. distilled water. A little chloroform should be added as a preservative.

This method is very satisfactory in practice, and is accurate to within 5 per cent. Unfortunately, not all the instruments upon the market are well standardized, and the comparison

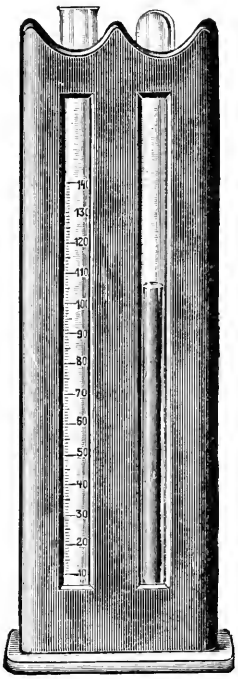


Fig. 72.—Sahli's hemoglobinometer.

tube does not keep its color unchanged indefinitely. Usually, however, the apparent fading is due to the fact that the hematin is in suspension and may settle out if the instrument lies unused for some time. This can be remedied by inverting the tube a number of times. Most tubes contain a glass bead to facilitate mixing.

(4) **Dare's hemoglobinometer** (Fig. 73) differs from the others in using undiluted blood. The blood is allowed to flow by capillarity into the slit between two small plates of glass. It is then placed in the instrument and compared with different portions of a circular disk of colored glass. The reading must be made quickly, before clotting takes place. This instrument is easy to use, and is one of the most accurate.

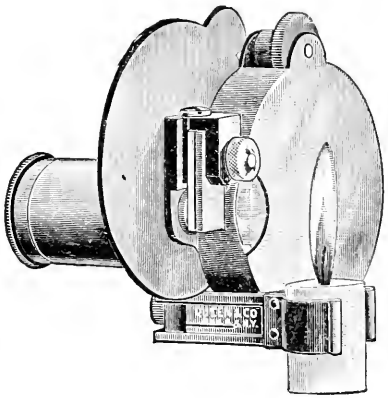


Fig. 73.—Dare's hemoglobinometer.

(5) **Hammerschlag Method.**—This is an indirect method which depends upon the fact that the percentage of hemoglobin varies directly with the specific gravity of the blood. It yields fairly accurate results except in leukemia, where the large number of leukocytes disturbs the relation, and in dropsical conditions.

Mix chloroform and benzol in a urinometer tube, so that the specific gravity of the mixture is near the probable specific gravity of the blood. Add a drop of blood by means of a pipet of small caliber. A pipet like that shown in Fig. 169, A, will be found satisfactory. If the drop floats near the surface, add a little benzol; if it sinks to the bottom, add a little chloroform. When it remains stationary near the middle, the mix-

ture has the same specific gravity as the blood. Take the specific gravity with a urinometer, and obtain the corresponding percentage of hemoglobin from the following table:

Specific Gravity.	Hemoglobin Per Cent.	Specific Gravity.	Hemoglobin Per Cent.
1.033–1.035	25–30	1.048–1.050	55–65
1.035–1.038	30–35	1.050–1.053	65–70
1.038–1.040	35–40	1.053–1.055	70–75
1.040–1.045	40–45	1.055–1.057	75–85
1.045–1.048	45–55	1.057–1.060	85–95

For accurate results with this method, care and patience are demanded. The following precautions must be observed:

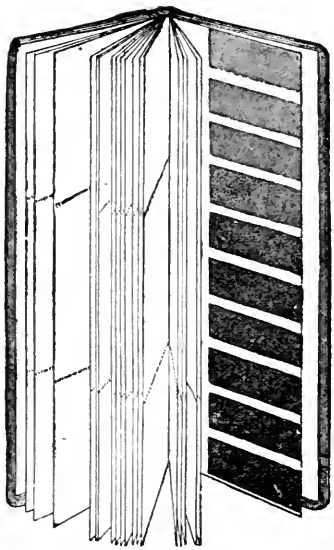


Fig 74.—Tallqvist's hemoglobin scale.

(*a*) The two fluids must be well mixed after each addition of chloroform or benzol. Close the tube with the thumb and invert several times. Should this cause the drop of blood to

break up into very small ones, adjust the specific gravity as accurately as possible with these, and test it with a fresh drop.

(*b*) The drop of blood must not be too large; it must not contain an air-bubble, it must not adhere to the side of the tube, and it must not remain long in the fluid.

(*c*) The urinometer must be standardized for the chloroform-benzol mixture. Most urinometers give a reading two or three degrees too high, owing to the low surface tension. Make a mixture such that a drop of distilled water will remain suspended in it (*i. e.*, with a specific gravity of 1.000) and correct the urinometer by this.

(6) **Tallqvist Method.**—The popular Tallqvist hemoglobinometer consists simply of a book of small sheets of absorbent paper and a carefully printed scale of colors (Fig. 74).

Take up a large drop of blood with the absorbent paper, and when the humid gloss is leaving, before the air has darkened the hemogobin, compare the stain with the color scale. The color which it matches gives the percentage of hemoglobin. Except in practised hands, this method is accurate only to within 10 or 20 per cent.

Of the methods given, the physician should select the one which best meets his needs. With any method, practice is essential to accuracy. The von Fleischl was for many years the standard instrument, but is now little used. For accurate work the best instruments are the von Fleischl-Miescher and the Dare. The former is essentially a laboratory instrument. The Dare is easy to use and to clean, and is probably the best for clinical work. The Sahli, although less easy to use and probably less accurate, is inexpensive and is very satisfactory, provided a well-standardized color-tube is obtained. The specific gravity method is useful when special instruments are not at hand. The Tallqvist scale is so

inexpensive and so convenient that it should be used by every physician at the bedside and in hurried office work; but it should not supersede the more accurate methods.

III. ENUMERATION OF ERYTHROCYTES

In health there are about 5,000,000 red corpuscles per cubic millimeter of blood. Normal variations are slight. The number is generally a little less—about 4,500,000—in women. Hawk finds the normal for athletes in training to be 5,500,000.

Increase of red corpuscles, or *polycythemia*, is unimportant. There is a decided increase following change of residence from a lower to a higher altitude, averaging about 50,000 corpuscles for each 1000 feet, but frequently much greater. The increase, however, is not permanent. In a few months the erythrocytes return to nearly their original number. Three views are offered in explanation: (*a*) Concentration of the blood, owing to increased evaporation from the skin; (*b*) stagnation of corpuscles in the peripheral vessels because of lowered blood-pressure; (*c*) new formation of corpuscles, this giving a compensatory increase of aëration surface.

Pathologically, polycythemia is uncommon. It may occur in: (*a*) Concentration of the blood from severe watery diarrhea; (*b*) chronic heart disease, especially the congenital variety, with poor compensation and cyanosis; and (*c*) idiopathic polycythemia, which is considered to be an independent disease, and is characterized by cyanosis, blood-counts of 7,000,000 to 10,000,000, hemoglobin 120 to 150 per cent., and a normal number of leukocytes.

Decrease of red corpuscles, or *oligocythemia*. Red

corpuscles and hemoglobin are commonly decreased together, although usually not to the same extent.

Oligocythemia occurs in all but the mildest symptomatic anemias. The blood-count varies from near the normal in moderate cases down to 1,500,000 in very severe cases. There is always a decrease of red cells in chlorosis, but it is often slight, and is relatively less than the decrease of hemoglobin. Leukemia gives a decided

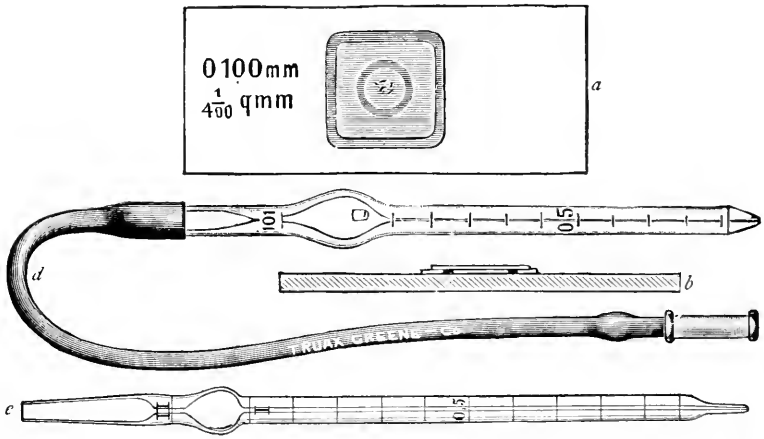


Fig. 75.—Thoma-Zeiss hemacytometer: *a*, Slide used in counting; *b*, sectional view; *d*, red pipet; *e*, white pipet.

oligocythemia, the average count being about 3,000,000. The greatest loss of red cells occurs in pernicious anemia, where counts below 1,000,000 are not uncommon.

Method of Counting.—The most widely used instrument for counting the corpuscles is that of Thoma-Zeiss. Bürker's hemacytometer is more accurate and has been very favorably received. The new Thoma-Metz instrument is convenient for routine working. The hematocrit is not to be recommended for accuracy, since in anemia,

where blood-counts are most important, the red cells vary greatly in size and probably also in elasticity. The hematocrit is, however, useful in determining the relative volume of corpuscles and plasma (see Volume Index, p. 245).

The **Thoma-Zeiss instrument** consists of two pipets for diluting the blood and a counting chamber (Fig. 75). The

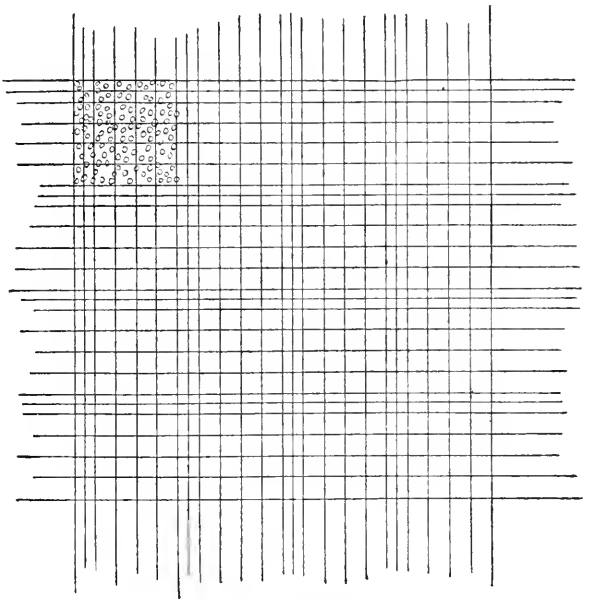


Fig. 76.—Thoma ruling of counting chamber, showing red corpuscles in left upper corner.

rubber tubes which come with the pipets are too short and too flexible and should be replaced. For this purpose nothing is so good as a rubber catheter. The counting chamber is a glass slide with a square platform in the middle. In the center of the platform is a circular opening, in which is set

a small circular disk in such a manner that it is surrounded by a "ditch," and that its surface is exactly one-tenth of a millimeter below the surface of the square platform. Upon this disk is ruled a square millimeter, subdivided into 400 small squares. Each fifth row of small squares has double rulings for convenience in counting (Fig. 76). This ruling, known as the Thoma, constitutes the central square millimeter of most of the more recent forms, such as the Zappert

Fig 77.—Method of drawing blood into the pipet (Boston).

and the Türck (see Fig. 82). A thick cover-glass, ground perfectly plane, accompanies the counting chamber. Ordinary cover-glasses are of uneven surface, and should not be used with this instrument.

It is evident that, when the cover-glass is in place upon the platform, there is a space exactly one-tenth of a millimeter thick between it and the disk; and that, therefore, the square millimeter ruled upon the disk forms the base of a space holding exactly one-tenth of a cubic millimeter.

Technic.—To count the red corpuscles, use the pipet with 101 engraved above the bulb. It must be clean and dry. Puncture the skin, wipe off the first drop of blood, and fill the pipet from the second, sucking the blood to the mark 0.5 or 1.0, according to the dilution desired. While doing this, hold the pipet at nearly right angles to the line of vision, so that the exact height of the column may be easily seen. The side of the tip should rest against the skin, but the end must be free. Air-bubbles will enter if the drop is too small or if the tip is not kept immersed. Should the blood go slightly beyond the mark, draw it back by touching the tip of the pipet to a moistened handkerchief. Quickly wipe off the blood adhering to the tip, plunge it into the diluting fluid, and suck the fluid up to the mark 101, slightly rotating the pipet meanwhile. This dilutes the blood 1:200 or 1:100, according to the amount of blood taken. Except in cases of severe anemia, a dilution of 1:200 is preferable. Close the ends of the pipet with the fingers, and shake vigorously until the blood and diluting fluid are well mixed, keeping the pipet horizontal meanwhile.

When it is not convenient to count the corpuscles at once, place a heavy rubber band around the pipet so as to close the ends, inserting a small piece of rubber-cloth or other tough, non-absorbent material, if necessary, to prevent the tip from punching through the rubber. It may be kept thus for twenty-four hours or longer.

When ready to make the count, clean the counting chamber and cover-glass, and place a sheet of paper over them to keep off dust. Mix the fluid thoroughly by shaking; blow 2 or 3 drops from the pipet, wipe off its tip, and then place a small drop (the proper size can be learned only by experience) upon the disk of the counting chamber. Adjust the cover immediately. Hold it by diagonal corners above the drop of fluid so that a third corner touches the slide and rests upon the edge of the platform. Place a finger upon this cor-

ner, and, by raising the finger, allow the cover to fall quickly into place. If the cover be properly adjusted, faint concentric lines of the prismatic colors—Newton's rings—can be seen between it and the platform when the slide is viewed obliquely. They indicate that the two surfaces are in close apposition. If they do not appear at once, slight pressure upon the cover may bring them out. Failure to obtain them is usually due to dirty slide or cover—both must be perfectly clean and *free from dust.* The drop placed upon the disk must be of such size that, when the cover is adjusted, it nearly or quite covers the disk, and that none of it runs over into the "ditch." There should be no bubbles upon the ruled area.

The following is a somewhat easier method of applying the cover: Place a drop of fluid upon the ruled disk. The size of the drop is of no great consequence, if only it be large enough. Place the cover-glass flat upon one side of the platform with its edge close to the drop of fluid, and hold it firmly down with the two index-fingers, or with the index-finger and middle fingers of the right hand. Now slide it firmly and quickly into place. If the drop of fluid is too large, the excess will be caught on the top of the cover. A moderately thin cover is best.

Allow the corpuscles to settle for a few minutes, and then examine with a low power to see that they are evenly distributed. If they are not *evenly distributed over the whole disk*, the counting chamber must be cleaned and a new drop placed in it.

Probably the most satisfactory objective for counting is the 8 mm. or the 4 mm. with long working distance. To understand the principle of counting, it is necessary to remember that the square millimeter (400 small squares) represents a capacity of one-tenth of a cubic millimeter. Find the number of corpuscles in the square millimeter, multiply by 10 to find the number in 1 c.mm. of the diluted blood, and finally,

by the dilution, to find the number in 1 c.mm. of undiluted blood. Instead of actually counting all the corpuscles, it is customary to count those in only a limited number of small squares, and from this to calculate the number in the square millimeter. Nearly every worker has his own method of doing this. The essential thing is to adopt a method and adhere to it.

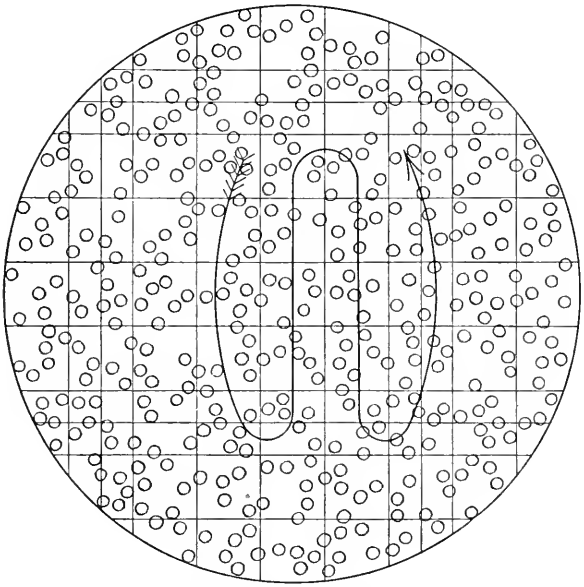


Fig. 78.—Appearance of microscopic field in counting red corpuscles. The arrow indicates the squares to be counted.

In practice a convenient procedure is as follows: *With a dilution of* 1 : 200, *count the cells in* 80 *small squares, and to the sum add* 4 *ciphers; with dilution of* 1 : 100, *count* 40 *small squares and add* 4 *ciphers.* Thus, if with 1 : 200 dilution, 450 corpuscles were counted in 80 squares, the total count would be 4,500,000 per c.mm. This method is sufficiently accurate

for all clinical purposes, provided the corpuscles are evenly distributed and 3 drops from the pipet be counted. It is convenient to count a block of 20 small squares, as indicated in Fig. 78, in each corner of the large square. Four columns of 5 squares each are counted. The double rulings show when the bottom of a column has been reached and also indicate the fourth column. In the writer's opinion it is easier to count in vertical than horizontal rows. If distribution be even, the difference between the number of cells in any two such blocks should not exceed twenty. In order to avoid confusion in counting cells which lie upon the border-lines, the following rule is generally adopted: *Corpuscles which touch the upper and left sides should be counted as if within the squares, those touching the lower and right sides, as outside; and vice versâ.*

Diluting Fluids.—The most widely used are Hayem's and Toisson's. Both of these have high specific gravities, so that, when well mixed, the corpuscles do not separate quickly. Toisson's fluid is probably the better for beginners, because it is colored and can easily be seen as it is drawn into the pipet. It stains the nuclei of leukocytes blue, but this is no real advantage. It must be filtered frequently because of the ready growth of fungi in it.

Hayem's Fluid.	
Mercuric chlorid	0.5
Sodium sulphate	5.0
Sodium chlorid	1.0
Distilled water	200.0

Toisson's Fluid.	
Sodium chlorid	1.0
Sodium sulphate	8.0
Glycerin	30.0
Distilled water	160.0
Methyl-violet, 5 B to give a strong purple color.	

Sources of Error.—The most common sources of error in making a blood-count are:

(*a*) Inaccurate dilution, usually from faulty technic, occasionally from inaccurately graduated pipets. Only an instrument of standard make can be relied upon.

(*b*) Too slow manipulation, allowing a little of the blood to coagulate and remain in the capillary portion of the pipet.

(*c*) Inaccuracy in depth of counting chamber usually due to imperfect application of the cover-glass, but sometimes to softening of the cement by alcohol or heat. The slide should not be cleaned with alcohol nor left to lie in the warm sunshine.

(*d*) Uneven distribution of the corpuscles. This results when the blood has partially coagulated, when it is not thoroughly mixed with the diluting fluid, or when the cover-glass is not applied soon enough after the drop is placed upon the disk.

(*e*) The presence of yeasts, which may be mistaken for corpuscles, in the diluting fluid

Cleaning the Instrument.—The instrument should be cleaned immediately after using, and the counting chamber and cover must be cleaned again just before use.

Transfer the rubber tube to the small end of the pipet and draw through it, successively, water, alcohol, ether, and air. This can be done with the mouth, but it is much better to use a rubber bulb or suction filter pump. When the mouth is used, the moisture of the breath will condense upon the interior of the pipet unless the fluids be shaken and not blown out. If blood has coagulated in the pipet—which happens when the work is done too slowly—dislodge the clot with a horsehair, never with a wire, and clean with strong sulphuric acid, or let the pipet stand over night in a test-tube of the acid. Even if the pipet does not become clogged, it should be occasionally cleaned in this way. When the etched graduations on the pipets become dim, they can be renewed by rubbing with a grease pencil.

Wash the counting-chamber and the cover with water and dry them with clean soft linen. Alcohol may be used to clean the latter, but never the former.

Bürker's hemacytometer (Fig. 79).—This modification of the Thoma-Zeiss instrument allows of greater accuracy. It consists of a counting slide with cover-glass, three pipets —(*a*) for measuring blood, (*b*) measuring diluting fluid, and (*c*) transferring the diluted blood to the slide—and one or more small flasks for mixing blood and diluting fluid.

The floor-piece of the counting slide, instead of being circular, as in the Thoma-Zeiss instrument, consists of a plate

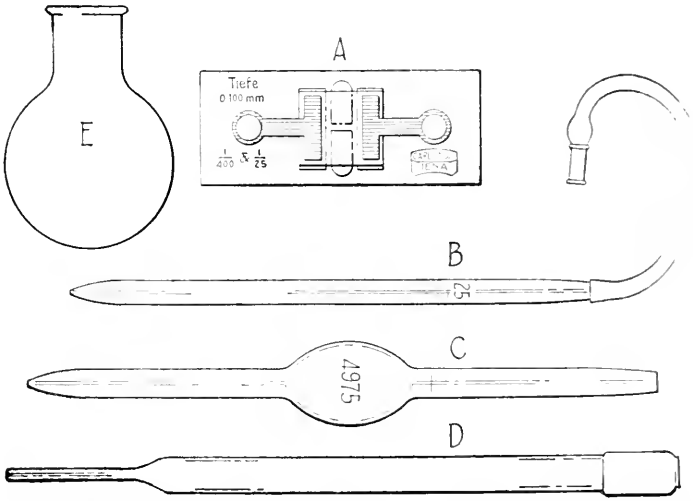


Fig. 79.—Bürker's hemacytometer with pipets for counting red-corpuscles: *A*, counting slide with cover-glass in place; *B*, pipet for measuring blood; *C*, pipet for measuring diluting fluid; *D*, pipet for transferring diluted blood to slide; *E*, mixing flask.

of glass 5 mm. wide and 25 mm. long, which extends across the slide. This is divided across the middle by a deep groove 1.5 mm. wide, and upon each portion is a ruled area. On each side of the floor-piece and separated from it by a ditch is a glass platform 0.1 mm. higher than the ruled areas. When the cover-glass is adjusted upon the platform, the ends of the floor-piece project beyond it. There are two clamps to hold the cover in place.

When the count is to be made, the correct amount of Hayem's fluid is placed in the mixing flask, and the blood, accurately measured in the blood-pipet, is added and well mixed, care being taken that none of the fluid touches the stopper of the flask. The cover-glass is then carefully adjusted so as to show Newton's rings, and is clamped in place. A drop of the diluted blood is placed on each of the projecting ends of the floor-piece by means of the special pipet. The fluid will run under the cover by capillary attraction. The slide is now placed on the microscope with the diaphragm wide open and viewed obliquely with the unaided eye. If the film of corpuscles is not uniform, the slide must be cleaned and filled again. The count is made in the usual way. As

Fig. 80.—Thoma-Metz hemacytometer and diagram showing ruling in ocular.

ordinarily supplied the instrument has a special ruling, but other rulings may be obtained.

The Bürker counting chamber can now be obtained with the regular Thoma mixing pipets.

Thoma-Metz Hemacytometer (Fig. 80).—This new instrument introduces certain conveniences into the routine counting of both red cells and leukocytes. Its special feature is that the ruling is engraved upon a disk in the ocular instead of upon the counting slide. This disk is ruled with a large circle and with a square, which in turn is subdivided into four smaller squares.

For the red count the squares are used. The four small squares have each the same value as the small squares of the

Thoma ruling ($\frac{1}{400}$ sq. mm.), and the count may be conducted as already described.

The circle is used for counting leukocytes. Its area corresponds to one-tenth of a square millimeter when the correct magnification is used. The leukocytes are counted as in the circle method described on p. 256.

A decided advantage of this instrument is that the ruled lines are always sharp and clear. The eye-lens of the ocular can be focused to suit different eyes. The chief disadvantage and a source of inaccuracy lies in the fact that the values of the ruled areas vary according to magnification. The makers say that values are correct as above given when the Leitz No. 6 objective (4-mm. focus) is used with tube length of 170 mm. Slight variations with other objectives can be compensated by altering the tube length. For accurate evaluation a square is ruled on the counting slide, and the tube length should be so adjusted that this square exactly coincides with the large square in the ocular.

IV. COLOR INDEX

This is an expression which indicates the amount of hemoglobin in each red corpuscle compared with the normal amount. For example, a color index of 1.0 indicates that each corpuscle contains the normal amount of hemoglobin; of 0.5, that each contains one-half the normal.

The color index is most significant in chlorosis and pernicious anemia. In the former it is usually much decreased; in the latter, generally much increased. In symptomatic anemia it is generally moderately diminished.

To obtain the color index, divide the percentage of hemoglobin by the percentage of corpuscles. The percentage of

corpuscles is found by multiplying the first two figures of the red corpuscle count by 2. This simple method holds good for all counts of 1,000,000 or more. Thus, a count of 2,500,000 is 50 per cent. of the normal. If, then, the hemoglobin has been estimated at 40 per cent., divide 40 (the percentage of hemoglobin) by 50 (the percentage of corpuscles). This gives $\frac{4}{5}$, or 0.8, as the color index.

V. VOLUME INDEX

The term "volume index" was introduced by Capps to express the average size of the red cells of an individual compared with their normal size. It is the quotient obtained by dividing the *volume* of red corpuscles (expressed in percentage of the normal) by the *number* of red corpuscles, also expressed in percentage of the normal.

The volume index more or less closely parallels the color index, and variations have much the same significance. The following are averages of the examinations reported by Larrabee in the "Journal of Medical Research":

	Red corpuscles per cubic millimeter.	Hemoglobin per cent. by Sahli instrument.	Color index.	Volume index.
Normal males	5,267,250	103.0	0.98	1.007
Normal females	4,968,667	106.0	1.06	1.001
Primary pernicious anemia	1,712,166	50.0	1.47	1.270
Secondary anemia	3,737,160	61.0	0.81	0.790
Chlorosis	3,205,000	34.5	0.55	0.695

Method.—The red cells are counted and the percentage of red cells calculated as for the color index.

The volume percentage is obtained with the hematocrit as follows: Fill the hematocrit tubes (Fig. 81) with blood, and before coagulation takes place insert them in the frame and centrifugalize for three minutes at about 8000 to 10,000

revolutions a minute. The red cells collect at the bottom and, normally, make up one-half of the total column of blood. Multiply the height of the layer of red cells (as indicated by the graduations upon the side of the tube) by 2 to obtain the volume percentage. When the examination cannot be made immediately after the blood is obtained, the method of Larrabee is available. This consists in mixing a trace of sodium oxalate with a few drops of blood to prevent coagulation, drawing this mixture into a heavy-walled tube of about 2-mm. caliber, closing the ends with a rubber band, and waiting until sedimentation is complete—usually about three days. The height of the column is then measured with a

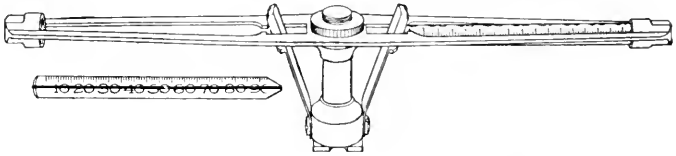


Fig. 81.—Daland hematocrit for use with the centrifuge.

millimeter scale and the percentage relation to the normal calculated.

After the volume of the red cells and the red corpuscle count are thus expressed in percentages, divide the former by the latter to find the volume index. Example: Suppose the volume percentage is 80 (the reds reaching to mark 40 on hematocrit tube) and that the red count is 50 per cent. of the normal (2,500,000 per c.mm.), then $\frac{80}{50}$, or 1.6, is the volume index.

VI. ENUMERATION OF LEUKOCYTES

The normal number of leukocytes varies from 5000 to 10,000 per cubic millimeter of blood. The number is larger in robust individuals than in poorly nourished

ones, and, if disease be excluded, may be taken as a rough index of the individual's nutrition. Since it is well to have a definite standard, 7500 is generally adopted as the normal for the adult. With children the number is somewhat greater, counts of 12,000 and 15,000 being common in healthy children under twelve years of age.

Decrease in Number of Leukocytes

Decrease in number of leukocytes, or *leukopenia*, is not important. It is common in persons who are poorly nourished, although not actually sick. The infectious diseases in which leukocytosis is absent (see p. 251) often cause a slight decrease of leukocytes. Chlorosis may produce leukopenia, as also pernicious anemia, which usually gives it in contrast to the secondary anemias, which are frequently accompanied by leukocytosis. Leukocyte counts are, therefore, of some aid in the differential diagnosis of these conditions.

Increase in Number of Leukocytes

Increase in number of leukocytes is common and of great importance. It may be considered under two heads:

A. Increase of leukocytes due to chemotaxis and stimulation of the blood-making organs, or *leukocytosis*. The increase affects one or more of the normal varieties.

B. Increase of leukocytes due to *leukemia*. Normal varieties are increased, but the characteristic feature is the appearance of great numbers of abnormal cells.

The former may be classed as a *transient*, the latter, as a *permanent*, increase.

A. Leukocytosis

This term is variously used. By some it is applied to any increase in number of leukocytes; by others it is restricted to increase of the polymorphonuclear neutrophilic variety. As has been indicated, it is here taken to mean a transient increase in number of leukocytes, that is, one caused by chemotaxis and stimulation of the blood-producing structures, in contrast to the permanent increase caused by leukemia.

By *chemotaxis* is meant that property of certain agents by which they attract or repel living cells—positive chemotaxis and negative chemotaxis respectively. An excellent illustration is the accumulation of leukocytes at the site of inflammation, owing to the positively chemotactic influence of bacteria and their products. A great many agents possess the power of attracting leukocytes into the general circulation. Among these are many bacteria and certain organic and inorganic poisons.

Chemotaxis alone will not explain the continuance of leukocytosis for more than a short time. It is probable that substances which are postitively chemotactic also stimulate the blood-producing organs to increased formation of leukocytes; and in at least one form of leukocytosis such stimulation apparently plays the chief part.

As will be seen later, there are several varieties of leukocytes in normal blood, and most chemotactic agents attract only one variety, and either repel or do not influence the others. It practically never happens that all are increased in the same proportion. The most satisfactory classification of leukocytoses is, therefore, based upon the type of leukocyte chiefly affected.

Theoretically, there should be a subdivision for each

variety of leukocyte, *e. g.*, polymorphonuclear leukocytosis, lymphocytic leukocytosis, eosinophilic leukocytosis, large mononuclear leukocytosis, etc. Practically, however, only two of these, polymorphonuclear leukocytosis and lymphocytic leukocytosis, need be considered under the head of Leukocytosis. Increase in number of the other leukocytes will be considered when the individual cells are described (see pp. 279–291). They are present in the blood in such small numbers normally that even a marked increase scarcely affects the total leukocyte count; and, besides, substances which attract them into the circulation frequently repel the polymorphonuclears, so that the total number of leukocytes may actually be decreased.

The polymorphonuclear neutrophiles are capable of active ameboid motion, and are by far the most numerous of the leukocytes. Lymphocytes are about one-third as numerous and have little independent motion. As one would, therefore, expect, marked differences exist between the two types of leukocytosis: polynuclear leukocytosis is more or less acute, coming on quickly and often reaching high degree; whereas lymphocytic leukocytosis is more chronic, comes on more slowly, and is never so marked.

1. Polymorphonuclear Neutrophilic Leukocytosis.—Polymorphonuclear leukocytosis may be either physiologic or pathologic. A count of 20,000 would be considered a marked leukocytosis; of 30,000, high; above 50,000, extremely high.

(1) **Physiologic Polymorphonuclear Leukocytosis.**—This is never very marked, the count rarely exceeding 15,000 per cubic millimeter. It occurs: (*a*) In the new-

born; (*b*) in pregnancy; (*c*) during digestion, and (*d*) after cold baths. There is moderate leukocytosis in the moribund state: this is commonly classed as physiologic, but is probably due mainly to terminal infection.

The increase in these conditions is not limited to the polymorphonuclears. Lymphocytes are likewise increased in varying degrees, most markedly in the newborn.

In view of the leukocytosis of digestion, the hour at which a leukocyte count is made should always be recorded. Digestive leukocytosis is most marked three to five hours after a hearty meal rich in protein. It is absent in pregnancy and when leukocytosis from any other cause exists. It is usually absent in cancer of the stomach, a fact which may be of some help in the diagnosis of this condition, but repeated examinations are essential.

(2) **Pathologic Polymorphonuclear Leukocytosis.**—In general, the response of the leukocytes to chemotaxis is a conservative process. It has been compared to the gathering of soldiers to destroy an invader. This is accomplished partly by means of phagocytosis—actual ingestion of the enemy—and partly by means of chemic substances which the leukocytes produce.

In those diseases in which leukocytosis is the rule the degree of leukocytosis depends upon two factors: the *severity of the infection* and the *resistance of the individual*. A well-marked leukocytosis usually indicates good resistance. A mild degree means that the body is not reacting well, or else that the infection is too slight to call forth much resistance. Leukocytosis may be absent altogether when the infection is extremely mild, or when

it is so severe as to overwhelm the organism before it can react. When leukocytosis is marked, a sudden fall in the count may be the first warning of a fatal issue. These facts are especially true of pneumonia, diphtheria, and abdominal inflammations, in which conditions the degree of leukocytosis is of considerable prognostic value.

The classification here given follows Cabot:

(*a*) *Infectious and Inflammatory.*—The majority of infectious diseases produce leukocytosis. The most notable exceptions are influenza, malaria, measles, tuberculosis, except when invading the serous cavities or when complicated by mixed infection, and typhoid fever, in which leukocytosis indicates an inflammatory complication.

All inflammatory and suppurative diseases cause leukocytosis, except when slight or well walled off. Appendicitis has been studied with especial care in this connection, and the conclusions now generally accepted probably hold good for most acute intra-abdominal inflammations. A marked leukocytosis (20,000 or more) nearly always indicates abscess, peritonitis, or gangrene, even though the clinical signs be slight. Absence of or mild leukocytosis indicates a mild process, or else an overwhelmingly severe one; and operation may safely be postponed unless the abdominal signs are very marked. On the other hand, no matter how low the count, an increasing leukocytosis—counts being made hourly—indicates a spreading process and demands operation, regardless of other symptoms.

Leukocyte counts alone are often disappointing, but are of much more value when considered in connection with a differential count of polymorphonuclears (see p. 283).

(*b*) *Malignant Disease.*—Leukocytosis occurs in about one-half of the cases of malignant disease. In many instances it is probably independent of any secondary infection, since it occurs in both ulcerative and non-ulcerative cases. It seems to be more common in sarcoma than in carcinoma. Very large counts are rarely noted.

(*c*) *Posthemorrhagic.*—Moderate leukocytosis follows hemorrhage and disappears in a few days.

(*d*) *Toxic.*—This is a rather obscure class, which includes gout, chronic nephritis, acute yellow atrophy of the liver, ptomain-poisoning, prolonged chloroform narcosis, and quinin-poisoning. Leukocytosis may or may not occur in these conditions, and is not important.

(*e*) *Drugs.*—This also is an unimportant class. Most tonics and stomachics and many other drugs produce a slight leukocytosis.

2. Lymphocytic Leukocytosis.—This is characterized by an increase in the total leukocyte count, accompanied by an increase in the percentage of lymphocytes. The word "lymphocytosis" is often used in the same sense. It is better, however, to use the latter as referring to any increase in the absolute number of lymphocytes, without regard to the total count, since an absolute increase in number of lymphocytes is frequently accompanied by a normal or subnormal leukocyte count, owing to loss of polymorphonuclears.

Lymphocytic leukocytosis is probably due more to stimulation of blood-making organs than to chemotaxis. It is less common, and is rarely so marked as a polymorphonuclear leukocytosis. When marked, the blood cannot be distinguished from that of lymphatic leukemia.

A marked lymphocytic leukocytosis occurs in pertussis, and is of value in diagnosis. It appears early in the catarrhal stage, and persists until after convalescence. The average leukocyte count is about 17,000, lymphocytes predominating. There is moderate lymphocytic leukocytosis in other diseases of childhood, as rickets, scurvy, and especially hereditary syphilis, where the blood-picture may approach that of pertussis. It must be borne in mind in this connection that lymphocytes are normally more abundant in the blood of children than in that of adults.

Slight lymphocytic leukocytosis occurs in many other pathologic conditions, but is of little significance.

B. Leukemia

This is an idiopathic disease of the blood-making organs, which is accompanied by an enormous increase in number of leukocytes. The leukocyte count sometimes reaches 1,000,000 per cubic millimeter, and leukemia is always to be suspected when it exceeds 50,000. Lower counts do not, however, exclude it. The subject is more fully discussed later (see p. 315).

Method of Counting Leukocytes

The leukocytes are counted with one of the hemacytometers already described (see pp. 234–244). Numerous modifications of the original ruling have been introduced, notably the Neubauer, the Zappert, and the Türck (Fig. 82), which give a ruled area of 9 sq.mm., the center having the Thoma ruling. They were devised for counting the leukocytes in the same specimen with the red corpuscles. The red cells are counted

in the central portion in the usual manner, after which all the leukocytes in the whole area of 9 sq. mm. are counted; and the number in a cubic millimeter of undiluted blood is then easily calculated. Leukocytes are

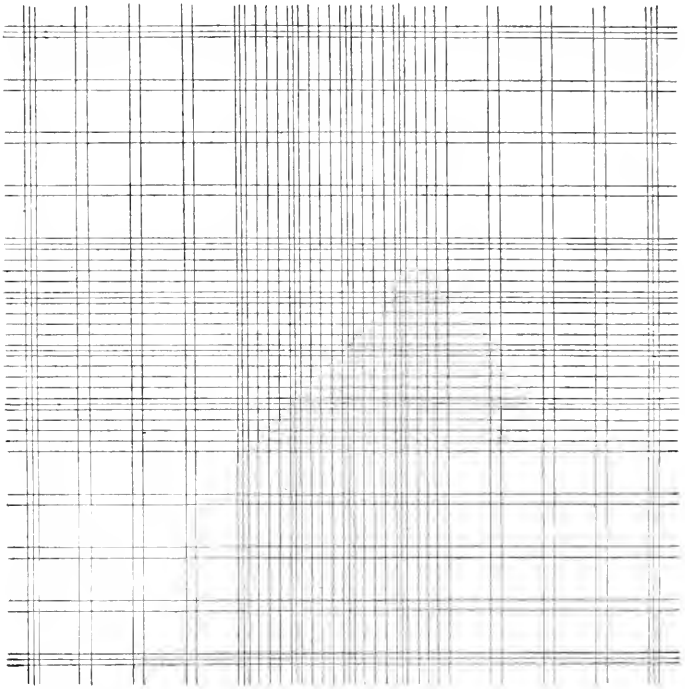


Fig. 82.—Türck ruling of counting chamber.

easily distinguished from red cells, especially when Toisson's diluting fluid is used. This method may be used with the older Thoma ruling by adjusting the microscopic field to a definite size, and counting a sufficient number of fields, as described later.

Although less convenient, it is more accurate to count the leukocytes separately, with less dilution of the blood, as follows:

Technic.—A larger drop of blood is required than for counting the erythrocytes, and more care in filling the pipet. Boggs has suggested a device (Fig. 83) which enables one to

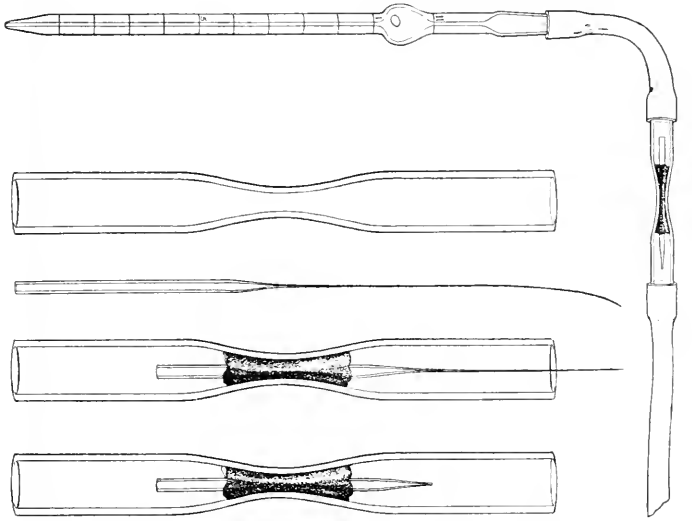


Fig. 83.—Boggs' "throttle control" for blood-counting pipet, and enlarged diagram showing construction of the throttle.

draw in the blood more slowly and hence more accurately. He cuts the rubber tube and inserts a Wright "throttle." This consists of a section of glass tubing in which a capillary tube drawn out to a fine thread is cemented with sealing wax. After sealing in place the tip is broken off with forceps, so that upon gentle suction it will just allow air to pass.

Use the pipet with 11 engraved above the bulb. Suck the blood to the mark 0.5 or 1.0, and the diluting fluid to the

mark 11. This gives a dilution of 1:20 or 1:10, respectively. The dilution of 1 : 20 is easier to make. Mix well by shaking in all directions except in the long axis of the pipet; blow out 2 or 3 drops, place a drop in the counting chamber, and adjust the cover as already described (see p. 237).

Examine with a low power to see that the cells are evenly distributed. Count with the 16-mm. objective and a high eye-piece, or with the long-focus 4 mm. and a low eye-piece. An 8-mm. objective will be found very satisfactory for this purpose. As one gains experience one will rely more upon the lower powers.

With the Thoma ruling count all the leukocytes in the square millimeter, multiply by 10 to find the number in 1 c.mm. of diluted blood, and by the dilution to find the number per cubic millimeter of undiluted blood. In every case at least 200 leukocytes must be counted as a basis for calculation, and it is much better to count 500. This will necessitate examination of several drops from the pipet. With the Zappert and Türck rulings a sufficient number can usually be counted in one drop, but the opportunity for error is very much greater when only one drop is examined.

In routine work the author's modification of the "circle" method is very satisfactory. It requires a 4-mm. objective, and is, therefore, especially desirable for beginners, who are usually unable accurately to identify leukocytes with a lower power. The student is frequently confused by particles of dirt, remains of red cells, and yeast cells which frequently grow in the diluting fluid. Draw out the sliding tube of the microscope until the field of vision is such as shown in Fig. 84. One side of the field is tangent to one of the ruled lines, A, while the opposite side just cuts the corners, B and C, of the seventh squares in the rows above and below the diameter line. When once adjusted, a scratch is made upon the draw-tube, so that for future counts the tube has only to be drawn out to the mark. The area of this microscopic field is one-

tenth of a square millimeter. With a dilution of 1 : 20, count the leukocytes in 20 such fields upon different parts of the disk without regard to the ruled lines, and to their sum add two ciphers. With dilution of 1 : 10, count 10 such fields, and add two ciphers. Thus, with 1 : 10 dilution, if 150 leukocytes

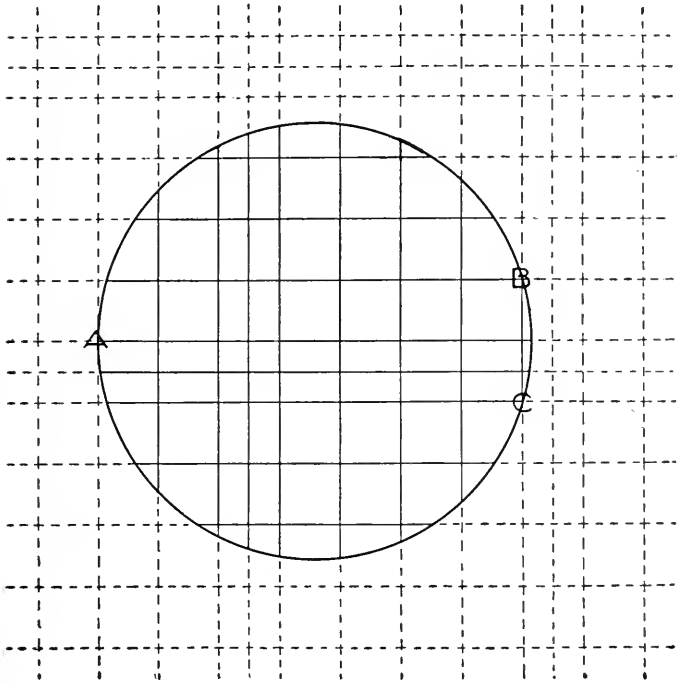


Fig. 84.—Size of field required in counting leukocytes as described in the text.

were counted in 10 fields, the leukocyte count would be 15,000 per cubic millimeter. To compensate for possible unevenness of distribution, it is best to count a row of fields horizontally and a row vertically across the disk. This method is applicable to any degree of dilution of the blood, and is simple to

remember: *one always counts a number of fields equal to the number of times the blood has been diluted, and adds two ciphers.* Evidence of the convenience of using a circle of this size is afforded by its adoption in the new Thoma-Metz instrument.

It is sometimes impossible to obtain the proper size of field with the objectives and eye-pieces at hand. In such case place a cardboard disk with a circular opening upon the diaphragm of the eye-piece, and adjust the size of the field by drawing out the tube. The circular opening can be cut with a cork-borer.

Diluting Fluids.—The diluting fluid should dissolve the red corpuscles so that they will not obscure the leukocytes. The simplest fluid is a 0.5 per cent. solution of acetic acid. More satisfactory is the following: glacial acetic acid, 1 c.c.; 1 per cent. aqueous solution of gentian-violet, 1 c.c.; distilled water, 100 c.c. These solutions must be filtered frequently to remove yeasts and molds.

VII. ENUMERATION OF BLOOD-PLAQUES

The average normal number of plaques is variously given as 200,000 to 700,000 per cubic millimeter of blood. Many of the counts were obtained by workers who used unreliable methods. Using their new method, Wright and Kinnicutt found the normal average to range from 263,000 to 336,000. Physiologic variations are marked; thus, the number increases as one ascends to a higher altitude, and is higher in winter than in summer. There are unexplained variations from day to day; hence a single abnormal count should not be taken to indicate a pathologic condition.

Pathologic variations are often very great. Owing to lack of knowledge as to the origin of the platelets and to the earlier imperfect methods of counting, the clinical

significance of these variations is uncertain. The following facts seem, however, to be established:

(*a*) In acute infectious diseases the number is subnormal or normal. If the fever ends by crisis, the crisis is accompanied by a rapid and striking increase.

(*b*) In secondary anemia plaques are generally increased, although sometimes decreased. In pernicious anemia they are always greatly diminished, and an increase should exclude the diagnosis of this disease.

(*c*) They are decreased in chronic lymphatic leukemia, and greatly increased in the myelogenous form.

(*d*) In purpura hæmorrhagica the number is enormously diminished.

Blood-plaques are difficult to count, owing to the rapidity with which they disintegrate, and to their great tendency to adhere to any foreign body and to each other.

Method of Kemp, Calhoun, and Harris.—Wash the finger well and allow a few minutes to elapse for the circulation to become normal. Prick the finger lightly with a blood-lancet, regulating the depth of the puncture so that the blood will not flow without gentle pressure. Quickly dip a clean glass rod into a vessel containing diluting and fixing fluid, and place two or three good-sized drops upon the finger over the puncture. Then exert gentle pressure above the puncture so that a small drop of blood will exude into the fluid. Mix the two by passing the rod lightly several times over the surface of the blended drop. (Some workers first place a drop of the fluid upon the finger and then make the puncture through it, this necessitating less care as to depth of the puncture.) Now transfer a drop of the diluted blood from the finger to a watch-glass which contains 2 or 3 drops

of the fluid, and mix well. From this, transfer a drop to the counting slide of the hemacytometer, and cover. An ordinary thin cover will answer for this purpose, and is preferable because it allows the use of a higher power objective. Allow the slide to stand for at least five minutes, and then with a 4-mm. or higher objective count the plaques and the red corpuscles in a definite number of squares. At least 100 plaques must be counted. The number of red corpuscles per cubic millimeter of blood having been previously ascertained in the usual manner (see p. 234), the number of plaques can easily be calculated by the following equation:

$$r : p :: R : P ; \text{ and } P = \frac{p \times R}{r}.$$

r represents the number of red corpuscles in any given number of squares; p, the number of plaques in the same squares; R, the total number of red corpuscles per cubic millimeter of blood; and P, the number of plaques per cubic millimeter.

Beginners are apt to take too much blood and not to dilute it enough. Best results are attained when there are only one or two plaques in a small square. With insufficient dilution, the platelets are more or less obscured by the red cells.

The following diluting and fixing fluid is recommended:

Formalin..... 10 c.c.;
Aqueous solution sodium chlorid (1 per cent.).... 150 c.c.
(Color with methyl-violet if desired.)

This fluid is cheap and easily prepared, and keeps indefinitely. It fixes the plaques quickly without clumping, and does not clump nor decolorize the reds. It has a low specific gravity, and hence allows the plaques to settle upon the ruled area along with the reds. Fluids of high specific gravity cause the plaques to float so that they do not appear in the same plane with the reds and the ruled lines.

Method of Wright and Kinnicutt.—This new method is simple, appears to be accurate, and certainly yields uniform results.

The plaques are counted with the hemacytometer already described, using a dilution of 1 : 100. The diluting fluid consists of 2 parts of an aqueous solution of "brilliant cresyl blue" (1 : 300) and 3 parts of an aqueous solution of potassium cyanid (1 : 1400). These two solutions must be kept in separate bottles and mixed and filtered immediately before using. After the blood is placed in the counting-chamber it is allowed to stand for ten minutes or longer in order that the plaques may settle. The plaques appear as rounded, lilac-colored bodies; the reds are decolorized, appearing only as shadows.

The leukocytes are stained and may be counted at the same time.

VIII. STUDY OF STAINED BLOOD

A. Making and Staining Blood-films

1. Spreading the Film.—Thin, even films are essential to accurate and pleasant work. They more than compensate for the time spent in learning to make them. There are certain requisites for success with any method: (*a*) The slides and covers must be perfectly clean: thorough washing with soap and water and rubbing with alcohol will usually suffice; (*b*) the drop of blood must not be too large; (*c*) the work must be done quickly, before coagulation begins.

The blood is obtained from the finger-tip or the lobe of the ear, as for a blood count; only a very small drop is required, usually about the size of a pin-head.

Ehrlich's Two Cover-glass Method.—This method is very widely used, but considerable practice is required to get good

results. Touch a cover-glass to the top of a small drop of blood, and place it, blood side down, upon another cover-glass. If the drop be not too large, and the covers be perfectly clean, the blood will spread out in a very thin layer. Just as it stops spreading, before it begins to coagulate, pull the covers quickly but firmly apart on a plane parallel to their surfaces (Fig. 85). It is best to handle the covers with forceps, since the moisture of the fingers may produce artifacts. The forceps must have a firm grasp.

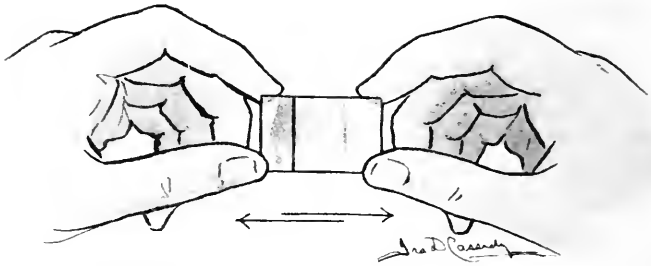


Fig 85.—Spreading the film: two cover-glass method.

This method is especially to be recommended for very accurate differential counts, since all the leukocytes in the drop will be found on the two covers. One of the covers is usually much better spread than the other.

Two-slide Method.—Take a small drop of blood upon a clean slide about $\frac{1}{2}$ inch from the end, using care that the slide does not touch the skin. Place the end of a second slide against the surface of the first at an angle of 45 degrees, and draw it up against the drop of blood, which will immediately run across the end, filling the angle between the two slides. Now push the "spreader slide" back along the other in the manner indicated in Fig. 86. The blood will follow. The

thickness of the smear can be regulated by changing the angle.

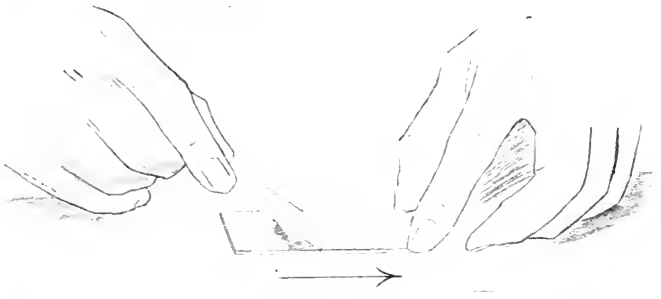


Fig. 86.—Spreading the film: two-slide method.

It is very easy to make large, thin, even films by this method.

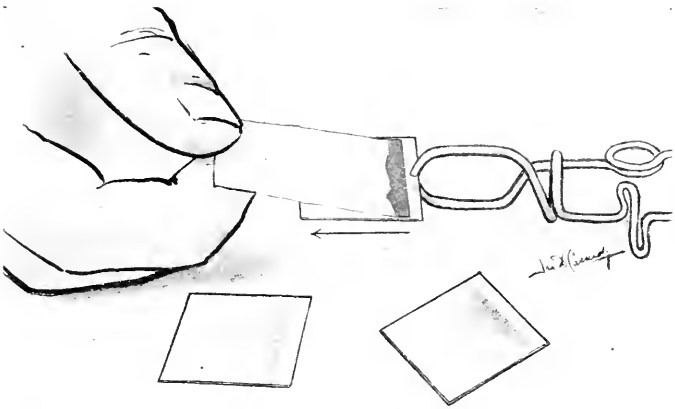


Fig. 87.—Spreading the film: cigarette-paper method applied to cover-glasses.

Cigarette-paper Method.—This gives excellent results in the hands of the inexperienced if directions are carefully followed, but its only advantage over the two-slide method is

that it may be used with covers as well as with slides. A very thin paper, such as the "Zig-zag" brand, is best. Ordinary cigarette paper and thin tissue-paper will answer, but do not give nearly so good results.

Cut the paper into strips about $\frac{3}{4}$ inch wide, *across the ribs*. Pick up one of the strips by the gummed edge, and touch its opposite end to the drop of blood. Quickly place the end which has the blood against a slide or a large cover-glass held in a forceps. The blood will spread along the edge of the paper. Now draw the paper evenly across the slide or cover. A thin film of blood will be left behind (Fig. 87).

The films may be allowed to dry in the air, or may be dried by gently heating high above a flame (where one can comfortably hold the hand). Such films will keep for years, but for some stains they must not be more than a few weeks old. They must be kept away from flies—a fly can work havoc with a film in a few minutes.

When slides are used the label can be written with a soft lead pencil directly on the blood-film, as has been suggested by von Ezdorf.

2. Fixing the Film.—In general, films must be "fixed" before they are stained. Fixation may be accomplished by chemicals or by heat. *Those stains which are dissolved in methyl alcohol combine fixation with the staining process.*

Chemic Fixation.—Soak the film one to five minutes in pure methyl alcohol or absolute ethyl alcohol, or one-half hour or longer in equal parts of absolute alcohol and ether. One minute in 1 per cent. formalin in alcohol is preferred by some, especially for the carbol-thionin stain. Chemic fixation may precede eosin-methylene-blue and other simple stains.

Heat Fixation.—This may precede any of the methods which do not combine fixation with the staining process; it *must* be used with Ehrlich's triple stain. The best method is to place the film in an oven, raise the temperature to 150° C., and allow to cool slowly. Without an oven, the proper degree of fixation is difficult to attain. Kowarsky has devised a small plate of two layers of copper (Fig. 88), upon which the films are placed together with a crystal of urea. The plate is heated over a flame until the urea melts, and is then set aside to cool. This is said to give the proper degree of fixation, but in the writer's experience the films have always been underheated. He obtains better results by use of tar-

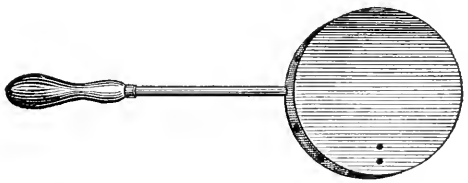


Fig 88.—Kowarsky's plate for fixing blood (Klopstock and Kowarsky).

taric acid crystals (melting-point, 168°–170° C.). The plate, upon which have been placed the cover-glasses, film side down, and a crystal of the acid, is heated over a low flame until the crystal has completely melted. It should be held sufficiently high above the flame that the heating will require five to seven minutes. The covers are then removed. Freshly made films of normal blood should be allowed to remain upon the plate for a minute or two after heating has ceased. Fresh films require more heat than old ones, and normal blood more than the blood of pernicious anemia and leukemia.

Blood films can be satisfactorily fixed for most purposes by covering with absolute alcohol, quickly dashing off the excess, and igniting the remainder.

3. Staining the Film.—The anilin dyes, which are extensively used in blood work, are of two general classes: basic dyes, of which methylene-blue is the type; and acid dyes, of which eosin is the type. Nuclei and certain other structures in the blood are stained by the basic dyes, and are hence called *basophilic*. Certain structures take up only acid dyes, and are called *acidophilic*, *oxyphilic*, or *eosinophilic*. Certain other structures are stained only by combinations of the two, and are called *neutrophilic*. Recognition of these staining properties marked the beginning of modern hematology.

(1) **Eosin and Methylene-blue.**—In many instances this stain will give all the information desired. It is especially useful in studying the red corpuscles. Nuclei, basophilic granules, and all blood parasites are blue; erythrocytes are red or pink; eosinophilic granules, bright red. Neutrophilic granules and blood-plaques are not stained.

(1) Fix the film by heat or chemicals.

(2) Stain about five minutes with a 0.5 per cent. alcoholic solution of eosin, diluted one-half with water.

(3) Rinse in water.

(4) Stain one-half to one minute with half-saturated aqueous solution of methylene-blue.

(5) Rinse well, dry, and mount. Films upon slides may be examined with an oil-immersion objective without a cover-glass.

(2) **Ehrlich's Triple Stain.**—This was the standard blood-stain for many years, but is now little used. It is probably the best for neutrophilic granules. It is difficult to make, and should be purchased ready pre-

pared from a reliable dealer. Nuclei are stained pale blue or greenish blue; erythrocytes, orange; neutrophilic granules, violet; and eosinophilic granules, copper red. Basophilic granules and blood-plaques are not stained.

Success in staining depends largely upon proper fixation. The film must be carefully fixed by heat: underheating causes the erythrocytes to stain red; overheating, pale yellow. The staining fluid is applied for five to fifteen minutes, and the preparation is rinsed quickly in water, dried, and mounted. Subsequent application of Löffler's methylene-blue for one-half to one second will bring out the basophilic granules and improve the nuclear staining, but there is considerable danger of overstaining.

(3) **Polychrome Methylene-blue-eosin Stains.**—These stains, mostly outgrowths of the original Romanowsky method, have largely displaced other blood-stains for clinical purposes. They stain differentially every normal and abnormal structure in the blood. Most of them are dissolved in methyl alcohol and combine the fixing with the staining process. Numerous methods of preparing and applying these stains have been devised. Three only need be given here: Wright's stain, Harlow's stain, and Jenner's stain.

Wright's Stain.—This is one of the best and is the most widely used in this country. The practitioner will find it best to purchase the stain ready prepared or to purchase the powder and dissolve it in methyl alcohol as needed. Most microscopic supply-houses carry it in stock. Wright's most recent directions for its preparation and use are as follows:

Preparation.—To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene-blue (B. X. or "medicinally pure") in the proportion of 1 gm. of the dye to each 100 c.c. of the solution. Heat the mixture in a steam sterilizer at 100° C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask, or flasks, of such size and shape that it forms a layer not more than 6 cm. deep. After heating, allow the mixture to cool, placing the flask in cold water, if desired, and then filter it to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed in a thin layer by transmitted yellowish artificial light. It does not show this color while it is warm.

To each 100 c.c. of the filtered mixture add 500 c.c. of a 0.1 per cent. aqueous solution of "yellowish water-soluble" eosin and mix thoroughly. Collect the abundant precipitate which immediately appears on a filter. When the precipitate is dry, dissolve it in methylic alcohol (Merck's "reagent") in the proportion of 0.1 gm. to 60 c.c. of the alcohol. In order to facilitate solution, the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle. This alcoholic solution of the precipitate is the staining fluid.

Application.—1. Cover the film with a noted quantity of the staining fluid by means of a medicine-dropper. There must be plenty of stain in order to avoid too great evaporation and consequent precipitation. When slides are used, the stain may be confined to the smeared area by two heavy grease pencil marks.

2. After one minute add to the staining fluid on the film the same quantity of distilled water by means of a medicine-dropper and allow the mixture to remain for two or three minutes, according to the intensity of the staining desired. A longer period of staining may produce a precipitate.

Eosinophilic granules are best brought out by a short period of staining.

The quantity of the diluted fluid on the preparation should not be so large that some of it runs off.

3. Wash the preparation in water for thirty seconds or until the thinner portions of the film become yellow or pink in color.

4. Dry and mount in balsam.

The stain is more conveniently applied upon cover-glasses than upon slides. Films much more than a month old do not stain well. In some localities ordinary tap-water will answer both for diluting the stain and for washing the film; in others, distilled water must be used. The difficulty here is probably that the tap-water is acid in reaction. This causes the nuclei to stain too palely. Other causes of pale nuclei are addition of too much or too little water and the development of formic acid from the methyl alcohol of the staining fluid.

When properly applied, Wright's stain gives the following picture (see Frontispiece): erythrocytes, yellow or pink; nuclei, various shades of bluish purple; neutrophilic granules, reddish lilac; eosinophilic granules, bright red; basophilic granules of leukocytes and degenerated red corpuscles, very dark bluish purple; blood-plaques, dark lilac; bacteria, blue. The cytoplasm of lymphocytes is generally robin's-egg blue; that of the large mononuclears may have a faint bluish tinge. Malarial parasites stain characteristically: the cytoplasm, sky-blue; the chromatin, reddish purple.

Harlow's Stain.—Probably the simplest of the polychrome methylene-blue-eosin stains, both in preparation and method of use, is that devised by W. P. Harlow, of

the University of Colorado. It differentiates granules particularly well, but is not so satisfactory for demonstrating *slight* grades of polychromatophilia, because it usually gives all the red cells a faint bluish tinge.

Preparation.—The stain consists of two solutions used separately:

No. 1.	Eosin, yellowish, water soluble (Grübler)	1 gm.;
	Methyl alcohol (Merck's reagent)	100 c.c.;
No. 2.	Methylene-blue ("B. X." or Ehrlich's rectified) (Grübler)	1 gm.;
	Methyl alcohol (Merck's reagent)	100 c.c.

Application.—(1) Stain the film without previous fixation for one minute with the eosin solution.

(2) Shake off the excess, allowing a very little to remain, and at once cover with the methylene-blue solution for one or two minutes.

(3) Rinse quickly in distilled water, dry, and mount.

It is well known that pathologic bloods will sometimes not stain well with fluids which are satisfactory for normal bloods. Doctors Peebles and Harlow have shown that the various polychrome methylene-blue-eosin stains can be modified to suit any blood by adding a little alkali or acid. The alkali used is a weak solution of "potassium hydrate by alcohol" in methyl alcohol; the acid, glacial acetic in methyl alcohol. In the case of the Harlow stain it is added to the methylene-blue solution only. The alkali solution also serves to "correct" old fluids which, by reason of development of formic acid in the methyl alcohol, do not stain sufficiently with the blue. In general a stain is satisfactory when both nuclei and neutrophilic granules are clearly defined.

Jenner's Stain.—This brings out leukocytic granules well, and is, therefore, especially useful for differential counting. It stains nuclei poorly and is much inferior to Wright's stain for the malarial parasite.

It may be purchased in solution, in the form of tablets, or as a powder, 0.5 gm. of which is dissolved in 100 c.c. neutral absolute methyl alcohol. The blood-film is flooded with the stain and after three to five minutes is rinsed with water, dried in the air, and mounted.

(4) **Carbol-thionin** is especially useful for the study of basophilic granular degeneration of the red cells. The method is described on p. 467. Nuclei, malarial parasites, and basophilic granules are brought out sharply. Polychromatophilia is also evident. Fixation may be by alcohol-formalin (see page 264) or saturated solution of mercuric chlorid.

(5) **Pappenheim's pyronin-methyl green** (see p. 467) can be used as a blood-stain and is very satisfactory for study of the red cells, and especially of the lymphocytes. All nuclei are greenish blue; basophilic granules, red; cytoplasm of lymphocytes, red. Polychromatophilia is well demonstrated, the affected cells taking more or less of the red color. Heat fixation is probably best.

B. Study of Stained Films

Much can be learned from stained blood-films. They furnish the best means of studying the morphology of the blood and blood parasites, and, to the experienced, they give a fair idea of the amount of hemoglobin and the number of red and white corpuscles. An oil-immersion objective is required.

1. Erythrocytes.—Normally, the red corpuscles are acidophilic. The colors which they take with different stains have been described. When not crowded together, they appear as circular, homogeneous disks, of nearly uniform size, averaging 7.5 μ in diameter (see Fig. 105). The center of each is somewhat paler than the periphery. The degree of pallor furnishes a rough index to the amount of hemoglobin in the corpuscle. As hemoglobin is diminished, the central pale area becomes larger and paler, producing the so-called "pessary forms" in which only the periphery of the cell is apparent. These forms indicate a low color index and are most abundant in chlorosis. Red cells are apt to be crenated when the film has dried too slowly.

Pathologically, red corpuscles vary in size and shape, staining properties, and structure.

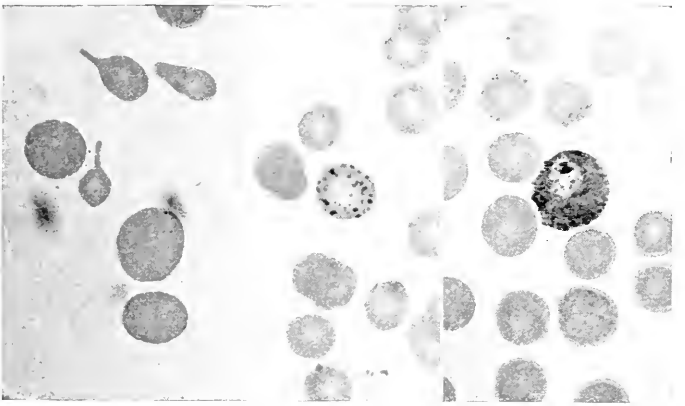
(1) **Variations in Size and Shape** (See Plate IX and Fig. 105).—The cells may be abnormally small (called *microcytes*, 5 μ or less in diameter); abnormally large (*macrocytes*, 10 to 12 μ); or extremely large (*megalocytes*, 12 to 25 μ). Abnormal variation in size is called *anisocytosis*.

Variation in shape is often very marked. Oval, pyriform, caudate, saddle-shaped, and club-shaped corpuscles are common (Fig. 89). They are called *poikilocytes*, and their presence is spoken of as poikilocytosis.

Red corpuscles which vary from the normal in size and shape are present in most symptomatic anemias, and in the severer grades are often very numerous. Irregularities are particularly conspicuous in leukemia and pernicious anemia, where, in some instances, a normal erythrocyte is the exception. In pernicious anemia there is a

decided tendency to large size and oval forms, and megalocytes are rarely found in any other condition.

(2) **Variations in Staining Properties** (See Plate IX).—These include polychromatophilia, basophilic degeneration, and malarial stippling. With exception of polychromatophilia they are probably degenerative changes.



A B C

Fig. 89.—Abnormal red corpuscles: A, Poikilocytosis; B, basophilic granular degeneration; C, malarial stippling, the cell also containing a tertian parasite (× 1000) (courtesy of Dr. W. P. Harlow).

(*a*) *Polychromatophilia.*—Some of the corpuscles partially lose their normal affinity for acid stains, and take the basic stain to greater or less degree. Wright's stain gives such cells a faint bluish tinge when the condition is mild, and a rather deep blue when severe. Sometimes only part of a cell is affected. A few polychromatophilic corpuscles can be found in marked symptomatic anemias. They occur most abundantly in malaria, leukemia, and pernicious anemia.

Polychromatophilia has been variously interpreted. It is thought by many to be evidence of youth in a cell, and hence to indicate an attempt at blood regeneration. There are probably several forms referable to different causes.

(*b*) *Basophilic Granular Degeneration* (*Degeneration of Grawitz*).—This is characterized by the presence, within the corpuscle, of basophilic granules which vary in size from scarcely visible points to granules as large as those of basophilic leukocytes (Fig. 89, B). The number present in a red cell commonly varies in inverse ratio to their size. They stain deep blue with carbol-thionin or Wright's stain; not at all with Ehrlich's triple stain. The cell containing them may stain normally in other respects, or it may exhibit polychromatophilia. Polychromatophilic cells generally contain the smaller granules, which may be so fine that the cell appears dusted with them.

Numerous cells showing this degeneration are commonly found in chronic lead-poisoning, of which they were at one time thought to be pathognomonic. Except in this disease, the degeneration indicates a serious blood condition. It occurs in well-marked cases of pernicious anemia and leukemia, and, much less commonly, in very severe symptomatic anemias.

(*c*) *Malarial Stippling*.—This term has been applied to the finely granular appearance often seen in red corpuscles, which harbor malarial parasites (see Frontispiece, Pl. VII, and Fig. 89). It was formerly classed with the degeneration just described, but is undoubtedly distinct. Not all stains will show it. With Wright's stain it can be brought out by staining longer and washing less than for the ordinary blood-stain. The minute granules,

"Schüffner's granules," stain reddish purple. They are sometimes so numerous as almost to hide the parasite.

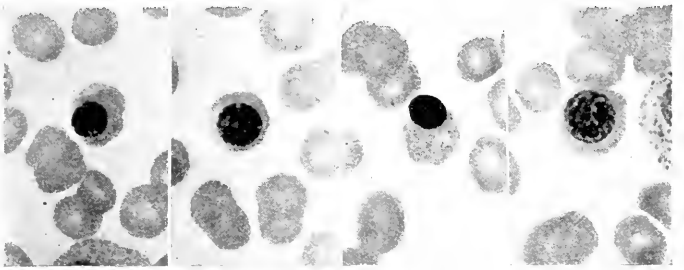


Fig. 90.—Normoblasts from cases of secondary anemia and leukemia (× 1000) (photographs by the author).

(3) **Variations in Structure.**—The most important is the presence of a nucleus (see Frontispiece, Pl. IX, and Fig. 90). Nucleated red corpuscles, or *erythroblasts*, are classed according to their size: *microblasts*, 5 μ or less in

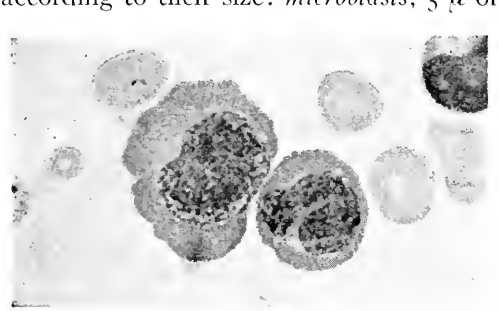


Fig. 91.—Megaloblasts from a case of pernicious anemia (× 1000) (courtesy of Dr. W. P. Harlow).

diameter; *normoblasts*, 5 to 10 μ; and *megaloblasts*, above 10 μ. Microblasts and normoblasts contain one, rarely two, small round, sharply defined, deeply staining nuclei, often located eccentrically. Occasionally the nucleus is

irregular in shape, "clover-leaf" forms being not infrequent. Sometimes it is completely broken up into fragments. Mitoses (see Fig. 96) are not uncommon in leukemia and pernicious anemia. The megaloblast (Fig. 91) is probably a distinct cell, not merely a larger size of the normoblast. Its nucleus is large, stains rather palely, has a delicate chromatin network, and often shows evidences of degeneration (karyorrhexis, etc.). In ordinary work, however, it is safer to base the distinction upon size than upon structure. Any nucleated red cell, but especially the megaloblast, may exhibit polychromatophilia.

Normally, erythroblasts are present only in the blood of the fetus and of very young infants. In the adult, their presence in the circulating blood denotes an excessive demand upon the blood-forming organs to regenerate lost or destroyed red corpuscles. In response to this demand, immature and imperfectly formed cells are thrown into the circulation. Their number, therefore, is an indication of the extent to which the bone-marrow reacts rather than of the severity of the disease. Normoblasts occur in severe symptomatic anemia, leukemia, and pernicious anemia. They are most abundant in myelogenous leukemia. While always present in pernicious anemia, they are often difficult to find. Megaloblasts are found in pernicious anemia, and with extreme rarity in any other condition. They here almost invariably exceed the normoblasts in number, which is one of the distinctive features of the disease. Microblasts have much the same significance as normoblasts, but are less common.

Cabot's ring bodies are ring- or figure-of-8-shaped

structures which have been observed in certain of the red cells in pernicious anemia, lead-poisoning, and lymphatic leukemia. They stain red with Wright's stain. Their nature is unknown, although they have been thought to be the remains of a nuclear membrane.

2. The Leukocytes.—An estimation of the number or percentage of each variety of leukocyte in the blood is called a differential count. It probably yields more helpful information than any other single procedure in blood examinations.

The **differential count** is best made upon a film stained with Jenner's, Harlow's, or Ehrlich's stain. Wright's stain differentiates the leukocytes somewhat less satisfactorily. Go carefully over the film with an oil-immersion lens, using a mechanical stage if available. The experienced worker will often use the lower powers (even the 16-mm. objective, as recommended by Simon) in routine work. Classify each leukocyte seen, and calculate what percentage each variety is of the whole number classified. For accuracy, 500 to 1000 leukocytes must be classified; for approximate results, 300 are sufficient. Track of the count may be kept by placing a mark for each leukocyte in its appropriate column, ruled upon paper. Some workers divide a slide-box into compartments with slides, one for each variety of leukocyte, and drop a coffee-bean into the appropriate compartment when a cell is classified. When a convenient number of coffee-beans is used (any multiple of 100), the percentage calculation is extremely easy.

The actual number of each variety in a cubic millimeter of blood is easily calculated from these percentages and the total leukocyte count. An increase in

actual number is an *absolute increase;* an increase in percentage only, a *relative increase.* It is evident that an absolute increase of any variety may be accompanied by a relative decrease.

A record is generally kept of the number of nucleated red cells seen during a differential count of leukocytes.

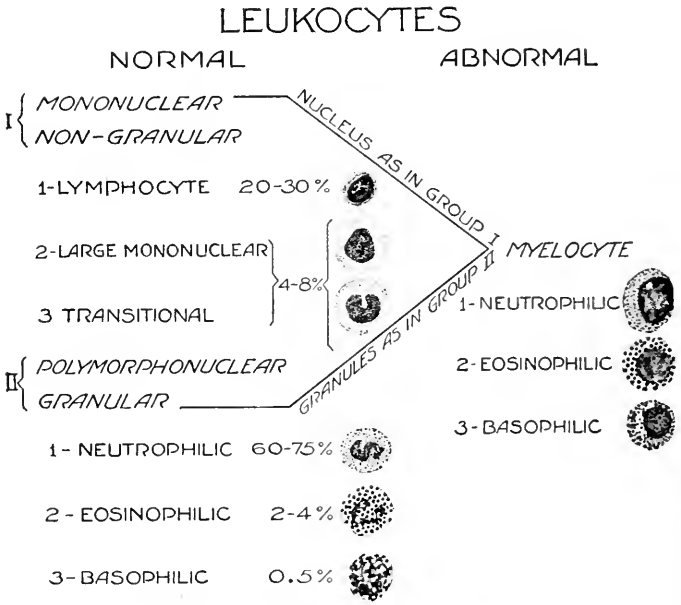


Fig. 92.—Outline of the classification of leukocytes.

The usual classification of leukocytes is based upon their size, their nuclei, and the staining properties of the granules which many of them contain. It is not altogether satisfactory, but is probably the best which our present knowledge permits.

The writer has found the table (Fig. 92) very help-

ful in impressing this classification upon the student. It makes no attempt to indicate histogenetic relationships. The leukocytes of normal blood fall into two groups, each including three types. The cells in Group I contain single, round, oval or horseshoe-shaped nuclei, and have few or no granules in their cytoplasm. The stippling of the cytoplasm shown in the diagram represents the finely granular appearance of protoplasm, not true granulation. The cells in Group II are polymorphonuclear and contain granules which are distinguished by their size and staining reactions. In its structure the chief abnormal leukocyte, the myelocyte, combines the two groups, being mononuclear like Group I and granular like Group II.

(1) **Normal Varieties.**—(*a*) **Lymphocytes** are small mononuclear cells without specific granules (see Frontispiece and Plate X). They are about the size of a red corpuscle or slightly larger (6–10 μ), and consist of a single, sharply defined, deeply staining nucleus, surrounded by a narrow rim of protoplasm. The nucleus is generally round, but is sometimes indented at one side. Wright's stain gives the nucleus a deep purple color and the cytoplasm a pale robin's-egg blue in typical cells. Larger lymphocytes are frequently found, especially in the blood of children, and are difficult to distinguish from the large mononuclear leukocytes. It is possible that the larger forms are young lymphocytes, which become smaller as they grow older. In the cytoplasm of a certain percentage of lymphocytes the Romanowsky stains show a variable number of reddish-purple (azurophilic) granules.

Lymphocytes are formed in the lymphoid tissues, including that of the bone-marrow. They constitute,

normally, 20 to 30 per cent. of all leukocytes, or about 1000 to 3000 per c.mm. of blood. They are more abundant in the blood of children.

The percentage of lymphocytes is usually moderately increased in those conditions which give leukopenia, especially typhoid fever, chlorosis, pernicious anemia, and many debilitated conditions. A marked increase, accompanied by an increase in the total leukocyte count,

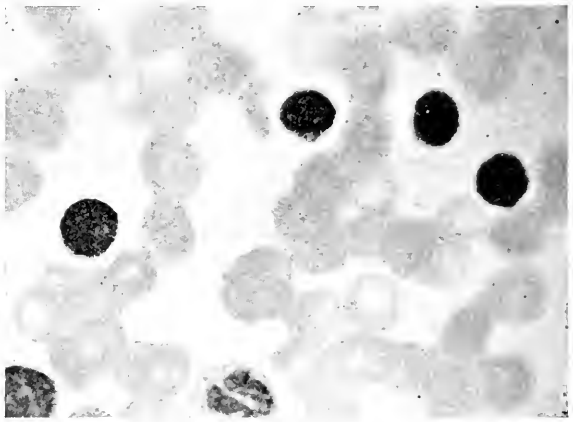


Fig. 93.—Lymphocytosis, case of pertussis (× 1000) (courtesy of Dr. W. P. Harlow).

is seen in pertussis (Fig. 93) and lymphatic leukemia. In the latter the lymphocytes sometimes exceed 98 per cent. Exophthalmic goiter commonly gives a marked relative lymphocytosis, while simple goiter does not affect the lymphocytes. In pulmonary tuberculosis a high percentage of lymphocytes is considered a favorable prognostic sign.

(*b*) **Large Mononuclear Leukocytes** (See Frontispiece). —These cells are two or three times the diameter of the

normal red corpuscle. Each contains a single round or oval nucleus, often located eccentrically. The zone of protoplasm surrounding the nucleus is relatively wide. With Wright's stain the nucleus is less deeply colored than that of the lymphocyte, while the cytoplasm is very pale blue or colorless, and sometimes contains a few reddish granules. The size of the cell, the width of the zone of cytoplasm, and the depth of color of the nucleus are the points to be considered in distinguishing between large mononuclears and lymphocytes. When large forms of the lymphocyte are present the distinction is often difficult or impossible. It is then advisable to count the two cells together as lymphocytes.

Large mononuclear leukocytes are probably developed from the endothelial cells of the blood-vessels. They constitute 2 to 4 per cent. of the total number of leukocytes: 100 to 400 per c.mm. of blood. An increase is unusual except in malaria, where it is quite constantly observed, and where many of the cells contain engulfed pigment.

(*c*) **Transitional Leukocytes** (See Frontispiece).—These are essentially large mononuclears with lobulated, deeply indented, or horseshoe-shaped nuclei. A few fine neutrophilic granules are sometimes present in their cytoplasm.

They are probably formed from the large mononuclears, and occur in the blood in about the same numbers. The two cells are counted together, constituting 4 to 8 per cent. of the leukocytes.

(*d*) **Polymorphonuclear Neutrophilic Leukocytes** (See Frontispiece).—There is usually no difficulty in recognizing these cells. Their average size (10 to 12 μ) is somewhat

less than that of the large mononuclears. The nucleus stains rather deeply, and is extremely irregular, often assuming shapes comparable to letters of the alphabet, E, Z, S, etc. (Fig. 94). Frequently there appear to be several separate nuclei, hence the widely used name, "polynuclear leukocyte." Upon careful inspection, however, delicate nuclear bands connecting the parts

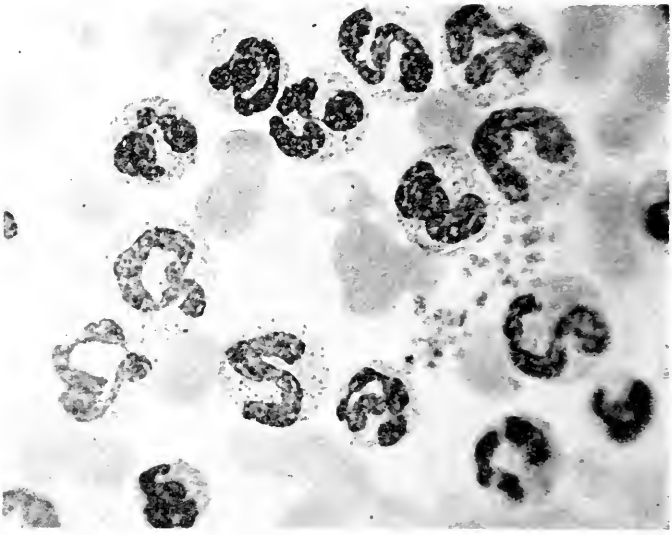


Fig. 94.—Marked polymorphonuclear neutrophilic leukocytosis (×1000) (courtesy of Dr. W. P. Harlow).

can usually be seen. The cytoplasm is relatively abundant, and contains great numbers of very fine neutrophilic granules (see Fig. 97, A). With Wright's stain the nucleus is bluish purple, and the granules reddish lilac.

Polymorphonuclear leukocytes are formed in the bone-marrow from neutrophilic myelocytes. They constitute 60 to 75 per cent. of all the leukocytes: 3000 to 7500 per

c.mm. of blood. Increase in their number practically always produces an increase in the total leukocyte count, and has already been discussed under Polymorphonuclear Leukocytosis. The leukocytes of pus, *pus-corpuscles*, belong almost wholly to this variety.

A comparison of the percentage of polymorphonuclear cells with the total leukocyte count yields more information than a consideration of either alone. In a general way the percentage represents the severity of the infection or, more correctly, the *degree of toxic absorption;* while the total count indicates the patient's *power of resistance*. With moderate infection and good resisting powers the leukocyte count and the percentage of polymorphonuclears are increased proportionately. When the polymorphonuclear percentage is increased to a notably greater extent than is the total number of leukocytes, no matter how low the count, either very poor resistance or a very severe infection may be inferred.

Gibson has suggested the use of a chart to express this relationship graphically (Fig. 95). Its arrangement is purely arbitrary, but it may be found helpful in interpreting counts. An ascending line from left to right indicates an unfavorable prognosis in proportion as the line approaches the vertical. All fatal cases show a rising line. A descending or horizontal line suggests a very favorable prognosis.

It is a matter of observation that in the absence of acute infectious disease or of inflammation directly in the blood-stream (*e. g.*, phlebitis, sigmoid sinusitis, septic endocarditis), a polymorphonuclear percentage of 85 or over points very strongly to gangrene or pus formation somewhere in the body. On the other hand, excepting

in children, where the percentage is normally low, pus is uncommon with less than 80 per cent. of polymorphonuclears.

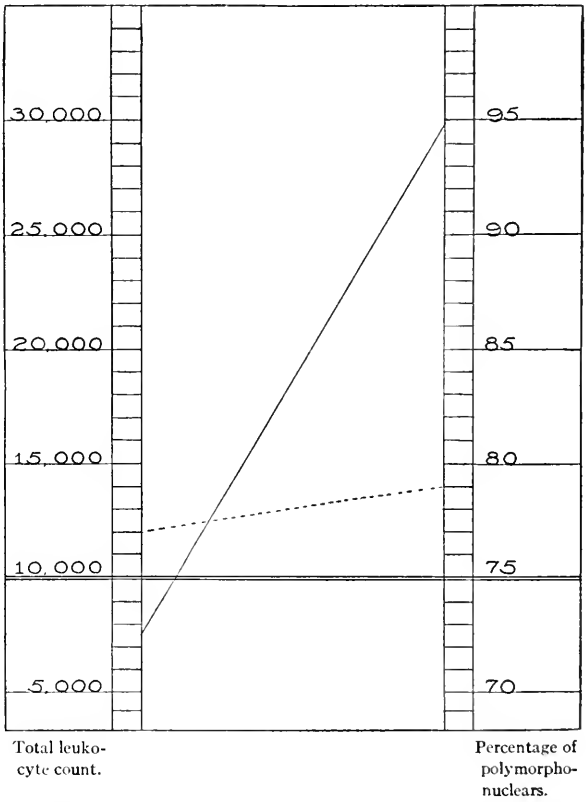


Fig. 95.—Gibson chart with blood-count in 2 cases of appendicitis: Dotted line represents a mild case with prompt recovery; the continuous line, a very virulent streptococcic case with poor resistance, peritonitis, and early death.

Normally, the cytoplasm of leukocytes stains pale yellow with iodin. Under certain pathologic conditions

the cytoplasm of many of the polymorphonuclears stains diffusely brown, or contains granules which stain reddish brown with iodin. This is called *iodophilia*. Extracellular iodin-staining granules, which are present normally, are more numerous in iodophilia.

This iodin reaction occurs in all purulent conditions except abscesses which are thoroughly walled off and purely tuberculous abscesses. It is of some value in diagnosis between serous effusions and purulent exudates, between catarrhal and suppurative processes in the appendix and Fallopian tube, etc. Its importance, however, as a diagnostic sign of suppuration has been much exaggerated, since it may occur in any general toxemia, such as pneumonia, influenza, malignant disease, and puerperal sepsis.

To demonstrate iodophilia, place the air-dried films in a stoppered bottle containing a few crystals of iodin until the films become yellow. Mount in syrup of levulose and examine with an immersion objective.

Arneth classifies polymorphonuclear leukocytes into five groups, according to the number of lobes which the nucleus shows. The percentage of cells in each group is fairly constant in health, but shows considerable variation in disease.

(*e*) **Eosinophilic Leukocytes, or "Eosinophiles"** (See Frontispiece).—The structure of these cells is similar to that of the polymorphonuclear neutrophiles, with the striking difference that, instead of fine neutrophilic granules, their cytoplasm contains coarse round or oval granules having a strong affinity for acid stains. They are easily recognized by the size and color of the granules, which stain bright red with stains containing eosin (see

Fig. 97, B). Their cytoplasm has generally a faint sky-blue tinge, and the nucleus stains somewhat less deeply than that of the polymorphonuclear neutrophile.

Eosinophiles are formed in the bone-marrow from eosinophilic myelocytes. Their normal number varies from 50 to 400 per c.mm. of blood, or 1 to 4 per cent. of the leukocytes. An increase is called *eosinophilia*, and is better determined by the actual number than by the percentage.

Slight eosinophilia is physiologic during menstruation. Marked eosinophilia is always pathologic. It occurs in a variety of conditions, the most important of which are: infection by animal parasites; bronchial asthma; myelogenous leukemia; scarlet fever, and many skin diseases.

(*a*) Eosinophilia may be a symptom of *infection by any of the worms*. It is fairly constant in trichiniasis, uncinariasis, filariasis, and echinococcus disease. In this country an unexplained marked eosinophilia warrants examination of a portion of muscle for *Trichinella spiralis* (see p. 415). The cells usually range between 10 and 30 per cent. of all the leukocytes, but may go much higher.

(*b*) *True bronchial asthma* commonly gives a marked eosinophilia during and following the paroxysms. This is helpful in excluding asthma of other origin. Eosinophiles also appear in the sputum in large numbers.

(*c*) In *myelogenous leukemia* there is almost invariably an absolute increase of eosinophiles, although, owing to the great increase of other leukocytes, the percentage is usually diminished. Dwarf and giant forms are often numerous.

(*d*) *Scarlet fever* is frequently accompanied by eosinophilia, which may help to distinguish it from measles.

(*e*) Eosinophilia has been observed in a large number of *skin diseases*, notably pemphigus, prurigo, psoriasis, and urticaria. It probably depends less upon the variety of the disease than upon its extent.

(*f*) **Basophilic Leukocytes or "Mast-cells"** (See Frontispiece).—In general, these resemble polymorphonuclear neutrophiles except that the nucleus is less irregular (usually merely indented or slightly lobulated) and that the granules are larger and have a strong affinity for basic

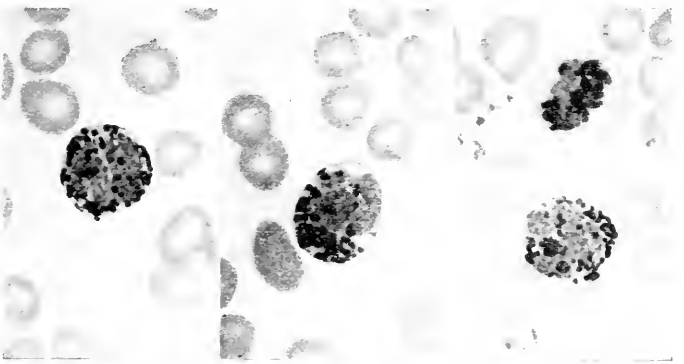
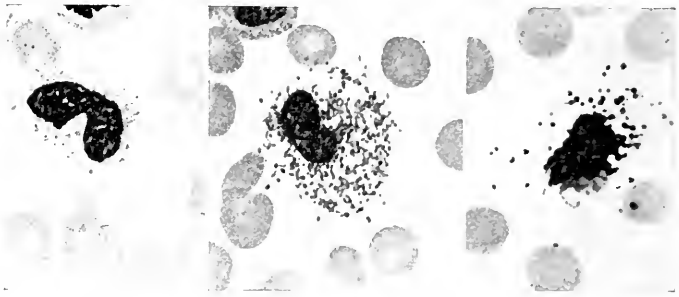


Fig. 96.—Basophilic leukocytes. At the right is also a normoblast undergoing mitosis (× 1000) (photographs by the author).

stains. They are easily recognized (Figs. 96 and 97, C). With Wright's stain the granules are deep purple, while the nucleus is pale blue and is often nearly or quite hidden by the granules, so that its form is difficult to make out. These granules are not colored by Ehrlich's stain.

Mast-cells probably originate in the bone-marrow from basophilic myelocytes. They are least numerous of the leukocytes in normal blood, rarely exceeding 0.5 per cent., or 25 to 50 per c.mm. A notable increase is lim-

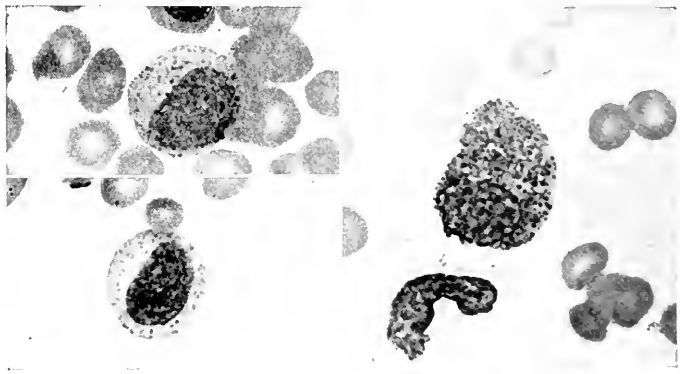
ited almost exclusively to myelogenous leukemia, where they are sometimes very numerous.



A B C

Fig. 97.—Ruptured leukocytes, showing relative size of granules: A, neutrophilic; B, eosinophilic; C, basophilic (×1000) (photographs by the author).

(2) **Abnormal Varieties.**—(*a*) **Myelocytes** (see Frontispiece and Fig. 98) are large mononuclear cells whose



A B

Fig. 98.—Myelocytes from blood of myelogenous leukemia: A, Neutrophilic; B, eosinophilic (×1000) (photographs by the author).

cytoplasm is filled with granules. Typically, the nucleus occupies about one-half of the cell, and is round or oval.

It is sometimes indented, with its convex side in contact with the periphery of the cell. It stains rather feebly. The average diameter of this cell (about 15.75 μ) is greater than that of any other leukocyte, but there is much variation in size among individual cells. Myelocytes are named according to the character of their granules—neutrophilic, eosinophilic, and basophilic myelocytes. These granules are identical with the corresponding granules in the leukocytes just described. They are, however, often less distinct and less sharply differentiated by the various stains than those of the corresponding polymorphonuclear cells. In some the granules are few in number, the cells departing but little from the structure of the parent myeloblast. Although the occurrence of two kinds of granules in the same cell is rare, a few basophilic granules are sometimes seen in young eosinophilic myelocytes. The basophilic myelocyte is usually small; and its nucleus is commonly so pale and so obscured by the granules that the cell is not easily distinguished from the mast-cell.

Myelocytes are the bone-marrow cells from which the corresponding granular leukocytes are developed. They in turn are derived from non-granular cells of the bone-marrow, the myeloblasts. Their presence in the blood in considerable numbers is diagnostic of myelogenous leukemia. The neutrophilic form is the least significant. A few of these may be present in very marked leukocytosis or any severe blood condition, as pernicious anemia. Eosinophilic myelocytes are found only in myelogenous leukemia, where they are often very numerous. The basophilic variety is less common, and is confined to long-standing, severe myelogenous leukemia.

(*b*) **Myeloblasts.**—These are the parent cells of the myelocytes, from which they differ chiefly in the absence of cytoplasmic granules. Their round or oval nuclei contain several nucleoli. The cytoplasm is somewhat basophilic.

Myeloblasts appear in the blood in considerable numbers in acute myelogenous leukemia and the terminal stages of chronic myelogenous leukemia, when the bone-marrow reverts to the embryonic type. They may be indistinguishable morphologically from the large lymphocytes of acute lymphatic leukemia, but can usually be distinguished by the oxydase reaction.

Indophenol Oxydase Test.—Fix cover-glass films in alcohol for five minutes. Float the covers for ten to twenty minutes face downward upon a freshly prepared solution containing equal parts of 1 per cent. aqueous solutions of dimethyl-para-phenylendiamin and of alpha-naphthol. Rinse in water and mount in water, glycerin, or a strong solution of water-glass.

The cytoplasm of cells containing oxydase—polymorphonuclears, large mononuclears, myelocytes, and myeloblasts—will be colored pale blue to dark blue. Lymphocytes, red corpuscles, and plaques should show no blue whatever.

(*c*) **Atypic Forms.**—Leukocytes which do not fit in with the above classification are not infrequently met, especially in high-grade leukocytosis, pernicious anemia, and leukemia. The nature of many of them is not clear, and their number is usually so small that they may be disregarded in making a differential count. Among them are:

(*a*) Border-line forms between polymorphonuclear neutrophiles and neutrophilic myelocytes.

(*b*) Small neutrophilic cells with a single round, deeply staining nucleus; they probably result from division of polymorphonuclear neutrophiles.

(*c*) "Irritation forms"—large non-granular mononuclear cells, whose cytoplasm stains fairly deep purple with Wright's stain and intense brown with Ehrlich's, and usually contains vacuoles. They appear in the blood under the same conditions as myelocytes.

(*d*) Degenerated forms: vacuolated leukocytes, or merely palely or deeply staining homogeneous or retic-

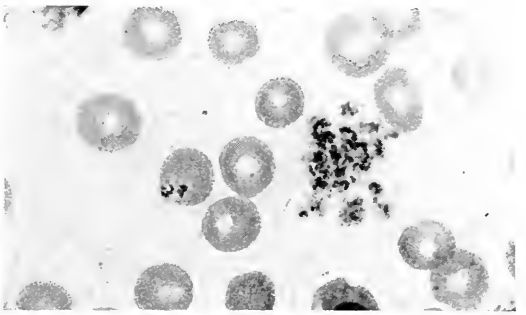


Fig. 99.—A cluster of blood-plaques and two plaques lying upon a red cell and simulating malarial parasites (×1000) (photograph by the author).

ulated masses of chromatin (the so-called "basket-cells," see Frontispiece).

3. Blood-plaques.—These are not colored by Ehrlich's stain nor by eosin and methylene-blue. With Wright's stain they appear as spheric or ovoid, reddish to violet, granular bodies, 2 to 4 μ in diameter. Occasionally a platelet as large as a red corpuscle is seen. When well stained a delicate hyaline peripheral zone can be distinguished. In ordinary blood-smears they are usually clumped in masses. A single platelet lying upon a red

corpuscle may easily be mistaken for a malarial parasite (see Frontispiece and Fig. 99).

Blood-platelets are being much studied at present, but, aside from the facts mentioned under their enumeration (see p. 258), little of clinical value has been learned. They have been variously regarded as very young red corpuscles (the "hematoblasts" of Hayem), as disintegration products of leukocytes, as remnants of extruded nuclei of erythrocytes, and as independent nucleated bodies. The most probable explanation of their origin seems to be that of J. H. Wright, who regards them as detached portions of the cytoplasm of certain giant-cells of the bone-marrow and spleen.

IX. BLOOD PARASITES

A. Bacteria

Bacteriologic study of the blood is useful in many conditions, but in general, the elaborate technic involved takes it out of the reach of the clinician. As applied to the diagnosis of typhoid fever, however, the technic of blood-cultures has been so simplified that it can be carried through by any one who is competent to do the simplest cultural work.

Typhoid bacilli can be detected in the blood in practically every case of typhoid fever in the first week of the disease; in about 80 to 85 per cent. of cases in the second week; and in decreasing percentages in the later weeks. The blood-culture, therefore, offers the most certain means of early diagnosis. It is in a sense complementary to the Widal reaction, the former decreasing and the latter increasing in reliability as the disease progresses.

The blood-culture gives best results before the Widal appears, as one would expect from the fact that the Widal test depends upon the presence of antibodies which destroy or, at least, injure the bacilli. The two methods together will establish the diagnosis in practically every case at any stage. Bacilli disappear from the blood in convalescence and reappear in a relapse.

Technic of Blood-cultures in Typhoid Fever.—The blood may be obtained in one of two ways:

(*a*) With a spring-lancet make a deep puncture in the edge (not the side) of the lobe of the ear, as for a blood-count. Allow the blood to drop directly into a short culture-tube containing the bile medium. By gentle milking, 20 to 40 drops can usually be obtained. This simple method of obtaining blood is especially applicable during the first week of the disease when bacilli are abundant. Contamination with skin cocci is possible, but does not usually interfere when the bile medium is used.

(*b*) In the later weeks of the disease a larger quantity of blood is needed. Prepare the skin on the front of the elbow as for a minor operation, or simply rub well with alcohol or paint with tincture of iodin. Tie a bandage tightly around the upper arm, have the patient open and close the fist a few times, and when the veins are sufficiently distended insert a hypodermic needle attached to a syringe into any vein that is prominent. The needle should go through the skin about $\frac{1}{4}$ inch from the vein with the bevel at its tip uppermost, and should enter the vein from the side in a direction opposite to the blood-current (Fig. 100). Unless too small a needle is used, blood will begin to rise in the syringe as soon as the needle has entered the vein. Suction is rarely necessary. When sufficient blood is obtained, the bandage is first removed, the needle is withdrawn, and the blood is allowed to

run into a tube of culture-medium. It is usually easy to secure 5 to 10 c.c. of blood. The procedure causes the patient surprisingly little inconvenience, seldom more than does an ordinary hypodermic injection. There is rarely any difficulty in entering the vein except in children, and in adults when the arm is fat and the veins are small. If desired, one of the veins about the ankle can be used. Instead of a syringe one can use a large glass tube which has been drawn out at the ends and one end ground to fit a "slip-on" needle. Either a large hypodermic needle or a small antitoxin needle may

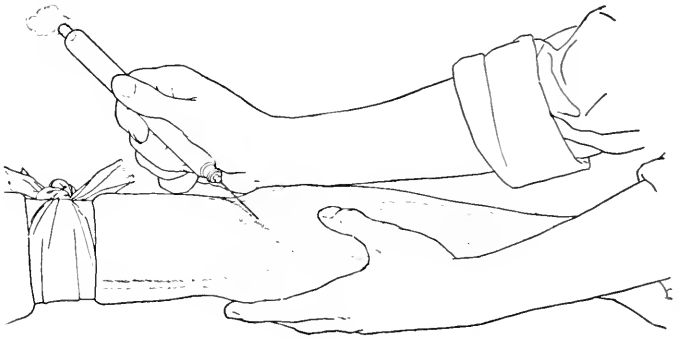


Fig. 100.—Method of obtaining blood for a blood-culture.

be used. These little instruments (Fig. 100) can be made by any glass-blower at a cost of about fifty cents, and several of them can be kept on hand in test-tubes sterilized ready for use.

As special culture-medium, ox-bile is generally used. It favors the growth of the typhoid bacillus and retards the growth of other organisms. A good formula is given on p. 463.

As soon as convenient after the blood is added, place the tubes in the incubator. After about twelve hours examine for motile bacilli. If none are found, transfer a few drops to tubes of bouillon or solidified blood-serum and incubate for

twelve hours longer. If motile, Gram-negative bacilli are found; they are almost certainly typhoid bacilli. Further study is, however, desirable, especially from a scientific point of view. The only bacilli which might cause confusion are the paratyphoid and colon bacilli, which can be distinguished by gas production in glucose media, indol production, and their effect upon litmus milk (see p. 462). The agglutination test for the identity of the bacillus is not available clinically, since freshly isolated bacilli do not agglutinate well.

B. Animal Parasites

Of the animal parasites which have been found in the blood, five are interesting clinically: the spirochete of relapsing fever; trypanosomes; malarial parasites; filarial larvæ; and the larvæ of *Trichinella spiralis*.

1. Spirochæta recurrentis is described on p. 373.

2. Trypanosoma gambiense.—Various trypanosomes are common in the blood of fishes, amphibians, birds, and mammals (see Fig. 115). They live in the blood-plasma and do not attack the corpuscles. In some animals they are apparently harmless; in others they are an important cause of disease. They are discussed more fully on p. 376.

The trypanosome of human blood, *Trypanosoma gambiense* (Plate VII), is an actively motile, spindle-shaped organism, two or three times the diameter of a red corpuscle in length, with an undulating membrane which terminates at the anterior end in a long flagellum. It can be seen with medium-power objectives in fresh blood, but is best studied with an oil-immersion lens in preparations stained as for the malarial parasite. It may be necessary to search many slides. Human trypanosomiasis is common in Africa. As a rule, it is a very

chronic disease. "Sleeping sickness" is a late stage when the organisms have invaded the cerebrospinal fluid. Infection is carried by the tsetse fly, *Glossina palpalis*.

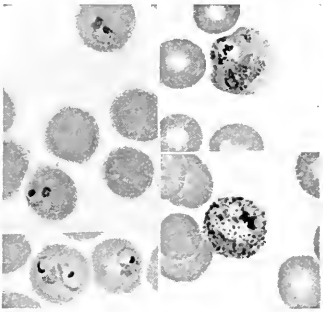
3. The Malarial Parasites.—These protozoa belong to the Sporozoa (see p. 381), order Hemosporidia, the members of which are parasites in the blood of a great variety of vertebrates. Three species, constituting the genus *Plasmodium*, are associated with malarial fever in man: *Plasmodium vivax*, *P. malariæ*, and *P. falciparum*, the parasites respectively of the tertian, quartan, and estivo-autumnal types of malaria.[1] The life histories of the three are so similar that they may well be described together.

(1) **Life Histories.**—There are two cycles of development: one, the *asexual*, in the blood of man; and the other, the *sexual*, in the intestinal tract of a particular genus of mosquito, *Anopheles*.

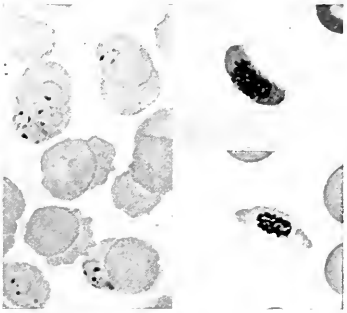
(*a*) *Asexual Cycle.*—The young organism enters the blood through the bite of the mosquito. It makes its way into a red corpuscle, where it appears as a small, pale, "hyaline" body. This body exhibits ameboid movement and increases in size. Soon dark-brown granules, derived from the hemoglobin of the corpuscle, make their appearance within it. When it has reached its full size —filling and distending the corpuscle in the case of the tertian parasite, smaller in the others—the pigment granules gather at the center or at one side; the organism divides into a number of small hyaline bodies, the spores or merozoites; and the red corpuscle bursts, setting spores and pigment free in the blood-plasma.

[1] A new species, occurring in India, has very recently been described by Stephens. The name *Plasmodium tenue* has been given.

Trypanosoma gambiense (slide presented by Professor F. G. Novy).



Tertian malarial parasites, one red cell showing malarial stippling.



Estivo-autumnal malarial parasites, small ring forms and crescents.

Spirochæta novyi.

Animal parasites of the blood; × 1000 (photographs by the author).

This is called segmentation. It coincides with, and by liberation of toxins causes, the paroxysm of the disease. A considerable number of the spores are destroyed by leukocytes or other agencies; the remainder enter other corpuscles and repeat the cycle. Many of the pigment granules are taken up by leukocytes. Migration of young parasites from corpuscle to corpuscle between paroxysms has recently been noted. In estivo-autumnal fever segmentation occurs in the internal organs and the segmenting and larger pigmented forms are not seen in the peripheral blood.

The asexual cycle of the tertian organism occupies forty-eight hours; of the quartan, seventy-two hours; of the estivo-autumnal, an indefinite time—usually twenty-four to forty-eight hours.

The parasites are thus present in the blood in great groups, all the individuals of which reach maturity and segment at approximately the same time. This explains the regular recurrence of the paroxysms at intervals corresponding to the time occupied by the asexual cycle of the parasite. Not infrequently there is multiple infection, one group reaching maturity while the others are still young; but the presence of two groups which segment upon the same day is extremely rare. Fevers of longer intervals—six, eight, ten days—are probably due to the ability of the body, sometimes of itself, sometimes by aid of quinin, to resist the parasites, so that numbers sufficient to cause a paroxysm do not accumulate in the blood until after several repetitions of the asexual cycle. In estivo-autumnal fever the regular grouping, while usually present at first, is soon lost, thus causing "irregular malaria."

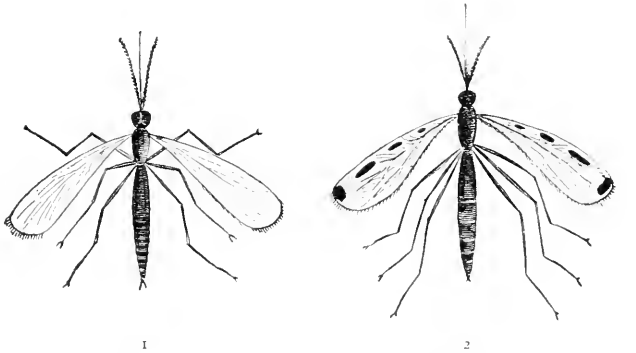
Bass has recently succeeded in cultivating the malarial parasite outside of the body.

(*b*) *Sexual Cycle.*—Besides the ameboid individuals which pass through the asexual cycle, there are present with them in the blood many individuals with sexual properties. These are called *gametes*. The female is a little the larger. The gametes do not undergo segmentation, but grow to adult size and remain inactive in the blood until taken up by a mosquito. Many of them are apparently extracellular, but stained preparations usually show them to be surrounded by the remains of a corpuscle. In tertian and quartan malaria they cannot easily be distinguished from the asexual individuals until a variable time after the blood leaves the body, when the male gamete sends out one or more flagella. In estivo-autumnal malaria the gametes take distinctive ovoid and crescentic forms, and are not difficult to recognize. They are very resistant to quinin and often persist in the blood long after the ameboid forms have been destroyed, but are probably incapable of continuing the disease until they have passed through the cycle in the mosquito.

When a malarious person is bitten by a mosquito, the gametes are taken with the blood into its stomach. Here the male sends out one or more flagella. These break off and seek out the females, which they penetrate much as the sperm penetrates the ovum. The female soon thereafter becomes encysted in the wall of the intestine. After a time it ruptures, liberating many minute rods, or sporozoites, which have formed within it. These migrate to the salivary glands, and are carried into the blood of the person whom the mosquito bites. Here they

enter red corpuscles as young malarial parasites, and the majority pass through the asexual cycle just described.

The sexual cycle can take place only within the body of the female of one genus of mosquito, *Anopheles*. The male does not bite. Absence of this mosquito from certain districts explains the absence of malaria. It is distinguished from our common house-mosquito, *Culex*, by the relative lengths of proboscis and palpi (Fig. 101), which can be seen with a hand-lens, by its attitude when



1 2

Fig. 101.—Mosquitoes: 1, *Culex;* 2, *Anopheles* (Bergey).

resting, and by its dappled wing (Fig. 102). Anopheles is strictly nocturnal in its habits; it usually flies low, and rarely travels more than a few hundred yards from its breeding-place, although it may be carried by winds. These facts explain certain peculiarities in malarial infection; thus, infection occurs practically only at night; it is most common near stagnant water, especially upon the side toward which the prevailing winds blow; and the danger is greater when persons sleep upon or near the ground than in upper stories of buildings. The insects

frequently hibernate in warmed houses, and may bite during the winter. A mosquito becomes dangerous in eight to fourteen days after it bites a malarious person, and remains so throughout its life.

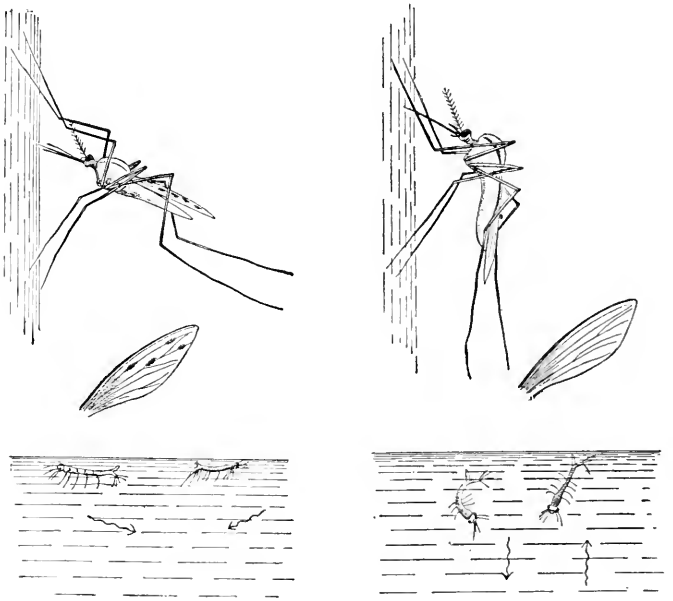


Fig. 102.—Showing, on the left, *Anopheles* in resting position, its dappled wing, and the position of its larvæ in water; on the right, *Culex* in resting position, its plain wing, and the position of its larvæ in water. The arrows indicate the directions taken by the larvæ when the water is disturbed (Abbott).

(2) **Detection.**—Search for the malarial parasite may be made in either fresh blood or stained films. If possible, the blood should be obtained a few hours before the chill—never during it nor within a few hours afterward, since at that time (in single infections) only the very young, unpigmented forms are present, and these

are the most difficult to find and recognize. Sometimes many parasites are found in a microscopic field; sometimes, especially in estivo-autumnal infection, owing to accumulation in internal organs, careful search is required to find any, despite very severe symptoms. Quinin causes them rapidly to disappear from the peripheral blood, and few or none may be found after its administration. In the absence of organisms, the presence of pigment granules within leukocytes—especially the large mononuclears—may be taken as presumptive evidence of malaria. Pigmented leukocytes (see Frontispiece) are most numerous after a paroxysm.

(*a*) *In Fresh Unstained Blood* (Plate VIII).—Obtain a small drop of blood from the finger or lobe of the ear. Touch the center of a cover-glass to the top of the drop and quickly place it, blood side down, upon a slide. If the slide and cover be perfectly clean and the drop not too large, the blood will spread out so as to present only one layer of corpuscles. Search with an oil-immersion objective, using very subdued light. The preparation may be kept for many hours if ringed with vaselin or melted paraffin.

The young organisms appear as small, round, ring-like or irregular, colorless bodies within red corpuscles. The light spots caused by crenation and other changes in the corpuscles are frequently mistaken for them, but are generally more refractive or have more sharply defined edges. The older forms are larger colorless bodies containing granules of brown pigment. In the case of the tertian parasite, these granules have active vibratory motion, which renders them conspicuous; and as the parasite itself is very pale, one may see only a large pale

corpuscle in which fine pigment granules are dancing. Segmenting organisms, when typic, appear as rosets, often compared to daisies, the petals of which represent the segments, while the central brown portion represents the pigment. Tertian segmenting forms are less frequently typic than quartan. Flagellated forms are not seen until ten to twenty minutes after the blood has left the vessels. As Cabot suggests, one should, while searching, keep a sharp lookout for unusually large or pale corpuscles, and for anything which is brown or black or in motion.

(*b*) *In Stained Films* (See Frontispiece and Plate VII). —Recognition of the parasite, especially the young forms, is much easier in films stained by Wright's or some similar stain than in fresh blood. The films must be well stained. It is useless to search preparations in which the nuclei of leukocytes are very pale. When parasites are very scarce, they may sometimes be found, although their structure is not well shown by the method of Ruge. This consists in spreading a very thick layer of blood, drying, placing for a few minutes in a fluid containing 5 per cent. formalin and 1 per cent. acetic acid, which removes the hemoglobin and fixes the smear, rinsing, drying, and finally staining. Carbol-thionin is very useful for this purpose. If Wright's stain be used in this method, it is recommended that the preparation be subsequently stained for a half-minute with borax-methylene-blue (borax, 5; methylene-blue, 2; water, 100).

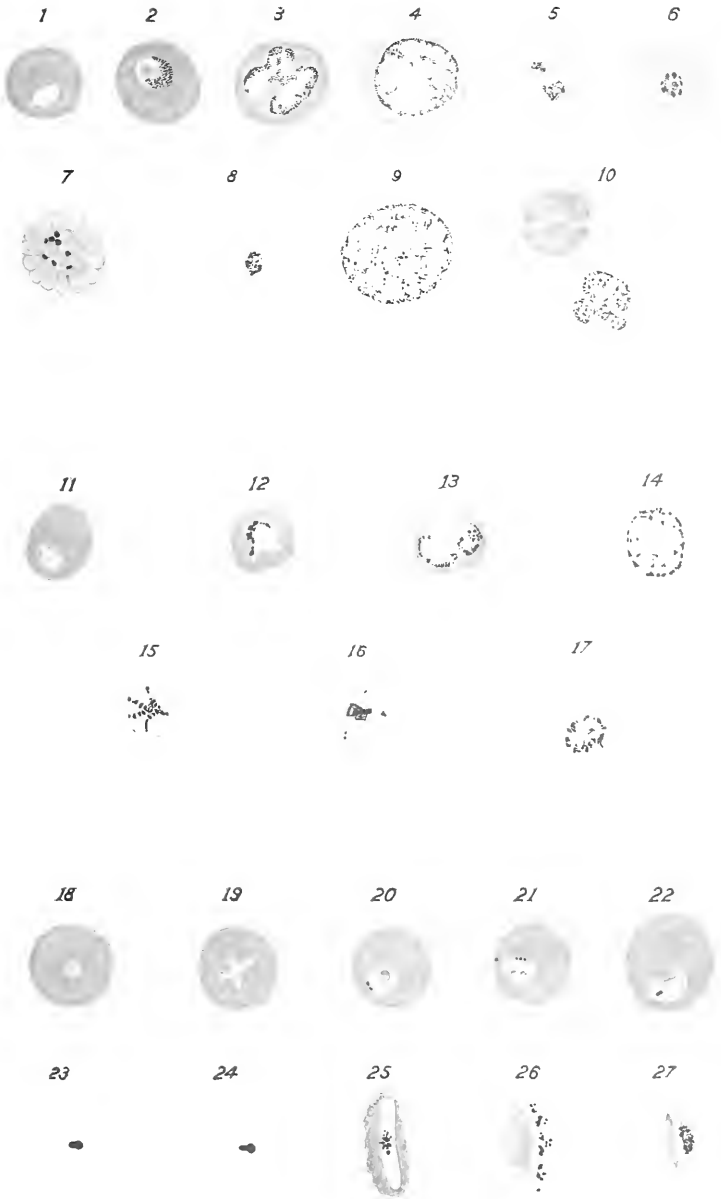
In films which are properly stained with Wright's fluid the young organisms are small, round, ring-like or irregular, sky-blue bodies, each with a very small, sharply defined, reddish-purple chromatin mass. Many structures

Explanation of Plate VIII

Various forms of malarial parasites (unstained) (Thayer and Hewetson).

1 to 10, inclusive, Tertian organisms; 11 to 17, inclusive, quartan organisms; 18 to 27, inclusive, estivo-autumnal organisms; 1, young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, extracellular pigmented form; 10, flagellate form; 11, young hyaline form; 12, 13, pigmented forms; 14, fully developed pigmented form; 15, 16, segmenting forms; 17, flagellate form; 18, 19, 20, ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

PLATE VIII



—deposits of stain, dirt, blood-plaques lying upon red cells (see Fig. 99), etc.—may simulate them, but should not deceive one who looks carefully for both the blue cytoplasm and the reddish-purple chromatin. A plaque upon a red corpuscle is surrounded by a colorless zone rather than by a distinct blue body. Young estivo-autumnal parasites commonly take a "ring" form (the chromatin mass representing the jewel), which is infrequently assumed by the other varieties. The older tertian and quartan organisms show larger sky-blue bodies with more reticular chromatin, and contain brown granules of pigment, which, however, is less evident than in the living parasite. The chromatin is often scattered through the cytoplasm or apparently outside of it, and is sometimes difficult to see clearly. Typical "segmenters" present a ring of rounded segments or spores, each with a small, dot-like chromatin mass. With the tertian parasite, the segments more frequently form an irregular cluster. The pigment is collected near the center or scattered among the segments. In estivo-autumnal fever usually only the small "ring bodies" and the crescentic and ovoid gametes are seen in the blood. The gametes are easily recognized. Their length is somewhat greater than the diameter of a red corpuscle. Their chromatin is usually centrally placed, and they contain more or less coarse pigment. The remains of the red cell often form a narrow rim around them or fill the concavity of the crescent.

While the parasites are more easily found in stained preparations, the varieties are more easily differentiated in fresh blood. The chief distinguishing points are included in the table on page 304.

VARIETIES OF THE MALARIAL ORGANISM

Tertian.	Quartan.	Estivo-autumnal.
Asexual cycle, forty-eight hours.	Seventy-two hours.	Usually twenty-four to forty-eight hours.
Substance pale, transparent, comparable to hyaline tube-cast.	Highly refractive, comparable to waxy tube-cast.	Highly refractive.
Outline indistinct.	Distinct.	Distinct.
Ameboid motion active.	Sluggish.	Active.
Mature asexual form large; fills and often distends corpuscle.	Smaller.	Young forms, only, in peripheral blood.
Pigment-granules fine, brown, scattered throughout. Very active dancing motion.	Much coarser, darker in color, peripherally arranged. Motion slight.	Very few, minute, inactive. Distinctly pigmented forms seldom seen.
Segmenting body rarely assumes typical "daisy" form. 15 to 20 segments.	Usually typical "daisy." 6 to 12 segments.	Not seen in peripheral blood.
Gametes resemble asexual forms.	Same as tertian.	Appear in blood as distinctive ovoids and crescents.
Red corpuscles pale and swollen.	Generally darker than normal.	Dark, often bronzed.

4. Filarial Larvæ.—A description of the filariæ whose larvæ appear in the blood will be found on p. 356.

The embryos can be seen in stained preparations (Fig. 103), but are best found in fresh unstained blood. A rather large drop is taken upon a slide, covered, and examined with a low power. The embryo can be located by the commotion which its active motion produces

among the corpuscles. This motion consists almost wholly in apparently purposeless lashing and coiling movements, and continues for many hours.

5. Larvæ of Trichinella spiralis.—The worm and its life-history are described on page 414. In 1909 Herrick and Janeway demonstrated that diagnosis of trichiniasis can frequently be made by detection of the larvæ in the blood during their migration to the muscles. Of the examinations which have been reported

Fig. 103.—Filarial larvæ in blood. Stained. Red corpuscles decolorized; a few leukocytes remain (×200) (photographs by the author).

since that time, about one-half have been positive. The earliest time at which the embryos were found was the sixth day after the onset of symptoms; the latest, the twenty-second day.

The method is very simple. One to 10 c.c. of blood are obtained from the ear or a vein, as described on page 293, and mixed with ten times its volume of 3 per cent. acetic acid. The mixture is centrifugalized, and large drops of the sediment are placed on slides, covered, and searched with a low-power objective. The larvæ are

not difficult to recognize. They are about 125 μ long and 6 μ broad.

X. TESTS FOR RECOGNITION OF BLOOD

The recognition of red blood-corpuscles microscopically is the surest and simplest means of detecting the presence of blood. In most pathologic material, however, the corpuscles are too much degenerated for recognition with the microscope, and one has to rely upon a test for hemoglobin or its derivatives. Of such tests, the two given in this section are probably the best. Each is reliable within its own sphere, but each has its limitation. The guaiac test is reliable only when *negative*. The same is true of its modifications, such as the benzidin test. The hemin test is reliable only when *positive*. When, however, proper care is taken to exclude fallacies, the guaiac test and its modifications are the most useful and reliable tests for clinical purposes, although they could not be accepted medicolegally.

The only reliable test for human blood as distinguished from that of animals is the precipitin test described on p. 503.

1. Guaiac Test.—The technic of this test has been given (see p. 157). It may be applied directly to a suspected fluid or, better, to the ethereal extract. Add a few cubic centimeters of glacial acetic acid to about 10 c.c. of the fluid; shake thoroughly with an equal volume of ether; decant, and apply the test to the ether. Jager states that the test is rendered much more sensitive if a few drops of ammonia or sodium hydroxid solution be added to the ether extract. In case of dried stains

upon cloth, wood, etc., dissolve the stain in distilled water and test the water, or press a piece of moist blotting-paper against the stain, and touch the paper with drops of the guaiac and the turpentine successively. The test may be applied to microscopic particles by running the reagents under the cover-glass.

The benzidin test (see p. 157) is similar to the guaiac test and has the same fallacies, but is said to be more sensitive.

Fig. 104.—Teichmann's hemin crystals (Jakob).

2. Teichmann's Test.—This depends upon the production of characteristic crystals of *hemin*. It is a sensitive test and, when positive, is absolute proof of the presence of blood. A number of substances—lime, fine sand, iron rust—interfere with production of the crystals; hence negative results are not always conclusive. Dissolve the suspected stain in a few drops of normal salt solution upon a slide. If a liquid is to be tested, evaporate some of it upon a slide and dissolve the residue in a few drops of the salt solution. Let dry, apply a

cover-glass, and run glacial acetic acid underneath it. Heat *very gently* until bubbles begin to form, replacing the acid as it evaporates. Allow to cool slowly. When cool, replace the acid with water, and examine for hemin crystals with 16-mm. and 4-mm. objectives. The crystals are dark-brown rhombic plates, lying singly or in crosses, and easily recognized (Fig. 104). Failure to obtain them may be due to too much salt, too great heat, or too rapid cooling. If not obtained at first, let the slide stand in a warm place, as upon a hot-water radiator, for an hour.

XI. SPECIAL BLOOD PATHOLOGY

The more conspicuous characteristics of the blood in various diseases have been mentioned in previous sections. Although the great majority of blood changes are secondary, there are a few blood conditions in which the changes are so prominent, or the etiology so obscure, that they are commonly regarded as blood diseases. These will receive brief consideration here. They fall into two general groups. In the one group (Anemia) the red cells and hemoglobin are chiefly affected; in the other (Leukemia) changes in the leukocytes constitute the conspicuous feature of the blood-picture.

A. Anemia

This is a deficiency of hemoglobin or red corpuscles, or both. It is either primary or secondary. The distinction is based chiefly upon etiology, although each type presents a more or less distinctive blood-picture. Secondary anemia is that which is symptomatic of some

other pathologic condition. Primary anemia is that which progresses without apparent cause.

1. Secondary Anemia.—The more important conditions which produce secondary or symptomatic anemia are:

(*a*) *Poor nutrition*, which usually accompanies unsanitary conditions, poor and insufficient food, etc.

(*b*) *Acute infectious diseases*, especially rheumatism and typhoid fever. The anemia is more conspicuous during convalescence.

(*c*) *Chronic Infectious Diseases.*—Tuberculosis, syphilis, leprosy.

(*d*) *Chronic exhausting diseases*, as heart disease, chronic nephritis, cirrhosis of the liver, and gastrointestinal diseases, especially when associated with atrophy of gastric and duodenal glands. The last may give an extreme anemia, indistinguishable from pernicious anemia.

(*e*) *Chronic poisoning*, as from lead, arsenic, and phosphorus.

(*f*) *Hemorrhage.*—Either repeated small hemorrhages (chronic hemorrhage), as from gastric cancer and ulcer, hemorrhoids, uterine fibroids, etc., or acute hemorrhage, such as may occur in typhoid fever, tuberculosis, or traumatism.

(*g*) *Malignant Tumors.*—These affect the blood partly through repeated small hemorrhages, partly through toxic products, and partly through interference with nutrition.

(*h*) *Animal Parasites.*—Some cause no appreciable change in the blood; others, like the hookworm and *Dibothriocephalus latus*, may produce a very severe

anemia, almost identical with pernicious anemia. Anemia in these cases is probably due both to toxins and to abstraction of blood. In malaria the parasites themselves directly destroy the red cells.

The blood-picture varies with the grade of anemia. Diminution of hemoglobin is the most characteristic feature. In mild cases it is slight, and is the only blood change to be noted. In very severe cases hemoglobin may fall to 15 per cent. or even lower. Red corpuscles are diminished in all but very mild cases, while in the severest cases the red corpuscle count is sometimes below 2,000,000. The color-index is usually decreased.

Although the number of leukocytes bears no relation to the anemia, leukocytosis is common, being due to the same cause.

Stained films show no changes in very mild cases. In moderate cases variations in size and shape of the red cells and polychromatophilia occur. Very severe cases show the same changes to greater degree, with addition of basophilic degeneration and the presence of normoblasts in small or moderate numbers. Megaloblasts in very small numbers have been encountered in extremely severe cases. They are especially abundant and may even predominate over the normoblasts in dibothriocephalus infection. Blood-plaques are usually increased.

Posthemorrhagic Anemia.—Within a few hours after an acute hemorrhage the volume of blood is nearly or quite restored by means of fluids from the tissues. Owing to the fact that some destruction of red corpuscles continues for a time, the anemia is most marked a few days after the hemorrhage. Hemoglobin and red

cells are diminished according to the amount of blood lost. The color-index is moderately low. There is moderate leukocytosis. Some of the red cells may show polychromatophilia and a few normoblasts may be found. In some cases great numbers of normoblasts appear rather suddenly—a so-called **blood crisis.** Normal conditions may be restored within a few weeks, although the color-index is apt to remain low for some time thereafter.

2. Primary Anemia.—The commonly described varieties of primary anemia are pernicious anemia and chlorosis, but splenic anemia may also be mentioned under this head.

(1) **Progressive Pernicious Anemia.**—It is frequently impossible to diagnose this disease from the blood examination alone. Severe secondary anemia, especially that due to gastro-intestinal cancer, intestinal parasites, and repeated small hemorrhages, sometimes gives an identical picture. Remissions, in which the blood approaches the normal, are common. All the clinical data must, therefore, be considered, together with a careful analysis of repeated blood examinations.

The disease is characterized by active destruction of red corpuscles with excessive activity of the erythroblastic bone-marrow, and the appearance of immature and abnormal red cells in the circulation.

Hemoglobin and red corpuscles are always greatly diminished. Several counts in which the red cells were below 150,000 have been recorded. In none of Cabot's 139 cases did the count exceed 2,500,000, the average being about 1,200,000. In more than two-thirds of the cases hemoglobin was reduced to less

extent than the red corpuscles; the color-index was, therefore, high. A low color-index probably indicates a mild type of the disease. The average hemoglobin value is about 25 to 30 per cent.

The leukocyte count may be normal, but is commonly diminished to about 3000, and is sometimes much lower.

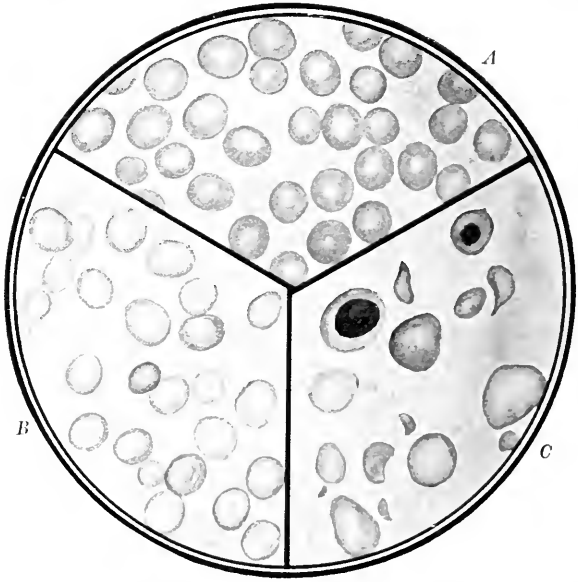


Fig. 105.—*A*, Normal blood; *B*, chlorosis; *C*, pernicious anemia. The plate shows the sharp contrast between cells rich in hemoglobin and the pale cells of chlorosis, and also the poikilocytes and marked variations in size noted in pernicious anemia. A normoblast and megaloblast also appear. Stained smears (from Greene's "Medical Diagnosis").

The decrease affects chiefly the polymorphonuclear cells, so that the lymphocytes are relatively increased. In some cases a decided absolute increase of lymphocytes occurs. Polymorphonuclear leukocytosis, when present, is due to some complication.

The red corpuscles show marked variation in size and

Explanation of Plate IX

Fig. 1.—Preparation from an advanced case of progressive pernicious anemia from unknown cause: *a*, Megaloblasts or gigantoblasts; the protoplasm shows marked polychromasia; *b*, stained granules in erythrocytes with normally stained protoplasm; *c* and *d*, polychromatophilic degeneration; *e*, megalocytes; *f*, normocytes.

Fig. 2.—Preparation from the same case taken some time later while the patient was subjectively and objectively in perfect health: *a*, Punctate erythrocytes with normal and anemic degenerated protoplasm; *b*, polynuclear leukocyte; *c*, normal red blood-corpuscles; *d*, somewhat enlarged erythrocytes.

Fig. 3.—Series of cells from a case of severe progressive pernicious anemia of unknown etiology; preparation made two days antemortem: *a*, Nucleated red blood-corpuscles characterized as normoblasts by the intense staining of the nuclei; *a'* and *a''*, karyokinetic figures in erythrocytes; the protoplasm finely punctate; *b*, beginning karyolysis in a megaloblast; *c*, erythroblasts with coarse granulation of the protoplasm; *d*, nuclear remains (?) and fine granulation of the protoplasm; *e* and *f*, finely punctate red blood-corpuscles; *g*, megalocyte with two blue nuclei; nuclear remains (?) in the polychrome protoplasm.

(Nothnagel-Lazarus.)

PLATE IX

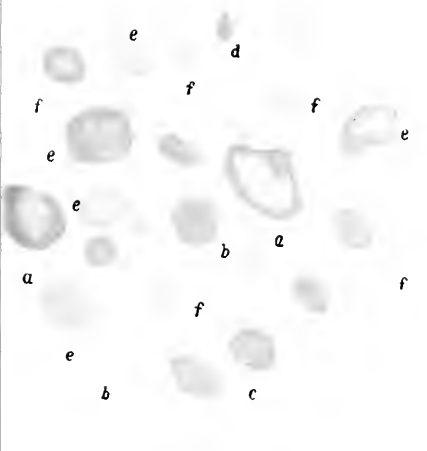


Fig. 1.

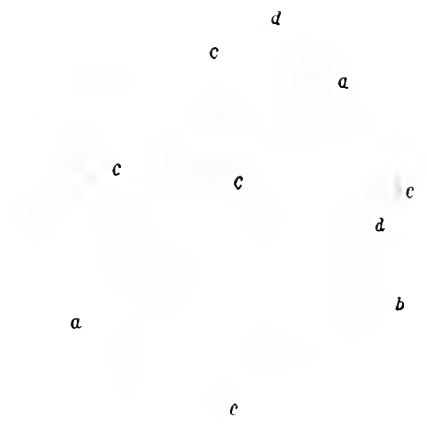


Fig. 2.

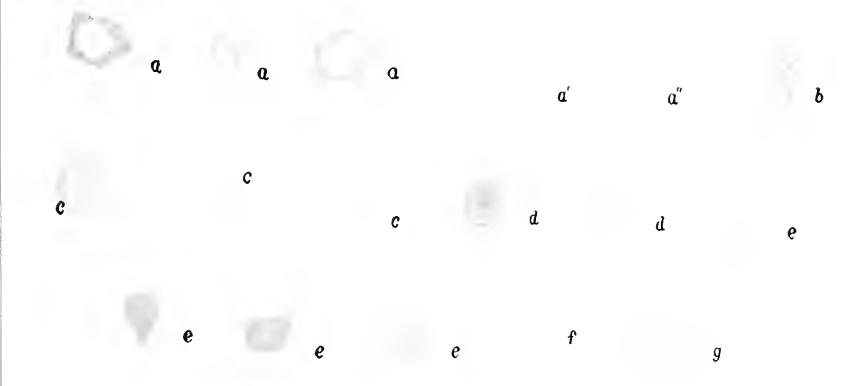


Fig. 3.

shape (Plate IX and Fig. 105). There is a decided tendency to large oval forms, and, despite the presence of microcytes, the average size of the corpuscles is generally strikingly increased. Polychromatophilia and basophilic degeneration are common. Nucleated red cells are always present, although *in many instances careful search is required to find them.* In the great majority of cases megaloblasts exceed normoblasts in number. This ratio is practically unknown in other diseases. Blood-plaques are diminished.

The chief points to be considered in diagnosis are the high color-index and the presence of megaloblasts.

The rare and rapidly fatal anemia which has been described under the name of **aplastic anemia** is probably a variety of pernicious anemia. Absence of any attempt at blood regeneration explains the marked difference in the blood-picture. Red corpuscles and hemoglobin are rapidly diminished to an extreme degree. The color-index is normal or low. The leukocyte count is normal or low, with relative increase of lymphocytes. Stained smears show only slight variations in size, shape, and staining properties of the red cells. There are no megaloblasts and few or no normoblasts.

(2) **Chlorosis.**—This is probably a disease of defective blood formation. It is confined almost exclusively to unmarried girls. The clinical symptoms furnish the most important data for diagnosis. The blood resembles that of secondary anemia in many respects.

The most conspicuous feature is a *marked* decrease of hemoglobin, accompanied by a *slight* decrease in number of red corpuscles. The color-index is thus almost invariably low.

The following figures represent about the average for well-marked cases: hemoglobin, 40 per cent.; red corpuscles, 4,000,000; color-index, 0.5. Much lower figures are frequent; while, upon the other hand, mild cases may show no loss at all in number of red cells.

As in pernicious anemia, the leukocytes are normal or decreased in number, with a relative increase of lymphocytes.

In contrast to pernicious anemia (and in some degree also to secondary anemia), the red cells are of nearly uniform size, are pale (see Fig. 105), and their average diameter is somewhat less than normal. Changes in size, shape, and staining reactions occur only in severe cases. Erythroblasts are rarely present. The number of plaques is generally decreased.

(3) **Splenic Anemia.**—This is an obscure form of anemia associated with great enlargement of the spleen. It is probably a distinct entity, although several types may exist. There is decided decrease of hemoglobin and red corpuscles, with moderate leukopenia and relative lymphocytosis. Osler's 15 cases averaged 47 per cent. hemoglobin and 3,336,357 red cells. Stained films show notable irregularities in size, shape, and staining properties only in advanced cases. Erythroblasts are uncommon.

B. Leukemia

Except in rare instances, diagnosis is easily made from the blood alone, usually at the first glance at the stained film. Two types of the disease are commonly distinguished: the *myelogenous* and the *lymphatic*. Atypical forms are not uncommon. The disease is

characterized by hyperplasia of the leukoblastic bone-marrow (myelogenous leukemia) or of the lymphoid tissues (lymphatic leukemia), together with overflow of many immature leukocytes and excessive numbers of normal types into the circulating blood. The more acute the process, the more immature are the cells which appear in the blood.

1. Myelogenous Leukemia (Plate X).—This is usually a chronic disease, although acute cases have been described.

Hemoglobin and red corpuscles show decided decrease. The red count is usually below 3,500,000. Accurate hemoglobin estimation is difficult because of the great number of leukocytes. The color-index is moderately low.

Most striking is the immense increase in number of leukocytes. The count in ordinary cases varies between 100,000 and 400,000. Counts over 1,000,000 have been met. During spontaneous remissions, during treatment with *x*-ray or benzol, and during intercurrent infections the leukocyte count may fall to normal.

While these enormous leukocyte counts are equaled in no other disease, and approached only in lymphatic leukemia and extremely high-grade leukocytosis, the diagnosis, particularly during remissions, depends more upon qualitative than quantitative changes. Although all varieties are increased, the characteristic and conspicuous cell is the myelocyte. This cell never appears in normal blood; extremely rarely in leukocytosis; and never abundantly in lymphatic leukemia. In myelogenous leukemia myelocytes usually constitute more than 20 per cent. of all leukocytes. Da Costa's lowest case

gave 7 per cent. The neutrophilic form is generally much more abundant than the eosinophilic. Both show considerable variations in size. Myeloblasts may be present in small numbers at any stage, and in the terminal stages they may be abundant. An increase in their number is of grave significance. Very constant in myelogenous leukemia is a marked absolute, and often a relative, increase of eosinophiles and basophiles. Polymorphonuclear neutrophiles and lymphocytes are absolutely increased, although relatively decreased.

The red cells show the changes characteristic of a severe secondary anemia, except that nucleated reds are commonly abundant; in fact, no other disease gives so many. They are chiefly of the normoblastic type. Megaloblasts are uncommon. Blood-plaques are generally increased.

In **acute myelogenous leukemia** the myeloblast may be the predominant cell, and the blood will then resemble that of acute lymphatic leukemia. The myeloblast can be distinguished from the large lymphocyte by the oxydase reaction (see p. 290) although cases occur in which the type of blood formation is so embryonic that the oxydase reaction fails.

2. Lymphatic Leukemia (Plate X).—**Chronic Form.**—There is generally greater loss of hemoglobin and red corpuscles than in myelogenous leukemia. The color-index is usually moderately low.

The leukocyte count is high, but lower than in the myelogenous type. Counts of 100,000 to 200,000 are about the average, but in many cases are much lower. This high count is referable almost wholly to increase of lymphocytes. They generally exceed 90 per cent.

PLATE X

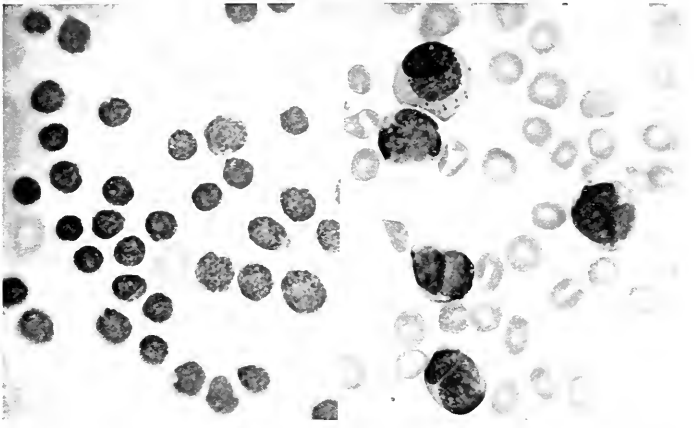


Fig. 1.—Blood in lymphatic leukemia; × 700. On the left, chronic form of the disease; on the right, acute form (courtesy of Dr. W. P. Harlow).

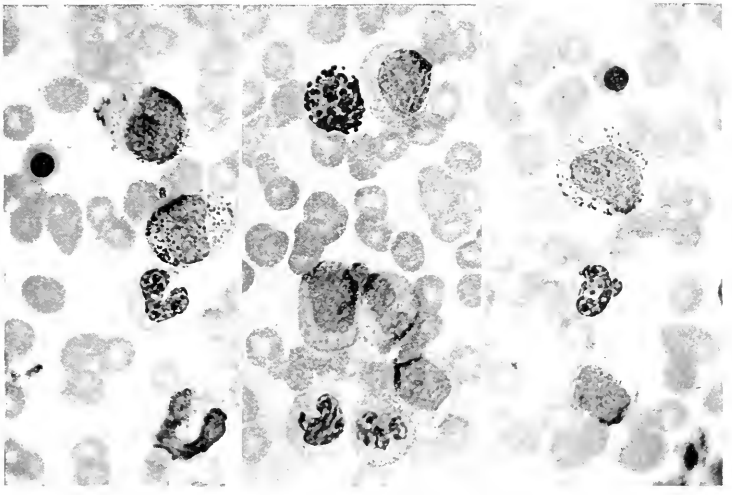


Fig. 2.—Blood in splenomyelogenous leukemia. Wright's stain. × 700 (photographs by the author).

of the total number and are chiefly of the small variety. During remissions the total count may fall to below normal, but the percentage of lymphocytes remains high. Myelocytes are rare.

The red corpuscles show the changes usual in severe secondary anemia. Erythroblasts are seldom abundant. Blood-plaques are decreased.

Acute Form.—The blood is similar to that of the chronic variety. The total leukocyte count is seldom so high, and the large type of lymphocyte predominates in most cases. The anemia is apt to be more severe and the normoblasts more abundant.

3. Anæmia Infantum Pseudoleukæmica.—Under this name von Jaksch described a rare disease of infancy, the proper classification of which is uncertain. There is enlargement of liver and spleen, and sometimes of lymph-nodes, together with the following blood changes: grave anemia with deformed and degenerated red cells and many erythroblasts of both normoblastic and megaloblastic types; great increase in number of leukocytes (20,000 to 100,000) and great variations in size, shape, and staining of leukocytes, with many atypic forms, and a few myelocytes.

From the work of more recent investigators it appears probable that von Jaksch's anemia is not a distinct disease, and that the reported cases have been atypical forms of leukemia, pernicious anemia, or even secondary anemia with leukocytosis. As is well known, all of these conditions are apt to be atypical in children.

The table on the following page contrasts the distinctive blood-changes in the more common conditions.

DIFFERENTIAL DIAGNOSIS OF BLOOD DISEASES

	Hemoglobin.	Red Corpuscle Count.	Color-index.	Leukocyte Count.	Stained Films.	
					Red Corpuscles.	Leukocytes.
Secondary anemia.	Diminished according to degree of anemia.	Normal in mild cases; diminished in all others.	Normal or slightly diminished.	Not necessarily affected; leukocytosis common.	Variations in size and shape in moderate cases; variations in staining reactions and normoblasts in severe cases.	Normal proportions or increase of polynuclears.
Pernicious anemia.	Diminished	Greatly diminished.	High.	Normal or diminished.	Marked variations in size, shape, and staining reactions. Average size increased. Tendency to large oval forms. Erythroblasts always present; megaloblasts exceed normoblasts.	Lymphocytes relatively, sometimes absolutely, increased.
Chlorosis.	Greatly diminished.	Slightly diminished.	Low.	Normal or diminished.	Nearly uniform size and shape; average size decreased; pale centers. Erythroblasts very rare.	Lymphocytes apt to be relatively increased.
Myelogenous leukemia.	Decidedly diminished.	Decidedly diminished.	Usually slightly diminished.	Extremely high.	Similar to secondary anemia, except normoblasts generally very numerous.	Large numbers of myelocytes (average, 20 per cent.). Absolute increase of eosinophiles and basophiles. Relative decrease of polynuclears and lymphocytes.
Lymphatic leukemia.	Markedly diminished.	Markedly diminished.	Usually slightly diminished.	Very high.	Similar to secondary anemia. Erythroblasts not numerous.	Lymphocytes exceed 90 per cent. Other varieties relatively decreased.

CHAPTER IV

THE STOMACH

LABORATORY methods may be applied to the diagnosis of stomach disorders in: I. Examination of the gastric contents removed with the stomach-tube. II. Certain other examinations which give information as to the condition of the stomach.

I. EXAMINATION OF THE GASTRIC CONTENTS

Stomach digestion consists mainly in the action of pepsin upon proteins in the presence of hydrochloric acid and in the curdling of milk by rennin. The fat-splitting ferment, lipase, of the gastric juice has very little activity under normal conditions of acidity.

Pepsin and rennin are secreted by the gastric glands as zymogens—pepsinogen and renninogen respectively—which are converted into pepsin and rennin by hydrochloric acid. Hydrochloric acid is secreted by certain cells of the fundus glands. It at once combines loosely with the proteins of the food, forming acid-metaprotein, the first step in protein digestion. Hydrochloric acid, which is thus loosely combined with proteins, is called "combined" hydrochloric acid. The acid which is secreted after the proteins present have all been converted into acid-metaprotein remains as "free" hydrochloric acid, and, together with pepsin, continues the process of digestion.

At the height of digestion the stomach-contents consist essentiallly of: (1) Water; (2) free hydrochloric acid; (3) combined hydrochloric acid; (4) pepsin; (5) rennin; (6) mineral salts, chiefly acid phosphates, of no clinical importance; (7) particles of undigested and partly digested food; (8) various products of digestion in solution. In pathologic conditions there may be present, in addition, various microscopic structures and certain organic acids, of which lactic acid is most important.

A **routine examination** is conveniently carried out in the following order:

(1) Give the patient a test-meal upon an empty stomach, washing the stomach previously if necessary.

(2) At the height of digestion, usually in one hour, remove the contents of the stomach with a stomach-tube.

(3) Measure and examine macroscopically.

(4) Filter. A suction filter is desirable, and may be necessary when much mucus is present.

(5) During filtration, examine microscopically and make qualitative tests for—(*a*) free acids; (*b*) free hydrochloric acid; (*c*) lactic acid.

(6) When sufficient filtrate is obtained, make quantitative estimations of—(*a*) total acidity; (*b*) free hydrochloric acid; (*c*) combined hydrochloric acid (if necessary).

(7) Make whatever additional tests seem desirable, as for blood, pepsin, or rennin.

A. Obtaining the Contents

Gastric juice is secreted continuously, but quantities sufficiently large for examination are not usually obtainable from the fasting stomach. In clinical work, therefore, it is desirable to stimulate secretion with food—

which is the natural and most efficient stimulus—before attempting to collect the gastric fluid. Different foods stimulate secretion to different degrees, hence for the sake of uniform results certain standard "test-meals" have been adopted.

1. Test-meals.—It is customary to give the test-meal in the morning, since the stomach is most apt to be empty at that time. If it be suspected that the stomach will not be empty, it should be washed out with water the evening before.

(1) **Ewald's test-breakfast** consists of a roll (or two slices of bread), without butter, and two small cups (300 to 400 c.c.) of water, or weak tea, without cream or sugar. It should be well masticated. The contents of the stomach are to be removed one hour afterward, counting from the beginning, not the end, of the meal. This test-meal has long been used for routine examinations. Its disadvantage is that it introduces, with the bread, a variable amount of lactic acid and numerous yeast-cells. This source of error may be eliminated by substituting a shredded whole-wheat biscuit for the roll. The shredded wheat test-meal is now widely used and is probably the most satisfactory for general purposes.

(2) **Boas' test-breakfast** consists of a tablespoonful of rolled oats in a quart of water, boiled to one pint, with a pinch of salt added. It should be withdrawn in forty-five minutes to one hour. This meal does not contain lactic acid, and is usually given when the detection of lactic acid is important, as in suspected gastric cancer. The stomach should always be washed with water the evening previous.

(3) **Riegel's test-meal** consists of 400 c.c. of bouillon,

a broiled beefsteak (about 150–200 gm.), and 150 gm. of mashed potato. Since it tends to clog the tube, it must be thoroughly masticated.

(4) **Fischer's test-meal** is similar, but probably preferable. It consists of an Ewald breakfast plus about ¼ pound lean, finely chopped Hamburger steak, broiled, and lightly seasoned. This and Riegel's may be removed in three to four hours. They give somewhat higher acidity values than the Ewald breakfast.

2. Withdrawal of the Contents.—The Boas stomach-tube, with bulb, is probably the most satisfactory form. It should be of rather large caliber, and have an opening in the tip and one or two in the side near the tip. When not in use it should be kept in a vesse of borax solution, and should be well washed in hot water both before and after using.

It is important confidently to assure the patient that introduction of the tube cannot possibly harm him; and that, if he can control the spasm of his throat, he will experience very little choking sensation. When patients are very nervous it is well to spray the throat with cocain solution.

The tube should be dipped in warm water just before using; the use of glycerin or other lubricant is undesirable. With the patient seated upon a chair, his clothing protected by towels or a large apron, and his head tilted forward, the tip of the tube, held as one would a pen, is introduced far back into the pharynx. He is then urged to swallow, and the tube is pushed boldly into the esophagus until the ring upon it reaches the incisor teeth, thus indicating that the tip is in the stomach. If, now, the patient cough or strain as if at stool, the contents of

the stomach will usually be forced out through the tube. Should it fail, the fluid can generally be pumped out by alternate compression of the tube and the bulb. If unsuccessful at first, the attempts should be repeated with the tube pushed a little further in, or withdrawn a few inches, since the distance to the stomach is not the same in all cases. The tube may become clogged with pieces of food, in which case it must be withdrawn, cleaned, and reintroduced. If, after all efforts, no fluid is obtained, another test-meal should be given and withdrawn after a somewhat shorter period, since, owing to excessive motility, the stomach may empty itself in less than the usual time.

Care must be exercised to prevent saliva running down the outside of the tube and mingling with the gastric juice in the basin.

As the tube is removed, it should be pinched between the fingers so as to save any fluid that may be in it.

The stomach-tube must be used with great care, or not at all, in cases of gastric ulcer, aneurysm, uncompensated heart disease, and marked arteriosclerosis. Except in gastric ulcer, the danger lies in the retching produced, and the tube can safely be used if the patient takes it easily.

B. Physical Examination

Under normal conditions 30 to 50 c.c. of fluid can be obtained one hour after administering Ewald's breakfast. More than 60 c.c. points to motor insufficiency; less than 20 c.c., to too rapid emptying of the stomach, or else to incomplete removal. Upon standing, it separates into two layers: the lower consisting of particles of food; the upper, of an almost clear, faintly yellow fluid.

The extent to which digestion has taken place can be roughly judged from the appearance of the food-particles.

The **reaction** is frankly acid in health and in nearly all pathologic conditions. It may be neutral or slightly alkaline in some cases of gastric cancer and marked chronic gastritis, or when contaminated by a considerable amount of saliva.

A small amount of **mucus** is present normally. Large amounts, when the gastric contents are obtained with the tube and not vomited, point to chronic gastritis. Mucus is recognized from its characteristic slimy appearance when the fluid is poured from one vessel into another. It is more frequently seen in stomach washings than in the fluid removed after a test-meal.

A trace of **bile** may be present as a result of excessive straining while the tube is in the stomach. Large amounts are very rarely found, and generally point to obstruction in the duodenum. Bile produces a yellowish or greenish discoloration of the fluid.

Blood is often recognized by simple inspection, but more frequently requires a chemic test. It is bright red when very fresh, and dark, resembling coffee-grounds, when older. Vomiting of blood, or *hematemesis*, may be mistaken for pulmonary hemorrhage, or *hemoptysis*. In the former the fluid is acid in reaction and usually dark red or brown in color and clotted, while in hemoptysis it is brighter red, frothy, alkaline, and usually mixed with a variable amount of mucus.

Particles of food eaten hours or even days previously may be found, and indicate deficient motor power.

Search should always be made for **bits of tissue** from the gastric mucous membrane or new growths. These,

when examined by a pathologist, will sometimes render the diagnosis clear.

C. Chemic Examination

A routine chemic examination of the gastric contents involves qualitative tests for free acids, free hydrochloric acid, and organic acids, and quantitative estimations of total acidity, free hydrochloric acid, and sometimes combined hydrochloric acid. Other tests are applied when indicated.

1. Qualitative Tests.—(1) **Free Acids.**—The presence or absence of free acids, without reference to the kind, is easily determined by means of Congo-red, although the test is not much used in practice.

Congo-red Test.—Take a few drops of a strong alcoholic solution of Congo-red in a test-tube, dilute with water to a strong red color, and add a few cubic centimeters of filtered gastric juice. The appearance of a *blue color* shows the presence of some free acid (Plate XI, B, B′). Since the test is more sensitive to mineral than to organic acids, a marked reaction points to the presence of free hydrochloric acid.

Thick filter-paper soaked in Congo-red solution, dried, and cut into strips may be used, but the test is much less delicate when thus applied.

(2) **Free Hydrochloric Acid.**—In addition to its digestive function, free hydrochloric acid is an efficient antiseptic. It prevents or retards fermentation and lactic-acid formation, and is an important means of protection against the entrance of pathogenic organisms into the body. It is never absent in health.

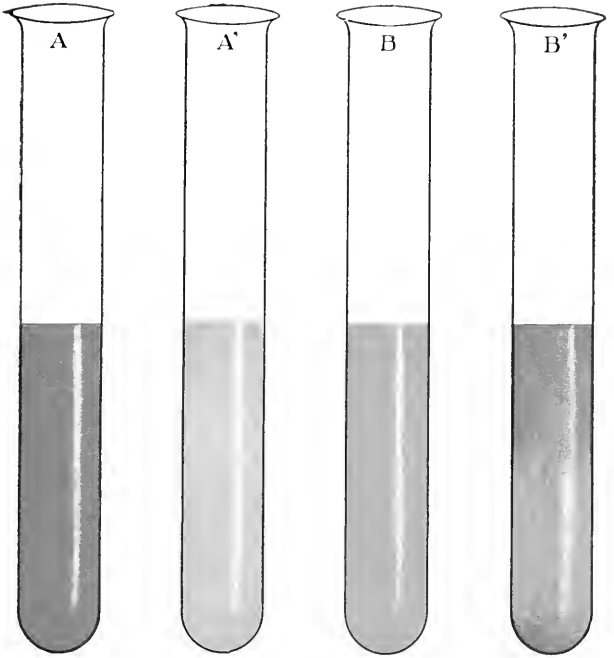
Amidobenzol Test.—To a little of the filtered gastric juice in a test-tube, or to several drops in a porcelain dish, add a drop of 0.5 per cent. alcoholic solution of dimethylamido-azobenzol. In the presence of free hydrochloric acid there will at once appear a *cherry-red color*, varying in intensity with the amount of acid (Plate XII, C). This test is very delicate; but, unfortunately, organic acids, when present in large amounts (above 0.5 per cent.), give a similar reaction. The color obtained with organic acids is, however, more of an orange red.

Boas' Test.—This test is less delicate than the preceding, but is more reliable, since it reacts only to free hydrochloric acid. It is probably the best routine test.

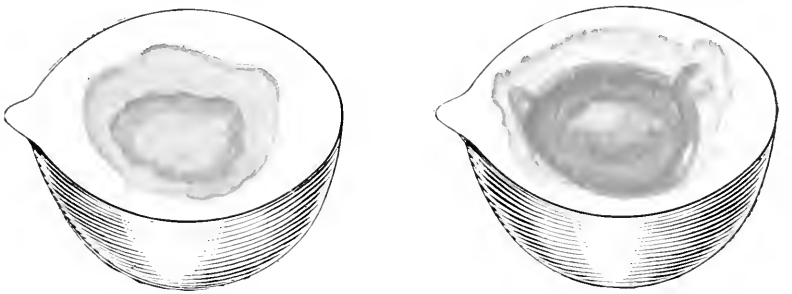
In a procelain dish mix a few drops of the gastric juice and the reagent, and slowly evaporate to dryness over a flame, *taking care not to scorch.* The appearance of a *rose-red color*, which fades upon cooling, shows the presence of free hydrochloric acid (Plate XI, 1).

Boas' reagent consists of 5 gm. resublimed resorcinol, and 3 gm. cane-sugar, in 100 c.c. alcohol. The solution keeps well, which, from the practitioner's view-point, makes it preferable to Günzburg's phloroglucin-vanillin reagent (phloroglucin, 2 gm.; vanillin, 1 gm.; absolute alcohol, 30 c.c.). The latter is just as delicate, is applied in the same way, and gives a sharper reaction (Plate XI, 2), but is unstable.

(3) **Organic Acids.**—Lactic acid is the most common, and is taken as the type of the organic acids which appear in the stomach-contents. It is a product of bacterial activity. Acetic and butyric acids are sometimes present. Their formation is closely connected with that of lactic acid, and they are rarely tested for. When abundant, they may be recognized by their odor



A, Uffelmann's reagent; A', A after the addition of gastric fluid containing lactic acid; B, water to which three drops of Congo-red solution have been added; B', change induced in B when gastric fluid containing free hydrochloric acid is added (Boston).



1, Resorcin-test for free hydrochloric acid; 2, Günzburg's test for hydrochloric acid (Boston).

upon heating. Butyric acid gives the odor of rancid butter.

Lactic acid is never present at the height of digestion in health. Although usually present early in digestion, it disappears when free hydrochloric acid begins to appear. Small amounts may be introduced with the food. Pathologically, small amounts may be present whenever there is stagnation of the gastric contents with deficient hydrochloric acid, as in many cases of dilatation of the stomach and chronic gastritis. The presence of notable amounts of lactic acid (more than 0.1 per cent. by Strauss' test) is strongly suggestive of gastric cancer, and is probably the most valuable single symptom of the disease.

As already stated, the Ewald test-breakfast introduces a small amount of lactic acid, but rarely enough to respond to the tests given here. In every case, however, in which its detection is important, the shredded-wheat biscuit or Boas' test-breakfast should be given, the stomach having been thoroughly washed the evening before.

Uffelmann's Test for Lactic Acid.—Thoroughly shake up 5 c.c. of filtered stomach fluid with 50 c.c. of ether for at least ten minutes. Collect the ether and evaporate over a water-bath. Dissolve the residue in 5 c.c. of water and test with Uffelmann's reagent as follows:

In a test-tube mix 3 drops concentrated solution of phenol and 3 drops saturated aqueous solution of ferric chlorid. Add water until the mixture assumes an amethyst-blue color. To this add the solution to be tested. The appearance of a *canary-yellow color* indicates the presence of lactic acid (Plate XI, A, A′).

Uffelmann's test may be applied directly to the stomach-contents without extracting with ether, but is then neither sensitive nor reliable, because of the phosphates, sugars, and other interfering substances which may be present.

Kelling's Test (*Simon's Modification*).—This is much more satisfactory than Uffelmann's. To a test-tube of distilled water add sufficient ferric chlorid solution to give a faint yellowish tinge. Pour half of this into a second test-tube to serve as a control. To the other add a small amount of the gastric juice. Lactic acid gives a distinct yellow color which is readily recognized by comparison with the control.

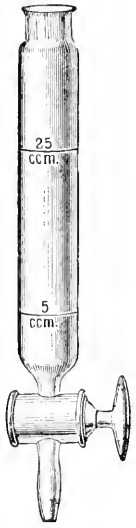


Fig. 106.—Separatory funnel for Strauss' lactic acid test (Sahli).

Strauss' Test for Lactic Acid.—This is a good test for clinical work, since it gives a rough idea of the quantity present and is not sufficiently sensitive to respond to the traces of lactic acid which some test-meals introduce. Strauss' instrument (Fig. 106) is essentially a separating funnel with a mark at 5 c.c. and one at 25 c.c. Fill to the 5 c.c. mark with filtered stomach fluid, and to the 25 c.c. mark with ether. Shake thoroughly for ten or fifteen minutes, let stand until the ether separates, and then, by opening the stop-cock, allow the gastric juice to run out. Fill to the 25 c.c. mark with water, and add 2 drops of a 10 per cent. solution of ferric chlorid. Shake gently. If 0.1 per cent. or more lactic acid be present, the water will assume a strong greenish-yellow color. A slight tinge will appear with 0.05 per cent.

(4) **Pepsin and Pepsinogen.**—Pepsinogen itself has no digestive power. It is secreted by the gastric glands,

and is transformed into pepsin by the action of a free acid. Although pepsin digests proteins best in the presence of free hydrochloric acid, it has a slight digestive activity in the presence of organic or combined hydrochloric acids.

The amount is not influenced by neuroses or circulatory disturbances. Absence or marked diminution, therefore, indicates organic disease of the stomach. This is an important point in diagnosis between functional and organic conditions. Pepsin is rarely or never absent in the presence of free hydrochloric acid.

Test for Pepsin and Pepsinogen.—With a cork-borer cut small cylinders from the coagulated white of an egg, and cut these into disks of uniform size. The egg should be cooked very slowly, preferably over a water-bath, so that the white may be readily digestible. The disks may be preserved in glycerin, but must be washed in water before using.

Place a disk in each of three test-tubes.

Into tube No. 1 put 10 c.c. distilled water, 5 grains pepsin, U. S. P., and 3 drops of the official dilute hydrochloric acid.

Into tube No. 2 put 10 c.c. filtered gastric juice.

Into tube No. 3 put 10 c.c. filtered gastric juice and 3 drops dilute hydrochloric acid.

Place the tubes in an incubator or in warm water for three hours or longer. At intervals observe the extent to which the egg-albumen has been digested. This is recognized by the depth to which the disk has become translucent.

Tube No. 1 is used for comparison, and should show the effect of normal gastric juice.

Digestion of the egg in tube No. 2 indicates the presence of both pepsin and free hydrochloric acid.

When digestion fails in tube No. 2 and occurs in No. 3, pepsinogen is present, having been transformed into pepsin

by the hydrochloric acid added. Should digestion fail in this tube, both pepsin and pepsinogen are absent.

(5) **Rennin and Renninogen.**—Rennin is the milk-curdling ferment of the gastric juice. It is derived from renninogen through the action of hydrochloric acid. Lime salts also possess the power of transforming renninogen into the active ferment.

Deficiency of rennin has the same significance as deficiency of pepsin, and is more easily recognized. Since the two enzymes are almost invariably present or absent together, the test for rennin serves also as a test for pepsin.

Test for Rennin.—Neutralize 5 c.c. filtered gastric juice with very dilute sodium hydroxid solution; add 5 c.c. fresh milk, and place in an incubator or in a vessel of water at about 40° C. Coagulation of the milk in ten to fifteen minutes shows a normal amount of rennin. Delayed coagulation denotes a less amount.

Test for Renninogen.—To 5 c.c. feebly alkalinized gastric juice add 2 c.c. of 1 per cent. calcium chlorid solution and 5 c.c. fresh milk, and place in an incubator. If coagulation occurs, renninogen is present.

(6) **Peptid-splitting Enzyme.**—It has been found that in cancer of the stomach there may be present a pathologic ferment which is capable of splitting peptids into amino-acids. No such ferment is present normally, the gastric juice being incapable of carrying digestion to the amino-acid stage. Neubauer and Fischer have utilized this fact for the diagnosis of gastric cancer by subjecting the dipeptid, glycyl-tryptophan, to the

action of the gastric fluid and testing for the presence of the amino-acid tryptophan. The method is as follows:

Place 10 c.c. of the filtered gastric juice and about 1 c.c. of glycyl-tryptophan in a test-tube, overlay with toluol to prevent bacterial action, and place in an incubator at about 38° C. At the end of twenty-four hours pipet off a few cubic centimeters and test for tryptophan as follows: Acidify with a few drops of 3 per cent. acetic acid, add a very little bromin vapor with a medicine-dropper, and shake. The appearance of a rose-red color shows the presence of tryptophan and hence of the peptid-splitting ferment. The color quickly disappears if too much bromin is added. If no color appears at first, add more bromin vapor in small quantities. Only when the fluid has become yellow from excess of bromin can the test be considered negative.

Before applying this method, the stomach fluid must be tested for pre-existing tryptophan, blood (see p. 333), and bile (see p. 154). Blood and pancreatic juice each contain peptid-splitting ferments, and pancreatic juice may be assumed to be present if bile is detected.

Glycyl-tryptophan can be purchased in bottles, each containing a little toluol and the correct amount of the dipeptid for one test. The gastric juice is introduced into the bottle to the level of a mark on its side and then incubated. Such an outfit is called a "ferment diagnosticum."

Instead of glycyl-tryptophan, Jacque and Woodyatt and others have used 20 c.c. sterilized filtered 2 per cent. solution of Witte's peptone for each 10 c.c. of stomach fluid. They then estimate amino-acids in 10 c.c. of the mixture before incubating and in 10 c.c. afterward, using a formalin method which is practically the same as that given for ammonia in urine (see p. 127). The difference between the two estimations expresses the degree of peptolysis.

The value of the test is impaired by Warfield's discovery of peptid-splitting ferments in the saliva. Later workers have shown that much, at least, of the peptolytic activity of the saliva is due to ferments of leukocytes and bacteria, which are capable of splitting proteins as well as peptids. The chief source of error, however, appears to be regurgitated trypsin, which may be present in the absence of bile. To exclude these sources of error Friedman and Hamburger propose a control test for proteolytic ferments, using edestin as substrate. If the edestin test is positive, the glycyl-tryptophan test cannot be relied upon. It is performed as follows:

Edestin Test.—The gastric juice is filtered, neutralized with normal Na_2CO_3 solution, using phenolphthalein as indicator, and then brought to an alkalinity equal to $\frac{N}{100}$ Na_2CO_3, in order to inactivate pepsin. Place 2 c.c. of a 0.1 per cent. solution of edestin[1] in 0.1 per cent. Na_2CO_3 in each of four test-tubes. To three tubes add 2 c.c., 1 c.c., and 0.5 c.c. of the faintly alkalinized gastric fluid, reserving the fourth tube as a control and adding to it only a drop of phenolphthalein solution. Place the four tubes in an incubator at 37° C. At the end of four hours exactly neutralize the contents of each of the tubes with 5 per cent. acetic acid. When the neutral point is reached all the undigested edestin will be precipitated. The degree of digestion is indicated by the amount of turbidity compared with that in the control tube. Absence of turbidity denotes complete digestion.

(7) **Blood** is present in the vomitus in a great variety of conditions. When found in the fluid removed after

[1] Edestin is a protein extracted from hemp seed. It can be purchased from Eimer and Amend, New York.

a test-meal, it commonly points toward ulcer or carcinoma. Blood can be detected in nearly one-half of the cases of gastric cancer. The presence of swallowed blood must be excluded.

Test for Blood in Stomach-contents.—Extract with ether to remove fat. To 10 c.c. of the fat-free fluid add a few cubic centimeters of glacial acetic acid and shake the mixture thoroughly with an equal volume of ether. Separate the ether and apply to it the guaiac test (see p. 157); or evaporate and apply the hemin test (see p. 307) to the residue. When brown particles are present in the fluid, the hemin test should be applied directly to them.

2. Quantitative Tests.—(1) **Total Acidity.**—The acid-reacting substances which contribute to the total acidity are free hydrochloric acid, combined hydrochloric acid, acid salts, mostly phosphates, and, in some pathologic conditions, the organic acids. The total acidity is normally about 50 to 75 *degrees* (see method below), or, when estimated as hydrochloric acid, about 0.2 to 0.3 *per cent.* With Riegel's or Fischer's test-meal the figures are a little higher.

Töpfer's Method for Total Acidity.—In an evaporating dish or small beaker (an "after-dinner" coffee-cup is a very convenient substitute) take 10 c.c. filtered stomach-contents and add 3 or 4 drops of the indicator, a 1 per cent. alcoholic solution of phenolphthalein. When the quantity of stomach fluid is small, 5 c.c. may be used, but results are less accurate than with a larger amount. Add decinormal solution of sodium hydroxid drop by drop from a buret, until the fluid assumes a rose-red color which does not become

deeper upon addition of another drop (Plate XII, A, A'). In ordinary titrations the end-point is the appearance of the first permanent pink, but owing to interaction of phosphates it is advised (Wood) to carry the titration of gastric juice a little farther, as here indicated. When this point is reached, all the acid has been neutralized. The end reaction will be sharper if the fluid be saturated with sodium chlorid. A sheet of white paper beneath the beaker facilitates recognition of the color change.

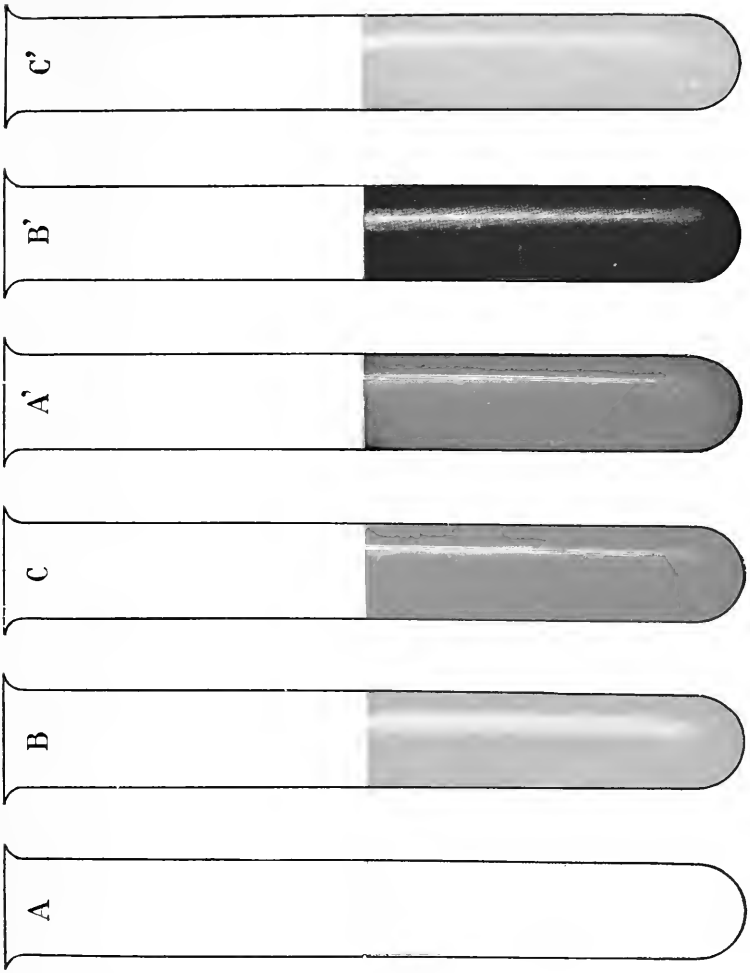
In clinical work the amount of acidity is expressed by the number of cubic centimeters of the decinormal sodium hydroxid solution which would be required to neutralize 100 c.c. of the gastric juice, each cubic centimeter representing one *degree* of acidity. Hence, multiply the number of cubic centimeters of decinormal solution required to neutralize the 10 c.c. of stomach fluid by 10. This gives the number of degrees of acidity. The amount may be expressed in terms of hydrochloric acid, if one remember that each degree is equivalent to 0.00365 per cent. hydrochloric acid. Some one suggests that this is the number of days in the year, the last figure, 5, indicating the number of decimal places.

Example.—Suppose that 7 c.c. of decinormal solution were required to bring about the end reaction in 10 c.c. gastric juice; then $7 \times 10 = 70$ *degrees* of acidity; and, expressed in terms of hydrochloric acid, $70 \times 0.00365 = 0.255$ *per cent.*

Preparation of decinormal solutions is described in textbooks on chemistry. The practitioner will find it best to have them made by a chemist, or to purchase from a chemic supply house. Preparation of an approximately decinormal solution is described on page 549.

(2) **Hydrochloric Acid.**—After the Ewald and Boas test-breakfasts the amount of **free hydrochloric acid** varies normally between 25 and 50 degrees, or about 0.1

PLATE XII



A, Gastric fluid to which a 1 per cent. solution of phenolphthalein has been added; B, gastric fluid to which a 1 per cent. solution of alizarin has been added; C, gastric fluid to which a 0.5 per cent. solution of dimethylamido-azobenzol has been added; A', A after titration with a decinormal solution of sodium hydroxid; B', B after titration with a decinormal solution of sodium hydroxid; C', C after titration with a decinormal solution of sodium hydroxid (Boston).

to 0.2 per cent. In disease it may go considerably higher or may be absent altogether.

When the amount of free hydrochloric acid is normal, organic disease of the stomach probably does not exist.

Increase of free hydrochloric acid above 50 degrees (*hyperchlorhydria*) generally indicates a neurosis, but also occurs in most cases of gastric ulcer and beginning chronic gastritis.

Decrease of free hydrochloric acid below 25 degrees (*hypochlorhydria*) occurs in some neuroses, chronic gastritis, early carcinoma, and most conditions associated with general systemic depression. Marked variation in the amount at successive examinations strongly suggests a neurosis. Too low values are often obtained at the first examination, the patient's dread of the introduction of the tube probably inhibiting secretion.

Absence of free hydrochloric acid (*achlorhydria*) occurs in most cases of gastric cancer and far-advanced chronic gastritis, in many cases of pernicious anemia, and sometimes in hysteria and pulmonary tuberculosis.

The presence of free hydrochloric acid presupposes a normal amount of **combined hydrochloric acid,** hence the combined need not be estimated when the free acid has been found. When, however, free hydrochloric acid is absent, it is important to know whether any acid is secreted, and an estimation of the combined acid then becomes of great value. The normal average after an Ewald breakfast is about 10 to 15 degrees, the quantity depending upon the amount of protein in the test-meal. Somewhat higher figures are obtained after a Riegel or Fischer test-meal.

Töpfer's Method for Free Hydrochloric Acid.—In a beaker take 10 c.c. filtered stomach fluid and add 4 drops of the indicator, a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol. A red color instantly appears if free hydrochloric acid be present. Add decinormal sodium hydroxid solution, drop by drop from a buret, until the last trace of red just disappears, and a canary-yellow color takes its place (Plate XII, C, C′). Read off the number of cubic centimeters of decinormal solution added, and calculate the degrees, or percentage of free hydrochloric acid, as in Töpfer's method for total acidity.

When it is impossible to obtain sufficient fluid for all the tests, it will be found convenient to estimate the free hydrochloric acid and total acidity in the same portion. After finding the free hydrochloric acid as just described, add 4 drops phenolphthalein solution, and continue the titration. The amount of decinormal solution used in both titrations indicates the total acidity.

Töpfer's Method for Combined Hydrochloric Acid.—In a beaker take 10 c.c. filtered gastric juice and add 4 drops of the indicator, a 1 per cent. aqueous solution of sodium alizarin sulphonate. Titrate with decinormal sodium hydroxid until the appearance of a bluish-violet color which does not become deeper upon addition of another drop (Plate XII, B, B′). It is difficult, without practice, to determine when the right color has been reached. A reddish violet appears first. The shade which denotes the end reaction can be produced by adding 2 or 3 drops of the indicator to 5 c.c. of 1 per cent. sodium carbonate solution.

Calculate the number of cubic centimeters of decinormal solution which would be required for 100 c.c. of stomach fluid. This gives, in degrees, *all the acidity except the combined hydrochloric acid*. The combined hydrochloric acid is then found by deducting this amount from the total acidity, which has been previously determined.

Example.—Suppose that 5 c.c. of decinormal solution were required to produce the purple color in 10 c.c. gastric juice; then $5 \times 10 = 50$ = *all the acidity except combined hydrochloric acid.* Suppose, now, that the total acidity has already been found to be 70 degrees; then $70 - 50 = 20$ *degrees* of combined hydrochloric acid; and $20 \times 0.00365 = 0.073$ *per cent.*

When free hydrochloric acid is absent, it is probably more helpful to estimate the **acid deficit** than the combined hydrochloric acid. The acid deficit shows how far the acid secreted by the stomach falls short of saturating the protein (and bases) of the meal. It represents the amount of hydrochloric acid which must be added to the fluid before a test for free hydrochloric acid can be obtained. It is determined by titrating with $\frac{n}{10}$ hydrochloric acid, using dimethyl-amido-azobenzol as indicator, until the fluid assumes a red color. The amount of deficit is expressed by the number of cubic centimeters of the decinormal solution required for 100 c.c of the stomach fluid.

(3) **Organic Acids.**—There is no simple direct quantitative method. After the total acidity has been determined, organic acids may be removed from another portion of the gastric filtrate by shaking thoroughly with an equal volume of neutral ether, allowing the fluids to separate, and repeating this process until the gastric fluid has been extracted with eight or ten times its volume of ether. The total acidity is then determined, and the difference between the two determinations indicates the amount of organic acids.

(4) **Pepsin.**—No direct method is available. The following are sufficient for clinical purposes:

(1) **Hammerschlag's Method.**—To the white of an egg add twelve times its volume of 0.4 per cent. hydrochloric acid (dilute hydrochloric acid, U. S. P., 4 c.c.; water, 96 c.c.), mix well, and filter. This gives a 1 per cent. egg-albumen solution. Take 10 c.c. of this solution in each of three tubes or beakers. To *A* add 5 c.c. gastric juice; to *B*, 5 c.c. water with 0.5 gm. pepsin; to *C*, 5 c.c. water only. Place in an incubator for an hour and then determine the amount of albumin in each mixture by Esbach's method. Tube *C* shows the amount of albumin in the test-solution. The difference between *C* and *B* indicates the amount of albumin which would be digested by normal gastric juice. The difference between *C* and *A* gives the albumin which is digested by the fluid under examination. Schütz has shown that the amounts of pepsin in two fluids are proportionate to the squares of the products of digestion. Thus, if the amounts of albumin digested in tubes *A* and *B* are to each other as 2 is to 4, the amounts of pepsin are to each other as 4 is to 16.

Certain sources of error can be eliminated by diluting the gastric juice several times before testing. The most important of these are that the law of Schütz holds good only for comparatively dilute solutions, and that the products of peptic activity inhibit digestion.

(2) **Mett's method** is generally preferred to the preceding. Put three or four Mett's tubes about 2 cm. long into a small beaker with diluted gastric juice (1 c.c. of the filtrate plus 15 c.c. twentieth-normal hydrochloric acid). Place in an incubator for twenty-four hours, and then measure as accurately as possible in millimeters the column which has been digested, using a millimeter scale and a hand lens or, better, a low power of the microscope and an eye-piece micrometer. Square the average length of this column (law of Schütz) and multiply by the degree of dilution, 16. The maximum figure obtained in this way is 256, representing a digested column of 4 mm.

Prepare Mett's tubes as follows:

Beat up slightly the whites of one or two eggs and filter. Pour into a wide test-tube and stand in this a number of capillary glass tubes, 1 to 2 mm. in diameter. When the tubes are filled, plug their ends with bread crumbs, and coagulate the albumin by heating in water just short of boiling. Dip the ends of the tubes in melted paraffin and preserve until needed. Bubbles, if present, will probably disappear in a few days. When wanted for use, cut the tubes into lengths of about 2 cm. Discard any in which the albumin has separated from the wall.

D. Microscopic Examination

A drop of unfiltered stomach-contents is placed upon a slide, covered with a cover-glass, and examined with the 16-mm. and 4-mm. objectives. A drop of Lugol's solution allowed to run under the cover will aid in distinguishing the various structures. As a rule, the microscopic examination is of little value.

Under normal conditions little is to be seen except great numbers of starch-granules, with an occasional epithelial cell, yeast-cell, or bacterium. Starch-granules are recognized by their concentric striations and the fact that they stain blue with iodin solutions when undigested, and reddish, due to erythrodextrin, when partially digested.

Pathologically, remnants of food from previous meals, red blood-corpuscles, pus-cells, sarcinæ, and excessive numbers of yeast-cells and bacteria may be encountered (Fig. 107).

Remnants of food from previous meals indicate deficient gastric motility.

Red Blood-corpuscles.—Blood is best recognized by the chemic tests already given. The corpuscles sometimes retain a fairly normal appearance, but are generally so degenerated that only granular pigment is left. When only a few fresh-looking corpuscles are present, they usually come from irritation of the mucous membrane by the tube.

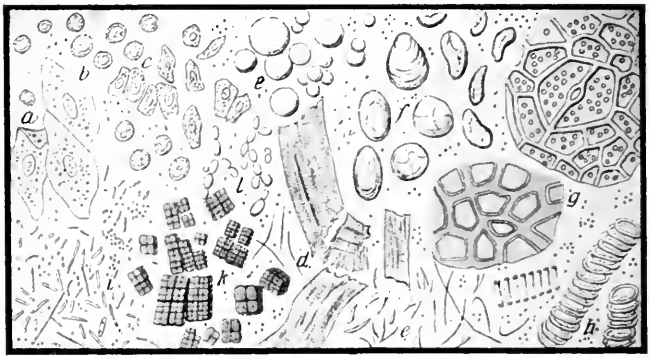


Fig. 107.—General view of the gastric contents: *a*, Squamous epithelial cells from esophagus and mouth; *b*, leukocytes; *c*, cylindric epithelial cells; *d*, muscle-fibers; *e*, fat-droplets and fat-crystals; *f*, starch-granules; *g*, chlorophyl-containing vegetable matters; *h*, vegetable spirals; *i*, bacteria; *k*, sarcinæ; *l*, budding (yeast) fungi (Jakob).

Pus-cells.—Pus is rarely encountered in the fluid removed after a test-meal. Considerable numbers of pus-corpuscles have been found in some cases of gastric cancer. The corpuscles are usually partially digested, so that only the nuclei are seen. Swallowed sputum must always be considered.

Sarcinæ.—These are small spheres arranged in cuboid groups, often compared to bales of cotton. They frequently form large clumps and are easily recognized. They stain brown with iodin solution. They signify fer-

mentation. Their presence is strong evidence against the existence of gastric cancer, in which disease they rarely occur.

Yeast-cells.—As already stated, a few yeast-cells may be found under normal conditions. The presence of considerable numbers is evidence of fermentation. Their appearance has been described (see p. 208). They stain brown with iodin solution.

Bacteria.—Numerous bacteria may be encountered, especially in the absence of free hydrochloric acid. The

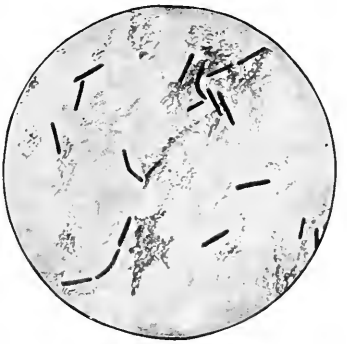


Fig. 108.—Boas-Oppler bacillus from case of gastric cancer (Boston).

Boas-Oppler bacillus is the only one of special significance. It occurs in the majority of cases of cancer, and is rarely found in other conditions. Carcinoma probably furnishes a favorable medium for its growth.

These bacilli (Fig. 108) are large (5 to 10 μ long), non-motile, and usually arranged in clumps or end to end in zig-zag chains. They stain brown with iodin solution, which distinguishes them from *Leptothrix buccalis* (see p. 433), which is not infrequently swallowed, and hence found in stomach fluid. They also stain by

Gram's method. They are easily seen with the 4-mm. objective in unstained preparations, but are best recognized with the oil lens, after drying some of the fluid upon a cover-glass, fixing, and staining with a simple bacterial stain or by Gram's method.

A few large non-motile bacilli are frequently seen; they cannot be called Boas-Oppler bacillus unless they are numerous and show something of the typical arrangement.

E. The Gastric Contents in Disease

In the diagnosis of stomach disorders the practitioner must be cautioned against relying too much upon examinations of the stomach-contents. A first examination is especially unreliable. Even when repeated examinations are made, the laboratory findings must never be considered apart from the clinical signs.

The more characteristic findings in certain disorders are suggested here:

1. Dilatation of the Stomach.—Evidences of retention and fermentation are the chief characteristics of this condition. Hydrochloric acid is commonly diminished. Pepsin may be normal or slightly diminished. Lactic acid may be detected in small amounts, but is usually absent when the stomach has been washed before giving the test-meal. Both motility and absorptive power are deficient. The microscope commonly shows sarcinæ, bacteria, and great numbers of yeast-cells. Remnants of food from previous meals can be detected with the naked eye or microscopically.

2. Gastric Neuroses.—The findings are variable. Successive examinations may show normal, increased,

or diminished hydrochloric acid, or even entire absence of the free acid. Pepsin is usually normal.

In the neurosis characterized by continuous hypersecretion (gastrosuccorrhea), 40 c.c. or more of gastric juice can be obtained from the fasting stomach. Should the fluid contain food-particles, it is probably the result of retention, not hypersecretion.

3. Chronic Gastritis.—Free hydrochloric acid may be increased in early cases. It is generally diminished in well-marked cases, and is often absent in advanced cases. Lactic acid is often present in traces, rarely in notable amount. Secretion of pepsin and rennin is always diminished in marked cases. Mucus is frequently present, and is very significant of the disease. Motility and absorption are generally deficient. Small fragments of mucous membrane may be found, and when examined by a pathologist, may occasionally establish the diagnosis.

4. Achylia Gastrica (Atrophic Gastritis).—This condition may be a terminal stage of chronic gastritis. It is sometimes associated with the blood-picture of pernicious anemia. It gives a great decrease, and sometimes entire absence of hydrochloric acid and ferments. The total acidity may be as low as 1 or 2 degrees. Small amounts of lactic acid may be present. Absorption and motility are usually not affected greatly.

5. Gastric Carcinoma.—As far as the laboratory examination goes, the cardinal signs of this disease are absence of free hydrochloric acid and presence of a peptid-splitting ferment, of lactic acid, and of the Boas-Oppler bacillus. These findings are, however, by no means constant.

It is probable that some substance is produced by the cancer which neutralizes the free hydrochloric acid, and thus causes it to disappear earlier than in other organic diseases of the stomach. The peptid-splitting ferment (see p. 330) is also probably a product of the cancer.

The presence of lactic acid is possibly the most suggestive single symptom of gastric cancer. In the great majority of cases its presence in notable amount (0.1 per cent. by Strauss' method) after Boas' breakfast, the stomach having been washed the evening before, warrants a tentative diagnosis of malignancy.

Carcinoma seems to furnish an especially favorable medium for the growth of the Boas-Oppler bacillus, hence this micro-organism is frequently present.

Blood can be detected in the stomach fluid by the chemic tests in nearly one-half of the cases, and is more common when the new growth is situated at the pylorus. Blood is present in the stool in nearly every case.

Evidences of retention and fermentation are the rule in pyloric cancer. Tumor particles are sometimes found late in the disease.

6. Gastric Ulcer.—There is excess of free hydrochloric acid in about one-half of the cases. In other cases the acid is normal or diminished. Blood is often present. The diagnosis must be based largely upon the clinical symptoms, and where ulcer is strongly suspected, it is probably unwise to use the stomach-tube.

II. ADDITIONAL EXAMINATIONS WHICH GIVE INFORMATION AS TO THE CONDITION OF THE STOMACH

1. Absorptive Power of the Stomach.—This is a very unimportant function, only a few substances being ab-

sorbed in the stomach. It is delayed in most organic diseases of the stomach, especially in dilatation and carcinoma, but not in neuroses. The test has little practical value.

Give the patient, upon an empty stomach, a 3-grain capsule of potassium iodid with a glass of water, taking care that none of the drug adheres to the outside of the capsule. At intervals test the saliva for iodids by moistening starch-paper with it and touching with yellow nitric acid. A blue color shows the presence of an iodid, and appears normally in ten to fifteen minutes after ingestion of the capsule. A longer time denotes delayed absorption.

Starch-paper is prepared by soaking filter-paper in boiled starch and drying.

2. Motor Power of the Stomach.—This refers to the rapidity with which the stomach passes its contents on into the intestines. It is very important: intestinal digestion can compensate for insufficient or absent stomach digestion only so long as the motor power is good.

Motility is impaired to some extent in chronic gastritis. It is especially deficient in dilatation of the stomach due to atony of the gastric wall or to pyloric obstruction, either benign or malignant. It is increased in most conditions with hyperchlorhydria.

The best evidence of deficient motor power is the detection of food in the stomach at a time when it should be empty, *e. g.*, before breakfast in the morning. A special test-meal containing easily recognized materials (*e. g.*, rice pudding with currants) is sometimes given and removed at the end of six or seven hours. When more than 60 c.c. of fluid are obtained with the tube one hour

after an Ewald breakfast, deficient motility may be inferred.

Ewald's salol test is scarcely so reliable as the above. It depends upon the fact that salol is not absorbed until it reaches the intestines and is decomposed by the alkaline intestinal juices.

The patient is given 15 grains of salol with a test-breakfast, and the urine, passed at intervals thereafter, is tested for salicyluric acid. A few drops of 10 per cent. ferric chlorid solution are added to a small quantity of the urine. A violet color denotes the presence of salicyluric acid. It appears normally in sixty to seventy-five minutes after ingestion of the salol. A longer time indicates impaired motor power.

3. To Determine Size and Position of Stomach.—After removing the test-meal, while the tube is still in place force quick puffs of air into the stomach by compression of the bulb. The puffs can be clearly heard with a stethoscope over the region of the stomach, and nowhere else.

If desired, the patient may be given a dram of sodium bicarbonate in solution, followed immediately by the same amount of tartaric acid, also in solution; or he may take the two parts of a Seidlitz powder separately. The carbon dioxid evolved distends the stomach, and its outline can easily be determined by percussion.

4. Sahli's Desmoid Test of Gastric Digestion.—Two pills, one containing 0.1 gram iodoform, the other 0.05 gram methylene-blue, are wrapped in little bags made of thin sheets of rubber and tied with a string of raw catgut, No. 00. The bags must be carefully folded and tied. Before use they should be placed for a time in water.

If they float or if any of the methylene-blue escapes and colors the water they are useless for the test.

The patient swallows the two bags with the aid of a little water during the noon meal, and the urine is tested at intervals thereafter. According to Sahli, the catgut is digested by gastric juice and not by pancreatic or intestinal juices. If gastric digestion is normal, iodin and methylene-blue can be detected in the urine in the afternoon or evening of the same day. The reaction may occur when digestion is very poor, provided gastric motility is diminished, but it is then delayed. If the reaction does not appear, gastric digestion has not occurred.

Methylene-blue is recognized in the urine by the green or blue color which it imparts. It is sometimes eliminated as a chromogen, in which case a little of the urine must be acidified with acetic acid and boiled to bring out the color.

To detect the iodin, some of the urine is decolorized by gently heating and filtering through animal charcoal. To 10 c.c. are then added 1 c.c. dilute sulphuric acid, and 0.5 c.c. of a 1 per cent. solution of sodium nitrite and 2 c.c. of chloroform. Upon shaking, a rose color will be imparted to the chloroform if iodin be present. Another method of testing for iodin is given on page 168.

CHAPTER V

THE FECES

As commonly practised, an examination of the feces is limited to a search for intestinal parasites or their ova. Much of value can, however, be learned from other simple examinations, particularly a careful inspection. Anything approaching a complete analysis is, on the other hand, a waste of time for the clinician.

The normal stool is a mixture of—(*a*) water; (*b*) undigested and indigestible remnants of food, as starch-granules, particles of meat, vegetable cells and fibers, etc.; (*c*) digested foods, carried out before absorption can take place; (*d*) products of the digestive tract, as altered bile-pigments, mucus, etc.; (*e*) products of decomposition, as indol, skatol, fatty acids, and various gases; (*f*) epithelial cells shed from the wall of the intestinal canal; (*g*) harmless bacteria, which are always present in enormous numbers.

Pathologically, we may find abnormal amounts of normal constituents, blood, pathogenic bacteria, animal parasites and their ova, and biliary and intestinal concretions.

The stool to be examined should be passed into a clean vessel, without admixture of urine. The examination should not be delayed more than a few hours, owing to the changes caused by decomposition. The offensive odor can be partially overcome with turpentine or 5 per cent. phenol. When search for amebæ is to be made, the

vessel must be warm, and the stool kept warm until examined; naturally, no disinfectant can be used. For other protozoa a saline cathartic may be given and the second stool examined. The first stool is usually too solid, and the later ones too greatly diluted.

I. MACROSCOPIC EXAMINATION

1. Quantity.—The amount varies greatly with diet and other factors. The average is about 100 to 150 gm. in twenty-four hours.

2. Frequency.—One or two stools in twenty-four hours may be considered normal, yet one in three or four days is not uncommon with healthy persons. The individual habit should be considered in every case.

3. Form and Consistence.—Soft, mushy, or liquid stools follow cathartics and accompany diarrhea. Copious, purely serous discharges without fecal matter are significant of Asiatic cholera, although sometimes observed in other conditions. Hard stools accompany constipation. Rounded scybalous masses are common in habitual constipation, and indicate atony of the muscular coat of the colon. Flattened, ribbon-like stools result from some obstruction in the rectum, generally a tumor or a stricture from a healed ulcer, most commonly syphilitic. When bleeding piles are absent, blood-streaks upon such a stool point to carcinoma.

4. Color.—The normal light or dark-brown color is due chiefly to hydrobilirubin, which is formed from bilirubin by reduction processes in the intestine, largely the result of bacterial activity. The stools of infants are yellow, owing partly to their milk diet and partly to the presence of unchanged bilirubin.

Diet and drugs cause marked changes: milk, a light yellow color; cocoa and chocolate, dark gray; various fruits, reddish or black; iron and bismuth, dark brown or black; hematoxylin, red, etc.

Pathologically, the color is important. A golden yellow is generally due to unchanged bilirubin. Green stools are not uncommon, especially in diarrheas of childhood. They are sometimes met in apparently healthy infants, alternating with normal yellow stools, and have little significance unless accompanied by symptoms. The color is due to biliverdin or, sometimes, to chromogenic bacteria. Putty-colored or "acholic" stools occur when bile is deficient, either from obstruction to outflow or from deficient secretion. The color is due less to absence of bile-pigments than to presence of fat. Similar stools, which manifestly consist largely of fat, are common in conditions like tuberculous peritonitis, which interfere with absorption of fats, and in pancreatic disease.

Notable amounts of blood produce tarry black stools when the source of the hemorrhage is the stomach or upper intestine, and a dark brown or bright red as the source is nearer the rectum. When diarrhea exists the color may be red, even if the source of the blood is high up. Red streaks of blood upon the outside of the stool are due to lesions of rectum or anus.

5. Odor.—Products of decomposition, chiefly indol and skatol, are responsible for the normal offensive odor. A sour odor is normal for nursing infants, and is noted in mild diarrheas of older children. In the severe diarrheas of childhood a putrid odor is common. In adults, stools emitting a very foul stench are suggestive of malignant

or syphilitic ulceration of the rectum or gangrenous dysentery.

6. Mucus.—Excessive quantities of mucus are easily detected with the naked eye, and signify irritation or inflammation. When the mucus is small in amount and intimately mixed with the stool, the trouble is probably in the small intestine. Larger amounts, not well mixed with fecal matter, indicate inflammation of the large intestine. Stools composed almost wholly of mucus and streaked with blood are the rule in dysentery, ileocolitis, and intussusception.

In the so-called mucous colic or membranous enteritis, shreds and ribbons of altered mucus, sometimes representing complete casts of portions of the bowel, are passed. These may appear as firm, irregularly segmented strands, suggesting tapeworms. The mucus sometimes takes the form of frog-spawn-like masses. In some cases it is passed at variable intervals, with colic; in others, with every stool, with only vague pains and discomfort. It is distinguished from inflammatory mucus by absence of pus-corpuscles. The condition is not uncommon and should be more frequently recognized. It is probably a secretory neurosis, hence the name "membranous enteritis" is inappropriate.

7. Concretions.—Gall-stones are probably more common than is generally supposed, and should be searched for in every case of obscure colicky abdominal pain. Intestinal concretions (enteroliths) are rare. Intestinal sand, consisting of sand-like grains, is especially common in neurotic conditions, such as mucous colitis. After ingestion of considerable amounts of olive oil, nodules of soap and fat often appear in the feces, and may be

mistaken by the patient for gall-stones, particularly when the oil has been given for cholelithiasis.

Concretions can be found by breaking up the fecal matter in a sieve (which may be improvised from gauze) while pouring water over it. It must be remembered that gall-stones, if soft, may go to pieces in the bowel.

8. Animal Parasites.—Segments of tapeworms and the adults and larvæ of other parasites are often found in the stool. They are best searched for in the manner described for concretions. The search should be preceded by a vermicide and a brisk purge. Patients frequently mistake vegetable tissue for intestinal parasites, and the writer has known physicians to make similar mistakes. Probably the most frequent sources of confusion are long fibers from poorly masticated celery or "greens," which suggest round worms; cells from orange, which suggest seat worms; and fibers from banana, which, because of the segmented structure and the presence of oval cells, suggest tapeworms and ova. Even slight familiarity with the microscopic structure of vegetable tissue will prevent the chagrin of such errors.

9. Curds.—The stools of nursing infants frequently contain whitish curd-like masses, due either to imperfect digestion of fat or casein or to excess of these in the diet. When composed of fat, the masses are soluble in ether, and give the Sudan III test. If composed of casein, they will become tough and fibrous-like when placed in formalin (10 per cent.) for twenty-four hours.

II. CHEMIC EXAMINATION

Complicated chemic examinations are of little value to the clinician. Certain tests are, however, important.

1. Blood.—When present in large amount blood produces such changes in the appearance of the stool that it is not likely to be overlooked. Traces of blood (occult hemorrhage) can be detected only by special tests. Recognition of occult hemorrhage has its greatest value in diagnosis of gastric cancer and ulcer. It is constantly present in practically every case of gastric cancer, and is always present, although usually intermittently, in ulcer. Traces of blood also accompany malignant disease of the bowel, the presence of certain intestinal parasites, and other conditions.

Detection of Occult Hemorrhage.—Soften a portion of the stool with water, shake with an equal volume of ether to remove fat, and discard the ether. Treat the remaining material with about one-third its volume of glacial acetic acid and extract with ether. Should the ether not separate well, add a little alcohol. Apply the guaiac or benzidin test to the ether as already described (see p. 157).

In every case iron-containing medicines must be stopped, and blood-pigment must be excluded from the food by giving an appropriate diet, *e. g.*, bread, milk, eggs, and fruit. At the beginning of the restricted diet give a gram of powdered charcoal or, better, 0.3 gram of carmin, in capsules, so as to mark the corresponding stool.

2. Bile.—Normally, unaltered bile-pigment is never present in the feces of adults. In catarrhal conditions of the small intestine bilirubin may be carried through unchanged. It may be demonstrated by the test for hydrobilirubin which follows, or, if a considerable amount is present, by filtering (after mixing with water if the stool be solid) and testing the filtrate by Gmelin's method, as described under The Urine.

3. Hydrobilirubin.—This substance, which is the principal normal pigment of the feces, is derived from bilirubin by reduction processes in the intestine, largely the result of bacterial activity. It is apparently identical with urobilin. Hydrobilirubin is absent from the stool in complete obstruction of the common or hepatic bile-duct.

Hydrobilirubin will give a red color if a little of the stool be rubbed up with saturated mercuric chlorid solution and allowed to stand twenty-four hours. The red color is likewise imparted to microscopic structures which are stained with hydrobilirubin. A green color in this test shows the presence of unchanged bilirubin.

4. Trypsin.—This ferment is normally present in the feces. The amount is greatest upon a diet rich in proteins, least upon a carbohydrate diet. When it is absent or greatly diminished, pancreatic insufficiency is to be inferred. The test is usually made upon a protein diet.

Gross' Test.—In a mortar thoroughly rub up a portion of the fecal mass with three times its bulk of 0.1 per cent. sodium carbonate solution. Filter. Mix 10 c.c. of the filtrate with 100 c.c. of a fresh solution consisting of 0.5 gram Grübler's pure casein, 1 gm. sodium carbonate, and 1000 c.c. distilled water. Add a little toluol to prevent bacterial activity and place in an incubator at about 38° C. At intervals remove a few cubic centimeters and test for casein by adding a few drops of acetic acid of about 1 per cent. strength. A white cloud appears as long as any casein remains undigested. With the patient upon a protein diet, there is normally a sufficient amount of trypsin to digest all the casein in ten to fifteen hours. Delay or complete failure of digestion shows diminution or absence of trypsin.

III. MICROSCOPIC EXAMINATION

Care must be exercised in selection of portions for examination. A random search will often reveal nothing of interest. A small bit of the stool, or any suspicious-looking particle, is placed upon a slide, softened with water if necessary, and pressed out into a thin layer with a cover-glass. A large slide—about 2 by 3 inches—with a correspondingly large cover will be found conve-

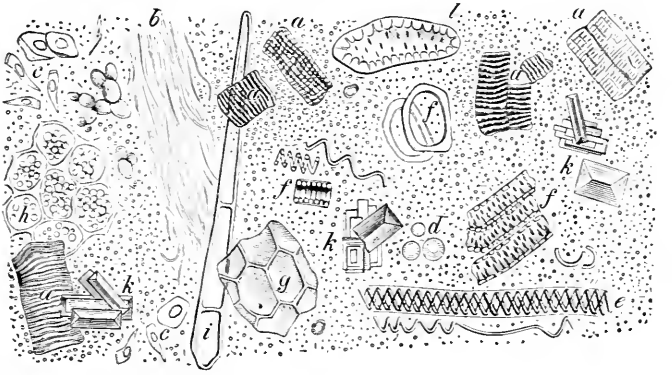


Fig. 109.—Microscopic elements of normal feces: *a*, Muscle-fibers; *b*, connective tissue; *c*, epithelial cells; *d*, white blood-corpuscles; *e*, spiral vessels of plants; *f–h*, vegetable cells; *i*, plant hairs; *k*, triple phosphate crystals; *l*, stone cells. Scattered among these elements are micro-organisms and débris (after v. Jaksch).

nient. Most of the structures which it is desired to see can be found with a 16-mm. objective. Details of structure must be studied with a higher power.

The bulk of the stool consists of granular débris. Among the recognizable structures met in normal and pathologic conditions are: Remnants of food, epithelial cells, pus-corpuscles, red blood-corpuscles, crystals, bacteria, and ova of animal parasites (Fig. 109).

1. Remnants of Food.—These include a great variety of structures which are very confusing to the student. Considerable study of normal feces is necessary for their recognition.

Vegetable fibers are generally recognized from their spiral structure or their pits, dots, or reticulate markings; *vegetable cells*, from their double contour and the chlorophyl bodies which many of them contain. These cells are apt to be mistaken for the ova of parasites.

Fig. 110.—Poorly digested muscle-fiber in feces showing striations (×200) (photograph by the author).

Vegetable hairs (Fig. 109, *i*) frequently look much like the larvæ of some of the worms. Anything like a careful examination will, however, easily distinguish them, because of the homogeneous and highly refractile wall, the distinct central canal which extends the whole length, and, especially, the absence of motion. *Starch-granules* sometimes retain their original form, but are ordinarily not to be recognized except by their staining reaction. They strike a blue color with Lugol's solu-

tion when undigested; a red color, when slightly digested. *Muscle-fibers* are yellow, and when poorly digested appear as short, transversely striated cylinders with rather squarely broken ends (Fig. 110). Generally, the ends are rounded and the striations faint, or only irregularly round or oval yellow masses are found. *Curds of milk* are especially important in the stools of children. They must be distinguished from small masses of *fat* (see p. 352).

Excess of any of these structures may result from excessive ingestion or deficient intestinal digestion.

2. Epithelial Cells.—A few cells derived from the wall of the alimentary canal are a constant finding. They show all stages of degeneration, and are often unrecognizable. A marked excess has its origin in a catarrhal condition of some part of the bowel. Squamous cells come from the anal orifice; otherwise the form of the cells gives no clue to the location of the lesion.

3. Pus.—Amounts of pus sufficient to be recognized with the eye alone indicate rupture of an abscess into the bowel. If well mixed with the stool, the source is high up, but in such cases the pus is apt to be more or less completely digested, and hence unrecognizable. Small amounts, detected only by the microscope, are present in catarrhal and ulcerative conditions of the intestine, the number of pus-cells corresponding to the severity and extent of the process.

4. Blood-corpuscles.—Unaltered red corpuscles are rarely found unless their source is near the anus. Ordinarily, only masses of blood-pigment can be seen. Blood is best recognized by the chemic tests (see p. 353).

5. Bacteria.—In health, bacteria—mostly dead—constitute about one-third of the weight of the dried stool.

They are beneficial to the organism, although not actually necessary to its existence. Under certain conditions they may be harmful. It is both difficult and unprofitable to identify them. The great majority belong to the colon bacillus group, and are negative to Gram's method of staining.

In some pathologic conditions the character of the intestinal flora changes, so that Gram-staining bacteria very greatly predominate. As shown by R. Schmidt, of Neusser's clinic in Vienna, this change is most constant and most striking in cancer of the stomach, owing to large numbers of Boas-Oppler bacilli, and is of considerable value in diagnosis. He believes that a diagnosis of gastric carcinoma should be very unwillingly made with an exclusively "Gram-negative" stool, while a "Gram-positive" stool, due to bacilli (which should also stain brown with Lugol's solution), may be taken as very strong evidence of cancer. A Gram-positive stool due to cocci is suggestive of intestinal ulceration. The technic is the same as when Gram's method is applied to other material (see p. 467), except that the smear is fixed by immersion in methyl-alcohol for five minutes instead of by heat. Pyronin is a good counterstain. The deep purple Gram-staining bacteria stand out more prominently than the pale-red Gram-negative organisms, and one may be misled into thinking them more numerous even in cases in which they are much in the minority. The number of Boas-Oppler bacilli can be increased by administering a few ounces of sugar of milk the day before the examination. The bacteria can be obtained comparatively free from food remnants by mixing a little of the feces with water, allowing to settle for a

short time, and making smears from the supernatant fluid.

Owing to the difficulty of excluding swallowed sputum, the presence of the **tubercle bacillus** is less significant in the feces than in other material. It may, however, be taken as evidence of intestinal tuberculosis when clinical signs indicate an intestinal lesion and reasonable care is exercised in regard to the sputum. Success in the search will depend largely upon careful selection of the portion examined. A random search will almost surely fail. Whitish or grayish flakes of mucus or blood-stained or purulent particles should be spread upon slides or covers and stained by the method given upon p. 205. In the case of rectal ulcers, swabs can be made directly from the ulcerated surface. With young children who swallow all their sputum an examination of the stool for tubercle bacilli may be the means of diagnosing tuberculosis of the lung.

6. Crystals.—Various crystals may be found, but few have any significance. Slender, needle-like crystals of fatty acids and soaps (see Fig. 37) and triple phosphate crystals (see Fig. 109) are common. Characteristic octahedral crystals of calcium oxalate (see Fig. 52) appear after ingestion of certain vegetables. Charcot-Leyden crystals (see Fig. 9) are not infrequently encountered, and strongly suggest the presence of intestinal parasites. Yellowish or brown, needle-like or rhombic crystals of hematoidin (see Fig. 37) may be seen after hemorrhage into the bowel. The dark color of the feces after administration of bismuth salts is due largely to great numbers of bismuth suboxid crystals. They resemble hemin crystals.

7. Parasites and Ova.—Descriptions will be found in the following chapter. The flagellates are usually best found in the second stool after a saline cathartic, the first stool being ordinarily too solid and the later ones too dilute.

To find ova when scarce, they must be concentrated. Stiles advises thoroughly mixing the stool with a quart or more of water, allowing to settle, pouring off the water almost down to the sediment, and repeating the process as long as any matter floats. The final sediment is poured into a conical glass and allowed to settle. Ova will be found in the fine sediment, which can readily be removed with a pipet. Other and more complicated methods have been devised, but this and Pepper's method for hookworm eggs (see p. 413) will probably answer all clinical needs.

IV. FUNCTIONAL TESTS

1. Schmidt's Test Diet.—Much can be learned of the various digestive functions from a microscopic study of the feces, especially when the patient is upon a known diet. For this purpose the standard diet of Schmidt is generally adopted. This consists of:

Morning	0.5 liter milk and 50 gm. toast.
Forenoon	0.5 liter porridge, made as follows: 40 gm. oatmeal, 10 gm. butter, 200 c.c. milk, 300 c.c. water, one egg, and salt to taste.
Midday	125 gm. hashed meat, with 20 gm. butter, fried so that the interior is quite rare; 250 gm. potato, made by cooking 190 gm. potato with 100 c.c. milk and 10 gm. butter, the whole boiled down to 250 c.c.
Afternoon	Same as morning.
Evening	Same as forenoon.

At the beginning of the diet, the stool should be marked off with carmin or charcoal (see p. 362). One should familiarize himself with the microscopic appearance of the feces of normal persons upon this diet.

Deficiency of starch digestion is recognized by the number of starch-granules which strike a blue color with iodin. With exception of those inclosed in plant cells none are present normally.

The degree of protein digestion is ascertained by the appearance of the muscle-fibers. Striations are clearly visible on any considerable number of the fibers only when digestion is imperfect (see Fig. 110). They usually disappear after the feces have stood for some time. According to Schmidt, the presence of nuclei in muscle-fibers denotes complete absence of pancreatic function. The presence of connective-tissue shreds indicates deficient gastric digestion, since raw connective tissue is digested only in the stomach. These shreds can be recognized macroscopically by examining in a thin layer against a black background, and microscopically by their fibrous structure and the fact that they clear up when treated with acetic acid. Digestion of fats is checked up by the amount of neutral fat.

Schmidt's nuclei test for pancreatic insufficiency consists in the administration of a $\frac{1}{2}$-cm. cube of fresh beef tied in a little gauze bag with the test-meal. When the bag appears in the feces it is opened and its contents examined microscopically by pressing out small bits between a slide and cover. A drop of some nuclear stain may be applied if desired. If the nuclei are for the most part undigested, pancreatic insufficiency may be assumed, since it is probable that nuclei can be digested

only by the pancreatic juice. Normally the nuclei are digested, provided the time of passage through the intestine is not less than six hours.

2. Sahli's Glutoid Test.—The Schmidt test diet involves some inconvenience for the patient, and interpretation of results requires much experience upon the part of the physician. A number of other methods of testing the digestive functions have been proposed. The glutoid test of Sahli is one of the most satisfactory. This is similar to his desmoid test of gastric digestion described on page 346. A glutoid capsule containing 0.15 gram iodoform is taken with an Ewald breakfast. The capsule is not digested by the stomach fluid, but is readily digested by pancreatic juice. Appearance of iodin in the saliva and urine within four to six hours indicates normal gastric motility, normal intestinal digestion, and normal absorption. Instead of iodoform, 0.5 gram salol may be used, salicyluric acid appearing in the urine in about the same time. For tests for iodin and salicyluric acid, see pages 346 and 347.

Glutoid capsules are prepared by soaking gelatin capsules in formalin. Sahli states that filled capsules can be purchased of A. G. Haussmann, in St. Gall, Switzerland.

3. Motility.—Ordinarily, fifteen to thirty hours are required for the passage of ingested material through the gastro-intestinal tract. In diarrheal conditions the time is usually much shortened, unless the pathologic process is in the colon. In intestinal stasis it may be much prolonged. The time of passage is acertained by giving 0.5 gm. of powdered charcoal or 0.3 gm. of carmine in a capsule with a meal and watching for the resulting discolored feces.

CHAPTER VI

ANIMAL PARASITES

ANIMAL parasites are common in all countries, but are especially abundant in the tropics, where, in some localities, almost every native is host for one or more species. Because of our growing intercourse with these regions the subject is assuming increasing importance in this country. Many parasites, hitherto comparatively unknown here, will probably become fairly common.

Some parasites produce no symptoms, even when present in large numbers. Others cause very serious symptoms. It is, however, impossible to make a sharp distinction between pathogenic and non-pathogenic varieties. Parasites which cause no apparent ill effects in one individual may, under certain conditions, produce marked disturbances in another. The disturbances are so varied, and frequently so indefinite, that diagnosis can rarely be made from the clinical symptoms. It must rest upon detection, by the naked eye or the microscope, of (*a*) the parasites themselves, (*b*) their ova or larvæ, or (*c*) some of their products.

Unlike bacteria, the great majority of animal parasites multiply by means of alternating and differently formed generations, which require widely different conditions for their development. The few exceptions are chiefly among the protozoa. Multiplication of parasites within the same host is thus prevented. In the case of the

hookworm, for example, there is no increase in the number of worms in the host's intestine, except through reinfection from the outside. The ova are carried out of the intestine and the young must pass a certain period of development in warm, moist earth before they can again enter the human body and grow to maturity. This also explains the geographic distribution of parasites. The hookworm cannot flourish in cold countries; malaria can prevail only in localities in which the mosquito, Anopheles, exists, and then only after the mosquitoes have become infected from a human being.

In general, this alternation of periods of development takes place in one of three ways:

(1) The young remain within the original host, but travel to other organs, where they do not reach maturity, but lie quiescent until taken in by a new host. A good example is *Trichinella spiralis.*

(2) The young or the ova which subsequently hatch pass out of the host, and either (*a*) go through a simple process of growth and development before entering another host, as is the case with the hookworm, or (*b*) pass through one or more free-living generations, the progeny of which infect new hosts, as is the case with *Strongyloides intestinalis.*

(3) The young or ova or certain specialized forms either directly (*e. g.*, malarial parasites) or indirectly (*e. g.*, tapeworms) reach a second host of different species, where a widely different process of development occurs. The host in which the adult or sexual existence is passed is called the *definitive* or final host; that in which the intermediate or larval stage occurs, the *intermediate* host. Man, for example, is the definitive

host for *Tænia saginata*, and the intermediate host for the malarial parasites and *Tænia echinococcus*.

A few words concerning the classification and nomenclature of living organisms in general will be helpful at this place. Individuals which are alike *in all essential respects* are classed together as a *species*. Closely related species are grouped together to form a *genus;* genera, which have certain characteristics in common make up a *family;* families are grouped into *orders;* orders, into *classes;* and classes, finally, into the *branches* or *phyla*, which make up the animal and vegetable *kingdoms*. In some cases these groups are subdivided into intermediate groups—subphyla, subfamilies, etc., and occasionally slight differences warrant subdivision of the species into *varieties*.

The scientific name of an animal or plant consists of two parts, both Latin or Latinized words, and is printed in italics. The first part is the name of the genus and begins with a capital letter; the second is the name of the species and begins with a lower case letter, even when it was originally a proper name. When there are varieties of a species, a third part, the designation of the variety, is appended. The author of the name is sometimes indicated in Roman type immediately after the name of the species. Examples: *Spirochæta vincenti*, often abbreviated to *Sp. vincenti* when the genus name has been used just previously; *Staphylococcus pyogenes albus; Necator americanus*, Stiles.

At the present time there is great confusion in the naming and classification of parasites. Some have been given a very large number of names by different observers, and in many cases different parasites have been

described under the same name. The alternation of generations and the marked differences in some cases between male and female have contributed to the confusion, different forms of the same parasite being described as totally unrelated species.

The number of parasites which have been described as occurring in man and the animals is extremely large. Only those which are of medical interest are mentioned here. They belong to four phyla—Protozoa, Platyhelminthes, Nemathelminthes, and Arthropoda.

PHYLUM PROTOZOA

These are unicellular organisms, the simplest types of animal life. There is very little differentiation of structure. Each contains at least one, and some several, nuclei. Some contain contractile vacuoles; some have cilia or flagella as special organs of locomotion. They reproduce by division, by budding, or by sporulation. Sometimes there is an alternation of generations, in one of which sexual processes appear, as is the case with the malarial parasites. The protozoa are very numerous, the subphylum Sarcodina alone including no less than 5000 species. Most of the protozoa are microscopic in size; a few are barely visible to the naked eye. The beginning student can gain a general idea of their appearance by examining water (together with a little of the sediment) from the bottom of any pond. Such water usually contains amebæ and a considerable variety of ciliated and flagellated forms.

The following is an outline of those protozoa which are of medical interest, together with the subphyla and classes to which they belong:

PHYLUM PROTOZOA

SUBPHYLUM I. **SARCODINA.**—Locomotion by means of pseudopodia.

CLASS **Rhizopoda.**—Pseudopodia form lobose or reticulose processes.

Genus.	*Species.*
Entamœba.	E. histolytica.
	E. coli.
	E. buccalis.

SUBPHYLUM II. **MASTIGOPHORA (FLAGELLATA).**—Locomotion by means of flagella.

CLASS **Zoömastigophora.**—Forms in which animal characteristics predominate.

Genus.	*Species.*
Spirochæta.	Sp. recurrentis.
	Sp. vincenti.
	Sp. buccalis.
	Sp. dentium.
	Sp. refringens.
Treponema.	T. pallidum.
	T. pertenue.
Trypanosoma.	T. gambiense.
	T. rhodesiense.
	T. cruzi.
	T. lewisi.
	T. evansi.
	T. brucei.
	T. equiperdum.
Leishmania.	L. donovani.
	L. tropica.
	L. infantum.
Cercomonas.	C. hominis.
Bodo.	B. urinarius.
Trichomonas.	T. vaginalis.
	T. intestinalis.
	T. pulmonalis.
Lamblia.	L. intestinalis.

Subphylum III. **SPOROZOA.**—All members parasitic. Propagation by means of spores. No special organs of locomotion.

Class **Telosporidia.**—Sporulation ends the life of the individual.

Genus.	*Species.*
Coccidium.	C. cuniculi.
Plasmodium.	P. vivax.
	P. malariæ.
	P. falciparum.
	P. tenue.
Babesia.	B. bigeminum.

Subphylum IV. **INFUSORIA.**—Locomotion by means of cilia.

Class **Ciliata.**—Cilia present throughout life.

Genus.	*Species.*
Balantidium.	B. coli.

SUBPHYLUM SARCODINA

Class Rhizopoda

These are protozoa the body substance of which forms changeable protoplasmic processes, or pseudopodia, for the taking in of food and for locomotion. They possess one or several nuclei.

1. Genus Entamœba.—(1) **Entamœba histolytica.**—This organism is found, often in large numbers, in the stools of tropical dysentery and in the pus and walls of hepatic abscesses associated with dysentery. Infection is more common in this country than is usually supposed. It is a grayish or colorless, granular cell, usually between 25 and 40 μ in diameter (Fig. 111). Its appearance varies according to its stage of development. In the vegetative stage, which is found in acute dysentery, there is a distinct homogeneous, refractile ectoplasm and a granular endoplasm containing one or more distinct vacuoles, a round nucleus which is ordinarily very indistinct, and frequently ingested red blood-

corpuscles and bacteria. When at rest its shape is spheric, but upon a warm slide it exhibits the characteristic *ameboid motion*, constantly changing its shape or moving actively about by means of distinct pseudopodia. This motion is its most distinctive feature, and should always be seen to establish the identity of the organism in this stage. It is lost when the specimen cools, and can usually not be re-established by

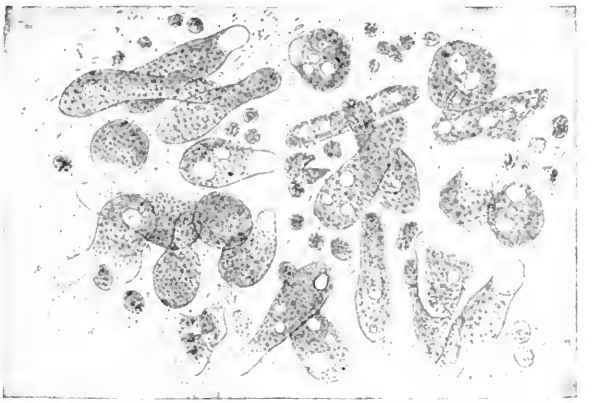


Fig. 111.—Entamœba histolytica in intestinal mucus, with blood-corpuscles and bacteria (Lösch).

warming. According to Simon, if neutral red in 0.5 per cent. solution be run under the cover-glass, it will be taken up by the entamebæ and other protozoa and will render them conspicuous without killing them ("vital staining"). Pauton recommends that the suspected material be mounted in a drop of aqueous methylene-blue solution and covered. Body cells will be stained blue, while the entamebæ remain colorless and stand out distinctly against a blue background.

In dysentery "carriers" and in chronic cases when the stools are formed and hard most or all of the parasites may become encysted. Their appearance in this stage of development is given in the table on pages 371, 372.

When the presence of entamebæ is suspected, the stool should be passed into a warm vessel and kept warm until and during the examination. A warm stage can be improvised from a plate of copper with a hole cut in the center. This is placed upon the stage of the microscope, and one of the projecting ends is heated with a small flame. Entamebæ are most likely to be found in grayish or blood-streaked particles of mucus. Craig recommends the liquid stool following a saline cathartic. Favorable material for examination can often be obtained at one's convenience by inserting into the rectum a large catheter with roughly cut lateral openings. A sufficient amount of mucus or fecal matter will usually be brought away by it.

No staining method is as useful in diagnosis as the study of the living parasite. For more scientific study Darling recommends the following method: Stain with Wright's (or Hasting's or Leishman's) stain in the usual way, and follow this with Giemsa's stain until the film has a purple cast. Then plunge the preparation into a small beaker of 60 per cent. alcohol to which 10 to 20 drops of ammonia have been added and keep it in motion until the desired differentiation is obtained, when the film will have a violet color.

(2) **Other Entamebæ.**—*Entamœba coli*, a similar but somewhat smaller organism, rarely over 25 μ in diameter, has frequently been found in the stools of healthy

persons. It has less distinct pseudopodia, less sharp differentiation between ectoplasm and endoplasm, less active motion and more distinct nucleus, and does not contain ingested red corpuscles or never more than one or two.

The principal points of distinction between *E. histolytica* and *E. coli* are included in the following table which is slightly modified from Craig[1]:

VEGETATIVE STAGE

This stage of *E. histolytica* is found in acute dysentery.

Entamœba histolytica.	*Entamœba coli.*
Averages larger. Unimportant.	Averages smaller.
Actively motile. Characteristic. Often moves from place to place.	Sluggishly motile. Seldom moves from place to place.
Ectoplasm hyaline, glass-like, sharply differentiated from endoplasm. Characteristic.	Ectoplasm not glass-like, poorly differentiated from endoplasm.
Nucleus usually indistinct, often invisible. Changes position with motion of parasite.	Nucleus distinct. Located near center.
Red blood-cells present in endoplasm when stool contains blood. Very characteristic.	No red blood-cells (or never more than one or two) in endoplasm when stool contains blood.

PRECYSTIC STAGE

E. histolytica may be found in this stage when symptoms of dysentery have practically disappeared. The parasite is reduced in size, is sluggishly motile, and becomes practically indistinguishable from *E. coli*. The distinction must be based upon the vegetative or cystic forms, a few of which can usually be found in the same stool.

[1] Craig: Archives of Internal Medicine, 1914, xiii, 917.

CYSTIC STAGE

In formed stools both entamebæ are commonly encysted. This, therefore, is the form of *E. histolytica* to be looked for between recurrences and in dysentery "carriers."

Entamœba histolytica.	*Entamœba coli.*
Cysts spheric or oval. Cyst wall single and delicate in young cysts; thicker and sometimes double outlined in older ones.	Similar, but double outline of wall more frequently observed and more distinct.
Diameter 10–20 μ; average, 12 μ.	Diameter 10–25 μ; average, 15 μ.
Cytoplasm of young cysts granular, often with a large vacuole. Pressure of chromidia (brightly refractive, spindle-shaped or irregular masses of chromatin) characteristic.	Similar, but chromidia very rare.
Fully developed cysts contain four distinct nuclei seen by focusing at different levels. Very characteristic.	Fully developed cyst contains eight to sixteen nuclei, eight being the normal number.

E. tetragena, which was described in 1907 by Viereck, is now regarded as identical with *E. histolytica*. Another, *E. buccalis*, has been found in decaying teeth. A number of similar organisms have been described as occurring in pus and in ascitic and other body fluids, but it is probable that in many cases, at least, the structures seen were ameboid body cells.

SUBPHYLUM MASTIGOPHORA (FLAGELLATA)

Class Zoömastigophora

The protozoa of this subphylum are provided with one or several whip-like appendages with lashing motion, termed flagella, which serve for locomotion and, in some cases, for feeding. They generally arise from the

anterior part of the organism. Some members of the group also possess an undulating membrane—a delicate membranous fold which extends the length of the body, and somewhat suggests a fin. When in active motion this gives the impression of a row of cilia. The flagellata do not exhibit ameboid motion, and, in general, maintain an unchanging oval or spindle shape, and contain a single nucleus. The cytoplasm contains numerous granules and usually several vacuoles, one or more of which may be contractile. Encystment as a means of resisting unfavorable conditions is common.

1. Genus Spirochæta.—The spirochetes appear to occupy a position midway between the bacteria and protozoa, but are more frequently described with the latter.

(1) **Spirochæta recurrentis.**—This spirochete was described by Obermeier as the cause of relapsing fever. It appears in the circulating blood during the febrile attack, and, unlike the malarial parasite, lives in the plasma without attacking the red corpuscles. The organism is an actively motile spiral, 16 to 40 μ long, with three to twelve wide, fairly regular turns. It can be seen in fresh unstained blood with a high dry lens, being located by the commotion which it creates among the red cells. For diagnosis, thin films, stained with Wright's or some similar blood-stain, are used (Fig. 112). In such preparations the spirals are not so regular.

Besides *Spirochæta recurrentis*, a number of distinct strains have been described in connection with different types of relapsing fever: *Sp. novyi* (Plate VII), *Sp. kochi*, *Sp. duttoni*, and *Sp. carteri*.

(2) **Spirochæta vincenti.**—In stained smears from the

ulcers of Vincent's angina (see p. 436) are found what appear to be two organisms. One, the "fusiform bacillus," is a slender rod, 6 to 12 μ long, pointed at both ends and sometimes curved. The other is a slender spiral organism, 30 to 40 μ long, with three to eleven comparatively shallow turns (see Fig. 161). These were formerly thought to be bacteria, a spirillum and bacillus living in symbiosis. The present tendency is to regard

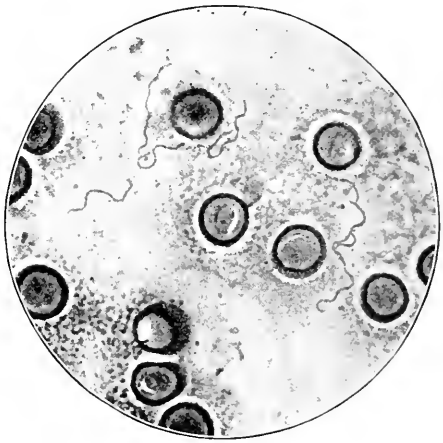


Fig. 112.—Spirochete of relapsing fever in blood (×1000) (Karg and Schmorl).

them as stages or forms of the same organism, and to class them among the spirochetes. The same organisms are quite constantly present in large numbers in ulcerative stomatitis and in noma. They are not infrequently found in small numbers in normal mouths.

(3) **Other Spirochetes.**—A number of harmless forms are of interest because of the possibility of confusing them with the more important pathogenic varieties. Of these, *Sp. buccalis* and *Sp. dentium* are inhabitants

of the normal mouth. The former is similar in morphology to *Sp. vincenti*. *Sp. dentium* (Fig. 113) is smaller, more delicate, has deep curves, and may be easily mistaken for *Treponema pallidum*. It, also, stains reddish with Giemsa's stain. In suspected syphilitic sores of the mouth it is, therefore, important to make smears from the tissue juices rather than from the surface (see p. 446). Thibaudeau has found it or a similar organism in as high as 41 per cent. of normal mouths. *Sp. refringens* is frequently present upon the surface

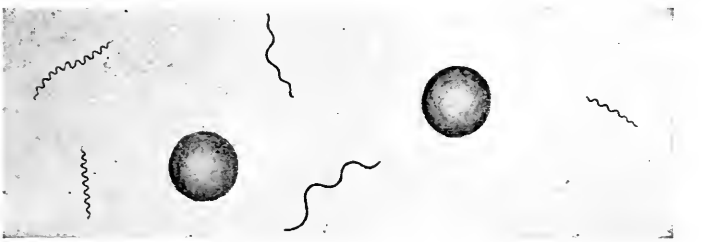


Fig. 113.—Spiral organisms: A, Treponema pallidum; B, Spirochæta refringens; C, Spirochæta dentium. Two red corpuscles are also shown (×1200).

of ulcers, especially about the genitals, and has doubtless many times been mistaken for *Treponema pallidum*. It can be avoided by properly securing the material for examination; but its morphology should be sufficient to prevent confusion. It is thicker than the organism of syphilis, stains more deeply, and has fewer and shallower curves (Fig. 113). Giemsa's stain gives it a bluish color.

2. Genus Treponema.—(1) **Treponema pallidum.**—This is the organism of syphilis. Its description and methods of diagnosis will be found on p. 445.

(2) **Treponema pertenue,** morphologically very similar to *Treponema pallidum*, was found by Castellani in yaws, a skin disease of the tropics.

3. Genus Trypanosoma.—Trypanosomes have been found in the blood-plasma of a great variety of vertebrates. Many of them appear to produce no symptoms, but a few are of great pathologic importance. As seen in the blood, they are elongated, spindle-shaped bodies, the average length of different species varying from 10 to 70 μ. Along one side there runs a delicate undulating membrane, the free edge of which appears to be somewhat longer than the attached edge, thus throwing it into folds. Somewhere in the body, usually near the middle, is a comparatively pale-staining nucleus; and near the posterior end is a smaller, more deeply staining chromatin mass, the micronucleus or blepharoplast. A number of coarse, deeply staining granules, chromatophores, may be scattered through the cytoplasm. A flagellum arises in the blepharoplast, passes along the free edge of the undulating membrane, and is continued anteriorly as a free flagellum. These details of structure are well shown in Plate VII.

The life history of the trypanosomes is not well known. In most cases there is an alternation of hosts, various insects playing the part of definitive host.

Trypanosomes have been much studied of late, and many species have been described. At least three have been found in man.

Trypanosoma gambiense is the parasite of African "sleeping sickness." Its detection in the blood is described on p. 295. A new species causing sleeping sickness in man has recently been described and has been

named *T. rhodesiense.* The chief point of distinction from *T. gambiense* is the situation of the nucleus close to or even posterior to the blepharoplast. It is transmitted by the fly *Glossina morsitans.*

Trypanosoma cruzi is a small form which has been found in the blood of man in Brazil.

Fig. 114.—Trypanosoma lewisi in blood of rat. The red corpuscles were decolorized with acetic acid (×1000) (photograph by the author from a slide presented by Prof. Novy).

Trypanosoma lewisi, a very common and apparently harmless parasite of gray rats, especially sewer rats, is interesting because it closely resembles the pathogenic forms, and is easily obtained for study. Its posterior end is more pointed than that of *T. gambiense* (Fig. 114).

Trypanosoma evansi, *T. brucei*, and *T. equiperdum* produce respectively surra, nagana, and dourine, which are common and important diseases of horses, mules, and cattle in the Philippines, East India, and Africa.

4. Genus Leishmania.—The several species which compose this genus are apparently closely related to the trypanosomes, but their exact classification is undetermined. They have been grown outside the body and their transformation into flagellated trypanosome-like structures has been demonstrated. Calkins places them in the genus *Herpetomonas*.

(1) **Leishmania donovani** is the cause of kala-azar, an important and common disease of India. The "Leishman-Donovan bodies" are round or oval structures, 2 to 3 μ in diameter, with two distinct chromatin masses, one large and pale, the other small and deeply staining. The parasites are especially abundant in the spleen,

Fig. 115.—Cercomonas hominis (about ×500): A, Larger variety; B, smaller variety (Davaine).

splenic puncture being resorted to for diagnosis. They are readily found in smears stained by any of the Romanowsky methods. They lie chiefly within endothelial cells and leukocytes. They are also present within leukocytes in the peripheral blood, but are difficult to find in blood-smears.

(2) **Leishmania tropica** resembles the preceding. It is found, lying intracellularly, in the granulation tissue of Delhi boil or Oriental sore.

(3) **Leishmania infantum** has been found in an obscure form of infantile splenomegaly in Algiers.

5. Genus Cercomonas.—(1) **Cercomonas hominis** has been found in the feces in a variety of diarrheal condi-

tions, and in from 10 to 25 per cent. of healthy persons in tropical regions. It is probably harmless. The body is 10 to 12 μ long, is pointed posteriorly, and has a flagellum at the anterior end (Fig. 115). The nucleus is difficult to make out. The feces should be examined in the fresh state, and preferably while warm, in order to observe the active motion of the organism.

6. Genus Bodo.—(1) **Bodo urinarius** is sometimes seen in the urine, darting about in various directions. It is probably an accidental contamination. It has a lancet-

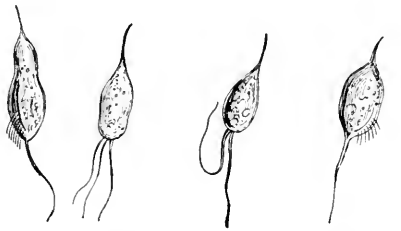


Fig. 116.—Trichomonas vaginalis (about ×1000) (after Kölliker and Scanzoni).

shaped body, about 10 μ long, and is somewhat twisted upon itself, with two flagella at the end.

7. Genus Trichomonas.—(1) **Trichomonas vaginalis.**—The acid discharge of catarrhal vaginitis sometimes contains this parasite in abundance. It is oval or pear shaped and has a cluster of flagella at one end (Fig. 116). The average size is about 12 by 20 μ, although there is considerable variation among individuals. As seen in fresh material it may suggest an actively motile pus-corpuscle. When in motion the flagella are not easily seen. No pathogenic significance is ascribed to it in the vagina, but a few cases have been reported in which it

was apparently the cause of a urethritis in the male. It is occasionally found in the urine. This and similar organisms, such as cercomonas and bodo, might be mistaken for spermatozoa by the totally inexperienced worker.

(2) **Other Trichomonads.**—Various forms have been described, regarded by some as identical with *T. vagi-*

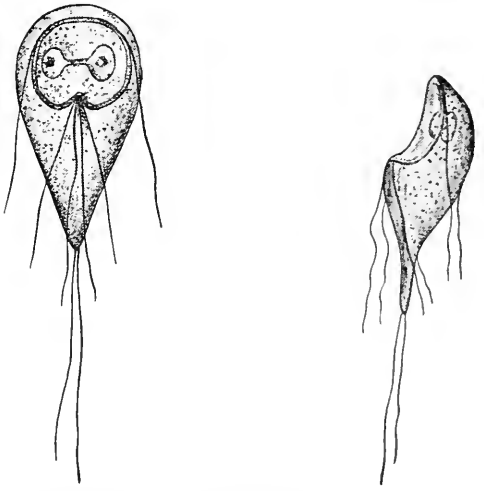


Fig. 117.—Lamblia intestinalis from the intestines of a mouse (about × 2000) (Grassi and Schweiakoff).

nalis, by others as distinct species. Among these are *T. intestinalis*, sometimes found in the feces in diarrheal conditions, and *T. pulmonalis*, which has been encountered in the sputum of persons suffering from pulmonary gangrene and putrid bronchitis.

8. Genus Lamblia.—(1) **Lamblia intestinalis** is a very common parasite in the tropics, but is generally considered of little pathogenic importance. It is pear shaped,

measures 10 to 15 μ or more in length, and has a depression on one side of the blunt end, by which it attaches itself to the tops of the epithelial cells of the intestinal wall. Three pairs of flagella are arranged about the depression and one pair at the pointed end (Fig. 117). Unless the stool is obtained by catharsis (see p. 360), encysted forms only may be found, and these may be difficult or impossible to recognize.

SUBPHYLUM SPOROZOA

Class Telosporidia

All the members of this class are parasitic, but only a few have been observed in man, and only one genus, *Plasmodium*, is of much importance in human pathology. Propagation is by means of spores, and sporulation ends the life of the individual. In some species there is an alternation of generations, in one of which sexual processes appear. In such cases the male individual may be provided with flagella. Otherwise, there are no special organs of locomotion.

1. Genus Coccidium.—(1) **Coccidium cuniculi.**—This is a very common parasite of the rabbit and has been much studied; but extremely few authentic cases of infection in man have been reported. The parasite, which when fully developed is ovoid in shape and measures about 30 to 50 μ in length and has a shell-like integument, develops within the epithelial cells of the bile-passages. Upon reaching adult size it divides into a number of spores or merozoites which enter other epithelial cells and repeat the cycle. A sexual cycle outside the body, which suggests that of the malarial parasite, but does not require an insect host, also occurs.

Infection takes place from ingestion of the resulting sporozoites.

2. Genus Plasmodium.—This genus includes the malarial parasites which have already been described (see p. 296).

3. Genus Babesia.—The proper position of this genus is uncertain. It is placed among the flagellates by some. The chief member is *Babesia bigeminum*, the cause of Texas fever in cattle. It is a minute, pear-shaped organism, lying in pairs within the red blood-corpuscles. An organism, *B.* (or *Piroplasma*) *hominis*, described as occurring in the red cells in "tick-fever" of Montana, is also placed in this genus, but its pathogenicity and even its existence are questionable.

SUBPHYLUM INFUSORIA

Class Ciliata

The conspicuous feature of this class is the presence of cilia. These are hair-like appendages which have a regular to-and-fro motion, instead of the irregular lashing motion of flagella. They are also shorter and more numerous than flagella. Most infusoria are of fixed shape and contain two nuclei. Contractile and food-vacuoles are also present. Encystment is common. Only one species is of medical interest. Certain ciliated structures, which have been described as infusoria, notably in sputum and nasal mucus, were probably ciliated body cells.

1. Genus Balantidium.—(1) **Balantidium coli.**—This parasite, formerly called *Paramœcium coli*, is an occasional inhabitant of the colon of man, where it sometimes penetrates into the mucous membrane and pro-

duces a diarrheal condition resembling amebic dysentery. It is an oval organism, about 60 to 100 μ long and 50 to 70 μ wide, is covered with cilia, and contains a bean-shaped macronucleus, a globular micronucleus, two contractile vacuoles, and variously sized granules (Fig. 118).

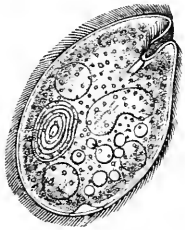


Fig. 118.—Balantidium coli (about × 350) (after Eichhorst).

Its ordinary habitat is the rectum of the domestic pig, where it apparently causes no disturbance. It probably reaches man in the encysted condition.

PHYLUM PLATYHELMINTHES

The old phylum Vermidea has been subdivided into three phyla, those which are of interest here being the Platyhelminthes and Nemathelminthes, the flat worms and the round worms respectively. Of these, many species are parasitic in man and the higher animals. In some cases man is the regular host; in others the usual habitat is some one of the animals, and the occurrence of the worm in man is more or less accidental. Such are called *incidental parasites*. Only those worms that are found in man with sufficient frequency to be of medical interest are mentioned here.

The most important means of clinical diagnosis of infection by either the flat worms or the round worms

is the finding of ova. In many cases the ova are so characteristic that the finding of a single one will establish the diagnosis. In other cases they must be carefully studied and a considerable number measured. While ova from the same species will naturally vary somewhat, the average size of a dozen or more is pretty constant. The measurements given here are mainly those accepted by Stiles or Ward.

PHYLUM PLATYHELMINTHES

(Flat Worms)

CLASS **Trematoda**.—Flukes. Unsegmented, leaf shaped.

Genus.	*Species.*
Fasciola.	F. hepatica.
Dicrocœlium.	D. lanceatum.
Opisthorchis.	Op. felineus.
	Op. sinensis.
Fasciolopis.	F. buski.
Paragonimus.	P. westermannii.
Schistosomum.	S. hæmatobium.
	S. mansoni.
	S. japonicum.

CLASS **Cestoda**.—Tapeworms. Segmented, ribbon shaped.

Genus.	*Species.*
Tænia.	T. saginata.
	T. solium.
	T. echinococcus.
Hymenolepis.	H. nana.
	H. diminuta.
Dipylidium.	D. caninum.
Dibothriocephalus.	D. latus.

Class Trematoda

The trematodes, commonly known as "flukes," are flat, unsegmented, generally tongue- or leaf-shaped worms. They are comparatively small, most species averaging between 5 and 15 mm. in length. They pos-

sess an incomplete digestive tract, without anus, and are provided with one or more sucking disks by means of which they can attach themselves to the host. Some are also provided with hooklets. Nearly all species are hermaphroditic, and the eggs of nearly all are operculated (provided with a lid), the only important exception being the several species of *Schistosomum*. Development takes place by alternation of generations, the intermediate generation occurring in some water animal: mol-

Fig. 119.—Fasciola hepatica, about two-thirds natural size (Mosler and Peiper).

lusks, amphibians, fishes, etc. Trematode infection is uncommon in this country.

1. Genus Fasciola.—(1) **Fasciola hepatica.**—The "liver fluke" inhabits the bile-ducts of numerous herbivorous animals, especially sheep, where it is an important cause of disease. It brings about obstruction of the bile-passages, with enlargement and degeneration of the liver —"liver rot." A species of snail serves as intermediate host. The worm is leaf shaped, the average size being about 2.8 by 1.2 cm. The anterior end projects like a beak (head-cone 3 to 4 mm. long) (Fig. 119). Ova appear in the feces. They are yellowish brown, oval, operculated, and measure about 130 to 140 by 75 to 90 μ.

2. Genus Dicrocœlium.—(1) **Dicrocœlium lanceatum** is often associated with the liver fluke in the bile-passages of animals, but is neither so common nor so widely distributed geographically. It has rarely been observed in man. It is smaller (length about 1 cm.) and more elongated. The eggs measure 38 to 45 μ long and 22 to 30 μ wide.

3. Genus Opisthorchis.—(1) **Opisthorchis felineus** inhabits the gall-bladder and bile-ducts of the domestic cat and a few other animals. Infection in man has been repeatedly observed in Europe, and especially in Siberia. The body is flat, yellowish-red in color, and almost transparent. It measures 8 to 11 by 1.5 to 2 mm. The eggs, which are found in the feces, are oval, with a well-defined operculum at the narrower end, and contain a ciliated embryo when deposited. They measure about 30 by 11 μ.

(2) **Opisthorchis sinensis,** like the preceding fluke, inhabits the gall-bladder and bile-ducts of domestic cats and dogs. It is, however, much more frequent in man, being a common and important parasite in certain parts of Japan and China. The number present may be very great; over 4000 were counted in one case. The parasite resembles *Op. felineus* in shape and color. It is 10 to 14 mm. long and 2.5 to 4 mm. broad. The eggs have a sharply defined lid and measure 25 to 30 by 15 to 17 μ. When they appear in the feces they contain a ciliated embryo. The intermediate host is unknown.

4. Genus Fasciolopis. (1) **Fasciolopis buski.**—This fluke is parasitic in the duodenum of man, and is widespread in the East, notably in India, China, and

Japan. A few imported cases have been reported in this country. When in considerable numbers it causes a bloody diarrhea accompanied by high fever. The usual length is about 30 mm.; width, 10 to 12 mm.; thickness, 1.5 to 4 mm. The eggs are thin shelled, with granular contents, possess a minute operculum, and measure about 125 by 75 to 80 μ.

5. Genus Paragonimus.—(1) **Paragonimus westermannii,** called the "lung fluke," is also a common parasite of man in Japan, China, and Korea. It inhabits the lung, causing the formation of small cavities. Moderate hemoptysis is the principal symptom. Ova are readily found in the sputum (Fig. 120); the worms themselves are seldom seen, except *postmortem*. The worms are faint reddish-brown in color, egg shaped, with the ventral surface flattened, and measure 8 to 10 by 4 to 6 mm. The ova are thin shelled, operculated, brownish yellow, and measure about 87 to 100 by 52 to 66 μ. Little is known of the development outside the body.

According to Ward, three distinct species have been confused under the name *P. westermannii:* the original form, *P. westermannii*, found in the tiger; the American lung fluke, *P. kellicotti*, thus far found only in cat, dog, and hog; and the Asiatic lung fluke of man, *P. ringeri*, described above.

6. Genus Schistosomum.—(1) **Schistosomum hæmatobium.**—This trematode, frequently called *Bilharzia hæmatobia*, is an extremely common cause of disease (bilharziasis or Egyptian hematuria) in northern Africa, particularly in Egypt.

Unlike the other flukes, the sexes are separate. The male is 12 to 14 mm. long and 1 mm. broad. The body

is flattened and the lateral edges curl ventrally, forming a longitudinal groove, in which the female lies (Fig. 122). The latter is cylindric in shape, about 20 mm. long and 0.25 mm. in diameter. The eggs are an elongated oval, about 120 to 190 μ long and 50 to 73 μ broad, yellowish in color, and slightly transparent. They possess no lid, such as characterizes the eggs of most of the trematodes,

Fig. 120.—Sputum of man containing eggs of the lung fluke, greatly enlarged. (After Manson.)

but are provided with a thorn-like spine which is placed at one end (Fig. 121). Within is a ciliated embryo.

In man the worm lives in the veins, particularly the portal vein and the veins of the bladder and rectum, leading to obstruction and inflammation. The eggs penetrate into the tissues and are present in abundance in the mucosa of the bladder and rectum. They also appear in the urine and, less commonly, in the feces. The mode of infection is unknown.

(2) **Schistosomum mansoni.**—It has long been observed that schistosomum eggs in the urine have usually a terminal spine, while in the feces the lateral spine is more common. It is now known that the lateral-spined egg is that of a distinct species, to which the name *Schistosomum mansoni* has been given. It is found in Africa along with *Schistosomum hæmatobium*, but is especially prevalent in the West Indies and Central America. The adult worms closely resemble the male and female of *S. hæmatobium*. They inhabit the rectal and portal veins, and ova appear in the feces, where they are very easily recognized from their size and the characteristic spine (see Figs. 123 and 135). They are light yellow in color, measure 112 to 162 by 60 to 70 μ, and are provided with a cleanly cut, sharply pointed spine, which is situated at the juncture of the last and third quarter

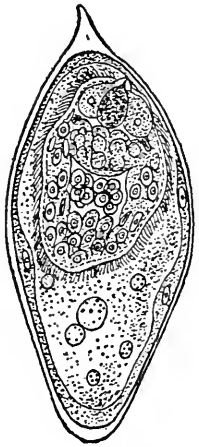


Fig. 121.—Egg of human blood fluke (*Schistosomum hæmatobium*), with contained embryo, passed in the urine (× 285). (After Looss.)

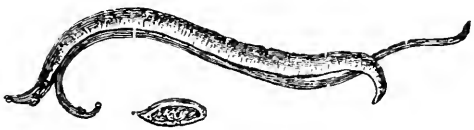


Fig. 122.—Schistosomum hæmatobium, male and female (about × 4), with egg (about × 70) (von Jaksch).

of the egg and is directed backward. Within the egg is a ciliated embryo (miracidium) which can be seen without difficulty.

(3) **Schistosomum japonicum** resembles *S. hæmatobium* morphologically, but both the male and female are smaller. The ova, which appear in the feces, are ovoid,

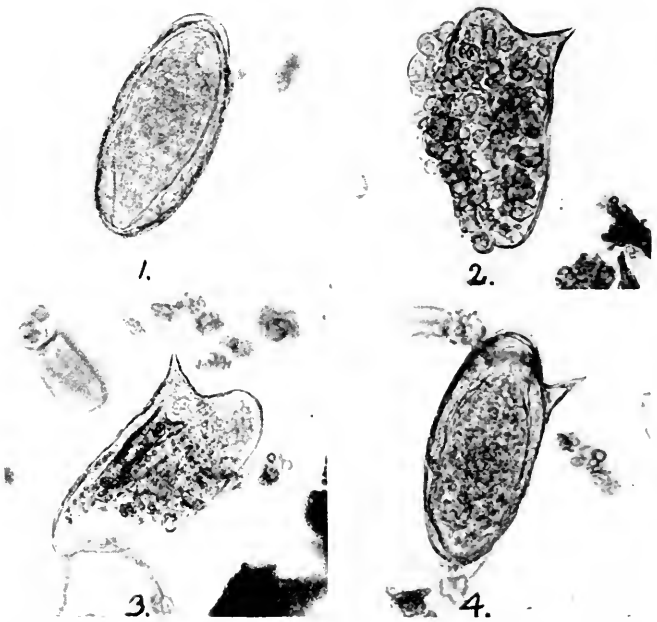


Fig. 123.– Ova of Schistosomum mansoni: 1, With spine out of focus; 2, in a clump of red blood-cells; 3, apparently unfertilized; 4, usual appearance (× 250). (Photographs by the author.)

thin shelled, and without lid or spine. They average 83 by 62 μ in size, and contain a ciliated embryo. The worm inhabits the portal and probably also other veins.

Class Cestoda

The cestodes, or tapeworms, are very common parasites of both man and the animals. In the adult stage

they consist of a linear series of flat, rectangular segments (proglottides), at one end of which is a smaller segment, the scolex or head, especially adapted by means of sucking disks and hooklets for attachment to the host. The series represents a colony, of which the scolex is ancestor. The proglottides are sexually complete individuals (in most cases hermaphroditic) which are derived from the scolex by budding. With the exception of the immature segments near the scolex, each contains a uterus filled with ova.

The large tapeworms, *Tænia saginata*, *T. solium*, and *Dibothriocephalus latus*, are distinguished from one another mainly by the structure of the scolex and the uterus. The scolex should be studied with a low-power objective or a hand lens. The uterus is best seen by pressing the segment out between two plates of glass.

All the tapeworms pass a larval stage in the tissues of an intermediate host, which is rarely of the same species as that which harbors the adult worm. From the ova which have developed in the proglottides of the adult worm, and which pass out with the feces of the host, there develop embryos, or *oncospheres*, each provided with three pairs of horny hooklets. When the oncosphere is taken into the intestines of a suitable animal, it penetrates to the muscles or viscera and there forms a cyst in which develop usually one, but sometimes many, scolices, which are identical with the head of the adult worm. When the flesh containing this cystic stage is eaten without sufficient cooking to destroy the scolices, the latter attach themselves to the intestinal wall and produce adult tapeworms by budding.

Ordinarily, only the adult stage occurs in man. In the case of *Tænia echinococcus* only the larval stage is found. *T. solium* may infect man in either stage, although the cystic stage is rare.

Since the head, or scolex, is the ancestor from which the worm is formed in the intestine, it is important, after giving a vermifuge, to make certain that the head has been passed with the worm. Should it remain, a new worm will develop.

The principal tapeworms found in man belong to the genera Tænia, Hymenolepis, and Dibothriocephalus.

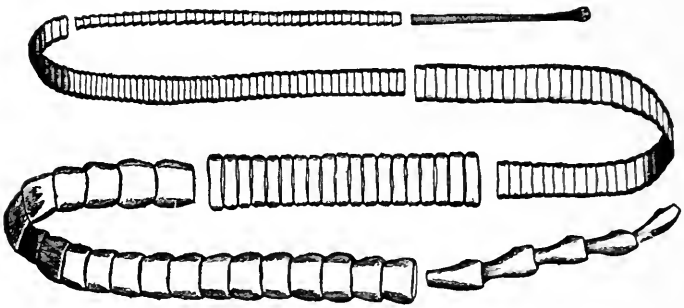


Fig. 124.—Tænia saginata (Eichhorst).

1. Genus Tænia.—(1) **Tænia saginata** (Fig. 124).—This, the beef tapeworm, is the common tapeworm of the United States, and is widely distributed over the world. Its length is generally about 4 to 8 meters. The scolex is about the size of a large pin-head (1.5 to 2 mm. in diameter), and is surrounded by four sucking disks, but has no hooklets (Fig. 125). The neck is about 1 mm. wide. The terminal segments, which become detached and appear in the feces, measure about 18 to 20 mm. long by 4 to 7 mm. wide. The uterus extends along the

midline of the segment and gives off twenty to thirty branches upon each side (see Fig. 133, 1).

The larval stage is passed in the muscles of various animals, especially cattle. It rarely or never occurs in man, hence there is little or no danger of infection from examining feces.

The scolex is ingested with the meat, its capsule is dissolved by the digestive juices, and it attaches itself to the intestinal wall by means of its suckers. It then

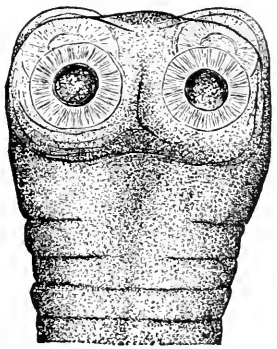


Fig. 125.—Head of Tænia saginata (Mosler and Peiper).

develops into the mature worm, which may grow very rapidly, even as many as thirteen or fourteen segments being formed in a day.

The ova are present in the feces of infected persons, sometimes in great numbers. When, however, segments are passed, the ova for the most part remain within them and comparatively few are found free in the feces. They are spheric or ovoid, yellow to brown in color, and have a thick, radially striated shell (Fig. 126). Within them the six hooklets of the embryo (oncosphere) can usually be made out as three pairs of parallel lines.

The size of the ova varies from 20 to 30 μ wide and 30 to 40 μ long. Vegetable cells, which are generally

Fig. 126.—Eggs of Tænia saginata, magnifications 100, 250, and 500 diameters (photographs by the author).

present in the feces, are often mistaken for them, although there is no great resemblance.

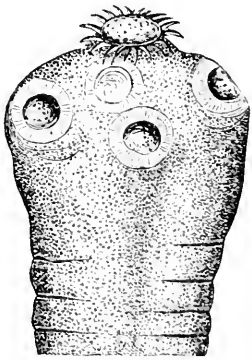


Fig. 127.—Head of Tænia solium (Mosler and Peiper).

(2) **Tænia solium,** the pork tapeworm, is very rare in this country. It is usually much shorter than *Tænia saginata*. The scolex is about 0.6 to 1 mm. wide, is

surrounded by four sucking disks, and has a projection, or rostellum, with a double row of horny hooklets, usually twenty-six to twenty-eight in number. (Fig. 127). The terminal segments measure about 5 to 6 by 10 to 12 mm. The uterus has only seven to fourteen branches on each side (see Fig. 133, 3).

The cysticercus stage occurs ordinarily in the muscles of the pig, but is occasionally seen in man, most frequently affecting the brain and eye (*Cysticercus cellulosæ*). There is, therefore, danger of infection from handling feces.

The ova so closely resemble those of *Tænia saginata* as to be practically indistinguishable. They average about 31 to 36 μ in diameter and are usually spheric.

Fig. 128.—Tænia echinococcus; enlarged (Mosler and Peiper).

(3) **Tænia echinococcus.** — The mature form of this tapeworm inhabits the intestines of the dog and wolf. The larvæ develop in cattle and sheep ordinarily, but are sometimes found in man, where they give rise to echinococcus or "hydatid" disease. The condition is unusual in America, but is not infrequent in Central Europe and is common in Iceland and Australia.

The adult parasite is 2.5 to 5 mm. long and consists of only four segments (Fig. 128). It contains many ova. When the ova reach the digestive tract of man the embryos are set free and find their way to the liver, lung, or other organ, where they develop into cysts, thus losing their identity. The cysts may attain the size of

a child's head. Other cysts, called "daughter-cysts," are formed within these. The cyst-wall is made up of two layers, from the inner of which develop larvæ which are identical with the head, or scolex, of the mature parasite. These are ovoid structures 0.2 to 0.3 mm. long. Each has four lateral suckers and a rostellum

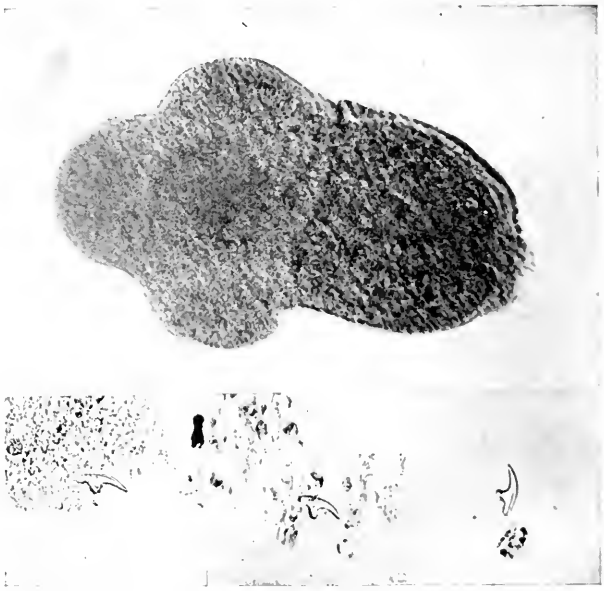


Fig. 129.—Scolex and hooklets of Tænia echinococcus in fluid from hepatic cyst (×300) (photographs by the author).

surmounted by a double circular row of horny hooklets. The rostellum with its hooklets is frequently invaginated into the body.

Diagnosis of echinococcus disease depends upon detection of scolices, free hooklets which have fallen off from degenerated scolices, or particles of cyst-wall which

are characteristically laminated and usually have curled edges. The lamination is best seen at the torn edge of the membrane. All of these structures can be found in fluid withdrawn from the cysts or, less frequently, in the sputum or the urine, when the disease involves the lung or kidney (see Figs. 59 and 129). The cysts are sometimes "barren," growing to a considerable size without producing scolices.

The cyst fluid is clear, between 1.009 and 1.015 in specific gravity, and contains a notable amount of sodium chlorid, but no albumin.

2. Genus Hymenolepis.—(1) **Hymenolepis nana,** the dwarf tapeworm (Fig. 130), is 1 to 4.5 cm. in length

Fig. 130.—Hymenolepis nana, about natural size (Mosler and Peiper).

Fig. 131.—Egg of hymenolepis nana, as seen in fresh feces. Enlarged. (After Ransom, from Stiles.)

and 0.5 to 0.7 mm. in breadth at the widest part. The head is globular and has a rostellum with a crown of 24 to 30 hooklets. There are about 150 segments. Diagnosis must, in general, depend upon the discovery of ova in the feces. These are spheric and have two distinct membranous walls. The outer membrane is about 39 μ in diameter. The inner averages 28 μ, and at each pole has a slight projection provided with indistinct filamentous processes, which may lie between the two

membranes in such a way as to simulate a third. The egg contains an embryo, of which only the three pairs of hooklets are clearly seen.

The worm is common in Europe and America. It is most frequent in children and is generally present in large numbers, producing considerable digestive and nervous disturbances. The mode of infection is unknown.

(2) **Hymenolepis diminuta** is a common intestinal parasite of rats. A few cases of infection in man have been reported in America. The parasite measures 1 to 6 cm. in length and is composed of 600 to 1300 segments. The scolex lacks hooklets. Ova are round or ovoid, with a distinct, sometimes radially striated outer membrane, 56 to 80 μ in diameter, and an inner membrane 24 to 40 by 20 to 35 μ. Between these membranes is a broad layer of gelatinous substance. The contained embryo has six hooklets.

3. Genus Dipylidium.—(1) **Dipylidium caninum,** sometimes called *Tænia elliptica*, is a very common tapeworm of dogs and cats. Its length is 15 to 35 cm. The head, globular in shape, is armed with hooklets. Terminal segments are shaped like melon seeds, 8 to 11 mm. long and 1.5 to 3 mm. broad. Ova are spheric, 43 to 50 μ in diameter, and thin shelled. They contain a six-hooked embryo, 32 to 36 μ in diameter.

The intermediate host is the flea or louse. Infection of human beings is not common, and is mostly confined to children, who are probably infected from getting lice or fleas of dogs or cats into their mouths.

4. Genus Dibothriocephalus.—(1) **Dibothriocephalus latus,** the fish tapeworm, sometimes reaches 20

meters in length, although it is generally not more than one-half or one-third as long. When several worms are present, they are much shorter, often only

Fig. 132.—Head of Dibothriocephalus latus (about × 9): *a*, *a*, Head grooves; *b*, neck (Blanchard).

1.5 or 2 meters. The head is oval, about 1 mm. broad and 1.5 mm. long. It is unprovided with either suckers or hooklets, but has two longitudinal grooves which

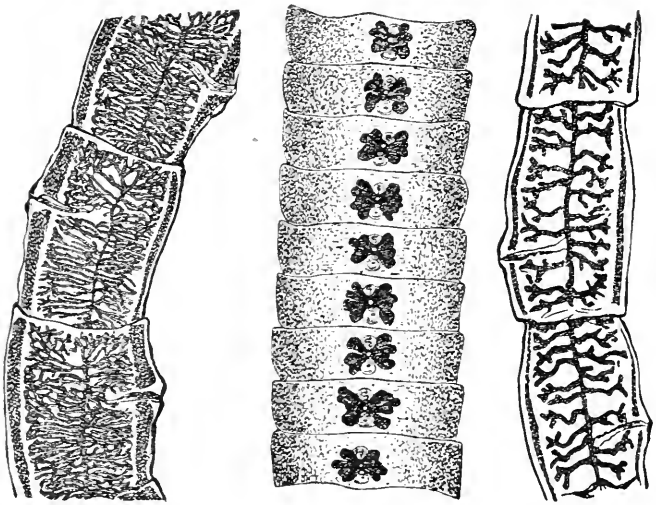


Fig. 133.—Segments of—(1) Tænia saginata; (2) Dibothriocephalus latus; (3) Tænia solium, showing arrangement of uterus.

serve the same purpose (Fig. 132). The length of the segments is generally less than their breadth, mature segments measuring about 3 by 10 or 12 mm. The

uterus, which is situated in the center of the segment, is roset shaped (Fig. 133, 2) and brown or black in color.

The number of segments sometimes exceeds 3000. As a rule they do not appear in the feces singly, but in chains of considerable length.

The larval stage is found in various fish, notably the pike, burbot, grayling, and certain trout. Infection of man prevails only in regions where these fish are found.

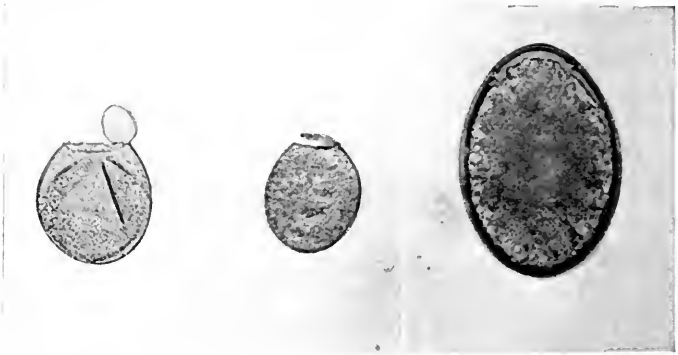


Fig. 134.—Ova of Dibothriocephalus latus (× 250 and 500). The lids were forced open by pressure upon the cover-glass (photographs by the author).

It is very common in Japan and in various countries of Europe, especially Ireland and the Baltic provinces of Russia. A number of cases of infection have been reported in this country, a few of which were undoubtedly acquired here. Any locality in which favorable fish are native becomes a possible center of infection if the worm is introduced by infected immigrants.

The ova are characteristic. They measure about 45 by 70 μ, are brown in color, and are filled with small spherules. The shell is thin and has a small hinged lid at one end. As the eggs appear in the feces the lid is

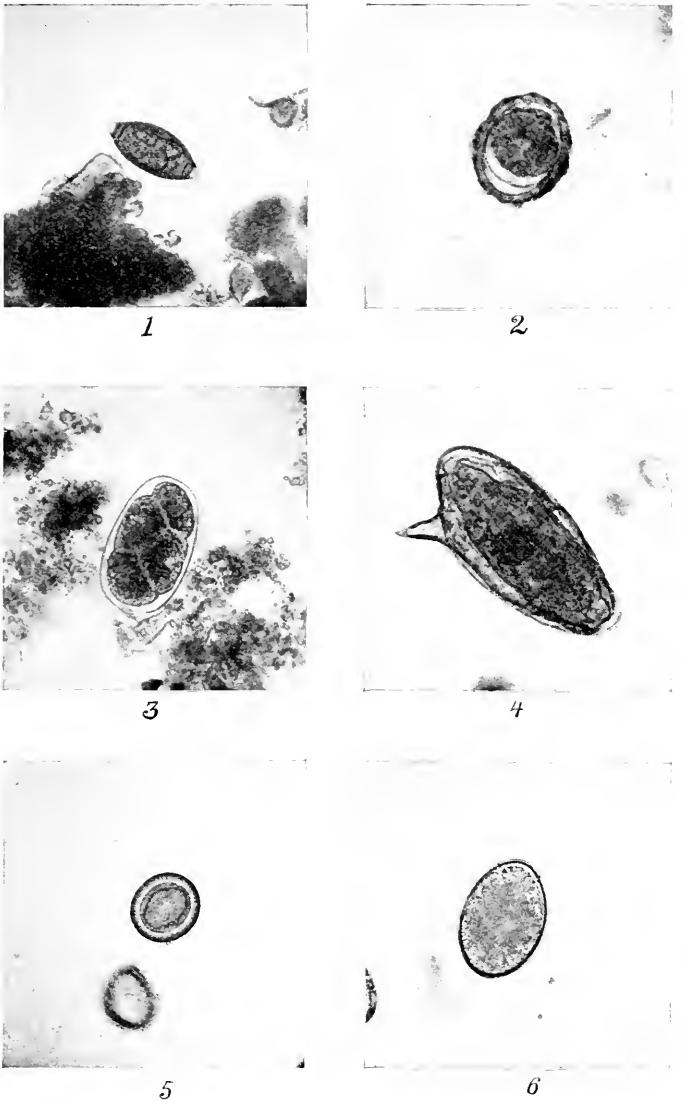


Fig. 135.—Showing comparative size of ova found in the feces: 1, Trichocephalus trichiurus; 2, Ascaris lumbricoides; 3, Necator americanus, four-cell stage; 4, Schistosomum mansoni; 5, Tænia saginata; 6, Dibothriocephalus latus, the line of the lid being out of focus (×250). (Photographs by the author.)

not easily seen, but it may be demonstrated by sufficient pressure upon the cover-glass to force it open (Figs. 134, 135). The only other operculated eggs met with in man are those of the fluke worms.

Dibothriocephalus latus is interesting clinically because it often causes a very severe grade of anemia, which may be indistinguishable from pernicious anemia.

PHYLUM NEMATHELMINTHES

(Round Worms)

CLASS **Nematoda.**—Unsegmented, cylindric or fusiform.

Genus.	*Species.*
Anguillula.	A. aceti.
Ascaris.	A. lumbricoides.
	A. canis.
Oxyuris.	O. vermicularis.
Filaria.	F. bancrofti.
	F. philippinensis.
	F. perstans.
	F. diurna.
	F. medinensis.
Uncinaria.	U. duodenalis.
Necator.	N. americanus.
Strongyloides.	S. intestinalis.
Trichinella.	T. spiralis.
Trichocephalus.	T. trichiurus.

Class Nematoda

The nematodes, or round-worms, are cylindric or fusiform worms, varying in length, according to species, from 1 mm. to 40 or 80 cm. As a rule, the sexes are separate. The male is smaller and more slender than the female. In a few cases the female is viviparous; in most cases she deposits ova which are characteristic, so that the finding of a single egg may establish the diagnosis. Except in a few instances the young are

different from the adult, and must pass a certain larval stage of development before again reaching a host. An intermediate host is, however, necessary with only a few species.

1. Genus Anguillula.—(1) **Anguillula aceti.**—This worm, commonly called the "vinegar eel," is usually present in vinegar. A drop of the vinegar, particularly of the sediment, will frequently show great numbers, all in active motion: males, about 1 or 1.5 mm. long; females, somewhat larger and frequently containing several coiled embryos; and young, of all sizes up to the adult (see Fig. 60).

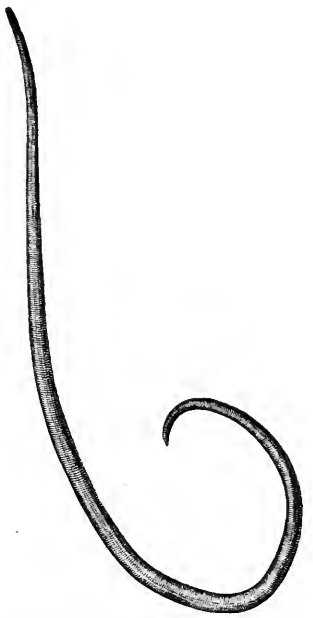


Fig. 130.—Ascaris lumbricoides (female) (Mosler and Peiper).

The vinegar eel is never parasitic, but is occasionally met with as a contamination in the urine (see p. 208), and has there been mistaken for the larva of filaria or strongyloides.

2. Genus Ascaris.—(1) **Ascaris lumbricoides.**—The female is 20 to 40 cm. long and about 5 mm. thick (Fig. 136); the male, 15 to 17 cm. long and 3 mm. thick. They taper to a blunt point anteriorly and posteriorly. At the anterior end are three small lips which can easily be seen with a hand lens. Their color is reddish or brown.

They are the common "round-worms" so frequently found in children. Their habitat is the small intestine. Usually several individuals are present and sometimes many.

The diagnosis is made by detection of the worms or ova in the feces. The latter are generally numerous and are easily recognized. They are elliptic, measuring about 49 by 65 μ, and have an unsegmented protoplasm. There is usually a crescentic clear space at each pole between the contents and the shell (Fig. 137). The

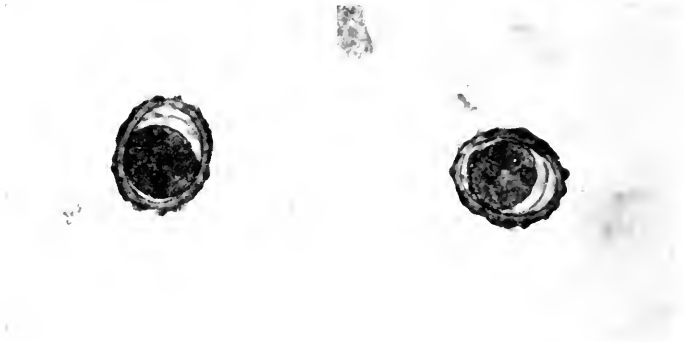


Fig. 137.—Ova of Ascaris lumbricoides in fresh feces (× 250) (photographs by the author).

shell is thick and has a roughly mammillated or sculptured surface (Fig. 138). When only females are present in the intestine, and occasionally at other times, one finds unfertilized eggs. These are generally much more elongated, have a thinner and smoother shell, have coarsely granular contents, and lack the crescentic clear spaces.

The eggs do not hatch in the intestine of the original host. They pass out in the feces and, after a variable period, usually about five weeks, come to contain an

embryo which remains within the shell until ingested by a new host. The embryo is very resistant and may remain alive within the shell for years even, according to Morris, when preserved in 2 per cent. formalin. Upon reaching the intestine of the new host it hatches out and develops into the adult worm in about a month.

(2) **Ascaris canis** is the very common "stomach worm" of cats and dogs. It is also known as *Ascaris mystax* and *Toxocara canis*. It is rare as a human parasite.

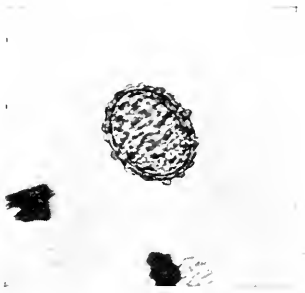


Fig. 138.—Egg of Ascaris lumbricoides, surface view (× 250) (photograph by the author).

The male is 4 to 9 cm. long; the female, 12 to 20. Individuals from dogs are generally larger than those from cats. The egg is spheric, 68 to 70 μ in diameter, and has a thin shell with comparatively smooth surface.

3. Genus Oxyuris.—(1) **Oxyuris vermicularis.**—This is the "thread-worm" or "pin-worm" which in the adult stage inhabits the colon and rectum, especially of young children. Its presence should be suspected in all unexplained cases of pruritus ani. The female is about 9 to 12 mm. long; the male, about 3 to 5 mm. (Fig. 139).

The worms are not infrequently found in the feces;

the ova, rarely. The latter are best found by scraping the skin with a dull knife at the margin of the anus, where they are deposited by the female, who wanders out from the rectum for this purpose, thus producing the troublesome itching. They are asymmetrically oval with one flattened side, are about 50 μ long by 16 to 20 μ wide, have a thin clear shell, and when desposited contain a partially developed embryo. The diagnosis is best made by giving a purgative and searching the stool for the adult worms.

Fig. 130.—Oxyuris vermicularis, male and female, natural size (after Heller).

Infection takes place through swallowing the ova. Auto-infection is likely to occur in children; the ova cling to the fingers after scratching and are thus carried to the mouth. Diagnosis can sometimes be made by finding the ova in the dirt beneath the finger-nails.

4. Genus Filaria.—(1) **Filaria bancrofti.**—The adults are thread-like worms, the male about 4 cm., the female about 8 cm., long. They live in pairs in the lymphatic channels and glands, especially those of the pelvis and groin, and often occur in such numbers as to obstruct the flow of lymph. This is the most common cause of elephantiasis. Infection is very common in tropical countries, especially in Samoa, the West Indies, Central America, and the Isthmus of Panama. It is said that in Samoa 50 per cent. of the natives are infected.

The female is viviparous, and produces vast numbers of larvæ, which appear in the circulating blood. The name *Filaria sanguinis hominis*, which is commonly applied to them, is incorrect, since they do not constitute a species. These larvæ are about as wide as a red corpuscle and 0.2 to 0.4 mm. long (see Fig. 103), and are very active, although, owing to the fact that they are inclosed in a loose transparent sheath, they do not move

Fig. 140.—Larva of Filaria bancrofti in chylous hydrocele fluid; length, 300 μ; width, 8 μ. A number of red blood-corpuscles also appear (studied through courtesy of Dr. S. D. Van Meter).

about from place to place. They are found in the peripheral blood only at night, appearing about 8 P. M., and reaching their maximum number—which is sometimes enormous—about midnight. If the patient change his time of sleeping, they will appear during the day. Infection is carried by a variety of mosquito, which acts as intermediate host. Diagnosis rests upon detection of larvæ in the blood, as described on p. 304.

The larvæ are sometimes found in urine and chylous fluids from the serous cavities. Their motion is then

usually less active than when in blood. That shown in Fig. 140 was alive sixty hours after removal of the fluid. Larvæ were present in the blood of the same patient.

A number of other filariæ whose larvæ appear in the blood are known, some of them only in the larval stage. Among these are *Filaria philippinensis* and *F. perstans*, which exhibit no periodicity, and *F. diurna* and *F. loa*, whose larvæ appear in the blood during the day. The adult of the last named is especially frequent in the orbit and beneath the conjunctiva.

(2) **Filaria medinensis,** the "guinea-worm," is a very interesting and important worm of Africa and southern Asia. It has been thought to be the "fiery serpent" which molested the Children of Israel in the Wilderness.

The larva probably enters the body through the skin or gastro-intestinal tract. It wanders about in the subcutaneous tissues until maturity, producing slight, if any, symptoms. The male has only recently been discovered. It is only 4 cm. long. It dies soon after the female is impregnated. The adult female is a very slender, yellowish worm, about 50 to 80 cm. long, its appearance somewhat suggesting a catgut suture. When gestation is complete the greater part of the female's body consists of a uterus filled with embryos. The female then travels to the feet or ankles of the host and there produces a red nodule and, finally, an ulcer, from the center of which her head protrudes. Through this great numbers of larvæ are discharged whenever it comes in contact with water. Little damage is done unless the worm is pulled out, when the larvæ are set free in the tissues and cause serious disturbances.

When discharged the larvæ seek out a small crustacean, cyclops, which serves as intermediate host.

5. Uncinaria duodenalis and Necator americanus.—These, the Old and the New World hookworm, respectively, are among the more harmful of the animal parasites. They inhabit the small intestine, often in great numbers, and commonly produce an anemia which is often severe and sometimes fatal. The presence of a few, however, may cause slight, if any, disturbance.

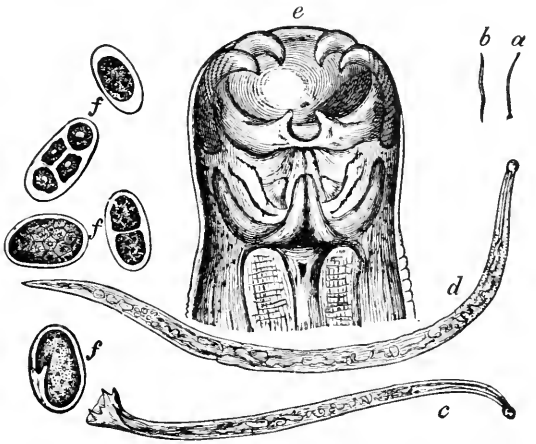


Fig. 141.—Uncinaria duodenalis: *a*, Male (natural size); *b*, female (natural size); *c*, male (enlarged); *d*, female (enlarged); *e*, head; *f*, *f*, *f*, eggs (after v. Jaksch).

Uncinaria duodenalis is common in southern Europe and in Egypt. The body is cylindric, reddish in color, and the head is bent sharply. The oral cavity has six hook-like teeth. The female is 12 to 18 mm. long and the tail is pointed. The male is 8 to 10 mm. long and the posterior end is expanded into an umbrella-like pouch, the caudal bursa. The eggs are oval and have a thin, smooth, transparent shell. As they appear in the

feces the protoplasm is divided into 2, 4, 8, or more rounded segments (Fig. 141). They measure 32 to 38 by 52 to 61 μ.

Necator americanus is very common in subtropical America, including the southern part of the United

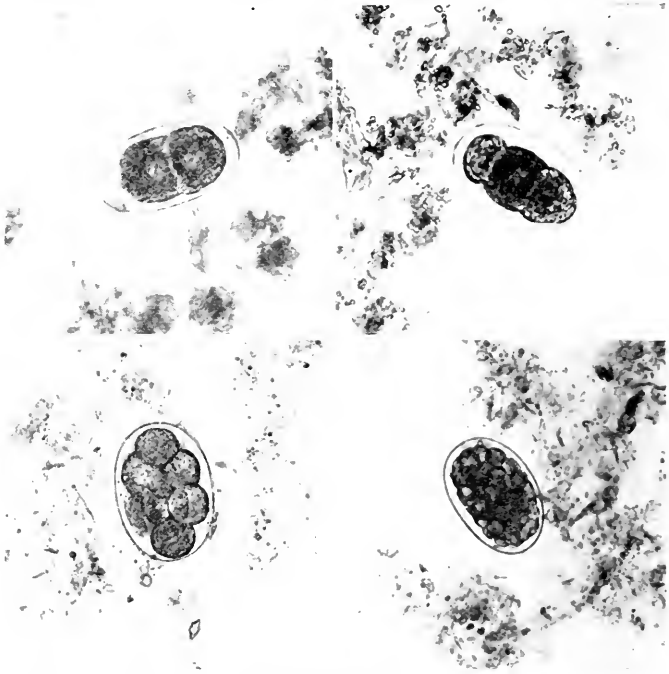


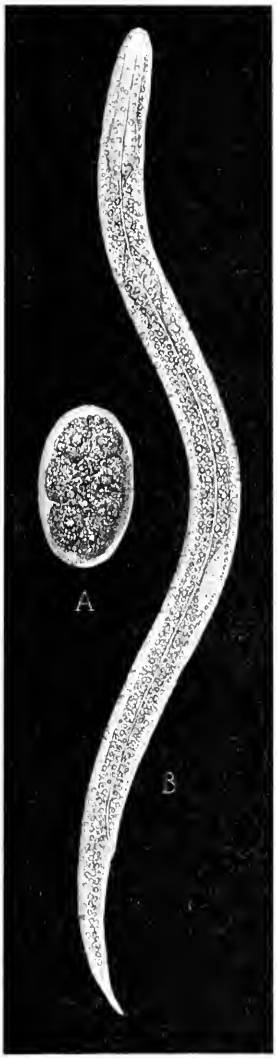
Fig. 142.—Ova of Necator americanus in feces. The egg, showing three cells, is a lateral view of a four-cell stage (× 250) (photographs by the author).

States and the West Indies. In Porto Rico 90 per cent. of the rural population is infected. Isolated cases, probably imported, have been seen in most of the Northern States. The American hook-worm is smaller than the Old World variety, the male being 7 to 9 mm. long, the

female 9 to 11 mm. The four ventral hook-like teeth are replaced by chitinous plates. There are also differences in the caudal bursa of the male, and in the situation of the vulva in the female. The ova (Fig. 142) resemble those of *Uncinaria duodenalis*, but are larger, 36 to 45 by 64 to 75 μ.

The life-history of the two worms is probably the same. The ova pass out with the feces, and, under favorable conditions of warmth and moisture, devolop an embryo which hatches within a few days. The resulting larvæ pass through a stage of development in warm moist earth, growing to a length of 0.5 to 0.6 mm., and moulting twice. They are then ready to infect a new host. In some cases they probably reach the host's intestine by way of the mouth, with food or water; but the usual route is probably that established by Loos. When moist earth containing the larvæ comes in contact with the skin, they penetrate into the subcutaneous tissues. This is favored by retention of mud between the toes of those who go barefooted. When the larvæ are abundant a dermatitis is induced ("ground itch"). From the subcutaneous tissue they pass by way of lymph- and blood-streams to the lungs. Here they make their way into the smaller bronchi, are carried by the bronchial mucus to the pharynx, and are swallowed. They thus ultimately reach the small intestine, where they develop into mature worms.

The diagnosis of hookworm infection, which is assuming increasing importance in this country, must rest upon detection of ova in the feces. The worms themselves seldom appear except after thymol and a cathartic. A small portion of the feces, diluted with water if nec-



essary, is placed upon a slide and the larger particles removed. The material is covered and searched with a 16-mm. objective. A higher power may rarely be necessary to positively identify an egg, but should not be used as a finder. The eggs (see Figs. 135, 142) are nearly always typical, showing a thin but very distinct shell, a clear zone, and a finely granular segmented protoplasm. A light spot, representing the nucleus, can usually be made out in each segment. After having once been seen the eggs are not easily mistaken. Larvæ are not found in fresh feces, but may hatch within twenty-four to forty-eight hours, and are then distinguished with difficulty from *Strongyloides intestinalis*. In severe infections eggs may be found in nearly every microscopic field; in most cases, even though comparatively mild, they can be found on the first

Fig. 143.—A, Egg of Strongyloides intestinalis (parasitic mother worm) found in stools of case of chronic diarrhea; B, Rhabditiform larva of Strongyloides intestinalis from the stools. (William Sydney Thayer, in Journal of Experimental Medicine.)

slide examined. It is seldom necessary to search more than half a dozen slides. From the estimate of Dock and Bass it seems probable that ova will average at least one to the slide if ten or more laying females are present in the intestine. Very old females may fail to produce eggs. When they are scarce, some method of sedimenting the feces may be tried (see p. 360).

Pepper's method of concentration is simple, but is not applicable to other ova than those of the hookworm. It is best first to sediment the feces. A layer of the diluted feces is placed on a slide and allowed to remain for some minutes. The slide is then gently immersed in water. The ova, which have settled to the bottom, cling to the glass and are not washed away as is other material. This may be repeated several times and numerous eggs collected.

6. Genus Strongyloides.—(1) **Strongyloides intestinalis.**—Infection with this worm is by no means so rare in this country as the few clinical reports would indicate. It is apparently widespread in the Southern States. It is very common in subtropical countries, notably in Italy and in southern China. It seems probable that the parasite is the cause of "Cochin China diarrhea," although some authorities regard it as harmless.

The adult female, which reproduces by parthenogenesis and is about 2 mm. long, inhabits the upper portion of the small intestine, but neither it nor the ova appear in the stool unless an active diarrhea exists. Ordinarily they hatch in the intestines, and when infection is severe larvæ can be found in the feces in large numbers. These are the "rhabditiform larvæ," which measure 450 to 600 μ by 16 to 20 μ (Figs. 143, 144). They

are actively motile, and are best found by making a small depression in the fecal mass, filling it with water, and keeping in a warm place (preferably an incubator) for twelve to twenty-four hours. The larvæ will collect in the water, and can be easily found by transferring a drop to a slide and examining with a 16-mm. objective. The inexperienced worker should make sure that the worms *move*, or he may be misled by the vegetable hairs which are generally present in the feces.

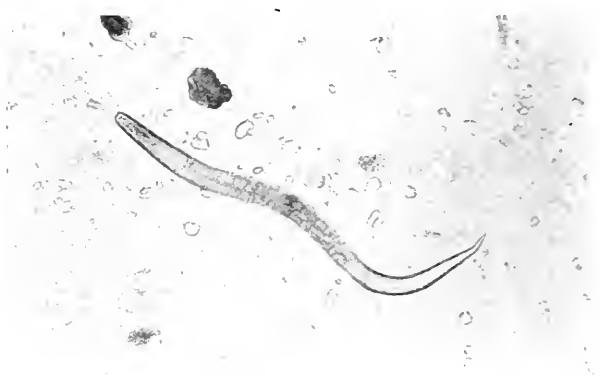


Fig. 144.—Rhabditiform larva of Strongyloides intestinalis in feces (× 150) (photograph by the author).

Certain of these hairs (notably those from the skin of a peach) closely resemble small worms (see page 356).

Outside the body the rhabditiform larvæ develop into a free-living, sexually differentiated generation. The young of this generation are the more slender "filariform larvæ," which constitute the infective form. Direct transformation of rhabditiform into filariform larvæ also occurs. Infection takes place by ingestion or by way of the skin.

7. Genus Trichinella.—(1) **Trichinella spiralis.**—

This is a very small worm—adult males, 1.5 to 1.6 by 0.04 mm.; females, 3 to 4 by 0.06 mm. Infection in man occurs from eating of insufficiently cooked pork, which contains encysted larvæ. Ordinary "curing" of pork does not kill them. These reach maturity in the small intestine. Soon after copulation the males die, and the females penetrate into the mucous membrane. They live in this situation about six weeks, giving birth to great numbers of young, averaging as high as 1500 from

Fig. 145.—Trichinella spiralis (larvæ) from head of right gastrocnemius muscle; seventh week of disease (16-mm. objective; eye-piece 4) (Boston).

a single female. The larvæ migrate to the striated muscles, chiefly near the tendinous insertions, where they grow to a length of about 0.8 mm., and finally become encysted. In this condition they may remain alive and capable of developing for as long as twenty-five years.

Trichiniasis is generally accompanied by a marked eosinophilia. The diagnosis is made by teasing out upon a slide a bit of muscle, obtained preferably from the outer head of the gastrocnemius, the insertion of the deltoid, or the lower portion of the biceps. The coiled larvæ

can easily be seen with a 16-mm. objective (Fig. 145). The larvæ can sometimes be found in the blood (see p. 305) before they have reached their final resting-place in the muscles. During the diarrheal stage adults may be present in the feces, and are found by diluting with water and decanting several times and examining the sediment in a very thin layer with a hand lens.

8. Genus Trichocephalus.—(1) **Trichocephalus trichiurus.**—This, the "whip-worm," is 3.5 to 5 cm. long. Its anterior portion is slender and thread-like, while the posterior portion is thicker (Fig. 146). It is widely distributed geographically, and is one of the most

Fig. 146.—Trichocephalus trichiurus: *a*, Female; *b*, male (natural size) (Heller).

common of intestinal parasites in this country. It lives in the large intestine, especially the cecum, with its slender extremity embedded in the mucous membrane. Whip-worms do not, as a rule, produce any symptoms, although gastro-intestinal disturbances, nervous symptoms, and anemia have been ascribed to them. They, as well as many other intestinal parasites, are probably an important factor in the etiology of appendicitis, typhoid fever, and other intestinal infections. The damage which they do to the mucous membrane favors bacterial invasion.

The number present is usually small. The worms themselves are rarely found in the feces. The ova, which

are not often abundant, are easily recognized with the 16-mm. objective. Although they are comparatively small, their appearance is striking. They are brown,

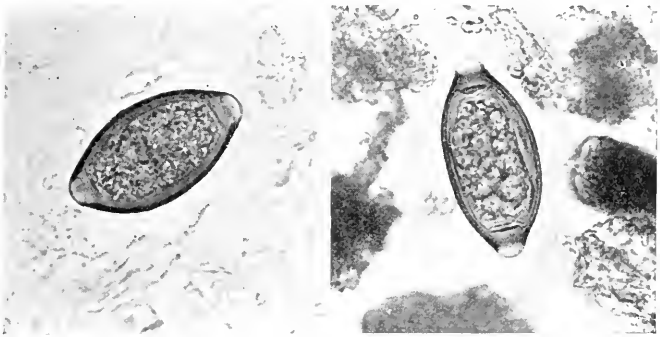


Fig. 147.—Ova of Trichocephalus trichiurus (×500) (photographs by the author).

ovoid in shape, 50 to 54 μ long by about 23 μ wide, and have a button-like projection at each end (Fig. 147).

PHYLUM ARTHROPODA

The arthropoda which are parasitic to man belong to the classes Arachnoidea and Insecta. They are nearly all external parasites, and the reader is referred to the standard works upon diseases of the skin for descriptions. The several species of the louse (*Pediculus capitis, P. vestimenti, P. pubis*), the itch mite (*Sarcoptes scabiei*), and the small organism (*Demodex folliculorum*) which lives in the sebaceous glands, especially about the face, are the most common members of this group.

A number of flies may deposit their ova in wounds or in such of the body cavities as they can reach, and the resulting maggots may cause intense irritation. Ova may be swallowed with the food and the maggots appear in the

feces. Probably most important is the "screw worm," the larva of *Chrysomyia macellaria*, infection with which is not rare in some parts of the United States. The ova are most commonly deposited in the nasal passages, and the larvæ, which may be present in great numbers, burrow through the soft parts, cartilage, and even bone, always with serious and often with fatal results.

Fig. 148.—Larva of linguatula serrata (de Faria and Travassos).

A few cases of human infection with *Linguatula serrata* have recently been reported from the Panama Canal Zone and from Brazil, and it may prove to be more common than has been recognized. The parasite belongs to the class Arachnoidea, which includes spiders, mites, ticks, etc. It is not at all rare in Europe. Related species are common in certain birds in North America. Man may be infected with either adult or larval stages, the former living in the nasal and accessory passages, the latter, encysted, in the internal organs, particularly the liver. The larvæ may be found in the feces, and, because of their serrations, may be mistaken for minute tapeworms (Fig. 148). They are white in color and measure about 4 to 6.5 mm. long and 0.9 to 1.5 mm. broad at the widest (anterior) part.

CHAPTER VII

MISCELLANEOUS EXAMINATIONS

PUS

Pus contains much granular débris and numerous more or less degenerated cells, the great majority being polymorphonuclear leukocytes—so-called "pus-corpuscles." Eosinophilic leukocytes are common in gonorrheal pus and in asthmatic sputum. Examination of pus is directed chiefly to detection of bacteria.

When very few bacteria are present, culture methods, which are outlined in Chapter VIII, must be resorted to. When considerable numbers are present, they can be detected and often identified in cover-glass smears. Several smears should be made, dried, and fixed, as described on p. 466.

One of these should be stained with a bacterial stain. Löffler's methylene-blue and Pappenheim's pyronin-methyl-green are especially satisfactory for this purpose. These stains are applied for one-half minute to two minutes or longer, without heating; the preparation is rinsed in water, dried, mounted, and examined with an oil-immersion lens. Another smear should be stained by Gram's method (see page 467). These will give information concerning all bacteria which may be present, and frequently no other procedure will be necessary for their identification.

The most common pus-producing organisms are *staphylococci* and *streptococci*. They are both cocci, or

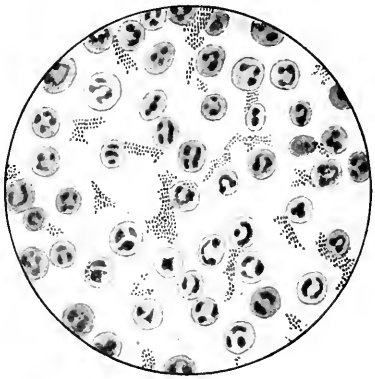


Fig. 149.—Staphylococcus pyogenes albus from an abscess of the parotid gland (Jakob).

spheres, their average diameter being about 1 μ. Staphylococci are commonly grouped in clusters, often compared

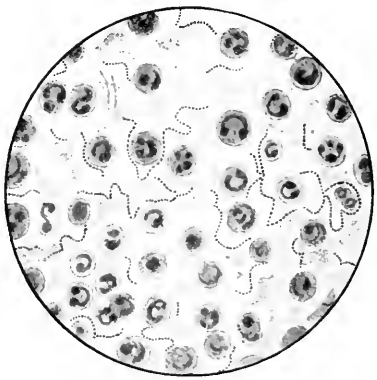


Fig. 150.—Streptococcus pyogenes from a case of empyema (Jakob).

to bunches of grapes (Fig. 149). There are several varieties which can be distinguished only in cultures.

Streptococci are arranged side by side, forming chains of variable length (Fig. 150). Sometimes there are only three or four individuals in a chain; sometimes a chain is so long as to extend across several microscopic fields. Streptococci are more virulent than staphylococci, and are less commonly met. Both are Gram-positive. Their cultural characteristics are given on p. 475.

Should bacteria resembling *pneumococci* be found, Buerger's or Smith's method for capsules (see p. 72) should be tried. When these are not available, capsules

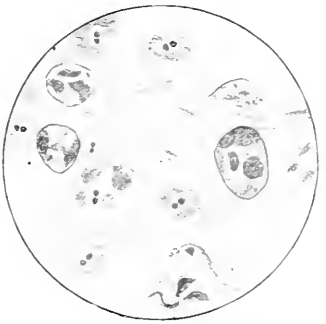


Fig. 151.—Diplococcus pneumoniæ from ulcer of cornea (obj. one-twelfth oil-immersion) (study through courtesy of Dr. C. A. Oliver) (Boston).

can usually be shown by the method of Hiss. The dried and fixed smear is covered with a stain composed of 5 c.c. saturated alcoholic solution gentian-violet and 95 c.c. distilled water, and heated until steam rises. The preparation is then washed with 20 per cent. solution of copper sulphate, dried, and mounted in Canada balsam.

Pneumococci may give rise to inflammation in many locations (see p. 71). When they form short chains, demonstration of the capsule is necessary to distinguish them from streptococci.

If tuberculosis be suspected, the smears should be stained by one of the methods for the *tubercle bacillus* (see pp. 64 to 66), or guinea-pigs may be inoculated. The bacilli are generally difficult to find in pus, and bacteria-free pus would suggest tuberculosis.

Gonococci, when typical, can usually be identified with sufficient certainty for clinical purposes in the smear stained with Löffler's methylene-blue or, much better, Pappenheim's pyronin-methyl-green. They are coffee-

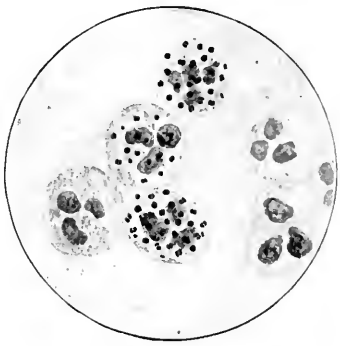


Fig. 152.—Gonococci in urethral pus (McFarland).

bean-shaped cocci which lie in pairs with their flat surfaces together (Fig. 152). They lie for the most part within pus-cells, an occasional cell being filled with them, while the surrounding cells contain few or none. A few are found outside of the cells. It is not usual to find gonococci when many other bacteria are present, even though the pus is primarily of gonorrheal origin. Whenever the identity of the organism is at all questionable, Gram's method should be tried. In rare instances it may be necessary to resort to cultures. The gonococcus

is distinguished by its failure to grow upon ordinary media (see p. 476).

Gonococci are generally easily found in pus from untreated acute and subacute gonorrheal inflammations—conjunctivitis, urethritis, etc.—but are found with difficulty in pus from chronic inflammations and abscesses, and in urinary sediments.

PERITONEAL, PLEURAL, AND PERICARDIAL FLUIDS

The serous cavities contain very little fluid normally, but considerable quantities are frequently present as a result of pathologic conditions. The pathologic fluids are classed as transudates and exudates.

Transudates are non-inflammatory in origin. They contain only a few cells, and less than 2.5 per cent. of albumin, and do not coagulate spontaneously. The specific gravity is below 1.018. Micro-organisms are seldom present.

Exudates are of inflammatory origin. They are richer in cells and albumin, and tend to coagulate upon standing. The specific gravity is above 1.018. The amount of albumin is estimated by Tsuchiya's method, after diluting the fluid, if much albumin is present. A mucin-like substance, called serosomucin, is likewise found in exudates. It is detected by acidifying with acetic acid, when a white cloudy precipitate results. Some transudates give a slight turbidity with acetic acid. Bacteria are generally present and often numerous. When none are found in stained smears or cultures, tuberculosis is to be suspected, and animal inoculation should be resorted to.

Exudates are usually classed as serous, serofibrinous,

seropurulent, purulent, putrid, and hemorrhagic, which terms require no explanation. In addition, chylous and chyloid exudates are occasionally met, particularly in the peritoneal cavity. In the chylous form the milkiness is due mainly to the presence of minute fat-droplets, and is the result of rupture of a lymph-vessel, usually from obstruction of the thoracic duct. Chyloid exudates are milky chiefly from proteins in suspension, or fine

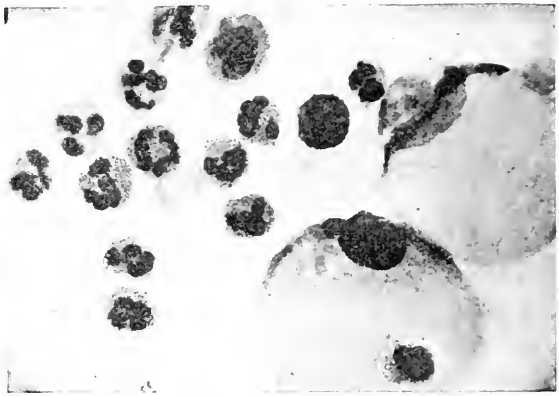


Fig. 153.—Cytodiagnosis. Polymorphonuclear leukocytes and swollen endothelial cells from acute infectious non-tuberculous pleuritis (Percy Musgrave; photo by L. S. Brown).

débris from broken-down cells. These exudates are most frequently seen in carcinoma and tuberculosis of the peritoneum.

Cytodiagnosis.—This is diagnosis from a differential count of the cells in a transudate or exudate, particularly one of pleural or peritoneal origin.

The fresh fluid, obtained by aspiration, is centrifugalized for at least five minutes; the supernatant liquid is poured off; and smears are made from the sediment and

dried in the air. The smears are then stained with Wright's, Harlow's or Jenner's stain, mounted, and examined with an oil-immersion objective.

Predominance of *polymorphonuclear leukocytes* (pus-corpuscles) points to an acute infectious process (Fig. 153). These cells are the neutrophiles of the blood. Eosinophiles and mast-cells are rare. In thin smears they are easily recognized, the cytoplasmic granules

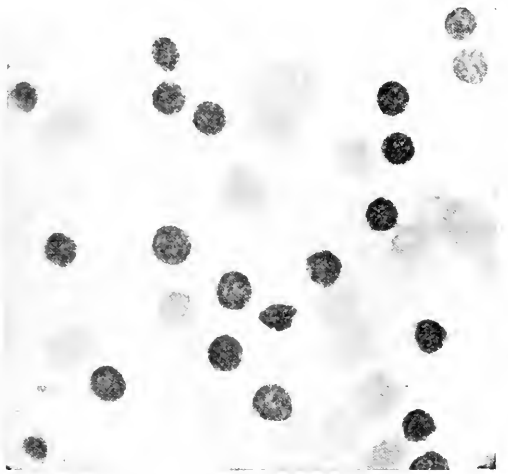


Fig. 154.—Cytodiagnosis. Lymphoid cells from pleural fluid; case of tuberculous pleuritis (Percy Musgrave; photo by L. S. Brown).

often staining characteristically with polychrome-methylene-blue-eosin stains. In thick smears, upon the other hand, they are often small and shrunken, and may be identified with difficulty, being easily mistaken for lymphocytes.

Predominance of *lymphocytes* (Fig. 154) generally signifies tuberculosis. They are the same as found in the blood. The cytoplasm is usually scanty, is often ragged,

and sometimes is apparently absent entirely. Tuberculous pleurisy due to direct extension from the lung may give excess of polymorphonuclears owing to mixed infection.

Predominance of *mesothelial cells*, few cells of any kind being present, indicates a transudate (Fig. 155). These

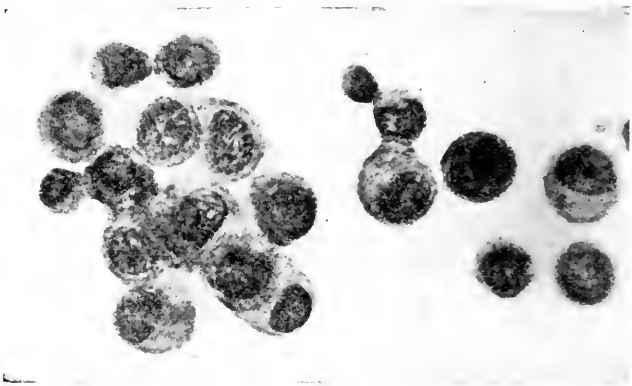


Fig. 155.—Cytodiagnosis. Mesothelial cells from transudate or mechanical effusion (Percy Musgrave; photo by L. S. Brown).

cells are large, with relatively abundant cytoplasm, and contain one, sometimes two, round or oval, palely staining nuclei. Mesothelial cells generally predominate in carcinoma, but are accompanied by considerable numbers of lymphocytes and red blood-corpuscles. Cancer cells cannot be recognized as such, although the presence of mitotic figures would suggest malignant disease.

CEREBROSPINAL FLUID

Examination of the fluid obtained by lumbar puncture has of recent years become a very important aid in diagnosis.

1. Macroscopic Examination.—The amount obtainable varies from a few drops to 100 c.c. Normally, the fluid is clear and limpid, resembling water. The reaction is alkaline. The specific gravity is 1.003 to 1.008. Not infrequently it is tinged with fresh blood from a punctured vessel. This should not be confused with the dull-red or brown color which is seen in hemorrhagic conditions like intraventricular and subdural hemorrhage and hemorrhagic meningitis. When the bleeding is extensive and recent it may give the appearance of practically pure blood.

In purulent meningitis the fluid may exhibit varying degrees of cloudiness, from slight turbidity to almost pure pus. In the less acute stage of the epidemic form it is sometimes quite clear.

After standing for twelve to twenty-four hours the fluid will often coagulate. This occurs especially in the various forms of meningitis, rarely in non-inflammatory conditions. In tuberculosis the coagulum is usually very delicate and cobweb-like and is not easily seen.

2. Chemical Examination.—Only a few constituents are of clinical importance.

(1) **Globulin.**—Traces are present normally. A notable increase occurs in acute inflammations and in syphilis and parasyphilitic affections. The two tests for globulin which follow are positive in 93 to 95 per cent. of all cases of paresis, and are, therefore, an important diagnostic consideration. When acute inflammation is excluded, they run practically parallel with the Wassermann reaction when the latter is applied to the spinal fluid. They must not be applied to fluid containing blood, owing to the presence of serum-globulin.

Noguchi's Butyric Acid Test.—In a small test-tube take 1 to 2 c.c. of the fluid and 5 c.c. of a 10 per cent. solution of butyric acid in normal salt solution. The original test calls for one-tenth these quantities, but they are too small for convenient manipulation. Heat to boiling and immediately add 1 c.c. of normal sodium hydroxid solution and boil again for a few seconds. A positive reaction, corresponding to a pathologic amount of globulin, varies from a distinct cloudiness to a heavy flocculent precipitate which generally appears within twenty minutes, but may be delayed for two hours. A slight opalescence may be seen in normal fluids.

Ammonium Sulphate Test.—Globulin is precipitated by strong solutions of ammonium sulphate. Ross and Jones apply the test after the manner of the ring tests for albumin in the urine. In a test-tube or horismascope take a few cubic centimeters of a saturated solution of ammonium sulphate and overlay with the suspected fluid. In the presence of an excess of globulin, a clear-cut, thin, grayish-white ring appears at the zone of contact of the two fluids within five minutes to three hours. This test appears to be only very slightly less reliable than the butyric acid test.

(2) **Sugar.**—The normal cerebrospinal fluid gives a distinct reaction with the copper tests (see pp. 138, 139), apparently due to glucose. A number of writers lay stress upon the absence of this reduction in meningitis. From a study of a recent series of cases, Jacob finds that: (1) No reduction of copper occurs in pyogenic meningitis (pneumococcus, streptococcus, etc.) or in acute meningococcic meningitis; (2) reduction occurs, but may be diminished in tuberculosis and in the more chronic cases of meningococcic meningitis; (3) reduction is normal in poliomyelitis.

(3) **Antimeningococcus-serum Test.**—Vincent and other French investigators have developed the following test, which they believe to be specific for epidemic cerebrospinal meningitis:

To a few cubic centimeters of the spinal fluid, which has been cleared by thorough centrifugation, are added a few drops of antimeningoccocus serum. The tube, along with a control tube of the untreated fluid, is then placed in an incubator at 52° C. for a few hours. A positive reaction consists in the appearance of a white cloud. The test is said to be reliable even when meningococci cannot be found. The serum must be free from phenol and other interfering substances.

3. Microscopic Examination.—This consists in a study of the bacteria, and of the number and kind of cells.

(1) **Bacteria.**—*Tubercle bacilli* can be found in the majority of cases of tuberculous meningitis. The delicate coagulum which forms when the fluid is allowed to stand in a cool place for twelve to twenty-four hours will entagle any bacilli which may be present. This clot may be removed, spread upon slides, and stained by one of the methods already given (see pp. 64 to 66). If desired, the coagulum may be treated with antiformin (see p. 68). In case no coagulum forms, the fluid should be thoroughly centrifugalized and the sediment stained, or, if much protein be present, it may be coagulated by heat, precipitated by the centrifuge, and treated with antiformin. It may be necessary to examine a considerable number of smears. In doubtful cases inoculation of guinea-pigs must be resorted to.

The *Diplococcus intracellularis meningitidis* is recognized as the cause of epidemic cerebrospinal fever, and can be detected in the cerebrospinal fluid of most cases, especially those which run an acute course. Cover-glass smears from the sediment should be stained by a simple

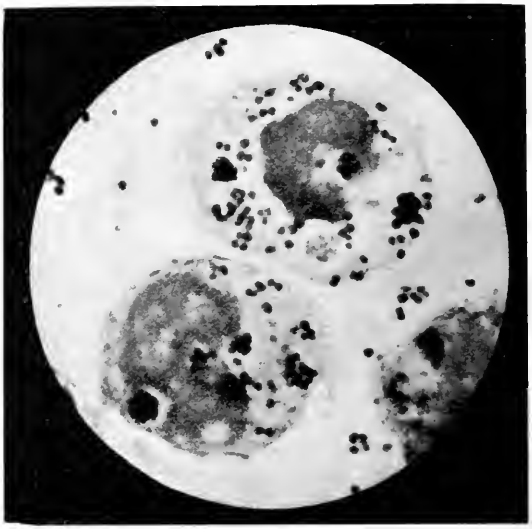


Fig. 156.—Diplococcus intracellularis meningitidis in leukocytes (×2000) (Wright and Brown).

bacterial stain and by Gram's method. The meningococcus is an intracellular diplococcus which often cannot be distinguished from the gonococcus in stained smears (Fig. 156). It also decolorizes by Gram's method. The presence of such a diplococcus in meningeal exudates is, however, sufficient for its indentification in clinical work.

Various organisms have been found in other forms of meningitis—the pneumococcus most frequently, the

influenza bacillus (Fig. 157) rarely. In some cases no micro-organisms can be detected even by culture methods.

(2) **Cytology.**—The total number of cells is counted with the hemacytometer. Unna's polychrome methylene-blue or a solution of methyl-violet or other nuclear dye is drawn into the leukocyte pipet to the 0.5 mark, and the fresh spinal fluid, which has been well shaken, is

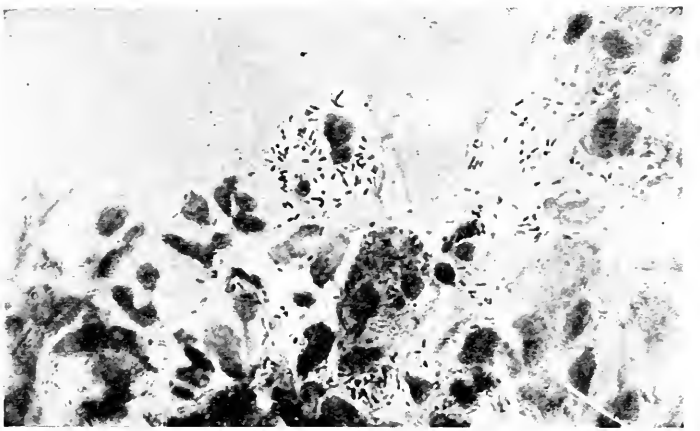


Fig. 157.—Influenza bacilli in spinal fluid. Case of meningitis (× 1000) (photograph by the author).

drawn up to the mark 11. After mixing, a drop is placed on the counting slide and covered. To reduce the error arising from the small number of cells present it is necessary to count a large area on several slides. Normally, the cells rarely exceed 5 or 7 per c.mm.; 10 is perhaps the maximum. The differential count is made as described on page 277. Ordinarily, only two kinds of cells are seen: lymphocytes and polymorphonuclear neutrophiles.

Lymphocytes predominate normally. An increase in

the total count, together with predominance of lymphocytes (over 70 per cent.), strongly suggests tuberculosis or syphilitic disease of the nervous system, such as paresis. It has been observed in the more chronic type of epidemic cerebrospinal meningitis, but not to the same extent.

In acute meningitis the total count is high and polymorphonuclears prevail.

ANIMAL INOCULATION

Inoculation of animals is one of the most reliable means of verifying the presence of certain micro-organisms in fluids and other pathologic material, and is helpful in determining the species of bacteria which have been isolated in pure culture.

Clinically, it is applied most frequently to demonstration of the tubercle bacillus when other means have failed or are uncertain. The guinea-pig is the most suitable animal for this purpose. When the suspected material is fluid and contains pus, it should be well centrifugalized, and 1 or 2 c.c. of the sediment injected by means of a large hypodermic needle into the peritoneal cavity or underneath the loose skin of the groin. Fluids from which no sediment can be obtained must be injected directly into the peritoneal cavity, since at least 10 c.c. are required, which is too great an amount to inject hypodermically. Solid material should be placed in a pocket made by snipping the skin of the groin with scissors, and freeing it from the underlying tissues for a short distance around the opening. When the intraperitoneal method is selected, several animals must be inoculated, since some are likely to die

from peritonitis caused by other organisms before the tubercle bacillus has had time to produce its characteristic lesions.

The animals should be killed at the end of six or eight weeks, if they do not die before that time, and a careful search should be made for the characteristic pearl-gray or yellow tubercles scattered over the peritoneum and through the abdominal organs, particularly the spleen and liver, and for caseous inguinal and retroperitoneal lymph-glands. The tubercles and portions of the caseous glands should be crushed between two slides, dried, and stained for tubercle bacilli. The bacilli are difficult to find in the caseous material.

THE MOUTH

Micro-organisms are always present in large numbers. Among these is *Leptothrix buccalis* (Fig. 158), which is

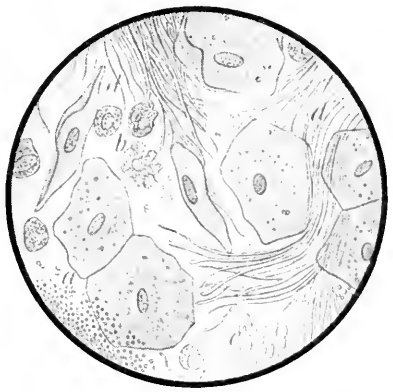


Fig. 158.—Gingival deposit (unstained): *a*, Squamous epithelial cells; *b*, leukocytes; *c*, bacteria; *d*, Leptothrix buccalis (Jakob).

especially abundant in the crypts of the tonsils and the tartar of the teeth. The whitish patches of *Pharyn-*

gomycosis leptothrica are largely composed of these fungi. They are slender, segmented threads, which generally, but not always, stain violet with Lugol's solution, and are readily seen with a 4-mm. objective. At times they are observed in the sputum and stomach fluid. In the former they might be mistaken for elastic fibers; in the latter, for Boas-Oppler bacilli. In either case, the reaction with iodin will distinguish them.

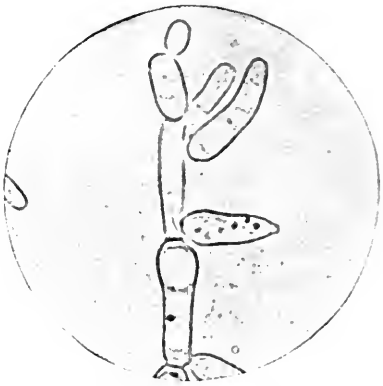


Fig. 159.—Thrush fungus (Endomyces albicans) (Kolle and Wassermann).

Thrush is a disease of the mouth seen most often in children, and characterized by the presence of white patches upon the mucous membrane. It is caused by the thrush fungus, *Endomyces albicans*. When a bit from one of the patches is pressed out between a slide and cover and examined with a 4-mm. objective, the fungus is seen to consist of a network of branching segmented hyphæ with numerous spores, both within the hyphæ and in the meshes between them (Fig. 159). The meshes also contain leukocytes, epithelial cells, and granular débris.

Acute pseudomembranous inflammations, which occur chiefly upon the tonsils and nasopharynx, are generally caused by the diphtheria bacillus, but may result from streptococcic infection. In many cases diphtheria bacilli can be demonstrated in smears made from the membrane and stained with Löffler's methylene-blue or 2 per cent. aqueous solution of methyl-green. They are

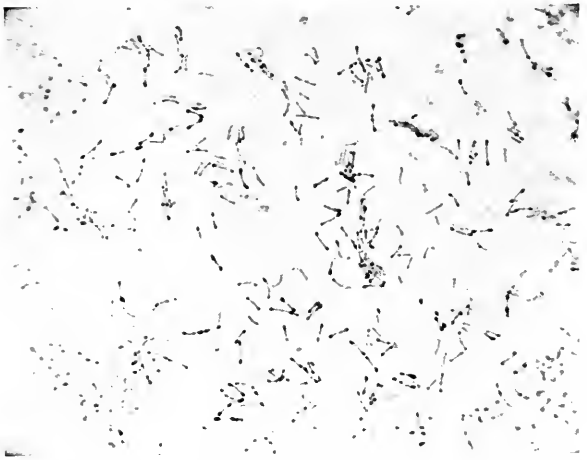


Fig. 160.—Bacillus diphtheriæ stained with methyl-green; culture from throat (×1000) (photograph by the author).

straight or curved rods, which vary markedly in size and outline, and stain very irregularly. A characteristic form is a palely tinted rod with several deeply stained granules (metachromatic bodies), or with one such granule at each end (Fig. 160). They stain by Gram's method. It is generally necessary, and always safer, to make a culture upon blood-serum, incubate for twelve hours, and examine smears from the growth.

Neisser's stain has long been the standard differential stain for the diphtheria bacillus. It colors the bodies of the bacilli brown and the metachromatic bodies blue.

1. Make films and fix as usual.

2. Apply the following solution, freshly filtered, for about one-half minute:

Methylene-blue	0.1 gm.;
Alcohol (96 per cent.)	2.0 c.c.;
Glacial acetic acid	5.0 "
Distilled water	95.0 "

3. Rinse in water.

4. Apply a saturated aqueous solution of Bismarck brown one-half minute.

5. Rinse, dry, and mount.

Ponder's Stain.—This new stain is preferred by many to Neisser's:

Toluidin blue (Gruebler)	0.02 gm.;
Glacial acetic acid	1.00 c.c.;
Absolute alcohol	2.00 "
Distilled water to	100.00 "

Cover the fixed film with the stain; turn the cover-glass over and examine as a hanging-drop preparation. Diphtheria bacilli are blue, with red granules.

Vincent's angina is a pseudomembranous and ulcerative inflammation of mouth and pharynx, which when acute may be mistaken for diphtheria, and when chronic is very apt to be mistaken for syphilis. Stained smears from the ulcers or membrane show large numbers of spirochetes and "fusiform bacilli," giving a striking and characteristic picture (Fig. 161). The "bacillus" is spindle shaped, more or less pointed at the ends, and

about 6 to 12 μ long. The spirillum is a very slender, wavy thread, about 30 to 40 μ long, and stains feebly. Diluted analin-gentian-violet makes a satisfactory stain. With methylene-blue the palely staining spirillum may easily be overlooked. Further description is given on p. 374.

Tuberculous ulcerations of mouth and pharynx can generally be diagnosed from curetings made after careful

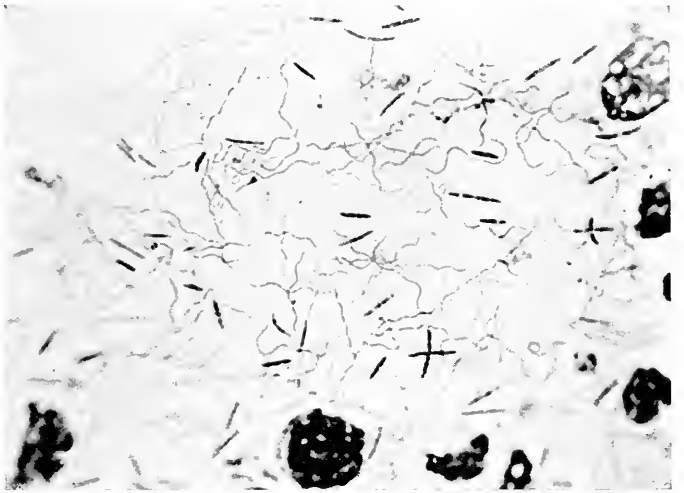


Fig. 161.—Spirochæta vincenti from case of ulcerative stomatitis stained with gentian-violet (×1200).

cleansing of the surface. The curetings are well rubbed between slide and cover, and the smears thus made are dried, fixed, and stained for tubercle bacilli. Since there is much danger of contamination from tuberculous sputum, the presence of tubercle bacilli is significant only in proportion to the thoroughness with which the ulcer was cleansed. The diagnosis is certain when the bacilli are

found within groups of cells which have not been dissociated in making the smears.

THE EYE

Staphylococci, pneumococci, and **streptococci** are probably the most common of the bacteria to be found in nonspecific conjunctivitis and keratitis. Serpiginous ulcer of the cornea is generally associated with the pneumococcus (see Fig. 151).

The usual cause of acute infectious conjunctivitis ("pink-eye"), especially in cities, seems to be the *Koch-Weeks bacillus*. This is a minute, slender rod, which

Fig. 162.—Conjunctival secretion from acute contagious conjunctivitis; polynuclear leukocytes with the bacillus of Weeks; P, phagocyte containing bacillus of Weeks (one-twelfth oil-immersion, ocular iii) (Morax).

lies within and between the pus-corpuscles (Fig. 162), and is negative to Gram's stain. In smears it cannot be distinguished from the influenza bacillus, although its length is somewhat greater.

The **diplobacillus of Morax and Axenfeld** gives rise to an acute or chronic blepharoconjunctivitis without follicles or membrane, for which zinc sulphate seems to be a specific. It is widely distributed geographically and

is common in many regions. The organism is a short, thick diplobacillus, is frequently intracellular, and is Gram-negative (Fig. 163). A delicate capsule can sometimes be made out.

Early diagnosis of gonorrheal ophthalmia is extremely important, and can be made with certainty only by detection of **gonococci** in the discharge. They are easily found in smears from untreated cases. After treatment is

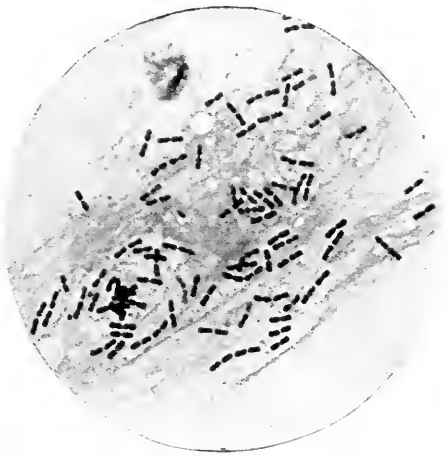


Fig. 163.—The diplobacillus of Morax and Axenfeld (from a preparation by Dr. Harold Gifford).

begun they soon disappear, even though the discharge continues.

Pseudomembranous conjunctivitis generally shows either **streptococci** or **diphtheria bacilli.** In diagnosing diphtheric conjunctivitis, one must be on his guard against the **Bacillus xerosis,** which is a frequent inhabitant of the conjunctival sac in healthy persons, and which is identical morphologically with the diphtheria bacillus.

Hence the clinical picture is more significant than the microscopic findings.

Various micro-organisms—bacteria, molds, protozoa—have been described in connection with trachoma, but the specific organism of the disease is not definitely known.

Herbert has called attention to the abundance of eosinophilic leukocytes in the discharge of **vernal catarrh.** He regards their presence in considerable numbers as very helpful in the diagnosis of this disease.

THE EAR

By far the most frequent exciting causes of acute otitis media are the pneumococcus and the streptococcus. The finding of other bacteria in the discharge generally indicates a secondary infection, except in cases complicating infectious diseases, such as typhoid fever, diphtheria, and influenza. Discharges which have continued for some time are practically always contaminated with the staphylococcus. The presence of the streptococcus should be a cause of uneasiness, since it much more frequently leads to mastoid disease and meningitis than does the pneumococcus. The staphylococcus, bacillus of Friedländer, colon bacillus, and *Bacillus pyocyaneus* may be met in chronic middle-ear disease.

In tuberculous disease the tubercle bacillus is present in the discharge, but its detection offers some difficulties. It is rarely easy to find, and precautions must always be taken to exclude the smegma and other acid-fast bacilli (see p. 69), which are especially liable to be present in the ear. Rather striking is the tendency of old squamous cells to retain the red stain, and fragments of such cells may mislead the unwary.

PARASITIC DISEASES OF THE SKIN

Favus, tinea versicolor, and the various forms of ringworm are caused by members of the fungus group. To demonstrate them, a crust or a hair from the affected area is softened with a few drops of 20 per cent. caustic soda solution, pressed out between a slide and cover, and examined with a 4-mm. objective. They consist of a more or less dense network of hyphæ and numerous round or oval refractive spores. The cuts in standard works upon diseases of the skin will aid in differentiating the members of the group.

MILK

A large number of analyses of human and cows' milk are averaged by Holt as follows, Jersey milk being excluded because of its excessive fat:

	Human Milk.		Cows' Milk.
	Normal variations, per cent.	Average, per cent.	Average, per cent.
Fat	3.00 to 5.00	4.00	3.50
Sugar	6.00 to 7.00	7.00	4.30
Proteins	1.00 to 2.25	1.50	4.00
Salts	0.18 to 0.25	0.20	0.70
Water	89.82 to 85.50	87.30	87.50
	100.00 100.00	100.00	100.00

The reaction of human milk is slightly alkaline; of cows', neutral or slightly acid. The specific gravity of each is bout 1.028 to 1.032. Human milk is sterile when secreted, but derives a few bacteria from the lacteal ducts. Cows' milk, as usually sold, contains large numbers of bacteria, the best milk rarely containing fewer than 10,000 per cubic centimeter. Microscopically, human milk is a fairly homogeneous emulsion of fat, and is practically destitute of cellular elements. Any

notable number of leukocytes indicates infection of the mammary gland.

Chemical examination of milk is of great value in solving the problems of infant feeding. The sample examined

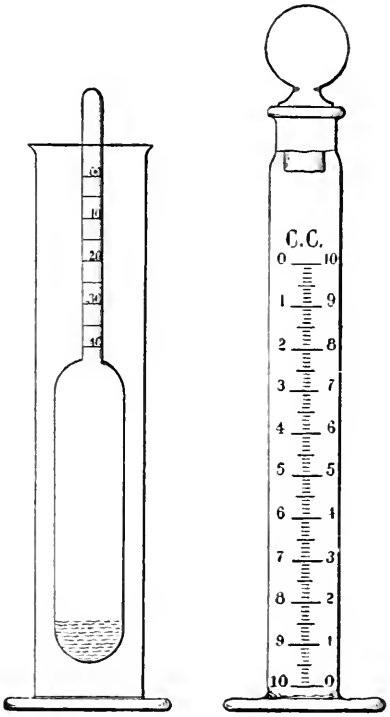


Fig. 164.—Holt's milk-testing apparatus.

should be the middle milk, or the entire quantity from one breast. The fat and protein can be estimated roughly, but accurately enough for many clinical purposes by means of Holt's apparatus, which consists of a 10-c.c. cream gage and a small hydrometer (Fig. 164).

The cream gage is filled to the o mark with milk, allowed to stand for twenty-four hours at room temperature, and the percentage of cream then read off. The percentage of fat is three-fifths that of the cream. The protein is then approximated from a consideration of the specific gravity and the percentage of fat. The salts and sugar very seldom vary sufficiently to affect the specific gravity, hence a high specific gravity must be due to either an increase of protein or decrease of fat, or both, and vice versâ. With normal specific gravity the protein is high when the fat is high, and vice versâ. The method is not accurate with cows' milk.

Fig. 165.—Centrifuge tube for milk analysis.

For more accurate work the following methods, applicable to either human or cows' milk, are simple and satisfactory:

Fat.—*Leffmann-Beam Method.*—This is essentially the widely used Babcock method, modified for the small quantities of milk obtainable from the human mammary gland. The apparatus consists of a special tube which fits the aluminum shield of the medical centrifuge (Fig. 165) and a 5-c.c. pipet. Owing to its narrow stem, the tube is difficult to fill and to clean. Exactly 5 c.c. of the milk are introduced into the tube by means of the pipet, and 1 c.c. of a mixture of equal parts of concentrated hydrochloric acid and amyl-alcohol is added and well mixed. The tube is filled to the o mark with concentrated sulphuric acid, adding a few drops at a time and agitating constantly. This is revolved in the centrifuge at 1000

revolutions a minute for three minutes, or until the fat has separated. The percentage is then read off upon the stem, each small division representing 0.2 per cent. of fat.

Proteins.—*T. R. Boggs' Modification of the Esbach Method.*—This is applied as for urinary albumin (see p. 134), substituting Boggs' reagent for Esbach's. The reagent is prepared as follows:

(1) Phosphotungstic acid..... 25 gm.;
Distilled water.....125 c.c.;

(2) Concentrated hydrochloric acid..... 25 "
Distilled water.....100 "

When the phosphotungstic acid is completely dissolved, mix the two solutions. This reagent is quite stable if kept in a dark glass bottle.

Before examination, the milk should be diluted according to the probable amount of protein, and allowance made in the subsequent reading. For human milk the optimum dilution is 1:10; for cows' milk, 1:20. Dilution must be accurate.

Lactose.—The protein should first be removed by acidifying with acetic acid, boiling, and filtering. The copper methods may then be used as for glucose in the urine (see p. 142); but it must be borne in mind that lactose reduces copper more slowly than glucose, and longer heating is, therefore, required; and that 10 c.c. of Fehling's solution (or 25 c.c. of Benedict's) are equivalent to 0.0676 gm. lactose (as compared with 0.05 gm. glucose).

Detection of Preservatives.—Formalin is the most common preservative added to cows' milk, but boric acid is also used.

To detect formalin, add a few drops of dilute ferric chlorid solution to a few cubic centimeters of the milk, and run the mixture gently upon the surface of some strong sulphuric acid in a test-tube. If formaldehyd be present, a bright red ring will appear at the line of contact of the fluids. This is not a specific test for formaldehyd, but nothing else likely to be added to the milk will give it.

To detect boric acid, Goske's method, as used by the Chicago Department of Health, is simple and satisfactory: Mix 2 c.c. of concentrated hydrochloric acid with 20 c.c. of the milk and place in a 50-c.c. beaker. In this suspend a long strip of turmeric paper (2 cm. wide), so that its end reaches to the bottom of the beaker. Allow to remain about half an hour. The liquid will rise by capillarity, and if boric acid be present a red-brown color will appear at the junction of the moist and dry portions of the paper. If this is touched with ammonia, a bluish-green slate color develops. A rough idea of the amount of boric acid may be had by comparing the depth of color with that produced by boric acid solutions of known strength.

SYPHILITIC MATERIAL

In 1905 Schaudinn and Hoffmann described the occurrence of a very slender, spiral micro-organism in the lesions of syphilis. This they named *Spirochæta pallida*, because of its low refractive power and the difficulty with which it takes up staining reagents. The name was later changed to *Treponema pallidum*. Its etiologic relation to syphilis is now universally admitted. It is found in primary, secondary, and tertiary lesions, but

is not present in the latter in sufficient numbers to be of value in diagnosis.

Treponema pallidum is an extremely slender, spiral, motile thread, with pointed ends. There is a flagellum at each end, but it is not seen in ordinary preparations. The organism varies considerably in length, the average being about 7 μ, or the diameter of a red blood-corpuscle; and it exhibits three to twelve, sometimes more, spiral curves, which are sharp and regular and resemble the

Fig. 166.—Treponema pallidum (×1000) (Leitz $\frac{1}{12}$ oil-immersion objective and Leitz dark-ground condenser).

curves of a corkscrew (Figs. 113, 166, 167). It is so delicate that it is difficult to see even in well-stained preparations; a high magnification and careful focusing are, therefore, required. Upon ulcerated surfaces it is often mingled with other spiral micro-organisms, which adds to the difficulty of its detection. The most notable of these is *Spirochæta refringens*, described on p. 375.

Treponema pallidum is most easily demonstrated in chancres and mucous patches, although the skin lesions

—papules, pustules, roseolous areas—often contain large numbers. Tissue-juice from the deeper portions of the lesions is the most favorable material for examination, because the organisms are commonly more abundant than upon ulcerated surfaces and are rarely accompanied by other micro-organisms. After cleansing, the surface is gently scraped with a curet or rubbed briskly with a swab of cotton or gauze. In a few moments serum will exude. The rubbing should not be so vigorous as to

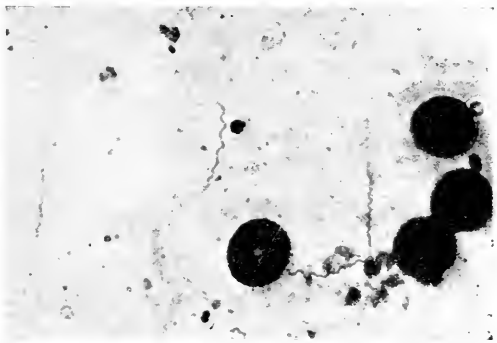


Fig. 167.—Treponema pallidum and Spirochæta refringens (×1200) (Leitz oil-immersion objective).

bring the blood, because the corpuscles may hide the treponema. Very thin cover-glass smears are then made from the serum.

Staining Methods.—**Giemsa's stain** is probably the most widely used. It is best purchased ready prepared. Smears are fixed in absolute alcohol for fifteen minutes. Ten drops of the stain are added to 10 c.c. of faintly alkaline distilled water (1 drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of the water), and the fixed smear is immersed in this diluted stain for one to three hours or longer. It is then

rinsed in distilled water, dried, and mounted. In well-stained specimens *Treponema pallidum* is reddish; most other micro-organisms, bluish. More intense staining may be obtained by gently warming the stain.

Wright's blood-stain, used in the manner already described (see p. 268) except that the diluted stain is allowed to act upon the film for fifteen minutes, gives good results.

Silver Method.—The silver impregnation method has long been used for tissues. Stein has applied it to smears as follows:

1. Dry the films in the incubator at 37° C. for three or four hours.

2. Immerse in 10 per cent. silver nitrate solution, in diffuse daylight, for some hours, until the preparation takes on a metallic luster.

3. Wash in water, dry, and mount.

The organisms are black against a brownish background.

India-ink Method.—A small drop of India-ink of good grade (Günther and Wagner's "Chin-Chin liquid pearl" or Grübler's "nach Burri" recommended) is mixed on a slide with 1 or 2 small drops of serum from the suspected lesion. The mixture is then spread over the slide and allowed to dry. After drying, it is examined with an oil-immersion lens. Micro-organisms, including *Treponema pallidum*, appear clear white on a brown or black background, much as they do with the dark ground condenser (see Fig. 166). If desired, the mixture of ink and serum may be covered with a cover-glass and examined in the fresh state, the living organisms being thus demonstrated. Because of its extreme simplicity this method has been favorably received. It cannot, however, be absolutely relied upon, since, as has been pointed out, many India-inks contain wavy vegetable fibrils which might easily mislead a beginner, and sometimes, indeed, even an experienced worker. Instead of India-ink, collargol, diluted 1 : 20 with water and thoroughly shaken, has been recommended.

Dark ground illumination (see p. 22) may be used to study the living organisms in fresh tissue juices. This offers a satisfactory means of diagnosis, but since the instrument is expensive the practitioner will rely upon one or more of the staining methods just enumerated.

Method of Oppenheim and Sachs.—Very thin air-dried films are stained for from thirty seconds to three minutes with phenol-gentian-violet (saturated alcoholic solution of gentian-violet, 10 c.c.; 5 per cent. phenol, 90 c.c.). Previous fixation is not necessary.

SEMEN

Absence of spermatozoa is a more common cause of sterility than is generally recognized. In some cases they are present, but lose their motility immediately after ejaculation.

Semen should be kept warm until examined. When it must be transported any considerable distance, the method suggested by Boston is convenient: The fresh semen is placed in a small bottle, to the neck of which a string is attached. This is then suspended from a button on the trousers, so that the bottle rests against the skin of the inguinal region. It may be carried in this way for hours. When ready to examine, place a small quantity upon a warmed slide and apply a cover. The spermatozoa are readily seen with a 4-mm. objective (see Fig. 58). Normally, they are abundant and in active motion.

Detection of semen in stains upon clothing, etc., is often important. The finding of spermatozoa, after soaking the stain for an hour in normal salt solution or dilute alcohol and teasing in the same fluid, is absolute proof that the stain in question is semen, although it is not possible to distinguish human semen from that of the

lower animals in this way. A little eosin added to the fluid will bring the spermatozoa out more clearly.

Florence's Reaction.—The suspected material is softened with water, placed upon a slide with a few drops of the reagent, and examined at once with a medium power of the microscope. If the material be semen, there will be found dark-brown crystals (Fig. 168) in the form of rhombic platelets resembling hemin crystals, or

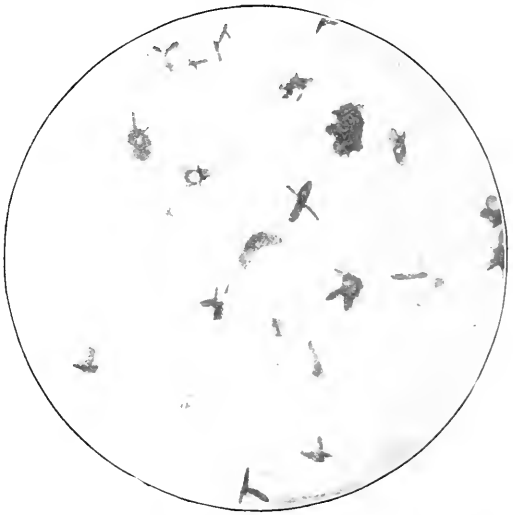


Fig. 168.—Seminal crystals (medium size) (×750) from a stain on clothing. A single thread ⅛ inch long was used in the test, the stain being three years and four months old (Peterson and Haines).

of needles, often grouped in clusters. These crystals can also be obtained from crushed insects, watery extracts of various internal organs, and certain other substances, so that they are not absolute proof of the presence of semen. Negative results, upon the other hand, are conclusive, even when the semen is many years old.

The reagent consists of iodin, 2.54 gm.; potassium iodid, 1.65 gm.; and distilled water, 30 c.c.

DIAGNOSIS OF RABIES

In view of the brilliant results attending prophylactic treatment by the Pasteur method, early diagnosis of rabies (hydrophobia) in animals which have bitten persons is extremely important.

The most reliable means of diagnosis is the production of the disease in a rabbit by subdural or intracerebral injection of a little of the filtrate from an emulsion of the brain and medulla of the suspected animal. The diagnosis can, however, usually be quickly and easily made by microscopic demonstration of Negri bodies. Whether these bodies be protozoan in nature and the cause of the disease, as is held by many, or whether they be products of the disease, it is certain that their presence is pathognomonic.

Negri bodies are sharply outlined, round, oval, or somewhat irregular structures which vary in size, the extremes being 0.5 and 18 μ. They consist of a hyalin-like cytoplasm, in which when properly stained one or more chromatin bodies can usually be seen. They are situated chiefly within the cytoplasm of the large cells of the central nervous system, the favorite location being the multipolar cells of the hippocampus major (Ammon's horn). In many cases they suggest red blood-corpuscles lying within nerve-cells.

Probably the best clinical method of demonstrating Negri bodies is the impression method of Langdon Frothingham, which is carried out as described on page 452.

(1) Place the dog's brain[1] upon a board about 10 inches square, and divide into two halves by cutting along the median line with scissors.

(2) From one of the halves cut away the cerebellum and open the lateral ventricle, exposing the Ammon's horn.

(3) Dissect out the Ammon's horn as cleanly as possible.

(4) Cut out a small disk at right angles to the long axis of the Ammon's horn, so that it represents a cross-section of the organ.

(5) Place this disk upon the board near the edge, with one of the cut surfaces upward.

(6) Press the surface of a thoroughly clean slide upon the disk and lift it suddenly. The disk (if its exposed surface has not been allowed to become too dry) will cling to the board, leaving only an impression upon the slide. Make several similar impressions upon different portions of the slide, using somewhat greater pressure each time. Impressions are also to be made from the cut surface of the cerebellum, since Negri bodies are sometimes present in the Purkinje cells when not found in the Ammon's horn.

(7) Before the impressions dry, immerse in methyl-alcohol for one-half to two minutes.

(8) Cover with Van Gieson's methylene-blue-fuchsin stain, warming gently for one-half to two minutes. This stain, as modified by Frothingham, is as follows. It must be freshly mixed each day:

Tap-water . . . . . 20 c.c.;
Saturated alcoholic solution basic fuchsin . . . . . 1 drop;
("Fuchsin f. Bac.," Grübler).
Saturated aqueous solution methylene-blue . . . . . 1 drop.
("Methylenblau f. Bac.," Grübler).

(9) Wash in water and dry with filter-paper. Examine with a low power to locate the large cells in which the bodies

[1] For Dr. Frothingham's method of removing a dog's brain see American Journal of Public Hygiene for February, 1908.

PLATE XIII

Nerve-cells containing Negri bodies.

Hippocampus impression preparation, dog. Van Gieson stain; × 1000. 1, Negri bodies; 2, capillary; 3, free red blood-corpuscles (courtesy of Langdon Frothingham).

are apt to be found, and study these with an oil-immersion lens.

The Negri bodies are stained a pale pink to purplish red, and frequently contain small blue dots (Plate XIII). The nerve-cells are blue, and red blood-corpuscles are colorless or yellowish-copper colored.

When the work is finished, the board with the dissected brain is sterilized in the steam sterilizer.

Demonstration of Negri bodies by this method is quicker and, probably, more certain than by the study of sections. It has the decided advantage over the smear method that the histologic structure is retained. One or more of the impressions generally shows the entire cell arrangement almost as well as in sections, and it is very easy to locate favorable fields with a 16-mm. objective.

CHAPTER VIII

BACTERIOLOGIC METHODS

Bacteriology has become so important a part of medicine that some knowledge of bacteriologic methods is imperative for the present-day practitioner. It has been the plan of this book to describe the various bacteria and bacteriologic methods with the subjects to which they seemed to be particularly related. The tubercle bacillus and its detection, for example, are described in the chapters upon Sputum and Urine; blood-cultures are discussed in the chapter upon Blood. There are, however, certain methods, notably the preparation of media and the study of bacteria by cultures, which do not come within the scope of any previous section, and an outline of these is given in the present chapter.

I. APPARATUS

Much of the apparatus of the clinical laboratory is called into use. Only the following need special mention:

1. **Sterilizers.**—Two are required.

The *dry*, or *hot-air sterilizer*, is a double-walled oven similar to the detached ovens used with gas and gasolene stoves. It has a hole in the top for a perforated cork with thermometer.

The *steam sterilizer* is preferably of the Arnold type, opening either at the top or the side. An *autoclave*, which

sterilizes with steam under pressure, is very desirable, but not necessary. An aluminum pressure cooker is a very satisfactory substitute for the autoclave. It costs about fifteen dollars.

2. **Incubator.**—This is the most expensive piece of apparatus which will be needed. It is made of copper, and has usually both a water- and an air-jacket surrounding the incubating chamber. It is provided with thermometer, thermo-regulator, and some source of heat, usually a Koch safety Bunsen burner. With a little ingenuity one can rig up a drawer or a small box, in which a fairly constant temperature can be maintained by means of an electric light. The degree of heat can be regulated by moving the drawer in or out, or holes can be made in which corks may be inserted and removed as needed. A Thermos bottle has been suggested as a temporary make-shift.

3. **Culture-tubes and Flasks.**—For most work ordinary test-tubes, 5 by $\frac{3}{4}$ inches, are satisfactory. For special purposes a few 3 by $\frac{1}{2}$ inch and 6 by $\frac{3}{4}$ inch tubes may be needed. Heavy tubes, which do not easily break, can be obtained, and are especially desirable when tubes are cleaned by an untrained assistant. The tubes are usually stored in wire baskets.

Flasks of various sizes are needed. The Ehrlenmeyer type is best. Quart and pint milk bottles and 2-ounce wide-mouthed bottles will answer for most purposes.

4. **Platinum Wires.**—At least two of these are needed. Each consists of a piece of platinum wire about 8 cm. long, fixed in the end of a glass or metal rod. One is made of about 22 gage wire and its end is curled into a loop 1 to 2 mm. in diameter. A loop 1 mm. in diameter is

sometimes called a "normal." The other wire is somewhat heavier and its tip is hammered flat.

5. **Pipets, etc.**—In addition to the graduated pipets with which every laboratory is supplied, there are a number of forms which are generally made from glass tubing as needed. One of the simplest of these is made as follows: A section of glass tubing, about 12 cm. long and 5 mm. in diameter, is grasped at the ends, and its

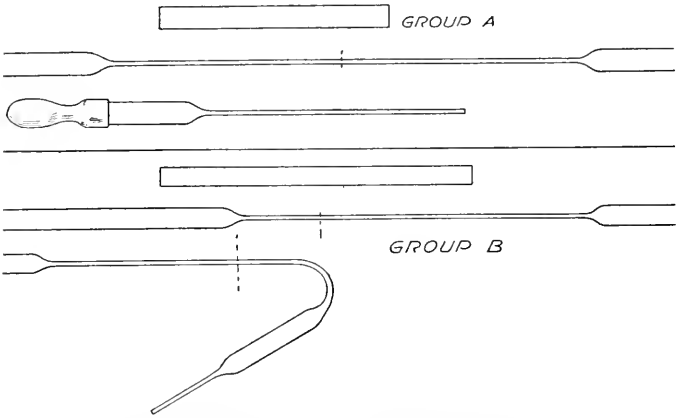


Fig. 169.—Process of making pipets (Group A) and Wright's capsule (Group B). The dotted lines indicate where the glass is to be broken.

center is heated in a concentrated flame. A blast-lamp is best, but a Bunsen burner will usually answer, particularly if fitted with a "wing" or "fish-tail" attachment. When the glass is thoroughly softened it is removed from the flame, and, with a steady, but not rapid pull, is drawn out as shown in Fig. 169. The slender portion is scratched near the middle with a file and is broken to make two pipets, which are then fitted with rubber nipples. Two conditions are essential to success: the

glass must be thoroughly softened and it must be removed from the flame before beginning to pull.

A nipple can be made of a short piece of rubber tubing, one end of which is plugged with a glass bead.

This pipet has many uses about the laboratory. When first made it is sterile and may be used to transfer cultures. With a grease-pencil mark about 2 cm. from its tip (see Fig. 172), it is useful for measuring very small quantities of fluid, as in making dilutions for the Widal test and in counting bacteria in vaccines. Mett's tubes for pepsin estimation may be made from the capillary portion. The capillary portion also makes a very satisfactory blood-lancet if the center is heated in a low flame and the two ends pulled quickly apart.

Another useful device is the Wright capusle, which is made as shown in Fig. 169. Its use is illustrated in Fig. 173. After the straight end is sealed the curved portion may be hooked over the aluminum tube of the centrifuge, and the contained blood or other fluid sedimented; but the speed should not be so great as to break the capsule.

II. STERILIZATION

All apparatus and materials used in bacteriologic work must be sterilized before use.

Glassware, metal, etc., are heated in the hot-air sterilizer at 150° to 180° C. for half an hour. Flasks, bottles, and tubes are plugged with cotton before heating. Petri dishes may be wrapped in paper in sets of three. Pipets and glass and metal hypodermic syringes are placed in cotton-stoppered test-tubes.

Culture-media and other fluids must be sterilized by steam. Exposure in an autoclave to a temperature of

110° C. (6 pounds' pressure) for one-half hour is sufficient. With the Arnold sterilizer, which is more commonly used, the intermittent plan must be adopted, since steam at ordinary pressure will not kill spores. This consists in steaming for thirty to forty-five minutes on three or four successive days. Spores which are not destroyed upon the first day develop into the vegetative form and are destroyed at the next heating. Gelatin media must not be exposed to steam for more than twenty minutes at a time, and must then be removed from the sterilizer and cooled in cold water, otherwise the gelatin may lose its power to solidify.

Cotton and **gauze** are sterilized by either hot air or steam, preferably the latter.

III. PREPARATION OF CULTURE-TUBES

New tubes should be washed in a very dilute solution of nitric acid, rinsed in clear water, and allowed to drain dry.

Tubes which contain dried culture-media are cleaned with a test-tube brush after boiling in a strong solution of washing-soda. They are then rinsed successively in clear water, acidulated water, and clear water, and allowed to drain.

The tubes are now ready to be plugged with raw cotton —the "cotton batting" of the dry goods stores. This is done by pushing a wad of cotton into each tube to a depth of about 3 cm. with a glass rod. The plugs should fit snugly, but not too tightly, and should project from the tube sufficiently to be readily grasped by the fingers. The tubes are next placed in wire baskets and heated in an oven for about one-half hour at 150° C. in order to mold the stoppers to the shape of the tubes. The heat-

ing should not char the cotton, although a slight browning does no harm. The tubes are now ready to be filled with culture-media.

IV. CULTURE-MEDIA

For a careful study of bacteria a great variety of culture-media is required, but only a few—bouillon, agar or solidified blood-serum, and gelatin—are much used in routine work. A great deal of work can be done with a single medium, for which purpose solidified blood-serum is probably best. The ordinary culture-media, put up in tubes ready for use, can be purchased through any pharmacy.

Preparation of Culture-media.—

Beef Infusion

Hamburger steak, lean	500 gm.;
Tap-water	1000 c.c.

Mix well; let soak about twenty-four hours in an ice-chest, and squeeze through cheese-cloth. This infusion is not used by itself, but forms the basis for various media. "Double strength" infusion, used in making agar-agar, requires equal parts of the meat and water.

Infusion Bouillon

Beef infusion	1000 c.c.;
Peptone (Witte)	10 gm.;
Salt	5 "

Boil until dissolved; bring to original bulk with water; adjust reaction, and filter.

Beef Extract Bouillon

Liebig's extract of beef	3 gm.;
Peptone	10 "
Salt	5 "
Tap-water	1000 c.c.

Heat until all ingredients are dissolved, cool, and beat in the whites of two eggs; bring slowly to the boiling-point again; boil briskly for five minutes and filter. It is not usually necessary to adjust the reaction.

Agar-agar

Preparation of this medium usually gives the student much trouble. There should be no difficulty if the directions are carefully carried out.

Agar-agar, powdered or in shreds..... 15 gm.;
Tap-water.....500 c.c.

Boil until thoroughly dissolved and add—

Peptone..... 10 gm.;
Salt..... 5 "

When these have dissolved, replace the water lost in boiling, cool to about 60° C., and add 500 c.c. double-strength beef infusion. Bring slowly to the boil, adjusting the reaction meanwhile, and boil for at least five minutes. Filter while *hot* through a moderately thick layer of absorbent cotton wet with *hot* water in a *hot* funnel. A piece of coarse wire gauze should be placed in the funnel underneath the cotton to give a larger filtering surface. This medium will be clear enough for ordinary work. If an especially clear agar is desired, it can be filtered through paper in an Arnold sterilizer.

Agar can also be made by boiling 15 gm. of powdered agar in 1000 c.c. of bouillon until dissolved, replacing the water lost in boiling, and filtering through paper in a sterilizer. It can be cleared with egg if desired.

Glycerin Agar-agar

To 1000 c.c. melted agar add 60 to 70 c.c. glycerin.

Gelatin

Dissolve 100 to 120 gm. "golden seal" gelatin in 1000 c.c. nutrient bouillon with as little heat as possible, adjust the reaction, cool, beat in the whites of two eggs, bring slowly to the boiling point, boil for a few minutes, and filter hot through filter-paper wet with hot water. Sterilize in an Arnold sterilizer for twenty minutes upon three successive days and cool in cold water after each heating.

Sugar Media

Any desired sugar may be added to bouillon, agar, or gelatin in proportion of 10 gm. to the liter. Dextrose is most frequently required. When other sugars are added, media made from beef-extract should be used, since those made from beef-infusion contain enough dextrose to cause confusion.

Löffler's Blood-serum

Dextrose-bouillon (1 per cent.) ..... 1 part;
Blood-serum ..... 3 parts.

Mix and tube. Place in an inspissator at the proper slant for three to six hours at 80° to 90° C. When firmly coagulated, sterilize in the usual way. A large "double-cooker" makes a satisfactory inspissator. The tubes are placed in the inner compartment at the proper slant, a lid with perforation for a thermometer is applied, and the whole is weighted down in the water of the outer compartment.

Blood-serum is obtained as follows: Beef or pig blood is collected in a bucket at the slaughter-house and placed in an ice-chest until coagulated. The clot is then gently loosened from the wall of the vessel. After about

twenty-four hours the serum will have separated nicely and can be siphoned off. It is then stored in bottles with a little chloroform until needed. Red cells, if abundant, darken the medium, but do no harm.

Solidified blood-serum is probably the most satisfactory medium for general purposes. Nearly all pathogenic organisms grow well upon it.

Hemoglobin Medium

The simplest way to prepare this is to smear a drop of blood, obtained by puncture of the finger, over the surface of an agar-slant, and to incubate over night to make sure of sterility. It is used chiefly for growing the influenza bacillus. It may be noted that the bacillus will not grow well on blood from a person who has recently recovered from influenza.

Litmus Milk

Fresh milk is steamed in an Arnold sterilizer for half an hour, and placed in the ice-chest over night. The milk is siphoned off from beneath the cream, and sufficient aqueous solution of litmus or, preferably, azolitmin is added to give a blue-violet color. It is then tubed and sterilized.

Potato

Cylinders about $\frac{1}{2}$ inch thick are cut from potato and split obliquely. These wedge-shaped pieces are soaked over night in running water and placed, broad ends down, in large tubes, in the bottom of which is placed a little cotton saturated with water. They are sterilized for somewhat longer periods than ordinary media.

DUNHAM'S PEPTONE SOLUTION

Peptone	10 gm.;
Salt	5 "
Water	1000 c.c.

Dissolve by boiling; filter, tube, and sterilize.

This medium is used to determine indol production. To a twenty-four- to forty-eight-hour-old culture is added 5 to 10 drops of concentrated chemically pure sulphuric acid and 1 c.c. of 1 : 10,000 solution of sodium nitrite. Appearance of a pink color shows the presence of indol. A pink color before the nitrite is added shows the presence of both indol and nitrites.

HISS' SERUM MEDIA

Blood-serum	1 part;
Water	3 parts.

Warm and adjust reaction to +0.2 to +0.8. Add litmus or azolitmin solution to give a blue-violet color. Finally, add 1 per cent. of inulin or any desired sugar. The inulin medium is very useful in distinguishing between the pneumococcus and streptococcus.

BILE MEDIUM

Ox- or pig-bile is obtained at the slaughter-house, tubed, and sterilized. This is used especially for growing typhoid bacilli from the blood during life. The following is probably as satisfactory as fresh bile and is more convenient:

Inspissated ox-bile (Merck)	30.0 gm.;
Peptone	2.5 "
Water	250.0 c.c.

Dissolve, place in tubes, and sterilize.

Reaction of Media.—The chemical reaction of the medium exerts a marked influence upon the growth of bacteria. It is adjusted after all ingredients are dissolved by adding sufficient caustic soda solution to overcome the acidity of the meat and other substances used. In general, the most favorable reaction lies between the neutral points of litmus and phenolphthalein, representing a very faint alkalinity to litmus. In routine work it is usually sufficient to test with litmus-paper. When greater accuracy is demanded, the following method should be used: After all ingredients are dissolved and the loss during boiling has been replaced with water, 10 c.c. of the medium are transferred to an evaporating dish, diluted with 40 c.c. of water, and boiled for three minutes to drive off carbon dioxid. One c.c. of 0.5 per cent. alcoholic solution of phenolphthalein is then added, and decinormal sodium hydroxid solution is run in from a buret until the neutral point is reached, indicated by the appearance of a permanent pink color. The number of cubic centimeters of decinormal solution required to bring this color indicates the number of cubic centimeters of *normal* sodium hydroxid solution which will be required to neutralize 100 c.c. of the medium. The standard reaction is +1.5, which means that the medium must be of such degree of acidity that 1.5 c.c. of normal solution would be required to neutralize 100 c.c. This corresponds to faint alkalinity to litmus. Most pathogenic bacteria grow better with a reaction of +1.0 or +0.8. Example: If the 10 c.c. which were titrated required 2 c.c. of decinormal solution to bring the pink color, the reaction is +2; and 0.5 c.c. of normal sodium hydroxid must be added to

each 100 c.c. of the medium to reduce it to the standard, +1.5.

Tubing Culture-media.—The finished product is stored in flasks or distributed into test-tubes. This is done by means of a funnel fitted with a section of rubber tubing with a glass tip and a pinch-cock. Great care must be exercised, particularly with media which solidify, not to smear any of them upon the inside of the mouth of the tube, otherwise the cotton stopper will stick. Tubes are generally filled to a depth of 3 or 4 cm. For stab-cultures a greater depth is required.

After tubing, all culture-media must be sterilized as already described. Agar-tubes are cooled in a slanting position to secure the proper surface for inoculation.

Storage of Culture-media.—All media should be stored in a cool place, preferably an ice-chest. Evaporation may be prevented by covering the tops of the tubes with tin-foil or the rubber caps which are sold for the purpose; or the cotton stopper may be pushed in a short distance and a cork inserted.

V. STAINING METHODS

In general, bacteria are stained to determine their morphology, their reaction with special methods (*e. g.*, Gram's method), and the presence or absence of certain structures, as spores, flagella, and capsules. Staining methods for various purposes and the formulæ of the staining fluids have been given in previous chapters and can be found by consulting the Index. The following will probably be most frequently used:

Methods for tubercle bacilli (see pp. 64–66 and 205).

Methods for capsules of bacteria (see pp. 72 and 421).

Methods for *Treponema pallidum* (see p. 447).
Method for gonococcus (see p. 422).
Löffler's alkaline methylene-blue (see p. 74).
Blood-stains (see pp. 266–271).

The **method of staining for morphology** is as follows, using any bacterial stain:

(1) Make a thin smear upon a slide or cover-glass. Heavy grease-pencil marks across the slide will limit the stain to any portion desired.

(2) Dry in the air, or by warming high above the flame, where one can comfortably hold the hand.

(3) "Fix" by passing the preparation, film side up, rather slowly through the flame of a Bunsen burner: a cover-glass three times, a slide about twelve times. If the film takes on a brownish discoloration, most marked about the edges, it has been scorched and is worthless. Smears can also be fixed by flaming with alcohol, as described for blood-films (see p. 265).

(4) Apply the stain for the necessary length of time, generally one-quarter to one minute.

(5) Wash in water.

(6) Dry by waving high above a flame or by blotting with filter-paper.

(7) Mount by pressing the cover, film side down, upon a drop of Canada balsam on a slide. Slides may be examined with the oil-immersion lens without a cover-glass.

Simple Bacterial Stains.—A simple solution of any basic anilin dye (methylene-blue, basic fuchsin, gentian violet, etc.) will stain nearly all bacteria. These simple solutions are not much used in the clinical laboratory, because other stains, such as Löffler's methylene-blue and

Pappenheim's pyronin-methyl-green stain, which serve the purpose even better, are at hand.

Pappenheim's Pyronin-methyl-green Stain.—This solution colors bacteria red and nuclei of cells blue. It is, therefore, especially useful for intracellular bacteria like the gonococcus and the influenza bacillus. It is a good stain for routine purposes, and is a most excellent contrast stain for Gram's method. It colors the cytoplasm of lymphocytes bright red, and has been used as a differential stain for these cells. The solution is applied cold for one-half to five minutes. It consists of saturated aqueous solution methyl-green, 3 to 4 parts, and saturated aqueous solution pyronin, 1 to $1\frac{1}{2}$ parts. It is a good plan to keep these solutions in stock and to mix a new lot of the staining fluid about once a month. If it stains too deeply with either dye, the proper balance is attained by adding a little of the other.

Carbol Thionin.—Saturated solution thionin in 50 per cent. alcohol, 20 c.c.; 2 per cent. aqueous solution phenol, 100 c.c.

This stain is especially useful in counting bacteria for standardization of vaccines (see p. 483). It can be used as a general stain. In blood work it is used for the malarial parasite and for demonstration of basophilic degeneration of the red cells. The fluid is applied for one-half to three minutes, after fixation by heat, or about a minute in saturated aqueous solution of mercuric chlorid or 1 per cent. formalin in alcohol.

Gram's Method.—This is a very useful aid in differentiating certain bacteria and should be frequently resorted to. It depends upon the fact that when treated successively with gentian-violet and iodin, certain

bacteria (owing to formation of insoluble compounds) retain the stain when subsequently treated with alcohol, whereas others quickly lose it. The former are called *Gram-positive;* the latter, *Gram-negative.* In order to render Gram-negative organisms visible, some contrasting counterstain is commonly applied, but this is not a part of Gram's method proper.

(1) Make smears, dry, and fix by heat.

(2) Apply anilin-gentian-violet or formalin-gentian-violet (see p. 74) two to five minutes.

(3) Wash with water.

(4) Apply Gram's iodin solution one-half to two minutes.

(5) Wash in alcohol until the purple color ceases to come off. This is conveniently done in a watch-glass. The preparation is placed in the alcohol, face downward, and one edge is raised and lowered with a needle. As long as any color is coming off, purple streaks will be seen diffusing into the alcohol where the surface of the fluid meets the smear. If forceps be used, beware of stain which may have dried upon them. The thinner portions of smears from pus should be practically colorless at this stage. If the smears resist decolorization the gentian-violet and iodin solution should be applied for a shorter time, say, one-half minute each.

(6) Apply a contrast stain for one-half to one minute. The stains commonly used for this purpose are an aqueous or alcoholic solution of Bismarck brown and a weak solution of fuchsin. In the writer's experience, Pappenheim's pyronin-methyl-green mixture is much more satisfactory; it brings out Gram-negative bacteria more sharply, and is especially desirable for intracellular Gram-negative organisms like the gonococcus and influenza bacillus, since the bacteria are bright red and nuclei of cells blue.

(7) Wash in water, dry, and mount in balsam.

The more important bacteria react to this staining method as follows:

Gram Staining (Deep purple).	Gram Decolorizing (Colorless unless a counterstain is used).
Staphylococcus.	Gonococcus.
Streptococcus.	Meningococcus.
Pneumococcus.	Micrococcus catarrhalis.
Bacillus diphtheriæ.	Bacillus of influenza.
Bacillus tuberculosis.	Typhoid bacillus.
Bacillus of anthrax.	Bacillus coli communis.
Bacillus of tetanus.	Spirillum of Asiatic cholera.
Bacillus aërogenes capsulatus.	Bacillus pyocyaneus.
	Bacillus of Friedländer.
	Koch-Weeks bacillus.
	Bacillus of Morax-Axenfeld.

Möller's Method for Spores.—Bodies of bacteria are blue, spores are red.

(1) Make thin smears, dry, and fix.
(2) Wash in chloroform for two minutes.
(3) Wash in water.
(4) Apply 5 per cent. solution of chromic acid one-half to two minutes.
(5) Wash in water.
(6) Apply carbolfuchsin and heat to boiling.
(7) Decolorize in 5 per cent. solution of sulphuric acid.
(8) Wash in water.
(9) Apply 1 per cent. aqueous solution of methylene-blue one-half minute.
(10) Wash in water, dry, and mount.

Huntoon's Method for Spores.—This is a new method which is simple and appears to be very reliable. Spores are deep red, bodies of bacteria are blue.

(1) Make a rather thick smear, dry, and fix in the usual way.

(2) Apply as much of the stain as will remain on the cover-glass, and steam over a flame for one minute, replacing the stain lost by evaporation.

(3) Wash in water. The film is bright red.

(4) Dip the preparation a few times into a weak solution of sodium carbonate (7 or 8 drops of saturated solution in a glass of water). Too long application of the carbonate will cause the spores to be blue.

(5) The instant the film turns blue, rinse well in water.

(6) Dry, mount, and examine.

Preparation of Stain.—

(1) Acid fuchsin (Grübler) ..... 4 gm.;
Aqueous solution acetic acid (2 per cent.) .... 50 c.c.

(2) Methylene-blue (Grübler) ..... 2 gm.;
Aqueous solution acetic acid (2 per cent.) ..... 50 c.c.

Mix the two solutions, let stand for fifteen minutes, and filter off the voluminous precipitate through moistened filter-paper. The filtrate is the staining fluid. It keeps several weeks, but requires filtration when a precipitate forms.

Löffler's Method for Flagella.—The methods for flagella are applicable only to cultures. Enough of the growth from an agar-culture (which should not be more than eighteen to twenty-four hours old) to produce faint cloudiness is added to distilled water. A small drop of this is placed on a cover-glass, spread by tilting, and dried quickly. The covers must be absolutely free from grease. To insure this, they may be warmed in concentrated sulphuric acid, washed in water, and kept in a mixture

of alcohol and strong ammonia. When used they are dried upon a fat-free cloth.

(1) Fix by heating the cover over a flame while holding in the fingers.

(2) Cover with freshly filtered mordant and gently warm for about a minute.

The mordant consists of:

Aqueous solution of tannic acid (20 per cent.)	10 c.c.;
Saturated solution ferrous sulphate, cold	5 "
Saturated aqueous or alcoholic solution gentian-violet	1 "

(3) Wash in water.

(4) Apply freshly filtered anilin-gentian-violet, warming gently for one-half to one minute.

(5) Wash in water, dry, and mount in balsam.

VI. METHODS OF STUDYING BACTERIA

The purpose of bacteriologic examinations is to determine whether bacteria are present or not, and, if present, their species and comparative numbers. In general, this is accomplished by: 1, Direct microscopic examination; 2, Cultural methods; 3, Animal inoculation.

1. Direct Microscopic Examination.—Every bacteriologic examination should begin with a microscopic study of smears from the pathologic material, stained with a general stain, by Gram's method, and often by the method for the tubercle bacillus. This yields a great deal of information to the experienced worker, and in many cases is all that is necessary for the purpose in view. It will at least give a general idea of what is to be expected, and may determine future procedure.

2. Cultural Methods.—(1) **Collection of Material.**—Material for examination must be collected under absolutely aseptic conditions. It may be obtained with a platinum wire—which has been heated to redness just previously and allowed to cool—or with a swab of sterile cotton on a stiff wire or wooden applicator. Such swabs may be placed in cotton-stoppered test-tubes, sterilized, and kept on hand ready for use. Fluids which contain very few bacteria, and hence require that a considerable quantity be used, may be collected in a sterile hypodermic syringe or one of the pipets described on p. 456. The method of obtaining blood for cultures is given on p. 293.

(2) **Inoculating Media.**—The material is thoroughly distributed over the surface of some solid medium, solidified blood-serum being probably the best for routine work. When previous examination of smears has shown that many bacteria are to be expected, a second tube should be inoculated from the first, and a third from the second, so as to obtain isolated colonies in at least one of the tubes. The platinum wire must be heated to redness *before* and *after* each inoculation. When only a few organisms of a single species are expected, as is the case in blood-cultures, a considerable quantity of the material is mixed with a fluid medium.

(3) **Incubation.**—Cultures are placed in an incubator which maintains a uniform temperature, usually of 37.5° C., for eighteen to twenty-four hours, and the growth, if any, is studied as described later. Gelatin will melt with this degree of heat, and must be incubated at about room-temperature.

(4) **Study of Cultures.**—When the original culture

contains more than one species, they must be separated, or obtained in "pure culture," before they can be studied satisfactorily. To accomplish this it is necessary to so distribute them on solid media that they form separate colonies, and to inoculate fresh tubes from the individual colonies. In routine work the organisms can be sufficiently distributed by drawing the infected wire over the surface of the medium in a series of streaks. If a sufficient number of streaks be made, some of them are sure to show isolated colonies. Another method of obtaining isolated colonies is to inoculate the water of condensation of a series of tubes, the first from the second, the second from the third, etc., and, by tilting, to flow the water once over the surface of the medium. One or more of these tubes will be almost sure to show nicely separated colonies.

In order to determine the species to which an organism belongs it is necessary to consider some or all of the following points:

(1) Naked-eye and microscopic appearance of the colonies on various media.

(2) Comparative luxuriance of growth upon various media. The influenza bacillus, for example, can be grown upon media containing hemoglobin, but not on the ordinary media.

(3) Morphology, special staining reactions, and the presence or absence of spores, flagella, and capsules. Staining methods for these purposes have been given.

(4) Motility. This is determined by observing the living organism with an oil-immersion lens in a hanging-drop preparation, made as follows: A small drop of a bouillon culture or of water of condensation from an

agar or blood-serum tube is placed upon the center of a cover-glass; and over this is pressed the concavity of a "hollow-ground slide" previously ringed with vaselin. The slide is then turned over so as to bring the cover-glass on top. In focusing, the edge of the drop should be brought into the field. Great care must be exercised not to break the cover by pushing the objective against it.

It is not always easy to determine whether an organism is or is not motile, since the motion of currents and the Brownian motion which affects all particles in suspension are sometimes very deceptive.

(5) Production of chemical changes in the media. Among these are coagulation of milk; production of acid in milk and various sugar media to which li mus has been added to detect the change; production of gas in sugar media, the bacteria being grown in fermentation tubes similar to those used for sugar tests in urine; and production of indol.

(6) Ability to grow without oxygen. For anaërobic methods, the reader is referred to larger works.

(7) Effects produced when inoculated into animals.

3. Animal Inoculation.—In clinical work this is resorted to chiefly to detect the tubercle bacillus. The method is described on p. 432.

For the study of bacteria in cultures, a small amount of a pure culture is injected subcutaneously or into the peritoneal cavity. The animals most used are guinea-pigs, rabbits, and mice. For intravenous injection the rabbit is used because of the easily accessible marginal vein of the ear.

VII. CHARACTERISTICS OF SPECIAL BACTERIA

Owing to the great number of bacterial species, most of which have not been adequately studied, positive identification of an unknown organism is often a very difficult problem. Fortunately, however, only a few are commonly encountered in routine work, and identification of these with comparative certainty presents no great difficulty. Their more distinctive characteristics are outlined in this section.

1. **Staphylococcus pyogenes aureus.**—The morphology and staining reactions (described on p. 420) and the appearance of the colonies are sufficient for diagnosis. Colonies on solidified blood-serum and agar are rounded, slightly elevated, smooth and shining, and vary in color from light yellow to deep orange. Young colonies are sometimes white.

2. **Staphylococcus pyogenes albus.**—This is similar to the above, but colonies are white. It is generally less virulent.

3. **Staphylococcus pyogenes citreus.**—The colonies are lemon yellow; otherwise it resembles the white staphylococcus.

4. **Streptococcus pyogenes.**—The morphology and staining reactions have been described (see p. 421). The chains are best seen in the water of condensation and in bouillon cultures. The cocci are not motile. Colonies on blood-serum are minute, round, grayish, and translucent. Litmus milk is usually acidified and coagulated, although slowly. The streptococcus rarely produces acid in Hiss' serum-water-litmus-inulin medium (see p. 463).

5. **Pneumococcus.**—The only organism with which this is likely to be confused is the streptococcus. The distinction is often extremely difficult.

Detection of the pneumococcus in fresh material has been described (see p. 71). In cultures it frequently forms long chains. Capsules are not present in cultures except upon special media. They show best upon a serum medium like that described for the gonococcus, but can frequently be seen in milk. Colonies on blood-serum resemble those of the streptococcus. The pneumococcus usually promptly acidifies and coagulates milk and acidifies and coagulates Hiss' serum-water with inulin. The latter property is very helpful in diagnosis.

6. **Gonococcus.**—Its morphology and staining peculiarities are given on p. 422. These usually suffice for its identification, cultural methods being rarely undertaken. In cultures the chief diagnostic point is its failure to grow on ordinary media. To grow it the most convenient medium is made by adding ascitic or hydrocele fluid (which has been obtained under aseptic conditions) to melted agar in proportion of 1 part of serum to 3 parts of agar. The agar in tubes is melted and cooled to about 45° C.; the serum is added with a pipet and mixed by shaking; and the tubes are again cooled in a slanting position. Colonies upon this medium are minute, grayish, and translucent.

7. **Diplococcus intracellularis meningitidis.**—It grows poorly or not at all on plain agar. On Löffler's blood-serum, upon which it grows fairly well, colonies are round, colorless or hazy, flat, shining, and viscid looking. It quickly dies out.

8. **Diphtheria Bacillus.**—The diagnosis is usually made from a study of stained smears from cultures upon blood-serum. Its morphology and staining peculiarities are characteristic when grown on this medium (see p. 435). The bacilli are non-motile and Gram-positive. The colonies are round, elevated, smooth, and grayish.

9. **Typhoid and Colon Bacilli.**—These are medium-sized, motile, Gram-negative, non-spore-bearing bacilli. Upon blood-serum they form rounded, grayish, slightly elevated, viscid looking colonies, those of the colon bacillus being somewhat the larger. They do not liquefy gelatin. They represent the extremes of a large group with many intermediate types. They are distinguished as follows:

Typhoid Bacillus.	*Colon Bacillus.*
Actively motile.	Much less active.
Growth on potato usually invisible.	Growth distinctly visible as dirty gray or brownish slimy layer.
No gas produced in glucose media.	Produces gas.
Growth in litmus milk produces no change.	Litmus milk pink and coagulated.
Produces no indol in Dunham's peptone medium.	Produces indol. (For test, see p. 463.)
Agglutinates with serum from typhoid-fever patient. (Recently isolated bacilli do not agglutinate well.)	Does not agglutinate with typhoid serum.

10. **Bacillus of Influenza.**—Diagnosis will usually rest upon the morphology and staining peculiarities, described on p. 75, and upon the fact that the bacillus will not grow on ordinary media, but does grow upon hemoglobin-containing media. It can be grown upon agar-

slants which have been smeared with a drop of blood from a puncture in the finger. Before inoculation these slants should be incubated to make sure of sterility. The colonies are difficult to see without a hand lens. They are very minute, discrete, and transparent, resembling small drops of dew.

11. **Bacillus of Tuberculosis.**—The methods of identifying this important organism have been given (see pp. 63, 205). Cultivation is not resorted to in clinical work. It grows very slowly and only on certain media. It is Gram-positive and non-motile.

CHAPTER IX

PREPARATION AND USE OF VACCINES

Bacterial vaccines, sometimes called "bacterins," which within the past few years have come to play an important rôle in therapeutics, are suspensions of definite numbers of dead bacteria in normal salt solution. While in many cases, notably in gonorrhea and tuberculosis, ready prepared or "stock" vaccines are satisfactory, it is usually desirable and often imperative for best results to use vaccines which are especially prepared for each patient from bacteria which have been freshly isolated from his own lesion. These latter are called "autogenous vaccines." Only through them can one be certain of getting the exact strain of bacterium which is producing the disease.

I. PREPARATION OF VACCINE

The method of preparing autogenous vaccines which is used in the author's laboratory is here described.

1. Preparation of Materials.—A number of 2-ounce wide-mouthed bottles are cleaned and sterilized. Each is charged with 50 c.c. freshly filtered normal salt solution (0.85 per cent. sodium chlorid in distilled water), and is capped with a new rubber nursing-nipple, without holes, inverted as shown in Fig. 170. A small section of hollow wire or a hypodermic needle is thrust through the cap near the edge to serve as an air vent, and the bottle

and contents are sterilized in an autoclave. If an autoclave is not at hand, successive steamings in an Arnold sterilizer will answer, provided it is not opened between steamings. After sterilization, the pieces of wire are pulled out and the holes sealed with collodion.

Most workers use a smaller bottle with less salt solution and with a cotton stopper; and, after the solution

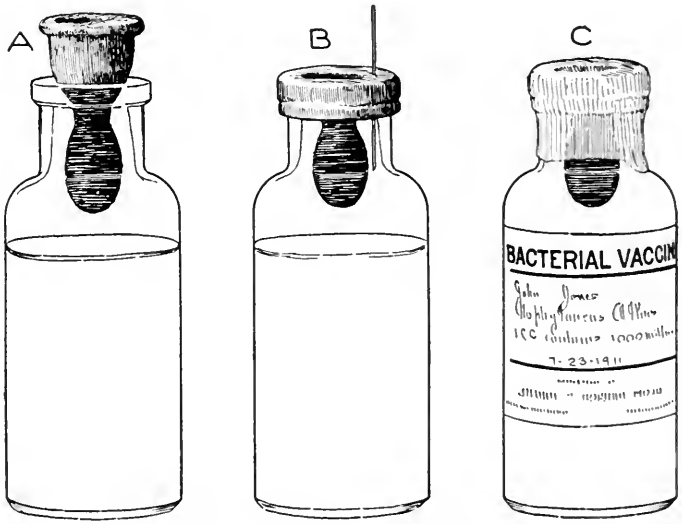


Fig. 170.—Vaccine bottles: A, Cap ready to be applied; B, ready for sterilization; C, finished vaccine.

has been sterilized, apply a specially made "vaccine bottle cap." The method which has been detailed above calls for an unnecessarily large quantity of fluid (which is no real objection), but has certain advantages: the nursing nipples are easily obtained at any pharmacy; the rubber is not put upon the stretch as is the case with some caps, and is, therefore, self-sealing; no cotton-lint

falls into the salt solution before the cap is applied; and the cap offers a concavity which may be filled with alcohol for sterilizing before the needle is plunged through.

A number of test-tubes, each charged with 10 c.c. of normal salt solution and plugged with cotton, are also prepared and sterilized.

2. Obtaining the Bacteria.—A culture on some solid medium is made from the patient's lesion, and a pure culture is obtained in the usual way. This preliminary work should be carried through as quickly as possible. If for any reason there is much delay, it is best to begin over again, the experience gained in the first trial enabling one to carry the second through more rapidly. When a pure culture is obtained, a number of tubes of blood-serum or agar—10 or 12 in the case of streptococcus or pneumococcus, 4 or 5 in the case of most other organisms—are planted and incubated over night or until a good growth is obtained.

3. Making the Suspension.—A few cubic centimeters of the salt solution from one of the 10-c.c. salt-tubes is transferred by means of a sterile pipet to each of the culture-tubes, and the growth thoroughly rubbed up with a stiff platinum wire or a glass rod whose tip is bent at right angles. The suspension from the different tubes, usually amounting to about 10 c.c., is then collected in one large tube (size about 6 by $\frac{3}{4}$ inches); and the upper part of the tube is drawn out in the flame of a blast lamp or Bunsen burner, as indicated in Fig. 171, *B*, a short section of glass tubing being fused to the rim of the tube to serve as a handle. It is then stood aside, and when cool the narrow portion is sealed off.

The resulting hermetically sealed capsule is next thoroughly shaken for ten to twenty minutes to break up all clumps of bacteria. Some small pieces of glass or a little clean sterile sand may be introduced to assist in this, but with many organisms it is not necessary.

4. Sterilization.—The capsule is placed in a water-bath at 60° C. for forty-five minutes. This can be done in

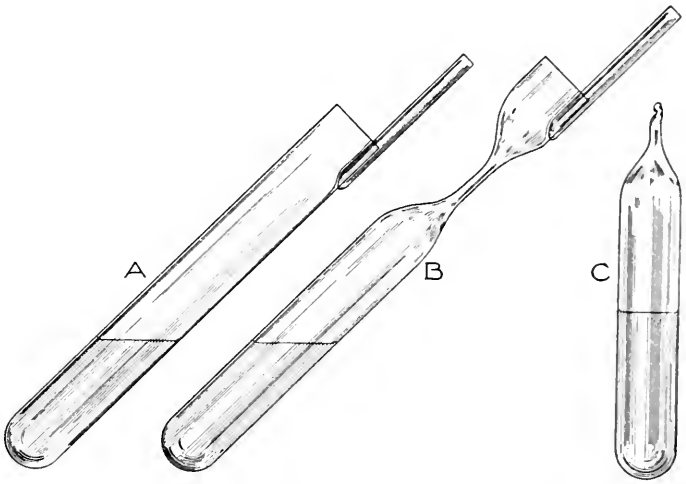


Fig. 171.—Process of making hermetically sealed capsules containing liquid.

an ordinary rice-cooker, with double lid, through which a thermometer is inserted. When both compartments are filled with water it is an easy matter to maintain a uniform temperature by occasional application of a small flame. The time and temperature are important: too little heat will fail to kill the bacteria, and too much will destroy the efficiency of the vaccine.

When sterilization is complete the capsule is opened,

a few drops are planted on agar or blood-serum, and the capsule is again sealed.

5. Counting.—When incubation of the plant has shown the suspension to be sterile it is ready for counting.

There must be ready a number of clean slides; a few drops of normal salt solution on a slide or in a watch-glass; a blood-lancet, which can be improvised from a spicule of glass or a pen; and two capillary pipets with squarely broken off tips and grease-pencil marks about 2 cm. from the tip (Fig. 172). These are easily made by drawing out a piece of glass tubing, as described on page 456.

It is necessary to work quickly. After thorough shaking, the capsule is opened and a few drops forced out upon a slide. Any remaining clumps of bacteria are broken up with one of the pipets by holding it against and at right angles to the slide, and alternately sucking the fluid in and forcing it out. The pipet is most easily controlled if held in the whole hand with the rubber bulb between the thumb and the side of the index-finger. A finger is then pricked until a drop of blood appears; and into the second pipet are quickly drawn successively:

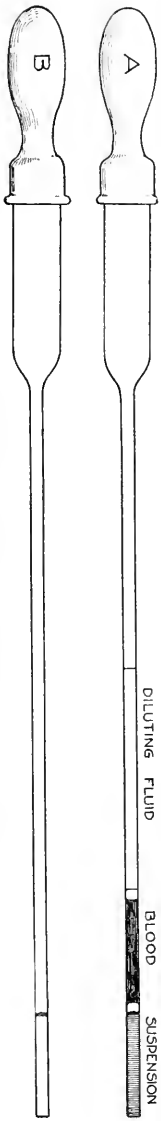


Fig. 172.—Capillary pipets: *A*, Filled for counting a vaccine by Wright's method; *B*, empty, showing grease-pencil mark. The slender portion should be narrower than here represented.

1 or 2 volumes normal salt solution (or, better, a 1 per cent. solution of sodium citrate which prevents coagulation); a small bubble of air; 1 volume of blood; a small bubble of air; and, finally, 1 volume of bacterial suspension. (A "volume" is measured by the distance from the tip of the pipet to the grease-pencil mark.) The contents of the pipet are then forced out upon a slide and thoroughly mixed by sucking in and out, care being taken to avoid bubbles; after which the fluid is distributed to a number of slides and spread as in making blood-smears.

The films are stained with Wright's blood-stain or, better, by a few minutes' application of carbol-thionin, after fixing for a minute in saturated mercuric chlorid solution. With an oil-immersion lens both the red cells and the bacteria in a number of microscopic fields are counted. The exact number is not important; for convenience 500 red cells may be counted. From the ratio between the number of bacteria and of red cells, it is easy to calculate the number of bacteria in 1 c.c. of the suspension, it being known that there are 5000 million red corpuscles in a cubic centimeter of normal human blood. If there were twice as many bacteria as red corpuscles in the fields counted, the suspension would contain 10,000 million bacteria per cubic centimeter.

The count can also be made with the hemacytometer, using a weak carbolfuchsin or gentian-violet, *freshly filtered*, as diluting fluid. Callison recommends the following diluting fluid:

Hydrochloric acid ..... 2 c.c.;
Mercuric chlorid (0.2 per cent. solution)..... 100 "
Acid fuchsin (1 per cent. aqueous solution), to color.

The color should be just deep enough not to obscure the ruled lines.

A very thin cover-glass must be used; and, after filling, the counting-chamber must be set aside for an hour or more to allow the bacteria to settle. Mallory and Wright advise the use of the shallow chamber manufactured by Zeiss for counting blood-plates, but many 2-mm. oil-immersion objectives have sufficient working distance to allow the use of the regular counting-chamber, provided a very thin cover is used.

6. Diluting.—The amount of the suspension which, when diluted to 50 c.c., will give the strength desired for the finished vaccine having been determined, this amount of salt solution is withdrawn with a hypodermic syringe from one of the bottles already prepared, and is replaced with an equal amount of suspension. One-tenth c.c. of trikresol or lysol is finally added and the vaccine is ready for use. To prevent possible leakage about the cap, the neck of the bottle is dipped in melted paraffin. The usual strengths are: staphylococcus, 1000 million in 1 c.c.; most other bacteria, 100 million in 1 c.c.

II. METHOD OF USE

Vaccines are administered subcutaneously, usually in the arm or abdominal wall or between the shoulder-blades. The technic is the same as for an ordinary hypodermic injection. The syringe is usually sterilized by boiling. The site of the injection may be mopped with alcohol or may be touched with a pledget of cotton saturated with tincture of iodin or liquor cresolis compositus. The rubber cap of the container is sterilized by filling the concavity with alcohol for some minutes, usually while the syringe is being sterilized, or simply placing a drop of liquor cresolis compositus upon it.

The bottle is then inverted and well shaken, when the needle is plunged through the rubber and the desired quantity withdrawn. The hole seals itself. The most satisfactory syringe is the comparatively inexpensive "Sub-Q Tuberculin" syringe graduated in hundredths of a cubic centimeter.

III. DOSAGE

Owing to variations, both in virulence of organisms and susceptibility of patients, no definite dosage can be assigned. Each case is a separate problem. Wright's original proposal was to regulate the size and frequency of dose by its effect upon the opsonic index, but this is beyond the reach of the practitioner. The more widely used "clinical method" consists in beginning with a very small dose and cautiously increasing until the patient shows either improvement or some sign of a "reaction," indicated by headache, malaise, fever, exacerbation of local disease, or inflammatory reaction at the site of injection. The reaction indicates that the dose has been too large. The beginning dose of staphylococcus is about 50 million; the maximum, 1000 million or more. Of most other organisms the beginning dose is 5 million to 10 million; maximum, about 100 million. Ordinarily, injections are given once or twice a week; very small doses may be given every other day.

IV. THERAPEUTIC INDICATIONS

The therapeutic effect of vaccines depends upon their power to produce active immunity: they stimulate the production of opsonins and other antibacterial substances which enable the body to combat the infecting

bacteria. Their especial field is the treatment of subacute and chronic localized infections, in some of which they offer the most effective means of treatment at our command. In most chronic infections the circulation of blood and lymph through the diseased area is very sluggish, so that the antibodies, when formed, cannot readily reach the seat of disease. Ordinary measures which favor circulation in the diseased part should, therefore, accompany the vaccine treatment. Among these may be mentioned incisions and drainage of abscesses, dry cupping, application of heat, Bier's hyperemia, etc., but such measures should not be applied during the twenty-four hours succeeding an injection, since the first effect of the vaccine may be a temporary lowering of resistance. Vaccines are of little value, and, in general, are contraindicated in very acute infections, particularly in those which are accompanied by much systemic intoxication, for in such cases the power of the tissues to produce antibodies is already taxed to the limit. It is true, nevertheless, that remarkably beneficial results have occasionally followed their use in such acute conditions as malignant endocarditis, but here they should be tried with extreme caution.

Probably best results are obtained in staphylococcus infections, although pneumococcus, streptococcus, and colon bacillus infections usually respond nicely. Among clinical conditions which have been treated successfully with vaccines are furunculosis, acne vulgaris, infected operation-wounds, pyelitis, cystitis, subacute otitis media, osteomyelitis, infections of nasal accessory sinuses, etc. Vaccine treatment of the mixed infection is doubtless an important aid in tuberculosis therapy,

and in some cases the result is brilliant. When, as is common, several organisms are present in the sputum, a vaccine is made from each, excepting the tubercle bacillus, of which autogeneous vaccines are not used in practice. To avoid the delay and consequent loss of virulence entailed by study and isolation of the several varieties, many workers make the bacterial suspension directly from the primary cultures. The resulting vaccines contain all strains which are present in the sputum in approximately the same relative numbers. Although open to criticism from a scientific standpoint, this method offers decided practical advantages in many cases.

It has been shown that vaccines are useful in preventing as well as curing infections. Their value has been especially demonstrated in typhoid fever. Three or four doses of about 100 million, 200 million, 400 million, and 600 million typhoid bacilli are given about five to seven days apart. Results in the army, where the plan has been tried on a large scale, show that such vaccination is effective.

V. TUBERCULINS

Tuberculins contain the products of tubercle bacilli or their ground-up bodies, the latter class being practically vaccines. They are undoubtedly of great value in the treatment of localized tuberculosis, particularly of bones, joints, and glands; and are of rather indefinite though certainly real value in chronic pulmonary tuberculosis, especially when the disease is quiescent. The best known are Koch's old tuberculin (T. O.), bouillon filtrate (B. F.), triturate residue (T. R.), and bacillary emulsion (B. E.). There seems to be little difference in

the actions of these, although theoretically T. R. should immunize against the bacillus and B. F. against its toxic products. The choice of tuberculin is much less important than the method of administration. The making of autogenous tuberculins is impracticable, hence stock preparations are used in practice.

Since the dose is exceedingly minute, the tuberculin as purchased must be greatly diluted before it is available for use. A convenient plan is to use the rubber-capped bottles of sterile salt solution described for vaccines (see p. 479), adding sufficient tuberculin to give the desired strength, together with 0.1 c.c. trikresol to insure sterility. The practitioner should bear in mind that while tuberculin is capable of good, it is also capable of great harm. Everything depends upon the dosage and plan of treatment. Probably a safe beginning dose for a pulmonary case is 0.00001 milligram of B. E., B. F., or T. R.; for gland and bone cases, about 0.0001 milligram. O. T. is now used chiefly in diagnosis. The intervals are about one week or, rarely, three days, when very small doses are given. The dose is increased steadily, but with *extreme caution;* and should be diminished or temporarily omitted at the first indication of a "reaction," of which, in general, there are three forms:

(*a*) *General.*—Elevation of temperature (often slight), headache, malaise.

(*b*) *Local.*—Increase of local symptoms, amount of sputum, etc.

(*c*) *Stick.*—Inflammatory reaction at site of injection.

Treatment is usually continued until a maximum dose of 1 milligram is reached, the course extending over a year or more.

VI. TUBERCULIN IN DIAGNOSIS

The tissues of a tuberculous person are sensitized toward tuberculin, and a reaction (see preceding section) occurs when any but the most minute quantity of tuberculin is introduced into the body. Non-tuberculous persons exhibit no such reaction. This is utilized in the diagnosis of obscure forms of tuberculosis, the test being applied in a number of ways:

1. **Hypodermic Injection.**—Koch's old tuberculin is used in successive doses, several days apart, of 0.001, 0.01, and 0.1 mg. A negative result with the largest amount is considered final. The reaction is manifested by fever within eight to twenty hours after the injection. The method involves some danger of lighting up a latent process, and has been largely displaced by safer methods.

2. **Calmette's Ophthalmo-reaction.**—One or two drops of 0.5 per cent. purified old tuberculin are instilled into one eye. Tuberculin ready prepared for this purpose is on the market. If tuberculosis exists anywhere in the body, a conjunctivitis is induced within twelve to twenty-four hours. This generally subsides within a few days. The method is not without some, though slight, risk of injury to the eye; and the test is absolutely contraindicated in the presence of any form of ocular disease. A second instillation should not be tried in the same eye.

3. **Moro Reaction.**—A 50 per cent. ointment of old tuberculin in lanolin is rubbed into the skin of the abdomen, a piece about the size of a pea being required. Dermatitis, which appears in twenty-four to forty-eight hours, indicates a positive reaction. The ointment can be purchased ready for use.

4. **Von Pirquet's Method.**—This is the most satis-

factory of the tuberculin tests. Two small drops of old tuberculin are placed on the skin of the front of the forearm, about 2 inches apart, and the skin is slightly scarified, first at a point midway between them, and then through each of the drops. A convenient scarifier is a piece of heavy platinum wire, the end of which is hammered to a chisel edge. A wooden tooth-pick with a chisel-shaped end is also convenient. This is held at right angles to the skin, and rotated six to twelve times with just sufficient pressure to remove the epidermis without drawing blood. In about ten minutes the excess of tuberculin is gently wiped away with cotton. No bandage is necessary. A positive reaction is shown by the appearance in twenty-four to forty-eight hours of a papule with red areola, which contrasts markedly with the small red spot left by the control scarification.

These tests have very great diagnostic value in children, especially those under three years of age, but are often misleading in adults, positive reactions occurring in many apparently healthy individuals. Negative tests are very helpful in deciding against the existence of tuberculosis.

VII. CUTANEOUS TEST FOR SYPHILIS

Noguchi has prepared a substance called *luetin*, which produces a cutaneous reaction in syphilis similar to the tuberculin skin reaction in tuberculosis. Luetin consists of ground cultures of *Treponema pallidum* sterilized and preserved with trikresol.

A small drop (0.05 c.c.) of luetin is injected into the skin (not under it) of one arm. A similar preparation

without the treponema is injected into the skin of the other arm as a control. A positive reaction usually begins within forty-eight hours and consists of an inflammatory induration, papule, or pustule. It is sometimes delayed three or even four weeks.

The test is specific for syphilis. It is present in late secondary, tertiary, latent, and hereditary syphilis, but is usually absent in primary and untreated secondary cases. In general paralysis and tabes dorsalis it is inconstant.

Compared with the Wassermann reaction it is more constant in tertiary and latent syphilis, while the Wassermann is more constant in primary and secondary cases. Unlike the Wassermann, the reaction does not disappear with treatment, but persists probably until a complete cure is effected.

CHAPTER X

SERODIAGNOSTIC METHODS[1]

I. IMMUNITY

With two exceptions the diagnostic methods here described depend on one or another law of immunity. These laws are customarily described in terms of Ehrlich's side-chain theory. It is not practicable to undertake a detailed discussion of the theory here, and I shall, accordingly, confine myself to such discussion and definition of the bodies concerned as will enable the reader to undertake the reactions himself with a reasonably intelligent conception of their mechanism.

Acquired immunity, that form of immunity resulting from an attack of a given disease, depends upon the formation within the body, under the influence of the disease-producing agent, or "antigen," of bodies possessing the power to neutralize the poisons produced by the antigen, or to destroy or otherwise affect the antigen itself. Since the action of these bodies is specific (*i. e.*, they act only on the particular antigen whose presence has led to their production), the search for them may be resorted to for diagnostic purposes whenever they can be found more easily than can the antigen itself. With certain exceptions, to be noted later, the presence of one or other of these bodies may be regarded as pathognomonic of the corresponding disease.

[1] By Ross C. Whitman, B.A., M.D., Professor of Surgical Pathology and Serology in the University of Colorado.

The several "immune bodies" act by means of different mechanisms, by virtue of which they may be classified in three groups—the three orders of receptors of Ehrlich's side-chain theory. With the first group we are not immediately concerned.

1. *Receptors of the First Order.*—These are receptors which serve simply as connecting links between the disease-producing agent (or, rather, of its toxin) and the tissues. Under the influence of the antigen they are produced in excess, and are finally set free in the circulation. Here they seize upon and, so to speak, saturate the free valence of the antigen, while it also is still free in the blood and lymph, in such a way as to leave the latter no chemical affinities by means of which it may combine with similar bodies still in relation with the cells. The antigen is thus rendered inert. This order of receptors includes only the antitoxins; for example, those of diphtheria and tetanus.

2. *Receptors of the Second Order.*—These have a combining group similar to that of the first order, and, in addition, a group possessing a ferment-like action, by means of which the characteristic action of the body is effected. The ferment, or zymophore, group is readily destroyed by heat, so that serum to be used for any of the purposes included in the group must not be heated. The group includes the agglutinins, responsible for the several applications of the Widal reaction; the precipitins, responsible for one of the biologic methods to be described later for the forensic identification of bloodstains; and the opsonins.

3. *Receptors of the Third Order.*—These bodies consist of two combining affinities only. One of these com-

bines with suitable analogous groups of the antigen, the other combines with a substance which is called complement because it "complements" or supplements or completes the specific action of the immune substance. Complement is normally present in the blood, but is unable to act upon the antigen without the mediation and aid of the immune body. The latter is, therefore, called the amboceptor or 'tween body. It is (relatively) thermostabile and keeps practically indefinitely under suitable conditions. It is to be remembered that this is the specific immune substance whose presence or absence is indicative of the presence or absence of the corresponding disease. The native, normally present complement is (relatively) thermolabile, being destroyed in a few minutes by a temperature of 54° to 56° C., and keeps only a few hours under the best conditions. It is non-specific, and within certain limits the complement of one species may be substituted for that of another. Thus, in the Wassermann reaction, complement containing fresh serum from guinea-pigs is usually substituted for the normally present complement of the patient's serum, after the latter has been destroyed.

This group contains the lysins and the bodies responsible for the various applications of the complement-deviation method to the diagnosis of syphilis (Wassermann reaction), gonorrhea, cancer, typhoid fever, forensic identification of blood, etc.

II. APPARATUS

Before the description of the several tests is taken up, I shall give, to save space, a list of general equipment needed for such work. Special apparatus needed for

some of the tests will be mentioned in connection with these.

1. *Centrifuge.*—While the usual small electric or water-driven instrument can be employed, a larger machine, capable of holding 4 or 8 tubes of about 50-c.c. capacity, is desirable.

2. *Scales*, about 0.1 to 100 gm. capacity.

3. *Microscope*, magnifying 50 to 750 diameters.

4. *Incubator* at 37° C.

5. *Water-bath*, to be regulated as required.

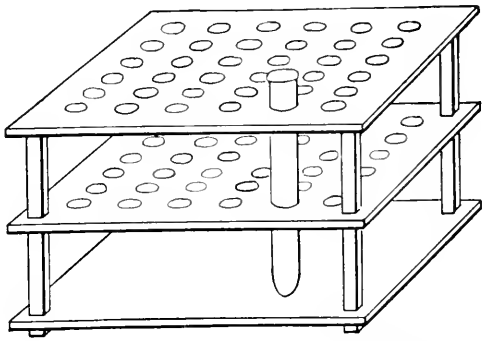


Fig. 173.—Convenient test-tube rack for serum work.

6. A large number of *test-tubes*, about $\frac{1}{2}$ x 5 inches.

7. *Test-tube racks* to accommodate the above. A double row of holes is very convenient. Still better is a special rack (Fig. 173), made of copper or zinc, with six rows of holes, six to each row. A sheet of metal midway between top and bottom contains holes to correspond, so that tubes are held without danger of tipping. The rack holds tubes enough for 18 Wassermann reactions, if but one antigen is used. A similar rack, made round, and with the two lower sheets of metal

small enough to go through the circular opening of the water-bath, while the top sheet is larger, so as to rest on the edge of the opening, is also very convenient.

8. *Volumetric pipets*, 0.1 c.c. in one-one-hundredths, and 10 c.c. in one-tenths. The graduation should start near the point where the emptying of the pipet is stopped by capillarity.

9. *Capillary pipets* (see Fig. 172), made from soft-glass tubing, as described on page 456. The tube should be of such a size that the ordinary medicine-dropper nipple will fit it snugly. Such pipets are useful for a variety of purposes. After being used once they should be thrown away.

10. *Glass Capsules.*—These may be purchased or, with a little practice, can be readily prepared from the same sort of tubing by drawing out a piece at both ends, and sealing in the flame. If desired, one end may be bent over to form a hook at the point where the narrowing begins (see Fig. 169).

11. An *all-glass syringe*, such as the Luer, about 5-c.c. capacity, with a fairly large needle, say 19 or 20 gage, preferably of platinum.

III. REACTIONS BASED UPON IMMUNE BODIES OF THE SECOND ORDER

A. The Widal Reaction

The test may be employed for the diagnosis of a variety of infections, *e. g.*, typhoid, paratyphoid, bacillary dysentery, the plague, Asiatic cholera, epidemic meningitis, etc. In clinical work it is used only for the diagnosis of typhoid and paratyphoid infections.

1. **Materials Required.**—The following especial equipment is needed:

(1) A *homogeneous suspension* of the *organism* or *organisms* suspected of causing the disease. Such suspensions may be purchased from the manufacturers of biologic preparations, or may be prepared by the worker himself. In the latter case twenty-four-hour-old agar-slant cultures (preferably attenuated by long-continued growth on culture-media) should be washed off with

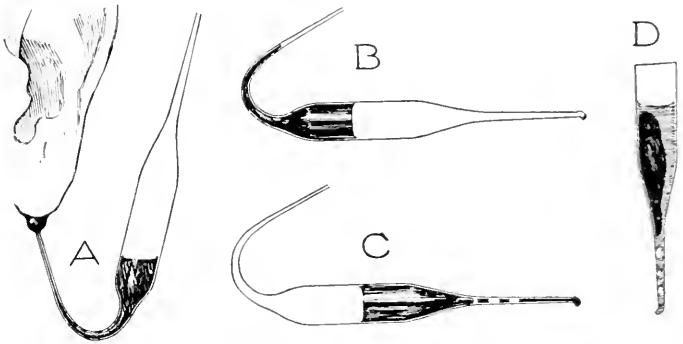


Fig. 174.—Method of obtaining blood in a Wright capsule: A, Filling the capsule; B, the bulb has been warmed and the capillary end sealed in the flame; C, cooling of the capsule has drawn the blood to the sealed end; D, the serum has separated, and the top of the capsule has been broken off.

normal salt solution (0.85–0.9 per cent. sodium chlorid), containing either 0.5 per cent. phenol or 0.1 per cent. formalin, and shaken until the suspension is as uniform as possible. Dilute by adding more of the salt solution until the suspension is but slightly milky, and preserve in the ice-box. Such a suspension will keep for months. Shake thoroughly before using. The suspension will settle less rapidly if 10 per cent. lactose is added to it. Suspensions which show any tendency to spontaneous agglutination cannot, of course, be used.

(2) Instead of the suspension of killed bacteria, *living young cultures* (not over twenty-four hours old) of attenuated organisms can be employed.

(3) About 0.1 c.c. of the *patient's serum*. This may be obtained by pricking the cleansed finger or ear rather deeply and collecting the blood in one of the capsules above mentioned, as is indicated in Fig. 174. More than one capsule should be at hand, so that a fresh one may be substituted if the first is plugged by fibrin before enough blood is obtained.

When the capsules are not at hand, blood may be obtained in little vials such as may be made by breaking off the lower $\frac{1}{2}$ inch of the tubes which have contained peptonizing powder. Vials in which hypodermic tablets are sold can be used, but are somewhat too narrow. They must, of course, be well cleaned. One of these is filled to a depth of about $\frac{1}{4}$ inch from a puncture in the ear, and is then set aside for a few hours. When the clot has separated it is picked out with a needle, leaving the serum.

Sufficient blood may also be collected by allowing drops to dry on glass or unglazed paper (without heating), to be afterward macerated in water. In this case, however, dilutions can only be made approximately.

(4) *Slides*, preferably hollow ground, *cover-glasses*, *vaselin*.

2. **Methods.**—Two methods of performing the test will be described:

(1) **Macroscopic Method.**—Separate the clot and serum in the capsule by centrifugation, nick the wall of the capsule a short distance above the serum with a file, and break the capsule at this point. Pipet off the serum, placing in a clean test-tube, and add 9 volumes of salt

solution. Counting the drops of serum as they fall from the capillary pipet, and adding nine times the number of drops of salt solution will give sufficiently accurate dilution. Now place a number of very small test-tubes in a rack, and add to each one *except the first* 0.5 c.c. of salt solution by means of a volumetric pipet. Then place in the first and second tubes *only* 0.5 c.c. of the diluted blood-serum. Shake the second tube, and with the pipet transfer 0.5 c.c. to the third tube. Shake this tube and transfer 0.5 c.c. to the fourth tube, and so on, to the end. Discard 0.5 c.c. from the last tube. One tube, to serve as control, should contain only 0.5 c.c. of salt solution, without any serum. The volumetric pipet should be thoroughly rinsed out with salt solution after each transfer. One thus arrives at a series of dilutions of the serum, as follows: 1–10, 1–20, 1–40, 1–80, 1–160, 1–320, 1–640, etc. Now add to each tube a like amount (0.3 to 0.5 c.c.) of the suspension of killed bacteria. This doubles the dilution of the serum in each of the tubes. Mix all the tubes thoroughly by shaking, and place the rack in a moderately warm place or in the incubator for eight to twelve hours. In those tubes in which the reaction is positive there will be found a sediment consisting of agglutinated bacteria at the bottom of the test-tube, with a clear supernatant fluid. The control tube and the negative tubes will be cloudy and without sediment.

Dead cultures of typhoid bacilli, together with all apparatus necessary for performing the macroscopic test, are put up at moderate cost by various firms under the names of typhoid diagnosticum, typhoid agglutometer, etc. Full directions accompany these outfits.

Recently Bass and Watkins have described a modification of the macroscopic method (using very concentrated suspensions of the bacilli) by which the test can be applied at the bedside. Agglutination occurs within a few minutes. The apparatus has been put upon the market by Parke, Davis & Co.

(2) **Microscopic Method.**—Arrange a series of dilutions of the blood-serum as above, or, if dried blood is used, macerate the dried clot with salt solution or tap-water. In the latter case, unless the size of the original drop of blood is known, the color is the only guide as to the degree of dilution. A light amber color will roughly correspond to a dilution of 1–50. From such a dilution others can be prepared. On the center of each of several clean cover-glasses place a loopful of each of the several dilutions, employing a platinum loop of about 2 mm. diameter. With the same loop add to each droplet of diluted serum a loop from a twelve- to twenty-four-hour-old bouillon culture of the organism in question, or of a suspension in salt solution prepared from a young agar-slant culture. This doubles the dilution of serum in each case. One cover-glass containing no serum should be prepared to serve as a control. Press over each cover-glass a hollow-ground slide previously ringed with vaselin. Turn the slide over so as to bring the cover-glass on top. Drying is prevented and the cover-glass held in place by the vaselin.

When hollow-ground slides are not at hand, a drop each of the diluted serum and the bacterial suspension may be placed in the center of a heavy ring of vaselin on an ordinary slide and a cover-glass applied to this. Vaselin containing an antiseptic must not, of course, be used for this purpose.

Place the slides in a moderately warm place or in the incubator at 37° C. for two hours. Examine under the oil-immersion lens or, better, the high-power dry lens of the microscope, *using very subdued light.* Yellow (artificial) light gives a clearer view than does white light. In the negative slides and in the control the organism will be found moving freely (if a motile species) and not clumped; while in the positive slides the organisms are found motionless and gathered in tangled

Fig. 175.—Showing clumping of typhoid bacilli in the Widal reaction. At one point a crenated red blood-corpuscle is seen (Wright and Brown).

masses and balls, *i. e.*, they are agglutinated (Fig. 175). Pseudoreactions, in which there are a few small clumps of organisms whose motion is not entirely lost, together with many freely moving organisms scattered throughout the field, should not mislead.

3. **Interpretation of Results.**—Experience has shown that not much significance attaches to reactions occurring in two hours with dilutions of serum less than 1–75 or 1–100. With killed organisms the dilution may

be somewhat lower than when living organisms are employed. On the other hand, recently isolated virulent cultures are, in general, more resistant to agglutination than old attenuated ones. A number of other disease conditions may give rise to a positive reaction with the typhoid bacillus, notably infections with closely related organisms, such as the colon bacillus. (In such cases, if tests are made with several species, the species agglutinated in the highest serum dilution may *generally*, but not always, be regarded as the cause of the infection.) Agglutination of typhoid bacilli may also, though rarely, occur in diseases of the liver, particularly those accompanied by jaundice, and in pneumococcus infections.

In typhoid the average time of first appearance of the reaction in the dilutions above recommended is the fourteenth to the fifteenth day of actual disease, which corresponds roughly to the eighth or tenth day of apparent disease. In doubtful cases the test should be repeated at frequent intervals, and no disappointment should be felt if, as sometimes (though rarely) happens, the reaction does not appear until the twentieth to the twenty-fifth day of the disease. It is evident, therefore, that its value for early diagnosis is much less than that of the blood-culture (see page 292). After the Widal reaction first appears it remains throughout the whole course of the disease and often persists for years.

B. Biologic Identification of Unknown Proteins

This includes the differentiation of human and animal blood, detection of meat adulteration, etc., by means of the **precipitin test** (method of Uhlenhuth).

1. **Materials Required.**—The following equipment is needed:

(1) *Blood-serum* of an animal (rabbit) highly immunized against the protein to be determined. Immunize several rabbits by several intravenous or intraperitoneal injections of, for example, human blood, or better, blood-serum. Placental blood may be used, or the blood may be obtained as for the Wassermann reaction. The doses should be 2 or 3 c.c. and should be given at four- or five-day intervals. After the fourth or fifth dose draw 2 or 3 c.c. of blood from an ear vein, separate the serum, and determine its strength as follows:

Prepare dilutions of (in this case) human blood-serum in the proportions 1–1000, 1–5000, 1–10,000, 1–20,000, etc., using physiologic salt solution as a diluent. Place 2 c.c. each of the several dilutions in a series of test-tubes. To each tube add 0.1 c.c. of the rabbit's serum, *without shaking*. A distinct cloud should appear in the lowest dilution (1–1000) within a minute or two, rapidly deepening to a heavy flocculent precipitate; the reaction develops somewhat more slowly in the higher dilutions, but no reaction is significant which occurs after more than twenty minutes.

If the titration results as above described, anesthetize the rabbit *while it is in a fasting condition*, as otherwise the serum is apt to be opalescent; remove the anterior breast wall under aseptic conditions; take out the left lung and open the heart, so as to allow the animal to bleed to death into its pleural cavity. Cover the body with sterile towels wet with antiseptic solution. After clotting has occurred, pipet the serum into sterile bottles, and add $\frac{1}{10}$ volume of 5 per cent. carbolic acid as a

preservative. If the serum is opalescent, it cannot be used; if cloudy, it must be filtered clear through a sterile Berkefeld filter. Sometimes the cloudiness can be removed by simple sedimentation. The titration above described should be repeated and verified before the serum is used for making the test proper.

Other sera immune to horse, dog, sheep, beef, fowl, etc., may, of course, be prepared in the same way.

(2) A *solution* of the *unknown substance* in physiologic salt solution. The stock dilution should be about 1–1000. If made from a dried clot this can only be approximate. The following criteria may be used:

(*a*) It should be almost completely colorless by transmitted light.

(*b*) It should give only a slight cloudiness when heated with a little nitric acid.

(*c*) It should, nevertheless, foam freely on shaking. The solution must be made perfectly clear—by filtration if necessary.

2. **Method.**—Arrange a series of test-tubes and charge them as follows:

Tube No. 1—2 c.c. of the unknown solution (diluted 1–1000) plus 0.1 c.c. of immune serum.

Tube No. 2—2 c.c. of normal salt solution plus 0.1 c.c. of immune serum.

Tube No. 3—2 c.c. of a 1–1000 dilution of known serum of the species corresponding to that suspected to be present in the unknown material plus 0.1 c.c. of immune serum.

Tube No. 4—2 c.c. of a 1–1000 dilution of a serum from a species different from that suspected to be present in the unknown material plus 0.1 c.c. of immune serum.

Tube No. 5—2 c.c. of the unknown solution alone.

When the first and third tubes give a positive reaction, as above defined, and all the others a negative

reaction, the presence of the protein of the species tested for is established. It must be remembered that *shaking must not be employed.* When only limited amounts of material are available, the test can be made by contact in capillary tubes.

Meat adulteration may be recognized by the same method. Usually it is a question of horse flesh sold as beef or as sausage. Remove about 30 gm. of the meat from the deeper portions of the specimen with a clean sterile knife, free as much as possible from fat, chop up on a clean board, and, if salted, extract several times in the course of ten minutes with sterile distilled water. Cover the 30 gm. of freshened chopped meat with about 50 c.c. of 0.85 per cent. salt solution, and allow it to stand three hours at room-temperature or over night in the ice-box. Pipet off the supernatant fluid, and clarify and dilute for use according to the criteria given above for preparing extracts of the unknown substance. If acid to litmus, it is to be neutralized before use by adding an excess of an insoluble alkali, such as magnesium oxid, and filtering.

The immune serum is prepared as above by injecting rabbits with, in this case, horse-serum. It must have a titer of at least 1–20,000. That is, it must give a reaction with the homologous serum in a dilution of the latter of that degree.

3. **Interpretation of Results.**—These reactions are very closely specific, and are fully established for forensic purposes. Doubt can only arise as between the proteins of very closely related species, and this can be practically always removed by the use of adequate controls.

C. Opsonins

That phagocytosis plays an important part in the body's resistance to bacterial invasion has long been recognized. According to Metchnikoff, this property of leukocytes resides entirely within themselves, depending upon their own vital activity. The studies of Wright and Douglas, upon the contrary, indicate that the leukocytes are impotent in themselves, and can ingest bacteria only in the presence of certain substances which exist in the blood-plasma. These substances have been named *opsonins*. Their nature is undetermined. They probably act by uniting with the bacteria, thus preparing them for ingestion by the leukocytes; but they do not cause death of the bacteria, nor produce any appreciable morphologic change. They appear to be more or less specific, a separate opsonin being necessary for phagocytosis of each species of bacteria. There are, moreover, opsonins for other formed elements—red blood-corpuscles, for example. It has been shown that the quantity of opsonins in the blood can be greatly increased by inoculation with dead bacteria.

To measure the amount of any particular opsonin in the blood Wright has devised a method which involves many ingenious and delicate technical procedures. Much skill, such as is attained only after considerable training in laboratory technic, is requisite, and there are many sources of error. It is, therefore, beyond the province of this work to recount the method in detail. In a general way it consists in: (*a*) Preparing a mixture of equal parts of the patient's blood-serum, a suspension of the specific micro-organism, and a suspension of

washed leukocytes; (*b*) preparing a similar mixture, using serum of a normal person; (*c*) incubating both mixtures for a definite length of time; and (*d*) making smears from each, staining, and examining with an oil-immersion objective. The number of bacteria which have been taken up by a definite number of leukocytes is counted, and the average number of bacteria per leukocyte is calculated; this gives the "phagocytic index." The phagocytic index of the blood under investigation, divided by that of the normal blood, gives the *opsonic index* of the former, the opsonic index of the normal blood being taken as 1. Simon regards the percentage of leukocytes which have ingested bacteria as a more accurate measurement of the amount of opsonins than the number of bacteria ingested, because the bacteria are apt to adhere and be taken in in clumps.

Because of its simplicity the clinical laboratory worker will prefer some modification of the Leishman method, which uses the patient's own leukocytes. It is, perhaps, as accurate as the original method of Wright, although variations in the leukocyte count have been shown to affect the result. Two pipets like those shown in Fig. 172 are used.

(1) Make a suspension of the specific organism by mixing a loopful of a young agar culture with 1 c.c. of a solution containing 1 per cent. sodium citrate and 0.85 per cent. sodium chlorid. Thoroughly break up all clumps by sucking the fluid in and forcing it out of one of the capillary pipets held vertically against the bottom of the watch-glass.

(2) Puncture the patient's ear, wipe off the first drop of blood, and from the second draw blood into the other pipet

to the grease-pencil mark, let in a bubble of air, and draw in the same amount of bacterial suspension.

(3) Mix upon a slide by drawing in and forcing out of the pipet.

(4) Draw the mixture high up in the pipet, seal the tip in the flame, and place in the incubator for fifteen minutes.

(5) Repeat steps 2, 3, and 4 with the blood of a normal person.

(6) After incubation, break off the tip of the pipet, mix the blood-bacteria mixture, and spread films on slides.

(7) Stain with Wright's or Harlow's blood-stain or carbol-thionin.

(8) With an oil-immersion lens count the bacteria which have been taken in by 100 leukocytes, and calculate the average number per leukocyte. Divide the average for the patient by the average for the normal person. This gives the opsonic index. If in the patient's blood there was an average of 4 bacteria per leukocyte, and in the normal blood 5 bacteria per leukocyte, the opsonic index would be $\frac{4}{5}$ or 0.8.

Wright and his followers regarded the opsonic index as an index of the power of the body to combat bacterial invasion. They claimed very great practical importance for it as an aid to diagnosis and as a guide to treatment by the vaccine method. This method of treatment consists in increasing the amount of protective substances in the blood by injections of normal salt suspensions of dead bacteria of the same species as that which has caused and is maintaining the morbid process, these bacterial suspensions being called "vaccines." Vaccine Therapy (Chapter IX) has taken a permanent place among our methods of treatment of bacterial infections, particularly of those which are strictly local, but the

opsonic index is now little used either as a measure of resisting power or as an aid to diagnosis and guide to treatment.

IV. REACTIONS BASED UPON IMMUNE BODIES OF THE THIRD ORDER

The reactions of this group comprise the various applications of the Wassermann reaction or, more properly, of the Bordet-Gengou phenomenon of complement-fixation or deviation. Since the reaction involves three active substances—viz., antigen (the substance inducing the immune reaction); the specific amboceptor, or immune substance; and the non-specific complement—it is possible to so adjust matters that, any two factors being known, the third may be determined either qualitatively or (roughly) quantitatively. Practically, the method is employed chiefly for determining the presence of the middle term, or amboceptor. It may be applied to the diagnosis of any disease the antigen of which is known and which can be obtained in suitable form. This includes syphilis, gonorrhea, malignant disease, tuberculosis, echinococcus, cysticercus infections, trichiniasis, typhoid and pneumococcus, meningococcus and staphylococcus infections, etc. In several of these, other and simpler methods are, however, available. The method as applied to the first four diseases above mentioned is given below.

The method employed is based upon the fact that if suitable quantities of antigen, amboceptor (*i. e.*, patient's serum containing the same), and complement are mixed together and warmed gently in the incubator, a supposedly chemical, firm union of the three takes place.

The mere fact of combining in this way produces, however, no visible change in the fluid. It is necessary, therefore, to test for free complement by adding the two other units of another immune system which also requires the presence of complement, and which will produce a visible reaction if free complement is present. A "hemolytic system" is used for this purpose. The mixture is then incubated a second time. If the three units of the first system have combined (in other words, if the patient's serum contains syphilis antibody), and not otherwise, the complement is "fixed" or "deviated" during the first incubation period, so that it is no longer available to assist in completing the second and visible reaction represented by the hemolytic system. As will be seen later, an elaborate system of controls is needed.

A. Complement Deviation Test for Syphilis

The Wassermann Reaction

Of the many modifications of the Wassermann reaction, but one, the standard form of the reaction, will be given.

1. **Materials Required.**—The following reagents are needed:

(1) *Syphilitic Antigen.*—The reaction, originally supposed to depend upon the presence in the patient's serum of true syphilis antibodies, is now known to depend instead on a disorder of lipoid metabolism characterized by the presence of serum-foreign lipoids in the serum. Accordingly, solutions of lipoids from various sources can be used for the test. The following may be recommended:

(*a*) Grind or chop the liver and spleen of a syphilitic fetus. Place in a suitable vessel and add 4 to 10 parts of absolute ethyl alcohol. (The amount of alcohol varies in the hands of different workers.) Extract for three or four days in the incubator or for one to two weeks at room-temperature, with frequent vigorous shakings. Filter through paper. The filtrate constitutes the stock solution, which is diluted with salt solution for use, as described later.

(*b*) Grind in a mortar with quartz sand one or more guinea-pig hearts, previously weighed, place in a suitable receiver, and add 10 c.c. of absolute alcohol for each gram of heart tissue. Complete the preparation as above. This solution can be purchased from the various biologic houses.

(2) *Antisheep Amboceptor.*—This can now be obtained so readily in the market that the somewhat elaborate method of preparation may be omitted here.

(3) *Sheep's Red Blood-cells.*—Where a slaughter-house is available, it constitutes the most convenient source of supply. A sterile bottle (about 100-c.c. capacity), containing some glass beads, bits of glass rod, or steel shavings, is carried to the slaughter-house. After the first gush of blood from the slaughtered animal has cleansed the wound, the bottle is filled not quite full with blood. It is then stoppered and the bottle kept in motion for ten or fifteen minutes or until defibrination is complete. For use, "wash" the cells thoroughly free from serum by filling centrifuge tubes about one-quarter full of defibrinated blood, and adding 0.9 per cent sodium chlorid solution to the top. Centrifugate thoroughly and pipet off the supernatant fluid. Again fill with

salt solution, mix, centrifugate, and remove the supernatant fluid. Repeat *at least* three times. Finally, prepare a 5 per cent. emulsion by adding 1 volume of the cells, thoroughly packed by centrifugation, to 19 volumes of salt solution. This is the standard against which the strength of all other solutions is measured or titrated, as described below.

(4) *Complement.*—Stun a fasting guinea-pig by a blow at the base of the skull, cut the throat, and collect the blood in a clean, dry dish. The serum may be allowed to separate spontaneously over night in the ice-box, or be separated just before use by centrifugation. *Serum more than twenty-four hours old is worthless as complement.*

(5) *Patient's Serum.*—About 5 c.c. of blood will suffice. A convenient method consists in applying an Esmarch bandage to the upper arm, after cleansing the flexor surface of the elbow with alcohol or tincture of iodin. If the patient opens and closes the fist vigorously a few times the veins become more prominent. Insert the needle of the syringe above described above or alongside the vein and at an acute angle to the skin surface. Once through the skin, a little practice will enable one to quickly find the way into the vein. Slow withdrawal of the plunger will quickly fill the syringe. If the vein is a large one the blood will flow into the syringe, driving the plunger ahead of it. Remove the bandage before withdrawing the syringe to avoid a hematoma. Withdraw the needle quickly, and have the patient or an assistant apply fairly firm pressure over the punctured vein for a minute or two. In the meantime empty the syringe into a scrupulously clean test-tube, and immediately wash

out the syringe and needle thoroughly with water, followed, especially if the needle is of steel, by alcohol. If blood is given time to clot in the needle or syringe the instrument is practically ruined. The needle should, of course, be sterilized by boiling before use. The syringe should be clean and *dry* (as otherwise hemolysis will take place), but need not be sterilized.

After an hour or two, separate the clot, if necessary, from the test-tube wall with a clean wire, and either complete the separation of the serum at once by centrifugation or place in the ice-box over night. Transfer the serum with a capillary pipet to a second clean test-tube.

Before the test is made the serum is "inactivated" (*i. e.*, the native complement present is destroyed) by immersing the tube for half an hour in the water-bath at 55° to 56° C.

Unless a considerable number of sera are to be examined simultaneously, known positive and known negative control sera must be prepared in the same way.

2. **The Titrations.**—The strength of the complement and antisheep amboceptor must be determined on each occasion of its use. The antigen must be titrated every few weeks.

(1) **Titration of the Complement.**—The complement may be used undiluted or in varying dilutions of from 40 to 10 per cent. The greater the dilution, of course, the greater the accuracy with which it can be titrated. Assuming that it is to be used in a 40 per cent. dilution (1 part of complement serum to $1\frac{1}{2}$ parts of salt solution), arrange a series of test-tubes somewhat as follows:

Tube No. 1—0.02 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.[1]
Tube No. 2—0.04 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.
Tube No. 3—0.06 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.
Tube No. 4—0.08 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.
Tube No. 5—0.10 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.
Tube No. 6—0.12 c.c. complement serum plus 1 c.c. 5 per cent. sheep blood-cells and 1½ units amboceptor.

Make up all tubes to a like volume (1.5 or 2 c.c.). Mix thoroughly by gentle shaking, and place in the incubator (preferably standing in a dish of water, since this insures rapid and uniform heating to incubator temperature) at 37° C. for one hour. The tube containing the smallest amount of complement which shows *complete* solution of the red cells (the solution bright red, perfectly clear, and free from sediment) contains one unit of complement. A slight excess over this

[1] One unit of amboceptor is the amount required to bring about solution of 1 c.c. of the 5 per cent. red cell emulsion, in the presence of 1 unit of complement, in one hour at incubator temperature. In the same way, 1 unit of complement is the amount required to bring about solution in the presence of one unit of amboceptor under the same conditions. In the above experiment the 1½ units of amboceptor is only approximate. It is assumed that the worker has purchased amboceptor in 1-c.c. vials, guaranteed to contain 1000 units, and actually containing a slight excess over that amount. For use this is diluted with 100 parts of salt solution: 0.1 c.c. will then contain something over 1 unit. On the first occasion of its use, 0.15 c.c. may be accepted for titration purposes, the aim being to use a moderate excess to allow for the chance of deterioration and slight variations in the strength of the blood emulsion. On each later occasion the approximate value is known from the last previous titration. Amboceptor dilutions keep well in the ice-box, but may undergo a very abrupt deterioration at the end of about six months.

amount, say $1\frac{1}{2}$ units, is used in making the test proper, to allow for the rapid deterioration which takes place and for the small amount of complement directly absorbed by the antigen.

(2) **Titration of the Amboceptor.**—Arrange tubes as follows:

Tube.	Complement.	Red cells (5 per cent.).	Amboceptor (1–100 dilution).
No. 1	$1\frac{1}{2}$ units [1]	1.0 c.c.	0.06 c.c.
No. 2	$1\frac{1}{2}$ "	1.0 c.c.	0.08 c.c.
No. 3	$1\frac{1}{2}$ "	1.0 c.c.	0.10 c.c.
No. 4	$1\frac{1}{2}$ "	1.0 c.c.	0.12 c.c.
No. 5	$1\frac{1}{2}$ "	1.0 c.c.	0.14 c.c.
No. 6	$1\frac{1}{2}$ "	1.0 c.c.	0.16 c.c.

Bring all tubes to a like volume, mix, and incubate for one hour. The tube containing the smallest amount of amboceptor which causes complete hemolysis contains one unit. *Two* units are used for the test proper.

(3) **Titration of the Antigen.**—The stock solution is to be diluted freshly for use with salt solution. This makes a milky fluid. The amount of dilution will vary with the strength of the stock solution as determined by the following tests. For the latter a 10 per cent. dilution may be employed.

Arrange test-tubes as follows:

Tube.	Antigen (10 per cent.).	Red cells (5 per cent.).
No. 1	0.1 c.c.	1.0 c.c.
No. 2	0.2 c.c.	1.0 c.c.
No. 3	0.3 c.c.	1.0 c.c.
No. 4	0.4 c.c.	1.0 c.c.
No. 5	0.5 c.c.	1.0 c.c.
No. 6	0.6 c.c.	1.0 c.c.

[1] As determined in the previous titration.

Bring all tubes to a like volume. Mix and incubate. The amount used in making the test proper must not be more than one-half the smallest amount which causes hemolysis in the above. A modified form of this titration is repeated each time the antigen is used.

Arrange at the same time with the above test-tubes as follows:

Tube.	Antigen (10 per cent.).	Complement.
No. 1	0.1 c.c.	1½ units
No. 2	0.2 c.c.	1½ "
No. 3	0.3 c.c.	1½ "
No. 4	0.4 c.c.	1½ "
No. 5	0.5 c.c.	1½ "
No. 6	0.6 c.c.	1½ "

Bring all tubes to a like volume, mix, and incubate. Then add to all tubes 1 c.c. of 5 per cent. red cell emulsion and 2 units amboceptor solution. Mix and reincubate. If the antigen is "anticomplementary" it will prevent hemolysis in one or more of the tubes. The amount used for the test proper must not exceed one-half the smallest amount showing such action.

The antigen must also be shown to react with known positive sera, and the amount required to produce a reaction determined. For this purpose an abundant supply of serum from a patient with active secondary syphilis (still better, from several such patients) is obtained, and the complete reaction carried out as described below, employing varying amounts of the antigen dilution, *e. g.*, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14 c.c., etc. For the test proper an amount is used in the greatest possible excess of that amount which gives a positive reaction, but which complies, however, with the require-

ments mentioned above as to hemolytic and anticomplementary action.

3. **Errors and Their Causes.**—(1) Dirty glassware unquestionably is responsible for most of the errors. No control can eliminate an error caused by a single dirty test-tube among a hundred clean ones. The hemolytic system will be completely destroyed in such a tube, with the likelihood of interpreting the result as a positive reaction, or as due to an anticomplementary patient's serum, etc., depending on what it happens to be used for. Glassware need not be sterile, but *must be absolutely clean.* Never allow used tubes to stand and dry out. Immediately the work is finished wash with soap and water, rinse thoroughly with clean water followed by dilute (10 per cent.) nitric acid, then caustic soda solution, then several changes of distilled water. Place in a basket and dry in a dry-air sterilizer if one is available. New glassware should be prepared for use in the same way. Tubes may be kept stored in the oven or set aside in a clean dust-proof cupboard. Tubes used for this purpose should never be used for any other. Other glassware should be cleaned with equal care, and preserved from any other use.

(2) The patient's serum may be "anticomplementary," *i. e.*, it may have the power to combine with or absorb complement in the absence of antigen. Serum which has been kept too long, or which has been inactivated at a temperature above 56° C., is apt to exhibit this property. The anticomplementary property may sometimes be made to disappear by renewed inactivation. If this fails, the serum must be discarded. A control of this property is included in setting up the test.

(3) The antigen may become anticomplementary or hemolytic, or both. When this happens it must be discarded. A control for this is set up each time the antigen is used.

(4) The hemolytic system may fail to function for a variety of reasons. A control of this is furnished by the titration of complement and amboceptor above described.

4. **The Test Proper.**—Assuming for the moment that all preparations have been completed and the titrations satisfactorily performed, one proceeds to set up the test proper. We may suppose that we are dealing with at least three sera—viz., the patient's serum and known positive and known negative controls. Arrange in a rack having two rows of holes test-tubes as shown on p. 520.

Bring all tubes to a like volume (1.5 or 2 c.c.) by the addition of salt solution. Mix by gentle shaking and place in the incubator for one hour. The rack should stand with the tubes immersed in water to about the level of the contents. Then add to all the tubes except the last one, already containing blood-cells, 2 units of antisheep amboceptor dilution and 1 c.c. of 5 per cent. sheep red cell emulsion. Mix as before and incubate for two hours. The tubes in the back row show for each serum tested whether any of them is anticomplementary. They should all show complete solution of the red cells. The last two tubes show whether the antigen is hemolytic or anticomplementary respectively. The first, containing complement, should show complete hemolysis. The second, containing only antigen and red cells, should show no solution. Assuming that these controls are all

	Unknown serum, inactivated.	Known positive serum, inactivated.	Known negative serum, inactivated.
Back row.	Tube No. 1. Serum..... 0.2 c.c. Complement..... 1.5 units Salt solution to make.. 2 c.c.	Tube No. 2. Serum..... 0.2 c.c. Complement..... 1.5 units Salt solution to make.. 2 c.c.	Tube No. 3. Serum..... 0.2 c.c. Complement..... 1.5 units Salt solution to make.. 2 c.c.
Front row.	Tube No. 4. Serum..... 0.2 c.c. Complement..... 1.5 units Antigen,[1] quantity indicated by titration. Salt solution to make.. 2 c.c.	Tube No. 5. Serum..... 0.2 c.c. Complement..... 1.5 units Antigen, quantity indicated by titration. Salt solution to make.. 2 c.c.	Tube No. 6. Serum..... 0.2 c.c. Complement..... 1.5 units Antigen, quantity indicated by titration. Salt solution to make.. 2 c.c.
Placed as convenient.	Tube No. 7. Twice the above amount of antigen plus 1.5 units complement plus salt solution to make..... 2 c.c.		
	Tube No. 8. Twice the above amount of antigen plus 1 c.c. 5 per cent. red cell suspension plus salt solution to make..... 2 c.c.		

[1] It will be found convenient to arrange the dilution of the stock antigen in such a way that the amount required for the test is contained in an easily measured volume unit, *e. g.*, 0.1 c.c. The same device may, of course, be adopted with reference to the complement.

satisfactory, one turns to the tubes in the front row. The known positive control shows no solution of the red cells, the complement having been deviated or bound during the first incubation, and hence being not available for the reaction with the red cells. For the opposite reason the known negative serum will show complete solution. The unknown serum will behave like the first or the second, according as it is positive or negative.

It is apparent that an excess of complement may convert a positive reaction into a negative, while a deficiency may cause a negative serum to behave like a (more or less) positive one. It is to avoid this contingency that the *unknown* quantity of complement present in the fresh patient's serum is removed by inactivation, to be replaced by an accurately measured amount of guinea-pig complement.

With **cerebrospinal fluid** the reaction is carried out in the same way, except that this fluid *must not be inactivated*, and is used in larger amounts. When enough fluid is available the test should be set up so as to give a reading for 0.4, 0.6, 0.8, and 1 c.c. When economy of material is necessary a reading should be obtained for 0.5 and 1 c.c. Further modifications may, of course, be imposed by the exigencies of the case.

Several degrees of the reaction are recognized and are customarily indicated as follows:

Complete inhibition (cells intact with colorless supernatant fluid),	++++	or 4 +.
Almost complete inhibition,	+++	or 3 +.
About one-half complete inhibition,	++	or 2 +.
Slight inhibition,	+	or 1 +.
No inhibition.	0.	

5. **Interpretation of Results.**—(1) Jaundice and marked alcoholism may convert a positive reaction into a negative one.

(2) Scarlet fever, leprosy, active malaria, and malignant tumors may cause a positive reaction.

(3) The reaction is negative in primary syphilis, but becomes rapidly and strongly positive as the general manifestations of the disease develop. During this stage only a strongly positive reaction should be regarded as significant. In late and especially in latent syphilis the reaction again grows weaker. More significance may, therefore, attach to weak reactions in such cases.

(4) A positive reaction quickly becomes negative under specific treatment, to recur if treatment is inefficient. Apparently cured cases may show a positive reaction six months to a year after a "provocative" dose of salvarsan.

(5) The behavior of the blood is no guide as to the condition of the central nervous system. Recent investigations have shown that the central nervous system becomes involved very early in practically all cases, and the organisms so located are peculiarly inaccessible to attack by present methods. No case may be regarded as cured until both blood and cerebrospinal fluid show a persistent normal condition.

Routine Methods.—The labor involved in carrying out the somewhat elaborate details of the method as above outlined may be materially lightened by systematizing the work somewhat as follows: On the day before the tests are to be made prepare an abundance of clean glassware and the red cell emulsion; see that the water-bath is properly reg-

ulated; and, if desired, bleed one or more guinea-pigs for complement, and place the blood in the ice-box. In the morning proceed as follows:

(1) Set up the complement titration, place in the incubator, and mark the time.

(2) Pipet off the sera to be tested into clean test-tubes, and place in the water-bath to inactivate. Mark the time.

(3) Arrange in the rack the tubes needed for the test, the antigen control, and the amboceptor titration.

(4) By the time inactivation is complete the complement titration will be nearly or quite finished. Forty-five minutes will suffice for the latter. Now set up the tests proper, with the controls and the amboceptor titration.

(5) At the end of the hour the titration may be read, and the indicated amount, and the red cells, added to all tubes. Two hours later the final result is read and recorded. Glassware should immediately be washed and put away for the next occasion. A little experience will enable one to make from twenty-five to fifty tests between 9 A. M. and 3 P. M.

B. Complement Deviation Test for Gonorrhea

Method of Schwartz and McNeil

The method as given below represents minor modifications of the original method suggested by experience in the writer's laboratory. The antigen is an autolysate of a large number of strains of the gonococci. It may be obtained from Parke, Davis & Co. For use, dilute with 9 parts of salt solution. The amount used for the test is one which gives a strong positive reaction with a known positive serum or with the antigonococcic serum of Torrey (also marketed by Parke, Davis & Co.), provided this amount is not anticomplementary. In our experience 0.15 c.c. of a 10 per cent. dilution has met these conditions.

The complement is used in a 10 per cent. dilution. Complement and amboceptor are titrated against 0.1 c.c. of 5 per cent. sheep cell emulsion, instead of 1 c.c. The same quantity of red cell emulsion is, of course, also used in making the test.

The patient's serum is used inactivated. In the original method the test is carried out with 0.05, 0.10, and 0.15 c.c. In our experience 0.05 c.c. is almost invariably negative, while 0.15 c.c. is almost invariably anticomplementary. We have, therefore, used only 0.1 c.c.[1]

In other respects the test is carried out exactly like the test for syphilis. The reaction is negative during the acute stages of the disease, but is useful in determining the presence of a focus of chronic infection. Its chief importance lies in the fact that it becomes negative in a short time (probably about two weeks) after a cure is completed.

C. Complement Deviation Test for Malignant Disease

Method of von Dungern

1. The *antigen* is prepared in either of the two following ways:

(*a*) Free tissue from a malignant tumor as much as possible of fat and necrotic tissue. Chop up and extract for one or two weeks with 20 volumes of chemically pure acetone, with frequent shaking. Filter. For use, evaporate a portion to dryness at 37° C. and redissolve in half the amount of absolute alcohol. Dilute with

[1] My assistant, Dr. T. F. Walker, has kindly furnished me with these data, based on an unusually extensive experience in my laboratory with the method.

salt solution according to the result of the titration. The dose for the test is the largest possible, provided that twice the amount is not anticomplementary. The dilution is arranged simply to contain the required amount in a convenient volume unit, *e. g.*, 0.5 c.c.

(*b*) Receive 10 c.c. of human blood (preferably from a general paralytic) in a graduated container containing 0.1 c.c. of a 2 per cent. sodium oxalate solution. Wash the cells at least three times with normal salt solution. After the last centrifugation, which should be as complete as possible, weigh the cells. This can be done readily by difference if the weight of the centrifuge tube or tubes is known. Add 20 volumes of chemically pure acetone for 1 part by weight of the cells. Extract for three days at room temperature, with occasional shaking. Filter, and evaporate the filtrate in a weighed dish to dryness in the incubator. Take up the residue with enough 96 per cent. alcohol to make a 1 per cent. solution. The residue will dissolve completely if the alcohol is added as soon as the acetone evaporates. For use, mix 1 part of the alcoholic solution slowly with 4 parts of salt solution, and shake thoroughly. Titrate as above. About 0.8 c.c. is the usual dose for the reaction. This form of antigen is preferred by von Dungern to the cancer extract described above.

2. The *serum to be tested* (and when possible, positive and negative controls) is obtained aseptically, and after separation from the clot should be kept two or three days in the ice-box before completing the test. Then add 2 volumes of $\frac{N}{50}$ caustic soda solution, chemically pure and kept free from absorbing carbonic acid gas

from the air, and prepared as needed by diluting one volume of $\frac{N}{10}$ soda with four volumes of normal salt solution, and inactivate for one-half hour at 54° C. (*not 56° C.*).

3. The *complement serum* is used without titration, 1 c.c. of a 5 per cent. solution.

4. Von Dungern uses a beef *hemolytic system*. I have found the customary sheep system quite as applicable.

Arrange two rows of test-tubes, in each pair of which, front and rear, place 0.6, 0.3, 0.15, 0.075 c.c., respectively, of the inactivated soda-serum mixture. To all tubes add 1 c.c. of 5 per cent. complement and to the front row add the titrated amount of antigen—*e. g.*, 0.8 c.c. Bring all the tubes to a like volume, mix, and expose for *three hours at room temperature*. Add to all tubes 2 units of amboceptor (1 unit being the smallest amount which completely hemolyses 1 c.c. of 5 per cent. red cell emulsion in two hours in the incubator in the presence of 1 c.c. of 5 per cent. complement dilution) and 1 c.c. of 5 per cent. red cell emulsion. The results are read after standing for three hours at room-temperature.

Inactivation eliminates heterologous reactions due to syphilis, and the addition of the alkali eliminates similar errors due to tuberculosis. A correct result may be expected in over 90 per cent. of the cases.

D. Complement Deviation Test for Tuberculosis

Method of Hammer

The antigen is a mixture of Koch's old tuberculin and an extract of tuberculous granulation tissue freed as much as possible from other tissue. Tissues from a

surgical lesion, such as the knee, are most suitable. Cover the tissue with 4 parts of alcohol and extract for three to five days. Filter, and dilute the filtrate with 3 parts of salt solution for use. Test 0.4, 0.2, and 0.1 c.c. of this against 0.1 c.c. of known positive serum. Or, cover the tissue with 9 parts of acetone and extract for ten days. Filter, and evaporate to dryness at 37° C. Take up the residue in an equal volume of alcohol and dilute for use with 10 volumes of salt solution. Titrate as above. In either case the dose used is the largest, twice which is not anticomplementary.

Now add to 9 volumes of the diluted extract 1 volume of old tuberculin, and repeat the titration as above. The dose is determined according to the same rule. A certain proportion of cases will react with one or other of the antigens alone, but the larger percentage of positive results will be obtained with the mixed antigen.

Arrange the tubes as for the Wassermann reaction. In all the tubes place 1 c.c. of 5 per cent. complement serum. To the front tubes add the titrated dose of antigen. In each pair of tubes, front and rear, place 0.1 c.c. of the several sera respectively, inactivated at 56° C. for thirty minutes. Bring all the tubes to a like volume, mix, and let stand for three hours at room-temperature. Add 2 units of amboceptor and 1 c.c. of 5 per cent. red cell emulsion. Mix, and place in the incubator for one hour. The tests are then ready for the final reading.

The three methods which follow do not rest on any of the laws of immunity above mentioned. They, therefore, must be classified separately.

V. COBRA-VENOM TEST FOR SYPHILIS

Method of Weil

Of the several cobra-venom reactions, the method of Weil, for the diagnosis of syphilis, possesses the greatest practical value, and is here given. It appears to depend upon the same disturbance of lipoid metabolism which is responsible for the Wassermann reaction. It is known that syphilis is characterized by a withdrawal of lipoids from their chief depots, viz., the central nervous system and the red blood-cells, with a marked increase of the same in the fluid part of the blood. Since it is also known that the hemolytic action of the cobra venom depends upon its activation by lecithin, in other words, upon a lecithin-venom complex in which the lecithin serves as complement, it may fairly be assumed that the loss of lipoids by the red cells is responsible for the *increased resistance to hemolysis by cobra venom* upon which Weil's reaction is based.

1. **Materials Required.**—(1) The *cobra venom* may be obtained from Poulenc Frères, Paris. Weil's stock solution is a 0.5 per cent. solution in 0.9 per cent. salt solution, made, of course, very accurately. It deteriorates very rapidly unless kept frozen. For this reason I have tried very successfully the solvent usually employed for the purpose of other reactions in this group, viz., a 1 per cent. solution of venom in equal parts of distilled water and chemically pure glycerin. Before it is used, this should be allowed to stand several days in the ice-box, where it keeps extraordinarily well.

(2) The *blood-cells to be tested.* Have ready normal salt solution to which 2 per cent. sodium citrate is

freshly added, and which has been cooled in the ice-box. Into about 10 c.c. of this, contained in a graduated centrifuge tube, discharge about 2 c.c. of the patient's blood. *Do not shake.* Wash at least four times with 0.9 per cent. salt solution. The last washing of all bloods in a series is done at the same speed and for the same length of time. Accurate and uniform dilution of the cells is, of course, an absolute essential to obtain comparable readings. Pipet off the last wash-water and make up to a 4 per cent. emulsion by adding 24 volumes of solution to the 1 volume of cells as read in the graded tube before they are disturbed. The salt solution used for washing and diluting should be ice cold and the final emulsion should be placed on ice several hours before the test is made.

2. **Method.**—From the stock solution of venom prepare the following solutions for the test: 1–10,000, 1–20,000, 1–30,000, 1–40,000. Arrange a suitable rack with 4 tubes for each test. In the respective tubes of each row place 1 c.c. of the several venom solutions and 1 c.c. of the cell emulsion. Incubate for one hour at 37° C. Mix thoroughly by gentle shaking and place in the ice-box over night. In the morning again mix thoroughly and make the final reading an hour later. The result will depend on comparison with known normal cells. Something like the following may be anticipated:

No hemolysis at 1–10,000	=	strongly positive.
Moderate hemolysis at 1–20,000	=	positive.
Partial hemolysis at 1–30,000	=	negative.
Complete hemolysis at 1–40,000	=	hypersensitive.

The test appears later in the disease than the Wassermann reaction, and yields a higher percentage of positive

results in late latent syphilis. Furthermore, it yields less quickly to treatment. It is unquestionably an important aid to diagnosis and treatment in the class of cases indicated.

VI. THE PROTECTIVE FERMENT REACTIONS

Method of Abderhalden

The reaction depends upon the following laws, briefly stated:

When a foreign protein, fat, or carbohydrate is injected directly into the circulation, ferments, which are able to digest the material injected, promptly appear in the circulation. These ferments are specific, *i. e.*, they act only upon the substance the presence of which has led to their appearance in the blood.

The cells of the body are vital units, and carry out with a certain measure of independence each a series of metabolic changes peculiar to itself. The body fluids, and perhaps the body cells, are adapted to dispose of metabolic products only when they receive them in a certain state, which may be regarded as the normal. If the cells of any organ discharge into the blood any product in a form to which the blood is not accustomed, such product behaves as a foreign substance. There results the concomitant appearance of the ferment above mentioned, and this whether the substance is a normal product of the cell which normally does not enter the blood, or whether it is the product of diseased and abnormal metabolism, and, therefore, equally foreign to both cell and blood. A substance may, therefore, be foreign to the organism as a whole, yet normal to many of its parts—normal to the liver, for example, and for-

eign to every other tissue. Hence, a distinction must be made between body-native and blood-native, organ-foreign and blood-foreign, etc.

The source of the ferments is unknown. They appear too quickly to permit the assumption that they originate by a process analogous to that which produces the immune substances. Abderhalden has recently advanced the hypothesis that they are secreted by the same cells which produce the substance to be digested, or, in the case of parenteral administration of body-foreign substances, by the cells producing the ferment which, under normal conditions, would digest the substance in the intestinal tract. I would add that in the former case the ferments may then be identical, in part at least, with those responsible for autolysis.

It may be flatly asserted at the outset that, in spite of the severe criticism to which the method has been subjected, the correctness of Abderhalden's thesis has been abundantly confirmed. In every case results which seem to show the contrary have been shown to depend on faulty technic. The technical difficulties involved are very great. In the following paragraphs I shall, for the sake of brevity, content myself with pointing out the precautions which must be rigidly observed, without pausing for the whys, merely reminding the reader of Abderhalden's dictum, that no one is competent to express an opinion as to the method who has not first convinced himself of his ability to obtain *invariably correct results*, to distinguish invariably, for example, between pregnant and non-pregnant individuals.

A special room must be provided with special apparatus, including glassware, sterilizer, incubator, etc., not

to be used for any other purpose. All utensils must be rigidly clean, as for the Wassermann reaction, and, in addition, must be sterilized as for bacteriologic work. Two methods are available, which may be used alone or in combination:

A. The Dialyzation Method

This method depends upon the fact that albumin is not dialyzable, while peptone and simpler products produced from it by digestion are dialyzable.

1. **Preparation of Materials.**—(1) **The Substrate.**—The preparation of all organs is the same, except that material rich in fat and lipoids, such as the liver, kidney, brain, and tubercle bacillus, must, in addition, be extracted with carbon tetrachlorid in a Sohxlet apparatus. Placental tissue is obtained fresh, other organs are obtained from autopsy material, preferably from cases of death by violence. The pathologic condition of organs used should be determined by careful examination, microscopic and otherwise.

Preparation of placental tissue may be taken as the type. Remove visible blood-clots, together with the membranes, cut into small pieces, and squeeze them out hard in running water (if the tap-water is soft it is better to use normal salt solution). The easiest way is to place the tissue on a sieve, and allow the water to run over it uninterruptedly, squeezing the individual pieces. In the case of bacteria and finely divided tissue, a centrifuge must be substituted for the sieve. From time to time place in a cloth and wring out. Discard pieces containing clotted blood and as much of the connective tissue as possible. Finally, crush the tissue in

a mortar and wash again in running water. The tissue finally becomes perfectly white, except liver, spleen, and kidney, which will always retain some color.

In the meantime heat to boiling about 100 volumes of distilled water in a clean enameled container. When the tissue has been completely freed of blood, add 5 drops of acetic acid for each liter of water and immediately throw in the entire mass of tissue. Boil steadily for ten minutes, pour the entire contents into a sieve, and bring another measure of water (from now on without adding acid) to boiling. In the meantime wash the tissue on the sieve for about five minutes in distilled water. When the water is again boiling, throw the tissue into it again, and again boil for ten minutes. Repeat this process five or six times. After the last boiling, heat only about 5 volumes of water instead of 100 and throw the tissue into it. Boil for five minutes, carefully avoiding scorching. Filter off some of the cooking water through a hard filter-paper. To 5 c.c. of the filtrate add at least 1 c.c. of 1 per cent. aqueous ninhydrin solution, as described below, and boil in the flame for one minute. If absolutely no violet color is visible after standing half an hour, and provided the tissue remains perfectly white, the preparation is complete. Otherwise the boiling for five minutes in 5 volumes of water, alternating with five minutes' washing in cold water, is repeated until the desired result is obtained. Then place in a sterile, wide-mouthed, glass-stoppered bottle, add a little sterile water, and a good deal of chloroform and toluol. A properly prepared organ will keep indefinitely if it does not become infected. Remove portions only with sterile forceps, and never put back a

portion once removed. The layer of toluol must touch the stopper, and bits of tissue must not extend above the toluol.

Before used for testing unknown sera the substrate must be shown to react negatively with a variety of known negative sera, and positively with known positive sera.

(2) **Dialyzing Membrane.**—The dialyzing shells No. 579A of Schleicher and Schüll are employed. Before use they must be shown to be impermeable to albumin and all equally permeable to silk peptone. Shells already proved can be purchased from Schöps, Halle an der Salle, but these must be retested before use, and again from time to time, to avoid error. These tests are made as follows:

(*a*) *To Show Impermeability to Albumin.*—Place 5 c.c. of perfectly fresh egg-white, from which the membranes and any floccules have been removed, in a cylinder graduate, and add 95 c.c. of water. Mix by vigorous shaking. In the meantime soak the shells about one-half hour in cold water. Place each shell in a clean 100-c.c. Ehrlenmeyer flask, and add to each 2.5 c.c. of the albumin solution. Use a graduated pipet for this purpose, introducing well into the mouth of the shell, and carefully avoiding touching the outside of the shell with the pipet. Close the upper end of the shell between finger and thumb, and rinse the outside of the shell thoroughly in running water. Then close the shell by pinching near its center, and rinse the upper end, *inside* and *outside*, in the same way. The hands should be thoroughly clean and recently washed, or perhaps

covered by clean sterile gloves. Still better, the shells should be handled only with clean forceps.

Now place each shell in a fresh Ehrlenmeyer flask, or large test-tube with a foot, containing 20 c.c. of sterile distilled water. Cover both the water and the contents of the shell about $\frac{1}{2}$ cm. deep with toluol, taking care that the level of fluid in both shall be the same. Cover the flask or test-tube with an inverted watch-glass and place in the incubator for about sixteen hours.

Arrange the flasks in order on the work table, and with a pipet, closed with the finger while it is quickly passed through the layer of toluol, transfer 10 c.c. of the fluid on the outside of the shell to a test-tube. A separate, absolutely clean and dry pipet must be used for each transfer. Add to each test-tube 2.5 c.c. of 33 per cent. caustic soda solution, and mix by careful shaking, but do not use the thumb as a stopper. The skin excretions may contain substances which will cause a reaction. Add to each tube about 1 c.c. of copper sulphate, about 0.2 per cent., so as to form a layer on the surface. Examine the line of contact by transmitted light. The slightest trace of violet or rose color shows that the shell is permeable to albumin and it must be discarded. To be on the safe side, discard any about which there is any doubt.

The ninhydrin reaction is less suited for determining permeability to albumin than the biuret reaction, because the former reacts strongly with digested protein, but only weakly with undigested protein. It must be remembered, however, that the ninhydrin reaction depends on the concentration of the decomposition products, and that it is additive. A reaction may occur

when concentrations of albumin and peptone, neither of which reacts alone, are brought together. Individuals unable to recognize slight traces of the biuret reaction must use some other test for the albumin. One may make use of a homologous immune serum of very high titer, as described above for determining meat adulteration, or may use the ninhydrin test, first having determined the maximum amount of albumin which can be added to known impermeable shells without obtaining a positive reaction.

(*b*) *To Show Uniform Permeability to Silk Peptone.*— Empty the shells not discarded as a result of the first test, place them in a sieve and wash in flowing water for half an hour, and place in boiling water for not more than half a minute. Boiling injures the shells, rendering them thicker and less permeable. Handle the shells only with forceps sterilized in the flame. Arrange in flasks as before, and add to each, using the same precautions, 1.5 c.c. of a 1 per cent. solution of silk peptone (Höchst). Wash as before and place in flasks or tubes containing 20 c.c. of sterile distilled water. Cover with toluol, close the flask, and incubate for about sixteen hours. Evaporation must be prevented.

Remove 10 c.c. of the dialysate as before, place in large test-tubes (10 by 1 inches), and add to each tube 0.2 c.c. of ninhydrin solution prepared as follows: The ninhydrin is packed in vials containing 0.1 gm. Empty a vial into a 10-c.c. volumetric flask. Rinse the vial several times with sterile distilled water, adding rinsings to the flask. Fill the flask nearly full and place in the incubator till dissolved. Cool and fill to the mark.

The solution is affected by light and does not keep well,

hence only small amounts should be prepared at one time.

Now place in each test-tube a glass rod about 10 cm. long by 4 mm. in diameter, previously cleaned and dried in the dry air sterilizer, and kept in a glass-stoppered bottle. They must be handled only with forceps. The rod prevents "bumping," and insures uniform evaporation during the next step. This step is almost the most important in the entire procedure. By means of a holder, place each tube in succession in the center of a *high* Bunsen flame; as soon as bubbles begin to form, move to the edge of the flame. Keep the tube boiling vigorously (halfway up the tube) for exactly one minute from the time bubbles begin to form. Since the intensity of the reaction depends on the concentration of the fluid and the amount of heat applied, it is of vital importance that the boiling of all tubes shall be identical. As soon as the heating is complete, make sure that all tubes contain the same amount of fluid. This is made easier by marking the 10-c.c. level on each tube before it is used. Allow all the tubes to stand for half an hour. A certain depth of color will be found to predominate. Discard all the shells the dialysate of which yields either a lighter or darker color than this standard. Again wash and boil the shells as above, place in a sterile flask, add sterile distilled water and equal volumes of toluol and chloroform, so as to fill the flask entirely full. They are now ready for use. Remove from the flask only with sterile forceps.

(3) The **patient's blood** must be drawn while fasting, *i. e.*, before breakfast. The serum must be devoid of formed elements, and must show no hemoglobin on

spectroscopic examination. Puncture the vein with a sterile and *absolutely dry needle*, and receive 15 to 20 c.c. of blood in a clean, dry, sterile tube. After the serum has separated *spontaneously* (five or six hours), transfer the serum to a centrifuge tube (also clean, dry, and sterile) and centrifugate five to ten minutes. Serum cannot be used when more than twelve hours old, and must be sterile.

2. **The Test Proper.**—Abderhalden lays down the following fundamental laws to be observed:

(1) Absolute cleanliness of room, work-bench, all glassware, and utensils. The latter must also be dry.

(2) Only freshly distilled sterile water must be used. All work is to be done on an aseptic and antiseptic basis.

(3) No bacteriologic or chemical work may be done in the room. The incubator, especially, must be used for no other purpose.

(4) Good light must be had. Artificial light should never be used. Not more than five or six tests should be attempted at one time.

(5) The worker must understand not only the method, but the underlying principles.

While the serum is separating, prepare and arrange all the apparatus. Remove with sterile forceps the amount of substrate needed. Cover with 5 volumes of water and boil for five minutes. Test 5 c.c. of the filtrate with 1.0 c.c. of 1 per cent. ninhydrin, as described above. The substrate may be used only when not a trace of color is visible after boiling one minute and standing thirty minutes.

Place as many proved shells as will be required in clean, dry Ehrlenmeyer flasks and add to each about

0.5 gm. of the substrate, crushing and tearing the latter with forceps to increase its surface area. One shell for each serum to be tested should be left without substrate. To each shell add about 1.5 c.c. of serum. The same amount of serum should be placed in all. Wash the shells as already described (see p. 534) and transfer to fresh flasks or tubes containing 20 c.c. sterile distilled water. Pour a large amount of toluol in each shell and over the water outside, and thoroughly wet the part of the shell protruding above the fluid with toluol. Place in incubator at 37° C. for sixteen hours. On removing, toluol must still be present inside and outside the shell. Arrange the (numbered) flasks without special order on the work-bench. Remove 10 c.c. of the dialysate from each in the manner already described. Place in large, clean, dry test-tubes. Add to each 0.2 c.c. of the ninhydrin solution, and a clean dry bumping rod, and boil one after another as above described. Allow all tubes to stand for one-half hour. Now determine which tubes show color, *and then* determine the proper place of each in the series.

If serum alone gives no color, while serum plus substrate shows color, even the slightest, the test is positive, *provided heating and evaporation have been exactly alike for both.* When the dialysate of the serum alone shows color (due, for example, to the presence of hemoglobin or products of digestion in the serum), the reaction may be regarded as a valid positive reaction, provided the serum plus substrate shows a *distinctly darker color.*

The biuret reaction may be used in place of, or with, the ninhydrin reaction by any worker who is able to recognize the slightest trace of this reaction.

B. The Polariscopic Method

1. **Preparation of the Organ Peptone.**—The tissue is freed of blood and, if necessary, of fats and lipoids in the manner already described (see p. 532). Remove water, as far as possible, by pressing between sheets of absorbent paper. Then place the tissue in *cold* 70 per cent. sulphuric acid (volume per cent.). Shake vigorously and cork well. Repeat the shaking from time to time. The tissue dissolves shortly, and the solution takes on a brownish color. The temperature must not be allowed to rise above 20° C. At the end of exactly three days place the bottle containing the solution in ice-water, and add slowly 10 volumes of distilled water, taking care that the temperature does not rise above 20° C. Now add slowly baryta in substance, still keeping the temperature down, until the filtrate (or centrifugate) gives a precipitate with neither dilute baryta water nor dilute sulphuric acid. Approximately 105 gm. of the solid baryta neutralize 100 c.c. of the 70 per cent. sulphuric acid. The first stages of the neutralization can be controlled with litmus-paper. As the end-point is approached, a small amount is filtered or centrifugated, as above indicated, and portions tested for excess of acid and base respectively by adding very dilute solutions of the base or acid to each. A precipitate on adding baryta water, which dissolves on heating gently with nitric acid, is a baryta salt of peptone, and calls for the addition of more baryta.

When no precipitate is obtained, filter through a double-plaited filter, or under negative pressure through a hard paper, with animal charcoal. Centrifugation is more rapid and may be used. For economy's sake, wash

the precipitate several times with distilled water and add the wash-water to the solution. The ninhydrin test can be employed to determine when all the peptone has been recovered from the precipitate.

In the meantime the evaporation of the solution under negative pressure is begun. A convenient apparatus is

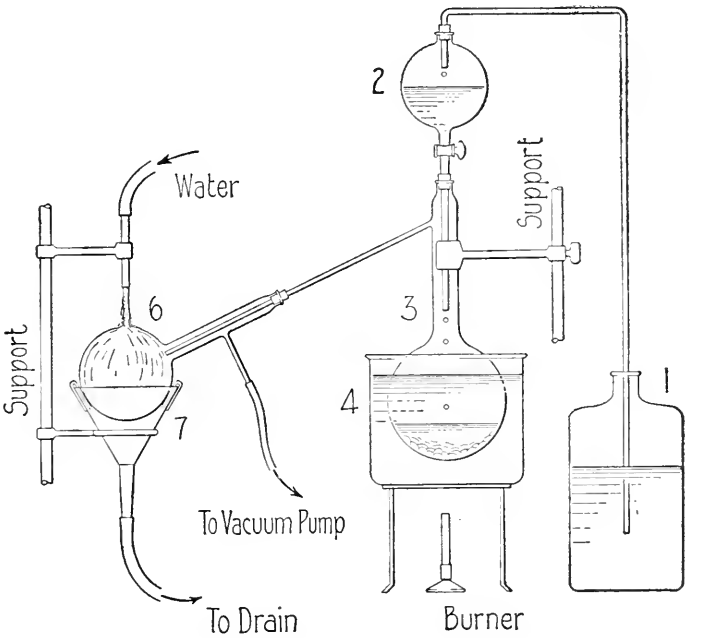


Fig. 176.—Abderhalden's apparatus for distilling under negative pressure at low temperature.

figured by Abderhalden, and reproduced here (Fig. 176). It permits the evaporation of the water under strong negative pressure at a temperature not to exceed 40° C. The separatory funnel (2) refills automatically from the reservoir (1). The solution, which is allowed to enter the

distilling flask (3) immersed in hot water (4) drop by drop, vaporizes at once, the vapor passing through the side arm, where it condenses in the second flask (6), over which flows a stream of cold water to be collected in the funnel (7). The flask is supported in the funnel by hooks. The negative pressure is furnished by the Chapman pump. High temperatures must be avoided throughout, unless one is certain that neither free acid nor free baryta is present. As the concentration increases, any trace of either present in the original solution is also concentrated, and may cause further hydrolysis of the peptone if the temperature is permitted to rise.

The final residue is bright yellow and syrupy. Mix with 100 volumes of methyl alcohol, boil, and filter hot through a plaited filter into about 5 volumes of ethyl alcohol. Place in ice-water. The addition of ether renders the precipitation more complete. Begin filtration as soon as the precipitate begins to grow flocculent. Do not permit the filter to become empty till the last funnelful has been added. Place the filter-paper with its contents in a vacuum desiccator. Drying is complete in two or three days. It may now be collected and weighed. Dissolve a small amount in enough 0.9 per cent. salt solution to make a 10 per cent. solution, and determine its rotating power at 37° C. with the polariscope. If the rotation is more than 1 degree, dilute with salt solution till it is reduced to about 0.75 degree. This is merely for the sake of economy of material.

2. **Proving the Peptone Solution.**—Assuming that we are dealing with placental peptone, place in a test-tube 1 c.c. of the undiluted serum of a non-pregnant individual

(a male). The serum must conform to the requirements laid down for serum to be used in the dialyzation method. Add 1 c.c. of the peptone solution, mix, and place in a 2-c.c. polariscope tube. Place in the incubator till the temperature reaches 37° C. Determine the rotating power at hourly intervals. If there is no change, the peptone certainly contains free acid, or free baryta, as the case may be. If there is a change, make readings at hourly intervals, to establish the normal curve, by entering the readings on a chart in which the abscissa corresponds to the readings, and the ordinate corresponds to the intervals between readings. The normal curve once established is compared with readings taken in the actual test only at four- to six-hour intervals. Placental peptone is now on the market. Other organ peptones must be prepared by the worker.

The individual worker must establish by trying the margin of error for successive readings of the same fluid in his own case. This is usually about 0.02 degree. For the test this is doubled. A change of 0.5 degree or more is required to constitute a positive reaction.

3. **The Test Proper.**—This is carried out in essentially the same manner as the testing of the peptone solution. The work must, of course, be done aseptically. The serum used must conform to the requirements laid down for serum to be used in the dialyzation method. In each of three 2-c.c. polariscope tubes place respectively 1 c.c. peptone solution plus 1 c.c. salt solution; 1 c.c. patient's serum plus 1 c.c. salt solution; and 1 c.c. peptone solution plus 1 c.c. patient's serum. Place all tubes in the incubator or water-bath at 37° C., and make readings at four- to six-hour intervals up to about

sixteen hours. Unless the polariscope is provided with some adequate means for maintaining a constant temperature, the tubes must be removed from the incubator, placed in the polariscope, and read in the shortest possible time. When the reading in the third tube, containing both peptone and serum, varies by 0.05 degree *or more* from that given by the other tubes, the reaction is positive.

The only **sources of error** in the method are: (*a*) Precipitates or cloudiness of the solution. This is rare, and excludes error by rendering any reading whatsoever impossible. (*b*) Errors arising from making the readings at other than a uniform temperature. The first reading should be made at the end of one hour; the second, two hours later, and subsequent readings at 4- to 6-hour intervals. No reading should be attempted after thirty-six to forty-eight hours. (*c*) Most important, errors arising in the observer, due to visual fatigue. Only a few readings should be attempted at the same time, and not more than thirty seconds should be given to making a single reading.

APPENDIX

I. APPARATUS, REAGENTS, AND STAINS

THE apparatus and reagents listed here are sufficient for all but the rarer tests described in the text. Those in smaller type are less frequently required. For ordinary routine work a much smaller list will suffice.

A. APPARATUS

Beakers and flasks, several sizes, preferably of Jena glass.

Blood lancet, or some substitute (see Fig. 67).

Bunsen-burner or alcohol lamp.

Buret, 25-c.c. capacity, preferably with Schellbach stripe.

Buret and filter-stand combined.

Centrifuge—hand, electric, or water-power (see Figs. 20, 21). With the last two a speed indicator is desirable. Radius of arm when in motion should be $6\frac{3}{4}$ inches. Plain and graduated tubes accompany the instrument; milk-tubes (see Fig. 165) must be purchased separately. The hematocrit attachment (see Fig. 81) is not much used.

Corks, preferably of rubber, with one and two holes.

Cover-glasses, No. 1 or No. 2 thickness—$\frac{7}{8}$-inch squares are most convenient.

Cover-glass forceps, preferably Stewart's (see Fig. 87).

Esbach's tube (see Fig. 29).

Evaporating dish.

Filter-paper: ordinary cheap paper for urine filtration; "ashless" quantitative filter-paper for chemical analyses.

Glass funnels.

Glass rods and tubing of sodium glass: for stirring rods, urinary pipets, etc.

Glass slides: the standard 1- by 3-inch size will answer for all work, although a few larger slides will be found convenient; those of medium thickness are preferable.

Graduates, cylindric form, several sizes. At least one of these (the 50 c.c. is most useful) should be certified.

Graniteware basin.

Hemoglobinometer: see pp. 226 to 232 for descriptions of the different instruments.

Hemacytometer: either Türck, Neubauer, or Zappert ruling is desirable (see Figs. 75, 76, 82).

Hypodermic syringe: the "Aseptic Sub-Q, Tuberculin," is probably the most useful type.

Incubator (see p. 455).

Labels for slides and bottles.

Litmus-paper, red and blue, Squibb's preferred.

Mett's tubes (see p. 339).

Microscope (see Fig. 1). Equipment described on p. 42.

Petri dishes.

Platinum wires (see p. 455).

Sterilizers: the Arnold type for steaming; oven for dry sterilization (see p. 454).

Stomach-tube.

Test-glass, conical, one side painted half-white, half-black.

Test-tubes, rack, and cleaning brush.

Ureometer, Doremus-Hinds' pattern (see Fig. 24).

Urinometer, preferably Squibb's (see Fig. 17).

Blood-fixing oven, or Kowarsky's plate (see Fig. 88).

Copper-foil and gauze.

Cotton, absorbent, for filtering, etc.

"Cotton-batting" for plugging tubes.

Culture-media. The selection depends upon the work to be done (see p. 459).

Holt's cream gage and hydrometer (see Fig. 164).

Horismascope (see Fig. 28).

Pipets, graduated, 5- to 50-c.c. capacity. For some serodiagnostic methods a few pipets of 10-c.c. capacity graduated in one-tenths, and 0.1-c.c. graduated in one-one-hundredths, will be required.

Ruhemann's tube for uric-acid estimation (see Fig. 27).

Saccharimeter (see Fig. 31).

Scales, about 0.1 to 100 gm. capacity.

Strauss' separatory funnel for lactic-acid test (see Fig. 106).

Suction filter.

Urinopyknometer of Saxe (see Fig. 18).

Widal reaction outfit: either living agar cultures of the typhoid bacillus, or the dead cultures with diluting apparatus, which are sold under various trade names.

Water-bath.

B. REAGENTS AND STAINS

All stains and many reagents are best kept in small dropping bottles, of which the flat top "T. K." pattern is probably most satisfactory. Formulæ are given in the text. Dry stains (Grübler's should be specified)

and most staining solutions and chemical reagents can be purchased of the Denver Fire Clay Co., Denver, Colorado; Bausch & Lomb Optical Co., Rochester, New York; or Eimer & Amend, New York. For the physician who does only a small amount of work the "Soloid" tablets manufactured by Burroughs, Wellcome & Co. are convenient and satisfactory. These tablets have only to be dissolved in a specified amount of fluid to produce the finished stain. Most of the stains and many of the reagents mentioned here come in this form.

Acid, glacial acetic. Other strengths can be made from this as desired.

Acid, hydrochloric, concentrated (contains about 32 per cent. by weight of absolute hydrochloric acid). Other strengths can be made as desired.

Acid, nitric, strong, colorless.

Acid, nitric, yellow. Can be made from colorless acid by adding a splinter of pine, or allowing to stand in sunlight.

Acid, sulphuric, concentrated.

Alcohol, ethyl (grain-alcohol). This is ordinarily about 93 to 95 per cent., and other strengths can be made as desired.

Aqua ammoniæ fortior (sp. gr. 0.9).

Bromin, or Rice's solutions (see p. 118), for urea estimation.

Chloroform.

Diluting fluid for erythrocyte count (see p. 240).

Diluting fluid for leukocyte count (see p. 258).

Dimethyl-amido-azobenzol, 0.5 per cent. alcoholic solution.

Distilled water.

Esbach's or Tsuchiya's reagent (see p. 134).

Ether, sulphuric.

Ferric chlorid: saturated aqueous solution and 10 per cent. aqueous solution.

Guaiac, powdered.

Haines' (or Fehling's or Benedict's) solution (see pp. 138, 139).

Lugol's solution (*Liquor Iodi Compositus*, U. S. P.). Gram's iodin solution (see p. 74) can be made from this by adding fourteen times its volume of water.

Obermayer's reagent (see p. 114).

Oil of cedar for immersion.

Phenylhydrazin, pure.

Phenol.

Phenolphthalein, 1 or 0.5 per cent. alcoholic solution.

Purdy's (or Fehling's or Benedict's) solution (see pp. 142–144).

Robert's reagent (see p. 132).

Sodium chlorid (table salt), saturated aqueous solution.

Sodium hydroxid (caustic soda), 40 per cent. solution; other strengths can be made from this as desired.

Sodium hydroxid, decinormal solution. The practitioner will find it best to purchase this solution ready prepared. Elmer and Amend, New York, and many other chemical supply houses carry it in stock. For ordinary clinical work 41 gm. of Merck's "sodium hydrate by alcohol" from a freshly opened bottle may be dissolved in 1000 c.c. water. This makes a normal solution and must be diluted with 9 volumes of water to make the decinormal solution.

Sodium nitrite, 0.5 per cent. solution for diazo-reaction. Must be freshly prepared.

Sulphanilic acid solution for diazo-reaction (see p. 161).

Stains.—It will be most satisfactory to have on hand a stock of dry stains (which keep well) and to make the solutions as needed. Ordinarily the smallest quantity obtainable in an unbroken package should be purchased. The following dry stains should be sufficient for the ordinary clinical laboratory: Eosin, w. g.; fuchsin, acid; fuchsin, basic; gentian-violet; methylene-blue, B. X. or Ehrlich's rectified; methyl-green; pyronin; Sudan III; Wright's stain. The most frequently used solutions are:

Carbolfuchsin (see p. 65).

Eosin, saturated aqueous solution.

Formalin-gentian-violet, or anilin-gentian-violet (see p. 74).

Gabbet's stain or Pappenheim's methylene-blue stain (see pp. 65, 66).

Löffler's alkaline methylene-blue solution (see p. 74).

Pappenheim's pyronin-methyl-green stain (see p. 467).

Stain for fat: Sudan III, saturated solution in 70 per cent. alcohol; or 1 per cent. aqueous solution osmic acid.

Wright's, Harlow's, or Jenner's stain for blood.

Tincture of guaiac, diluted to a light sherry-wine color (keep in a dark glass bottle).

Turpentine, "ozonized" (see p. 157).

Acid, boric, for preserving urine (see p. 86).

Acid, oxalic.

Acid, salicylous (salicyl aldehyd), 10 per cent. alcoholic solution.

Alcohol, amylic.

Alcohol, ethyl, absolute.

Alcohol, methyl (pure).

Ammonium sulphate, C. P.

Antiformin (see p. 68).

Barium chlorid mixture (see p. 112).

Benzidin.

Boas' reagent or Günzburg's (see p. 326).

Benzol.

Boggs' reagent (see p. 444).

Calcium chlorid, 1 per cent. solution.

Canada-balsam in xylol: necessary only when permanent microscopic preparations are made.

Carbon disulphid.

Charcoal, animal.

Chromium trioxid.

Congo-red, strong alcoholic solution.

Copper sulphate.

Diluting fluid for blood-platelet count (see pp. 260, 261).

Egg-albumen disks in glycerin (see p. 293).

Ether, acetic, pure.

Florence's reagent (see p. 450).

Formalin (40 per cent. solution of formaldehyd gas).

Hydrogen dioxid.

India-ink ("*nach Burri*," Grübler) (see p. 448).

Iodin crystals.

Iron sulphid.

Lead acetate (sugar of lead); used in 10 per cent. solution to clarify urine.

Lead acetate, tribasic.

Lime-water.

Müller's fluid saturated with mercuric chlorid (see p. 72).
Orcinol.
Pepsin, U. S. P.
Phenylhydrazin hydrochlorid.
Potassium ferrocyanid, 10 per cent. solution.
Potsssium oxalate (neutral).
Potassium persulphate.
Resorcinol.
Ruhemann's reagent (see p. 125).
Silver nitrate crystals; also 12 per cent. aqueous solution, and "ammoniated" solution (see p. 123).
Sodium alizarin sulphonate, 1 per cent. aqueous solution.
Sodium carbonate.
Sodium chlorid, 2 per cent. solution; from this normal salt solution (0.8 per cent.) can be made as desired.
Sodium hyposulphite.
Sodium nitroprussid.
Sodium sulphate.
Staining solutions:
- Carbol-thionin.
- Ehrlich's triple stain for blood.
- Fuchsin, weak solution; can be made when desired by adding a little carbolfuchsin to a test-tube of water.
- Gentian-violet, saturated alcoholic solution.
- Giemsa's stain (see p. 447).
- Methylene-blue and borax solution (see p. 302).
- Methylene-blue, saturated aqueous solution for blood.
- Van Gieson's stain for Negri bodies (see p. 452).

Sulphur, powdered.
Talc, purified (Talcum Purificatum, U. S. P.).
Toluol.
Trichloracetic acid solution (see p. 131).
Uranium nitrate, 5 per cent. aqueous solution.
Xylol.
Zinc, arsenic-free.

II. WEIGHTS, MEASURES, ETC., WITH EQUIVALENTS

METRIC

Meter (unit of length): Millimeter (mm.) = $\frac{1}{1000}$ meter. Centimeter (cm.) = $\frac{1}{100}$ meter. Kilometer = 1000 meters. Micron (μ) = $\frac{1}{1000}$ millimeter.

Gram (unit of weight): Milligram (mg.) = $\frac{1}{1000}$ gram. Kilogram (kilo.) = 1000 meters.

Liter (unit of capacity): Cubic Centimeter = $\frac{1}{1000}$ liter. Same measure as milliliter (ml.).

1 Millimeter = { 0.03937 ($\frac{1}{25}$ approx.) in. ; 1000 microns.

1 Centimeter = { 0.3937 ($\frac{8}{20}$ approx.) in. ; 0.0328 feet.

1 Meter = { 39.37 in. ; 3.28 feet.

1 Micron (μ) = { $\frac{1}{25000}$ in. ; 0.001 millimeter.

1 Gram = { 15.43 grains. ; 0.563 dram ; 0.035 ounce ; 0.0022 pound } Avoir. ; 0.257 dram ; 0.032 ounce ; 0.0027 pound } Apoth.

1 Kilogram = { 35.27 ounce (Avoir.). ; 2.2 pound (Avoir.).

1 Liter = { 1.056 (1 approx.) quart. ; 61.02 cu. inches. ; 1000 cu. centimeters.

1 Sq. Millimeter = 0.00155 ; 1 Sq. Centimeter = 0.1550 ; 1 Sq. Meter = 1550 } sq. in. ; 1 Sq. Meter = 10.76 sq. feet.

1 Cu. Millimeter = 0.00006 ; 1 Cu. Centimeter = 0.0610 } cu. in. ; 1 Cu. Centimeter = 0.001 liter.

1 Cu. Meter = { 35.32 cu. feet. ; 61025.4 cu. in.

1 Inch = 25.399 millimeters. ; 1 Sq. Inch = 6.451 sq. centimeters. ; 1 Cu. Inch = 16.387 cu. centimeters.

1 Foot = 30.48 centimeters. ; 1 Sq. Foot = 0.093 sq. meter. ; 1 Cu. Foot = 0.028 cu. meter.

AVOIRDUPOIS WEIGHT

1 Ounce = { 437.5 grains. ; 16 drams.

1 Pound = 16 ounces.

1 Grain = 0.065 ($\frac{3}{50}$ approx.) ; 1 Dram = 1.77 ($1\frac{3}{4}$ approx.) ; 1 Ounce = 28.35 (30 approx.) ; 1 Pound = 453.59 (500 approx.) } grams. ; 1 Pound = 27.7 cu. inches. ; 1 Pound = 1.215 lb. Troy.

APOTHECARIES' MEASURE

1 Dram = 60 minims. ; 1 Ounce = 8 drams. ; 1 Pint = 16 ounces. ; 1 Gallon = 8 pints.

1 Dram = 3.70 ; 1 Ounce = 29.57 ; 1 Pint = 473.1 ; 1 Gallon = 3785.4 } cu. centimeters. ; 1 Gallon = 231 cu. inches.

APOTHECARIES' WEIGHT

1 Scruple = 20 grains.
1 Dram = 3 scruples = 60 grains.
1 Ounce = 8 drams = 480 grains.
1 Pound = 12 ounces.

1 Grain = 0.065
1 Dram = 3.887
1 Ounce = 31.10
1 Pound = 373.2
} grams.

To convert	*minims*	into	*cubic centimeters*	multiply by	0.061
" "	*fluidounces*	"	*cubic centimeters*	" "	29.57
" "	*grains*	"	*grams*	" "	0.0648
" "	*drams*	"	*grams*	" "	3.887
" "	*cubic centimeters*	"	*minims*	" "	16.23
" "	*cubic centimeters*	"	*fluidounces*	" "	0.0338
" "	*grams*	"	*grains*	" "	15.432
" "	*grams*	"	*drams*	" "	0.257

TEMPERATURE

CENTIGRADE.	FAHRENHEIT.	CENTIGRADE.	FAHRENHEIT.
110°	230°	37°	98.6°
100	212	36.5	97.7
95	203	36	96.8
90	194	35.5	95.9
85	185	35	95
80	176	34	93.2
75	167	33	91.4
70	158	32	89.6
65	149	31	87.8
60	140	30	86
55	131	25	77
50	122	20	68
45	113	15	59
44	111.2	10	50
43	109.4	+5	41
42	107.6	0	32
41	105.8	—5	23
40.5	104.9	—10	14
40	104	—15	—5
39.5	103.1	—20	—4
39	102.2		
38.5	101.3	0.54° =	1°
38	100.4	1 =	1.8
37.5	99.5	2 =	3.6
		2.5 =	4.5

To convert Fahrenheit into Centigrade, subtract 32 and multiply by 0.555.

To convert Centigrade into Fahrenheit, multiply by 1.8 and add 32.

INDEX

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