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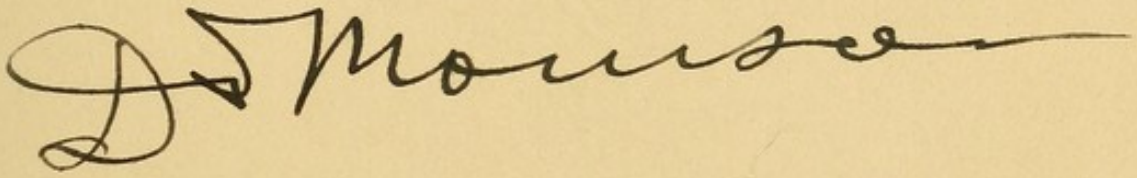

DIAGNOSTIC METHODS

WEBSTER

DIAGNOSTIC METHODS

CHEMICAL, BACTERIOLOGICAL AND MICROSCOPICAL

A Text-book for Students and Practitioners

BY

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WITH 37 COLORED PLATES
AND 164 OTHER ILLUSTRATIONS

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TO THE MEMORY

OF

MY FATHER

DR. JOHN RANDOLPH WEBSTER

THIS VOLUME IS

LOVINGLY DEDICATED

PREFACE.

In the present work the writer has attempted to bring together, for the use of the student and practitioner, the generally accepted facts regarding the various phases of clinical medicine, which may be rather more closely studied by the application of laboratory methods than without their use. It is to be especially emphasized that laboratory work must go hand in hand with the more direct clinical examination of the patient, as the former can be interpreted only in the light of the latter. While it is true that, in some cases, the laboratory findings may be of even more value than those of physical examination, yet it is to be understood that the function of the Clinical Laboratory is, more largely, perhaps, an accessory one, the application of methods of physical diagnosis usually pointing out the way toward a successful solution of the clinical problem by special laboratory methods, which yield numerous confirmatory or differential points not at all clearly defined by the methods of direct clinical examination.

The aim of the author has been to present the direct bearing of the various methods outlined upon the clinical history of the case and to point out the special interpretation of the findings in any given examination. Particular attention has been directed to the selection of methods, both for the simpler clinical and more complex scientific requirements. As no method, no matter how exact it may be or how sound its basic principle, will yield reliable results in the hands of the inexperienced, the writer has paid much attention to the details of such methods and has endeavored to direct the thought of the worker to the possible obstacles to be overcome before he is able to properly perform these examinations and interpret his results.

The writer makes no claim for originality except, possibly, in the matter of arrangement of subject-matter and selection of methods and ideas, which have been established by others after years of earnest research. His endeavor has been to be as catholic as possible in his reading and as selective as his judgment permitted, to the end that the student or practitioner be saved the burden of sifting the wheat from the chaff.

Numerous text-books, monographs, and special articles have been freely used in the preparation of the text, the writer attempting in each case to give due credit for such reference. It is possible that some direct use of material has been made which has not received deserved recognition. If so, the writer,

here acknowledges his indebtedness to them as well as to those whom he has directly quoted.

It has seemed desirable to omit extensive reference to the literature, as a bibliography to be of working value must be much more extensive and complete than is possible within the scope of this book. The writer has, however, inserted at the end of each chapter a list of the more important larger works, which he has found useful in correlating the general subjects included in the several sections.

In conclusion, the writer wishes to express his deep obligation to his colleagues for their many valuable suggestions as to the subject-matter of the text: to Prof. W. S. Haines for assistance in revising the section on Urine; to Dr. J. M. Washburn for many additions and revisions of the section on Blood; to Dr. O. J. West for many practical points throughout the whole work; to Dr. W. A. Pusey for photographs of Blastomycetes under various conditions; to Dr. Brown Pusey for slides showing various organisms in the conjunctival exudates; and to Dr. N. Gildersleeve, of the University of Pennsylvania, for illustrations of megalosporon and microsporon. Further, the writer wishes to express his thanks to Miss Hill for the excellent work done by her in preparing the original drawings which appear throughout the work.

RALPH W. WEBSTER.

100 STATE ST., CHICAGO,

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DIAGNOSTIC METHODS.

CHAPTER I.

THE SPUTUM.

I. General Considerations.

At the present time the examination of the sputum is more or less limited to the search for various specific organisms, especially the bacilli of tuberculosis and of pneumonia. This is very much to be regretted, as frequently the appearance, amount, consistency, color, and other characteristics are of great aid and have led our older brothers to correct diagnoses before the days of microscopic examination.

The sputum, strictly speaking, should be considered as the material which comes from the respiratory passage anywhere along its course. It may be, therefore, of laryngeal, bronchial, or alveolar origin. More commonly, however, we find the sputum considered by the general practitioner and by the laity as anything which is expectorated, so that specimens which consist of nothing more than salivary secretion are frequently sent to laboratories for examination. In some cases, especially of inflammation of the naso-pharynx or perforation from neighboring organs, this buccal secretion may be mixed with material from the nose, mouth, ear, or esophagus. For these reasons, if for no others, it is absolutely essential that frequent examinations of sputum, in the strict sense, be made before a negative diagnosis of a suspected condition may be given.

As a rule, any sputum at all should be considered pathological, as normal persons raise little or nothing from the lungs at any time. By this is not meant that serious disease may obtain when a small amount of sputum exists, as patients suffering with catarrhal conditions of the naso-pharynx frequently have an accumulation of material, which has settled in the bronchial tubes over night and is raised in the morning. Those of us who are unfortunate enough to live in atmospheres which are loaded with soot and dirt frequently raise a certain amount of sputum which arises from an increased activity of the mucous membrane of the respiratory passages to compensate for the dryness and irritation which these foreign substances have caused. This morning sputum is small in amount and is in the form of large, tough, elastic masses which very much

resemble boiled sago. These masses are at times extremely dark in color, due to the dirt which has been taken in with the inhaled air. In such sputum we find much mucus, degenerated epithelium, pus cells and various micro-organisms. These organisms are rarely of pathologic significance, although many pathogenic types may be present.

The sputum should be collected in receptacles which may be completely and easily disinfected. The best receivers for the sputum are the ordinary paper spit-cups which can be burned as occasion may demand. The practice of expectorating upon cloths is only to be advised when these cloths are immediately burned. In the spit-cup should always be contained a certain amount of disinfectant, such as dilute carbolic acid or corrosive sublimate solution, so that no chance of transference of infection may arise. Neglect of such precautions has frequently led to serious consequences in the case of the healthy members of the family. When sputum is to be collected for examination, the material raised by coughing should be received in a wide-mouthed bottle which contains no disinfectants, as these agents coagulate the protein material of the infecting organism and in some cases change its staining characteristics. Immediately after examination of such material it should, of course, be treated with the disinfecting solution.

II. Physical and Chemical Characteristics.

Amount.

Some general idea of the amount of sputum expectorated is always advisable. It is rarely necessary, however, to make any collection of the material during the 24 hours' period, as one can usually gain the information by questioning the patient or nurse as to the amount passed from time to time. The amount of sputum expectorated in 24 hours depends very much upon the nature of the pathologic condition. In cases of so-called dry bronchitis, diffuse bronchitis, early tuberculosis, and occasionally of lobar pneumonia, the sputum is so viscid that there is practically none obtained. In cases of chronic bronchitis, tuberculosis with cavity formation, and bronchiectasis we find large amounts; while in cases of lung abscess or of perforating pleurisy, blood or pus may flow from the mouth in very large quantities. The absolute amount of sputum may, therefore, vary from a cubic centimeter to a liter or more. An extensive expectoration will naturally have more or less serious effect upon the patient's general nutrition, so that we are not surprised to find cases in which as high as 5 per cent. of the total nitrogen eliminated passes from the system in the sputum (Lenz).

Consistency.

As a rule, the consistency of the sputum varies from that of a liquid to a highly tenacious material, inversely as the amount of sputum. This latter statement holds in most cases with the exception of pneumonia, in which we

have an extremely tenacious sputum and, also, a very abundant one. Just what substance induces this extreme tenacity is uncertain, but it may be generally said that mucin is the causative factor, although the tenacious pneumonic sputum shows very little mucin. According to Kossel, the tenacity may be due to the presence of nuclein derivatives. In the early stages of acute bronchitis, or bronchial asthma, and in whooping-cough the sputum is usually very tenacious and ropy; while in edema of the lungs, pulmonary abscess, putrid bronchitis, and pulmonary gangrene it is very watery and contains large numbers of pus-cells.

Reaction.

The fresh specimen of sputum is usually alkaline in reaction. However, in cases in which the sputum has remained in the lungs for some time, as in cavity formation, the reaction is acid.

Color.

The color of the sputum may range from that of a colorless material to one showing any of the tints of the rainbow. These colors are due to admixtures of various abnormal products with the sputum. A bright red sputum is significant of the presence of blood, according to Traube, unchanged red blood-cells necessarily being present. The amount of blood may vary from a light streaking of the sputum to one showing a deep red, rusty, or prune-juice color. Such bloody sputa are found after trauma, pneumonia, gangrene, hemorrhagic infarction of the lungs, chronic passive congestion, as well as adventitious mixture of sputum with nasal or pharyngeal material containing blood. The blood may be due to rupture of a vessel and may then constitute the condition known as pulmonary hemorrhage. This condition of hemoptysis is frequently confounded with that of hematemesis and is differentiated by the fact that the blood in hemoptysis is frothy, bright red in color, alkaline in reaction, and usually associated with mucopurulent material; while the blood in hematemesis is frequently dark and grumous, usually clotted and acid in reaction. In sputa which are tinted by changed hemoglobin, the color is most varied. Many oxidation products of hemoglobin are found in the sputum, depending upon the time which the sputum has lain in the lungs. Thus, for instance, in pneumonia we find a rusty, prune-juice colored sputum, whose color seems to be due to an unknown derivative of hemoglobin. The shade of color in pneumonia may, however, range from red through brown to green. In cases of mitral disease associated with passive congestion of the lungs we find, frequently, a light brown color, due to the presence of hematoidin granules in the epithelial cells.

In many cases of abscess of the liver which perforates into the lung or in catarrhal jaundice, bile pigments may be found in the sputum which may give rise to various colored sputa from red through blue to green. Although, as shown in a later table, the bile pigments and hemoglobin are very closely related chemically, yet the clinical significance of the appearance of bile pigment

in such cases as the above is very important. In many cases of true jaundice the sputum, in case any exists, may show a distinct grass-green color due to the presence of oxidized bile pigments. This same green color frequently appears in the sputum, however, in cases of croupous pneumonia during lysis. In this latter case the color is due, probably, to the same pigment which is oxidized before expectoration. In these cases of pneumonia with green sputum and no jaundice, a fresh involvement of the lung is usually associated with a rusty sputum. Besides jaundice and pneumonia we may have, as causes of green sputum, certain chloromata of the lungs as well as the development of chromogenic bacteria within the lungs. This action of chromogenic bacteria is not always observed when the sputum is expectorated, but may appear only after the sputum has stood. The presence of the bacillus pyocyaneus may give a sputum which is brilliant blue or greenish in tint.

Sputa very frequently show changes in color as well as consistency due to various substances inhaled. Thus we find a distinctly black sputum, in cases of anthracosis, in coal-miners and in many city residents. So frequent is this occurrence that the lung tissue may even be invaded by the coal pigment. The writer has been told by one of our most prominent pathologists that he has never seen, postmortem, a lung of a city resident which did not contain large amounts of coal pigment. This would partly substantiate the statement made by some writers that the pigment in the sputum is only the dust which had been taken up by the phagocytic cells as this dust is on its way to the lungs. Workers in bronze and brass as well as other metals frequently show a sputum tinged red with ferric oxide, arising from a condition of the lung known as siderosis. Stone-cutters frequently show much stone-dust in the sputum which is characteristic of the condition of chalicosis, stone-cutters' phthisis, or grinders' rot. Workers in flour mills and in bakeries frequently expectorate doughy masses, while those in cotton mills show the presence of cotton fibers in their sputum. Finally it may be said that the color of the sputum may be changed by the presence of certain foods, such as milk, eggs, and chocolate, while tobacco users frequently have a sputum tinged dark brown.

Odor.

Ordinarily the sputum has no odor unless it has stagnated either in the receiving cup or in the system. Such old sputum sometimes has a very distinct putrefactive odor. The odor of sputum in tuberculosis and bronchiectasis is peculiarly heavy and sweet, while that in putrid bronchitis is often extremely offensive. In cases of perforating empyema a peculiar cheese-like odor is observed. While these odors are not characteristic in themselves, they are usually more or less significant of the condition with which they are commonly associated.

Character of the Sputum.

The character of the sputum has reference more to the apparent composition of the sputum than to its consistency. Air is usually present in the

sputum in various amounts, so that one may judge, from the size of the air-bubbles, of the size of the bronchi from which the sputum came. The sputum from cavities and large bronchi contains no air and, therefore, sinks in water. This is the so-called *sputum fundum petens*.

Sputum known as mucoid sputum is glairy, transparent, and tenacious, becoming cloudy on the addition of acetic acid due to the precipitation of mucin. This type of sputum is found particularly in acute bronchitis and in asthma.

A mucopurulent sputum is one containing both pus and mucoid material. Small amounts of pus give a whitish color, either to the whole or to portions of the sputum, the pus being observed in masses or in streaks through the mucoid material. Larger quantities of pus give a yellowish or occasionally a yellowish-green tinge to the sputum. In this latter type the pus and mucus seem to be mixed homogeneously. In the sputum from cavities we find the mucopurulent material arranging itself flatly like a coin, constituting the so-called "*nummular*" sputum.

Purulent sputum is found in cases of ruptured empyema, abscess of the lung, and in some cases of bronchiectasis. This purulent sputum differs from the mucopurulent type in the fact that the pus is much more abundant and is almost in the pure state, being mixed with a small amount of tenacious mucus.

In some cases, especially in edema of the lungs, a sputum is obtained, known as serous sputum, which is colorless and quite frothy. This sputum resembles very closely the ordinary salivary secretions and should not be confused with it.

In many cases, especially in putrid bronchitis, gangrene of the lung, and bronchiectasis, the sputum on being voided into a cylinder will separate into three distinct layers, occasionally into four. The upper layer is of frothy mucus; a second layer, which is not always present, consists of certain albuminous material which hangs in long shreds down into the third layer, which consists of the sero-pus and is usually opaque and watery. The bottom layer contains the morphological elements, pus, tissue shreds, and bacteria.

Besides the varieties of sputum named above, the admixture of blood may give rise to sputum which is known as sanguinous sputum or as sanguino-mucopurulent or purulent sputum.

Chemical Properties.

The chemical properties are of little importance and are practically never investigated in a study of the sputum. The chief chemical examination is applied to the detection and estimation of the amount of albumin and of mucin in certain specimens of sputum. The estimation of the soluble albumin will be discussed fully in the section on urine, so that we may, for the present, pass this subject by with the statement that pneumonia, pulmonary edema, and perforating empyema are associated with a marked increase in the albumin of the sputum. It is sometimes of importance to know, in a general way, whether mucin or albumin is increased, so that in such cases the Zenoni test may prove

valuable. This test is as follows: Some of the sputum is spread upon a slide or cover-glass and is treated with alcohol for 15 minutes. It is then stained with a half-saturated aqueous solution of safranin, which will show the albumin distinctly red and the mucin yellow. Considerable mucin is found in chronic bronchitis, while in the pneumonic and tubercular cases the amount is much less. Wanner states that a trace of albumin in a case of suspected incipient tuberculosis will frequently distinguish it from one of chronic bronchitis, while a large amount of albumin indicates pneumonia or pulmonary edema.

Besides these albuminous principles, the sputum, especially in cases of gangrene and putrid bronchitis, contains a ferment very much resembling in its action the trypsin of the pancreatic juice (Stolnikow). This ferment seems to indicate a highly destructive process in the lung tissue. Other chemical substances, such as glycogen and fatty acids, are frequently found in the sputum, but may be passed with mere mention. The so-called myelin granules or globules, which appear in the alveolar cells of the sputum, consist largely of fatty principles, such as protagon, lecithin and cholesterin.

III. Macroscopic Examination.

While much that is included in the previous section would come properly under the head of the macroscopic examination of the sputum, the writer has reference more, in this connection, to the appearance of macroscopic elements as distinguished from those which are purely microscopic.

(1). Cheesy Masses.

Frequently one finds in the sputum small cheese-like particles which vary in size from that of a pin-point to that of a pea, the large majority being about the limit of ordinary vision. These cheesy masses are fragments of necrotic tissue and appear in the larger form in cases of abscess or gangrene of the lung, while in tuberculosis they are always small unless the cavity, from which the material is derived, is markedly necrotic. The color of these masses varies from a yellow to a black. Those fragments which come from an abscess are of yellow color due to the presence of much pus, the darker ones contain decomposition products of hemoglobin, while many of them are tinged a deep black with coal pigment. If the sputum be squeezed between two glass plates these cheesy particles or fragments can sometimes be more distinctly seen. They are preent in largest numbers in the so-called "nummular" sputum from a tuberculous cavity. This nummular sputum derives its name from the fact that such material floats upon the surface in a coin-like mass, and then sinks to the bottom.

(2). Dittrich's Plugs.

These masses are similar to the small caseous particles above mentioned and are frequently expectorated by perfectly normal individuals. The true

plugs are distinct casts of the bronchi or bronchioles and vary in size from pin-point to that of a bean, the majority being about the size of a mustard seed. The smaller ones are opaque and yellowish-white in color, while the larger ones have a distinct gray tinge. They are usually expectorated free from pus or mucus, so that they frequently give rise to anxiety, especially in those of a hypochondriac tendency. These plugs have a distinctly disagreeable odor, which is more evident if they are crushed on the glass plates. Microscopic examination of these masses shows large clumps of bacteria, fatty acid crystals, free fat globules, and cellular detritus. Occasionally a few leucocytes are found, but these are rare, while pigment granules, either of hematogenous or extraneous origin, are sometimes observed. While such plugs are especially numerous in cases of putrid bronchitis and bronchiectasis, they are frequently found in the crypts of the normal tonsil as well as in cases of follicular tonsillitis or of ozena.

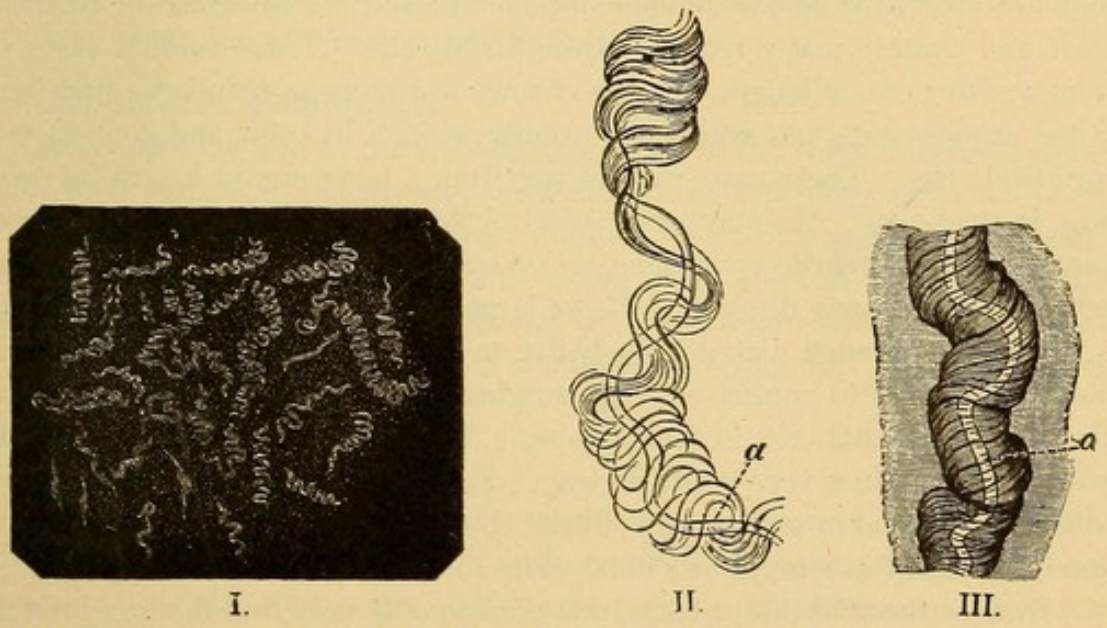


FIG. 1.—Curschmann's spirals (*Tyson after Curschmann*). I, Natural size; II and III, enlarged; *a*, *a*, central thread.

(3). Curschmann's Spirals.

These structures are found in the sputum in practically every case of true bronchial asthma and have been reported in acute bronchitis, croupous pneumonia, chronic pulmonary tuberculosis, and occasionally in chronic bronchitis. They are not present in every paroxysm of asthma, but are more frequently found just at the end of the paroxysm and are absent when the sputum becomes mucopurulent. They seem to be derived through true exudation from the bronchioles, as Curschmann says, a bronchiolitis exudativa. These structures are recognizable, to a certain extent, by the naked eye, but for their absolute identification a microscopic examination is essential. They are composed of a spirally-twisted network of very delicate fibrils, in the meshes

of which are numerous epithelial cells and eosinophile leucocytes. Along with these cellular bodies one frequently finds large numbers of diamond-shaped crystals, known as the Charcot-Leyden crystals, which will be discussed later. This spirally-twisted mass (the mantle) seems to be wound around a central light thread. While this is the structure of the complete spiral, we frequently find variations pointing apparently to two distinct forms. The first is the spirally-twisted strand of mucus with the enclosures above mentioned. The second is the tight spiral mass of mucus wound around a central fibre. This central fibre is very refractive and is homogeneous in structure, varying in size from 1/2 to 18 microns in diameter. The length of these spirals is from 1 to 2 cm. and their breadth about 1 mm.

(4). Fibrinous Casts.

By the term fibrinous cast we refer more directly to true bronchial casts, which are composed of fibrin. These are observed in pneumonia, in which case they are derived from the smaller bronchioles and are brownish or reddish in color and contain many red and white blood-cells. These smaller casts vary from 1/2 to 3 cm. in length. In the chronic fibrinous bronchitis we find the so-called arborescent casts which are usually whitish in color and contain many epithelial cells. These casts vary in size from 1 to 15 cm. in length by several mm. in thickness. We may have an acute form of fibrinous bronchitis accompanying various febrile conditions, so that similar casts may appear in almost any of the infectious diseases. These larger casts are fairly firm, usually have a lumen, and branch dichotomously five to ten times. Microscopic examination shows them to consist of large numbers of longitudinal fibres containing blood and epithelial cells in their meshes. They may be stained with the Weigert fibrin stain in a very beautiful way. Such staining methods show that not all of the material in such casts is fibrin, so that it may be necessary to rename these structures as simple bronchial casts rather than as fibrinous forms.

Besides these fibrinous casts, one occasionally may find distinct bronchial casts composed of the mycelium of various fungi. Thus mycelial threads of the aspergillus have been reported by Osler, Devillers and Renon.

(5). Concretions.

This name is applied to anything, expectorated with the sputum, which has the appearance or consistency of a stone. These concretions are formed in dilated portions of the bronchi or in cavities by the calcification of the stagnated contents. Although concretions consisting of cartilaginous or osseous material are frequently found postmortem, yet there seems to be little mention in the literature of any such formations being expectorated.

(*a*). Bronchioliths.

These concretions are formed by the deposition of calcium salts in the stagnated contents of the bronchus or of a cavity. They may be derived from the smaller or larger bronchi, but rarely form arborescent shapes, being usually

irregular and varying in size from a pin-head to that of a walnut. They are usually single, but may be multiple. They vary in consistency from that of chalk to a stone, and may be expectorated in small numbers over long periods of time.

(*b*). **Pneumoliths.**

These lung stones are in the majority of cases of tuberculous origin. They usually arise by the calcification of caseous areas which later ulcerate into a bronchus and are expectorated unless too large. They may, also, arise by the calcification of a pulmonary cavity or of a bronchial lymph-gland. These lung stones consist of the carbonates, phosphates, and sulphates of calcium and magnesium, in some one or more salts predominating, while in others still different combinations may exist. These pneumoliths either have a chalky or a calcareous consistency and vary in size from that of a pin-head to that of a tennis-ball. These lung stones are usually expectorated *en masse* or in the form of smaller portions of a larger stone. In some cases these smaller stones may reach the number of 500 (Portal).

(6). **Echinococcus Membranes.**

Rarely one may find in the sputum fragments of the walls of echinococcus cysts or their contents. These may come from a perforating cyst of the liver, kidney, or lung. The presence of the laminated membrane and of the parasitic scolices and hooks makes it possible to arrive at an absolute diagnosis of the origin of such material. The membrane is thick, tough, and of a porcelain-like color and may show a laminated or fibrillated structure. The parasite is discussed in a later section.

(7). **Foreign Bodies.**

Examination of the sputum may reveal the presence of material which has lodged in the air-passages and been retained for long periods of time. Such bodies are coins, fish-bones, and cherry-stones. Heyfelder reports a case of the expectoration of a wooden cigar-holder 11 1/2 years after its disappearance.

IV. Microscopic Examination.

The microscopic examination of the sputum is almost the only one to which it is subjected at the present day. This is to be regretted, as much may be learned from a careful macroscopic examination. However, a microscopic examination reveals evidence which points to an absolute diagnosis more frequently than does the macroscopic examination. Before making the microscopic examination, it is wise to place the sputum in a flat-bottomed dish (Petri dish) which has half of its base blackened, so that the more suspicious particles may be selected for microscopic investigation. Some experience in this work is necessary, as one is frequently called upon to recognize material which is purely extraneous and has absolutely nothing to do with the sputum. Such material practically always comes from the buccal cavity and consists of frag-

ments of various food-stuffs, such as bread, fruit pulp, meat fibers, vegetable tissue, and portions of tobacco leaf. Naturally, such material should not mislead one, but it very frequently does. The fragments of meat tissue contain elastic tissue fibers and may lead one to state that such material is present in the sputum, thus giving expression to the possibility of a diagnosis of incipient tuberculosis.

A portion of the sputum selected for examination is taken up with a platinum loop and spread in a thin layer upon a glass slide. It is then dried by

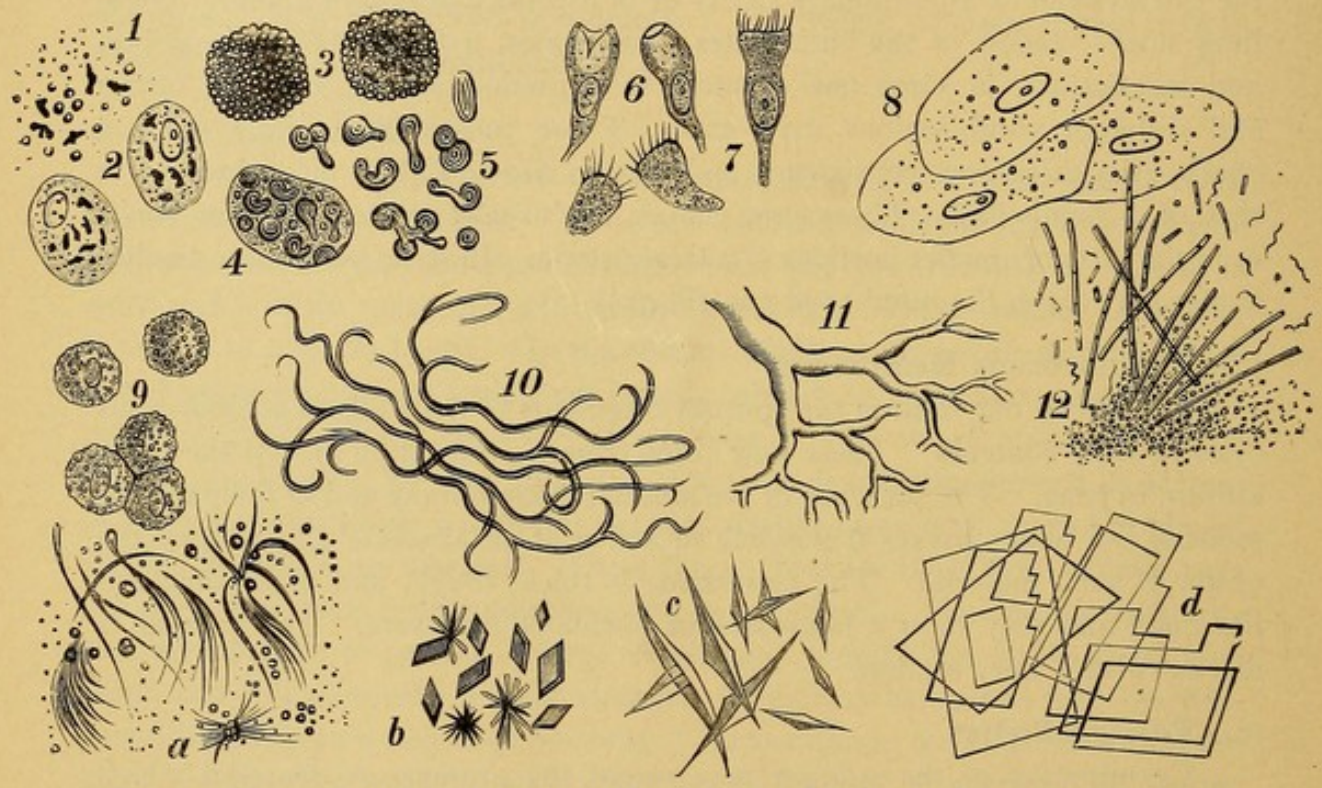


FIG. 2.—Objects found in the sputum (*Landois*). 1, Detritus and dust-particles; 2, pigmented alveolar epithelium; 3, fatty degenerated and partially pigmented alveolar epithelium; 4, alveolar epithelium showing myelin-degeneration; 5, free myelin forms; 6, 7, desquamated ciliated epithelium, partly changed and deprived of its cilia; 8, squamous epithelium from the mouth; 9, leucocytes; 10, elastic fibers; 11, fibrinous cast of a small bronchus; 12, leptothrix buccalis, together with cocci, bacilli, and spirochete; *a*, fatty acid crystals and free fatty granules; *b*, hematoidin; *c*, Charcot's crystals; *d*, cholesterin.

passing the slide several times through the flame, care being taken not to burn the specimen. The smear is allowed to cool and is then stained either with Löffler's methylene blue for general purposes or with special stains for the various specific organisms.

(*a*). Pus-cells (Leucocytes).

There is practically no specimen of sputum which does not contain leucocytes in larger or in smaller numbers. The true pus-cell is the polymorphonuclear neutrophile and appears in the sputum frequently filled with fat globules, or pigment granules. In cases of asthma, the eosinophile cells are very abundant, while basophile cells may occasionally obtain. Although these cells

are so frequent in asthma, a diagnosis should not rest upon such evidence alone, as there seems to be a form of bronchitis, possibly of the tubercular variety, which has been named "eosinophilic bronchitis" from the large number of such cells observed. In pulmonary tuberculosis, which is not associated with a mixed infection, it is very common to find the small mononuclear leucocyte (lymphocyte) in place of the polynuclear type. This finding is of such frequent occurrence that the writer is often led to search many slides for tubercle bacilli in case the organisms have not been found in the earlier specimens examined. The cytology of the sputum has not been as carefully worked out as it should be, so that for the present we must dismiss this phase of the subject with mere mention.

The thin smears of sputum may be treated like blood-smears and stained with the same stains when one desires to study the cellular types present.

(*b*). Red Blood-cells.

These cells are frequently found in the sputum and may have much significance. They may occasionally arise from contamination with nasal or buccal discharges, but the true bronchial or pulmonary sputum usually shows them only in cases of hemorrhage or exudation. The rusty sputum of pneumonia contains large numbers of such cells and the hemorrhage from a tubercular cavity may be very extensive. These cells are occasionally well-preserved, but at times they are difficultly recognizable. They may be very much distorted in shape, so that their color and staining properties must be relied upon for differentiation.

(*c*). Epithelial Cells.

Various types of epithelial cells are found in the sputum. Pavement epithelium may come from the mouth, pharynx, and upper larynx. Cylindrical epithelium may be derived from the nose or the bronchi. These cylindrical cells may be ciliated, but rarely does one find these ciliated forms except in cases of asthma and bronchitis. Alveolar epithelial cells are present in normal sputa as they are constantly desquamated from all of the epithelial surfaces. These cells assume a large variety of forms and frequently show various types of degeneration. They are very numerous in bronchitis and in general inflammatory conditions of the lungs, but may occur in almost any condition, associated with irritation along the respiratory tract. These cells contain large numbers of granules which are probably referable to coal pigment. Such cells give the sputum a grayish or green color. An abundance of such cells in the sputum was designated in earlier times as "*phthisis melanotica*." Occasionally these alveolar cells are filled with fat globules. In other cases one finds the so-called myelin globules, which are irregular in shape, often showing concentric lines, with very little refractility, and of a dull greenish or blue appearance. The cell may be invisible and only the myelin appear as a large irregular mass. Much doubt exists as to the origin of these globules, but it is more probable that they represent simply the fatty products of degenerated protoplasm.

These cells are especially frequent in the normal morning sputum, in acute or chronic influenza, and in the so-called desquamative catarrhal pneumonia, when they appear as small lumps resembling boiled sago. Occasionally free myelin globules are found in the sputum. These globules stain poorly with the aniline dyes, are stained yellow with iodin, but do not stain black with osmic acid or red with Sudan-III.

Frequently these alveolar cells contain pigment granules, derivatives of hemoglobin. This material is in the form of amorphous granules of a brown color and seems to be identical with hemosiderin, but may become iron-free, when it more closely resembles hematoidin. Cells containing such granules are especially numerous in chronic heart disease and are styled, therefore, "*heart-disease cells*." They occur, however, in any condition in which red blood-cells escape into the alveoli and are found, therefore, in pneumonia, infarction of the lung, and hemorrhagic pulmonary tuberculosis.

(*d*). Elastic Tissue.

The presence of elastic tissue in the sputum is indicative of destruction of the lung tissue and in many cases is found in the sputum before one can detect tubercle bacilli. It has, therefore, some importance in the early diagnosis of tuberculosis. When this elastic tissue is grouped in masses it is usually visible to the naked eye. However, one more frequently relies upon microscopic examination for its detection. The method of Clark, as applied to the detection of elastic tissue, is usually the best one to follow. The sputum is placed upon a glass plate about 14 inches square and pressed out by a smaller one, about 6 inches square, into a thin layer. The plates are then placed upon a dark background and examined with a hand lens. Instead of these glass plates one may use Petri dishes.

The elastic tissue fibers appear either in the form of distinct strands sometimes grouped in an elongated network, in that of the alveolar type, in which the fibers preserve the outline of the alveoli and are long and branching, or in that from the arteries in which we may have a distinct sheet-like arrangement. These fibers are characterized by their undulating outline, their curling ends, their sharp edges and uniform diameter, their frequent branching, and their intense refractility. These characteristics are brought out both with the low and with the high power of the microscope. The fibrous tissue fibers are differentiated from elastic fibers by the fact that the former are present in bundles of fine wavy lines without the coarse black refractive appearance of elastic tissue. Chains of bacteria, especially the leptothrix forms, frequently interlace in such a way as to simulate the alveolar structure of elastic tissue. These chains differ, however, from elastic tissue in their refractility, in the absence of the wavy outline, and in their denser arrangement. The elastic tissue fibers derived from food substances have the same characteristics as the lung elastic fiber, with the exception that they are not arranged in the aveolar form and may at times be coarser and more irregular in outline than the pulmonary elastic

tissue. Vegetable cells and fibers, as well as fatty acid crystals, which may occur in the sputum, should not mislead one into assuming the presence of elastic tissue.

Should one wish to stain elastic tissue, he may use the orcein stain of Unna-Tänzer. This stain consists of one gram of orcein dissolved in a mixture of 80 c.c. of 95 per cent. alcohol and 35 c.c. of distilled water, 40 drops of strong hydrochloric acid being added after solution is complete. In using this stain the elastic fibers are treated with a few c.c. of the dye and then warmed for five minutes, after which the preparation is decolorized with acid alcohol. The elastic tissue fibers will be stained a brownish violet by this process.

If the elastic tissue is very small in amount we usually resort to chemical means for the isolation of this material. Ten c.c. of sputum are mixed with an equal volume of 10 per cent. sodium hydrate solution and the mixture is boiled until it becomes homogeneous. Four volumes of water are then added, the entire mass well mixed, and either allowed to stand or to settle by centrifugation. In this way the constituents of the sputum are destroyed with the exception of the elastic tissue which has, however, become swollen and paler and does not have its characteristic appearance.

About 90 per cent. of cases showing elastic tissue in the sputum are of tuberculous origin, according to Dettweiler and Setzer. As the healing process begins and proceeds this elastic tissue gradually diminishes in amount, so that a constant presence or an increase in the amount indicates a progressive condition. It is seen in abscess of the lungs, in bronchiectasis, in pulmonary infarct, and occasionally in pneumonia. It is found in cases of gangrene of the lungs, although there are many statements that it is digested by the trypsin-like ferment so common in such conditions. Osler states that he has never seen a case of gangrene of the lung in which elastic tissue fibre could not be found.

(*e*). Crystals.

Crystals are never found in the freshly formed sputum, being indicative of stagnation of the material within the body or of decomposition after being expectorated.

(1). Fatty Acid Crystals.

These crystals occur most frequently in the sputum of gangrene, putrid bronchitis and of chronic tuberculosis. They occur as distinct needles, either singly or in groups, may be short and relatively thick with pointed ends, or they may be long and very closely resemble elastic fiber. Pressure upon the cover-glass will produce varicosities in these crystals which do not appear in the case of elastic tissue. They are soluble in alkalies and in ether and melt into fat globules if the slide be warmed.

(2). Cholesterin.

Crystals of cholesterin are found in the sputum of chronic lung abscesses, empyema and chronic tuberculosis. They are not as frequent as are the fatty

acid crystals, but are usually found associated with them when they are present. These crystals have a distinct rhomboid form with notched angles.

(3). **Hematoidin.**

Crystals of hematoidin occur very rarely in sputum, and then only when extravasation has taken place into the alveoli. They are found very rarely after direct hemorrhage unless the extravasated blood remains for some time in the alveoli. These crystals occur especially in abscess of the lungs, empyema, or perforating liver abscess. They are rhomboid or needle-shaped crystals, ruby-red in color, and may show small curved filaments projecting from the angles of the larger forms.

(4). **Leucin and Tyrosin.**

These substances are formed only by the decomposition of protein material and are found, therefore, in the putrid sputum of an empyema, or from a perforating liver abscess, and in the very early discharges of true lung abscess. The tyrosin is found in the form of long refractive needles, frequently arranged in bundles, while the leucin appears as distinctly spherical masses with concentric striations and radiating lines.

(5). **Magnesium-ammonium Phosphate.**

These crystals appear in the sputum under the same condition as do the preceding tyrosin and leucin crystals. They are usually the typical coffin-lid crystals so common in decomposed urine, but may at times assume an irregular structure.

(6). **Calcium Oxalate.**

These crystals occur in the sputum in conditions associated with decomposition and appear either in the typical octahedral crystal with a cross connecting the corners or as the more atypical dumb-bell shaped crystal.

(7). **Charcot-Leyden Crystals.**

These crystals are apparently derived from the eosinophile cells as they are more frequently present only in conditions in which the eosinophiles are very numerous, hence the term "leucocyte crystals." They increase in the sputum either from stagnation within the system or after being expectorated. They are associated in asthmatic attacks with the spirals of Curschmann, being frequently included in the meshes of the spiral.

These crystals form straight, pointed, colorless, hexagonal, double pyramids, resembling a very much elongated diamond. They have sharp elongated points with clear-cut edges, are very brittle, are colorless, show little refractility, and vary greatly in size. They may occur singly or in groups, forming either clusters or distinct Greek-cross types. They have been supposed to be identical with the spermin crystals of Böttcher, but the hexagonal type of the crystal as well as the facts that they do not show marked double refraction by polarized light and have but a single optical axis should serve to differentiate

them from the spermin crystals. They are colored yellow with Florence's reagent and may be stained with the polychrome and other blood dyes. (See Semen.)

(*f*). Bacteria.

(1). Saprophytes.

The bacteria found in the sputum are very numerous and under normal conditions are purely saprophytic. We may even at times find many truly pathogenic organisms in the sputum which are of no clinical significance, although one may be led into making a diagnosis without sufficient clinical evidence. These saprophytes may occur in the fresh sputum or develop therein after the specimen has stood for some time. In either case their chief effect is to bring about gradual decomposition of the sputum. The various chromogenic bacteria are of particular interest as their development along the respiratory tract may so change the color of the sputum that the examiner may be led astray.

Among the ordinary saprophytes found in the sputum we find representatives of the streptothrix and the leptothrix groups. Flexner and Warthin and Olney have reported the presence of a streptothrix, the streptothrix eppingeri, in the sputum of cases showing the clinical symptoms of pulmonary tuberculosis. These organisms are about four times as thick as the tubercle bacillus; when stained they are resistant to decolorization by acids, but are slowly decolorized by strong alcohol. Stained specimens are easily made with the use of methylene-blue dyes or of Gram's method. The leptothrix group is particularly abundant in the mouth and is found in large numbers in the lungs in cases of pulmonary gangrene.

Yeast fungi occur in the sputum at times, but rarely in the fresh specimens. They are oval or elliptical cells and are very refractive, sometimes resembling very closely fat droplets. Their appearance may vary from that of a simple oval cell without distinct limiting membrane to those with d stinct membrane and vacuoles. These cells are especially characterized by their tendency to throw out projections or buds at various points of their periphery. They vary in size from 1 to 40 microns in diameter. While these yeast fungi are usually extraneous, cases are reported (Busse) in which pathogenic yeasts have been found in anomalous pulmonary conditions. These organisms stain with the ordinary aniline dyes and appear in some instances to be acid-fast (resisting decolorization by acid).

Various types of molds are found in the sputum. Some of these appear to be distinctly pathogenic, while the majority are merely saprophytic. These molds are found in the true sputum only in cases associated with destructive processes of the lungs. It has been doubted that they could cause primary infections, but there are at present many reports of cases showing that some of hem at least may be distinctly answerable for primary infections.

Among these pathogenic molds we find certain types of the Mucor, of

the 130 varieties of which six are known to be distinctly pathogenic. Besides this type we find, as the most important pathogenic mold of the sputum the aspergillus fumigatus, 16 cases of pulmonary affection (pneumonomycosis asperg llina) having been traced by Sticker to this fungus. Other types of the

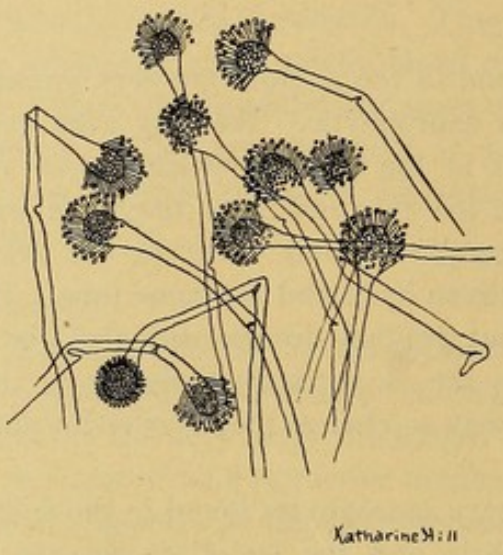


FIG. 3.—Aspergillus fumigatus.

aspergillus are the flavus, niger, and the sub-fuscus. The penicillium glaucum and the Oïdium albicans are occasionally found in the sputa.

Among the bacteria which may be purely saprophytic we may find almost any of the pus-forming organisms. More frequently, however, when these organisms are present in excessive numbers a contamination or a direct pathogenic

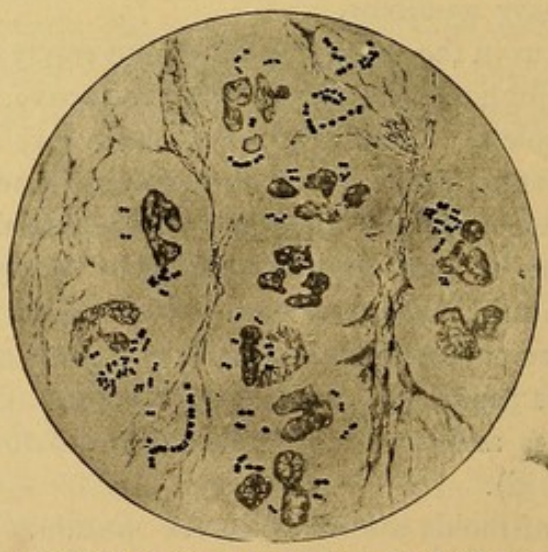


FIG. 4.—Micrococcus catarrhalis. (*From Emery's "Chemical Bacteriology."*

influence should be suspected. A form known as the micrococcus tetragenus occurs both as a pathogenic and a harmless organism. It consists, as its name implies, of four cocci arranged in a square within a mucous capsule. It stains with the ordinary dyes and is Gram-positive. This organism is found, in its

pathogenic state, in bronchitis, tubercular cavities, and hemorrhagic infarctions. The harmless form differs from the pathogenic type in the fact that it cannot be cultivated.

The sarcinæ are rarely found in the sputum. These organisms are somewhat smaller than those occurring in the stomach and are probably purely saprophytic in the sputum. They are found, however, in cases of putrid bron-

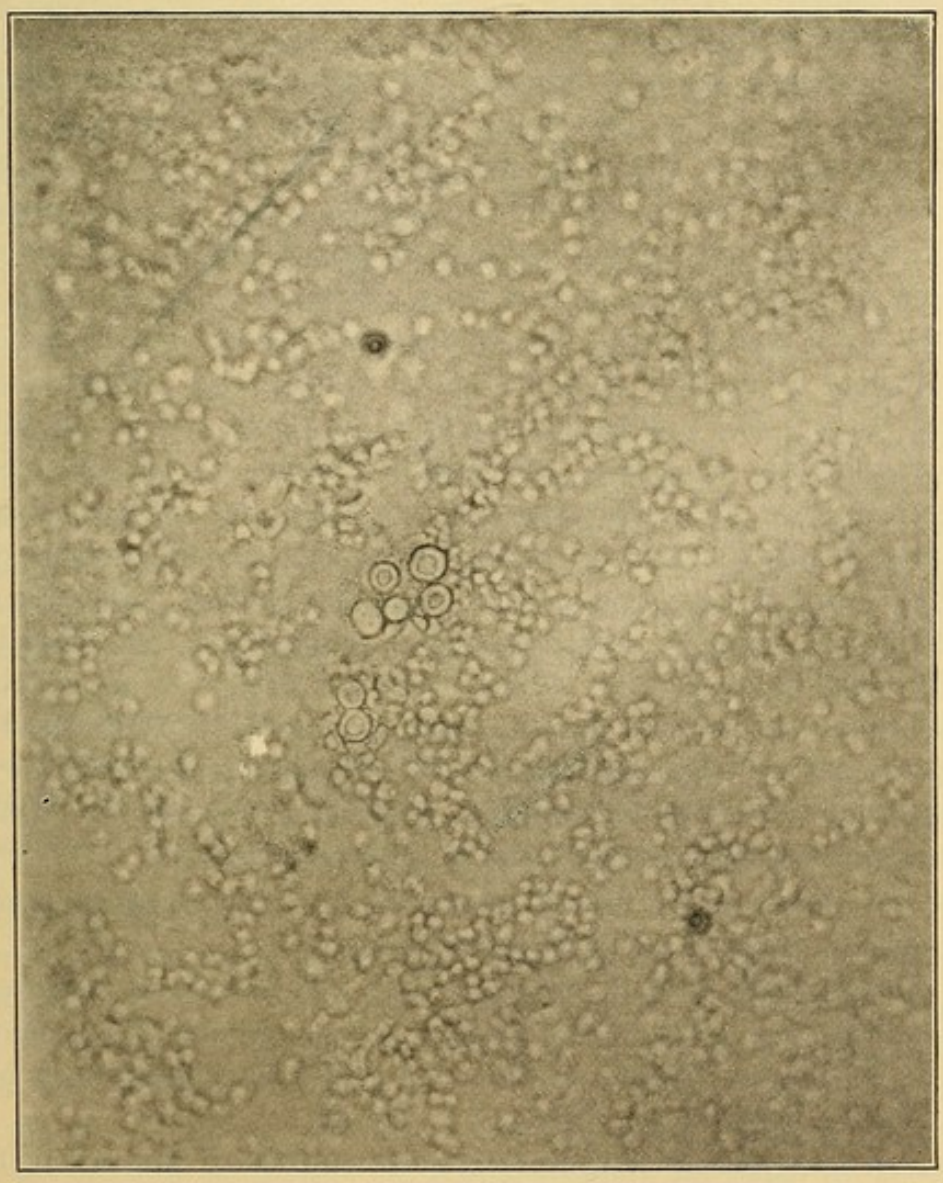


FIG. 5.—Budding forms of blastomycetes found in sputum.
(*From photograph by W. A. Pusey.*)

chitis, especially when this occurs in emphysematous lungs, in gangrene, tuberculosis, and pneumonia.

The micrococcus catarrhalis is observed quite frequent'y in the sputum, being derived largely from the nasal cavity. This organism resembles very closely the ordinary staphylococcus, but is larger and more often grouped in lateral pairs, which simulate the gonococcus. It stains readily with aniline dyes and is Gram-negative.

In cases of general systemic blastomycosis, Eisendrath and Ormsby have found the blastomycetes in the sputum. They recommend the examination of the unstained specimens after the addition of 10 per cent. sodium hydrate solution. In such preparations examined with a high-power dry lens, the typical refractile blastomycetes are observed. (See Parasitic Diseases.)

(2). Pathogenic Types.

(a). Tubercle Bacillus.

This organism is the most important pathogenic type found in the sputum. Its detection is usually easy and should be attempted in all suspicious cases, as an early diagnosis may frequently save the life of the patient. In the days before the organism was recognizable, physicians based their diagnosis of consumption upon the macroscopic appearance of the sputum. While such examinations frequently lead to a presumptive diagnosis of tuberculosis, nothing can settle the question except the microscopic examination of the sputum. This statement needs some modification in several ways. In the first place, specimens of tubercular sputum may not show the presence of the bacilli so that several examinations of sputum, collected at different periods, must be made. In the second place, the sputum may be examined in the very early stage of the disease and no tubercle bacilli be found, but in such cases the presence of elastic tissue fiber would be very significant of tubercular changes. In these days we have, fortunately, recourse to other diagnostic measures in case tubercle bacilli cannot be found in the sputum. I have reference here to the use of tuberculin, either introduced in the form of an injection as done by Koch, dropped into the eye as advocated by Calmette, as advised by Pirquet applied after the manner of vaccination, or used as an inunction as suggested by Moro. This test is usually certain and can be relied upon to settle the diagnosis.

The full details of the use of tuberculin, both for diagnostic and therapeutic purposes, must be found in other works.

In examining the sputum microscopically the fine cheesy particles previously mentioned are selected and smears of such material made upon glass slides. It is always advisable to make at least five such smears, as one may find no tubercle bacilli in the first ones examined.

Some of the most suspicious-looking sputa contain no tubercle bacilli, while, on the other hand, a distinctly mucoid one may show very large numbers. The character of the sputum can, therefore, have little more than a presumptive importance, but it is to be said that one accustomed to examining sputum will instinctively decide that a specimen of sputum will or will not contain the specific organism. Thus the writer recalls the examination of 20 slides made from one specimen of sputum before a single tubercle bacillus was found. The most favorable sputa, in the writer's experience, seem to be those which consist of a large amount of cheesy mucopurulent material, which forms as a distinct layer above the more liquid portion and sends into this lower layer various long threads of necrotic material.

In cases in which no tubercle bacilli have been found after repeated examination, it is advisable, especially if the sputum looks suspicious, to boil a portion of the sputum with dilute sodium hydrate solution which will destroy the larger portion of the material, leaving the tubercle bacilli and the elastic tissue behind. The material may then be centrifuged (care being taken to see that the specific gravity of the fluid is lower than 1010 or higher than 1080) and the deposit examined carefully for the organism in the former case, or the organisms may be collected from the surface in the latter. In case all such measures fail it is probable that no tubercular condition of the lungs is present. This statement must be made with a reserve that a second and, preferably, a third examination of a fresh specimen of suspected sputum be made and inoculation of a guinea-pig undertaken.

The worker must not imagine that an organism appearing to resemble the tubercle bacillus in morphological and staining characteristics is always the tubercle bacillus. Thus there are, at least, two organisms which should be differentiated from the tubercle bacillus, and occasionally a third should be held in mind. These three organisms are the bacillus of leprosy, the smegma bacillus, and the timothy bacillus. They show almost the same staining characteristics as the tubercle bacillus and must be closely differentiated. The most important of these, from a differential standpoint, is the smegma bacillus, which is so frequently found upon the tonsils, the tongue, and the teeth. Methods of differentiation will be outlined after the tubercle bacillus is discussed.

Staining Characteristics.

The methods of staining the tubercle bacillus depend upon the property, possessed by this organism, of taking up the aniline dyes with great difficulty, but, when once stained, of becoming just as resistant against decolorization.

Ziehl-Neelsen Method.

The smears are made upon glass slides and are fixed by passing several times through a flame. The smear is covered with carbol-fuchsin solution (a mixture of 90 parts of 5 per cent. carbolic acid water and 10 parts of a concentrated alcoholic solution of fuchsin), and is then heated over a flame for one to three minutes in such a way that the staining solution steams, but does not boil. If the staining solution is heated too strongly the smear decolorizes less readily so that it is very good practice never to boil the staining solution. Where several slides are to be examined, the writer has found the copper plate of Ehrlich very useful. The slides are laid upon the plate, are covered with the stain, and allowed to heat for ten minutes. The usual technic is, however, to heat a single slide at one time, more of the stain being added as the first evaporates. Some workers find that the immersion of the smear in cold carbol-fuchsin for 24 hours gives somewhat clearer pictures, but the time is too long for the ordinary laboratory diagnosis. Having thus stained the smear with the carbol-fuchsin solution, it is then decolorized. The tubercle bacillus is not only acid-fast, but also alcohol-fast, so that we use decolorizing agents containing both

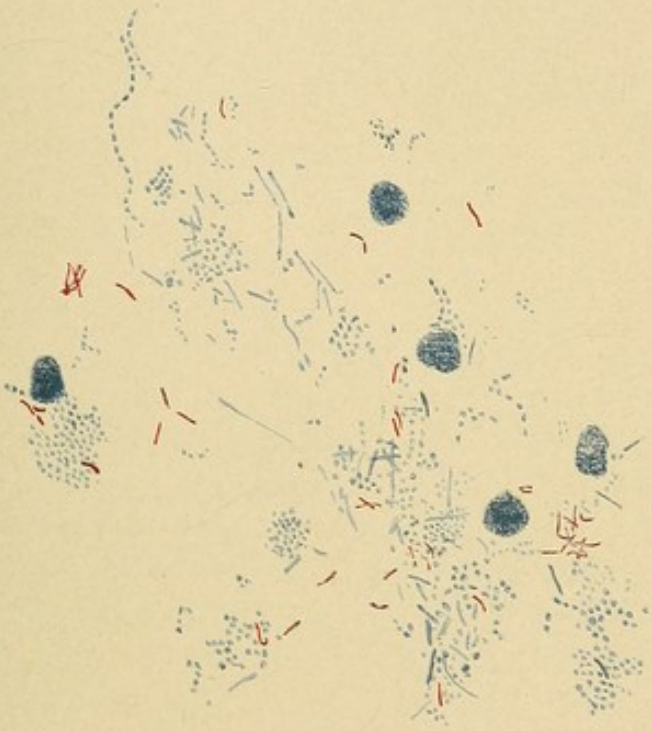
acid and alcohol. There are many acid-fast organisms known, such as the bacillus lepræ, the smegma bacillus, the timothy bacillus, the butter bacillus and many saprophytic bacilli found in water, soil, and manure. Few or none of these organisms are absolutely both alcohol and acid-fast, so that the use of the combined decolorizer will usually differentiate the tubercle bacillus. Many decolorizing agents have been advised, but the writer finds the use of a 10 per cent. solution of sulphuric acid in 95 per cent. alcohol very reliable. This decolorizing agent does not burn the specimen nor does it prevent the morphological characteristics from appearing in a clear-cut way. Some workers advise the use of 2 per cent. hydrochloric acid in 80 per cent. alcohol, while others use 25 per cent. nitric acid, followed by alcohol. The technic of decolorization is as follows: Wash the smear, which has been stained with carbol-fuchsin, in water and flood the specimen with the decolorizing solution until only the faintest pink color is seen in the smear. It frequently happens that the thicker portions of the smear resist this decolorizing so that it may be necessary either to make a new smear or to examine merely the portions which have been decolorized. After decolorization is complete the specimen is washed in water and counter-stained with Löffler's methylene blue (saturated alcoholic solution of methylene blue 30 c.c., 100 c.c. of a 1 to 10,000 aqueous potassium hydrate solution) for a few seconds, after which the specimen is washed with water, dried between filter-paper, and examined with the oil immersion lens.

In such preparations the tubercle bacilli are seen as bright red rods, somewhat bent, sometimes much curved and occasionally showing distinct branching forms. In a few preparations one may find the curves of the bacillus so marked that a very close resemblance to the spirillar forms obtains. These organisms occasionally show a distinct beading, giving the appearance of bright red cocci. The size of these organisms varies from 1 1/2 to 3 1/2 microns and about 2/10 microns in width. They may be single or arranged in clumps, sometimes in the form of distinct crosses, sometimes parallel, and very frequently forming acute angles by the joining of two bacilli.

Gabbet's Method.

This method is much more simple than the preceding, but is not as reliable. By this method the decolorization and counter-staining are carried out in one operation. The smears are prepared as previously described and stained with the carbol-fuchsin solution. The excess of the staining solution is drained off without washing and is replaced by Gabbett's methylene blue solution (methylene blue 2 grams, sulphuric acid 25 c.c., water 75 c.c.). This solution is allowed to act for one to three minutes and is then washed off with water and the specimen dried and examined. The tubercle bacilli will appear as bright red rods as previously described, while the other organisms as well as the various cellular types will be stained blue.

This method is not as reliable as is the former, owing to the fact that the alcohol-fast bacilli resist decolorization and may confuse one in making the



Katharine Hill.

TUBERCLE BACILLI IN SPUTUM. ZIEHL-NEELSEN METHOD.

diagnosis. Moreover, the use of the strong acid may cause decolorization of some of the tubercle bacilli and will, therefore, give rise to wrong ideas. These methods are the ones usually followed in routine laboratory work for the detection of the tubercle bacillus. The combined decolorization with alcohol and acid being the only reliable method, one should use the Gabbett's method only for obtaining somewhat clearer pictures.

Pappenheim's Method.

The technic of this method is as follows: The preliminary staining is carried out with carbol-fuchsin solution as previously outlined. The specimen is then drained and covered with the decolorizing solution, which is made by dissolving one gram of rosolic acid in 100 c.c. of absolute alcohol, saturating the mixture with methylene blue and adding 20 parts of glycerin. This solution is drained off slowly and the process repeated several times. The slide is then washed in water, dried between blotting-paper and examined with the immersion lens. The tubercle bacilli are stained red and the other organisms blue.

Spengler's Sedimentation Method.

Mix equal parts of sputum and luke-warm water, that has been alkalinized with sodium carbonate solution. Add 0.1 to 1 gram of pancreatin powder and shake. To this mixture add 0.2 to 1 gram of crystallized carbolic acid and place in the incubator at 37° C. This digestion must not be allowed to continue too long as the tubercle bacilli will also be partially digested, and thus lose their staining peculiarities. Pour off the supernatant fluid and examine, as previously described, the sediment which forms. If the sediment be very large in amount add more alkalinized water and pancreatin and incubate further.

This method is similar to that of Jousset as described under the head of Inoscopy in the section on Exudates. It is to be especially recommended in cases in which the sputum is so thick and tenacious as to interfere with the preparation of thin smears. The results are very excellent, providing the digestion in the incubator is properly regulated. This part of the technic will require considerable experience before the exact time can be determined in each individual case.

It seems to be very generally agreed that the use of both alcohol and acid as decolorizing agents prevents mistake in diagnosis of the tubercle bacilli. We must, however, remember that there is a possibility that every acid-alcohol fast bacillus is not the tubercle bacillus, so that in doubtful cases one should always resort to the inoculation of the guinea-pig.

Value of Examinations

Brown[1] in a recent article has summed up the value of the sputum examination for tubercle bacilli. He gives as his reasons for believing that one should

[1] *Montreal Med. Jour.*, vol. 30, 1901, p. 769; *Jour. A. M. A.*, vol. 40, 1903, p. 514.

be guarded in forming an opinion of the prognosis of certain cases the following points: (1) Many of the tubercle bacilli may not be stained at all. (2) Old foci may give off very few and young foci no bacilli at all. (3) By the occlusion of a bronchus the contents of a focus may be shut off entirely for a time and thus the expelled sputum may contain a large number of tubercle bacilli. (4) The organisms may be present one day and not again for months. (5) The organisms may be abundant in one part of a specimen and none be found in others. (6) Some patients with fatal tuberculosis (caseous pneumonia or acute miliary tuberculosis) may have no bacilli in the sputum, while in other cases the organisms are present even before physical signs obtain. (7) In severe cases w th bronchitis the secretion of the bronchus will dilute the sputum and give the appearance of a reduction in the number of organisms.

While the number of bacilli in the sputum may thus vary, it is usually in direct ratio to the severity of the disease, although for the reasons above mentioned too much reliance should not be placed upon the number of organisms found. Brown recommends the use of a somewhat modified Gaffky's table in judging of the prognosis in any particular case. The cases are classified as follows, being designated by the Roman numerals:

I. Only one to four bacilli in whole preparation.
II. Only one on an average in many fields.
III. Only one on an average in each field.
IV. Two to three on an average in each field.
V. Four to six on an average in each field.
VI. Seven to twelve on an average in each field.
VII. Thirteen to twenty five on an average in each field.
VIII. About fifty on an average in each field.
IX. About 100 on an average in each field.

There has been some attempt to base a prognosis upon the form and grouping of the tubercle bacilli, the short rods indicating a rapid growth while the longer form shows a slower development. The continued expectoration of large numbers of bacilli would indicate a cavity, while the sudden increase in numbers associated with an increase in the cellular elements would point to lung disintegration. A steady decrease over a long period of time would indicate improvement, but it must be remembered that occlusion of the bronchus may shut off large numbers of these organisms from the sputum. It should be stated as a working rule that the finding of a single or a very few organisms in the sputum should be looked upon with suspicion, but that an absolute diagnosis should be made only after repeated examination has shown the presence of the tubercle bacilli. The worker will find in the study of preparations stained as above that artefacts are very common so that a hurried examination is never warranted.

The sputum in tubercular cases rarely shows tubercle bacilli in pure culture. One usually finds large numbers of streptococci, staphylococci, micro-

cocci catarrhalis, and frequently influenza bacilli and pneumococci. Pus cells may be few or many, while the large number of leucocytes are more frequently of the mononuclear type. Blood-cells may or may not be present, while elastic tissue fibre is very frequent, appearing in many cases before tubercle bacilli can be demonstrated. It should be remembered that mixed infections, which are the rule in tubercular conditions, may be so marked that few if any tubercle bacilli appear in the sputum. This statement is especially true when the sputum has been allowed to stand for some time before examination. The writer has frequently found sputa showing fairly large numbers of tubercle bacilli becoming practically negative for these organisms if the sputum be allowed to stand exposed to the air for 48 hours. The explanation is that the other organisms so far outgrow the tubercle bacillus that they prevent any further development of this latter organism and bring about such degeneration that the staining qualities of the tubercle bacillus are markedly affected. This fact has been taken advantage of in the clinical use of injections of pus organisms into tubercular joints.

(*b*). **Lepra Bacillus.**

The bacillus lepræ, first described by Hansen, is a small slender bacillus from 4 to 6 microns in length and surrounded by a slimy envelope. These bacilli behave toward staining reagents very much like the tubercle bacillus, but are less resistant toward acid and alcohol than is the tubercle bacillus, so that a differentiation is possible provided decolorization is rather severe. The stained bacilli often show clear spots or appear as if made up of stained granules.

These organisms may be found in many cases of leprosy in the sputum or nasal secretion, so that in doubtful cases a differentiation is necessary. While these organisms stain much more easily than do the tubercle bacilli and are more easily decolorized, it may be necessary to resort to inoculation experiments to make the absolute differentiation.

(*c*). **Smegma Bacillus.**

This bacillus may be found normally in the saliva, coating of the tongue, the tartar of the teeth, and in the crypts of the tonsils. Pathologically, it may be found in cases of simple bronchitis, in the sputum in cases of gangrene of the lungs, and in the suppurative discharges from the ears. While these organisms are much more commonly confused with the tubercle bacillus when the urine is examined than when the sputum is investigated, yet they must be borne in mind in every sputum examination. It may be necessary to resort to inoculation experiments to decide the question, but ordinarily the use of the differentiating stain of Pappenheim (previously described) as well as the following method of Bunge and Trantenroth may be used. After fixation of the smear, the fat is removed by soaking the specimen in absolute alcohol. The preparation is now covered with a 5 per cent. solution of chromic acid for 15 minutes, after which it is washed with water. The smear is stained with carbol-fuchsin, decolorized with 16 per cent. sulphuric acid for three minutes, and is then counter-stained

for five minutes in a concentrated alcoholic solution of methylene blue. This method is said to give the tubercle bacillus as distinct red, while the smegma bacillus is blue.

While both of these methods of differentiation are usually applicable, yet one occasionally finds the smegma bacillus resisting the action of 16 per cent. sulphuric acid for 30 minutes and occasionally of strong alcohol for 12 hours. It is, therefore, necessary in such cases to resort to the court of last appeal, the guinea-pig test.

(*d*). **The Timothy Bacillus.**

This organism is present in the mouth reaching it through the medium of butter and milk, which may contain it in large numbers. These bacilli resist the decolorizing action of both alcohol and acid to almost the same extent as do the tubercle bacilli, but they usually appear as somewhat longer and thicker rods. Strangely enough, this organism produces a lesion in guinea-pigs which resembles very closely that of true tuberculosis, so that the inoculation test will not always be conclusive unless other animals are inoculated with material from the first one, in which case no lesions develop in the later animals. Fortunately, the cultural peculiarities of this organism are markedly different from those of the tubercle bacillus, as the former develops readily on the ordinary culture media.

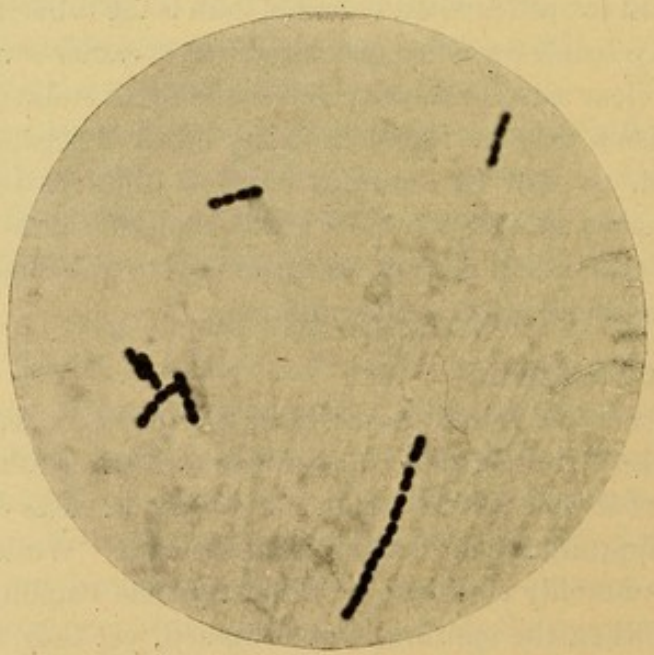


FIG. 6.—Diplococcus pneumoniæ. (*Williams.*)

(*e*). **The Pneumococcus (diplococcus pneumoniæ).**

The organism, discovered by Fränkel and elaborated by Weichselbaum, is generally recognized as the etiologic factor in cases of acute croupous pneumonia. It is found in large numbers in the sputum and occurs in the form of two short conical or lance-head shaped bacilli with their bases in contact and the pair surrounded by a delicate capsule. This organism stains with the ordi-

nary dyes as well as with Gram's stain. Frequently it is arranged in chains or groups so that the capsule must be stained in order to differentiate it from the ordinary streptococcus or staphylococcus. Such method of differentiation, while necessary in many cases for purely bacteriological purposes, may usually be dispensed with in clinical work as the form of the organism is usually clear-cut and as a distinct unstained capsule frequently appears in the sputum. These organisms occur so frequently in the mouths of healthy individuals that their mere demonstration under the microscope has little diagnostic importance.

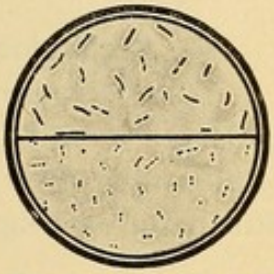


FIG. 7.—Friedländer's bacillus (above): pneumococcus (below). (*Greene.*)

Friedlander's Bacillus (bacillus mucosus capsulatus) is occasionally found in some cases of lobar pneumonia, and may be considered the etiologic factor in such conditions. These bacilli are encapsulated and stain readily with the ordinary dyes and are usually Gram-positive when found in the sputum.

(*f*). The Influenza Bacillus.

This bacillus, known as Pfeiffer's bacillus, is found in the bronchial sputum, especially in the pulmonary type of this disease. The most characteristic sputum is greenish-yellow in color with lumps of pus in nummular form. The organisms are found in such sputum as small, short bacilli measuring 2/10 to 3/10 micron in breadth by 5/10 micron in length. They usually occur singly, but may form chains. In the stained specimens these organisms show distinct polar stainings, appearing frequently as diplococci. They are stained with dilute carbol-fuchsin solution, faintly with the ordinary methylene blue solution, or are identified by their Gram-negative characteristics. The best counter-stain used in the Gram method is either Bismarck brown or safranin, the organisms appearing both intra- and extracellular.

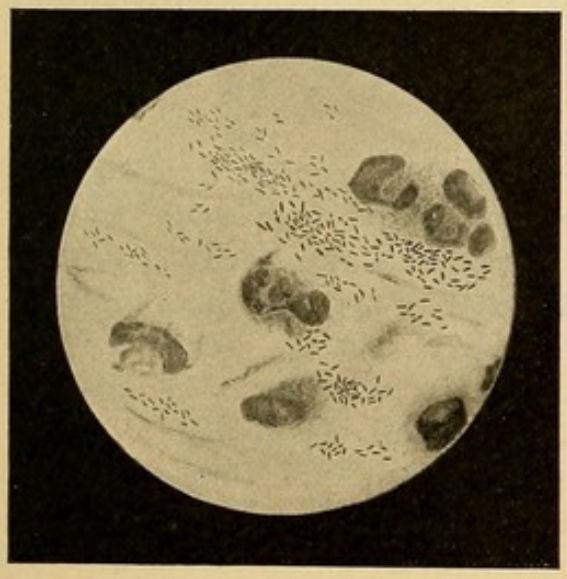


FIG. 8.—Bacillus influenzæ in sputum. (*Abbott.*)

(*g*). The Bacillus Pertussis.

This organism, discovered by Bordet and Gengou, and elaborated by Klimenko, has been frequently found in the sputum in cases of whooping-cough. It resembles very closely the influenza bacillus, appearing as short, plump, ovoid bacilli, with rounded ends and lying singly or in small groups between the pus and epithelial cells. It stains feebly with the usual dyes and is Gram-negative. This organism is rarely intracellular and may thus be distinguished from the influenza bacillus.

(*h*). **Typhoid Bacillus.**

This organism has been found in the sputum in typhoid fever cases showing a coexistent bronchitis or pneumonia. The sputum is usually hemorrhagic in character and shows the bacilli as short, thick rods, staining with the ordinary dyes and negative to Gram's stain. For the absolute identification of this organism cultural and agglutination tests are essential.

(*i*). **Staphylococcus and Streptococcus Pyogenes..**

These organisms are found in practically every sputum examined and can be identified only by the use of cultural methods. They stain well with any of the aniline dyes and are Gram-positive. Their presence in the sputum has little pathologic significance.

(*j*). **The Bacillus Pestis.**

This bacillus of bubonic plague was discovered by Kitasato and Yersin in 1894. It is a short, thick bacillus, measuring from 0.8 to 2 microns in length and from 0.4 to 0.8 micron in thickness. A capsule may be usually made out and the stained organism frequently resembles a diplococcus, owing to the intense polar staining with intermediate faint staining. These organisms are Gram-negative.

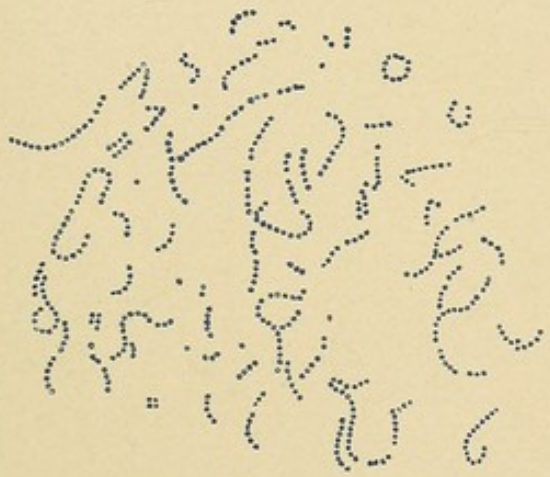
The bacillus pestis is found in the sputum of persons suffering from the pneumonic type of this disease and should be recognized owing to the markedly infective character of the material. It may be necessary to absolutely identify the organism by inoculation and cultural experiments.

(*k*). **The Bacillus Anthracis.**

The sputum of cases of pulmonary anthrax may contain large numbers of these bacilli. These organisms are from 5 to 10 microns in length and from 1 to 1 1/2 in breadth. They are frequently grouped in long segmented threads, the segments varying in length, but usually being two or three times as long as broad. Occasionally these bacilli may be single, but are usually multiple. They form oval spores in the middle of the short segments. The organism stains with the ordinary dyes and is also Gram-positive. For absolute identification cultural and inoculation experiments, the latter into white mice, may be necessary, but the morphological characteristics of this organism will usually identify it.

(*l*). **The Bacillus Mallei.**

This organism of glanders is found in the sputum in the pulmonary form of this disease. Morphologically, there is nothing characteristic in the appearance of this organism beyond the fact of the presence of faintly staining areas in the protoplasm of the rather long bacilli. These organisms stain by Gram's method as well as with the ordinary aniline dyes. For a final diagnosis inoculation into a güinea-pig should be made.



Katharine Hill

STREPTOCOCCUS PYROGENES (METHYLENE BLUE STAIN.)

(*m*). **Actinomycosis Hominis (Ray Fungus).**

This fungus, which gives rise to the condition known as lumpy jaw in cattle, occasionally infects man, causing pulmonary conditions much resembling tuberculosis. The mucopurulent sputum in such conditions contains elastic tissue and small sulphur-yellow granules which are visible to the naked eye and are the characteristic findings of such cases. Macroscopically these granules are yellowish, grayish, greenish, or brownish in color, and are sometimes abundant and sometimes scarce. They are very friable, and when gently crushed beneath the cover-glass and examined microscopically appear to have broken up into hyaline rounded masses at the margins of which, on close inspection, fine radial

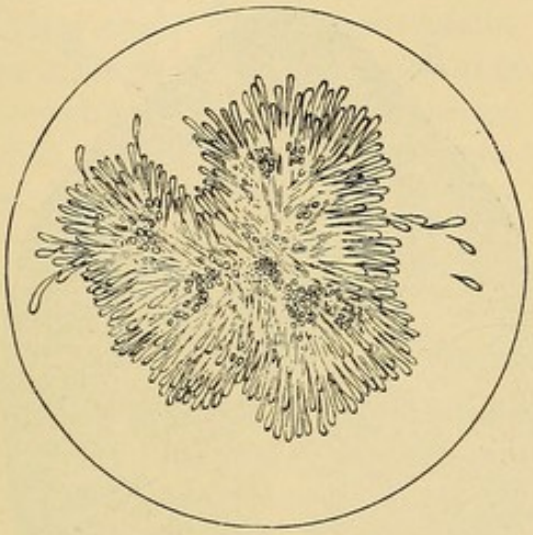


FIG. 9.—Actinomyces. (*Williams.*)

striations or filaments or hyaline club-shaped bodies, all closely set together, may be seen. The club-shaped bodies are variable in size and are composed of a hyaline refringent substance. In the granules obtained from the lesions in man the club-shaped formations are much less frequently observed than those obtained from the lesions in cattle (Mallory and Wright). If cover-glass preparations be made and stained with Gram's method, one will usually find isolated and matted filaments, many of which may be seen to branch, in addition to longer and shorter fragments of filaments and fine detritus of the same. If clubs are present in the granules they may be found scattered throughout the preparations.

(*g*). **Animal Parasites.**

(*a*). **Amebæ.**

Artault has described a unicellular ameboid body which resembles very closely a leucocyte when stained, but, while motile, differs in refractility and staining quality. This he calls the ameba pulmonalis. In cases of perforating liver abscess the true amebæ coli may be found in the sputum, and, according to Flexner, in cases of abscess of the jaw communicating with the mouth. These amebæ will be discussed fully in the chapter on Feces, to which the reader is

referred. It should be noted here that these organisms may not be numerous so that many slides may have to be examined. Nothing should be called an ameba unless it shows true ameboid movement.

(*b*). Flagellates.

Flagellated organisms, such as the trichomonas pulmonalis and the cercomonads, are found in the sputum associated with Dittrich's plugs in cases of gangrene, putrid bronchitis, and tubercular cavity formation. The trichomonas is probably identical with the trichomonas vaginalis or intestinalis which will be discussed later.

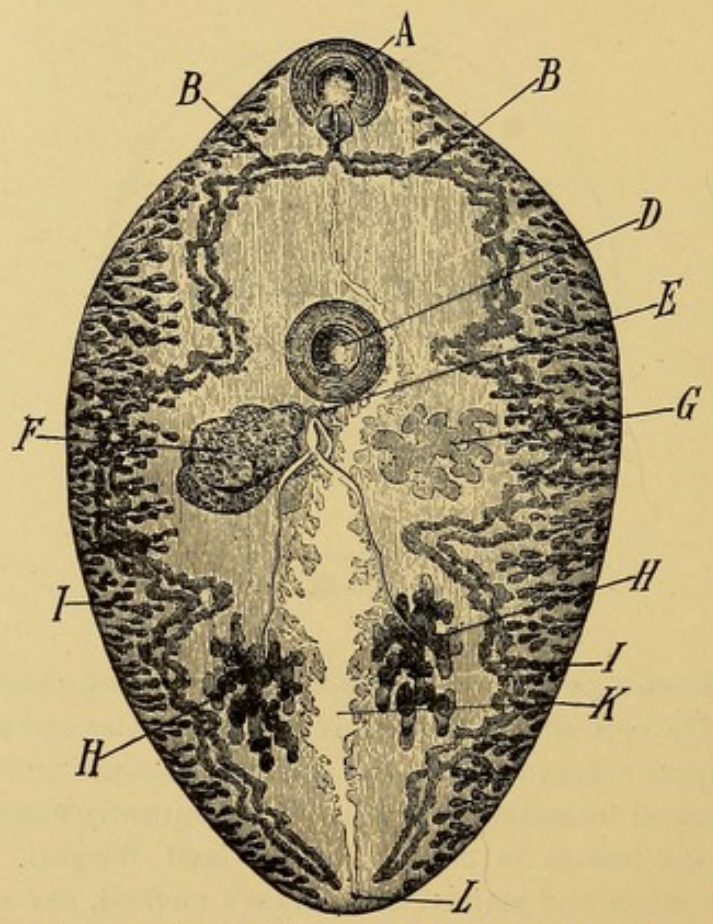


FIG. 10.—Paragonimus westermanii; (ventral view); 10×1. *A*, oral sucker; *B*, ceca; *D*, acetabulum; *E*, genital pore; *F*, uterus; *G*, ovary; *H*, testicles; *I*, vitelline glands; *K*, excretory canal; *L*, excretory pore. (*Tyson after Braun.*)

(*c*). Cestodes.

Not infrequently the lung is the seat of infection with the tenia echinococcus. In such cases various foreign bodies, such as fragments of membranes, scolices, hooklets, and cysts, may be found in the sputum. Such formations may, also, be found in cases of liver abscess perforating into the lung. Any one of the above formations is characteristic of this condition. The parasite will be discussed fully in the section on Feces, to which the reader is referred.

The sputum in such cases is usually purulent or mucopurulent and may be tinged with blood. This sputum may be expectorated over a long period

of time and may even contain tubercle bacilli from a coexistent tuberculosis. A distinguishing point between the sputum of pulmonary echinococcus disease and that of a perforating liver abscess is that the sputum in the latter is usually bile stained.

(*d*). Trematodes.

The most common of this class of animal parasites is the ordinary "lung fluke," which has been called also distoma pulmonale, distoma Westermanii, distoma Ringeri, and Paragonimus Westermanii. The eggs of this parasite are much more frequently found in the sputum than are the parasites themselves, so that the diagnosis will rest with the finding of these ova. These eggs measure from 80 to 100 microns in length and 40 to 60 microns in width. They are brownish in color, oval in shape, have a smooth thin shell and a lid near one end which is quite characteristic The parasite is from 8 to 10 mm. long, 4 to 6 mm. wide, and is very markedly rounded anteriorly, being nearly as thick as broad.

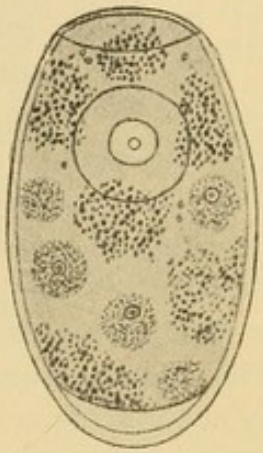


FIG. 11.—Ovum of paragonimus westermanii, from sputum: 1000 × 1. (*Tyson after Braun.*)

The sputum in such cases is usually small in amount, is very tenacious, and is reddish or rusty due to admixture of blood with the mucus Frequently no blood is found, in which case the sputum will still be of a yellowish or brown color due to the eggs themselves. The sputum, also, contains many spirals, which resemble very closely the Curschmann spiral and, also, the Charcot-Leyden crystals.

The eggs of another species of distoma, the distoma hematobium, have been found in the sputum by Manson. (See Blood.)

V. THE SPUTA IN DISEASE.

(1). Pulmonary Tuberculosis.

It has been truly said by Brown that pulmonary tuberculosis has no characteristic form of sputum The amount voided may vary from the very slight type of fibroid tuberculosis to the very abundant sputum of cavity formation. It is to be said that the amount of pus will usually depend upon the extent of the secondary infection, although caseous degeneration may lead to the expectoration of large amounts of material resembling pus.

In he early cases of pulmonary tuberculosis we may find a small amount of sputum which is expectorated only in the morning. This may be very tenacious and resemble very much the sago-like sputum previously mentioned. Sooner or later depending upon the extension of the disease, there will appear small caseous particles which are very suggestive. As ulcerative processes proceed, the sputum becomes more profuse, yellowish or greenish in color, and

muco-purulent in character. In any stage of this ulcerative tuberculous condition we may find blood in amounts ranging from a few blood-cells to a sputum loaded with blood from a hemorrhagic focus. Likewise we will find elastic tissue in more or less amount and tubercle bacilli varying from a few to many in each field. The color of the tubercular sputum may range through all the shades of the spectrum, the greenish shade being associated with a most marked decomposition. As stated previously, the most suspicious looking sputa frequently contain no tubercle bacilli, so that frequent search must be made for these organisms which are the only diagnostic point of the sputum in this condition.

(2). **Croupous Pneumonia.**

The early sputum of acute lobar pneumonia is usually yellowish-red in color and very tenacious in consistency. In some cases the sputum is mucoid and abundant for a few days, but soon takes on the characteristic reddish color from the presence of unchanged red blood-cells. Its consistency is so great that the receptacle may be inverted without allowing any material to run out. The characteristic rusty sputum, which is found when the exudation into the alveoli is taking place, is homogeneous, glairy, very tenacious, and deep red in color. This rusty sputum, while characteristic of pneumonia, is sometimes replaced by one ranging in color from a yellow to a green. These colors are due to different oxidation products of hemoglobin, and are, perhaps, more frequently observed in the stage of resolution when the sputum becomes less tenacious and more abundant. The greenish sputa in pneumonic conditions have some importance. This coloration may be due to a coincident jaundice or may arise from delayed resolution, especially when the exudate has been particularly hemorrhagic. It is, moreover, sometimes an indication that a true tubercular condition has intervened and hence that the prognosis must be guarded.

The so-called prune-juice sputum usually indicates a severe type of the disease, while at times it may signify merely a beginning resolution.

Fibrinous coagula are found, according to Osler, in every case in which search is made. These may vary from very small bronchial casts to very large branching types. Curschmann's spirals as well as the Charcot-Leyden crystals are frequently observed. The characteristic organism of this condition, the diplococcus lanceolatus of Fränkel, is usually found, but has only incidental importance, as it is so frequently present in the sputum of normal individuals.

(3). **Bronchopneumonia.**

The sputum of this disease is rarely characteristic. It partakes of both the type of a bronchitic and a pneumonic sputum. It may, therefore, contain much mucus and pus, may be viscid, may be streaked with blood, but is rarely so distinctly rusty as in the croupous type of this disease. As the disease is so limited in extent it is more or less rare to find an abundant sputum or to observe fibrinous coagula. Microscopical examination shows various organisms, but nothing diagnostic.

(4). **Acute Bronchitis.**

The sputum in this condition is very scanty in the early stages, is usually very tenacious and is expelled with difficulty. This early bronchitic sputum is known as "*sputum crudum*" and consists of practically pure mucin, containing within its meshes a few leucocytes, red cells, bronchial epithelial cells, and a few myelin drops.

After a few days the sputum is increased in amount, becomes less viscid, and assumes the type of a distinct muco-purulent sputum. This sputum, called the *sputum coctum*, contains numerous pus-cells, is yellow or yellowish-green in color, shows the presence of large numbers of red cells, as a rule, and an increase of the polynuclear leucocytes over the mononuclear form. These mononuclear forms are more characteristic of the sputum of true tubercular conditions. Fat may be found, either in isolated drops or in large masses. As improvement in the condition occurs, the sputum becomes more abundant, and more distinctly purulent, and then gradually diminishes until it ceases. The sputum of acute bronchitis may give much information as to the course of the disease, as the transition from the viscid mucoid sputum through the abundant purulent stage to the final cessation is quite characteristic.

(5). **Chronic Bronchitis.**

(*a*). **Simple Chronic Bronchitis.**

In most of these cases the sputum is either very little in amount or is much more abundant than in the acute forms. Such cases of simple chronic bronchitis are usually those following the acute type of the disease in which we find the expectoration, for long periods of time, of a tenacious, viscid, and scanty sputum. Later it may become more abundant and muco-purulent, and may have a dark color and a distinctly foul odor.

In the type of chronic bronchitis associated with cardiac disease we find large amounts of blood which may be fresh or changed, giving the typical prune-juice appearance. In such sputum we frequently find large numbers of the so-called "heart-disease cell" which have been previously described.

(*b*). **Putrid Bronchitis.**

This condition is brought about by dilatation of the bronchial tubes following a chronic bronchitis. The sputum lies stagnant in these dilated bronchi so that it decomposes to a great extent. The sputum in such cases is very abundant, is of an ash-gray or brown color, is markedly purulent, and has a very disagreeable odor. On standing it separates into the three layers which have been previously discussed. In such conditions no elastic tissue fiber is found, so that we have here a differentiation from gangrenous or tuberculous pulmonary conditions. The sputum in this condition is very similar to that found in bronchiectasis, which is usually associated with decomposition of the sputum. Whether a diagnosis is possible between a straight putrid bronchitis and bronchiectasis is doubtful, if one relies merely upon the sputum. The sputum in bronchiectasis occurs usually in the morning and is then very profuse. It

shows, however, the characteristics of the sputum of putrid bronchitis, but is more commonly associated with the presence of pus, while 50 per cent. of cases show more or less profuse hemorrhage.

(*c*). **Fibrinous Bronchitis.**

The chief characteristic of this condition is the expectoration of more or less perfect bronchial casts. These may be single or may be distinctly branching, showing the arrangement of the entire bronchial tree. This condition occurs quite frequently associated with many febrile diseases, but in the discussion at this point we have reference to the idiopathic type of the disease. The sputum in this latter class of diseases is mucoid and very abundant in the earlier stages. After a few days there is expectorated, following a severe coughing spell, a bronchial cast. This expectoration is usually tinged with blood. Such casts may be expectorated over long periods of time and their form may vary as previously described.

(6). **Bronchial asthma.**

The sputum in bronchial asthma is, perhaps, more characteristic than that of any other pulmonary condition. During the paroxysm of asthma there may be no sputum, or it may be scanty, consisting of the glairy mucoid plugs known as the pearls of Laennec. The sputum contains many eosinophile cells and many alveolar epithelial cells with myelin degeneration. The mucoid sputum usually contains large numbers of the spirals of Curschmann along with the Charcot-Leyden crystals. In some cases of asthma one finds small cylindrical casts of bronchi. Some of these branch while the majority are straight and may taper at one end into the central fiber of a true spiral.

(7). **Influenza.**

The sputum of the pulmonary type of his condition shows in the early stages as a very scanty tenacious expectoration. Later it increases in amount, becomes muco-purulent and often blood-streaked, and is greenish-yellow in color. This sputum contains large numbers of Pfeiffer's bacilli, which have been previously discussed.

(8). **Gangrene of the Lung.**

The sputum in this condition is very profuse, is greenish-brown in color, is very offensive in odor, and is extremely fluid in character. It contains shreds of elastic tissue which serve to distinguish it from the sputum of putrid bronchitis or bronchiectasis. This sputum separates, as do other forms of sputum which have undergone stagnation and decomposition, into three distinct layers.

Microscopic examination shows fragments of necrotic tissue varying from very minute particles to those several cm. in length. Very few epithelial cells or leucocytes are found, but red blood-cells are more or less frequent. The bacterial content is usually very high, but no hing characteristic is found among these organisms.

(9). **Abscess of the Lung.**

The most characteristic feature of true abscess of the lung or of liver abscess which has perforated into the lung is the sudden appearance of a large amount of pure pus containing fragments of lung tissue This material usually has the normal odor of pus, but may become offensive, although never as markedly so as in gangrene or putrid bronchitis. The sputum of the perforating liver abscess is usually distinguished from that of the true liver abscess by the so-called "anchovy-sauce" appearance. The color may vary, due to the presence of various types of bile pigment. Microscopically, bilirubin crystals may be found.

(10). **Perforating Empyema.**

The sputum of such conditions is composed almost entirely of pus and is thin and liquid. It contains many hematoidin crystals, but very little elastic tissue fiber or other tissue fragments. The odor is usually described as that of old cheese in the beginning, but soon becomes offensive ow ng to decomposition.

(11). **Pneumonoconiosis.**

The sputum in these various conditions will depend upon the pigment with which the lung has been infiltrated. The expectoration is usually mucopurulent, very profuse, and is laden with coal-dust (anthracosis), iron-dust (siderosis), with stone-dust, chalk-dust or plaster of Paris (chalicosis), and with starch granules (amylosis).

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CHAPTER II.

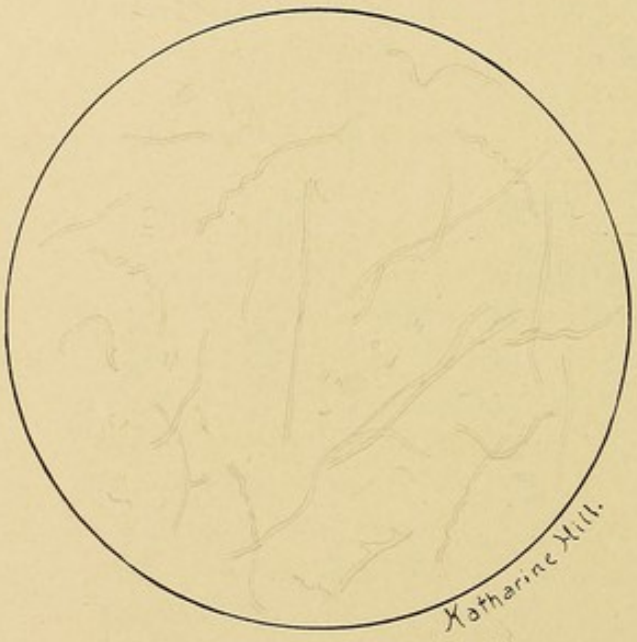
ORAL, NASAL, AURAL, AND CONJUNCTIVAL SECRETIONS.

I. Oral Secretions.

(1). General Considerations.

The oral secretion is a mixture derived from the various buccal glands, the submaxillary, sublingual, parotid, and mucous glands. To this secretion has been given the name saliva. It is a colorless, odorless, and tasteless fluid, which appears somewhat stringy and frothy, separating on standing into two layers, the upper one of which is clear and the lower one cloudy. The function of this secretion is to moisten the mouth and throat and, also, to aid in swallowing the food as well as to partially digest the starchy food through the action of a specific ferment (ptyalin) which it contains. The normal daily amount of saliva secreted is usually about 1,500 c.c., this quantity varying under the influence of many factors, both physiologic and pathologic. The specific gravity ranges between 1,002 and 1,009 giving a total solid content of 3 to 12 grams. Its reaction is alkaline, corresponding to 0.006 to 0.048 per cent. of sodium hydrate. While the reaction of the saliva is normally always alkaline, we occasionally find an acid reaction, especially in children and in the early morning hours, due to the production of lactic acid by the bacteria which are always present in the mouth. Likewise we find an acid reaction especially in conditions associated with acidosis, such states very frequently leading to dental caries and to many other irritative conditions of the mouth. The recent work of Talbot along this line is especially interesting.

The chemical composition of the saliva does not have any great clinical significance with the exception of the presence of the sulphocyanates, the nitrites, and the characteristic ferment ptyalin. These substances seem to have some importance both from a diagnostic and symptomatic standpoint, so that a few remarks may be timely. The presence of potassium sulphocyanate (KCNS) is more or less characteristic of normal saliva and may be detected as follows: Collect a few c.c. of saliva before meals and allow this to filter. Add a few drops of hydrochloric acid and then a drop or two of ferric chlorid solution, when a distinct red color will be observed, whose depth will depend upon the amount of sulphocyanate present. It has been stated that heat should be applied in this test, but the writer has never found it necessary as the characteristic reaction almost invariably appears in the cold. This color disappears on the addition of mercuric chlorid solution, which fact may serve to differentiate it from the similar one given by the saliva of opium habitues and due to meconic



LEPTOTHRIX AND SPIROCHETA BUCCALIS. (UNSTAINED SPECIMEN.)

acid. Very little pathologic significance has been attached to variations in the amount of the sulphocyanate in the saliva, but it is interesting to note that in many cases of diabetes as well as in cases of severe stomatitis this substance is frequently absent.

The nitrites may be detected by the more delicate tests used in water analysis, as their amount is usually not sufficient for the ordinary qualitative tests. A very good test is the use of the Griess-Ilosvay reagent (1/2 gram of sulphanilic acid is dissolved in 150 c.c. of dilute acetic acid and treated with 1/10 gram of naphthylamin dissolved in 20 c.c. of boiling water. On standing, a blue sediment forms which is separated and dissolved in 150 c.c. of dilute acetic acid). On treating 10 c.c. of saliva with a few drops of this reagent and heating, a red color will develop in the presence of nitrites.

The most important constituent of saliva is the ptyalin which has a definite hydrolytic action upon starch, converting this polysaccharid into maltose through the intermediate stages of erythrodextrin and achroödextrin. This action may be readily seen by treating a little starch paste with a few c.c. of filtered saliva and placing the vessel in the incubator for 10 to 15 minutes. At the end of this time iodin solution is added when a distinct red color or an entire absence of color will be noticed. It is to be remembered here that starch, treated with iodin, is colored blue, so that a change of the starch is evident by the color reaction. Recent work by Litmanowicz[1] has shown that the diastatic power of saliva is unaffected by physiologic or pathologic variations in general body functions.

(2). Microscopic Examination.

On allowing saliva to stand it separates into two distinct layers, the upper one clear and containing the liquid portion, while the lower is cloudy and contains the morphological elements. In the microscopic examination of this lower layer we observe many epithelial cells in the form of large, irregular, squamous cells which are derived from the mucous membrane of the mouth and tongue. The number of these cells present depends, of course, upon the erosion to which the mouth has been subjected by various irritants either of the food or of disease. The characteristic cells of the saliva are the salivary corpuscles, which resemble the leucocytes, but are larger and more granular. Occasionally red blood-cells are seen, but these have no direct significance other than to denote ulcerative or markedly irritative conditions somewhere in the nasopharynx. Beside these constituents of the saliva, we find, microscopically, many micro-organisms of the mold, yeast, and bacterial types. The bacteria are always present in the mouth as they are taken in with the air, food, and drink. Few of these have any direct significance, although the spirochæta buccalis should be borne in mind, especially when an examination is being made of a mucous patch for the spirochæta pallida. The former is differentiated from the latter by the fact that its ends lie upon a line drawn longitudinally through

[1] Zentralbl. f. d. ges. Phys. u. Path. des Stoffw. Bd. 4, 1909, S. 81.

the center of its spirals, while such a line drawn through the pallida lies above and below its ends. Moreover, it should be remembered that the smegma bacillus is an occasional habitant of the mouth and throat and may occur in specimens of sputum, giving rise to the assumption of the presence of tubercle bacilli unless proper means of identification are used. Simon has pointed out an interesting fact that the majority of the micro-organisms which are constantly present in the mouth cannot be cultivated on artificial media, while the temporary invaders easily develop. Many pathogenic bacteria have been found in the mouth of the healthy subject. This is interesting clinically as showing the constant danger to which we are all subject, in case our resistance becomes lowered. The writer recalls that the most virulent culture of pneumococci obtained from 200 throats, both diseased and normal, was from his own at the time when he was in perfect condition and showed no symptoms thereafter. Beside the pneumonia organism, streptococci and diphtheria bacilli are frequently found in the mouths of perfectly healthy individuals. Molds and yeast fungi are rarely found in the saliva during health, but they are frequently present in pathological conditions.

(3). **Pathologic Changes.**

The normal daily secretion of the saliva is, as stated above, about 1,500 c.c. The composition of the secretions of the various glands, which contribute to the mixed secretion, differs rather widely, the one from the other. We may, therefore, have changes, not only in amount of saliva, but, also, in the quality, depending on the diseased condition of one or more of these glands. The quantity of saliva is diminished in inflammation of the salivary glands, such as in parotitis, in all febrile diseases, in diabetes, and in nephritis. The secretion is also diminished by the therapeutic use of preparations of belladonna and of opium. It is increased by certain poisons, such as pilocarpin and mercury, by excessive irritation with acids and alkalies, and, also, by irritations arising from carious teeth. Occasional cases have been reported of a greatly increased amount of saliva through some obscure nervous reflex, while such a condition is not unusual in pregnancy. An increased flow of saliva is known as salivation or *ptyalism*. In determining whether or not salivation really exists, observation will frequently show increased amounts of saliva at all times. In some cases, however, it is necessary to measure the amount and, also, to make later chemical examinations of the saliva. The best way of obtaining saliva free from contamination is to wash the mouth thoroughly with a solution of sodium bicarbonate, brush the teeth thoroughly with the same solution, and then rinse out the mouth with cold water. On now touching the inner surface of the teeth or the edge of the tongue with a glass rod that has been dipped into dilute acid, saliva will be seen pouring into the mouth from many points. This saliva is then collected in clean receptacles and the quantity measured.

Variations in the reaction of the saliva are not uncommon in pathologic conditions. In various intestinal diseases with which we may have an associ-

ated stomatitis, an acid reaction is frequently noted. Also in fevers, diabetes, starvation, and other conditions giving rise to acidosis (overloading of the system with acid products) the reaction of the saliva is always acid. Strauss and Cohn believe that the saliva is practically always alkaline, even under pathological conditions.

Coating of the Tongue.

A coating of the tongue is practically always abnormal, as the normal appearance is a bright reddish color with no visible deposits. A change in the normal appearance of the tongue has so long been indicative, in the minds of the profession, of disturbed conditions not only in the mouth, but in the stomach and the bowels, that one should always take into consideration any such change. In severe infectious fevers a brownish coating with a furred appearance is practically always seen. This consists of remnants of food and of incrusted blood, along with large numbers of micro-organisms and dark desquamated epithelial cells. The white coating contains no blood and is more indicative of simple gastro-intestinal disturbance than is the brown coating. The so-called "tartar" which forms upon the teeth seems to consist of deposited calcium carbonate and contains many actively motile spirochete as well as large segmented leptothrices, along with leucocytes and epithelial cells.

Pharyngomycosis Leptothrica.

In many pathological conditions of the throat, such as tonsillitis, diphtheria, and thrush, we frequently find the tonsillar and other buccal structures covered with a coating which is, in many cases, a distinct membrane containing the pathogenic organisms in large numbers. Many perfectly normal subjects complain of the formation, in the tonsillar crypts, of plugs of material which are easily removed by pressure. These are frequently found in patients subject to tonsillitis, but, also, in those showing no pathological conditions of the tonsils and are closely related to Dittrich's plugs, which have been discussed.

In the pyoid masses of pharyngomycosis leptothrica, one finds large numbers of lymphocytes, epithelial cells and long segmented fungi, the leptothrices buccalis, which are colored bluish-red by a solution of iodopotassic iodid. In such conditions the polynuclear neutrophiles are present in only small numbers. In some cases patches of these fungi extend over quite an area of the tonsils so that the appearance may be one of the formation of a diphtheritic membrane, although microscopic examination will at once clear up the diagnosis.

Diphtheria.

One of the most important examinations of the oral cavities consists in the detection of the diphtheria bacillus (Klebs-Löffler bacillus), as an early diagnosis of this disease frequently enables the physician to institute antitoxin treatment. Such an examination should never be omitted in any case of suspected sore throat, especially where any membranous patches are present.

By means of a stout platinum loop or a swab of cotton a piece of membrane or a portion of the exudate is scraped from the throat. This material is then spread over the surface of Löffler's blood serum and is allowed to incubate at 37° C. for six to eight hours. This period of incubation is of some importance as it has been definitely shown that at the end of six to eight hours the diphtheria organism is the only one which will attract much attention, while if left for a longer time, other organisms, especially the streptococcus and staphylococcus, will so far outgrow the diphtheria bacillus that this latter may be unrecognizable unless the incubation be carried 36 hours, when the diphtheria bacillus then assumes the ascendency. From this culture, cover-glass preparations are then made and stained for one to five minutes in Löffler's alkaline methylene blue solution. They are then rinsed in water, dried, and examined with the immersion lens. Frequently attempts are made to diagnose diphtheria by examinations of smears from the fresh exudate. Such cultureless smears rarely show the characteristic appearances of the diphtheria bacilli, so that the writer would advise the use of a preceding culture in all cases. The characteristic cultural peculiarities upon different media must be ooked for in works on bacteriology.

Neisser's Stain.

This siain is supposed to differentiate the diphtheria bacillus from all others. The smear is stained for five minutes with a methylene blue solution (methylene blue, Grübler, 1 gram, 20 c.c. of 96 per cent. alcohol, glacial acetic acid 50 c.c., and water 950 c.c.). The stain should be filtered before use. The specimen is heated gently during the staining process and the dye renewed as the stain evaporates. Wash in water and stain for two minutes with an aqueous solution of Bismarck brown (2 grams in 1 liter of distilled water). The polar bodies will be stained a deep blue, while the body of the bacillus will take a light brown color

Microscopically, the stained organism appears as a slightly curved rod, but especially characteristic are the bizarre forms, such as rods with alternate staining and nonstaining portions, rods with distinct deeply staining polar bodies, club-shaped or "narrow-waisted' rods, many of which lie together in distinctly parallel lines.

Diphtheria bacilli may be found in the throat for weeks after all symptoms have disappeared so that it is wise to enforce isolation of the patient until a negative examination for hese organisms is obtained.

Occasionally in examination of smears from the throat, true diphtheria bacilli may be confounded with pseudo-diphtheria bacilli, and in examination of other specimens, such as those taken from the eye, the bacillus xerosis may be confusing. These different organisms are best differentiated by the study of their action n fermenting or not fermenting certain sugars. According to Knapp, the pseudo-bacilli will ferment none of the sugars, the diphtheria bacilli will ferment dextrose, mannite, maltose, and dextrin, but not saccharose, while



Katharine Hill.

DIPHTHERIA BACILLI SHOWING POLAR STAINING. (NEISSER METHOD, COUNTER STAINED WITH SUFRANIN.)

the xerosis bacillus ferments dextrose, mannite, maltose, and saccharose (cane sugar), while it does not ferment dextrin. The details of the methods for these tests must be found in bacteriological works.

Vincent's Angina (Ulceromembranous Angina and Stomatitis).

In this condition, smears taken from the throat, as well as the free saliva will be found to contain many organisms of two especial types, the first, spirilla, and the second, long fusiform bacilli. Usually both of these types are found together, but occasionally the spirilla are absent. The spirilla usually measure from 36 to 40 microns in length and 1/2 micron in breadth, while the bacilli

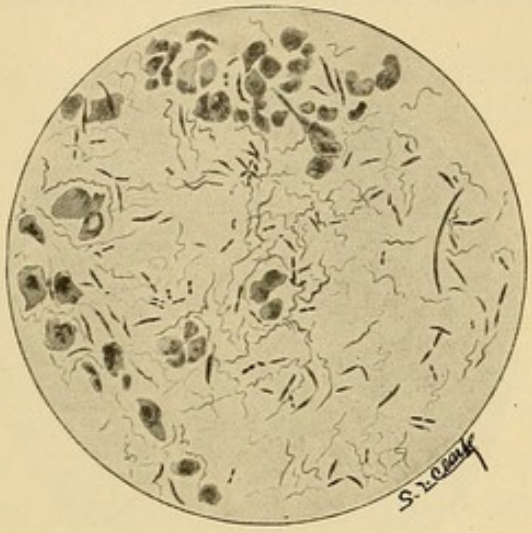


FIG. 12.—Vincent's spirillum and bacillus. (*Coplin.*)

are 6 to 12 microns in length and are somewhat thicker in the center than at the end. These organisms may be readily stained with Löffler's methylene blue, gentian violet, or dilute carbol-fuchsin, but they decolorize with Gram's method. They have so far shown negative cultivation and inoculation results. They are regarded by some as representing definite stages in the development of a trypanosome.

Catarrhal Stomatitis.

This condition is characterized by an increased quantity of saliva, which is rather strongly acid in reaction. Microscopic examination shows many epithelial cells leucocytes, and an occasional red blood-cell.

Should this condition change to the ulcerative form of stomatitis, which is so frequently found following scurvy or mercurial poisoning, the saliva becomes very fetid, dark in color, and alkaline in reaction. The microscope shows many epithelial cells, leucocytes, red blood-cells, necrotic tissue, and many non-specific bacteria.

Gonorrheal Stomatitis.

In this condition the usual changes of infection are observed along with the appearance of he gonococci in the smears. Boston reports several cases

of supposed gonorrheal stomatitis in which cultural methods showed the absence of this organism, although the smears showed the presence of intracellular Gram-negative diplococci. Such reports are not surprising in view of the fact that so many saprophytic diplococci are found which may or may not stain by Gram's method.

Thrush.

This is a condition most commonly seen in children, but may occur in adults, especially in those with tubercular tendencies. The saliva in this condition is usually acid and somewhat increased in amount. Microscopic examination of the membrane shows many epithelial cells, leucocytes, and much

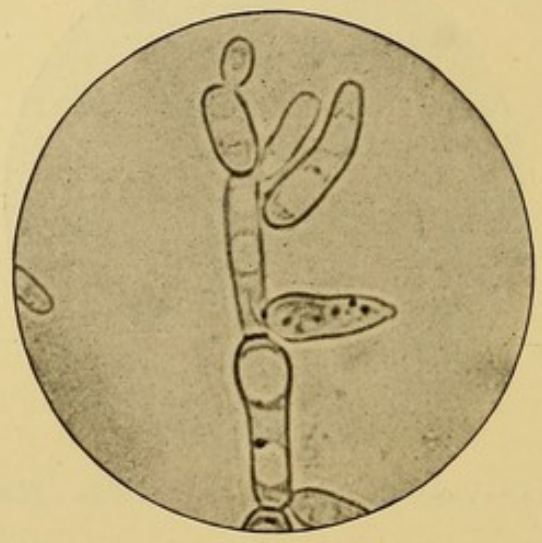


FIG. 13.—Oïdium albicans. (*Kolle and Wassermann.*)

granular detritus with a network of branching band-like formations, showing distinct segments. The contents of the segments are clear and usually contain two highly refractive granules, one at each pole. This organism is known as the Oïdium albicans. It stains well with the ordinary aqueous methylene blue solution.

II. NASAL SECRETION.

This secretion does not present many points for study and seems to be of pathologic significance only in infectious conditions. Normally, the nasal secretion is comparatively scanty, clear, tenacious, odorless, salty in taste, and alkaline in reaction. It is largely composed of mucus, showing squamous and ciliated epithelium in abundance, occasionally leucocytes, large numbers of bacteria and Charcot-Leyden and triple phosphate crystals. The bacterial content of the nasal secretion is made up of both pathogenic and non-pathogenic organisms, the tubercle bacilli having been frequently obtained from a normal mucous membrane.

Pathologic Changes.

In most acute infections, as well as in the so-called acute colds, the nasal secretion is at first diminished in amount, but soon becomes very profuse. This secretion shows the same appearance as does the normal fluid, but as ulceration ensues may be heavily loaded with pus-cells and bacteria. The chronic suppurative process in the nose may affect any or all of the accessory sinuses, so that we may have very severe conditions arising from simple ulceration. Frequently the ulcerative and membranous conditions spoken of above may extend from the mouth to the nose, so that distinct diphtheritic membranes are frequently found in the nasal cavities.

Hay Fever.

In this condition the nasal secretion is found to be increased to a large extent at certain times of the day and much diminished at others, depending upon the paroxysms of the disease. Nothing of pathological importance has been found, however, in the examination of the nasal secretion in this condition, no specific organism having been identified.

Meningitis.

In some cases of meningitis the cerebrospinal fluid passes into the nasal cavity as a result of caries of the bones of the skull. This fluid may be distinguished by the fact that it contains practically no albumin, but does show the presence of a reducing substance which may or may not be sugar. This fluid may also contain the diplococcus intracellularis meningitidis of Weichselbaum. While this organism is not found in all cases of epidemic meningitis, yet it is found in many, so that the nasal secretion may be of some diagnostic importance from this standpoint. The above diplococcus is similar, both in morphological and staining characteristics, to the gonococcus, and presents many difficulties in differentiation (see cerebro-spinal fluid).

In the course of glanders, leprosy, plague, pneumonia, typhoid fever, influenza, and many other infectious diseases, the characteristic bacilli of these conditions may be found in the nasal secretion, but this has little of diagnostic importance unless inflammatory conditions are associated with the other clinical symptoms of the disease.

Occasionally concretions are found in the nose, but these rarely reach a large size and do not have very great pathologic significance. They are largely composed of vegetable fibers taken in by inhalation and cemented by mucus which is hardened by the deposition of lime salts.

In the condition known as ozena the nasal secretion is found to contain many large diplococci (Löwenberg). Klemperer and Schierer believe that the organism of ozena is probably Friedländer's bacillus, as it is very plentiful in the nasal secretions of this disease.

III. The Aural Secretion.

Normally no secretions appear in the external ear, with the exception of that of cerumen, while the secretion of the middle ear and of the internal ear is normally inaccessible to examination. We find, therefore, that the chief importance which is attached to the clinical examination of the aural secretions, is entirely a pathological one. In catarrhal and inflammatory conditions of the external auditory canal, one finds naturally very large numbers of organisms, with which the disease may or may not be associated. In the chronic inflammatory processes of the middle ear, the more important organisms found are the pneumococcus, streptococcus pyogenes, staphylococcus pyogenes, bacillus pyocyaneus, the bacillus of Friedländer, the bacillus coli communis, the diplococcus intracellularis, the typhoid bacillus, and especially the diphtheria bacillus. As disease of the middle ear is so commonly associated with disease of the naso-pharynx, it is possible to find in the discharge from the ear any organism which is causing trouble either in the nose or in the throat. Hamilton has shown the almost constant presence of the pseudo-diphtheria bacillus in the discharge of the running ears following scarlet fever.

It is not an uncommon thing to find certain inflammatory processes of parasitic origin in the external auditory canal. This condition is known as otomycosis and is frequently caused by the aspergillus niger. Besides this organism, many other fungi belonging to this group have been found in the external ear. Among these we find the aspergillus flavus and fumigatus, the aspergillus nidumus, the mucor septatus, the eurotium malignum, and the penicillium minimum. These parasites are very readily detected by removing a small portion of the mycotic mass and spreading it thinly on a slide. Add a small drop of water, apply a cover-glass, and examine under a high-power dry lens. For the details of structure of such organisms, bacteriological works should be consulted.

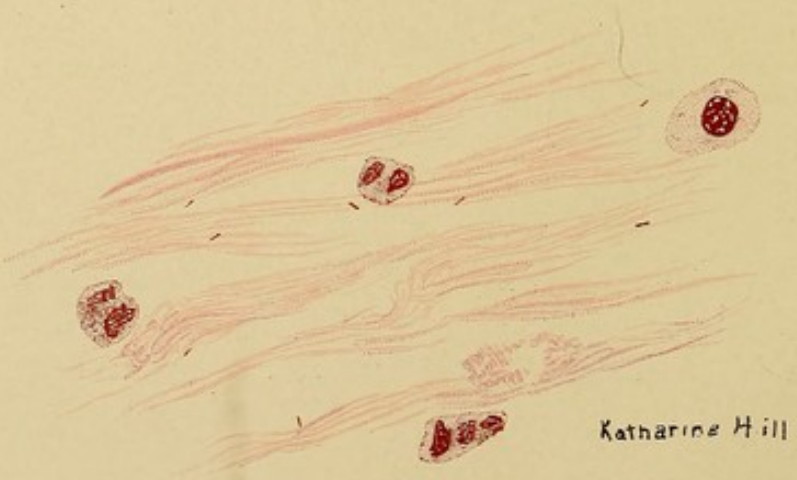
Besides these fungi we occasionally find in the external auditory canal larvæ of various insects. These larvæ may develop later into the full-grown insect and may be removed from the ear by the movements of the animal. In other cases such larvæ have incited inflammatory processes which are occasionally troublesome, as in the case reported by Richardson.

IV. The Conjunctival Secretions.

Under normal conditions the secretion of the conjunctivà and of the lacrimal gland concerns us only very little. It is in the course of an inflammatory process in which these secretions may be greatly increased and greatly changed by the inflammation that any clinical importance attaches to them. In inflammatory conditions of the conjunctiva we find certain organisms which require identification in order that proper treatment may be instituted and the proper prognosis given. It is to be recalled that the pseudo-diphtheria bacillus is practically always found in smears made from the conjunctival secretion, yet it is rarely, if ever, pathogenic in this situation.



MORAX-AXENFELD DIPLOBACILLUS. (GRAM'S STAIN.)
Courtesy of Dr. Brown Pusey.



KOCH-WEEKS BACILLUS. (GRAM'S STAIN.)
Courtesy of Dr. Brown Pusey.

Pathologic Changes.

Diphtheritic Conjunctivitis.

In cases of conjunctivitis which are traceable to infection with the diphtheria organism, we frequently find the formation of an extensive membrane which consists of epithelial cells, leucocytes, and large numbers of streptococci along with the diphtheria bacilli. Clinically, a membrane formation on the conjunctiva may arise from infection of this tissue with organisms other than the diphtheria bacillus; hence, it is wise in all suspicious cases to submit a portion of the membrane to both direct microscopic and cultural examinations. The appearance of the organism has been previously described.

Infectious Conjunctivitis.

In acute infectious conjunctivitis, various organisms have been found, the most common ones being the Koch-Weeks bacillus, the pneumococcus, and the gonococcus. Occasionally one finds the staphylococcus, streptococcus, colon bacillus, influenza bacillus, Morax-Axenfeld bacillus, the diphtheria bacillus, and other organisms.

In some regions the Koch-Weeks bacillus is frequently found as the etiologic factor in acute infectious conjunctivitis, while in others it is rarely, if ever, observed. This is an organism of the influenza group, a small, thin, Gram-negative bacillus, which is, so far as known, pathogenic only for the human conjunctiva (see cut). It grows best on media containing a slight amount of human blood, especially in symbiosis with the xerosis bacillus. This latter organism is differentiated from the Klebs-Löffler bacillus only by the application of the fermentation tests spoken of under Examination for Diphtheria Bacilli in the Throat.

The most common bacterial cause of chronic conjunctivitis is the bacillus of Morax-Axenfeld, usually seen as a diplobacillus, several groups of which may at times be arranged in chains (see Plate V). It is a Gram-negative organism and grows well on Löffler's blood-agar, which it digests, forming on the surface at the beginning of its growth, very characteristic small pits.

Gonorrheal Conjunctivitis.

This form of conjunctivitis is much more common than is generally supposed, so that the identification of the organism is of great importance. In the development of this type of conjunctivitis, large or small amounts of pus are invariably present between the folds of the conjunctiva. A portion of this pus may be collected by means of a cotton swab or a platinum loop and smeared thinly over a slide. This smear is then fixed in the flame and is stained for the gonococcus by both the methylene blue and Gram stains. The technic of this latter staining process, as well as the characteristic appearance of the organism, will be discussed in a later section

Trachoma.

Lately, a great deal of attention has been given to some bodies, which are found in trachoma and which are, possibly, the long-sought cause of this infec-

tious disease of the conjunctiva. These organisms are known as the Prowazek-Greef bodies and are shown in the accompanying cut. They are best stained by the Giemsa stain (see Blood), smears being made in the usual manner.

The present status of these bodies is that they are almost always found in the acute stages of this disease and have not been observed in the conjunctival smears when trachoma does not clinically exist.

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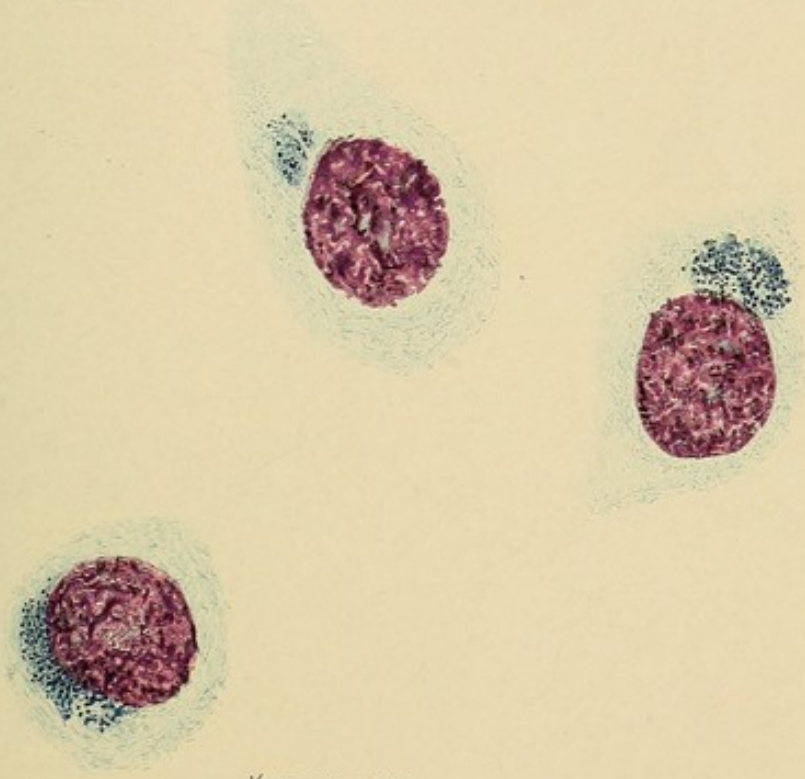

TRACHOMA BODIES OF PROWAZEK-GREEFF. (GIEMSA STAIN.)
Courtesy of Dr. Brown Pusey.

CAPTER III.

GASTRIC CONTENTS.

I. General Considerations.

The gastric juice is the product of the secretory activity of the glands of the stomach. Different series of glands contribute separate elements to the secretion, so that we find much variation, under pathologic conditions, in the composition of this fluid.

The stomach should be regarded as a dilated and specialized portion of the general digestive tube, its walls consisting of the following four coats: mucous, submucous, muscular, and fibrous. From the standpoint of secretory activity the internal or mucous coat is the most important. This mucous membrane is covered throughout its entire length by a single layer of simple columnar epithelium. It follows the various folds or *rugæ* dipping down in places to line the orifices and ducts of the tubular glands which are of such importance in the digestive activity of the stomach. The gastric glands are of two kinds, the peptic or fundus glands, situated in the middle and cardiac thirds of the stomach, and the pyloric glands, found in the pyloric third of the stomach.

Peptic Glands.

These glands are slightly wavy simple tubular depressions, in which a duct, a neck, and a fundus are recognizable. In exceptional cases the fundus is divided, while in nearly all it is tortuous or spiral its extremity being often sharply bent at right angles to the general axis of the tube (Piersol). In these peptic glands are found two types of cell. The first, known as the central, chief or adelomorphous cells, bound the lumen of the gland and form the bulk of the glandular epithelium. These cells are either polyhedral or columnar in form and each contains a spherical nucleus situated within the granular protoplasm. These cells do not stain readily with aniline dyes. The chief function of these central cells of he peptic glands is to secrete the rennet and lipase which are present in the gastric uice. The second type of cell in the peptic gland is known as the parietal, acid or oxyntic cell and is situated in the periphery of the gland immediately below the basement membrane. These cells are more oval or angular in form, are larger than the chief cells, are more finely granular in structure and stain deeply with the aniline dyes They are directly concerned with the secretion of hydrochloric acid.

The Pyloric Glands.

These glands are characterized by their relatively long wide ducts into which the several divisions of the body open; the tubular compartments are

wavy and tortuous and frequently end in slightly expanded extremities. The duct is lined by tall columnar epithelium, the cells becoming lower and broader as they approach the neck and toward the fundus. The cells contain finely granular protoplasm and do not secrete mucus but a thin albuminous liquid. Parietal or acid cells do not occur in the pyloric gland, being confined to the true peptic gland (Pierso[1]).

It will thus be seen that the active portions of the gastric juice are secreted by the fundus glands, the pyloric glands contributing nothing except a small amount of the ferments and liquid portion, the mucus being largely derived from the goblet cells which line the entire stomach and the wider portion of the glandular ducts. It would lead me too far astray to discuss the formation of the fermen s in the cells, but it is well in passing to state that these ferments do not exist in the cells as such, but rather in the form of zymogens or pro-zymogens which become active only in the presence of the free hydrochloric acid.

The free hydrochloric acid of the gastric juice is formed in he parietal cells of the peptic gland. The mechanism of this formation is not absolutely established, but it seems probable that this free acid arises from the chlorids taken up from the blood by these cells. Just what is the active agent in causing the conversion of the chlorids into free acid seems to be in doubt, but it may be either the continuous action of carbonic acid or, as Maly assumes, the interaction of the sodium phosphate (Na_2HPO_4) with the chlorids of the cell. It is also probable that the osmotic influences may be very great in the production of this free hydrochloric acid as Koeppe advocates. This acid is present at all times in the normal stomach, being found even in cases of extreme starvation.

The recent work of Pawlow[1] has shown that various factors influence the quantity and quality of the normal gastric juice. He asserts that the "appetite is the first and mightiest exciter of the secretory nerves of the stomach, a factor which embodies in itself a something capable of impelling the empty stomach of the dog in the sham feeding experiment to secrete large quantities of the strongest juice. A good appetite in eating is equivalent from the outset to a vigorous secretion of the strongest juice; where there is no appetite this juice is also absent." Moreover, under natural conditions, the stimulation of food is a very important factor. The administration of a diet causes a secretion of gastric juice which is directly proportionate, both in amount and activity, to the diet taken. We find, according to Chigin,[2] that the greatest digestive power is shown by the juice excreted after the administration of bread, although the total acidity is greatest following an intake of meat. If we compare equivalent weights of food material we find that flesh requires the most gastric juice and milk the least; but taking equivalents of nitrogen, bread needs the most and flesh the least. In this connection it is well to remember that the gastric secretion varies from hour to hour. Thus the most active juice occurs with flesh

[1] The Work of the Digestive Glands. London, 1902.
[2] *Loc. cit.* Pawlow.

in the first hour, with bread in the second and third hour, and with milk in the fifth to the sixth hour. The point of all this is that the rate and time of secretion of the gastric juice is always characteristic for each diet.

Moreover, it has been found that the hydrochloric acid first secreted combines at once with the proteins of the various food stuffs, so that we may find no free hydrochloric acid in the gastric contents, although the secretion may be normal and may show a very high degree of total acidity. Usually however, we find the presence of free hydrochloric acid in amounts ranging from 0.2 to 0.3 per cent.

II. Methods of Obtaining the Gastric Contents.

Unless the patient is one who can easily eject the contents of the stomach by vomiting, it is necessary to resort to the introduction of the so-called stomach-tube for the removal of the contents. This stomach-tube consists of a long, soft rubber tube about 75 cm. in length, having a lumen 6 to 7 mm. in diameter and provided with either two oval lateral openings or with three, one being at the end of the tube. Before introduction of the stomach-tube, it should be moistened with warm water and should be thoroughly cleaned. It is frequently a wise precaution, in general work where all classes of patients are to be examined, to have separate tubes which may be used by patients affected with tubercular or syphilitic conditions. While this may seem unnecessary, it is not impossible to bring about an infection in a patient free from these conditions. In those patients who require frequent washing-out of the stomach or frequent examination of the stomach contents, it is wise to order separate tubes for each.

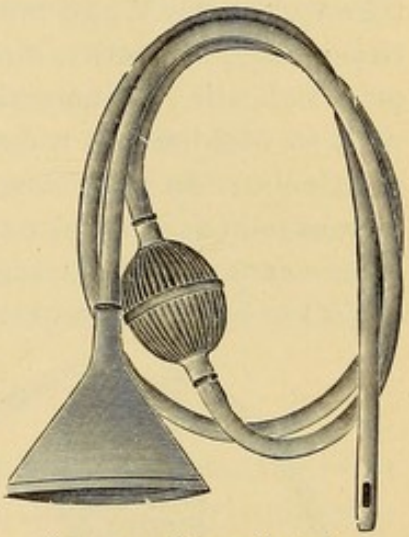

Fig. 14.—Stomach tube

Introduction of the Tube.

The patient must be in a sitting posture, a towel or a rubber sheet being placed about his neck to prevent soiling of the clothes with the saliva or material which is occasionally brought up during the passage of the tube. False teeth should be removed and anything interfering with the passage of the tube should be avoided. In patients who are hypersensitive, a 10 per cent. solution of cocain is applied to the pharynx. The head of the patient is now bent slightly forward, never backward as some advise, and the mouth slightly opened, care being taken never to use a depressor on the tongue. The rubber tube, held as one would a pen, is passed gently backward over the tongue until its tip strikes the posterior wall of the pharynx, when it turns downward and may be readily introduced into the stomach, by slight forcing. As the tube reaches the esopha-

gus, many patients complain of a sense of suffocation, which is not real but apparent. The tube interferes in no way with the normal respiratory movements and hence the patient should be cautioned to breathe normally and not forget to breathe. If the patient will swallow normally, the passage of the tube is greatly facilitated. It occasionally happens that highly nervous patients have great difficulty in swallowing this tube, so that it may be necessary to defer the withdrawal of the contents to a second or even a third period. It is never wise to excite a patient by forcing matters at any stage of the investigation. If any sign of cyanosis or marked pallor is evident the tube should be immediately withdrawn and a second attempt made at some later time. When the tube has reached the floor of the stomach, which is in normal cases about 40 cm. from the incisor teeth, a distinct resistance to further passage of the tube will be noticed. This point should be carefully observed as the forcing of the tube beyond this point may produce rupture of the stomach wall or may cause the tube to "buckle." In this latter condition it will be impossible to withdraw the stomach contents. Many of the tubes used for gastric examination have a mark indicating the normal length of tube from the incisor teeth to the stomach wall, so that one has a definite idea when he has introduced the tube to the right point. In some cases the gastric juice will commence to flow from the tube as soon as it is properly introduced, but in the majority of cases some help is necessary to start the siphonage. Frequently all that is needed is to ask the patient to bear down with his abdominal muscles or to cough a little. In other cases aspiration is necessary. This may be done by the mouth, but this method does not seem advisable. Better practice is to employ an ordinary Politzer bag or a Boas bulb for starting the fluid in the tube. This is very readily done by compressing the bulb and applying it, while compressed, to the end of the tube in such a way that the suction will be sufficient to draw the material into the tube. Once started, the material flows quite readily, but it may be necessary to use aspiration several times as the tube may become clogged wi h tenacious mucus or particles of food stuff. The material as it flows from the tube is collected in appropriate vessels and set aside for future work.

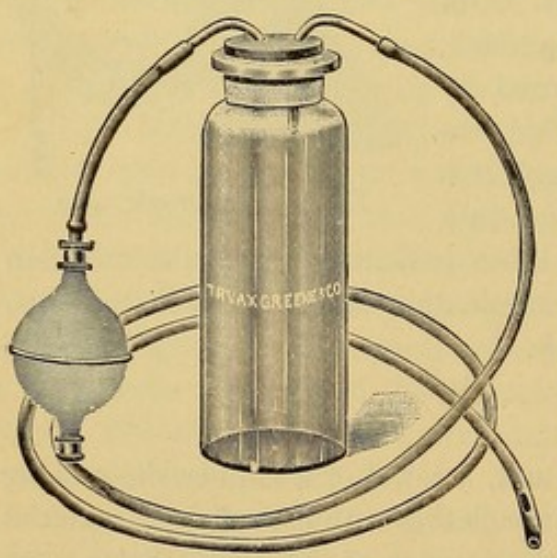

FIG. 15.—Turck's aspiration apparatus.

If it is desired to wash out the stomach, either to obtain the total gastric contents or for the purpose of mere lavage, a funnel is attached to the external end of the stomach-tube and about 500 c.c. of water are allowed to flow through the tube into the stomach. In this operation the funnel is held either on a level with the patient's mouth or a very little bit above. By depressing and

inverting the funnel over a suitable vessel, before all the water has left it, return flow will soon set in and the stomach will be practically emptied by siphonage. In some cases it becomes necessary to add more water, but in no case should any be added after the patient complains of a feeling of distress.

In collecting the stomach contents one should avoid as far as possible any admixture with the saliva which is more freely excreted at this time than normally. This is best done by wrapping a cloth about the tube so that the material may be absorbed as it runs along the side of the tube. After one has obtained the gastric contents, the tube is compressed with the fingers and is rapidly withdrawn, care being taken to keep up the compression so as to hold in the tube material which has not already passed into the receiving vessel. This residual material is added to the portion first received.

In cases in which water has been introduced to wash out the stomach after the gastric contents have been obtained, one should be careful to note the amount of fluid poured into the stomach so that he may be able to judge of the amount again received. In this way only may he arrive at approximate results regarding the total acidity of the contents withdrawn.

Not every case with which the practitioner meets is amenable to such manipulation. We find as especial contraindications to the use of the stomach-tube, uncompensated valvular lesions of the heart, arteriosclerosis, aneurysm, advanced pulmonary tuberculosis, marked emphysema, acute febrile diseases, severe hemorrhage, especially from ulcer or carcinoma, and excessively developed nervous antipathy.

Test Meals.

As the secretion of the gastric juice is so dependent upon administration of food, it has become the custom to use certain combinations of food principles, which will excite gastric activity and enable us to obtain a juice which will give us more or less definite ideas of its composition in the condition investigated. It must be remembered that marked idiosyncracy toward certain foods exists, so that we may not use in all cases the same sort of a diet for exciting the gastric juice. The results obtained in pathologic conditions are compared with those obtained from normal individuals under the influence of the same diet. In this way we are able to say, with some degree of certainty, that a suspected case shows normal or abnormal gastric relations. These diets, the so-called test meals, are always given to the fasting stomach and are removed after a suitable time by the use of the stomach-tube. The time best suited for the administration of these meals is in the morning, as the stomach has had occasion during the night to empty itself of most of its contents.

Ewald Test Meal.

This meal, which is, perhaps, the most frequently employed in general work, consists of a roll or piece of bread or toast without butter and a cup of water or tea without milk or sugar. In approximate figures this will represent 35 grams of wheat bread and 400 c.c. of water or tea. The bread should be

well masticated so that the later withdrawal of the contents may not be interfered with by the plugging up of the openings in the tube. The contents are removed one hour later and consist normally of 30 to 50 c.c., depending both upon the skill of the operator and upon the condition of the stomach. Hypermotility of the stomach will diminish the quantity of contents received, while a hypomotility will increase the quantity.

Boas Test Meal.

This meal consists of a dish of oatmeal prepared by concentrating to 500 c.c. a liter of water to which a tablespoonful of oatmeal is added. This meal was advised to prevent the introduction into the stomach of lactic acid which is a normal constituent of bread. While this small amount of lactic acid introduced in the Ewald meal has little significance, yet in doubtful cases it is well to avoid it. The contents of the stomach are withdrawn one hour later when the amount may be very small. If the stomach shows normal digestive powers most of the material will be then passed into the intestine, while an appreciable amount of material would indicate either a dilatation of the stomach or pyloric obstruction.

Riegel Test Meal.

This test meal has the advantage of permitting the patient to use a diet which is more normal than either of the ones previously mentioned. This diet is more important in America, where we are not accustomed to the continental breakfasts, than it is in Germany where the Ewald and Boas meals are more usual.

The Riegel meal is given in the middle of the day at a time when the patient is accustomed to such a meal. It consists of about 400 c.c. of soup, 200 grams of beef-steak, and either two slices of white bread or 150 grams of mashed potato along with one glass of water. This meal is withdrawn at the end of three to four hours. It has the advantage of allowing us to judge of the length of time which the food remains in the stomach under normal conditions and, also, to form an opinion of the rate and amount of digestion which has taken place. This meal incites a more nearly normal gastric juice than does the Ewald or Boas meal, but it is such that clogging of the stomach tube by paticles of undigested food frequently occurs.

Fischer Test Meal.

This meal, introduced by an American physician, has the advantage of more nearly approaching an American breakfast than the others. It consists of the bread and tea of the Ewald meal along with a quarter of a pound of finely chopped lean beef broiled and seasoned. The contents are removed at the end of three hours. Fischer has shown by comparing results after his meal with those of the Ewald breakfast that those with his are much more constant and somewhat higher than with the latter.

Salzer Test Meal.

This is in reality a double meal and is given as follows: For breakfast the patient receives 30 grams of lean cold roast meat, finely chopped, 250 c.c. of milk, 60 grams of rice, and one soft-boiled egg. Four hours thereafter a second meal is given, consisting of 35 to 70 grams of stale wheat bread and 400 c.c. of water. The contents are then removed one hour after this second meal. Under normal conditions of digestion and motility the stomach contents should show no remnants of the first meal.

Sahli Test Meal.

This meal was introduced to enable the worker to examine quantitatively the material withdrawn. The inconstant composition of the ordinary test meals makes it rather difficult to judge of the digestive power of the stomach. Sahli has introduced, therefore, a soup prepared as follows: Twenty-five grams of flour and 15 grams of butter are placed in a pan and browned over a fire. Three-hundred and fifty c.c. of water are then added and the whole boiled for five minutes (the loss in volume being replaced by fresh water) after which it is seasoned with a little salt. In this soup the fat is in the form of a very fine emulsion and the taste is so pleasant that a more nearly normal stimulus to gastric secretion is offered. The patient is now allowed to take 300 c.c. of this soup, while the remaining 50 c.c. are retained for a determination of the fat content. The contents are withdrawn, one hour after the meal, from the stomach which must have been thoroughly washed out prior to the administration of the meal.

We then determine the absolute amount of fat remaining in the stomach after the test digestion and compare this amount with that introduced. As we cannot be sure that the entire stomach contents have been withdrawn, we must know the residual amount of gastric juice. For this purpose one resorts to the method of Matthieu, which will be discussed later (p. 53).

The amount of fat both in the original soup and in the withdrawn stomach contents is then determined and the total gastric juice calculated. This method of fat determination will be given in detail under Milk, to which the reader is referred.

Calculation of Results.

"The following calculations are possible from a consideration of the residue from the acidity of the gastric filtrate, and from the difference be ween the amount of fat found in the ingested flour soup and that found in the expressed contents.

"By the addition of the value X, found in the calculation of the residue (p. 53), to the amount of contents expressed after one hour, there is obtained the volume of the contents which were actually present in the stomach at the end of that period. This we designate as *To*. From the absolute fat-content of *To*, there can be determined how much of the volume can be ascribed to the ingested flour soup. The amount of fat remaining in the stomach serves, there-

fore, as a measure for the amount of soup remaining. This is designated as *Su*. Representing this mathematically, we have the proportion To: Su: : F: f, in which F represents the percentage fat-content of the soup and f that of the expressed contents. To—Su will give, of course, the volume of gastric juice in the expressed contents. If the acid-content of *To* has been determined, it is possible from these data to proceed further and to calculate what acidity was possessed by the pure gastric juice as it was excreted. Thus, if 75 c.c. of pure gastric juice are present in the stomach contents, whose volume amounts to 150 c.c. with 2 per cent. acidity, then the acid-content of the pure gastric juice is evidently 4 per cent. The determination of this acidity may be expressed by the proportion, To: A: : Ma: a, in which *To* represents the amount of expressed contents including the residue, *Ma* the amount of secretion contained in the expressed contents (To—Su), *A* equals the acidity of the pure secretion in per cent, and *a* the percentage acidity of the expressed contents" (Sahli).

It is important to remember in selecting a test meal for any given case that the tastes of no two persons are alike and that no two persons will react identically toward the stimulation of the same test meal. We should in every case endeavor to pay more attention to the administration of meals similar to those to which the patient is accustomed and, also, to give them at the time when such meals are ordinarily taken. The results can only be comparative and have, in this sense, some value. Too much rigidity in administration of such meals will lead frequently to mistakes in diagnosis, so that one should learn to vary his test meals rather than to rely upon a single one in all cases. Another point to be borne in mind is that the meals should be removed at the time of optimum secretion, which may not in all cases be at the end of one hour with the Ewald meal. For this reason a single examination of the stomach contents should not be implicitly relied upon in making a diagnosis.

III. Macroscopic Examination.

The gastric juice is a clear, colorless, easily filtered, levorotatory fluid having a distinctly acid reaction, an acid taste, and a characteristic odor. Its specific gravity, when the stomach is empty, ranges between 1004 and 1006.5; after the ingestion of food from 1010 to 1020 and more than 1020 when the production of acid is diminished (Landois). Its cryoscopic point is —0.38 degree to —0.444 (Roth and Strauss).

Amount.

The figures for the total amount of gastric juice secreted in 24 hours are variable. Beaumont gives 180 grams per diem while Bidder and Schmidt give a figure corresponding to about one-tenth of the body weight. The amount of fluid obtained one hour after an Ewald meal, is from 20 to 50 c.c., although larger amounts ranging from 200 to 500 c.c. indicate either diminished motility

or hypersecretion, on the one hand, or dilatation associated with pyloric stenosis, on the other. It is to be remembered that the quantity of juice secreted is influenced by the appetite and by the amount and character of the food taken, as well as by the age and sex of the patient and the time of day at which the food is taken. The largest amounts of gastric juice are found in cases of hypersecretion when it is not uncommon to find a liter or more of gastric juice in the non-digesting stomach.

In order to determine the total amount of gastric juice secreted, one cannot rely upon the quantity removed as there is always a slight residuum. The method of Matthieu and Rémond is commonly used for such determinations. This gives results very nearly exact, at least for clinical purposes. With this method the gastric contents are removed, as nearly as possible, after an Ewald meal. A definite quantity of water, usually 300 c.c., is then poured into the stomach through the tube and is thoroughly mixed by moving the funnel up and down and by pressure upon the stomach. As much as possible of this added fluid and the remaining gastric juice is removed and collected in a separate vessel. The acidity of the undiluted as well as of the diluted stomach contents is then determined by titration. From the difference in these two values conclusions may be drawn as to the degree of dilution and to the residual amount of stomach contents which was not expressed. The amount expressed plus the residual amount equals the total gastric contents.

The following is the method of calculation according to Matthieu:

Let a = acidity of the undiluted gastric contents.

Let b = acidity of the diluted gastric contents.

Let x = amount of the test meal remaining in the stomach after expression.

Let 300 c.c. = the amount of water introduced into the stomach for dilution. Then

$$a : b : : x + 300 : x$$

$$ax = b\,(x + 300)$$

$$x = \frac{300\,b}{a - b}$$

An absolutely accurate result, in the study of gastric activity, can be obtained only when the total quantity of gastric juice is known. It is, therefore, necessary in stating, for instance, the acidity of a stomach contents to calculate the total available acidity rather than the mere degree of acidity. It is self-evident that a stomach contents expressed in the ordinary way, which shows an acidity of 40°, may have this acidity in a total quantity of 50 c.c., while an acidity of 40°, with a total quantity of 200 c.c., would represent actually four times as much hydrochloric acid available. It would seem, therefore, to the writer that the method of representing acidity in terms of degrees without any reference to the amount of gastric contents is absolutely irrational.

Color.

Gastric juice is normally a practically colorless liquid, although at times it may be somewhat opaque and, therefore, much whiter in color. Variations in this colorless fluid are observed after test meals due to admixture of various food products, so that we may have distinctly brownish colorations due to the tea or particles of toasted bread, while in the test meals consisting of meat the color may be more of a reddish tone.

Pathologically, we may find a distinct red color due to the presence of blood. This bright red color comes from the presence of fresh blood from a hemorrhagic gastric ulcer or may be derived from abrasions of other portions of the alimentary tract. If the blood has been thoroughly mixed with the stomach contents for some time it may appear in the form of a brownish-black deposit, the so-called coffee-ground material. The blood in these cases is in the form of hematin and must be tested for as later outlined. This coffee-ground appearance is particularly evident in cases of gastric carcinoma.

The color of the gastric contents may be either a yellow or a green, due to the presence of bilirubin in the former case and biliverdin in the latter. This biliary pigment should be detected by the tests outlined under Urine. The presence of bile in the gastric contents is indicative of duodenal occlusion.

In cases of intestinal occlusion below the duodenum we occasionally find fecal matter in the gastric contents. This is characterized by the brownish-black coloration and by its intense odor.

Odor.

The normal gastric juice is practically odorless or very slightly sour. Admixtures of material coming from the intestines cause a very intense odor, while the material rising from abscesses along the alimentary tract above the stomach will frequently give rise to a very offensive odor. In the vomitus obtained under various pathological conditions the odor may be very characteristic. Thus, in uremia we may find a distinct odor of ammonia, in alcohol intoxication a distinct alcoholic odor is evident, while in cases of stagnation of gastric contents an intensely strong odor is observed. In cases of dilatation we frequently find the organic acids so much increased in amount that distinct odors are noticeable.

Consistency.

The normal stomach contents are usually watery in character, but may vary due to admixture with extraneous material. After test meals or following a vomiting spell we may find portions of unchanged protein or carbohydrate material. The amount of bread taken with an Ewald meal should be so far digested in one hour as to form a puree-like mass which settles out on standing. Various food residues are, of course, present in the vomitus so that the consistency and appearance may give us much valuable information regarding the digestive process. In cases of mucous catarrh or in those showing either a diminution or an increase in the amount of hydrochloric acid we may find after

a test meal the presence of large amounts of tough, slimy, mucoid material, which may be so abundant as to practically make it impossible to filter the contents. The consistency of such material may be almost that of a paste, or may be simply that of a thick syrup which on pouring from the vessel onto the filter will form distinct mucoid threads. The presence of an increased amount of mucus is of some diagnostic importance and should, therefore, be looked for under all circumstances.

Gastric Contents from Fasting Stomach.

The stomach is practically never empty, always containing a certain amount of acid fluid. Boas considers anything between 10 and 100 c.c. as a normal amount of material for the fasting stomach. Anything above this amount would mean either motor insufficiency or hypersecretion. One may differentiate these two conditions by washing out the stomach at night, when the material withdrawn in the morning will be extremely scanty if the condition is one of motor insufficiency. Riegel regards any material in the fasting stomach as pathological.

This fluid from the fasting stomach is thin, has a specific gravity of 1004 to 1005, contains some free hydrochloric acid, no lactic acid, and no bacteria. It is very commonly bile-stained, may be alkaline from the presence of pancreatic juice and may contain large amounts of mucus. As such material is always found in the fasting stomach it is well to make it a rule to wash out the stomach the night before giving a test meal.

Vomitus.

In those cases which are associated with frequent vomiting we may obtain much valuable information from the examination of the ejected material. It is in these cases that one finds, frequently, much difficulty in passing the stomach-tube. The amount of material vomited will depend, of course, upon the motility of the stomach. In cases of dilatation or of stenosis we frequently find two or three quarts of material, while in conditions associated with hypermotility we may have simply a scanty highly mucoid vomitus. The presence of food particles will give much information as to the digestive power of the stomach. If undigested meat fibers are found in the vomitus, ejected three hours after eating, one may assume more or less disturbance of protein digestion. If particles of unchanged bread are found, three hours after taking, the disturbance in protein digestion is probably more marked than in the case of the meat fibers. If an individual vomits bits of food more than seven hours after a meal, some impairment of motility must exist, according to Sahli, for after that interval even a hearty meal should have completely left the stomach. The vomiting of an acid liquid containing no food particles is quite characteristic of hypersecretion of gastric juice.

The degree of acidity of the vomitus as well as the amount of hydrochloric acid present very frequently enables us to judge of the activity of the juice.

These figures will not be as reliable as are the ones obtained after a test meal, but may serve in cases in which the stomach-tube cannot be passed.

Frequently one finds a vomitus which is quite foamy and smells strongly of the volatile fatty acids. In such conditions we may assume a diminution in the amount of hydrochloric acid, which normally prevents the occurrence of any such decomposition, or we may ascribe this condition to simple stagnation of the gastric contents. Such contents will show microscopically the presence of large numbers of sarcinæ ventriculi, yeast fungi, and various bacteria.

The blood in the vomitus varies from a slight streaking of the material to a fluid which shows intimate mixing with the gastric contents. In cases of recent hemorrhage, which is particularly common in ulcer of the stomach, an abundant admixture of fresh arterial blood or of dark coagulated blood is observed. Brown or black coffee-ground-like material is particularly suggestive of carcinoma, although the same condition may result from erosion of the gastric mucous membrane when associated with hyperacidity or hypersecretion.

An admixture of bile, producing a yellowish or greenish discoloration, may occur with any type of vomiting, but more especially from an empty stomach and in that associated with duodenal obstruction. A biliary vomiting is frequently observed in peritonitis and may be due to the fact that there is no counterpressure from the gastric contents to prevent regurgitation from the duodenum. This green vomitus may not always be due to the presence of bile, but may come from contamination with various chlorophyll-containing organisms.

Almost all types of vomitus contain mucus. In some cases we find abundant tough, slimy masses which seem to be indicative of mucous catarrh of the stomach or of a diminution in the amount of hydrochloric acid.

Fecal vomiting is a sign either of complete motor insufficiency of the intestine as found at times in peritonits, or indicates intestinal obstruction, either in the lower part of the small intestine or in the large bowel. The brownish-black color of this vomitus and the distinct odor render it very characteristic.

Asiatic cholera and cholera nostras are associated with a vomitus which is abundant, alkaline in reaction, contains white flakes of mucus and epithelial cells, and large numbers of bacteria, both Koch's spirillum and the Finkler-Prior spirillum and various other unidentified types. This vomitus of cholera is known as the "*rice-water*" vomitus.

The time at which vomiting occurs is frequently of great importance from the diagnostic standpoint. If it be at the height of digestion and during intense pain the condition is probably one of ulcer. If during or shortly after eating we may have either gastritis, a neurosis, or cancer. If it is frequent in the morning before breakfast and seems to be independent of eating the condition is probably one of dilatation. While these statements are not infallible, yet they are applicable in the majority of cases.

Gastric Contents After Test Meals.

The amount of material obtained after a test meal has some diagnostic importance. As previously stated, one obtains after an Ewald or Boas meal from 20 to 50 c.c., of contents, but these figures may vary to as high as 500 c.c. Hypersecretion or motor insufficiency are the chief causes of such increased amounts, the former being more probable if a large amount of free hydrochloric acid is present along with the excessive amount of fluid. A larger proportion of solid undigested material is observed in cases of pure motor insufficiency, but we frequently have a combination of both conditions. Absolute proof of the diminished motility is found in the presence of more than a trace of food in the stomach seven to eight hours after a meal.

The general appearance of the material obtained after a test meal will be practically those previously discussed.

IV. Microscopic Examination.

The microscopical examination of the gastric contents is usually made on material withdrawn from the stomach after test meals, but the vomitus is occasionally examined. The gastric juice is practically never free from remnants of food, such as meat threads or starch granules, although nothing has been taken for many hours. Moreover, small masses of mucus, which occasionally assume a snail-like spiral form, and saliva which is recognized by the presence of large flat epithelial cells and the so-called salivary corpuscles are quite frequent. A few bacilli and yeast cells are almost always observed.

As such elements as the ones above mentioned are present in all gastric juice, we must not attach undue importance to the presence of small amounts of such material in the contents obtained after a test meal. After the Ewald meal one rarely finds anything beyond the presence of numerous starch granules and more or less mucoid material, along with bacteria of the various types which flourish particularly in the buccal and gastric cavities.

In cases associated with diminished motility of the stomach we may find remains of food which has been introduced many hours previously. In such specimens we observe numerous fat globules or fatty acid crystals, many vegetable fibers and cells and a few red blood-cells which have come from slight abrasion of the mucous membrane of the pharynx by the stomach-tube. These red cells usually are much altered in appearance by the hydrochloric acid and do not show their ordinary hemoglobin color, but take on a more brownish tint, which is due to the presence of hematin.

Boas-Oppler Bacillus.

This organism is found quite commonly in patients suffering with carcinoma of the stomach, and is almost always absent in nonmalignant disease. It is found more frequently in the gastric contents at a time when lactic acid is present in large amounts, so that in the incipient stages of carcinoma these

organisms may be absent. These bacilli are very long (3 to 10 microns), 1 micron broad, and are frequently joined end to end forming very long chains. They are readily stained with the usual aniline dyes and by Gram's method and, on treatment with iodin, take on a brown color which distinguishes them from the large mouth bacillus (leptothrix buccalis), which stains blue with iodin. This organism is not absolutely pathognomonic of carcinoma of the stomach, but is found in 75 to 85 per cent. of all cases, being rarely present in dilatation or benign stenosis of the pylorus.

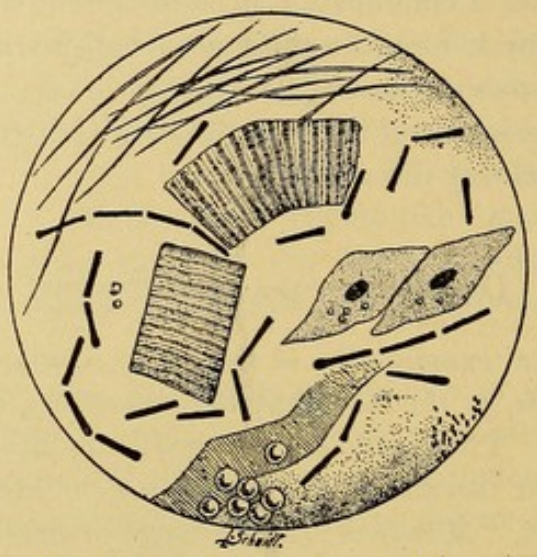

FIG. 16.—Boas-Oppler bacilli. (*Hemmeter.*)

Sarcinæ.

Occasionally in normal gastric juice and especially in cases of dilatation with marked fermentation one finds the so-called *sarcinæ ventriculi* which are cocci arranged in squares or tetrahedra which resemble, very much, cotton bales. These organisms have no pathologic significance, but are indicative of stagnation of gastric contents. Along with these sarcinæ one may find large numbers of yeast cells.

Protozoa.

These unicellular parasites have been occasionally found in the gastric contents. Flagellates, amebas, and monads seem to be more frequent than the other types of protozoa. They seem to be more commonly found in cases of carcinoma of the stomach; quoting from Simon, "from the available data there can be no question that the presence of protozoa in the stomach contents is suggestive of nonobstructive carcinoma."

Fragments of Tissue.

Frequently small shreds of mucous membrane are found in the expressed gastric contents. One finds these in cases of chronic gastritis, ulcer, hyperchlorhydria, and especially in cancer. These tissue fragments should be studied carefully under the microscope, as not infrequently a diagnosis of cancer is possible from such examination.

Crystals.

Various types of crystal are occasionally noted in the gastric contents, among which may be mentioned bile acids, cholesterin, fatty acids, leucin, tyrosin, and calcium oxalate. If the reaction of the juice is alkaline triple phosphate crystals may appear.

V. Chemical Examination.

The chemical examination of the gastric juice is the most important of all laboratory methods in the diagnosis of various pathologic gastric conditions. As previously stated, the acidity of gastric juice is referable to the presence of free and combined acids. The free acidity is traceable largely to hydrochloric acid, although organic acids, such as lactic, acetic, and butyric, may increase the free acidity under abnormal conditions. Besides this free acidity, we have hydrochloric acid which is bound chemically to the protein substances and does not react with tests for free acidity. There are also present in the gastric juice acid salts, especially the sodium dihydrogen phosphate (NaH_2PO_4).

Besides these factors which have to do with the reaction of the gastric juice, we find certain ferments which act only in the presence of the free hydrochloric acid. The first of these, pepsin, has the power of acting upon albumin in an acid medium and converting it, through various stages, into lower splitting products of albumin. This peptic digestion will be discussed in detail later. A second ferment, known as rennin, lab, or chymosin, has the power of curdling milk by coagulating the casein. A third ferment, lipase, acts upon fat, especially when this is present in a finely divided form. This lipolytic action is not ordinarily great, but should nevertheless be remembered. The experiments of Sahli show that this action is negligible during the period covered by the administration of his test meal.

Although many statements have been made to the contrary, the gastric juice, through the agency of the hydrogen ions of its free hydrochloric acid, acts upon certain polysaccharides, especially cane sugar, hydrolyzing them into the simpler monosaccharides. Careful determinations indicate that the speed of inversion is about the same as that of an equal strength of hydrochloric acid, so that we do not need to assume any ferment action.

Besides these substances gastric juice contains a small amount of albumin, carbohydrates, and various inorganic salts. None of these constituents have any importance from a clinical standpoint and will be disregarded.

(1). Total Acidity.

As previously stated, the total acidity of the gastric juice is referable to the presence of free and combined hydrochloric acid, organic acids, and acid salts. This factor may be readily determined by titrating 10 c.c. of gastric juice with tenth-normal sodium hydrate, using phenol-phthalein as an indicator. This indicator is colorless in the presence of acid and becomes red at the point of neutralization, being used as a 1 per cent. alcoholic solution. On adding a

few drops of this solution to the filtered gastric contents, a white cloud will be observed due to the precipitation of the reagent by the water of the gastric juice. The titration is carried to the point at which the addition of sodium hydrate produces a definite pink color which remains permanent and does not deepen on the addition of further alkali. If sodium chlorid be added to the point of saturation of the gastric contents, the end point becomes somewhat sharper owing to the diminished dissociation which the disodium hydrogen phosphate undergoes into sodium dihydrogen phosphate in the presence of increased sodium ions. This precaution is rarely taken, however, as the clinical result is never so accurately determined as is the scientific factor.

The total acidity varies between rather wide limits. Normally it ranges from 75 to 100°, being made up of approximately 50° of free hydrochloric acid, 25° of combined hydrochloric acid, and 25° of organic acids and acid salts. The chief variation under normal conditions is an increase in the combined hydrochloric acid and a decrease in the organic acids and acid salts.

In pathologic conditions we may find the total acidity high with very little free hydrochloric acid, or we may find the total acidity low, with a normal amount of hydrochloric acid present.

Sahli has stated the variations in the acid factors of the stomach contents as follows: If the total acidity is high and the hydrochloric acid is normal, the high acidity can be due only to a deficient motility and absorption and hence we find an increase in the organic acids. Such a gastric juice may show lactic acid, but will more probably give the tests for the other organic acids. A low total acidity with an excess of hydrochloric acid shows that the motility and absorptive powers of the stomach are good. If the total acidity be moderate and free acid small in amount, a poor motility may be assumed. Generally speaking, when much lactic acid is present we find low HCl and only combined HCl; that is, diminished secretion and diminished motility. No lactic acid is found when the HCl is normal or increased.

(2). Free Hydrochloric Acid.

A number of tests have been devised for the detection of free hydrochloric acid in the stomach and its differentiation from lactic and acetic acids. It should be remembered that the tests outlined below are not specific tests for hydrochloric acid, but are common to all mineral acids. Many of these tests react also with the organic acids, providing they are present in sufficient concentration. The efficiency of any acid is due merely to the ionic decomposition which it suffers when in solution; in other words, is due to the presence of free hydrogen ions. Hydrochloric acid appears to be more efficient in the digestive processes than do the organic acids, owing simply to its greater degree of dissociation. The tests commonly employed in clinical work are based upon the reaction which certain coloring matters undergo when treated with free hydrochloric acid. These tests can be, therefore, only approximate and must be used with discretion in scientific work, although in clinical work they are near

enough for all purposes. Were we able to express completely the stomach contents and thus to obtain material which would give us absolute data, we would then require better clinical methods. What should be measured in testing stomach contents for free acidity is the number of hydrogen ions which the gastric juice contains, since this is the important factor in the efficiency of the juice. This may be determined directly by measuring the speed of any catalytic reaction due to hydrogen ions, the most convenient one being the rate of inversion of cane sugar. Several indirect methods have been devised for determining the presence of free hydrochloric acid as distinguished from free organic acids, combined hydrochloric acid, or acid phosphates.

It is wise to have some quick method by which one may determine the presence of a free acid in the gastric juice. This may be done with litmus which will, however, not show the presence of free hydrochloric acid to the exclusion of other acids or acid salts. To determine whether the acid reaction is due to free acid it is customary to employ the Congo-red paper. This consists simply of filter-paper which has been saturated with an alcoholic solution of Congo-red and dried. On treating gastric juice with such paper we obtain a blue color in the presence of free acid. This paper reacts with a blue color to any free acid so that one should never assume the presence of free hydrochloric acid when he obtains a blue coloration. Although many writers state that a blue coloration is never given by free organic acids, the writer has seen too many cases in which distinct action was referable either to free lactic or free acetic acids to agree with this statement. The tests which are applicable to the detection of free mineral acids in general may be used as indicative of free hydrochloric acid, as this is the only mineral acid which one would ordinarily find in the gastric contents.

Qualitative Tests.

(*a*). Töpfer's Test.

The test is based upon the coloration which a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol takes when treated with gastric juice containing free hydrochloric acid. A few c.c. of filtered gastric juice are placed in a dish and one or two drops of the above solution added. In the presence of free mineral acids a carmin red color is obtained. This reagent is a very delicate one and does not react to organic acids unless they are present in amounts exceeding 0.5 per cent. The coloration with free hydrochloric acid varies in intensity with the amount of acid present, and may range from a deep orange to an intense carmin. According to Simon, lactic acid does not give the typical red color with this reagent, especially if albumoses are present, unless it be in a concentration of at least 1 per cent. This reagent will detect the presence of 0.02 parts of hydrochloric acid per thousand.

(*b*). Gunzburg's Test.

The reagent employed in this test consists of 2 grams of phloroglucin and 1 gram of vanillin dissolved in 30 c.c. of absolute alcohol. This yellowish solu-

tion should be kept in dark bottles, as it gradually changes to a dark red and then to brown when exposed to the light. Boas claims that the reagent becomes more delicate and stable if one dissolves the phloroglucin and vanillin in 100 c.c. of 80 per cent. alcohol.

Two or three drops of this solution are added to an equal amount of the gastric juice contained in a porcelain dish and the mixture evaporated over a a water-bath. In the presence of free mineral acid a rose-red color is developed, varying in intensity with the amount of acid present. This mixture must not be boiled or heated too rapidly as the resulting color will then be brown or brownish-red and may mislead one into believing that no free hydrochloric acid is present.

This test does not react to organic acids or to acid salts, nor is it interfered with by the presence of products of food digestion. It may, therefore, be used with the unfiltered gastric juice. This test reacts with the rose-red color in the presence of 0.05 parts of HCl per thousand.

(*c*). **Boas' Test.**

This reagent consists of 5 grams of resorcin and 3 grams of cane sugar dissolved in 100 grams of 95 per cent. alcohol. It has the same delicacy as Günzburg's test and is more stable. The test is applied in the same way as the preceding, taking particular care to use a low flame in evaporating, and gives a rose-red or vermilion color in the presence of mineral acids. This color gradually fades on cooling and is not given by organic acids or acid salts.

(*d*). **Tropeolin Test.**

The reagent for this test is a saturated alcoholic solution of tropeolin 00. This test is applied in the same way as the preceding and gives a lilac-blue color in the presence of free acid. This test is not as delicate as the preceding, reacting only in the presence of 0.3 parts of free hydrochloric acid per thousand, and has the objection that it strikes the blue color much more easily with the organic acids.

Other tests have been advocated for the qualitative detection of free hydrochloric acid, but they are not as delicate as the above and have nothing to justify their existence. Of the tests given, the Günzburg test seems to be the most reliable, although the Töpfer's test is clinically sufficient and has the advantage of being much less expensive and more convenient than is the former reagent.

Quantitative Methods.

The quantitative estimation of free hydrochloric acid is of great importance in the study of all pathologic conditions of the stomach. Any determination made with our present methods must have reference to the fact that the acidity of the gastric juice is due almost entirely to the free hydrochloric acid. While this is not absolutely true, yet the organic acids are rarely present in sufficient amounts to react with the indicators in conditions in which hydrochloric acid

is normal or increased in amount. However, in the conditions associated with diminished amount of hydrochloric acid one must be on his guard in the interpretation either of the qualitative or quantitative tests for free hydrochloric acid. The writer has seen several cases in which all of the indicators, with the exception of Günzburg's reagent, showed positive results for hydrochloric acid, the cases being those in which no free hydrochloric acid was actually present. Bearing this in mind we may determine the acidity, referable to free hydrochloric acid, by the titration of a known amount of the filtered gastric juice with tenth-normal sodium hydrate solution, using as indicators the solutions mentioned under the head of qualitative tests.

Mintz Method.

As the Günzburg reagent is the most delicate and reliable of all the tests for free hydrochloric acid, it is wise to use this reagent as an indicator. The test as usually followed by the writer is to add 20 to 30 drops of the Günzburg reagent to 10 c.c. of the gastric juice. On adding the sodium hydrate solution no color change will be visible, as the reaction takes place only when the solution is warmed. This warming cannot be done directly as the evaporation would necessarily have to proceed to the point at which loss of hydrochloric acid might occur. Following the recommendation of Sahli, the rod with which the solutions are stirred is warmed before being used. A distinct red color will be evident along the sides of the rod as the neutralization point is reached. The older method of Fleiner, consisting in the removal of a drop or two of the gastric juice after the sodium hydrate solution had been added and the evaporation of this mixture in a small dish, is too time-consuming and introduces the error of loss of substance at each trial. The writer would recommend, therefore, either the use of the warm glass rod or a combination of the Congo-red paper as an approximate guide to the point of neutralization and a determination of the final result with the Günzburg method.

Töpfer's Method.

This method is the simplest, is the most generally used, and at the same time is one of the most delicate of all the clinical quantitative methods for free hydrochloric acid. It consists in the use of dimethyl-amido-azobenzol as an indicator, the titration of the filtered gastric juice being done with tenth-normal sodium hydrate solution. Although this reagent does give, under some conditions, a red color in the absence of free hydrochloric acid when the organic acids are largely increased, yet such conditions are so rarely found in clinical work that the result of test-tube experiments cannot be applied to clinical cases. The experiments of Simon show that lactic acid must be present to the extent of 1 per cent. before any cherry-red color is obtained, providing albumoses are present. As these latter substances are always found in the gastric juice after intake of protein material, one can readily see that lactic acid need not be considered. We find, however, conditions associated with

fermentative processes in the stomach in which acetic and butyric acids are present in fairly large amounts. These acids will give a red color with the indicator, but should not mislead as their strong odor in such concentrations permits of easy recognition.

In the titration the sodium hydrate solution is added from a buret to the filtered gastric juice to which one or two drops of indicator are added for every 10 c.c. of juice. In the presence of free hydrochloric acid this indicator strikes a distinct cherry-red tone and thus enables the worker to decide at once as to the presence or absence of the acid. No evaporation is necessary, hence the test has the advantage of simplicity and does not occasion any loss of substance. As the sodium hydrate solution is added the reddish tint of the mixture changes to a distinct yellow. The titration must be carried to the point at which every trace of red disappears and the color becomes a pure yellow. This reaction requires considerable experience, hence the writer would advise the student to make his titration with a known solution of hydrochloric acid so that he may become familiar with the end point.

Much confusion exists in the literature regarding the use of filtered or of unfiltered juice for these titration tests. It is the writer's custom to use the filtered contents, as an exact measurement of the quantity of juice taken can rarely be made otherwise, owing to the presence of food particles which will raise the meniscus in the measuring glass to the desired point without giving in reality an actual measurement of the amount of true juice desired. As the hydrochloric acid is in solution what should be tested is a known quantity of the solution and not a mixture of the solution with the food particles. Another source of error in using the unfiltered gastric juice is the possibility of the combination of the sodium hydrate used in titration with the undigested protein material, thus giving a somewhat higher result than is really present. Using the Günzburg reagent, the results are lower than with the Töpfer reagent, as the removal of the substance in the former test introduces a distinct error.

Amount of Free Hydrochloric Acid.

The researches of Ewald, Szabo, and Boas show that free hydrochloric acid is present in the stomach to the extent of two to three parts per thousand (0.2 to 0.3 per cent.). This amount of acid is present only at the height of digestion as the hydrochloric acid first secreted is bound to the protein material in the form of combined hydrochloric acid, so that if a stomach contents be examined at the height of digestion and no free hydrochloric acid be found, one may assume that a disturbance of this function of the stomach is present. The time at which this physiologic excess will appear depends upon the kind of food upon which the gastric juice has acted. After an Ewald breakfast this excess should be present in from 45 to 60 minutes, while after a Riegel meal in from two to three hours. These points should be borne in mind as the removal of such meals before these periods will always give a deficit in the amount of free hydrochloric acid.

Certain clinical conditions lead to an excretion of gastric juice which is normal, increased, or diminished in amount of free hydrochloric acid. We must, therefore, have a method of interpretation of the free HCl acidity of the stomach. It is customary to report the results of titration of a given specimen of gastric juice in one of two ways:

(1). We may represent the acidity referable to free hydrochloric acid by the number of c.c. of tenth-normal sodium hydrate necessary to neutralize 100 c.c. of filtered gastric juice, using dimethyl-amido-azobenzol as an indicator. This is called degree or percentage of acidity. Thus, if 5 c.c. of tenth-normal sodium hydrate were used to neutralize 10 c.c. of filtered gastric juice, the degree or percentage of acidity would be, obviously, 50.

(2). We may report the actual amount of free hydrochloric acid present. This is the more scientific way, as we have a much better means of comparison with the normal standards. One c.c. of tenth-normal sodium hydrate neutralizes 0.00365 grams of free HCl. If, now, we multiply this factor by the number of c.c. necessary to neutralize 100 c.c. of filtered gastric juice, we obtain a figure representing the absolute amount of free HCl in the gastric content. Thus, if the gastric juice showed an acidity of 50 degrees, we would have 0.1825 (50 x 0.00365) grams of HCl. The usual text-book statement is that the free hydrochloric acid is normally about 40 degrees, but the writer is accustomed to consider an acidity of 50 to 55° as much more nearly normal than one of 40 .

Euchlorhydria.

This is a condition in which the amount of free hydrochloric acid is between 0.1 and 0.2 per cent. In speaking of percentage one must not confuse the two types of percentage reckoning. Thus, 10 per cent. or 10° of HCl represents only 0.0365 gram or true per cent. of hydrochloric acid. The lower figure of 0.1 per cent. is given for euchlorhydria owing to the fact that normal variations may permit of this low point, although the higher figure of 0.2 per cent. is the more usual. When this euchlorhydria exists in the presence of clinical symptoms pointing to gastric disturbance, we usually have to do with a neurosis. Gastritis may be absolutely ruled out, a carcinoma excluded except when the new growth has taken place upon an old ulcer and an ulcer practically always ruled out, although this latter may show an euchlorhydria. This condition may be associated with a certain amount of atony along with more or less marked dilatation.

Hypochlorhydria.

This is a condition associated with excretion of the gastric juice showing 0.1 or a lower per cent. of hydrochloric acid. It is found especially in subacute or chronic gastritis, in incipient carcinoma, in fevers, severe anemias, many mental diseases, passive congestion due to valvular heart lesions, many cases of chronic nephritis, and dilatation of the stomach. Some rare cases of ulcer of the stomach show a low degree of free hydrochloric acid, but this is not usual.

Anachlorhydria.

This is a condition characterized by the excretion of the gastric juice showing the complete absence of free hydrochloric acid. This condition has been supposed to be pathognomonic of gastric carcinoma. There are, however, many cases of cancer which show either a hypo- or a hyperchlorhydria, and there are, also, many other conditions which show an anachlorhydria. Thus we find this condition in a large majority of cases of advanced chronic gastritis, in the severe anemias, especially of the pernicious type, in neurasthenia and hysteria, in many severe febrile diseases, and in atrophic gastritis.

Hyperchlorhydria.

This conditions exists when we have the excretion of a gastric juice showing more than 0.2 per cent. hydrochloric acid. This figure may run anywhere from 0.2 to 0.9 per cent. It is a very common occurrence in nervous individuals, in ulcer of the stomach, some cases of chlorosis, in some chronic cachexias, in the early stages of chronic gastritis, in carcinoma which is grafted on to an old ulcer, in continuous hypersecretion, in chronic passive congestion of the stomach, and in cases of migraine.

It will thus be seen that the hydrochloric acid of the gastric juice varies under clinical conditions to quite an extent. While an increased amount of hydrochloric acid is usually present in ulcer of the stomach, we must not necessarily make our diagnosis on this point alone. Likewise in carcinoma of the stomach we should not exclude this condition if the examination of the stomach contents does not show a lessened amount of hydrochloric acid or even a total absence, as many cases of carcinoma may show all varieties of chlorhydria. It is to be said that here as in practically all other laboratory examinations a single determination is not conclusive. A series of examinations should be made so that one may have comparative figures. In this way only may one be sure of his ground.

(3). **Combined Hydrochloric Acid.**

As the hydrochloric acid first secreted by the gastric glands combines with the protein of the food material, it is necessary to have some method by which we may determine just how much of this material has been formed in the stomach. The physiologically active hydrochloric acid consists of both the free and combined acid so that we may have only a slight amount of the free acid, but a relatively large amount of the combined. Not infrequently we find cases which show no free hydrochloric acid, but quite a percentage of the combined acid, indicating that a certain amount of acid has been secreted by the gastric juice. This combined hydrochloric acid has, therefore, a certain clinical importance and should be investigated in every case. No direct methods are known for its determination so that we must resort to indirect methods.

Method of Martius and Luttke.

This method, as modified by Reissner, is much more applicable to scientific work than to clinical investigations, as it is too complicated and time-consuming

for the ordinary practitioner. It is based upon the facts that the free hydrochloric acid as well as the acid combined with protein material escape upon incineration of the gastric juice, while the inorganic chlorin in combination with inorganic bases remains in the ash. If the total amount of chlorin present in the filtered gastric juice be determined (a), and then the amount of chlorin in the ash (b) investigated, subtraction of the latter (b) from the former portion (a) will give the amount of chlorin referable to free and combined hydrochloric acid except a small loss referable to volatilization of ammonium chlorid. If the gastric juice be neutralized with sodium hydrate before it is incinerated and the chlorin in this ash determined, the amount of this chlorin (a′) subtracted from (a) represents the ammonium chlorid volatilized. Hence a′ − b equals the free and combined HCl. By now determining the amount of free hydrochloric acid according to Töpfer's method we may at once calculate the combined hydrochloric acid by subtracting this result from the amount of free and combined hydrochloric acid previously obtained. The method of determining the chlorin will be fully discussed in the section on urine to which the reader is referred.

Method of Töpfer.

This method embraces three separate determinations. In the first place the total acidity of the gastric juice is determined by titration of 10 c.c. of filtered gastric juice with tenth-normal sodium hydrate, using phenolphthalein as an indicator. This result is termed *a*. This indicator has the advantage of reacting toward anything of an acid nature, and will give us, therefore, the different factors which go to make up the total acidity of the gastric juice, namely, the free and combined acids, the organic acid, and the acid salts.

Having determined this factor (*a*), a second portion of 10 c.c. of gastric juice is titrated with tenth-normal sodium hydrate solution using a 1 per cent. aqueous solution of alizarin (alizarin monosulphonate of sodium) as an indicator. Two or three drops of this indicator are added to 10 c.c. of filtered gastric juice when the mixture becomes distinctly yellow. The titration is carried to the point of production of a pure violet color which does not deepen on the further addition of alkali. As this reaction demands the recognition of a change from yellow through a faint violet to a deep violet color the worker must have considerable practice before he is able to accurately determine the end point. The result is termed *b*. No trace of red should be present, a pure violet color being the true reacting point. This color may be observed by treating a few drops of alizarin solution with a 1 per cent. solution of sodium carbonate. Alizarin reacts with free acid, both mineral and organic, and with acid salts, but not with organically bound HCl. If, therefore, we subtract the figure obtained when alizarin is used as an indicator (*b*) from that obtained with phenolphthalein (*a*) the result will be the combined hydrochloric acid ($a - b$).

If now we add this combined hydrochloric acid to the free hydrochloric acid, which has been obtained by titration of the gastric juice using dimethyl-

amido-azo-benzol as an indicator, (c), we obtain the total physiologically active hydrochloric acid ($c+(a-b)$). The difference between the total acidity and this factor gives us the amount of organic acid and acid salts present. ($a-(c+(a-b)$). If but a small amount of gastric juice be available for chemical examination, recourse may be had to a modification suggested by Einhorn. This is a double titration of the same portion of juice. A few c.c. (5) of filtered gastric juice are treated with a few drops of dimethyl-amido-azobenzol and the solution titrated for free HCl with sodium hydrate. When the point of neutralization of the free acid is reached a few drops of phenolphthalein solution are added and the titration continued to the point of neutralization of total acidity. These indicators do not interfere at all with one another as their reacting points are usually widely different. The writer has found this method reliable and convenient.

Hydrochloric Acid Deficit.

In those cases in which the gastric contents shows no free hydrochloric acid, it is customary to determine the HCl deficit. By this is meant the amount of hydrochloric acid which must be added to the gastric contents before it shows a reaction for free acid. This amount will naturally depend on the amount of combined HCl already present, the amount of protein in the gastric contents and the amount of alkali secreted. Sahli suggests the expression "*saturation deficit*" for this figure. Ten c.c. of unfiltered gastric juice are titrated with tenth-normal hydrochloric acid, using dimethyl-amido-azobenzol as an indicator and titrating to the point of production of the red color. The result is expressed in terms of degrees as under the representation of the free hydrochloric acid. This factor enables one to follow the course of the disease, showing how little hydrochloric acid is excreted for combination with the proteins of the food.

(4). **Organic Acids.**

The organic acids, outside of the lactic acid, have very little clinical significance. The food practically always contains a certain amount of fatty acids which appear in the stomach contents and contribute to the total acidity. In the normal digestion of the carbohydrates, lactic acid is practically always formed, so that excess of this acid would indicate excessive fermentative processes in the stomach, due to a combination of diminished amount of hydrochloric acid along with a lessened motility of the stomach. Other acids, such as butyric and acetic acids are formed in this same process of carbohydrate fermentation, so that the organic acids may represent a large portion of the total acidity. Besides this, bacterial decomposition, in the absence of hydrochloric acid, plays a rôle in the production of these fatty acids. The fat-splitting ferment, lipase, may produce these organic acids in fairly large amounts.

Total Organic Acid.

It is sometimes of importance to know just how much organic acid is present in the stomach contents. This may be done directly by the Hehner-Maly

method, which is based upon the fact that, if a mixture of organic and inorganic acids be neutralized and then incinerated, the organic acids will be converted into carbonates while the inorganic acids remain as neutral salts. If the alkalinity of these carbonates be then determined and this factor subtracted from the total acidity we obtain directly the organic acids. This is possible owing to the fact that the degree of alkalinity of the carbonates is equal, in terms of tenth-normal solutions, to the acidity referable to the organic acids. The technic is as follows: The total acidity of 10 c.c. of gastric juice is determined by titration with tenth-normal sodium hydrate solution as previously described. The neutralized solution is evaporated to dryness in a platinum dish and is then incinerated. The ash is dissolved in distilled water and the alkaline solution titrated with tenth-normal oxalic acid solution. As 1 c.c. of the tenth-normal oxalic acid solution is equivalent to 1 c.c. of tenth-normal sodium hydrate solution, we subtract the factor obtained in the latter titration from that of the former and obtain directly the degree of acidity due to mineral acids. The number of c.c. of tenth-normal oxalic acid used represents directly the total organic acids present.

In this method the acid salts are included in the factor referable to mineral acids, so that we may subtract from this factor the degree of acidity, attributable to free hydrochloric acid, and obtain the amount of acid salts present. In some cases fatty acids are present which are not soluble in water and consequently are not neutralized by the addition of the sodium hydrate solution. These acids may be extracted from the neutralized solution with ether and may then be neutralized and added to the neutral aqueous solution. The mixture is now evaporated as before and incinerated. This estimation of the higher fatty acids requires the use of the unfiltered gastric contents. However, such acids do not play a large clinical rôle and may be ordinarily omitted.

(*a*). Lactic Acid.

The ordinary foods such as milk, bread, and meat contain a certain amount of lactic acid, so that any test for the presence of lactic acid can be of value only when the meal contains very little of such foods or when the portion taken in with the food has disappeared from the stomach. After the Boas meal there is always less lactic acid than after the Riegel meal, so that the former is much preferable when a special test is to be made for the presence of lactic acid. Boas has shown that under physiological conditions no appreciable amount of lactic acid is formed during the process of digestion. At the height of digestion practically no lactic acid is demonstrable in the stomach contents. This may be due to its absorption, on the one hand, or, on the other, to the fact that the hydrochloric acid interferes with the delicacy of the reactions. Pathologically, lactic acid is found in any condition associated with stagnation of the gastric contents as a result of motor insufficiency, provided the amount of hydrochloric acid is below the normal amount. As this condition of affairs is found most frequently in cases of carcinoma of the

stomach, an excess of lactic acid is very strongly suggestive of malignancy, although it must be remembered that such an excess may appear in cases of benign stenosis and gastric insufficiency. If the stomach be washed out the evening before giving a test meal, preferably the Boas meal, and lactic acid be found in appreciable amounts, carcinoma is the probable diagnosis. This finding of increased lactic acid and diminished hydrochloric acid is not always observed in every case of carcinoma of the stomach. In some cases periods of increased production of hydrochloric acid alternate with increased formation of lactic acid, and in some cases, especially those in which the carcinoma has developed upon the base of an old ulcer, no lactic acid may be present, but hydrochloric acid may be found in large amounts.

Uffelmann's Test.

This test is, perhaps, more commonly employed for the detection of lactic acid than is any other, but the writer prefers the Kelling test. Uffelmann's reagent consists of 20 c.c. of 1 per cent. carbolic acid solution, to which are added one drop of dilute ferric chlorid solution and sufficient water to form a transparent amethyst-blue solution. This solution is not permanent and must, therefore, be made fresh before each test. If a few drops of the filtered gastric juice be added to 5 c.c. of this reagent, the solution will be decolorized in the presence of lactic acid, taking on a beautiful canary-yellow or greenish-yellow tint. The mere decolorization of this solution is not sufficient for a positive test. A pure lemon yellow or canary color must be present before one may assume the presence of lactic acid. Even when this color appears one must eliminate such factors as the acid sodium phosphate, cane sugar, glucose, alcohol, and various organic acids, such as tartaric, citric, or oxalic, before he can say that lactic acid is present. A considerable excess of hydrochloric acid in the gastric juice may prevent the appearance of this color and likewise a yellowish tint of the stomach contents may obscure the result. Under such conditions it is necessary to extract the gastric contents with ether, which takes up the lactic acid, leaving the interfering substances behind. The ethereal solution is then evaporated, the residue taken up with distilled water, and the test applied to this solution.

Kelling's Test.

This test is in reality a modification of the previous one and consists in the addition of a few drops of filtered gastric juice to a very dilute solution of ferric chlorid. As used in the writer's laboratory, the method is as follows: To a test-tube full of water are added one or at most two drops of a 10 per cent. solution of ferric chlorid. The mixture is thoroughly shaken and divided into two portions, one of which serves as a control. On now adding a few drops of filtered gastric juice to one of these portions a distinct canary-yellow color will appear in the presence of lactic acid. The color of the two solutions should be compared so that any change in the one, to which gastric juice was

added, may be observed. This test has the same objections as the Uffelmann test, so that it is always wise to extract the gastric juice with ether before applying either one of these tests.

Strauss' Method.

This method is, perhaps, the very best clinical method at our disposal, as it shows lactic acid when present in pathological amounts. It does not, however, give a quantitative result, nor does one seem necessary in the ordinary clinical work. Into a special separatory funnel (see cut) are introduced 5 c.c. of the gastric juice. The funnel is then filled to the 25 c.c. mark with alcohol-free ether and well shaken. The ethereal layer will take up the lactic acid from the gastric contents. After the fluids have settled the gastric juice and ether are allowed to run out to the mark 5 by opening the stop-cock, after which distilled water is added to make up the 25 c.c. volume. Two drops of 10 per cent. ferric chlorid solution are then added with a medicine dropper and the mixture well shaken. The water will now extract the lactic acid from the ether. The aqueous layer is colored an intense greenish-yellow if more than 0.1 per cent. of lactic acid is present, while smaller amounts will show a slight greenish tinge. This test may be negative if the lactic acid present is completely combined with the proteins of the gastric juice. In such cases hydrochloric acid may be added to liberate this lactic acid before shaking out with ether.

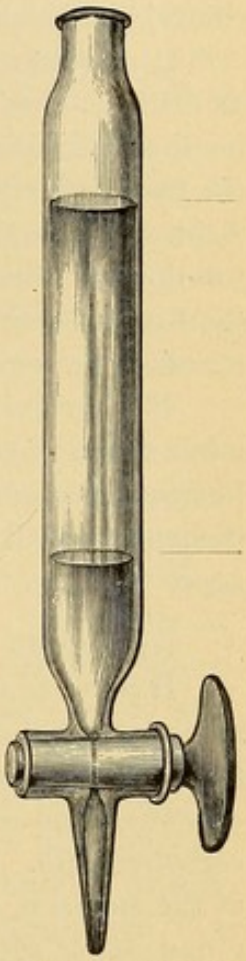

FIG. 17.—Strauss' separator funnel. (*Hemmeter.*)

Other qualitative tests as well as several quantitative tests have been given for lactic acid. Quantitative determinations of lactic acid do not seem to be of any great clinical importance, as any marked reaction for this substance is indicative of a pathologic condition whose extent may bear no relation whatever to the amount of lactic acid present. A general idea of the amount of lactic acid may be obtained by evaporating 10 c.c. of gastric juice, acidulated with a few drops of sulphuric acid, to the consistency of a syrup and then extracting this residue several times with acid-free and alcohol-free ether. The ether may be removed by evaporation and the residue taken up with water. This watery solution may now be titrated with tenth-normal sodium hydrate, each c.c. of alkali used representing 0.009 gram of lactic acid.

The method of Boas, while very exact, is much too complicated and time-consuming for clinical work so that the writer will refer to other books for a description of this test. The principle of the method is based upon the fact that when lactic acid is heated with a strong oxidizing agent it is decomposed into acetic aldehyd and formic acid. If now the aldehyd be distilled off and

transformed into iodoform by the addition of alkaline iodin solution, this iodoform may be quantitatively determined.

(*b*). **Butyric Acid.**

This acid does not occur in the gastric contents, under physiological conditions, unless much milk or carbohydrate food has been introduced. Flügge has shown that butyric acid may be derived from lactic acid and consequently may be present under the same conditions in which lactic acid is found. As butyric acid may be introduced from without and may have been formed in the mouth, one should be careful in drawing conclusions as to the clinical significance of butyric acid.

If present in any large amount, butyric acid may be usually recognized by its distinct odor which is that of rancid butter. This test may not be sufficient for the recognition of butyric acid so that it is advisable to shake out the gastric juice with ether, evaporate, and take up with water as described under Lactic Acid. If a small pinch of powdered calcium chlorid be added to this watery solution and the mixture warmed, butyric acid will separate from the fluid in the form of small fat drops which float on the surface and have a characteristic odor of rancid butter.

If a portion of the dried ethereal extract of the gastric juice be treated with a few drops of concentrated sulphuric acid and a little alcohol, the odor of ethyl-butyrate is perceptible on slight warming. This odor is the peculiar one of pineapples and is very easily recognized. This test is known as the pine-apple test.

(*c*). **Acetic Acid.**

It is not an infrequent occurrence to find acetic acid in rather large amounts in pathological conditions, although it is rare physiologically except when introduced with the food. The pathologic acetic acid is formed by the bacterial decomposition of the alcohol which is produced by the action of yeast upon carbohydrates. As yeast fungi are so frequently present in cases of dilatation of the stomach, associated with stagnation of its contents, acetic acid may be found under such conditions and may constitute a large portion of the total acidity.

In testing for acetic acid the aqueous extract of the ethereal residue of the gastric juice is carefully neutralized with sodium carbonate solution. If a few drops of ferric chlorid solution be added to this neutralized solution a deep red color will appear if acetic acid be present. If this solution be boiled a reddish precipitate of basic ferric acetate is formed. This neutralization of the aqueous solution is an essential point in this test, as the presence of free acid will prevent the appearance of any precipitate and the presence of free alkali will cause the formation of ferric hydroxid which will mislead, as the coloration is very much the same. The writer has seen several cases in which acetic acid was mistaken for lactic acid when the Kelling test was applied. On adding gastric juice containing a large amount of acetic acid to the dilute

ferric chlorid solution a change in color is observed, but in no case do you get the distinct canary-yellow color which can be possibly referable to acetic acid.

(5). Gastric Ferments.

(*a*). Pepsin.

The enzyme pepsin is the most important of the ferments occurring in the gastric juice. As previously stated this ferment is excreted in the form of the zymogen (pepsinogen or propepsin) by the chief cells of the fundus glands. It becomes active, that is, converted into pepsin, by the free hydrochloric acid of the gastric juice. This ferment acts only in acid media and is destroyed by the presence of minute traces of alkali. Its action is continuous, a small portion being capable of digesting large amounts of albumin, providing the products of this digestion are gradually removed. Should the products of ferment activity remain in the stomach an undue length of time, this ferment will cease to be active owing to the accumulation of the products of its own activity. Pepsin acts in the presence of many other acids, but the concentration of these acids must be higher than in the case of hydrochloric acid. Thus, a 0.2 to a 0.4 per cent. hydrochloric acid gives the best results with pepsin, while a 1 to a 1.018 per cent. lactic acid is necessary to bring about good results.

Very little data exists as to the amount of pepsin or of its zymogen so that we are forced to draw our conclusion regarding a normal or abnormal amount of this ferment from the rate at which known amounts of albuminous material are digested. Pepsin acts only upon protein substances, giving rise to a series of decomposition products which will be discussed later. Normally 25 c.c. of gastric juice will dissolve (digest) 0.05 to 0.06 gram of serum albumin in one hour, the same amount of coagulated egg-albumin in three hours, and a similar amount of fibrin in one and one-half hours.

A diminution in the amount of pepsin must be referable to a direct disease of the secreting gland, as general abnormalities do not affect this function as much as they do the production of free hydrochloric acid. Pepsin is usually present when the free hydrochloric acid is either increased or diminished, but in cases of carcinoma, atrophic gastritis, and in occasional cases of pernicious anemia we may find no pepsin and no hydrochloric acid. Such a condition is known as achylia gastrica and occurs sometimes as a direct pathologic condition without a known etiology. It frequently happens that pepsin is present when no hydrochloric acid is found. In such conditions no digestion will take place in the stomach unless hydrochloric acid be added or the organic acids be present in very large amounts.

Qualitative Tests.

The digestive power of the filtered gastric contents will depend, of course, upon the amount of pepsin and the amount of free acid present. Artificial digestion experiments are at present the only methods by which we may test the amount of pepsin. The substances used in these digestion experiments

are egg-albumin and fibrin. The fibrin may be prepared by beating freshly drawn ox-blood with a glass rod until the coagula are distinctly formed. These stringy masses are washed thoroughly in water to remove the coloring matter of the blood, are then cut into small pieces of uniform size and are kept in alcohol for a few days. These hardened masses are placed for one to two days in a neutral concentrated solution of carmin. They are then washed in water, thoroughly pressed, and are preserved in glycerin to which a little carmin has been added. Before being used they should be washed in water to remove the glycerin and free coloring matter. The egg-albumin is prepared for these experiments by boiling an egg until the albumin is distinctly coagulated. This material is then cut into cylinders of about 5 mm. in diameter with a cork borer and are then sectioned into disks 1 mm. thick. These disks may be preserved in glycerin.

In testing for the presence of pepsin 25 c.c. of gastric juice, which must contain free hydrochloric acid, is placed in a flask which contains a few pieces of fibrin or a disk of coagulated egg albumin. This flask is then placed in the incubator from 37 to 40° and allowed to remain until the protein is completely dissolved. If pepsin is present the fibrin will show signs of digestion by swelling up in from 15 to 30 minutes, the egg-albumin in from one-half to one hour. Within an hour and a half the fibrin should be practically dissolved, while the egg-albumin will require about three hours.

If no hydrochloric acid is present in the gastric juice a few drops of 10 per cent. hydrochloric acid are added to 25 c.c. of gastric juice and the test performed in the same manner. A positive result will indicate the presence of the zymogen, pepsinogen.

Quantitative Examination.

Certain laws have been discovered regarding the action of ferments in general and these are applicable to pepsin. Schütz has found that the relative quantities of pepsin in digesting mixtures containing the same quantity of hydrochloric acid are proportional to the squares of the quantities of albumin digested in the same time, or, in other words, the activity of a ferment varies as the square root of its amount. Nirenstein and Schiff[1] have found that this law applies only for the less concentrated pepsin solutions. If the quantity of pepsin in the digesting fluid is so large that more than 3.6 mm. of albumin (see Mette's Test) are digested in 24 hours, the above law does not give reliable values for the quantity of pepsin. The gastric juice under such circumstances must be diluted before this law applies. Sahli rightly calls attention to an important reason for diluting the gastric juice before testing for pepsin, namely, the constant presence of the products of peptic digestion which inhibit further peptic activity. The gastric juices which contain a diminished amount of hydrochloric acid are the richest in these inhibiting substances and should be carefully studied. The presence of these substances gives rise to conditions

[1] Arch. f. Verdauungskr., Bd. 8, 1903.

which make it impossible to arrive at accurate conclusions if the pepsin value is calculated from the pure gastric juice.

Hammerschlag's Method.

Ten c.c. of a 1 per cent. filtered solution of egg-albumin in 0.4 per cent. HCl are poured into two test-tubes. As fresh egg-albumin contains about 13 per cent. of dry protein, it should be diluted about 13 times to make a 1 per cent. solution. To one of the test-tubes 5 c.c. of gastric juice are added, to the other 5 c.c. of distilled water, both being placed in the incubator at body temperature for one hour. At the end of this time the albumin in each tube is estimated by Esbach's method (see Urine). The difference between the precipitate of albumin in the two tubes is equal to the amount of albumin which has been digested and forms, therefore, a measure of the peptic activity of the gastric juice, the square root of the amount of pepsin being proportionate to the quantity of albumin dissolved. This test is open to the objection that the albumin of the gastric juice, as well as the albumoses are precipitated by the reagent. In one hour not all of the egg-albumin will be digested, normally only about 90 per cent.

Mette's Method.

This method[1] is, perhaps, the one most frequently used and the one which gives much more accurate results than the others advised. The whites of several eggs are mixed, in order to avoid accidental variations in the egg-albumin, and are filtered. The gas should be removed from this material by the use of a suction pump as far as possible. A number of glass capillary tubes, each from 10 to 30 cm. in length and 1 to 2 mm. in diameter, are then filled by suction with this albumin. They are then laid in the bottom of a vessel which is placed for five minutes in boiling water in order to coagulate the albumin. The tubes are taken out, wiped carefully, and the ends sealed with paraffin or sealing wax. It occasionally happens that these tubes contain air-bubbles, which will, however, disappear in a few days. These longer tubes may be kept in stock for a considerable period of time. In performing the test for pepsin by the use of these tubes of coagulated albumin, the longer tubes are cut into lengths of about 2 cm. and are placed in a small dish or watch-glass with 5 c.c. of the gastric juice, which must necessarily be acid in reaction. These dishes containing the gastric juice and filled glass tubes are then placed in the incubator for 10 to 24 hours. At the end of this time the length of the digested column at each end of each tube is measured and the average length of the column of albumin digested estimated. The square of this digestion length is the measure of the relative amount of pepsin in the gastric juice. The unit upon which one may base comparative results of the relative amount of pepsin is that quantity of pepsin by which 1 mm. of albumin in a Mette's tube will be digested in 24 hours with an acidity of 0.18 per cent. free HCl. The length

[1] J. A. D., Petersburg, 1889.

which theoretically pure pepsin would give is 64 mm. the units represented by the pepsin of the gastric juice being anywhere from 0. to 256.

Nirenstein and Schiff, for the reasons previously mentioned, advise the dilution of a gastric juice before applying this test, believing that a dilution of 16 will give more nearly exact quantitative results. Their method is as follows: One c.c. of the filtered gastric juice is diluted with 16 c.c. of 0.18 per cent. HCl. The procedure is then the same as in Mette's method, the results being multiplied, of course, by 16 in order to obtain the actual number of units in the gastric juice. In some cases it has been found that a dilution of 1 to 32 gives better results, but this is rather unusual. The results of these workers show that striking differences exist in individual gastric secretions, figures ranging between 0 and 256 units being obtained. This points to the fact that the pepsin concentration is independent of the amount of acid in the gastric juice. It may be necessary, therefore, to pay more attention in the future to the determination of the pepsin values than in the past. The quantitative estimation of pepsinogen may be carried out by either one of the methods previously outlined for pepsin by rendering the gastric juice acid with hydrochloric acid up to 1 to 2 parts per thousand.

Method of Thomas and Weber.

This method[1] is based upon the digestion of an acid casein solution in 0.2 per cent. hydrochloric acid by the gastric juice. The acid casein solution is prepared by dissolving 100 grams of finely powdered dry casein in 1,900 grams of a solution of hydrochloric acid containing 5.04 grams HCl. A measured quantity of the gastric juice is added to a definite amount of the acid casein solution and placed in the incubator at body temperature. After the end of one hour the digestion mixture is poured into 100 c.c. of 20 per cent. sodium sulphate solution, in which the non-digested casein is completely precipitated. This is collected on a weighed filter and washed with distilled water until no trace of sulphate reaction is evident. It is then dried with alcohol and ether and weighed. The difference in weight between this undigested residue and that contained in the original amount taken gives the amount of casein digested.

This method, as modified by Volhard, consists in the titration of the acidity of the filtrate from the solution to which the sodium sulphate has been added. The total acidity is higher, the more the casein is in the uncoagulated form and the increase in the acidity will vary as the square root of the amount of pepsin.

The writer has had no experience with the ricin method of Jacoby-Solms and must, therefore, neglect a discussion of it.

(*b*). **Chymosin (Rennin).**

The normal gastric juice contains a second ferment, chymosin, which has the function of coagulating milk independently of the presence of acid. The zymogen of this ferment becomes active, however, only in the presence of

[1] Central. f. Stoffw. u. Verdauungskr., Bd. 2, 1901, S. 365.

acids; that is, the zymogen is converted into rennin by acids. In this process of coagulating milk, insoluble casein is formed from the caseinogen of the milk by the combined action of the rennin ferment and calcium salts, while the curdling of milk is due to the precipitation of unchanged caseinogen by acids. It is evident, therefore, that this process resembles very closely that of the coagulation of the blood, as calcium salts are absolutely essential for its success.

Leo's Method.

Three to five drops of gastric juice are added to 5 or 10 c.c. of fresh uncooked neutral or amphoteric milk and the mixture placed in the incubator for 15 to 20 minutes. If rennin is present in normal amounts coagulation will be observed. In this process one may not be sure as to whether the curdling is due to the action of rennin or to that of the acid. Rennin action occurs typically only when no change in the reaction of the milk has taken place.

Riegel's Method.

Three to 5 c.c. of neutralized gastric juice are added to 5 to 10 c.c. of fresh milk. This mixture is placed in the incubator and left for 15 minutes, when distinct coagulation will occur in the presence of rennin. If the milk be boiled previous to treatment the result is not so typical.

Quantitative methods for the determination of rennin are at present uncertain and are even of doubtful utility. So little is known about the variations in the rennin ferment of the gastric juice that an exact determination would add little to the clinical history. According to Glässner, pepsin and rennin are both diminished in cases of tumor of the fundus, while pepsin is diminished and rennin normal in tumors of the pylorus.

(*c*). **Lipase.**

While it is undoubted that lipase occurs in the gastric secretion, its action is very slight. Normally, gastric digestion is not much concerned with the splitting of fat into lower products, but, as Volhard has shown, this action does occur. In testing for the presence of lipase in gastric contents it is necessary that the examination be made after the stomach is thoroughly washed out following the administration of a test meal free from fat.

This ferment may be detected qualitatively by adding a small piece of fresh neutral butter to the gastric juice and placing the mixture in the incubator for one hour. At the end of this time a distinct odor of butyric acid will be observed. Quantitative methods for the determination of this ferment do not seem advisable, although Volhard as well as Stade have used such a method for scientific purposes.

It may be said that owing to this lipolytic action statements have arisen that the Sahli test meal gives erroneous results. Careful work by Seiler shows that the amount of fat decomposed under the conditions of the test meal is so slight that it may be neglected. Volhard found that after two hours from 30 to 36 per cent. of the fat was split up into fatty acids, which aid in dissolving the bile and in forming an emulsion with the neutral fat in the intestinal canal.

The Products of Protein Digestion.

It is generally stated that the action of the pepsin and hydrochloric acid of the gastric juice upon protein material passes through the following stages: The albumin is first converted into acid-albumin (syntonin), then into albumoses, of which there are four (prot-albumose, hetero-albumose, dys-albumose, and deutero-albumose), and ultimately into peptone. This statement is true so far as ordinary digestion in the stomach is concerned. However, it is well-established that pepsin-hydrochloric acid will convert albumin into the products given with trypsin digestion, providing the pepsin be allowed to exert its action over a long enough period of time. Such a condition does not exist normally, but that it exists in certain pathologic conditions is evidenced by the fact that the writer has found amino-acids and hexone bases in the contents of a dilated stomach.

It is practically never necessary to test the stomach contents for such material in clinical work, so that the writer will refer to text-books on physiologic chemistry for a description of the products of gastric protein digestion.

The Products of Carbohydrate Digestion.

The pure gastric juice, owing to its acid content, inverts sugars to a certain extent. The amount of this inversion depends on the number of free hydrogen ions arising from the acid, and is only very slight under normal conditions.

As previously described, the saliva converts starch into soluble substances through the stage of soluble starch, erythrodextrin, achroödextrin and finally maltose. The action of the ptyalin of the saliva is inhibited by the free acid of the gastric juice, but the action of this salivary ferment is so rapid that from 50 to 75 per cent. of the starch is converted into a soluble form. While these products are not ordinarily tested for in the gastric contents, the fact of their formation and presence must be remembered. An excess of starchy material in the food will lead to an increased amount of such decomposition products in the stomach when the acid of the gastric juice is not present in sufficient amount to inhibit the action of the ptyalin of the saliva.

Blood.

Blood is not a normal constituent of the gastric contents, but is found frequently in conditions associated with erosion, in ulcer, and in carcinoma. The appearance of the blood in cases of ulcer is usually that of fresh bright red blood, which may, however, be changed to a brownish substance due to the action of the excess of acid commonly present in this condition. In carcinoma the blood is more intimately mixed with the stomach contents and appears in the form of brownish-black clumps, constituting the so-called "coffee-ground" material. The tests for the presence of blood will be given in detail under Feces, to which the reader is referred.

Gases.

The stomach usually contains a certain amount of gas which may have been swallowed, may have passed into the stomach from the duodenum, or

may have been produced in the stomach by processes of fermentation. The examination for these gases is not of great clinical importance, but a general idea of the different kinds of gases present seems essential. During the processes of normal digestion, nitrogen, oxygen, and carbon dioxid may occur from the protein digestion, while hydrogen, marsh-gas, and olefiant gas may arise from the carbohydrate hydrolysis. In abnormal processes of digestion we may find ammonia and hydrogen sulphid arising from decomposing protein material. The work of Boas seems to indicate that the hydrogen sulphid is more commonly present in cases of benign gastric dilatation and is rare in carcinoma. This hydrogen sulphid does not seem to be produced either in the presence of free hydrochloric or of lactic acid. The presence of hydrogen sulphid can, however, not be considered as a specific substance in the stomach contents, as Dauber has shown that almost every stomach contains bacteria which may produce this gas from sulphur-containing bodies. In cases of dilatation of the stomach, providing the motility be sufficiently diminished, we find fermentation with resulting gas production even though hydrochloric acid be present. Such a condition never occurs if the motility is normal, utterly regardless of the amount of hydrochloric acid, as in cases of diminished hydrochloric acid lactic acid will usually prevent such a process.

One may show the presence of the gases in the stomach contents by filling a fermentation tube with the well-mixed unfiltered gastric contents and placing it in the incubator for some time. If there is no gas within 24 hours it may be wise to wait at least 48 hours to permit of the proper diffusion of the gas. If gas is formed its nature may be determined by the ordinary chemical tests. This test has some value in determining the degree of stagnation of the stomach contents, but it must be remembered that a small amount of gas is contained in the normal stomach.

Other substances, such as acetone, have been found in the gastric contents in pathologic conditions, but tests for these substances are rarely of importance. In conditions associated with the presence of acetone in the stomach contents this substance is usually detected in the breath, so that the examination of the stomach contents is unnecessary.

Functions of the Stomach and its Contents.

The stomach is to be regarded as a specialized portion of the alimentary tube in which the first stages of digestion of protein material take place. This occurs under the combined action of the hydrochloric acid and pepsin, the resulting products being gradually passed into the duodenum through the pylorus. An increased acidity of the gastric juice may be associated with a distinct spasm of the pylorus so that food material cannot pass into the duodenum. On the other hand, a lessened degree of acidity is associated with hypermotility, the contents passing rapidly into the intestine, where it is acted upon by the pancreatic ferment. Beside the function of digestion, principally of the protein foods, the stomach serves as a reservoir to hold the food material, allowing it to

pass only in small portions into the bowel at any one time. Owing to the presence of hydrochloric acid, the gastric juice is antiseptic, rendering inert many but not all types of bacteria. Moreover, the hydrochloric acid activates the zymogens and thus permits of action upon all types of food material. The work of Pawlow has shown that the acid of the gastric juice is one of the most powerful stimulators of pancreatic secretion. The mechanism of correlation between the stomach and bowel is more easily understood if this point be borne in mind. The acid chyme coming from the stomach is poured out only in small portions at a time so that the pancreatic juice secreted may act upon the smaller portions as they are passed into the bowel.

VI. Motility of the Stomach.

It is probably true that disturbances in the motility of the stomach are in reality of more importance than are those in the secretory activities. Under normal conditions of motility the food material passes into the intestine and is digested there, although no previous gastric digestion has taken place. If the motility be much impaired, stagnation of food with resulting dilatation of the stomach will occur, which will give rise to more or less serious disturbance. The motor disturbances are of three types, (1) vomiting, (2) hypermotility, and (3) motor insufficiency. The most important of these is the latter, as the former has little influence upon actual digestion in the stomach, although the patient may suffer for want of adequate nutrition; while in hypermotility the gastric disturbance will not be much noticed, owing to the fact that the food is rushed into the duodenum where it is digested. The consequences of motor insufficiency may be either disorders of secretion, decomposition, or both. Simple pathologic conditions which hinder the emptying of the stomach, such as ulcers, cicatrices, spasm of the pylorus, and simple atony, are usually associated with hyperacidity, while malignant conditions usually show a diminished secretion. It is quite rare to find a case of motor insufficiency without disorder of secretion, rarer at all events than disorders of secretion without motor disturbances (Schmidt). Motor insufficiency is quite commonly followed by decomposition of the gastric contents and may even be considered the chief cause of such decomposition. Under these conditions of disturbed secretory and digestive activity associated with motor insufficiency we find the absorptive power of the stomach very much affected.

Hypermotility is seen in many cases of hyperacidity, but it must be remembered that primary hyperacidity may cause spasm of the pylorus and hence bring on a distinct motor insufficiency. We cannot, therefore, judge of the motility of the stomach from the degree of acidity of the gastric contents. An enlarged stomach is not necessarily associated with motor insufficiency. Cases of megalogastria are more or less frequent in which the motility is practically normal. When a dilatation is associated with motor insufficiency it is clinically styled an ectasia or ectasis, being known as atonic

gastric ectasis if the condition is due to weakness of the muscle, while it is styled hypertonic gastric ectasis if due to pyloric stenosis. Normally, no food should be found in the stomach within seven to eight hours after taking, no matter how large the meal. We have, therefore, a method by which we may judge of the motive power of the stomach.

Leube's Method.

Leube administers a Riegel test meal and washes out the stomach with a liter of water six hours after. If only very slight traces of food are found in the washings the motor power is regarded as normal.

Boas' Method.

Boas administers a simple evening meal consisting of meat, bread and butter, and tea, washing out the stomach the following morning. If any food material is found the motor insufficiency is considerable. If the stomach be washed out previous to the administration of the evening meal no food should be found in the stomach in the morning.

Method of Ewald and Sievers.

This test is based upon the observation that salol is decomposed into carbolic and salicylic acids only in an alkaline medium. As the salicylic acid is eliminated in the urine in the form of salicyluric acid, it is possible to determine the rate of passage of salol from the stomach to the small intestine. It seems necessary to state that the assumptions on which this test are based are partially wrong. In the first place salol is split into its constituents by gastric juice within 15 minutes, although the degree of dissociation is slight. Moreover, a certain amount of absorption of salicylic acid takes place from the stomach so that a reaction may be obtained in the urine within 15 minutes in cases in which no hypermotility exists.

One gram of salol is given to the patient immediately after a meal. The urine is then collected every 15 minutes for two hours and tested by the addition of a small amount of ferric chlorid solution, which will give a violet color in the presence of salicyluric acid.

Under normal conditions, according to Ewald, a positive reaction occurs in from 45 to 75 minutes. A further delay above 75 minutes is indicative of motor insufficiency, the degree of insufficiency bearing some relation to the time of appearance of this reaction. Should no result be obtained after 24 hours a stenosis of the pylorus is highly probable.

As the writer has so frequently found a reaction for salicyluric acid in the urine within 15 minutes, which is due not to the action of the hydrochloric acid or the ferments of the gastric juice, but to the moisture, temperature, and bacteria, he is accustomed to use the time at which a reaction for this substance disappears from the urine rather than the time at which it makes its first appearance, as the basis of judgment regarding the motility of the stomach. Normally, no reaction for salicyluric acid should occur in the urine after 24 hours, although

Huber states that it may take 26 to 27 and hence limits his time to these latter figures. It might be wise to follow the suggestion of Sahli and determine both the time of appearance and disappearance.

Sahli has called attention to the fact that we are not justified in assuming a pyloric stenosis in case food material is found in the stomach even several days after being taken. He adds that the emptying of the stomach is regulated by the intestine rather than by the stomach itself, since nutritive substances reaching the intestine effect a reflex closure of the pylorus (von Mering's reflex) until the intestine has completed its work. The motor activity of the stomach should, therefore, be examined under conditions in which this reflex does not occur. This he determines by estimating the length of time required by the stomach to empty itself of a half-liter of water, the stomach being thoroughly washed out previously.

Winternitz Test.

Winternitz has recommended the use of iodipin instead of salol for testing the motility of the stomach. This substance is not affected by the gastric contents, but is acted upon in the intestine by the pancreatic secretion and bile in such a way that iodin is set free. This may be tested for in the saliva by adding to it a little starch paste, when a distinct blue color will be observed within 15 to 45 minutes.

VII. Absorptive Power of the Stomach.

The absorptive power of the stomach is not of great importance clinically, as the greatest part of absorption occurs from the intestinal tract. However, tests for such power have a certain associated value and are, therefore, usually made. For this purpose Penzoldt and Faber advance a method depending upon the principle that under physiologic conditions potassium iodid is rapidly absorbed by the gastric mucous membrane and is immediately eliminated in the saliva. A capsule containing two to three grains of potassium iodid is given to a patient shortly before a meal. The saliva is then tested as follows for the presence of potassium iodid at intervals of two to three minutes. The saliva is slightly acidified with nitric acid and treated with a few drops of starch paste when the characteristic blue color of iodid of starch will be formed by the action of the iodin liberated from the potassium iodid by the nitric acid. Under physiological conditions the first trace of iodin will appear in the saliva within ten minutes of its administration upon an empty stomach. Under pathological conditions a delayed reaction may be observed in almost all diseases of the stomach, especially in dilatation and in carcinoma. The test will naturally be delayed in case the stomach is filled with food. This test has little value, as it may appear or not in all types of gastric disease. Von Mering[1] has found that potassium iodid is not absorbed at all from the stomach even within

[1] Klin. Jahrb., vol. 7, 1899.

two or three hours, so that the iodin appearing in the saliva may be due to absorption from the intestine. If his results are confirmed, this test becomes useless as one indicating the absorptive power of the stomach, but may then be invaluable as an indicator of the motor power of the stomach.

VIII. Indirect Examination of the Stomach Contents.

As not all cases of disease of the stomach permit of examination by removal of the contents through the stomach-tube, methods have been advanced to permit of indirect determination as to the activity of the stomach contents. These methods do not permit of accurate determination of the acidity or of the ferments of the juice, but do give much information regarding the normal digestive powers and motility of the stomach.

Gunzburg's Method.

A tablet of 0.2 gram of potassium iodid is placed in a piece of the thinnest possible strongly vulcanized rubber tubing measuring about 2.5 cm. in length. The ends of the tubing are folded and the package tied with three threads of fibrin which have been hardened in alcohol. The package is now tested by placing it in warm water for several hours and examining the water for potassium iodid. The patient swallows one of these packages three-quarters of an hour after an Ewald meal, the saliva being tested for potassium iodid at intervals of 15 minutes. In the presence of free hydrochloric acid in normal amounts the threads of fibrin are dissolved and the potassium iodid is absorbed, giving a reaction in the saliva in from one to one and three-quarters hours. In cases of hypochlorhydria the reaction is delayed, a delay of six hours indicating a practical absence of free hydrochloric acid.

This test very frequently gives reliable results, but the threads of fibrin soon become brittle and break on swallowing the package so that a reaction for potassium iodid under these conditions would have no value.

Sahli's Desmoid Reaction.

Sahli[1] has recently introduced the "*Desmoid bag*" for use in estimating the functional activity of the stomach. These bags are made of the ordinary rubber-dam used by dentists and contain a pill of 0.05 gram of methylene blue and 0.1 gram of iodoform. The bag is tied, in a manner especially outlined by Sahli, with cat-gut which has been allowed to dry but has been untreated chemically. This gut, according to Sahli, is digested only by the gastric juice and not by the pancreatic juices. This pill is administered to the patient immediately following the noon meal and the urine and saliva tested at intervals of one hour, beginning three hours after administration of the pill. The digestion of the gut by the gastric juice liberates the pill and

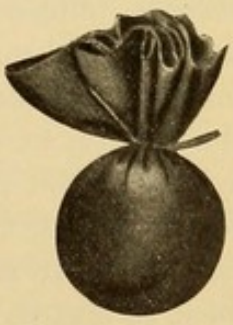

Fig. 18.—Sahli's Desmoid bag.

[1] Correspbl. f. Schweiz. Aertz., 1905.

permits of the absorption of both the methylene blue and the iodoform. The methylene blue will appear in the urine coloring it green within six hours, while the iodin will be found in the saliva within two hours. Should the color of the urine not be distinctly green, this tint may be more clearly brought out by adding a few drops of acetic acid and boiling. Variations from the periods indicated above denote a hyperacidity or a hypoacidity of the gastric juice according as the time of appearance of the reactions is lessened or increased. As the gut is digested only by the gastric juice a nonappearance of either reaction would indicate an anachlorhydria.

The writer has used these desmoid bags in a large number of cases and has found them fairly reliable, giving results which have, in many cases, been confirmed by chemical analysis. As these bags are not obtainable in the market, he has been forced to make them himself and has found that the technic of Sahli must be followed very closely, especially as regards the tying of the gut.

Other methods, such as those of Dunham, Turck, and Einhorn, have been advocated, but possess no advantages over those outlined. The writer will refer, therefore, to other works for their description.

IX. The Gastric Juice in Disease.

(1). Hyperchlorhydria.

By hyperchlorhydria is meant the secretion of an abnormally acid gastric juice whose acidity is due to an excess of free hydrochloric acid. This secretion is much more marked during digestion, being less frequent on an empty stomach. Under these conditions we usually find an increased total acidity along with the increase of free hydrochloric acid. A condition which is characterized by a high total acidity with a very high amount of organic acid would not, of course, be considered in this connection. A hyperacidity or hyperchlorhydria exists when we have more than 0.2 per cent. (60°) of free hydrochloric acid.

This condition may be due to pathologic changes in the mucosa or to direct nervous influences. Cases of pure hyperchlorhydria are occasionally very stubborn and may be associated with almost any variety of abnormal gastric function. While we find hypermotility of the stomach in many cases of hyperchlorhydria, we very frequently note a diminished motility due to spasm of the pylorus. This condition brings about a stagnation of the stomach contents and a consequent increase in the fermentative processes. The acidity in such cases may run as high as 200° or over and the digestive powers of the gastric juice, as regards protein substances, may be much increased, the carbohydrate digestion being correspondingly diminished. These facts point to the reason for the administration of an increased protein diet in such cases, the protein combining with the hydrochloric acid and thus taking a portion of the excess from the field of action.

While this condition is not a distinct entity, yet we find many cases which come under the heading of idiopathic hyperchlorhydria and which are not

associated with other pathologic conditions. Some of these cases are purely functional and clear up promptly under proper treatment, while others are of nervous origin and are remedied only when the etiologic factor is eliminated. In this latter type of hyperchlorhydria the degree of acidity varies with the nervous symptoms, giving rise to the term "*heterochylia.*"

(2). **Hypersecretion (Gastrosuccorrhea).**

By hypersecretion is meant an excessive secretion of gastric juice which is out of proportion to the physiologic stimulus. This hypersecretion occurs even when no stimulus is present, is always pathological, and, according to Riegel, always produces pathological results. A hyper- or continuous secretion may be determined by finding a fairly large amount of gastric juice in the fasting stomach under conditions which rule out stenosis and stagnation. The stomach is washed out before the patient retires, the contents being withdrawn the following morning. If a quantity of highly acid fluid is obtained, a hypersecretion is proven. The quantity taken from the fasting stomach should never be more than 100 c.c., while Strauss regards 40 c.c. as an indication of hypersecretion. This secretion, to be called a hypersecretion, must contain no food remnants, no sarcinæ nor yeast cells, but should be distinctly acid.

This condition is probably a functional neurosis, being constant or intermittent and a part of a general neurosis, a secretory neurosis, or the result of organic nervous disease, such as the gastric crises of tabes dorsalis. Reichmann has reported cases of the periodic or intermittent type, during the intervals between the attack the digestion of the patient being normal. Such cases are known as Reichmann's disease.

The chronic cases are of long duration and have a gradual onset. The patient complains of much discomfort, feeling of weight or depression in the stomach, pain during digestion, vomiting, especially at night, and a gastric contents with a large amount of free hydrochloric acid. Dilatation of the stomach sooner or later comes on as the result of spasm of the pylorus induced by the hyperacidity. In these dilated stomachs we find, of course, products of fermentation and many yeast cells and sarcinæ.

(3). **Achylia Gastrica.**

This condition may arise either from a functional disturbance of the mucosa or a true atrophy of the mucosa. This latter state, known as atrophic gastritis, may be the end stage of a chronic gastritis or the result of carcinoma. When this condition is not due to direct gastric disturbance, it is more frequently seen in connection with pernicious anemia, in which the general nutrition is very much below par. The local condition may not be suspected, as the hypermotility, so common in achylia gastrica, may prevent attention being drawn to the stomach.

For a diagnosis of achylia, the test meal of Ewald gives very good results. Examination of the gastric contents shows that the food is little changed, the

total acidity very low (1 to 6°), no free hydrochloric acid, gastric ferments much diminished or entirely absent, and lactic acid only in small amounts. The motility of the stomach is usually little impaired, so that the retention of food is unusual.

(4). **Acute Gastritis.**

The stomach contents of acute gastritis shows a diminished total acidity, little or no free hydrochloric acid, organic acids relatively increased, much mucus and undigested food. This condition is usually brought on by direct irritation and is generally easily remedied by total abstinence. The material for chemical examination is usually obtained in these cases from the vomitus, as the passing of the stomach-tube is very rarely tolerated.

(5). **Chronic Gastritis.**

All grades of this condition may exist up to complete atrophy of the mucosa. Examination of the stomach contents shows practically no digestion of the food material, much mucus intimately mixed with the food particles, the secretion usually diminished, free hydrochloric acid diminished or absent, ferments much reduced, protein digestion small, starch digestion little affected, microscopic examination showing the presence of many epithelial cells and leucocytes. There are some cases of chronic gastritis in which a hyperacidity of the juice is evident, but these are rare, the usual findings being one of diminished acidity. The motility of the stomach in these cases is sometimes normal, sometimes increased, or may be diminished. One of the most characteristic findings in this condition is the presence of a large amount of mucus containing either leucocytes or their nuclei and epithelial cells from the walls of the stomach. If there is little acid present in the gastric contents the mucus may swell up and appear greater in volume. As a general rule, it may be said that the mucus and the hydrochloric acid vary inversely as their amounts.

(6). **Nervous Dyspepsia.**

This condition of nervous dyspepsia is part of a general neurosis and may show no characteristic findings in the stomach contents. The degree of acidity may range from a normal to either a hyper- or a hypoacidity, while the amount of ferments present will not usually vary. The findings in nervous dyspepsia are not at all constant, varying at different examinations. We have, therefore, more or less distinct methods of differentiation between this condition and chronic gastritis. In the first place, the acidity of chronic gastritis remains constant for several examinations while that of nervous dyspepsia is variable. The ferments are diminished in cases of gastritis, while they are normal in nervous dyspepsia. Much mucus is found in gastritis while little or none is present in dyspepsia. The cases of nervous dyspepsia are partially associated with distinct errors in eating, the American frequently bolting his food in such a way as to make it difficultly digestible. The influence of nervous conditions over gastric function has been very well expressed by Emerson

when he says, "a neurasthenic will often worry his subliminal gastric sensations into the sphere of consciousness."

(7). Ulcer of the Stomach.

The diagnosis of ulcer of the stomach depends, to a large extent, upon the clinical symptoms of the disease rather than upon the examination of the stomach contents. While the symptoms of this disease, increasing dyspepsia, pain, vomiting, blood in the vomitus, and hyperacidity of the vomitus, are well-known, the stomach-tube should rarely ever be used in obtaining the contents of the stomach, owing to the danger of perforation in such cases.

The vomitus of such cases is usually ejected from one to three hours after a meal and contains well-digested food. Blood may or may not be present and may be either fresh red blood or dark in color from the formation of hematin. The total acidity of the gastric contents is usually increased, hydrochloric acid constituting a large part of this total acidity. A single examination of the gastric contents will rarely determine anything about an ulcer, so that repeated examinations of the vomitus must be made to obtain a general idea of the acidity. In these cases blood is usually present in the feces and may be detected as outlined later.

When an ulcer is complicated by a beginning carcinoma, we may find all types of variation in the acidity of the stomach contents.

(8). Carcinoma of the Stomach.

In no other condition of the stomach is an absolutely certain early diagnosis to be so much desired as in carcinoma of the stomach. The chemical features of the gastric juice in this condition may be very suggestive or may be negative. The clinical history of the case along with the age of the patient are probably of more importance in making a diagnosis of carcinoma than are variations in the chemical composition of the juice. However, such changes may usually be found and are, therefore, considered as at least of presumptive evidence. The local symptoms of carcinoma of the stomach are sometimes as variable as are the changes in the gastric juice, so that every possible point in diagnosis should be taken advantage of, if for no other purpose than to exclude this condition.

Perhaps the most important sign of carcinoma is the absence of free hydrochloric acid. Although this condition is present in about 85 per cent. of cases, it cannot always be traceable to carcinoma, as it may occur in atrophic gastritis and advanced chronic gastritis. This lack of free hydrochloric acid is due to the union of this acid with some body which in itself does not show an alkaline reaction. Von de Velden suggests that the secretion from the cancer is the active agent in neutralizing the hydrochloric acid. Moreover, the products of protein digestion might have some power in neutralizing the acid, Emerson having shown that hexone bases are present as a result of the action upon the protein of a ferment derived from the tumor itself. Certainly, in cases of carcinoma the total nitrogen of the stomach contents is much increased, so that

such bodies are probably a very great factor in the diminution of the hydrochloric acid. This reduction in hydrochloric acid is also influenced by changes in the mucosa to such an extent that the active secretion is diminished. The failure of free hydrochloric acid is usually a very early symptom, but it must be remembered that hydrochloric acid may be present in normal amounts or even in increased amounts when the carcinoma is small and occupies the pyloric region or when this growth develops on the base of an old ulcer. The acidity may vary markedly from day to day, sometimes showing no free hydrochloric acid and sometimes a considerable amount. This variation is of considerable practical importance. It is to be said, moreover, that absence of free hydrocholoric acid may be noted in cases of pernicious anemia, and carcinoma of gall-bladder, pancreas, or duodenum. In cases of carcinoma of the esophagus the disappearance of hydrochloric acid from the gastric contents seems necessarily to be the result of the secretion of the tumor neutralizing the gastric juice. Along with this diminution in free hydrochloric acid the total acidity is also diminished.

The presence of an increased amount of lactic acid is a very valuable sign in cancer. About 90 per cent. of patients show the presence of lactic acid when there is no free hydrochloric acid, but when there is a large amount of combined HCl pointing to a free secretion of this acid. Lactic acid may not be present in cases of carcinoma, especially those in which the growth is upon the base of an old ulcer, or it may be present in conditions other than carcinoma, such as chronic gastritis, associated with atrophy of the mucosa and dilatation of the stomach, especially when a benign stenosis of the pylorus exists. It must be stated, however, that we usually find an increased secretion of hydrochloric acid in cases of simple benign pyloric stenosis, so that lactic acid cannot be formed in the presence of this increased hydrochloric acid. According to Riegel, the chief cause of the lactic acid formation is the combination of motor insufficiency with a hypoacidity associated with a diminished secretion, both of acids and ferments. This diminution in the amount of ferments is in no way specific for cancer, as it is in reality due to the chronic gastritis which is set up by the tumor. As lactic acid cannot be formed in the presence of an appreciable amount of free HCl, we do not always find after a test meal a large excess of this acid, especially in those cases in which considerable combined HCl is present. It seems advisable, therefore, in testing for the presence of lactic acid, to examine the contents of the fasting stomach in the morning after it has been well washed out the preceding evening. Whether we are to assume that lactic acid is formed by the action of the organisms in the stomach upon food material, whether it be a normal product of digestion, or whether it is a product of the activity of a ferment derived from the tumor, must be left for future investigation.

The vomited material or the material obtained by washing out the stomach shows very little digestion of the protein elements, while the carbohydrates are well hydrolyzed. The amount of material in the stomach will be large or small,

depending upon the degree of stenosis, so that it is nothing unusual to obtain several pints of material containing undigested protein residue. Microscopic examination of this material may show cellular masses washed off from the tumor or may show these fragments embedded in masses of blood. Blood is a usual finding in cases of carcinoma and may be detected by the methods discussed under Feces. Sarcinæ and yeasts are rare, the former occurring more frequently in cases of marked dilatation. The Boas-Oppler bacilli are more or less constant findings in carcinoma, occurring in about 80 per cent. of the cases and only very rarely in any other condition. The characteristics of these bacilli have been previously discussed.

Salomon's Test.

This test is based upon the fact that albumin is secreted from the carcinoma itself and passes into the gastric contents. The patient is placed upon an absolutely protein-free diet for 24 hours and the stomach carefully washed out at the end of this time with 400 c.c. of physiological salt solution. A few hours thereafter the contents of the stomach are removed and the remaining material washed out with 400 c.c. of physiological salt solution. The total nitrogen and the albumin are then estimated, the former by the Kjeldahl method and the latter by the Esbach method (see Urine). The nitrogen ranges from 10 to 70 mg. per 100 c.c. in cases of carcinoma, while in other conditions it varies from 0 to 16 mg. per 100 c.c. The Esbach reaction gives a distinctly appreciable precipitate for albumin, anything over 0.5 parts per 1000 being considered indicative of carcinoma. While this test is not infallible, yet the writer has found it present in so many cases of carcinoma that he is inclined to make a very strong presumptive diagnosis on the basis of this test.

Gluzinski's Test.

This test is based upon variations in the amount of free hydrochloric acid under different conditions. Gluzinski makes a test for free hydrochloric acid upon the stomach contents withdrawn in the morning from the fasting stomach, also on that withdrawn 45 minutes after a test breakfast, and upon the material obtained four hours after a full meal. He finds a relative insufficiency of the HCl secretion and considers it a valuable indication of the grafting of a cancer on the bed of an old ulcer.

The differentiation of a carcinoma of the stomach from a chronic gastritis would be based both upon the clinical and laboratory findings. A greater degree of emaciation is usually seen in cases of carcinoma than in those of gastritis. A palpable tumor would indicate a carcinoma, while no such condition exists in gastritis. Lactic acid is usually present in carcinoma and is almost invariably absent in gastritis.

Little food material is obtained in the washings from the stomach affected with chronic gastritis, while in carcinoma it is frequently possible to obtain more or less food from the fasting stomach. The presence of the Boas-Oppler bacilli would be confirmatory evidence in favor of carcinoma.

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CHAPTER IV.

THE FECES.

I. General Considerations.

The feces are composed of substances of different origin, which may be divided as follows: (1) Food remnants, either undigestible constituents or digestible but unabsorbed elements; (2) secretions of the alimentary tract; (3) decomposition products and bacteria; (4) formed and unformed elements derived from the intestinal wall, and (5) foreign bodies, such as hair, wood fiber, parasites, parasitic ova, and enteroliths. It is not possible to draw a sharp line between a truly physiologic and pathologic composition of the feces. In each individual case this division will depend upon a number of factors, among which may be mentioned composition of the food, method of taking the food, individual functional capacity of the intestines, frequency of the bowel movements, and general systemic conditions. The condition of the food remnants will give much information regarding the functional capacity of the bowel, although examinations of this factor are at present made to a very slight extent. Under normal conditions, about one-third of the dry substance of the feces arises from the bacteria; this amount increasing under certain pathologic conditions which will be later discussed. As products of bacterial activity we observe the formation of hydrobilirubin from bilirubin as well as the reduction of certain medicaments and the rare formation of certain diamins, which will be treated later. This bacterial activity will vary much under pathologic conditions and may have much to do with the symptomatology of the case investigated. The ordinary decomposition products are derived from the carbohydrates and proteins of the food; from the former are produced, by fermentation, volatile fatty acids, lactic acid, succinic acid, alcohol, carbon-dioxid, hydrogen, and methane, while from the latter are formed by putrefaction indol, skatol, phenol, ammonia, and hydrogen sulphid. The fats are decomposed only to a very slight extent. The normal products of digestion in the intestinal tract as well as the factors bringing about these changes will be discussed later.

Normal Feces.

For proper comparisons between the feces of various individuals the food must be the same. Starvation feces, meat feces, and milk feces are typical types, but are not normal in the strict sense. For comparison, however, an arbitrary norm must be established in order to judge of slight variations which have to do with special differences in utilization of food and which are not

observable by the eye except under certain conditions (as, for instance, fatty stools). Through the work of Praussnitz and of Schmidt and Strasburger a new field for study of normal and abnormal feces has been opened up.

A normal feces should be one which consists almost entirely of remnants of the digestive juices and intestinal secretion arising from a purely digestible, properly prepared, and assimilable food. It contains approximately 8.6 per cent. of nitrogen, 16 per cent. of ether extract, and 15 per cent. of ash calculated on the dry basis. Any appreciable variation from this composition would indicate a diminution of the functional activity of the bowel. In the investigation of pathological cases this composition is scarcely to be assumed, as the



FIG. 19.—Normal Feces. (*Landois.*)

a, Muscle fibers; *b*, tendon; *c*, epithelial cells; *d*, leucocytes; *e-i*, various forms of plant-cells, among which are large numbers of bacteria; between *h* and *b* are yeast-cells; *k*, ammonium-magnesium phosphate.

diet is more or less restricted, the appetite is capricious, the intestinal activity is variable, and the feces are, therefore, different from those of the healthy. It must not be understood from this that a feces showing the above composition is necessarily normal in other respects. One may not rely entirely on the chemical composition, but must largely consider the macroscopic and microscopic findings. In order that we may have a sound basis upon which to judge of intestinal activity it seems wise to have some sort of test diet which may be given to suspected cases.

Diet of Schmidt and Strasburger.

This diet[1] is so selected that it can be used by the healthy as well as by those with intestinal trouble; its amount is sufficient to satisfy the maximal calorie requirement of the individual while at rest; it contains the three chief groups of food material in definite relation to each other; is as free as possible from remnant-leaving food, and can be easily obtained and prepared. The starchy food present is of the amount and kind which have been shown most

[1] Die Fæces des Menschen, Berlin, 1905.

favorable for the prevention of excessive fermentation in the bowel. The daily diet is as follows: One and five-tenths liters of milk, 100 grams of zwieback, two eggs, 50 grams of butter, 125 grams of beef (raw weight), 190 grams cooked potato, and a gruel of 80 grams of oatmeal. This is distributed through the day as may best suit the patient. This diet contains 102 grams of protein, 111 grams of fat, and 191 grams of carbohydrate, yielding 2,234 raw calories. In cases which show a diarrhea, due to the milk, one may substitute instead of 1/2 liter of milk the same amount of cocoa made from 20 grams of cocoa powder, 10 grams of sugar, 100 grams of milk, and 400 grams of water. Small variations in the amount of milk, sugar, butter, and even of eggs may be permitted, but the outline, as regards meat, zwieback, potato, and gruel should be rigidly adhered to.

This diet should be administered for three days or longer if necessary to obtain a stool which comes from it. In order to judge of the first appearance of the stool from this diet, the patient should be given a capsule containing 0.3 gram of powdered carmin, both preceding and following the diet. Instead of carmin, one may use cork, charcoal, or silicic acid.

Folin's Diet.

This diet[1] is especially serviceable in case one wishes to follow the metabolism in any special case. Its easy application and its fairly constant values for nitrogen, phosphorus, chlorin, and sulphur make it invaluable.

The standard diet, which is given to the patient daily for several days is as follows:

Whole milk,	500 c.c.
Cream (18 to 22 per cent. fat),	300 c.c.
Eggs (whole),	450 gm.
Horlick's malted milk,	200 gm.
Sugar,	20 gm.
Sodium chlorid,	6 gm.
Water,	2100 c.c.

This diet contains approximately 119 gm. of protein, 148 gm. of fat, and 225 gm. of carbohydrate, yielding 2787 raw calories. The intake of one day is nitrogen 18.9 gm., 5.9 gm. of P_2O_5, 3.8 gm. of SO_3, and 6.2 gm. of Cl. It is not to be compared with that of Schmidt and Strasburger for estimating the intestinal activity, but is more reliable if metabolism relations are to be studied.

The normal motility of the feces is from 6 to 20 hours, while on a milk diet it may vary from 36 to 48 hours. This factor may be of importance in judging of intestinal obstruction. The isolation of a stool under a special diet is of great importance, as the success of an investigation will depend upon the obtaining of a feces arising from a specified diet.

[1] Jour. of Physiol., vol 13, 1905, P. 45.

Obtaining Intestinal Juice.

Boas has shown that it is often possible both in healthy and sick patients to obtain enough of the secretion from the upper part of the small intestine to permit of examination. The technic is as follows: The stomach-tube is introduced and the stomach washed out with a 1 per cent. solution of sodium carbonate. The patient lies upon his back while the region under the right costal arch, between the mammary and parasternal lines, is massaged from right to left for several minutes. The stomach-tube is again introduced and whatever is forced back through the relaxed pyloric sphincter is removed in the ordinary way. This method frequently admits of obtaining 40 to 50 c.c. of a neutral, alkaline, or weakly acid fluid. The juice may then be tested for the presence of the various ferments known to exist in such material.

Recently Hœlscher has reported a study of the enzymes of the pancreatic and intestinal juices which he obtained through a jejunal fistula, which was kept open for several weeks. He was able to show the usual properties of intestinal juice as outlined in works on physiologic chemistry. An interesting portion of his work was the apparent proof that salol is both hydrolyzed and absorbed in the stomach.

Functions of the Intestinal Juices.

The writer cannot attempt in this place to go into detail regarding the dynamics of the process of digestion as ocurring in the intestines. This is or should be well known to every student of medicine and needs no elaboration in this place.

The pancreatic juice as excreted into the intestine is an alkaline fluid containing three ferments, trypsin which hydrolyzes protein, amylopsin which acts upon the carbohydrates, and lipase (steapsin) which aids in the digestion of fat. The trypsin appears to be excreted in the form of a zymogen which is activated by a second ferment, enterokinase, derived from the intestinal mucosa, while the lipase and amylopsin are active when secreted. It has been shown by Pawlow that the passage of free hydrochloric acid into the duodenum is a direct stimulant to the excretion of pancreatic juice. Bayliss and Starling believe, however, that the stimulus to the pancreatic secretion is not the free acid but is a ferment, secretin, which is formed by the action of the hydrochloric acid upon the intestinal mucosa. While the trypsin acts upon protein bodies, splitting them through various stages into the ultimate products, amino-acids and hexone bases, there is a second ferment, erepsin, discovered in the intestinal mucosa by Conheim, which acts upon the intermediate splitting products of protein, such as the albumoses and pepton, carrying this conversion to the same lengths as does trypsin. It is interesting to note that we find in the intestinal juice of the infant a ferment, lactase, which hydrolyzes lactose into the simpler saccharids.

The presence of the bile, which reaches the duodenum through the common duct, is necessary for the proper digestion and absorption of the fatty substances of the food. Variations in this constituent may reflexly cause disorders

in the secretion of the stomach, one finding very frequently a hyperacidity associated with obstructive jaundice. Whether the bile may be assumed to have a disinfecting power must be left for the future. Were this influence actually present, it is hard to understand, why, in cases of long-standing exclusion of bile, we do not find catarrhal conditions as we do in cases of marked putrefactive processes in the bowel.

Estimation of Intestinal Digestion.

A careful chemical examination of the feces coupled with a macroscopic and microscopic investigation, will give much information regarding the degree of intestinal digestion and absorption. However, such methods are time-consuming and not easily performed by the general practitioner. For this reason Sahli has introduced a method, similar to his stomach method, of investigating such activity. He employs glutoid capsules, which are made from gelatin hardened with formaldehyd. These capsules either do not dissolve in the gastric juice at all or only after considerable time, although they are quickly soluble in the intestinal juice. In these capsules are placed material which will not diffuse through the capsule wall and whose absorption may be studied from an examination of the saliva or urine. Sahli uses either iodoform or salol. In the former case 0.15 grams of iodoform are placed in a glutoid capsule, and given with an Ewald test meal. Under the best conditions (normal gastric motility, normal intestinal digestion, and normal intestinal absorption) the iodin reaction may be expected to appear, according to Sahli, in the saliva within four to six hours; that is, within one and one-fourth hours after the capsule has been dissolved by the pancreatic juice. Instead of iodoform salol may be used, being given in the amount of one-half gram along with an Ewald test meal. The reaction for salicyluric acid may be obtained in the urine within one and one-half hours after the capsule has been taken.

Sahli gives the following results obtained by the use of this capsule: When the stomach contains neither free hydrochloric acid nor pepsin, the reaction is not delayed so long as gastric motility is good. In cases of diarrhea due only to an increased peristalsis without any marked disturbance of intestinal digestion, the reaction is either normal or even somewhat hastened. In other types of diarrhea characterized by an involvement of the intestinal chemistry or intestinal absorption, the reaction is either absent or distinctly delayed or the capsules may be found undigested in the feces. This method also aids in differentiating an icterus due to occlusion of the ductus choledochus at its point of entrance into the intestine, in which case the digestion of the capsule may be interfered with, from one where the obstruction to the bile flow is higher up near the liver. This test may be of some presumptive evidence in the diagnosis of pancreatic carcinoma, although all cases of pancreatic carcinoma do not necessarily occlude the duct, in which case a positive result would obtain. Galli reports a case of carcinoma of the pancreas in which the glutoid capsule was dissolved, although no pancreatic juice was present in the bowel.

II. Macroscopic Examination.

(1). Method.

The macroscopic examination of the feces embraces not only the study of the physical characteristics, but also a recognition of various normal and abnormal substances. It s convenient, when examining the feces, to employ some form of a washing apparatus to separate the coarser from the finer particles.

Boas has introduced a special sieve for such work which, however, does not have much advantage over the ordinary flour-sifter which Einhorn advises. Strauss' method of washing the feces by a current coming from below seems to be much the best of any of these types of apparatus. In the absence of any other equipment, an ordinary house-hold sieve of various-sized mesh will answer the purpose. Small amounts of mucus or connective tissue fragments may be recognized by rubbing up a portion of the stool in a mortar with a little water, when these substances will float upon the surface. In some cases it may even be advisable to place the feces in tall glass jars in which the stool is mixed with water and allowed to arrange itself in layers.

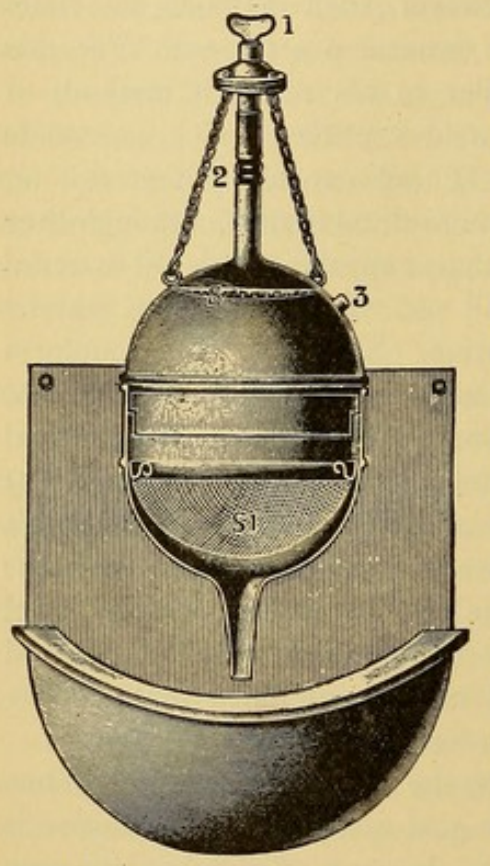


Fig. 20.—Boas' stool-sieve. (*Hemmeter.*)

If the stool is of ordinary consistency, it should be spread out in a more or less thin layer so that the larger particles may be easily recognized. If it be, however, a watery stool the contents are thoroughly mixed and examined as such.

(2). Amount.

The amount of feces excreted will depend upon (1) the quantity and quality of the food; (2) the remnants of intestinal juices and débris; (3) the condition of the digestive organs, and (4) the bacteria. These factors are all important, each being dependent to a certain extent upon the other.

The average moist weight may be considered as varying from 100 to 250 grams with a dry weight of 20 to 40 grams. This amount may, however, reach as high as 20 kilograms as in a case reported by Lynch.

Number of Stools.

The number of stools which may be passed in 24 hours is subject to very wide variation, even under physiological conditions, but is usually constant in the same individual. At least one stool a day should be considered normal.

although many persons are accustomed to have only one movement of the bowels in 48 hours and others in longer periods. We must, therefore, consider each individual case before judging as to abnormality in the number of stools. Lynch[1] has reported a case of a patient having only one stool each 100 days, while Geib and Jones[2] discuss a case in which there was no stool during an entire year the patient at the end of that time voiding 32 liters of feces.

A diarrhea is said to exist when the stools are frequent and fluid. The frequency may vary from 2 to 50 in 24 hours, although a single liquid stool in 24 hours may constitute a diarrhea in a patient unaccustomed to having a movement every day. A normal stool is never fluid, so that the character of the stool is of more importance than the number, as individual peculiarities must be considered. A diarrhea may be due to increased peristalsis, to increased intestinal secretion, a diminished gastric secretion, or a decreased absorption from the bowel. The most extreme grades of diarrhea are observed in Asiatic cholera, dysentery and the summer diarrhea of infants, although a marked diarrhea does occur in enteritis, peritonitis, intestinal tuberculosis, and uremia. Some infectious diseases are more frequently associated with diarrhea than are others, but little of diagnostic importance is obtained from this symptom.

By constipation is meant the infrequent and irregular movement of the bowels, associated with symptoms which are relieved by administration of laxatives. The habits of the individual must be taken into consideration before judging as to a real or apparent constipation. This condition is physiologically a result of a sedentary life or of a diet lacking in elements which will stimulate intestinal peristalsis. Pathologically, we find constipation in cases of dilated stomach, occlusion of the bowel, atony of the bowel wall, in conditions causing increased cerebral pressure, and in obstruction by pressure from without the bowel. It must be stated here that we frequently have an apparent diarrhea merely as a symptom of constipation high up in the bowel. Such being the case, it is better practice to administer a laxative in such conditions than it is to give an astringent.

(3). Consistency and Form.

The consistency and form of the normal stool vary considerably depending upon the nature of the food ingested. The stool is much softer with a purely vegetable diet, of which about 85 per cent. is water, than with an animal diet, of which only 65 per cent. is water. One differentiates, as regards consistency, a well-formed, a mushy, and a fluid stool, between which types there are many gradations, some stools being partly formed and partly fluid. Many factors may influence the amount of water present in the stool, such as (1) a lessened absorption of water from the intestinal canal; (2) intake of a large amount of water, and (3) an increased secretion from the intestinal glands.

The consistency of the normal stool varies from day to day and can be

[1] Thesis, Buenos Aires, 1896.
[2] Jour A M.A.,Vol. 38, 1902, P. 1304

constant only when the patient is placed upon a standard diet. It may be abnormally too fluid or too solid, in the latter case being frequently voided in the form of very hard masses, known as "scybala." As a general rule, it may be stated that the greater the absorption of water from the intestine the more firm will the feces be and, in consequence, the more frequently will these scybala form. Stools are frequently observed of which the consistency is normal but of which the size of the cylinder is quite small. Such small "lead-pencil" sized stools have been supposed to be indicative of stricture of the lower bowel, but this condition is not necessarily present.

Besides the water-content of the feces, the amount of fat, mucus, and vegetable residue has much to do with the consistency of the stool. One may differentiate the fat from water in the stool by placing a small portion of the feces upon a slide and pressing a cover-slip down upon it. If the cover-glass remains when the pressure is removed increased fat may be assumed, while if the softness of the feces be due to increased water the cover-glass will spring away from the feces.

In some cases, especially those associated with achylia gastrica, the stools are very frothy, indicating a marked bacterial decomposition. Such stools should not be confused with those of the ordinary diarrhea or with the characteristic "rice-water" stools of Asiatic cholera, in which particles of mucus are readily detected. Such stools are never found associated with large fat contents.

(4). **Odor.**

The peculiar odor of the normal stool is referable to the presence of indol and skatol, which arise from the putrefaction of protein material in the large intestine. Along with these we may find other substances, such as hydrogen sulphid, methane, and phosphine. The odor of the stool is much more marked following a meat diet than that after a vegetable diet; it is very slight on a milk diet and is practically lost in the fasting condition. If the processes in the intestine are of such a nature that fermentation of the carbohydrates exceeds the putrefaction of the proteins, the stool shows a distinct sour odor traceable to the presence of butyric or acetic acid. The odor in cases of acute and chronic diarrhea is frequently very slight, while that of the loose, watery discharges of cholera is peculiar and sperm-like, referable to the presence of cadaverin. In the diarrhea of children a distinct putrid odor may be present, although this is not necessarily the case. The so-called acholic stools have in themselves, according to Schmidt, very little odor, showing this property only when complicated by decomposition processes arising from the lack of bile. In cases of severe dysentery and carcinoma of the large intestine an intensely disagreeable odor is observed which differentiates these conditions from those associated with other types of decomposition.

(5). **Color.**

The color of the stool varies from a light brown to even a black, depending upon the kind of food, the residue of the intestinal secretions, the presence of

pathologic products from the intestinal wall, and the administration of therapeutic agents. The dark color of the normal stool is due to the presence of hydrobilirubin, which is formed from the secreted bilirubin by the reducing processes in the intestines. Bilirubin is found normally in the stool of a nursing child, being converted either into biliverdin or hydrobilirubin under abnormal conditions. This change from a light yellow infantile stool to a greenish one has much importance to the pediatrician. A well-formed stool is always darker in color than is the thin stool, which is equivalent to saying that the stool is darker the longer it remains in the intestine. The color of a stool under a meat diet is dark brown, with a vegetable diet a lighter brown, and following a milk diet a light yellow. This color which is traceable to the diet is shown best in those cases in which bile is excluded from the bowel. The dark color of the meat feces is probably traceable to the conversion of the blood-coloring matter into hematin and not to the formation of sulphid of iron which is so commonly stated. Food products may color the feces a characteristic shade. Thus, coffee may give a dark brown color, cocoa a brownish-red color, red wine a smoky black-brown color which has a shade of green. Chlorophyll-containing plants, such as spinach and lettuce, give rise to greenish shades.

Occasionally the reduction process in the intestines may go so far as to convert the bilirubin into leucohydrobilirubin instead of into hydrobilirubin. This hydrobilirubin is identical with the urobilin found in the urine. Such stools may be practically colorless when voided, but will be converted into the dark brown normal-colored stool on contact with the air. Besides these pigments, biliprasin has been isolated from the feces by Fleischer, while Müller obtained cholecyanin.

The variations in color of the stool following administration of therapeutic agents is frequently characteristic. Thus, after the use of calomel one observes a distinct green coloration due to the conversion of the bilirubin into biliverdin. Bismuth preparations color the stool a distinct black, due to the formation of the oxid or sulphid of bismuth. Rhubarb, senna, santonin, and gamboge cause a distinctly yellow coloration which will change to a reddish tone in the presence of alkali. Iron compounds produce shades ranging from dark brown to black, while methylene blue gives rise to the production of a blue-green color and sandal-wood a reddish-violet color.

Pathologic Variations in Color.

Cases showing the presence of large amounts of mucus or of pus in the feces are characterized by a gray-white or yellowish-gray coloration of the feces. Such cases are seen in membranous colitis and rupture of an abscess, especially of the appendiceal variety, into the intestinal tract. In cases of syphilitic or carcinomatous ulceration of the colon or rectum this characteristic color tone may be more or less influenced by the presence of blood.

Stools showing the presence of a large amount of fat are clay colored. That this coloration is due to the excess of fat, rather than to the absence of

bile, may be shown by extracting the feces with alcohol and ether, in which extraction the bile will be taken up along with the fat and will color the solvents. These acholic stools as they have been called occur both in cases associated with biliary obstruction and in those showing no obstruction. It would be better practice, therefore, to style these stools colorless, instead of acholic stools. Strümpell was able to obtain stools of a light brown color by feeding patients a diet containing small amounts of fat, thus proving that the increased fat was more important than the diminution of bile, although this latter does account for some cases. This clay-colored stool may also be found in diarrhea, while in Asiatic cholera and dysentery the stools may be absolutely colorless. They have also been found in cases of leukemia, carcinoma of the stomach or intestine, tuberculous enteritis, and chronic tuberculous peritonitis. The cause of this lack of color may be the same unknown cause that produces the formation of leucohydrobilirubin spoken of above.

In some cases we find a distinct golden-yellow or even a green color of the feces. This is due to the presence of unaltered bile, on the one hand, and biliverdin, on the other. Biliverdin is usually found in abnormal decomposition processes in the intestine of the infant, while unaltered bile may appear in cases of increased intestinal peristalsis, in which the contents of the bowel are rushed onward before absorption can take place. This is probably the partial explanation of the green stool following the administration of calomel. Normally, bilirubin is not found in the intestine below the ascending colon. Such being the case, it may be possible to judge of the point of irritation in a diarrheal attack by the fact that the higher up in the bowel the point of disturbance the more of this pigment will be found in the feces. Bilirubin indicates, therefore, an enteritis especially of the small, but also of the large intestine. This bile pigment may be found most frequently on cellulose material, mucus, muscle fibers, and fat. It is readily detected by rubbing up 2 or 3 c.c. of the feces with a concentrated aqueous solution of pure mercuric chlorid. This mixture is allowed to stand 24 hours and is examined microscopically thereafter. The fragments to which the pigment is attached will stain red if due to hydrobilirubin, while those stained with bilirubin will show a green color. Naturally, in this examination chlorophyll-containing fragments must be excluded. The most favorable material for such examination is the mucus. A green color of the stools may also be observed due to infection with the bacillus pyocyaneus.

Blood may give rise either to a distinct red color, a brownish-red shade, or even a black tint. If the blood be adherent to the scybalous masses or to the well-formed feces, it is usually derived from the rectum or anus and indicates hemorrhoids; if it be evenly distributed with the food material and is changed from a bright red to a brownish color, it indicates a hemorrhage in the stomach or high up in the small intestine, especially if the stool be solid; while an evenly mixed bloody feces of fluid character will usually point to the colon as the seat of the trouble. As a general rule, it may be said that the darker the color the more remote from the rectum will be the hemorrhage. Tarry black blood is

seldom of low origin, usually indicating trouble in the stomach or duodenum, while fluid scarlet blood usually arises from the colon or rectum, although in some cases of typhoid fever the blood may be a bright red, although the hemorrhage may be fairly well up in the bowel. In cases of intussusception the blood may appear mixed with serum, but with no fecal matter.

In deciding as to the importance of blood in the feces one must naturally exclude that arising from food or from hemorrhages above the gastro-intestinal tract. Thus blood coming from the mouth, nose, throat, or lungs may pass into the stomach and out with the feces, making a mistake in diagnosis very possible. Further, blood arising from vaginal discharges, which may be mixed with the feces at the time of defecation, should be excluded.

The detection of blood in the feces is more or less simple and direct in the fresh state, but when intimately mixed with the feces its recognition is a matter of some difficulty. In many cases of hidden or occult bleeding blood is never detected microscopically or macroscopically.

It is practically useless to search for blood-cells in the feces, as rarely are perfect cells found, unless the blood is present in very large amount, many specimens showing no cells. The chemical tests outlined in the chapter on Blood serve very well for its detection with certain modifications.

Guaiac Test (Van Deen's Test).

A small portion of the stool is rubbed up with water and one-third of its volume of glacial acetic acid added. This mixture is well shaken in a test-tube and a few cubic centimeters of ether added. After thoroughly shaking this mixture, it is allowed to settle, when the ether, in the presence of blood, will have assumed a brownish color. In case the ethereal extract is not clear, a few drops of alcohol may be added. On adding to this ethereal extract a mixture consisting of equal parts of fresh tincture of guaiac and ozonized turpentine, a blue ring will form at the point of contact or a blue coloration will be seen throughout the mixture if the tube be shaken.

This test is much more reliable in its negative phase than in its positive phase. The writer has frequently found positive tests for blood arising from the employment of tubes previously used with copper solutions or with nitric acid, so that he would advise the worker to invariably use either new or thoroughly clean test-tubes when testing for the presence of blood. Moreover, this test is given by other substances, which are enumerated in the section on Blood and may react positively in the absence of blood. If the patient has been eating potatoes or rice, has been taking iron, or the feces contain much pus, a distinct reaction may be present. In this test as well as in all of the other tests outlined, the presence of hematin arising from the meat of the diet must be excluded. This can be done only by placing the patient upon an absolutely meat-free diet for several days preceding the examination.

The Schaer-Klunge Test.

This test is very similar to the preceding, but is much more delicate, being

positive after the ingestion of only three grams of blood. It is even more important when using this test to exclude all hemoglobin and chlorophyll-containing foods for some days preceding the examination. The stool is rubbed up with water and treated as in the preceding test with acetic acid and ether. To this ethereal extract is then added a mixture of 1 c.c. of ozonized turpentine and 1/2 c.c. of fresh 3 per cent. alcoholic aloin solution. This may be prepared by dissolving what aloin will lie on the point of a spatula in 1/3 of a test-tube of 75 per cent. alcohol. At the line of contact a distinct red ring will be observed in the presence of blood in from three to five minutes. Careful work with this test has shown that fat interferes to some extent with its delicacy. It is, therefore, customary to treat the feces with an equal volume of ether and to shake thoroughly to remove all the fat present. The ethereal solution is then poured off and the remaining fecal material mixed with one-third its volume of glacial acetic acid and 10 c.c. of ether, being then thoroughly shaken and set aside.

A portion of this brownish ethereal extract, which contains the hematin formed by the action of the glacial acetic acid upon the hemoglobin of the blood, is poured into a thoroughly clean test-tube and treated as above described with the turpentine and aloin solution. If the tube be shaken after the red contact ring has formed the whole mixture will assume a cherry-red color. If the tube is allowed to stand for a few minutes the aloin solution may sink to the bottom, forming a distinct red layer beneath that of the ether and turpentine. A reaction to be positive should appear within 10 minutes as the aloin itself will gradually turn red under the conditions of the experiment if left for a much longer period. This test, as previously stated, is much more delicate than the guaiac test, especially when the feces are first extracted with ether. Charcoal instead of carmin should be used o mark the feces. The writer has found this test very reliable and very easy of application.

Weber's Test.

This test has the advantage of excluding practically every other factor which might influence the tests previously given. A portion of the feces is extracted with ether to remove the fat and is then separated from the ethereal solution. This extracted feces is then rubbed up with water and treated with glacial acetic acid and ether. The ether, as in the preceding test, takes up the hematin which is now detected by the spectroscope, showing the characteristic spectrum, namely, an intense narrow band in the red between C and D and a somewhat more definitely marked group of three broader bands, (1) in the yellow, (2) at the boundary between yellow and green, and (3) at the boundary between the green and blue, this last band being difficultly recognizable.

In order to avoid confusion with the spectrum of methemoglobin or of chlorophyll, one may convert the hematin into reduced hematin (hemochromogen) by the addition of alcoholic potassium hydrate, water, and ammonium sulphid solution. The spectrum of this latter substance is characterized by the two bands in the green.

This test is probably the most sensitive and should be more frequently employed. It is very simple of application, is very reliable and has practically no fallacy, especially when the hematin is converted into the hemochromogen. This test is, however, not so delicate as the aloin test, so that small amounts of blood, as found in occasional cases of occult bleeding, may escape detection.

Weber originally used the guaiac test with a modification of extracting the fat from the feces before testing with guaiac and turpentine. Schumm states that if the feces be thoroughly extracted with alcohol and ether most of the fat and urobilin will be removed and that under these circumstances Weber's test, either with the guaiac tincture and turpentine or with the spectroscope becomes much more valuable.

Adler's Test.

O. and R. Adler have introduced the use of benzidine as a test for the presence of blood. The stool is extracted with a mixture of alcohol and ether for the reasons above mentioned. It is then treated with glacial acetic acid and with ether as described in the other tests. This acid ethereal extract, which contains the hematin, is then treated with 2 c.c. of a saturated alcoholic benzidine solution and 2 c.c. of hydrogen peroxid (3 per cent.). In the presence of blood an intense green color appears. This test is almost too delicate for clinical work, as it shows the presence of 1 part of blood in 100,000 parts of water. However, it has a great value, as a negative test with this reagent will absolutely rule out of consideration the presence of blood. It is especially necessary in applying this test that absolutely every trace of hemoglobin be removed from the diet, so that any blood appearing in the feces may have some diagnostic importance.

(6). **Mucus.**

From the diagnostic standpoint the recognition of much mucus in the feces is of the greatest importance. Any amount of visible mucus should be considered pathological, although it is to be remembered that mucus may be increased physiologically as the result of hypersecretion, in wh ch case it will appear as a slimy coating of the scybalous masses or as small adherent particles. Boas regards the mucus found after strong cathartics as normal, but this is to be questioned as the irritation may be sufficient to set up a mild hypersecretion. The mucus expelled with the meconium and, according to Lynch, even that passed by infants up to the second week of life should be considered normal. The fecal mucus is a true mucin, being precipitated by acetic acid, but dissolving in 10 per cent. HCl.

The larger portions of mucus may be easily recognized with the naked eye, but the smaller bits are more clearly brought out by rubbing up the feces with water and holding the material, in a thin layer, toward the light. If the feces be well formed, the mucus may be separated from the exterior of the cylinder, as it is never found in the interior of a firm feces. In mushy stools the mucus is intimately mixed with the fecal material, usually in the form of smaller

particles, the exterior type of mucus being of much larger flakes. Nothnagel has reported a jelly-like consistency of a mushy stool in a case of jejunal diarrhea in which the mucus was not derived from the intestinal wall, but came, probably, from the bile. It is important that one be able to recognize mucus, as mistakes have been made in the presence of swollen vegetable tissue, fruit pulp, echinococcus membranes, and even of parasites.

The ordinary form in which mucus appears in the feces is in clumps, flocculi, or shreds with irregular margins. These pieces may vary in size from those just visible to those several inches in length. In some cases strips, tubes, ribbons, or maccaroni-like pieces are observed, which are especially frequent in enteritis membranacea or mucous colitis. The amount of mucus passed may vary from a few flakes to an enormous mass, Bories having seen 120 grams in one movement. Occasionally one may see, especially in the stools of infants, masses resembling cooked sago granules or "frog spawn," which Kitagawa has identified as mucinous material.

The consistency of fecal mucus varies from that of a jelly-like mass to one having the density of thin leather. The larger the piece the firmer it appears, although exceptions do occur. Pure mucus is usually glassy or jelly-like, certain inclusions changing its consistency. Cellular inclusions in the mucus change it to a paper-like mass, while some specimens appear tenacious due to absorption of protein material or to a diminution of its water-content. These inclusions, as well as those of fat or bacteria, will diminish the usual transparency of the mucus.

On remaining long in the bowel, mucus may take on the normal brown color of the feces due to hydrobilirubin, a dark orange shade from bilirubin, a greenish hue from biliverdin, or a red to reddish-brown tinge from blood pigments. The usual state of the mucus, however, is colorless.

It may be stated generally that the most of the macroscopically recognizable mucus of the feces arises from the large intestine. Owing to its easy digestibility the mucus from the mouth, stomach, and upper intestines passes out only under conditions of great motility. The secretion of mucus in the small bowel is much less than in the large intestine. Of all recognizable forms of fecal mucus only the smallest particles arise from the small bowel, and then only when they are found in a fluid feces. The higher the secreting point the smaller will the particles be, as a rule. These upper intestinal flecks contain much detritus of digestion and half-digested cells or free nuclei and crystals in cellular form, frequently a few bilirubin granules being observed.

The mucus from the large intestine, especially from the sigmoid, is large in amount, is jelly-like in appearance, and has ordinarily no inclusions. In cases of mucous colitis the mucus may be passed in the forms of the large strips or bowel-casts, appearing as pure transparent material grayish-white or bloody in color and having no inclusions. No fecal matter may be present. Such a condition is a pure secretion neurosis and becomes a true inflammation only when cell-inclusions are observed. It is to be said that mucus, even with a

small inclusion of pus or blood, does not necessarily point to ulceration. On the other hand, one must not judge from the absence of mucus that no catarrh exists, as one frequently finds variations in such excretions.

Microscopic mucus is much rarer than the macroscopic type, although both may be present at the tame time. Microscopically, mucus appears as a structureless mass, characterized by irregular lines running through it and by a difficultly recognizable margin, showing a more or less transparent ground substance in which may or may not be found epithelial cells, pus-cells, blood-cells, bacteria, protozoa, food remnants, and crystals. If the mucus comes from the higher sections of the bowel the food remnants will predominate, while in that from the lower bowel the cellular elements are in excess.

Mucus is stained with difficulty. For a successful stain the reaction must be neutral and very little admixture with foreign material is permissible. Thionin colors mucin a specific violet, while the other tissue elements are stained blue. Methylene blue and methyl violet stain it but slightly, while other aniline dyes color only the enclosed cells. Iodin may give a diffuse yellow.

(7). Pus.

Macroscopically visible masses of pus of a gray-white color are occasionally found in the stools, but these may hardly be distinguished from mucus particles without the microscope. If in large amounts, attention should be directed to the perforation of an abscess into the bowel. Such pus is more or less intimately mixed with the fecal material. The passage of pus from the small intestine and even from the cecum is associated with such a marked decomposition that it can be recognized only with the greatest difficulty. The nuclei may still persist, but these resemble very closely those of the food cells.

A few isolated leucocytes are usually present in the feces, as a result of diapedesis through the mucous membrane. A pure pus excretion is never seen in cases of uncomplicated catarrh, but in ulcerative processes of the large intestine and in many affections of the small bowel (dysentery, ulcerative colitis, syphilis, carcinoma, tuberculosis, and typhoid) pus-cells may be found in small masses.

Casein flocculi should be differentiated from pus masses by microscopic examination, which will show fat droplets mixed with the albumen.

(8). Food Remnants.

The appearance of macroscopic amounts of food remnants in the feces is known as *lientery* and is dependent upon the nature and amount of the food, its method of preparation, the degree of its mastication, and upon the condition of the digestive organs.

Much less undigested material remains on a meat diet than after a vegetable ration. In the former case we may find small bits of bone, cartilage, tendon, hair, feathers, skin, fish scales, and connective tissue; while in the latter we may observe cellulose-containing cells, such as those of cereal, cotyledonous and

leguminous vegetables, skins of fruits, nuts, and vegetables, grape seeds, cherry stones, etc.

Cooking has a great influence upon the digestibility of any food. Boiling seems to be the best method, although much of the nutritive material is taken up by the cooking water. The method of roasting is such as to produce a more nutritive but a less digestible substance, unless it is carried only to the point of slight coagulation of the protein. The outside layers of roasted meats seem to be practically indigestible, according to the work of van Ledden-Hulsebosch. Smoking seems to be the least desirable form of preparing meats. Vegetables become more digestible through the cooking process, owing to the bursting of the indigestible cellulose membrane. Those vegetables, such as lettuce, cucumbers, onions, turnips, cabbage, and radishes, which are eaten raw, appear in the feces absolutely unchanged.

While great individual differences exist in the power of utilizing digestible or undigestible food, yet we must assume that any appreciable residue, especially following the Schmidt-Strasburger diet, is pathological. The digestive insufficiency may begin in the mouth as a result of too little mastication. The importance of thorough mastication has been especially emphasized by Fletcher. Lack of digestive power of the stomach will have but little influence on intestinal digestion, providing the pancreatic secretion is sufficiently active, with the exception that raw or smoked connective tissue will be undigested and will appear in the feces. Lientery is much less frequent in motor disturbances of the stomach than in those of the intestines. Perhaps the greatest amount of undigested residue is seen in cases of perforation of an ulcer or carcinoma of the stomach into the intestine, a direct communication being established between the stomach and colon.

Naturally, in cases of increased intestinal motility, one will find more food residue in the feces than under normal conditions. This motor insufficiency is especially answerable for the appearance of undigested starchy remnants, as, even under the most severe conditions, starch digestion is not interfered with to any extent in the bowel, providing mastication and preparation of food have been sufficient and the succus entericus has not been so diminished by catarrhal processes as to permit of fermentation. The direct irritation from the hard particles of cellulose may cause an increased peristalsis and, hence, directly lead to lientery.

Marked disturbance of protein and fat digestion, evidenced by an intense lientery, will occur if the pancreatic secretion be insufficient. This is even more marked if gastric disturbance coexists. A lack of bile will cause an exclusive disturbance of fat digestion, which is seldom manifested by the appearance of macroscopic particles of fat, but is characterized by the typical clay color of the stool.

The final factor which influences lientery is the insufficiency of the absorptive power of the intestine. This increases the food residue by retarding digestion, according to the law that an accumulation of the products of ferment

activity will prevent further action of the ferment, as well as by holding within the bowel the products already digested.

Only in exceptional cases can one judge of the specific factor at the bottom of a lientery, a boundary line between normal and abnormal being drawn with difficulty. It is, however, important that one be able to recognize food remnants both macroscopically and microscopically. The writer recalls a case in which the residue of orange and banana pulp was mistaken for a new parasite. This recognition of the nature of a fecal residue is especially important in the examination of the infantile stool. Normally, the nursing child shows no food residue in the feces, but frequently white flecks or clumps are observed, which may consist of casein but perhaps more frequently of fat or of soaps. The proper recognition of such particles will require both chemical and microscopic examination, so that one should not jump to the conclusion that all such substances are casein, as is too frequently the case.

The following discussion of the appearances of the various food residues will include both the macroscopical and microscopical examinations, as these are inseparable in practical work.

(*A*). Protein Residues.

(*a*). Muscle Fibers.

The appearance of muscle fibers in increased amount in the stool is known as *azotorrhea*. The muscle fibers appear as isolated pieces of different size and shape. The smallest pieces have a circular or oval contour, the medium-sized particles are jagged, while the larger masses have parallel sides and angular surfaces. These fibers are colored yellow or yellowish-brown by the hydrobilirubin, but may be tinged with bilirubin or by foreign pigments. The color will depend both upon the amount of pigment and upon the time the fibers have lain in the bowel.

The smaller pieces may be entirely homogeneous, although these as well as the larger bits show both transverse and longitudinal striations. If the fibers are well digested, the longitudinal striations may be the only ones showing, and even these may disappear. No nucleus is visible unless the pancreatic secretion is entirely lacking. No specific micro-chemical reactions are known for these fibers. They may be tested with any of the color reactions for protein material.

Connective tissue and elastic tissue fibers are occasionally associated with muscle fiber and may be recognized by their appearance.

(*b*). Casein.

This is found especially in the infant stool and is always pathological. These masses, known as curds, are more or less round clumps which vary in size from that of a pin-head to that of a hazel-nut. They are either pale or golden-yellow, the larger masses being a pure white inside. These curds always have a distinct yellow tone exteriorly, but the interior is pure white. If these particles are pressed between a slide and cover-glass they spread out like white

cheese and show absolutely no structure microscopically. These masses show the protein reactions.

Leiner's Test.

A small amount of fecal matter containing these curds is spread on a slide and dried in the air. It is then fixed by heat and stained with a mixture of equal parts of a 0.75 per cent. solution of acid fuchsin and methyl green in 50 per cent. alcohol (dilute mixture 10 times with water). At the end of 15 minutes the slides are placed in distilled water and left for one hour. Casein and paracasein will take a pale blue or violet color, while similar substances will show a greenish tone.

Microscopically, one may differentiate from these true casein particles (curds), certain products which are more or less normal in the stools of the child. These masses are smaller than the curds, being not usually over pinhead size, are more yellow in color, and appear under the microscope as clumps of fatty acid crystals or of fat droplets and bacteria bound together by mucus.

(*B*). **Fat Residues.**

Fatty substances are present in all stools, if there be any in the diet, either in the form of neutral fat, free fatty acids, or soap, especially of calcium and magnesium. If present in macroscopically recognizable amounts, the condition is known as *steatorrhea*.

Neutral fat may be present in the form of white colorless clumps of different size and irregular in shape, some being globular while others are distinctly angular. The more usual form is the refractive, opaque, more or less yellow globule. Occasionally the fat may appear as a melted oil which hardens over the surface of a formed stool or gives the appearance of vaselin to a semisolid stool. Neutral fat may be recognized by its ready solubility in ether and its black color on treatment with osmic acid and deep red color when acted upon by Sudan III or Scharlach R.

Fatty acids appear either as scales or as crystals of varying form. The scales may not be distinguishable from those of pure fat except by their easy solubility in cold alcohol. The crystals are thin, delicate, curved needles, which run to a distinct point and are grouped in thick masses. Other types of fatty acid needles may occur, such as the small lancet-shaped plate or those closely resembling the soap crystal. These crystals are colorless and are soluble in cold alcohol, showing no stain with the above reagents for free fat. The fatty acid scales are, however, stained by these dyes.

The soaps likewise appear in the feces as scales or as crystals. The scales are less refractive, more firm, usually more angular, and may be colorless or yellow-brown from hydrobilirubin or yellow from bilirubin. The crystals appear most frequently as uncolored needles, which are shorter, plumper, not so pointed as the fatty acid crystals, and are arranged in clusters. Schmidt has described a peculiar form of crystal, which he styles the "cracknel" form, a round type with a sunken center and a raised border. These crystals are

insoluble in ether, do not melt on warming, and are uncolored with stains. Treated with acids they form fatty acid crystals.

Any condition which interferes with the proper absorption of fat by the mucosa or lymphatics will lead to *steatorrhea*. This condition may be observed, physiologically, after the ingestion of large amounts of fat, as in the oil treatment of gall-stones, but we are little concerned with such findings. It is seen, pathologically, in cases of atrophy of the mucosa, in amyloid disease of the intestines, tuberculous ulceration, tubercular peritonitis, tabes mesenterica, and even in catarrhal enteritis.

The peculiar, glistening, gray-white, pasty, acholic stool of steatorrhea is seen more frequently, however, in cases of biliary obstruction and of pancreatic disease. The fat in these cases of biliary stasis is in the usual form, that is three-fourths of the ether extract of the bile-free feces is present as fatty acids and soaps and one-fourth as neutral fat. In cases of acholia 50 to 80 per cent. of the fat will be unabsorbed instead of the normal 5 to 10 per cent.

In the fatty stools of pancreatic disease, the fat is largely present as fatty acid, no soap being found. The association of steatorrhea with the absence of decomposition products, few bacteria, and the presence of maltose in the feces is much more indicative of pancreatic disease than is steatorrhea alone (Le Nobel).

(*C*). Carbohydrate Residues.

Starch may be present, in the normal feces, enclosed within plant cells which have resisted digestion, but well-preserved starch granules are never found normally. A few partially digested or complete colorless granules may indicate no abnormality, but many will point to a hyperacidity or to a disturbance in the small intestine, especially to an insufficiency of the succus entericus leading to the so-called "fermentative dyspepsia" (Schmidt).

Starch may be detected by the addition of Lugol's iodin solution to a small portion of the feces spread upon a slide. A blue coloration will indicate starch, while a red tone will show the presence of erythrodextrin.

The varieties of cellulose-containing substances found in the feces are as numerous as are the types of such food material. These have been discussed previously and cannot be here elaborated. Certain microchemical tests may prove valuable in the identification of such cellulose material.

Cellulose treated with sulphuric acid and then with iodin solution gives a blue color due to the conversion of cellulose into amyloid.

If a solution of zinc chlorid be allowed to act upon a suspected mass of cells, which have been previously treated with Gram's iodin solution, a purple coloration with be produced. If a neutral or weakly alkaline solution of Congo-red be added to cellulose, it will be strained a distinct red. Cellulose is soluble only in an ammoniacal solution of cupric oxid, known as Schweitzer's reagent.

(9). Biliary Constituents.

As a rule, the unchanged biliary acids, glycocholic, taurocholic, and

cholalic acids, are absorbed from the bowel so that they do not appear in appreciable amounts in the feces. Schmidt believes that cholalic acid is present in slight amounts in all feces and that all the bile acids may be increased in pathologic conditions.

Bile pigments, on the other hand, are always present, chiefly in the form of hydrobilirubin, although bilirubin may occur. In pathologic conditions we may find, beside these two pigments, biliverdin, bilifuscin, bilicyanin, and bilihumin. The tests for these pigments as well as for the free bile acids and their salts will be considered in detail in the section on Urine, to which the reader is referred.

In cases of cholelithiasis gall-stones of varying size may be found in the feces, although not in every case. It is, therefore, of the greatest importance that the feces be carefully examined in suspected cases for the presence of these concretions. This is especially the case whenever a severe, colicky, abdominal pain of doubtful origin exists. The feces should be well mixed with water and passed through a fine sieve which will retain any of the suspected particles. A single examination of the feces is not sufficient in such cases unless the stones be found. In cases of negative findings, the stool should be searched for at least two weeks following a suspected attack. According to Naunyn, only the very firm stones will leave the bowel, the softer ones breaking up into small bits in the bowel.

These gall-stones vary from the size of a pin-head to that of a pigeon's egg and are found as small crumbling masses or as hard stones which either have a jagged surface or a smooth surface with characteristic facets, indicating the presence of many such stones. These stones consist for the most part of cholesterin mixed with the biliary pigment, or of compounds of calcium with the biliary pigments. These calcareous stones may be combinations of the various bile pigments and are always the hard facetted type, while the cholesterin stones are softer and may be colorless or tinged with bile pigment. The nucleus of these stones is usually a mass of organic detritus, in some cases being made up of clumps of bacteria, such as the typhoid bacillus and bacillus coli communis.

A gall-stone may be usually recognized from its fractured surface, but frequently it becomes necessary to submit it to chemical examination for purposes of identification. From the clinical standpoint it is a matter of more or less indifference as to the composition of a gall-stone, so that the writer will refer to works on physiological chemistry for the methods of detection of the various constituents.

In this examination for gall-stones the worker must not be deceived by the presence of extraneous substances, such as seeds, cherry-stones, fats and soaps of high melting point and of masses of impacted feces. In the treatment of gall-stones by the use of olive oil many of these soft fatty translucent masses appear in the feces which are in no way associated with gall-stones, although one may be deceived n supposing them to be true stones.

(10). **Intestinal Sand and Concretions.**

By intestinal sand we have reference to the small granules or masses of inorganic salts which appear in the feces. These masses are very small, may be spherical or irregular, are usually hard, usually have a reddish-brown or green color and consist of inorganic compounds mixed with organic detritus, especially with fat and bacteria. While most of this material is made up of ingested substances, we may have in cases of neurasthenia and especially in those associated with mucous colitis the excretion of large numbers of these particles as the result of a true secretory neurosis. Myer and Cook[1] have recently shown that the banana may be a source of this intestinal sand.

The massing together of this intestinal sand may form intestinal concretions or *enteroliths*. As a rule, however, these intestinal concretions have as a nucleus some foreign body around which calcium and magnesium salts of phosphoric and carbonic acid or ammonium magnesium phosphate have been deposited. These enteroliths are usually hard, heavy, and round, being colored, as a rule, brownish. A second form of intestinal calculus, known as *coproliths*, are irregular in shape, usually softer than the enteroliths, and consist of inorganic material, mixed with inspissated feces. Neither of these types of intestinal concretion have any great pathologic significance, although the possibility must be granted that some of them might either form or lodge in the appendix and thus be accountable for an acute appendicitis.

(11). **Tissue Fragments.**

The examination of feces for tissue fragments is more or less unsatisfactory, owing to the fact that these fragments are difficultly recognizable after being partially digested by the juices of the intestinal canal. It not infrequently happens, however, especially in carcinoma of the lower bowel, that such fragments may be obtained and a diagnosis made possible by microscopic examination. A diagnosis of malignancy would better not be made unless these tissue fragments show typical cellular arrangement, or at least, typical arrangement of the nuclei. The writer has seen a diagnosis of carcinoma made on the basis of a strip of mucus with cell enclosures and would, therefore, warn the worker to be on his guard against the possibility of such an absurd mistake.

III. Microscopic Examination.

This examination includes the search for many substances which have been described under the macroscopic examination, so that these need not be here considered. There are, however, some elements, both morphological and crystalline, which have not been treated.

For any success whatever in the microscopic examination of the feces, great care must be taken in the selection of the material for examination. This is especially true when searching for parasites or their ova, as well as for the

[1] Amer. Jour. Med. Sci., vol. 137, 1909, p. 383

differentiation between various food particles and secretions from the intestinal wall. If the stool be soft and mushy it should be thoroughly mixed by stirring and suspicious particles looked for macroscopically. Fresh specimens as well as stained preparations are then made and examined both with the low and high power. In examining feces for bacteria it is always well to mix the feces with water and centrifuge for a few minutes. The bacteria will remain suspended in this process and the coarser fecal material will be deposited. The fluid is then poured off and mixed with equal parts of alcohol, the mixture being again centrifuged when the organisms will be found in the sediment. This procedure is the one adopted by Strasburger in estimating the proportion of bacteria in the dry stool.

Various microchemical reactions are carried out with isolated fecal material and frequently are of great importance. In this procedure a few drops of reagent are allowed to flow under a cover-glass which covers the specimen. The staining material may be drawn under the cover-glass by placing a piece of ordinary filter-paper on the opposite side of the cover-slip.

If the examination for amebæ is to be made it is essential that the stool be kept warm, as the characteristic differentiating point of an ameba is its active motility. This can be done by placing the feces in a bottle which is surrounded by warm water until the examination is made. It is wise, also, to make the examination upon a warm stage, although this is not absolutely essential unless extended search has to be made for the parasite. There are no specific stains for the feces, the ordinary Löffler's methylene blue serving very well for a general staining of bacteria, while characteristic stains must be used when searching for special organisms.

Morphological E ements.

Epithelial cells are present in every specimen of feces. These may be squamous in form and are usually found in the mucoid particles of the stool. They are especially present in rectal carcinoma and in ulcerative conditions of the lower bowel. The cylindrical type of epithel um is much the commonest form and is found, also, in association with the mucus. Many well preserved cells may be present if their source is in the lower bowel, but, as a rule, these cells show all types of degeneration. They occur in catarrhal inflammations of the intestinal mucosa and are rarely associated with pus-cells unless ulceration has taken place. If the irritation is in the small bowel these cells are always more or less digested and contain bilirubin particles in cellular arrangement, while if from the lower bowel the cell is usually intact.

The presence of red blood-cells is determined more by the chemical examination than by he microscopical. It may occasionally happen that these cells may be seen, but a good result will depend upon a fortunate selection of the material for examination. A few scattered leucocytes are practically always found in the feces, and assume pathologic importance only when present in large amounts as pus-cells, whose occurrence has been previously discussed.

Crystals.

Besides the crystals of free fatty acids and soaps previously mentioned we find large numbers of other crystals in the feces. Among these we observe neutral phosphates of calcium and of magnesium which appear as wedge-shaped crystals occasionally forming rosettes in the former case while in the latter the crystals are more in the form of rhombic plates. The ammonium magnesium phosphate crystals are practically always present in the feces, appearing either as the typical coffin-lid crystal or as irregular fern-like masses. These triple phosphate crystals were at one time supposed to be characteristic of typhoid stools, but like so many other things their importance has been exaggerated. Various other crystals of calcium compounds are observed, such as calcium carbonate, calcium sulphate, and calcium oxalate, along with calcium salts of unknown fatty acids, which have been described by Nothnagel as irregular, oval, or circular masses, either fissured or showing concentric striations, and being always bile-stained. The lactate of calcium is seen in the form of radiating needles arranged in sheet-like masses in the stools of children on a milk diet.

Cholesterin crystals are found as thin, transparent, rhombic plates with notched corners. These crystals do not always appear in the typical shape, so that they should be tested by the addition of concentrated sulphuric acid when the cholesterin crystals will change from a yellow to a blood-red, violet, green, and finally blue color. The Charcot-Leyden crystal appears in typical form in the feces as a colorless, diamond, double pyramid-shaped crystal. The presence of these crystals is practically characteristic of *helminthiasis*, although it may indicate the presence of any parasite from the least harmful to the most pernicious. Hematoidin crystals occur as reddish-yellow rhombic plates or as groups of needles or amorphous masses in stools showing the presence of blood chemically. These have no especial significance and are not found as frequently as are the other blood pigments.

After the use of bismuth preparations we find the oxid of bismuth appearing in the form of black irregular rhombic crystals with notched edges. Charcoal appears in the form of irregular black masses which are larger and not so rhombic in form as are the bismuth crystals.

The examination of the feces for the various bacteria as well as the parasites and parasitic ova will be discussed in a special section. Various substances appearing in the feces have been mistaken for parasites or their ova and these also will be discussed in the later sections.

IV. Chemical Examination.

The chemical examination of the feces would naturally embrace both the qualitative and quantitative estimation of the products of digestion and decomposition as well as the estimation of the undigested portion of the food along with the products derived from the gastrointestinal canal itself. Clini-

cally, such work is rarely carried out and has questionable value from a diagnostic standpoint. However, in following the metabolism in any special case it is essential that the absorbed and unabsorbed portion of the food be known. This applies more particularly to the proximate food principles, protein, fat, and carbohydrate, although in some cases the estimation of the inorganic intake and excretion is of first importance. The writer cannot attempt in the scope of this work to go into great details regarding the chemical examination of the feces, but must limit himself to a few selected topics.

Reaction.

The normal reaction of the feces does not vary much from a neutral one, although it has a tendency to be slightly alkaline, owing to the presence of the alkaline secretions of the intestinal tract. The alkalinity of these secretions is diminished both by the combination of the alkali salt with the digestion products and by the absorption of some of this alkaline material into the blood. This reaction may at times be acid owing to the formation of lactic and butyric acids in the fermentative processes, while an increased alkalinity may be observed in cases of markedly increased ammoniacal decomposition. A pure meat diet gives, as a rule, an alkaline feces, while a pure carbohydrate or fat diet will have a tendency to form an acid feces.

The reaction of the feces under pathologic conditions is of little importance, although in typhoid fever the reaction is somewhat more strongly alkaline than in almost any other condition. So much depends upon the diet as well as upon the condition of the digestive organs that no conclusions at present may be drawn from the reaction of the feces.

Total Solids.

The normal amount of feces passed in 24 hours ranges from 100 to 250 grams, of which about 75 per cent. is water and 25 total solids. Although the determination of the total solids of the feces has little of importance in itself, it is essential that one should know the dry weight of the feces in order that he may properly calculate the amount of the various substances in the dry feces.

In determining the total solids in the feces, the specimen, preferably the 24-hour specimen or each movement separately, is placed in an evaporating dish, covered with a small amount of alcohol, and heated over a water-bath with frequent stirring. Small amounts of alcohol should be added as evaporation proceeds in order to hasten the drying process. It is, moreover, necessary to add a small amount (10 to 15 c.c.) of dilute sulphuric acid and to mix it thoroughly with the feces. This combines with the free ammonia, forming a non-volatile salt, and thus prevents loss of nitrogen. If the stool be rich in fats, it is wise to add a weighed amount of dry washed sand to make the mass more porous and thus permit of quicker drying. When the water has been driven off on the water-bath, the specimen may be placed in the drying oven at 105° and left for several hours, after which it is placed in the desiccator and dried to constant weight. Knowing the weight of original substance taken and the weight of the

dry substance, it is very easy to determine the percentage of total solids in the feces.

An increased total solids of the feces is observed in most cases of constipation, while in diarrhea the solid residue is much diminished. Increased separation of fluid into the bowel and diminished absorption from the bowel are the factors regulating the fluid content. Nothing can be determined, however, by the examination of the amount of total solids, as regards the pathologic conditions accountable for an increase or a decrease.

Total Nitrogen.

The determination of the total nitrogen must always be made in estimating the metabolism in any given case. In so doing it is likewise essential that one should know the absolute nitrogen intake, as without such a factor nothing can be learned from the excretion either in the urine or in the feces. The daily excretion of nitrogen in a fasting condition varies from 1 to 4 grams, on a mixed diet this may run from 3 to 7 grams, while on a vegetable diet the nitrogen of the feces may be as high as 10 grams, a pure meat diet yielding between 2 and 6 grams. In pathologic conditions this amount is practically always increased, due both to lack of digestive power and to decrease in the absorptive function of the intestines. Outside of metabolic experiments the absolute amount of nitrogen of the feces has no great diagnostic importance beyond showing some perversion of intestinal activity. In insufficiency of the pancreatic secretion, we are apt to find the greatest loss of nitrogen by way of the feces.

The method for determination of total nitrogen will be given in detail in the section on Urine to which the reader is referred.

The constituents which go to make up this total nitrogen are the various undigested protein bodies, such as albumin, globulin, nucleoprotein, nucleoalbumin, and gelatin, along with partially or completely digested products of protein origin, bacteria, and secretions from the intestines. These latter bodies embrace albumoses, peptone, the various amino-acids, and the hexone bases along with a certain amount of ammonium salts. The chemical properties of these substances, as well as the methods of their chemical detection and estimation must be looked for in works on physiological chemistry. The bacteria form a large percentage of the total nitrogen. It is rare, even in metabolic work, that the nitrogen partition of the feces is determined. Much more importance attaches, with our present knowledge, to such division of the nitrogenous material of the urine, which will be discussed by the writer at a later point. Likewise the products of abnormal decomposition of protein material, taking place in the bowel with the formation of such products as indol, skatol, and phenol, are rarely searched for in the feces. Our clinical knowledge of the importance of these substances is confined largely to their detection and estimation in the urine so that they must be passed for the time being. In the study of the metabolism of various cases of cystinuria, which is associated with

abnormal protein disintegration, certain diamines, such as cadaverin and putrescin, have been isolated from the feces by Udransky and Baumann, but these cannot be discussed at this time.

Fat.

The chemical estimation of the amount of fat is of importance only in metabolic work, as in the more direct clinical examinations this substance is detected by macroscopic and microscopic methods. It is, however, essential that the loss of fat by way of the feces should be known before a proper metabolic balance can be struck.

The dried feces is treated with a small amount of 1 per cent. acid-alcohol and evaporated to dryness in order to convert any soaps which may be present into the fatty acids. The dry residue thus treated is then placed in a Soxhlet apparatus and extracted with ether for at least 72 hours. The ether, which has taken up the free fat and the fatty acids, is then evaporated and the residue of fats weighed. From this weight the percentage of fat may be determined, and, knowing the original dry weight of the total 24-hour feces, the total fat lost by way of the feces may be readily calculated.

The amount of fat in the feces will depend much upon the amount and quality of the diet. In fasting conditions the amount is usually about 1 gram. On diets poor in fat the amount in the feces may exceed that of the diet, thus indicating a loss of fat from the body. It seems to be a general rule that the higher the melting point of the fat of the diet the greater will be the loss in the feces. Thus on a diet containing only butter as a fat about 4 per cent. of the intake will be lost, while with pork fat the loss may be as high as 13 per cent. It is impossible to say, *a priori*, just what the fat content of a normal feces would be. With the ordinary diet of our country it would range from 2 to 7 per cent. of the intake, which should be somewhere about 100 grams of fat per day, representing a loss of from 2 to 7 grams daily.

Carbohydrates.

As previously stated, carbohydrates may appear in the feces either in the form of starch or of cellulose. Only in exceptional conditions do we find any of the monosaccharids present. Disaccharids, such as lactose and maltose, may be occasionally found, but only when there is a combination of insufficiency, both of a salivary and pancreatic ferment. The presence of any appreciable amount of starch granules must be considered pathological, while the amount of cellulose will depend upon the amount in the food as well as upon the preparation of the food and its mastication.

Nothing is to be learned, from the clinical standpoint, by the determination of the absolute amount of carbohydrate in the feces. In the study of the utilization of food substances by the system and in general metabolic work, it is, however, necessary to know just how much of the carbohydrate intake is absorbed. This may be determined indirectly by subtracting from the weight of the dry feces the sum of the protein, fat, and ash. This result will represent a

much higher figure for carbohydrates than the one obtained by direct determination. In the direct determination much difference exists between the soluble forms and the insoluble cellulose. In the latter case we have to do with a useless form of carbohydrate and should, therefore, direct our attention rather toward the estimation of the amount of undigested or partially digested starch. We should determine the amount of cellulose and subtract this factor from the total carbohydrate obtained in the previous subtraction. This corrected figure will represent more nearly the true amount of carbohydrate than will the former, although for scientific purposes a direct determination is essential.

The starch may be estimated by taking a weighed amount of the dry feces and treating with 50 c.c. of 10 per cent. HCl. This is then boiled for one-half hour in order to convert the starch and digested portions of starch into the monosaccharids. This acid solution is then filtered and washed with sufficient water to make the total approximately the same as that of the original solution. The filtrate is then neutralized with sodium hydrate and made up to exactly 100 c.c. in a volumetric flask. The sugar in this solution is then determined by the methods outlined under Urine. This method, although not absolutely accurate will yield results sufficiently correct for ordinary purposes. More accurate results will be given by the Volhard-Pflüger method to be discussed later. The amount of glucose, as determined by either of these methods, will give, if multiplied by 0.94, the amount of starch in the feces taken. The percentage may then be readily calculated and the total quantity of the 24-hour excretion of starch determined.

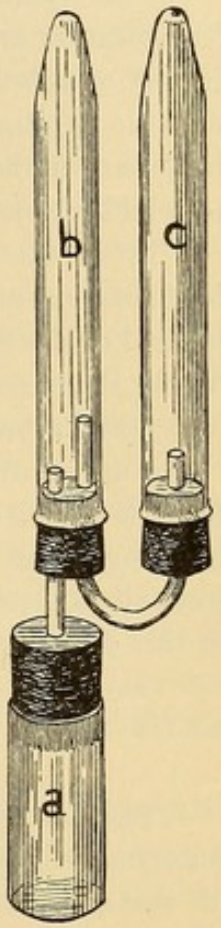


FIG. 21.
Schmidt's fermentation apparatus.

The fermentation method of Schmidt was advanced to permit of rough estimation of the presence of pathological amounts of starch, which, under ordinary circumstances, should have been digested. The principle of the method is the estimation of the amount of gas produced by the action of the intestinal bacteria upon the sugar which is formed from the starch by the intestinal amylolytic ferments. Prior to this determination the patient is placed upon the test diet outlined on page 93. The test is made as follows: Approximately 5 grams of the moist stool are placed in the vessel *a* which is then filled with water and the contents thoroughly mixed (see cut). The stopper is then placed in the fermentation flask in such a way that no air-bubbles are left. The tube *b* is filled with tap-water and closed with a small stopper without the inclusion of any air-bubbles. The tube *c* is now placed in position as shown in cut and the whole apparatus put in an incubator for 24 hours. The tube *c* has a small opening in the top so that water may be readily forced from the tube *b* into *c* by pressure of the gas produced. According to Schmidt 0.1 gram of starch

will cause the tube *b* to show about one-half its volume of gas. Normally, a positive result is said to occur when the tube is one-fourth to one-third filled with gas. The material in the fermentation flask *a* should be tested with litmus-paper after the test is complete to obtain the reaction of the mixture. If the gas formation is, as it should be, due to carbohydrate fermentation there will be a slight increase in the acidity of the mixture, while if it be due to protein putrefaction the reaction will show a slightly increased alkalinity.

The various decomposition products of carbohydrate digestion and fermentation are rarely of importance in fecal examinations. These products consist of volatile fatty acids, lactic acid, saccharic acid, alcohol and aldehyde, and the conjugated glycuronic acids. Tests for these various substances will be found in various parts of this work or in works on physiological chemistry to which the reader is referred.

From the clinical standpoint the gases produced by the processes of fermentation and putrefaction in the bowel have little value. The same is to be said of the quantitative estimation of the inorganic constituents of the feces. These latter may be determined by methods outlined in quantitative analysis and may be passed by for the present.

The various ferments found in the intestinal canal may be detected in the feces, but such examinations are at present of little clinical value. Methods of isolating these ferments and of determining their activity belong more to physiological chemistry than to clinical diagnosis and will, therefore, be neglected in this discussion.

V. Bacteriology of the Feces.

The bacteria of the intestinal canal are of many types, many of which are purely saprophytic, while others may or may not be pathogenic. The number of these bateria is usually enormous, Sucksdorff finding on an average 53,124,000,000 in the 24-hour specimen of normal feces. One may determine the amount of these bacteria by the method of Strasburger, who uses the following technic. Two c.c. of feces are rubbed up in a porcelain mortar with 30 c.c. of 1/2 per cent. hydrochloric acid. This mixture is then placed in centrifugal tubes and whirled for one minute. The bacteria will remain in suspension in the liquid which is poured off from the sediment. The sediment is again rubbed up with a little hydrochloric acid and again centrifuged, the liquid being added to the first portion. This procedure may be repeated until the fluid no longer becomes turbid on centrifuging. This acid solution, holding in suspension the bacteria, is then mixed with an equal portion of ordinary alcohol and placed in a beaker which is allowed to remain on a constant water-bath at 40° for 24 hours. At the end of this period a portion of the fluid may be evaporated and more alcohol added. This mixture is then placed in the centrifuge tubes and whirled for several minutes. The bacteria are now deposited and the supernatant fluid is poured off and mixed so that it may be

again centrifuged. The bacterial sediment is washed with alcohol and ether in the centrifuge tube and is then placed in a weighed dish. This is dried at 100° and again weighed. In order to determine the amount of bacteria as compared with the total feces, the dry weight of 2 c.c. of the fresh feces is determined as previously outlined. Knowing these factors, the percentage of bacteria in the dry feces may be easily calculated. Strasburger found that this was approximately one-third the weight of the dry stool and represented about 8 grams per day. As this dry feces is used for the determination of total nitrogen, we must bear in mind that the bacteria of the feces will represent about one-half of the total nitrogen of the feces. This fact is especially important in metabolic work.

The method of counting the bacteria in the feces by cultural and plate methods has little clinical value and will be passed in this discussion. It is necessary, however, in attempting to isolate definite organisms from the feces, that cultural methods be adopted, and these will be briefly mentioned under the discussion of the pathogenic organisms. It is to be said that the bacterial flora of the intestine is so varied that very careful and long-continued work is necessary to isolate a special organism. From the stand-point of preventive medicine it should always be remembered that the feces contain large numbers of typhoid bacilli and cholera spirilla, so that measures should be taken to properly treat such ejecta as soon as voided. While the majority of the bacteria of the intestines are saprophytic, being introduced with the food or drinking-water, yet we find the bacillus coli communis as a normal habitant of the intestine. This organism is usually harmless, but may, under certain conditions, become distinctly pathogenic, many obscure cases of gall-bladder infection, for instance, being traceable to this organism.

The presence of non-pathogenic organisms seems to be an essential for proper performance of intestinal function. This idea, originally advanced by Pasteur, has been denied by Schottelius, Nuttall, and Thierfelder, although their experiments extended only over periods of 17 days. These intestinal bacteria not only aid digestion, but also prevent a certain amount of abnormal decomposition, owing to the fact that they inhibit the development of foreign types to a large extent, especially under normal conditions. These normal bacteria may under certain conditions, however, become pathogenic, but only when the intestinal wall loses its continuity. In case the normal bacteria of the intestine become too numerous, the products formed by their activity upon protein material may be absorbed and bring about certain toxic effects. It is interesting in this connection to find that the bacteria are diminished in amount in chronic constipation, according to Strasburger. The toxic symptoms must, therefore, be referable to the absorption of other than bacterial products of decomposition, or the products have increased toxicity. For the best conditions to exist the symbiotic relations of the intestinal bacteria should be such that there are neither too few nor too many bacteria. Just how this is to be brought about is the problem of the clinician.

The writer will not attempt a description of the various types of bacteria occurring in the feces, but will limit his remarks to the discussion of a few of the more important pathogenic types.

(*a*). The Cholera Spirillum.

This organism, known as the comma bacillus, is about 2 microns long and 1/2 micron thick. It is very actively motile and has a single delicate flagellum at one end. It stains easily with the ordinary bacterial stains and is decolorized by Gram's method. The cultural peculiarities of this spirillum may be learned from any text-book on bacteriology. This organism is usually recognizable in the stools of Asiatic cholera, which are the characteristic "*rice-water*" stools. The blood serum of patients affected with cholera will show

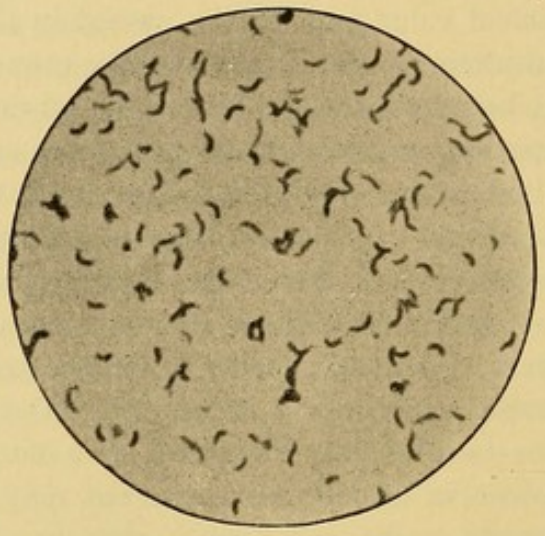


FIG. 22.—Cholera spirilla. (*Pitfield.*)

very characteristic agglutination of cultures of these organisms. The blood is usually used in a dilution of 1 to 15, the reaction being observed in from 5 to 20 minutes.

Closely related to this comma bacillus of Koch is the bacillus of Finkler-Prior. This latter organism may be distinguished from the spirillum of Asiatic cholera by its morphology, the organism being larger and thicker than the comma bacillus, and by the appearance of the stab cultures on gelatin. The cholera spirillum forms a typical funnel-shaped depression, while the bacillus of Finkler-Prior shows a stocking-like depression. The Finkler-Prior bacillus is found in cases of cholera nostras. It may be necessary for the absolute identification of these organisms to apply bacteriolytic tests with serum of animals immunized against a specific type of organism.

(*b*). Typhoid Bacillus.

This organism, discovered by Eberth, is so similar in morphology to numerous other organisms, especially to the bacillus coli communis, that simple staining methods do not suffice for its detection. The characteristic stool of typhoid fever is a copious watery stool, having a strong odor and an alkaline

reaction. This stool is known as the "*pea-soup*" stool, and may be tinged with blood and contain many pus-cells.

The typhoid bacilli are medium-sized organisms with rounded ends, generally short, but sometimes long or thread-like and frequently showing faintly-stained sharply-defined areas in their protoplasm. They are actively motile and have both polar and lateral flagella. This organism stains with the ordinary dyes and is decolorized by Gram's method.

Several methods have been advanced to permit of isolation of the typhoid bacillus from the feces. The writer selects, therefore, those that have proven the most satisfactory in his work.

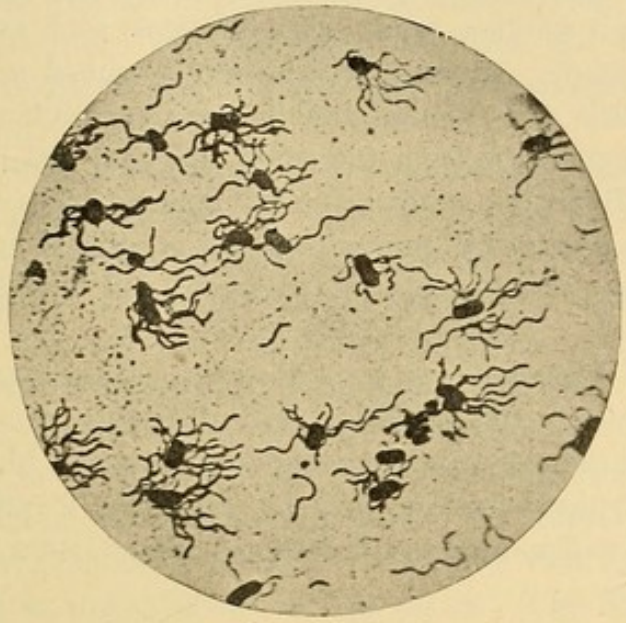


FIG. 23.—Bacillus typhosus, stained to show flagella (*Oertel after Fränkel and Pfeiffer.*)

Method of Drigalski and Conradi.[1]

Three pounds of minced beef are mixed with 2 liters of water and allowed to stand overnight. The beef is then pressed and the juice boiled for one hour and filtered. To the filtrate are added 20 grams of Witte's peptone, 20 grams of nutrose, and 10 grams of sodium chlorid. Boil this mixture one hour and filter. To the filtrate 60 grams of agar are added and the mixture boiled for three hours, one of which should be in the autoclave. Slightly alkalinize the mixture to litmus-paper, filter, and boil for one-half hour. To this hot agar solution, which should be now about 60°, add the following litmus-lactose solution. Two hundred and sixty c.c. of litmus solution is boiled for 10 minutes, after which 30 grams of chemically pure lactose are added and the mixture boiled for 15 minutes longer. This litmus-lactose solution is added while boiling to the hot agar solution, the mixture being well shaken and again faintly alkalinized to litmus. Four c.c. of a hot sterile 10 per cent. solution of sodium carbonate and 20 c.c. of a freshly prepared 0.1 per cent. solution

[1] Zeitsch. f. Hyg. u inf. Krankh., Bd. 39, 1902, S. 283.

of crystal-violet B. (Höchst) in warm sterile distilled water are then mixed in. This medium may be poured directly into plates or kept in flasks. It soons hardens to a firm mass and does not become dry readily.

The principle upon which the use of this medium depends is that in the presence of both lactose and protein the colon bacillus will first attack the milk-sugar, while the typhoid bacillus will act upon the protein. In the presence of litmus the colonies of colon bacilli become distinctly red, while those of the typhoid bacillus are blue. The crystal-violet inhibits the growth of many of the other organisms, especially of the acid-producing type.

If the stool be fluid, as is usually the case, one series of two plates is inoculated with the undiluted stool, another with a stool diluted with 10 volumes of sterile normal salt solution, while other dilutions, such as 1 to 100 and 1 to 1000 may also be made. If the stool is solid it is rubbed up into a homogeneous mass with sterile salt solution and the various dilutions made as above. In making the inoculations from the stools, the material is rubbed over the surface of the medium, the plates being left open to allow the surface to dry. The dry plates are then placed in the incubator at 37° and examined at the end of 24 hours. Any contamination of the media will be killed by the crystal-violet.

It frequently happens that certain strains of the paratyphoid bacillus develop blue colonies in this medium. It is, therefore, necessary for absolute differentiation that the agglutination test described under blood be carried out. In this way the typhoid bacillus may be absolutely identified, especially in the presence of its well-known morphological characteristics.

Hiss Method.[1]

Like the preceding method this requires a special media for the identification of the typhoid organism Five gram of Liebig's extract of beef and 5 grams of sodium chlorid are dissolved in 1 liter of boiling water. Five grams of agar are then added and 80 grams of gelatin after the agar is thoroughly dissolved. Enough hydrochloric acid or sodium hydrate is then added to bring the reaction of the mixture up to 0.5 per cent. of normal acid. To this medium is then added one or two eggs well beaten in 25 c.c. of water, the mixture boiled for 45 minutes and filtered. To the filtrate is added 10 grams of glucose. This material is then placed in tubes and properly sterilized.

In testing the feces for typhoid organisms by the use of this tube material of Hiss, a small portion of the fluid stool is taken up with a straight platinum wire and a stab culture made. The typhoid organism is practically the only one which has the power of uniformly clouding this medium without showing streaks or gas-bubbles.

Hiss has a second medium which he uses for plate methods. This consists of 10 grams of agar-agar, 25 grams of gelatin, 5 grams of beef extract, 5 grams of sodium chlorid, and 10 grams of glucose in one liter of water. This prepara-

[1] Jour. of Med. Res., vol. 8, 1902, p. 148.

tion is made up by adding the materials in the same order and with the same clearing as in the tube medium. This media should not contain less than 2 per cent. of normal acid. In this plate media the baccillus typhosus is the only one showing thread-forming colonies.

(*c*). The Bacillus of Dysentery.

Bacillary dysentery is distinctly different from the amebic type of dysentery. The bacillus dysenteriæ, or Shiga's bacillus, is now generally recognized as the specific organism of this type of dysentery.

Flexner found a similar bacillus in the dysentery of the Philippines, while Kruse has found practically the same type in Germany. In the United States Flexner and Harris find an organism which answers the description of the ordinary Shiga bacillus. The difference between the different organisms described is very slight, so that one may generally consider them as varieties of the same species.

The Shiga bacillus is a short rod with rounded ends, very much resembling in morphology the typhoid bacillus. It does not seem to have very active motility as far as progression is concerned, although it does show a high degree of molecular motility. It stains with the usual dyes and is decolorized by Gram's method. The only sure method of identification of the various types of the dysentery bacillus seems to be the agglutination test.

The fecal material is best obtained by curettage of the rectum, as these bacilli seem to be present in the mucus and are thus more easily concentrated. A bouillon culture is then made from these particles and agar slants are made as soon as possible. At the same time agar plates are prepared and placed in the incubator for 24 hours. The Shiga bacillus is not a rapid grower so that p actically no colonies of this organism will develop within 24 hours. The colonies which do develop at the end of 24 hours are marked on the plates and these are again incubated for 24 hours. From the colonies which develop at the end of 48 hours, tubes of glucose agar and litmus mannite agar are inoculated. If any of the tubes show fermentation at the end of 24 hours they are placed aside as not indicative of the Shiga bacillus. From these tubes showing no formentation, litmus milk, litmus mannite, and bouillon are inoculated. The Shiga bacillus will first render the milk slightly acid, later changing it to alkaline, the litmus mannite remains unchanged with the true Shiga bacillus.

(*d*). The Tubercle Bacillus.

The examination of the stools for tubercle bacilli is not always satisfactory. The enormous number of bacteria of the feces may prevent a recognition of the tubercle bacillus even though it is present. In the feces we find certain organisms which are acid-fast, such as the timothy bacillus, which is very closely related in morphology and staining characteristics to the tubercle bacillus. We should be on our guard, therefore, lest we make a wrong diagnosis from the occas'onal presence of acid-alcohol-fast organisms.

If these organisms are present on repeated examination and there are clinical symptoms pointing to such a trouble of the bowel, one may give a presumptive diagnosis of tuberculosis, remembering that the tubercle bacilli may have come from swallowed tubercular sputum.

In selecting the material for examination it is wise to pick out the particles of mucus, especially those which are blood-stained or purulent. Frequently one may facilitate his search by rubbing up the stool with water and centrifuging it as in Strasburger's method. These organisms will show the morphological and staining characteristics described under Sputum.

VI. Parasitology of the Feces.

In the examination of the feces for parasites one should obtain the feces as fresh as possible. This is especially the case where the examination for protozoa such as the ameba is to be made. The feces should be kept in a warm vessel prior to the examination, as the motility of these unicellular organisms is shown only with great difficulty after they have become cold. The other types of protozoa are less sensitive to changes in temperature and show their active motility, providing the feces be examined soon after voiding. The formed feces are not as suitable for examination for protozoa as are the more fluid stools, the types present in the former case more frequently being the resting forms and not the true actively motile organisms. It seems to be generally accepted that these organisms are the more easily found the more fluid, more mucoid, and more alkaline the feces. The particles for examination should be preferably the masses of mucus which can be found by careful search in the liquid stool. The organisms are best examined in their fresh condition, as the staining agents usually require fixation of the specimen and consequent death of the parasite. The following discussion of the intestinal parasites is taken directly from Tyson's modification of Braun's work.

(1). Protozoa.

The protozoa are unicellular animal organisms. These, although living occasionally symbiotically, are more usually found as isolated single organisms. A few of these organisms are sufficiently large to be detected by the naked eye, but the majority are minute and require the finer microscopic detection. They consist essentially of a mass of protoplasm (cytoplasm or sarcode), with differentiation for functional purposes (organelles) of a variable character, constancy, and prominence. In the ameba, for instance, the sarcode may be separable into an internal distinctly granular portion known as the endosarc, and a peripheral clearer portion known as the ectosarc; a cell-membrane in some instances is a well-marked feature, while in others it is absent; and in some of the free-living protozoa special external coverings of chitinous, siliceous, or chalky composition enclose the protozoon. Of the various parts the nucleus is, after the cytoplasm, the most constant, varying much in appear-

ance, shape, size and number in the individual form (single nucleus of variable size and shape; double or dimorphic nucleus, a macronucleus of vegetal character, a micronucleus with creative function; polymorphous nucleus, multiple nuclear granules more or less widely distributed in the cytoplasm).

Not uncommon examples of specialization are met in the contractile vacuoles, in pigment spots, in mouth-like ingestion foci and their pits on the surface of many forms with relatively firm cell membrane, in the anus-like excretory points of the same form, or the peripheral motor organelles, in the sucking tubes of the suctoria, and in the hook-like fixation apparatus of the gregarines.

Motile protozoa move in a variety of ways. The naked rhizopods move by a peculiar rolling due to currents in the internal substance of the cell or by the protrusion of the cell-substance as extensions or pseudopoda, these movements being always accompanied by change in the cellular shape of the animal. Ciliates and flagellates move through the activity of the special cuticular appendages known as ciliæ and flagella.

(*a*). Rhizopoda (Sarcodina).

Amœbina.

(α) Amœba coli (entamœba histolytica).

The ameba was first discovered in the large intestine by Lambl, although we are indebted to Lösch for the first accurate description of this organism. He did not believe it to be the cause of dysentery, but regarded it as a secondary invader. Since that time much work has been done, a controversy having arisen regarding its specificity. In a great many cases of dysentery this organism is not found, but in its stead bacteria, especially the Shiga bacillus, are present in large numbers. This bacillary dysentery is something entirely different from the amebic type of this disease. It is true that in certain cases of dysentery as well as in normal individuals, amebæ are found which are differentiated with great difficulty from the true amœba coli. There seem to be two distinct types: one pathogenic, to which Councilman and Lafleur give the name amœba dysenteriæ, which Lösch styles amœba coli, and which Schaudinn designates entamœba histolytica; a second type, which is non-pathogenic, has been styled by the first writers amœba coli Lösch and by Schaudinn entamœba coli. These two organisms differ to some extent in the relation of their cytoplasm, a well-developed ectoplasm being marked off in the entamœba histolytica as an especial plasmazone and being much more refractile than is the endoplasm.

One may sum up the points relative to the pathogenicity of the entamœba histolytica as follows: (1) It appears in a form of dysentery which is anatomically characterized by peculiar ulcerations which are markedly different from the diphtheritic inflammatory processes of the bacillary dysentery. (2) The more recent the case the more numerous are these parasites; (3) they are deposited in the dysenteric ulcers and tend to pass into the deeper

tissue appearing as true tissue parasites; (4) they frequently deposit themselves in the liver causing abscesses which contain these organisms in large numbers and practically no other infectious material; (5) by injection of amebæ-containing feces into the large intestine of animals typical amebic dysentery may be caused. As these organisms have thus far resisted successful attempts at pure cultures, experiments cannot, of course, be made with the single organism. It is to be borne in mind that symbiosis with bacteria is apparently necessary for the development of amœbæ.

The entamœba histolytica is an actively motile, roundish, pear-shaped, oval, or irregular unicellular organism having an endosarc which is typically

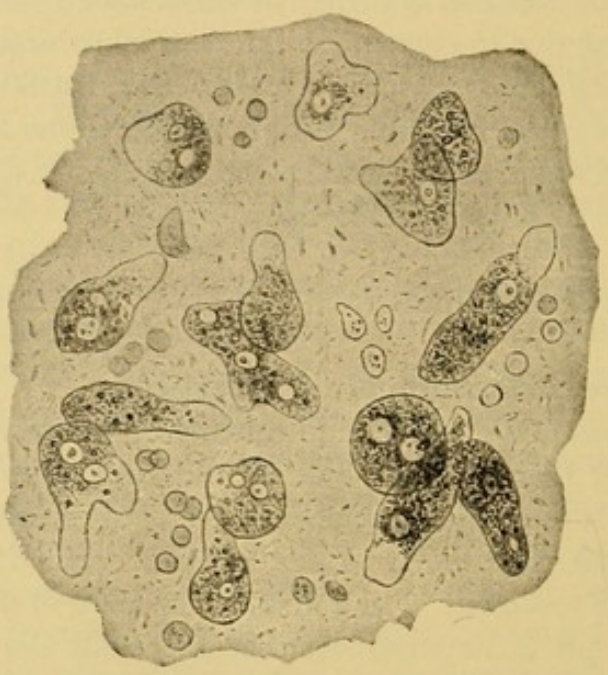


FIG. 24.—Amœba coli. (*Hemmeter.*)

granular and may contain leucocytes, red blood-cells, bacteria, particles of food, or pigments which the parasite has ingested, and shows the clear hyalin ectosarc, which is, perhaps, best seen in the pseudopoda. The pseudopoda are the typical motile portions of the parasites, these projections being thrown out from any point of the periphery, the protoplasm seeming to flow into them and drawing the animal after it. The parasite may not move, but will change its external appearance by throwing out these pseudopoda in various directions. The nucleus is a homogeneous, little refractile, chromatin-poor, spherical mass, about 6 microns in diameter (the diameter of the organism itself ranging between 10 and 50 microns, the average being 35). This nucleus is not always clearly visible, appearing much more frequently in the animal killed by corrosive sublimate. In the granular endosarc one frequently sees several vacuoles which may or may not pulsate, the general opinion being that pulsation is absent, although change in shape is frequent.

The stools of this amebic dysentery are thin and watery, show an alkaline reaction, and have a peculiar lime-like odor. Much mucus, blood, and occa-

sionally many pus-cells are found for which reason the mucus should be selected for examination in case these organisms are suspected. Frequently one may obtain better specimens for examination by examining mucus which is obtained with a rectal tube.

(*β*). **Entamœba coli. (amœba coli Lösch).**

This parasite varies in size between 10 and 15 microns. The hyaline protoplasm of the pseudopoda is not distinctly differentiated from the ectoplasm. It is opaque, gray in color, and its nucleus is sharply defined, being characterized by its richness in chromatin. The movements of this organism are not as rapid as those of the entamœba histolytica, which does not show as active phagocytic power. According to Craig, about 65 per cent, of normal persons show these nonpathogenic entamœba coli in the feces, especially after a dose of Epsom salts.

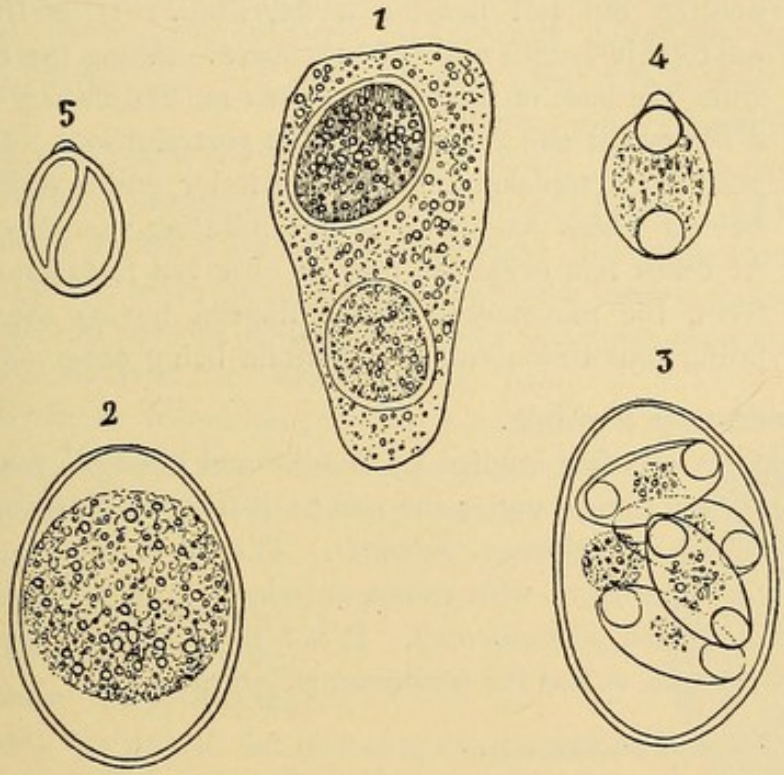


FIG. 25.—Coccidium hominis, from intestine of rabbit: 1, A degenerate epithelial cell containing two coccidia; 2, free coccidium from intestinal contents; 3, coccidium with four spores and residual substance; 4, an isolated spore; 5, spore showing the two falciform bodies—× 1140. (*Tyson after Railliet.*)

(*b*). **Sporozoa.**

Coccidium Hominis (coccidium perforans; cystospermium hominis).

This organism appears in the feces as an oval or spherical parasite about 22 microns long and showing a thin periphery. A large number of nuclei are usually observed.

The infection with these organisms seems to arise from rabbits in whose intestines these parasites develop in large numbers.

(*c*). Flagellates.

(α). Trichomonas intestinalis.

This organism was first studied by Marchand and Zunker and later elaborated by Grassi, Roos, and Janowski. It is probably identical with the one known as trichomonas vaginalis which may live in the vagina, the urethra, large and small intestine, the stomach, and may be found in the sputum. Various forms have been described as being found in the intestine, but they are in all probability the same organism; among these we find *protoryxomyces coprinarius*, *monocercomonas hominis*, *cimænomonas hominis*, *trichomonas hominis*, *cercomonas coli hominis*, and *cercomonas seu Bodo urinarius*.



FIG. 26.—Trichomonas intestinalis (*Tyson.*)

This is a colorless protozoon of a pyriform or spindle shape, rounded in front and bearing three flagella which are apt to be merged at the base and easily lost, the posterior end pointed but not bearing a flagellum. It is from 20 to 25 microns in length and 8 to 12 broad. Along the body, starting from the base of the flagella, runs an undulating membrane in a somewhat spiral manner to the posterior end. It has a finely granular cytoplasm and at its anterior end a vesicular nucleus, behind which one or more non-pulsating vacuoles may be seen. At times this organism may be observed to assume an ameboid form, the movements of the flagella having then ceased and projections resembling pseudopoda being observed.

(β). Cercomonas hominis.

This organism was first studied by Davine and has been found by many other workers. It is known under the names of *cercomonas intestinalis*, *monocercomonas hominis*, *cimænomonas hominis*. The adult organism is a small, colorless, pyriform parasite, with round anterior end provided with one long flagellum and a pointed posterior end. It is 8 to 10 microns long and has no undulatory membrane, as has the trichomonas intestinalis.

(γ). Megastoma entericum.

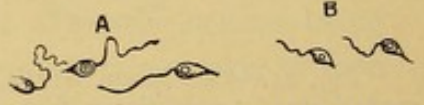


FIG. 27.—Cercomonas hominis; A, larger and B, smaller varieties. (*Tyson.*)

This organism was first found by Lambl in the feces of children. It is known under several names among which are *Lamblia intestinalis*, *hexamitus duodenalis*, *dimorphous muris*, and *megastoma intestinale*. This parasite is a colorless pear-shaped protozoon with a rounded anterior end and a pointed posterior end bearing a pair of flagella. The anterior end has one side concave with a raised border or lip, one pair of flagella, arising at the anterior border of this disk-like concavity, and two pairs together from its posterior margin. The cytoplasm is finely granular and the dumb-bell shaped nucleus is anteriorly about the middle level of the concavity. Vacuoles are absent and solid inclusions are never observed. The length of these organisms is from

15 to 16.5 microns, while the width is from 10 to 12.5 microns. The number of these organisms found in the feces may be very large. The surest points for their diagnosis seem to be the concavity and the dumb-bell shaped nucleus. The source of infection for man is the drinking of infected water.

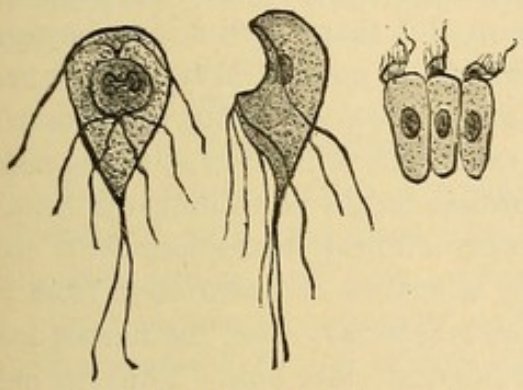


FIG. 28.—Megastoma entericum, showing disc-surface and lateral views in larger figures, and three epithelial cells with attached examples to the right. (*Tyson.*)

(*d*). **Infusoria.**

Balantidium coli (paramœcium coli).

This parasite is colorless, ovoid in shape, 70 to 100 microns long and 50 to 70 microns broad, having a finely granular cytoplasm containing fragments taken from the intestinal material, and a clear ectoplasm showing numerous longitudinal striations. It is covered completely with actively motile cilia which are more dense about the funnel-shaped mouth which extends about one-fourth the length of the body. The nucleus is kidney-shaped and is usually accompanied by one or more accessory nuclei, while two or more contractile vacuoles are seen which pulsate to a slight extent.

This parasite has been found in connection with various types of diarrheal affection and also in persons entirely free from intestinal symptoms. It may be present in the stools in very large numbers, being found especially in the colon, but in severe cases also in the jejunum. Whether or not these organisms are pathogenic has been a subject of much strenuous debate.

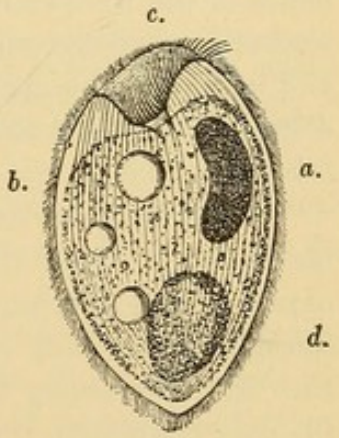


FIG. 29.—Balantidium coli: *a*, Nucleus; *b*, vacuoles; *c*, cytostome, with pit and peristome; *d*, ingested material. (*Tyson after Leuckart.*)

(2). **Entozoa (Enthelmintha).**

(*A*). **Platodes (Flat Worms).**

(a). **Cestodes (Tape-worms).**

The cestodes are naked, flat worms of elongated ribbon shape, endoparasitic, at least in their adult stage, and in many instances in all stages, without a digestive canal, and always more or less distinctly divided into segments. The entire parasite, or *strobile*, may be looked upon as a colony of individuals united in ribbon fashion from their mode of origin, for convenience in their development and functional performance; the various segments being derived by a process of constriction from the originally acquired parasite, which is spoken of as the *head*, *nurse*, or *scolex* of the strobile. A characteristic of the cestodes is the differentiation of two developmental stages: the first, *cysticercus stage*, in which the connective tissue or parenchymatous organs are invaded, and,

secondly, the development of the sexually mature animal in the intestine. The scolex obtains entrance as a larva into the intestinal tract of the host, becomes attached by a special fixation apparatus to the mucous membrane, and there develops into the adult parasite, forming the anterior extremity of the strobile in the developed worm. The head is usually a very small and inconspicuous object of globular, pyriform or club shape with a short posterior extension, spoken of as the *neck*. In the middle of the frontal face of the head there is often a small prominence, known as the *rostellum*, about which may be arranged in one or more rows, as one of the means of fixation of the parasite, small hooklets as a crown. As more constant means of fixation the head is provided with two or four suckers, rounded or linear depressions with more or less definite lips. Back of the head by a process of constriction from the neck, the segments, also known as links or *proglottids*, arise, the newest form always being placed between the neck and the next older link. Thus the older segments are always separated more and more from the head by each newly formed proglottid, each as it grows older and recedes further from the neck developing in size; the length of the strobile being thus dependent upon the two factors, growth of the individual length and the new formation of segments. These new segments as they are first formed are usually very short and proportionately broader, but as they increase in size with age they generally enlarge especially in their long diameter, and come to be more or less square. The number of these links may vary from three or four to several thousands, the length of some worms being 100 or more feet. The structure of each link and hence of the whole strobile includes an interior or matrix of an indeterminate connective reticular material, from which the various organs appear to develop and in which they are imbedded; over which are to be recognized exteriorly a delicate cuticle and beneath the latter, two layers of so-called muscle, the outer layer longitudinal and the inner transverse and circular.

Aside from the common parts the various links may be looked upon as individuals. There is no digestive canal, all nutrition being obtained by the parasite from absorption of dissolved material from the fluids in the infested intestine. The only really highly organized parts are the generative organs, each link containing both male and female organs. The terminal links are the ones containing the most ova, while the links nearest the head are usually but partially developed. In their development the ova fill the canal of the oviduct more and more often causing the appearance of side pockets of more or less branching character. The terminal ripe links either actually containing the ova or, after discharge of more or less of the original number, are apt to become separated from the strobile and be carried with the fecal matter from the intestine. Either with or without intermediate development the embryo is in some way, by water or in solids, carried into the alimentary canal of a second host. Arrived in this situation, both by its own activity and by passive convection by blood and lymph streams, the embryo penetrates the intestinal wall and becomes deposited in one or other situation as a larva, bladder-worm,

or *cysticercus*. This larval or cysticercus form is surrounded by a delicate connective tissue outer wall, derived from the host by a process of reactive inflammation, within which lies the true bladder-worm. This is essentially the head of the future parasite. This cysticercus, after remaining a variable period in the tissues, is devoured with the flesh of its host by a third (definitive) host.

(*a*). Tæniidæ.

(1). Tænia solium.

This parasite, which is derived from infection through the cysticercus cellulosæ of pork, has been also named *tænia cucurbitina*, *tænia dentata*, *cystotænia solium*, and *pork tape-worm*. The average length of the strobile is 2 to 3 meters, occasionally reaching twice this measurement. The head is somewhat spherical or slightly tetragonal from the four rather prominent cup-like suckers with thick lips. The head varies from 1/2 to 1 mm. in diameter, while the suckers range from 1/4 to 1/2 mm. in diameter. It is provided with a short, thick, rostellum bearing a double crown of hooklets, usually 28 in number. The neck is thin, about 3 cm. in length and is unsegmented. The proglottids number between 800 and 900, the fully grown and ripe segments measuring from 9 to 12 mm long and 5 to 6 mm. broad. The uterus consists of a large, median, longitudinal trunk with from 7 to 10 coarsely dendritic branches on each side. The ova are round or oval, the shell very thin but surrounded by an embryonic layer which is thick and shows distinct radiating lines. These eggs are usually of a brownish color and may show on their interior the hooklets of the embryo.

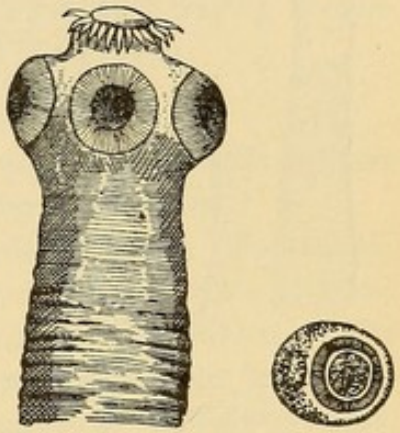


FIG. 30.—Head and neck, and ovum × 300, of tænia solium. Embryophore surrounded by vitellus. (*Tyson after Gould.*)

This parasite in its adult stage is practically limited to the small intestine of man, while the larval form has been found in swine, monkeys, dogs, etc. It has been shown that careful cooking and prolonged and thorough salting and drying of the meat will destroy the vitality of the cysticercus cellulosæ. It is, therefore, plain that any meat should be more or less thoroughly cooked before being eaten.

(2). Tænia saginata.

This parasite is the *beef tape-worm* and is also known as the *tænia mediocanellata*, *tænia inermis*, and *tænia dentata*. The adult worm varies from 3 to 8 meters in length, has a head from 1 to 2 mm. in diameter, tetragonal in shape without hooklets or rostellum, with four cup-shaped suckers each 0.8 mm. in diameter and placed at the corner of the frontal face. The right segments are from 18 to 20 mm. long and 5 to 7 mm. broad. The uterus shows

a distinct median longitudinal trunk with 20 to 35 lateral single or dichotomously branching and slender diverticula. The eggs are spherical with a thin shell surrounded by a thick radially striated embryonic shell. These eggs are from 30 to 40 microns long by 20 to 30 microns wide.

(3). Tænia cucumerina.

Infection with this parasite is relatively rare in the United States. It is found almost exclusively in children, the infection occurring through dogs and cats, the larval form of the parasites being found in the body lice and fleas. This organism has other synonyms, among them being *tænia canina*, *tænia moniliformis*, *tænia elliptica*, *dipylidium caninum*, and *dipylidium cucumerinum*. The parasite is from 15 to 35 cm. in length. The head is small, rhomboidal, with a clavate rostellum surrounded by three or four crowns of hooklets (48 to 60 in number), suckers rather large with radially-marked borders, and neck very short. The segments are from 80 to 150 in number, the older ones being 8 to 11 mm. in length and one to three mm. in breadth and often showing a reddish-brown color. The links frequently swell out in the middle so that the parasite has an appearance not unlike a chain of beads.

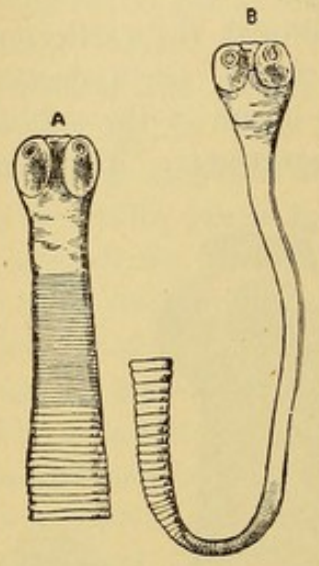


FIG. 31.—Head and neck of tænia saginata: A, retracted; B, extended. (*Tyson after Gould.*)

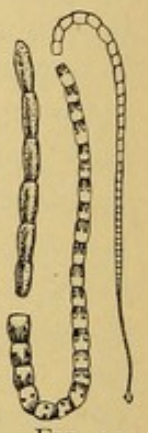


FIG. 32. Tænia cucumerina. (*Tyson after Leuckart.*)

A single uterus is common to the two oviducts, consisting of a network of tubes in which the ova lie in groups, filling small saccules, each containing 10 or 15 ova and surrounded by a reddish material which gives the color to the worm. The ova are spherical, 43 to 50 microns in diameter, and have a double wall. Within the wall one observes an embryo armed with hooklets.

(4). Tænia nana.

This worm is known as the "*dwarf tape-worm*" and is perhaps best known in Italy and Southern Europe, although it has been found in many cases in the eastern and southern portions of the United States. It has been called the *tænia ægyptica*, *hymenolepis nana*, *hymenolepis murina*, and *diplacanthus nana*. The infection with the ova is probably through the use of unfiltered water tainted with human or murine dejecta. It is most frequently seen in children, and inhabits the ileum, usually from the middle toward the ileocecal valve.

The parasite is from 10 to 15 mm. in length and from 0.5 to 0.7 mm. broad, is provided with a subglobular head measuring 0.2 to 0.3 mm. in transverse diameter, shows four large rounded suckers and a large rostellum retractile into an infundibulum. The rostellum is surrounded by a single row of characteristic hooklets, 24 to 30 in number and 14 to 18 microns in

length. The neck is rather long and slender, being followed by about 150 small proglottids, which are broader than long (0.4 to 0.9 mm. broad by 0.14 to 0.3 long).

The ova are characteristic. They are round or oval in shape, 32 to 36 by 42 to 56 microns in size, and have two distinct membranes. At each pole

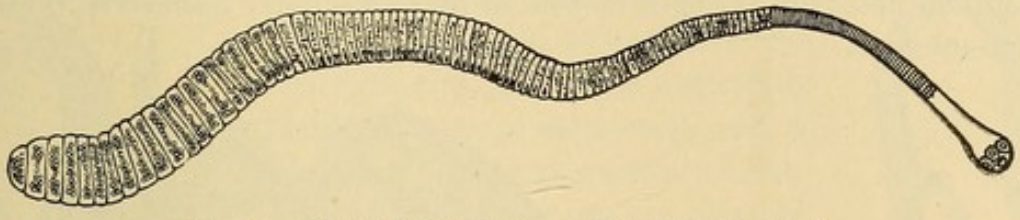


FIG. 33.—Tænia nana: × 10. (*Tyson after Gould.*)

of the inner membrane is seen a small protuberance from which springs a number of clear refractile threads, which are distributed in a waving fashion through the substance intermediate to the outer and inner walls.

(5). Tænia diminuta.

SYNONYMS—*Hymenolepis diminuta; hymenolepis flavopunctata; tænia leptocephala; tænia flavopunctata; tænia minima;* and *tænia varerina.*

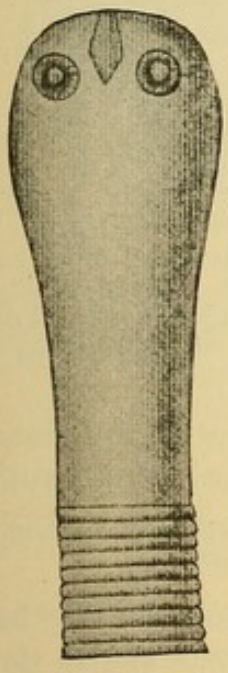


FIG. 34.—Head and neck of tænia diminuta. (*Tyson after Braun.*)

The parasite is 10 to 60 mm. long, head small, globular, with four globose suckers situated close to apex. rostellum small, pyriform, and devoid of hooklets. The segments number 800 to 1300 and are broader than long. Gravid uterus nearly fills the segments, showing as transverse line when not ripe.

The ova are round or slightly oval, yellowish in color, double-walled, inner wall showing slight protuberances at poles, a layer of albuminous material being seen between the walls.

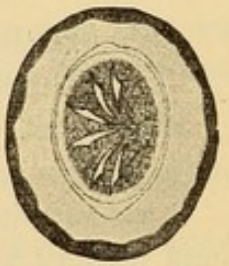


FIG. 35.—Ovum of tænia diminuta. (*Tyson after Braun.*)

(6). Tænia echinococcus.

This parasite in its adult stage is met with in the upper part of the small intestine of dogs, wolves, and jackals. The larval form, known as the *hydatid cyst*, is found in man, although more frequently in the ox, hog, horse, dog, cat, rabbit, etc. This disease is rare in America, and is acquired through association with the dog more frequently than in other ways. The most common seat of hydatid disease is in the liver.

The parasite is 2 to 5 mm. long, has a small subglobular head measuring 0.3 mm. in transverse diameter and bearing a rostellum with a double row of very characteristic hooklets (28 to 50 in number) and four prominent cup-

shaped suckers. The neck is short and rather thick; proglottids three or four in number, the last of which is usually longer than the rest of the worm put together. Uterus consists of a thick longitudinal median trunk with a few short lateral branches.

Ova spheroidal with thin radially striated shells and containing a granular hexacanthus embryo. Length of ova 30 to 36 microns, transverse diameter 25 to 30 microns.

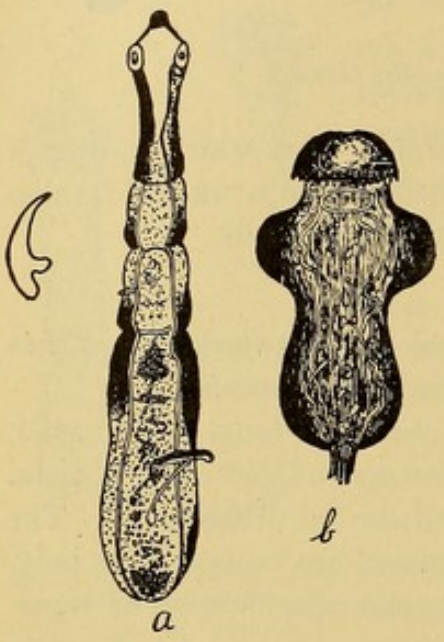


FIG. 36.—Tænia echinococcus: *a*, Adult; *b*, head from echinococcus cyst. On left a detached hooklet, as seen in fluid from cyst. (*Tyson after Coplin and Bevan.*)

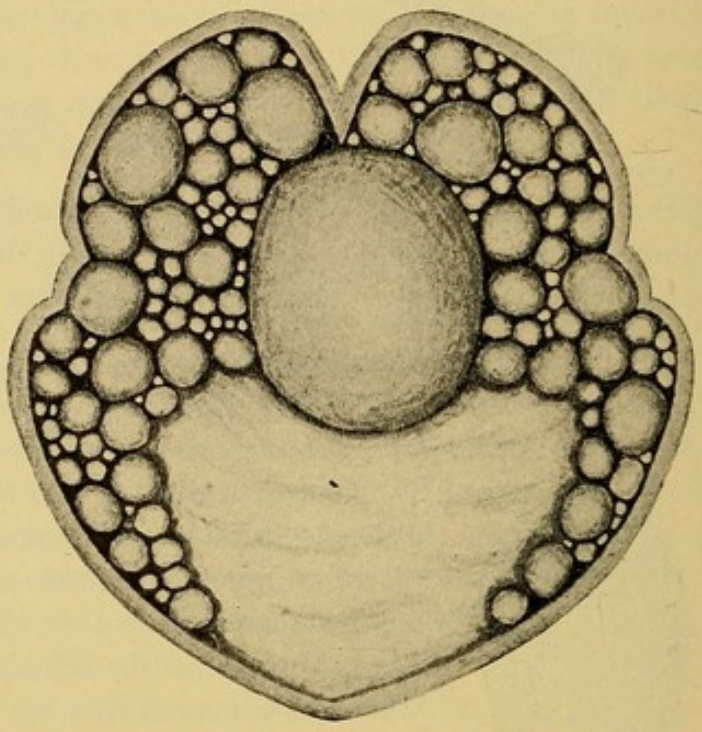


FIG. 37.—Hydatid cyst, showing daughter cysts. In the lower part of field is a whitish mass containing parts of the walls of ruptured daughter cysts. The thick wall of the mother cyst is well shown. From liver of man, × ⅔. (*Coplin.*)

(*b*). Bothriocephaloidea.

(1). Bothriocephalus Latus.

SYNONYMS.—*Dibothriocephalus latus; tænia lata; dibothrium latum; bothriocephalus latissimus; fish tape-worm.*

This parasite is most commonly met in the human intestine, but may be found in dogs and cats. It is most common in central Europe and in the maritime countries of Europe, British Islands, and Japan. The examples found in America occur in foreigners, as a rule. The ova, which are usually in large numbers in the feces, require for their further development immersion in water. The liberated embryo is then taken up by fresh-water fish and conveyed to man.

Strobile 2 to 10 meters (20 in a few cases) in length, marked in ripe segments by brownish central rosette (uterus with ova). Head elongated, almond shaped, 2 to 5 mm. long and 0.7 mm. transversely, with two lateral grooves or bothridia as suckers. Neck variable according to degree of contraction. The

segments number 3000 to 4000 and begin about 50 cm. from the head. The anterior links are poorly defined, in their growth increasing slowly in length but markedly in breadth. The ripe links measure 2 to 4 mm. in length and 10 to 12 in width, with opaque brownish rosettes in the middle line. Uterus formed of a number of plicated tubes in the form of a rosette.

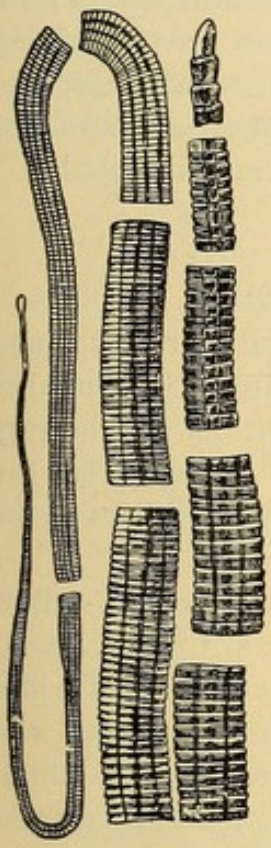


FIG. 38.—Bothriocephalus latus. (*Tyson after Leuckart.*)

The ova are brownish in color, ellipsoidal in shape, 68 to 71 microns in length and 44 to 45 in transverse diameter, have a thin shell, and a lid which may be opened or closed. The contents of the ova are coarsely granular or mulberry-like.

Infection with this worm is not always single, as high as 100 worms having been reported in the same individual. Many cases of infection with this parasite are associated with a high-grade anemia, which is distinguishable from pernicious anemia only by the effects of removal of the parasite.

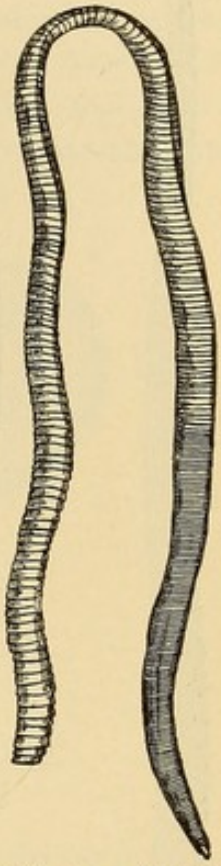


FIG. 39.—Dibothriocephalus cordatus: adult. (*Tyson after Leuckart.*)

(2). Dibothriocephalus cordatus.

This is a tape-worm of the same genus as the above and is parasitic in seals, being transmitted from them to man. It varies in length from 80 to 115 cm. Proglottids about 600, 7 to 8 mm. broad. The head is heart-shaped, 2 mm. long and broad. The ova are similar to those of the latus, but are a little larger in size.

(3). Bothriocephalus sp. Ijima et Kurimoto.

SYNONYMS.—*Diplogonoporus grandis; Krabbea grandis.*

Strobile measures up to 10 meters in length. Proglottids short and broad, head, neck, and number of segments unknown. Uterus rosette-shaped with several loops on each side. Ova brownish, operculated, oval, 63 microns in length and 48 to 50 in width. Intermediate host unknown, probably fish.

(β). Trematodes (Fluke-worms).

The various forms of distoma, which belong to this class, are more properly hepatic parasites, although they and their ova may at times appear in the intestines and feces. A detailed discussion of these parasites will be taken up in the chapter on Parasites.

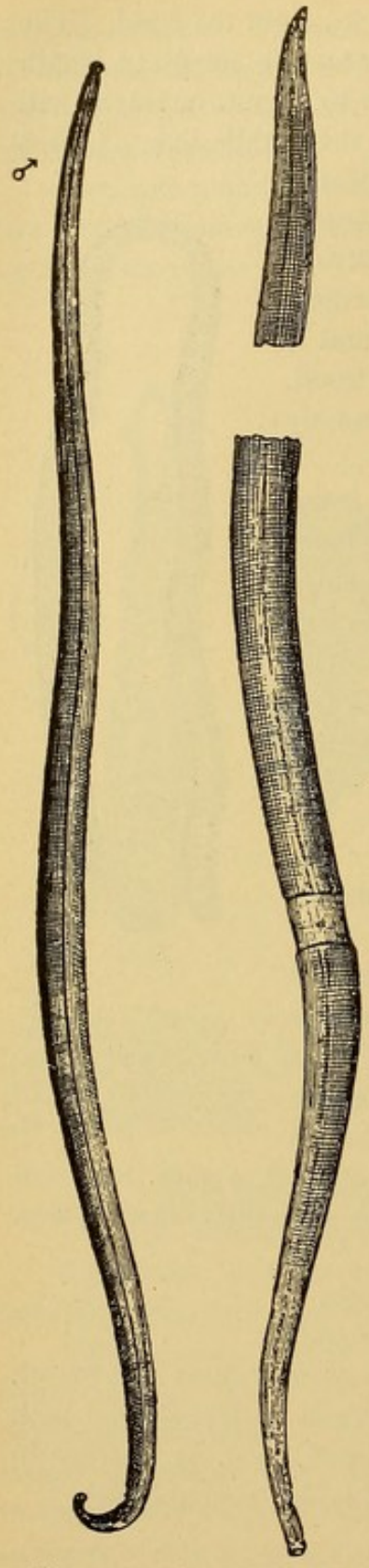


FIG. 40.—Ascaris lumbricoides: to left, male in lateral aspect; to right, female, ventral aspect, natural size. (*Tyson after Railliet.*.)

(*B*). Nematodes (Round Worms).

The nematode worms are unsegmented, elongate, circular or nearly so in their transverse section, cylindrical or more or less delicately fusiform and tapering toward each end. They are with but few exceptions parasitic and include many important examples, which are parasitic in man. Intermediate hosts, so essential for the intermediate development of the flukes and tapeworms, are practically absent in the nematodes.

(*a*). Ascaridæ.

(1). Ascaris lumbricoides.

This is the common round worm or maw-worm seen so frequently in children. The number in a single host is usually small, but may be very large. Its habitat is the small intestine, but the eggs may occur in the vomitus as well as in the feces.

The male worm is whitish to reddish-yellow in color; 15 to 17 cm. long, 3 to 3.5 mm. thick; elongate, fusiform; cuticle finely ringed; oral orifice terminal with three lips (one dorsal and the other two meeting in the median ventral line), each with fine denticulations on margins; at base of superior lip two papillæ, one only at base of other two lips; posterior end terminating conically, curved ventrally, with two slightly curved, short, equal spicules projecting from the subventral cloaca; 70 to 75 papillæ on the ventral face of posterior end, of which seven pairs are postanal.

The female parasite is 20 to 25 cm. long, 5 to 5.5 mm. thick; anterior end and general appearance as in male; posterior end tapering, ending in a conical, pointed, straight tail, vulva at level of first third of body length (in a slightly depressed annular band); anus subterminal.

The ova are elliptical in shape, 50 to 75 microns long and 40 to 58 microns broad; shell thick and transparent; stained yellowish with fecal material when in feces, but colorless in uterus; protoplasm unsegmented and coarsely granular; covered with a mammilated albuminous envelope, which may be lost.

(2). **Ascaris mystax.**

SYNONYMS.—*Ascaris canis; ascaris lumbricus canis; ascaris teres; ascaris caniculæ; ascaris cati; ascaris tricuspidata; ascaris felis; ascaris werneri; ascaris marginata; ascaris alata;* and *fusaria mystax.*

The male parasite is whitish or slightly brownish; 40 to 60 mm. long, 1 mm. thick; anterior end usually curved, with lateral cuticular expansions making the end look somewhat arrow-like; mouth terminal with three nearly equal lips with denticulate margins; at base of superior lip two papillæ, on inferior lip one ordinary and two minute papillæ; posterior end curled and with

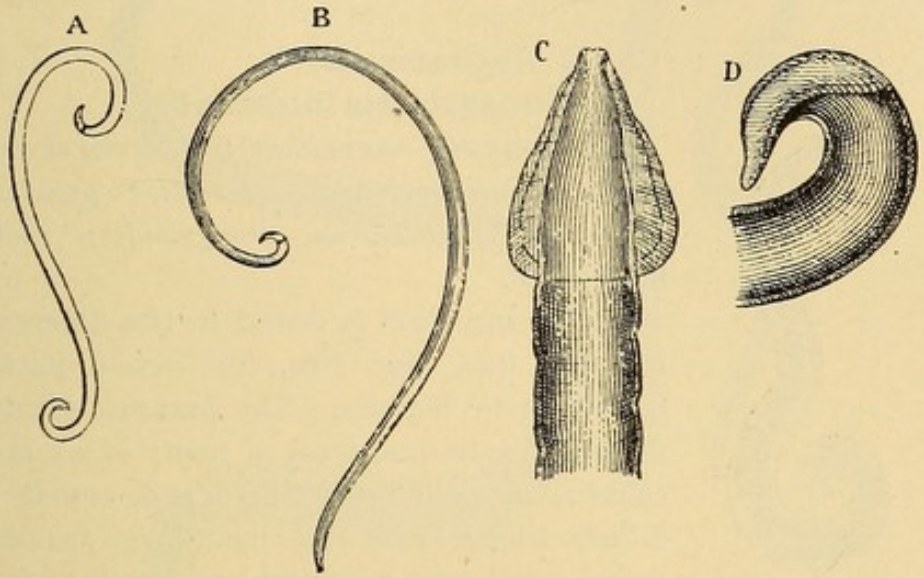


FIG. 41.—Ascaris mystax.

A, Male; B, female; C, anterior extremity, enlarged and shown from dorsum to exhibit the lateral wing-like cuticular expansions; D, same showing in profile. (*Tyson after Railliet.*)

lateral cuticular alar expansions, and on each side of cloacal aperture 26 papillæ, of which five are postanal.

The female worm is 120 to 180 mm. long; anterior end and general appearance as in male; posterior end straight, terminating obtusely; vulva at anterior fourth of body length; anus subterminal.

The ova are almost spherical; 68 to 72 microns in diameter; shell thin with thin albuminous envelope showing an alveolated surface.

(3). **Oxyuris vermicularis.**

SYNONYMS.—*Ascaris vermicularis; fusaria vermicularis; ascaris græcorum; pin-worm; thread-worm; seat-worm.*

The male is whitish in color; 3 to 5 mm. long, 0.3 to 0.4 mm. thick; cuticle transversely striated and at head end showing a vesicular swelling along the dorsal and ventral median lines; lateral lines distinct; mouth terminal, with three retractile lips; esophagus with distinct bulb; posterior end conical, curved ventrally, with six pairs of papillæ and slight cuticular expansion on each side; one spicule hooked at free end.

The female is 10 mm. long, 0.6 mm. thick; anterior end and general appearance as in male; posterior end straight, extended to a long mucronate tail; vulva at anterior third of body length.

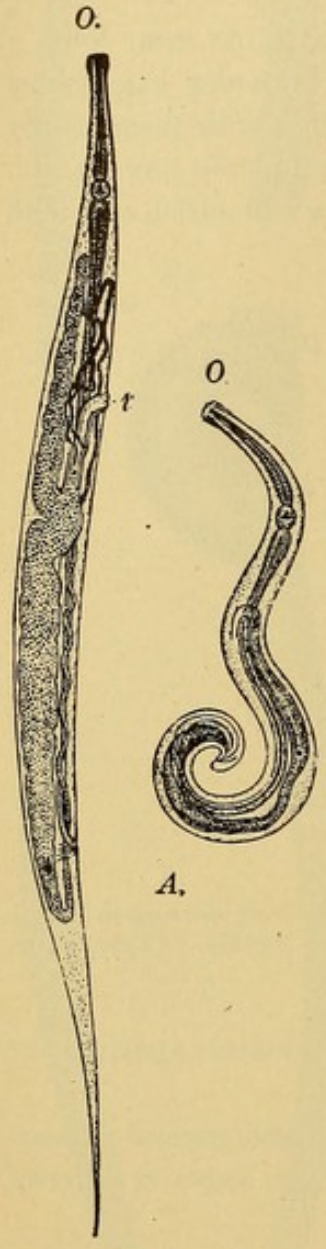


FIG. 42.—Oxyuris vermicularis: to left, female; to right, male (considerably enlarged); *A*, anus; *O*, mouth; *v*, vulva. (*Tyson after Braun.*)

The ova are oval, flattened on one side with a characteristic asymmetry, 50 microns long and 16 to 20 broad; shell thin; colorless.

This parasite inhabits the rectum and colon, but it may travel even into the stomach. The ova are rarely found in the feces, except in the mucus or about the anus.

(*b*). Angiostomidæ.

Strongyloides intestinalis.

SYNONYMS.—*Anguillula intestinalis et stercoralis; leptodera intestinalis et stercoralis; pseudorhabditis stercoralis; rhabdonema strongyloides; rhabdonema intestinalis.*

This organism is found in two different forms, the first diöic and free, the second parasitic, as parthogenetic females. The parasitic form lives in the upper intestinal tract of man; is 2.5 mm. long, cylindrical, with pointed tail end, cuticle smooth; mouth simple with four lips; long, slender, cylindrical esophagus reaching one-fourth of the length of the worm; anus close to tail; vulva at posterior third, containing yellowish-green oval ova, 50 to 58 microns long and 30 to 34 microns broad. The larvæ develop in the intestine and are passed in the fecal material. These larvæ are at first from 200 to 240 microns in length, but increase to two or three times this length. The larvæ differ essentially from the parent in having a rhabditiform esophagus. In the discharged feces at about 30° C. these develop with one moulting of the cuticle to a free-living generation with separate sexes.

In this free sexual generation the worms are smooth, cylindrical, and tapering, with pointed tail-ends; the mouth is the same as in the parasitic form; esophagus rhabditiform with its anterior portion long and with the posterior pyriform and containing a Y-shaped chitinous armature; anus at base of tail; male with tail curved and two spicules, body length 0.7 mm.; female 1 mm. long, with straight pointed tail, vulva a little back of the middle; ova few, yellowish, ellipsoid, thin-shelled, 70 by 45 microns in size, sometimes hatching in the uterus. The larvæ of this generation look much as their

free parents, are at first 0.22 mm. in length, but grow to 0.55 mm., then moult and assume a filariform or strongyloid character like that of the parasitic grandparent. These gain access to the intestine of a new host in an unknown manner or shortly die.

These worms may be found throughout the upper gastrointestinal tract, especially in the duodenum and upper part of the jejunum. The time elapsing

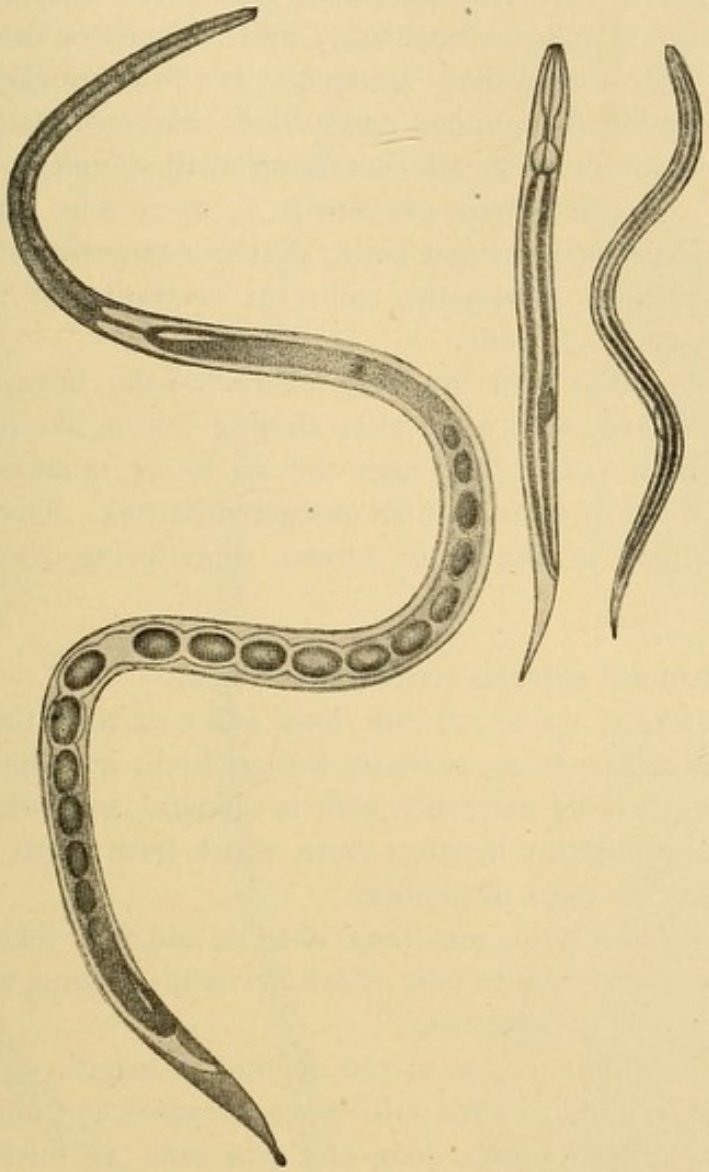


FIG. 43.—Strongyloides intestinalis; on the left, a gravid female from human intestine (natural size 2.5 m.m). In the middle, a rhabditiform larva from fresh fecal matter, × 120; to the right, a filariform larva from culture, × 120. (*Tyson after Braun.*)

between infections with the filariform larvæ of the sexual generation and the appearance of the rhabditiform embryos of the parasitic type in the stools is between two and three weeks. The parthogenetic female types are usually called the strongyloides intestinalis, while the free sexual form is styled strongyloides stercoralis.

This form is found widely distributed in Indo-China, the East Indies, Africa, Europe, and North and South America. The mode of transmission to

the second host is probably through the means of unfiltered water or of unclean, uncooked vegetables.

(*c*). Trichotrachelidæ.

(1). Trichiuris trichiura.

SYNONYMS.—*Ascaris trichiura; trichocephalus trichiurus; trichocephalus hominis; trichocephalus dispar; trichocephalus mastigodes; whip-worm.*

The male worm is 35 to 45 mm. long; whitish; anterior three-fifth slender and thread-like; posterior two-fifth thicker, cylindrical, terminally rounded and curled; anus terminal; single spicule in a tubular sheath containing small spinules.

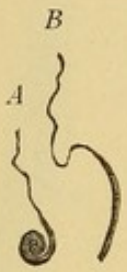


FIG. 44.—Trichiurs trichiura, natural size: A. Male; B, female. (*Tyson.*)

The female parasite is 35 to 50 mm. long; shape as in male for front and body; posterior extremity straight, bluntly pointed terminally; vulva at beginning of thick posterior portion of body.

The ova are very characteristic, brown, oval, thick-walled, with a colorless shining button-like protuberance at each pole. The eggs are 50 to 54 microns long and 23 microns wide, with an unsegmented yolk. Occasionally these eggs show variations in shades of brown, some being very much lighter than others.

(2). Trichinella spiralis (trichina spiralis).

The male worm is 1.4 to 1.6 mm. long and 0.04 mm. thick; cylindrical in shape; anterior end tapering, posterior end gradually and slightly thickening and terminating in a bifid extremity with two lateral somewhat conical tail appendages; cloacal aperture between these, which form a sort of bursa; back of cloacal aperture two pairs of papillæ.

The female worm is 3 to 4 mm. long; anterior end as in the male; posterior end nearly of same thickness to tail, which is rounded; anus terminal; vulva at anterior fifth of body; viviparous.

The larvæ when born are 90 to 100 microns in length, obtuse anteriorly, posteriorly prolonged to a pointed tail; when encysted as "muscle trichinæ" the larvæ measure about 1 mm. long and 0.04 mm. in thickness, tapering anteriorly, more thick and obtuse posteriorly with complete organization as in the adult and showing the characters of the different sexes.

This parasite or its larvæ are rarely if ever found in the stools. In its adult sexual stage it infests the intestinal tract of man and a number of mammalians, gives origin to a large number of larval worms, after which the adults die. The larvæ pass through the muscular wall of the intestine and are carried by the blood-current into various muscles of the host. Here they pass an indefinite encysted stage, a capsule forming around them and becoming calcified. Herrick and Janeway[1] have recently reported the finding of the embryo of this parasite

[1] Arch. of Int. Med. vol. 3, 1909, p. 263.

in the circulating blood of a patient. The blood was laked with 3% acetic acid, was centrifuged, and the sediment examined as outlined by Staübli. The chief means of infection of man with the trichinella spiralis is through the eating of insufficiently cooked pork, especially ham.

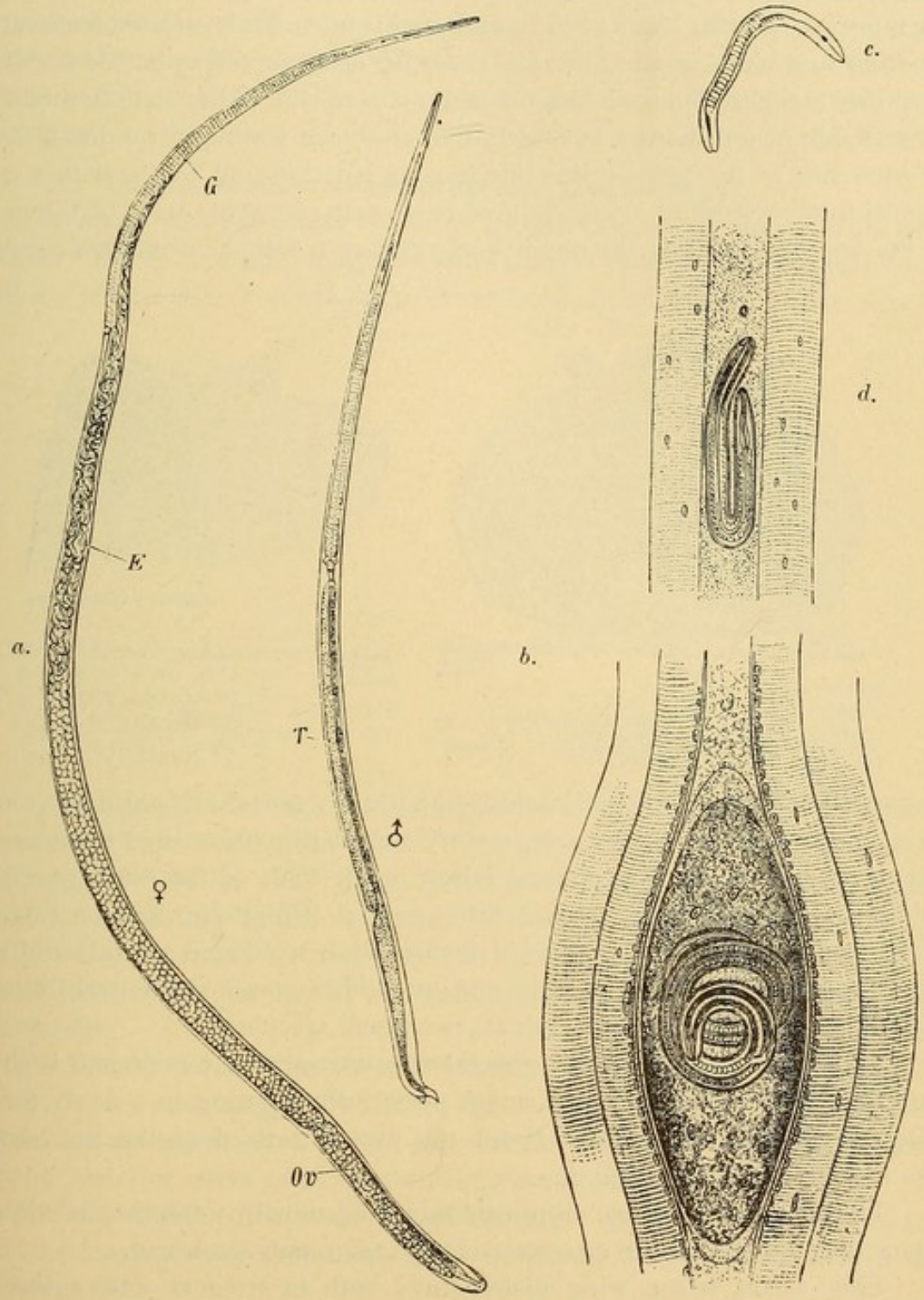


FIG. 45.—Trichinella spiralis: *a*, Gravid female "intestinal trichiura"; *E*, embryos; *G*, vulva; Ov, ovar ; *b*, adult male, "intestinal trichiura"; *T*, testicles; *C*, young larva; *d*, larva in musculature; *e*, encapsulated larva in muscle. (*Tyson after Braun*)

(*d*). Strongylidæ.

(1). Uncinaria duodenalis.

SYNONYMS.—*Anchylostoma duodenale; strongylus quadridentatus; dochmius anchylostomum; sclerostoma duodenale; strongylus duodenalis; dochmius duodenalis,* and *European hook-worm.*

The male parasite is whitish or blotched posteriorly with brownish when the intestine contains blood; 8 to 10 mm. long; cuticle finely striated transversely; tapering to a blunt point anteriorly and with head curved upon the dorsum so as to give a slightly hooked anterior end; on each side of the median line on the ventral side of oral border two hook-like chitinous teeth and on dorsal border on each side of the median line one less curved chitinous tooth; with a dorsal conical tooth extending along back of oral cavity from the base of the cavity; in the oral cavity about the esophageal opening a delicate armature consisting

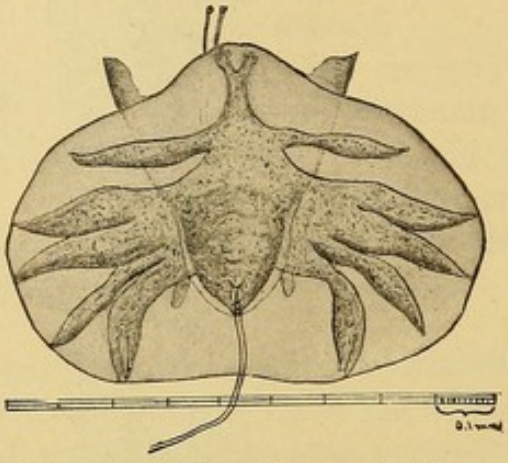


FIG. 46.—Tail, with expanded bursa, of male uncinaria duodenalis. (*Tyson.*)



FIG. 47.—Anterior end, showing mouth parts, of uncinaria duodenalis, dorsal view. (*Tyson.*)

of two dorsal and two ventral lancet-like pieces; posteriorly the body ends in an abruptly pointed tail in a copulatory bursal expansion of the cuticle, this having one dorsal and two lateral lobes; in the folds of the bursa one dorsal subdivided muscular ray, each division ending tridigitally, and on each side symmetrically placed an undivided dorsolateral, a divided lateral, undivided lateroventral, subdivided ventral and undivided small subventral muscular rays; cloacal aperture superterminal; two equal spicules.

The female worm has the general appearance of the male and is shaped anteriorly like it; 12 to 18 mm. long; posteriorly tapering to a finely pointed tail; anus subterminal; vulva about the posterior third of the body length; two uterine and ovarian tubes.

The ova are colorless, elliptical, thin-shelled with unsegmented or early segmenting material, 50 to 60 microns long and 30 microns broad.

This worm has a wide distribution both in tropical and subtropical countries. Its habitat is in the duodenum, jejunum, and upper part of the human ileum. Infection with this organism is known as *uncinariasis or anchylostomiasis.* A particularly severe type of anemia is set up by this para-

site both through the influence of loss of blood and the elaboration of toxic hemolytic material by the parasite. The eggs of this organism are frequently found in the stools and should be carefully searched for in every case of severe anemia. The mode of infection by these organisms may be direct ingestion of dirty water or unclean vegetables, but it is probable that the most frequent method of infection is through the skin, these larvæ attaching themselves to the feet of people walking in infested sand or water. The larvæ penetrate the skin and make their way through the blood- and lymph-currents to the lungs, whence they penetrate to the air-passages and are supposed to be carried upward toward the mouth by the bronchial mucus and are then swallowed.

(2). **Uncinaria americana (American Hook-worm).**

The male parasite differs from the former organisms discussed in being of smaller size (6 to 9 mm. long and more slender than the duodenalis), in the smaller size and more conical shape of the head, in having no hooklets on the

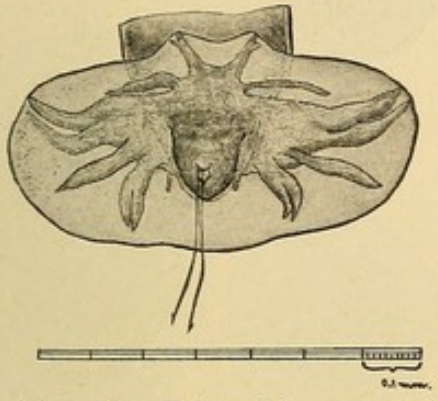


FIG. 48.—Tail, with expanded bursa, of male uncinaria americana. (*Tyson.*)



FIG. 49.—Anterior end of uncinaria americana, showing mouth parts (dorsal view). (*Tyson.*)

oral rim, but instead on each side a large ventral and smaller dorsal chitinous lip, extending from the the rim toward the median line; in a greater prominence and projection into the oral cavity of the dorsal conical tooth; in the smaller size of the copulatory bursa, its dorsal lobe being subdivided and the ventral margin being extended so as to form an indefinite ventral lobe; and showing the dorsal muscular ray of the bursa divided, each division ending in a bipartite tip.

The female worm differs from the duodenalis in being shorter and more slender (8 to 15 mm. long), with similar differences of the anterior end as above outlined for the male; vulva just in front of the middle of body length instead of at the posterior curve as in the uncinaria duodenalis.

The ova of this parasite are somewhat larger (68 to 70 microns long and 38 to 40 microns broad) than those of the uncinaria duodenalis, but are otherwise similar.

Stiles has found that these parasites are the common cause of the frequent "anemia of the South." Smith believes that uncinariasis exists in every case in which "ground-itch" has occurred within eight years and that the disease

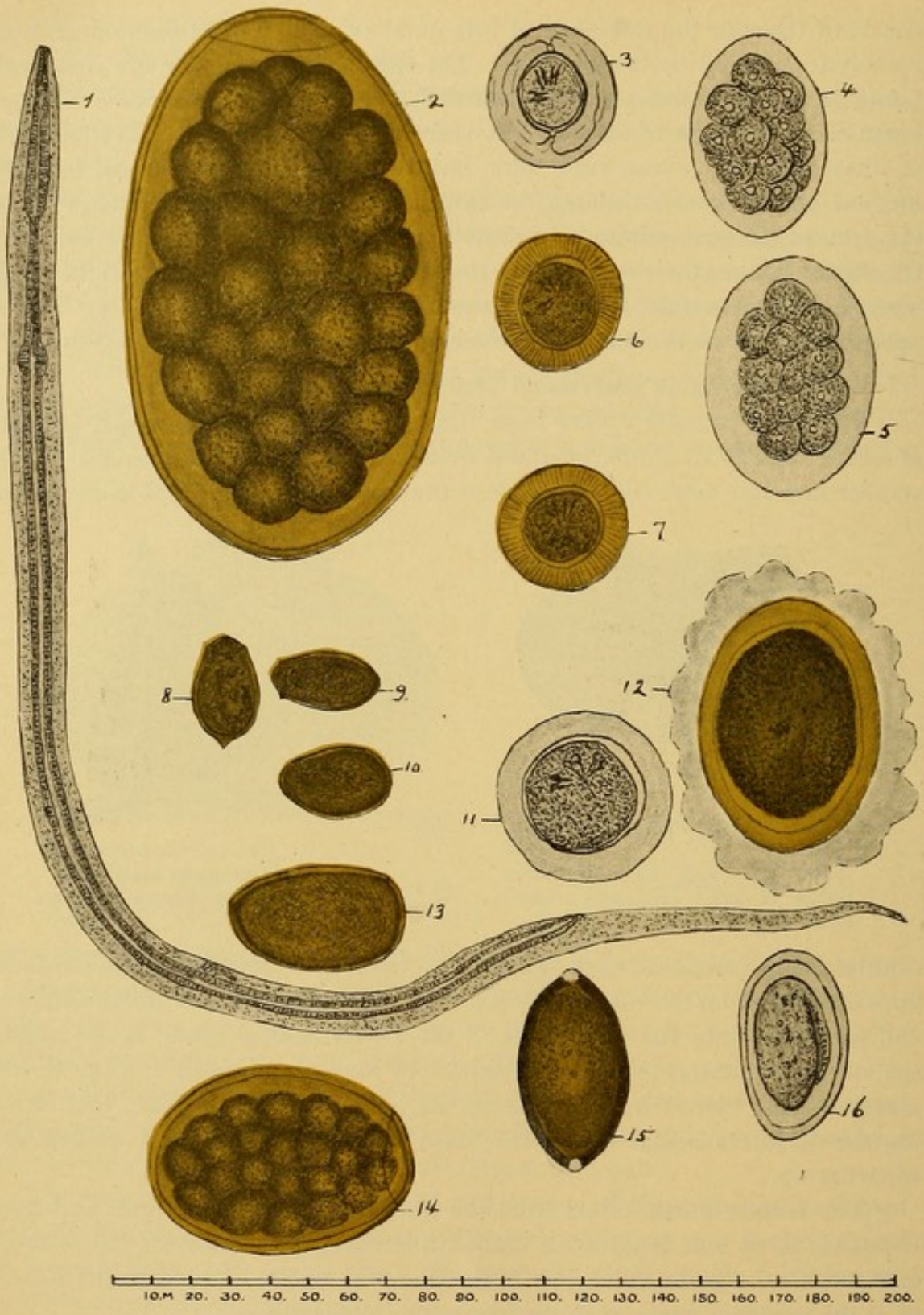
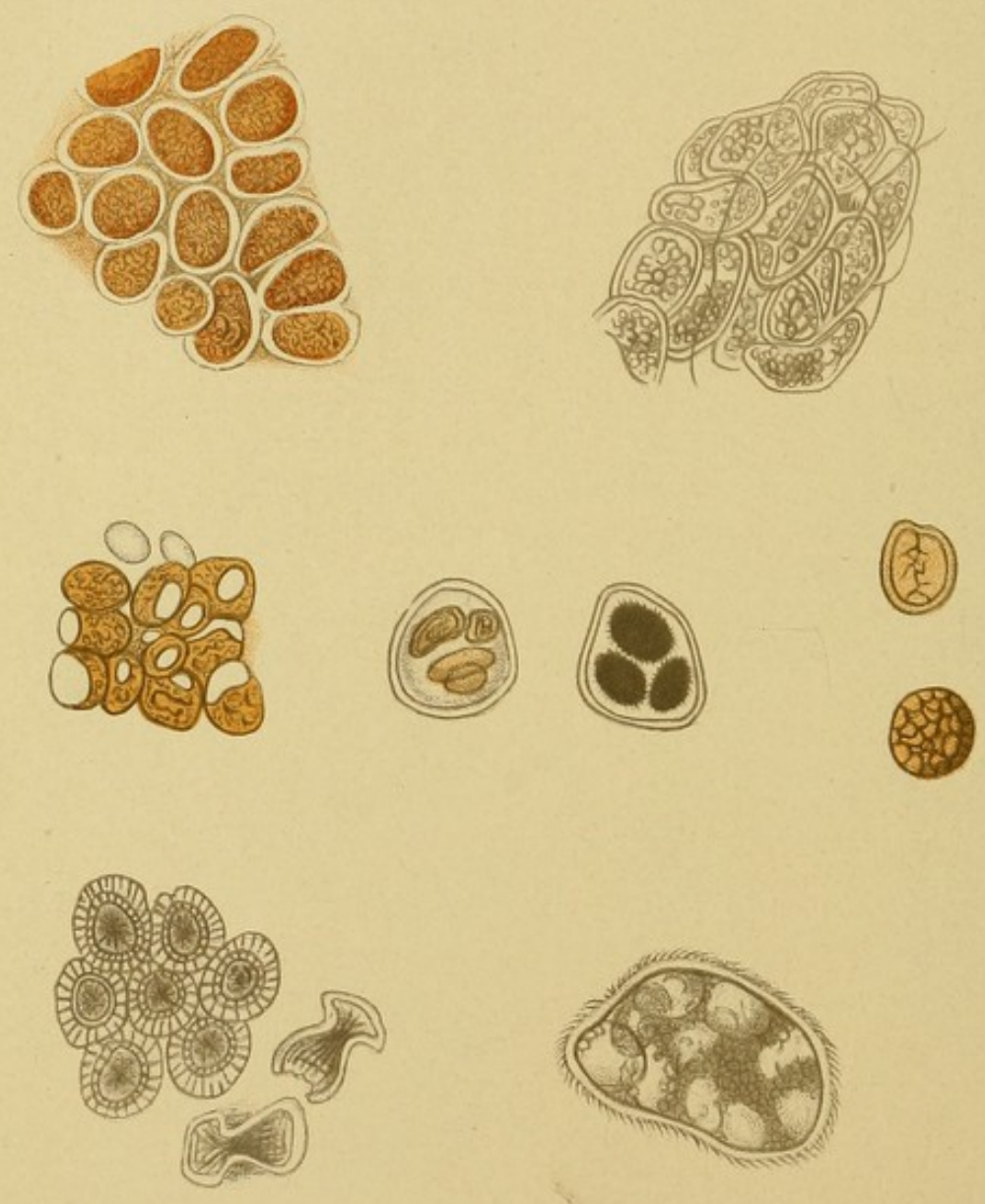


FIG. 50.—Parasitic bodies, ova, and larvæ met in human feces; color approximate only. (*Tyson.*)

1. Larval strongyloides intestinalis.
2. Ovum of fasciola hepatica.
3. Ovum of tænia nana.
4. Ovum of uncinaria duodenalis.
5. Ovum of uncinaria americana.
6. Ovum of tænia saginata.
7. Ovum of tænia solium.
8. Ovum of opisthorchis sinensis.
9. Ovum of opisthorchis felineus.
10. Ovum of cotylogonimus heterophyes
11. Ovum of tænia cucumerina.
12. Ovum of ascaris lumbricoides.
13. Ovum of dicrocœlium lanceatum.
14. Ovum of bothriocephalus latus.
15. Ovum of trichiuris trichiura.
16. Ovum of oxyuris vermicularis.



Katharine Hill.

VEGETABLE CELLS FOUND IN FECES. (AFTER SCHMIDT AND STRASBURGER.)

is rarely ever present in those who have not had this condition during that period. This would point to the general transmission of infection through the skin. The uncinaria duodenalis has long been known as the cause of the so-called Egyptian chlorosis, tunnel-workers' anemia, brick-layers' anemia, and other conditions necessitating work in low-lying watery places.

Pseudoparasites.

It not infrequently happens that extraneous substances are found in the feces which very closely simulate a parasite or its ova in appearance. These substances are, for the most part, food residues and should be carefully differentiated by applying tests for cellulose, which will show in all vegetable cells.

Stiebel has described, under the name of *diacanthos polycephalus*, a fragment of the woody portion of a bunch of raisins. Sultzer describes a mulberry seed as a vesicular worm under the name of *ditrachyceros rudis*. Bastiani considers the larynx of a bird which he found in the fecal material as being a biped worm, under the name of *sagitula hominis*. Scopoli regards a fragment of the trachea of a bird as an entozoon form under the name of *physis intestinalis*. A pupil of Moquin-Tandon describes a strip of lettuce under the name of *striatula*, regarding it as a worm intermediate between the ascaris and the oxyuris (Guiart and Grimbert).

Perhaps the most frequent pseudoparasite of the feces is the pulp of an orange, it shows in the feces in the form of large oblong masses terminated by two slender extremities, one of which ends in a sort of parenchyma. These vesicular masses are the large cells which secrete the orange juice and which are generally found intact in the fecal material. These have been frequently mistaken for hydatids or for parasitic ova. Certain spores, such as those of the truffle or of lycopodium, have not infrequently been mistaken for parasitic eggs. Moreover, one may find in the fecal material the pollen of the coniferous plants which very closely simulates parasitic ova. The spines which form the down on certain fruits, such as raspberries, strawberries, peaches, and quinces, so closely resemble parasites that careful study is in some cases essential.

It is, therefore, wise in all cases before pronouncing a finding as one of a parasite to be perfectly sure of your ground.

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CHAPTER V.

PARASITES.

I. General Considerations.

In the previous portions of the work, the writer has introduced those parasites which are more particularly related to the parts under discussion. There are, however, a large number of organisms which do not fall naturally within the scope of any of the chapters outlined for this book. These will, therefore, be discussed in general without much regard to distinct classifications, as many of the subdivisions have been treated previously. This discussion is taken, for the most part, from Tyson.

II. Trematodes (Fluke-worms).

Flukes are naked and unsegmented flat worms, usually of the shape of a leaf or of the tongue (occasionally pyramidal or elongated and more or less cylindrical), provided with incomplete digestive canal (without anus), possessing one or more suckers and occasionally hooklets; with but few exceptions hermaphroditic and, as a rule, presenting a complicated series of metamorphoses in their development.

In structure it is customary to speak of the surface upon which the genital pore opens as the ventral; this surface commonly shows also the orifices of the mouth and one or more suckers. On the dorsal surface in many occurs the opening of a small canal, spoken of as Laurer's canal, of unknown function. The surface of the body is covered by a fairly thick and firm cuticle, often provided over variable areas with small spines or tubercles. Beneath the cuticle over its internal surface is spread the superficial muscular layer (not showing the structure of muscle of higher animals, however), with longitudinal, circular, and diagonal fibers, while within this along the borders is met the parenchymatous muscle. The general internal tissue of the body, spoken of as the parenchyma, is a fine reticular connective tissue which closely surrounds the various organs.

The suckers of trematodes vary in number and arrangement on the anterior and posterior extremities, the ventral surface and its borders, and in a few cases also on the dorsal surface. Usually the oral opening is surrounded by such a sucker and in addition, in the forms likely to be met with in man, on the ventral surface some distance posterior to the oral sucker is a second, known as the ventral sucker or acetabulum, in the median line. Not infrequently in the lining of these suckers, on their lips, or on the cuticle close to the lips, chitinous hooklets are to be found.

The alimentary system consists of a mouth, opening in the oral sucker and situated terminally or on the ventral surface of the anterior end of the worm. This cavity continues into a dilated tube with thick walls, the pharynx, this extending posteriorly by a short, straight, and usually narrow esophagus, which divides in the anterior portion of the body into the two intestinal tubes or ceca. At the posterior extremity of the body is a small orifice, the excretory pore. which serves as outlet for a series of more or less complex canals for the convection of the fluid waste, the arrangement representing a low nephridial apparatus, while the mouth serves as an anus. The reproductive system is highly developed, showing numerous minor variations in the different genera and species.

Adult flukes are parasitic upon a wide range of animal life, including the higher animals, fish, amphibia, reptiles, and birds, living as ectoparasites and endoparasites. The varieties affecting man are comparatively few. The most common parts of the human body to be infested are the intestines, gall-ducts, respiratory tubes, and blood-vessels.

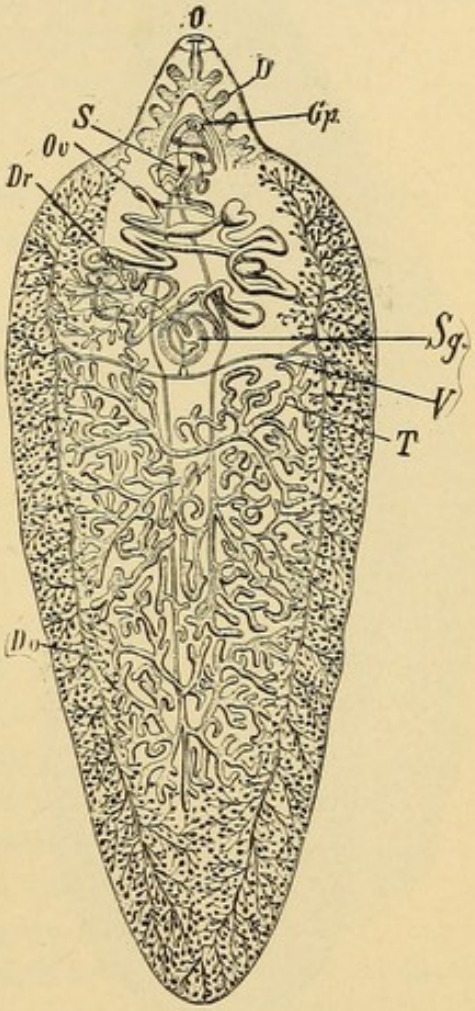

FIG. 51.—Showing the sexual glands of fasciola hepatica; 5×1. *O*, oral sucker; *D*, intestinal ceca; *Do*, vitelline glands; *Dr*. ovary; *Ov*. uterine canal; *T*, testicles; *Sq*, "shell gland"; *V*, transverse vitelline duct; *Gp*, genital pore; *S*, ventral sucker. (*Tyson after Braun.*)

(*a*). Fasciolidæ.

(1). Fasciola hepatica.

SYNONYMS.—*Distomum hepaticum; distomum caviæ; fasciola humana, cladocœlium hepaticum; common liver-fluke.*

A comparatively large fluke, measuring 20 to 50 mm. long and 8 to 13 mm. wide, of leaf-shape, with anterior extremity prolonged into a small cone; greatest width of body about the anterior third of length; light brown color; cuticle provided with alternating transverse rows of spines, extending on ventral surface to the posterior level of testes, but not as far posteriorly on the dorsal surface; the oral sucker at the anterior end of cephalic cone, inclining to ventral surface, 1 mm. in diameter; ventral sucker near anterior end behind cephalic cone, 1.6 mm. in diameter; well developed larynx and short esophagus; intestinal branches extending nearly to the posterior extremity of the worm, approaching the median line posteriorly with few median and numerous lateral branches; excretory pore at posterior extremity, with well developed

system of excretory tubes; genital pore in the median line anterior to the ventral sucker; two large highly branched testes, mostly posterior to the transverse vitelline duct; ovary single, branched, lying in front of testes and to one side of the median line; the uterus coiled into a rosette, showing as a brown spot just back of the ventral sucker on the ventral surface; vitelline glands numerous, ranging along each lateral border from the level of the ventral sucker to the posterior extremity of the worm; vitelline ducts running transversely at about the end of the anterior third of the body. The ova are yellowish-brown, oval, operculated, and measure 130 to 145 microns in length and 70 to 90 microns in width.

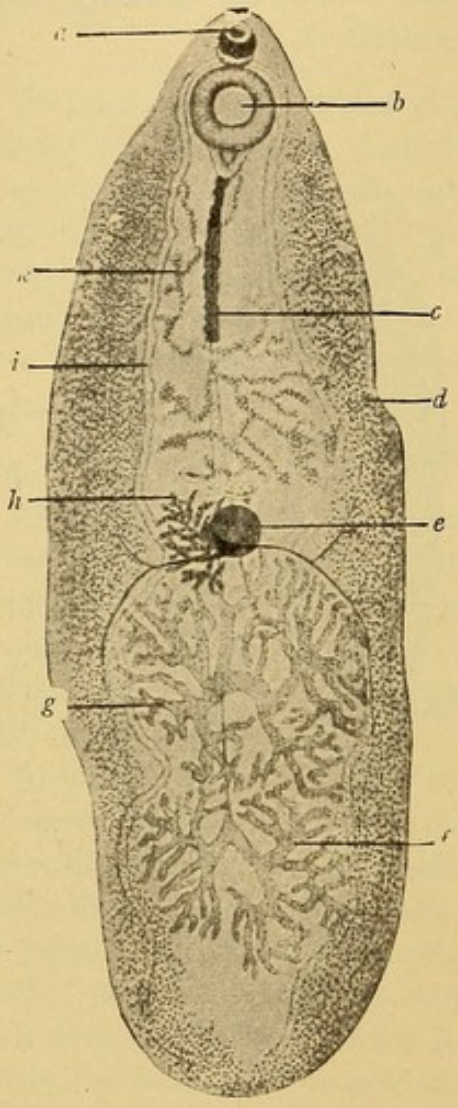

FIG. 52.—Fasciolopsis buski: *a*, oral sucker; *b*, acetabulum: *c*, cirrus pouch; *d*, vitelline glands; *e*, "shell gland"; *f* and *g*, posterior and anterior testicles; *h*, ovary; *i*, cecum; *k*, uterus. (*Tyson after Braun.*)

This fluke is a common one in most mammalians and has a wide geographical distribution over the world, being found not infrequently in America. Its usual habitat is the gall-ducts, but it has been seen in the gall-bladder, intestines, in the portal and other venous channels and in subcutaneous cysts. The ova appear in the feces and are the chief means of diagnosis of this condition. The embryo which develops from the ovum completes its developmental cycle in the body of a snail, the Limnæa truncatula. From the body of the snail the cercariæ escape in the water and become attached to grass or other aquatic material, which is taken in by the animal. Infection in man is quite rare, about 32 cases having been reported in the literature.

(2). Fasciolopsis buski.

SYNONYMS.—*Distomum buski; distomum crassum.*

The length of this organism is variable, ranging between 24 and 70 mm.; breadth 5.5 to 14 mm.; lance-shaped; narrowing more rapidly anteriorly than posteriorly, maximal width about the middle of the length; no cephalic cones; brownish in color; cuticle without spines; oral sucker small and placed on ventral surface of anterior extremity; ventral sucker two or three times as large as the oral, placed near anterior end and showing a saccular distention extending posteriorly; very short esophagus, back of the fairly developed pharynx; the two ceca without branches; genital pore at the anterior quarter of the

acetabulum; serous pouch large; testicles branched; in posterior part of body one back of the other; uterus in anterior half of body, tortuously coiled; ovary at middle of length of body, to the right of median line; Laurer's canal present; vitelline follicles numerous along lateral margin from the level of the ventral sucker to the posterior extremity; the transverse vitelline ducts at the equator of body. The ova are brownish, ovoid, operculated, and measure 125 microns in length and 75 in width.

Very little is known of the intermediate stage and of the host of Busk's intestinal fluke. This has been found in the small intestine of man and probably arises from eating infected food.

(3). Opisthorchis Felineus.

SYNONYMS.—*Distomum couns; distomum lanceolatum* (Siebold)*; distomum sibiricum; distomum tenuicolle; the European cat-fluke.*

Variable in size according to the state of contraction, 8 to 11 mm. long and 1.5 to 2 mm. broad; yellowish-red and nearly transparent; flat and lanceolate; anterior end constricted and attenuated into a cone; posterior end more obtuse; cuticle without spines; oral sucker toward the ventral surface at anterior extremity; ventral sucker at base of cone about one-fourth of body length; pharynx and esophagus of equal length; ceca comparatively straight and unbranched, reaching nearly to the posterior extremity and often seen filled with blood; excretory pore terminal; its tubular vesicle winding in the median line between the testes and branching in front of the anterior testes; testes in the posterior part of body, the anterior four-lobed, the other five-lobed; cirrus and pouch absent; genital pore in the median line in front of the ventral sucker; slightly lobate ovary in the median line anterior to the testes; receptaculum seminis prominent; uterus anterior to the ovary and testes coiled in the middle third of the body; vitelline follicles occupy about the middle third of the body, beginning anteriorly at the level of the ventral sucker. The ova are oval in shape, operculated, and measure 30 microns in length and 11 in width.

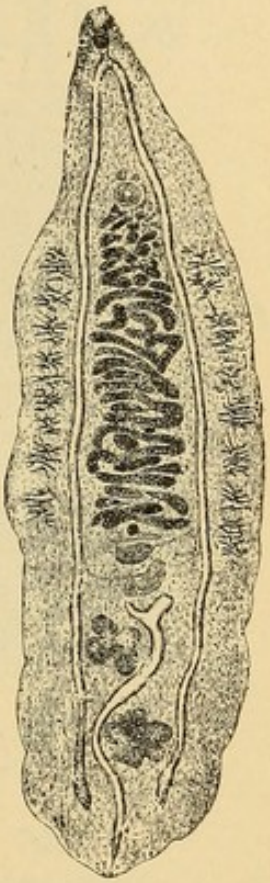

FIG. 53.—Opisthorchis felineus; from liver of cat. 10 × 1. (*Tyson after Braun.*)

This worm has been found in the gall-ducts of man and other animals, especially in Russia, Siberia, Hungary, and Japan.

(4). Opisthorchis Sinensis.

SYNONYMS.—*Distomum sinense; distomum spathulatum; distomum hepatis endemicum seu perniciosum; distomum hepatis innocuum; distomum japonicum; the Japanese or Chinese liver-fluke.*

This parasite measures 10 to 20 mm. in length and 2 to 5 mm. in breadth;

long and lanceolate; reddish and nearly transparent when fresh; cuticle without spines; oral larger than ventral sucker, on the ventral face of anterior extremity; ventral sucker about one-fourth of body length posterior to former; pharynx and esophagus small and a bifurcation of the latter close to oral sucker; ceca unbranched, reaching close to the posterior extremity; excretory system as in the previous species; in the posterior fourth of body the two testes, one in front of the other, with from four to six dendritic branches; no cirrus or pouch; genital pore in the median line just in front of the acetabulum; ovary trilobed and placed just anterior to a large gourd-shaped receptaculum seminis both in the median line and anterior to testes; uterus well-developed and coiled in the middle area of the body between the ovary and ventral sucker; vitelline follicles in the marginal fields along the middle third of the body. The ova are brown, oval, operculated, and measure 27 to 30 microns in length and 15 to 17 in width.

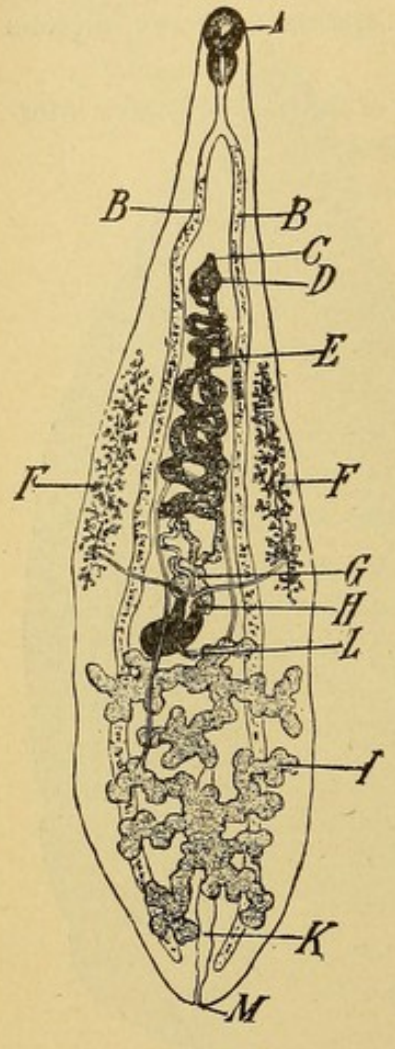

FIG. 54.—Opisthorchis sinensis: ventral surface, stretched; *a*, oral sucker; *b*, ceca; *c*, genital pore; *d*, acetabulum; *e*, uterus; *f*, vitelline glands; *g*, ovary; *h*, receptaculum seminis; *l*, Laurer's canal; *i*, testicles; *k*, excretory canal; *m*, excretory pore. (*Tyson after Braun.*)

This fluke is comparatively common in Japan and eastern Asia and has been reported in America. It infests the gall-ducts and has been found both in the pancreatic duct and intestine. Little is known of the intermediate hosts.

Many other types of these distomata are met with in the liver of various animals and are occasionally found in man. Among these we find the distomum lanceolatum, distomum heterophyes, distomum conjunctum, and amphistomum hominis (gastrodiscus hominis.)

III. Nematodes (Round Worms).

Eustrongylus Gigas.

SYNONYMS.—*Ascaris canis et martis; ascaris visceralis et renalis; strongylus gigas; strongylus renalis; eustrongylus visceralis.*

The male worm is red in color; 14 to 40 cm. in length and 4 to 6 mm. in thickness; slightly tapering anteriorly; mouth terminal; with a hexagonal orifice surrounded by six lips bearing papillæ; cuticle thin and transparent, finely striated transversely; about 150 papillæ along the longitudinal lines laterally; caudal extremity with an oval plate-like expansion serving as a bursa, its margin bearing small papillæ and slightly indented dorsally and ventrally; single sexual spicule.

The female parasite shows the general appearance and head end as in the male; 20 to 100 cm. in length and 5 to 12 mm. in thickness; caudal extremity obtuse, straight, with anus subterminal; vulva 50 to 70 mm. posterior to mouth; single ovarian and uterine tube plicated from near the anterior end along the intestine nearly to the anus, then returning to the vulva near the anterior end.

The ova are brown, ellipsoid, with thick shell marked by external cribriform depressions, 64 to 68 microns in length and 40 to 44 microns in breadth.

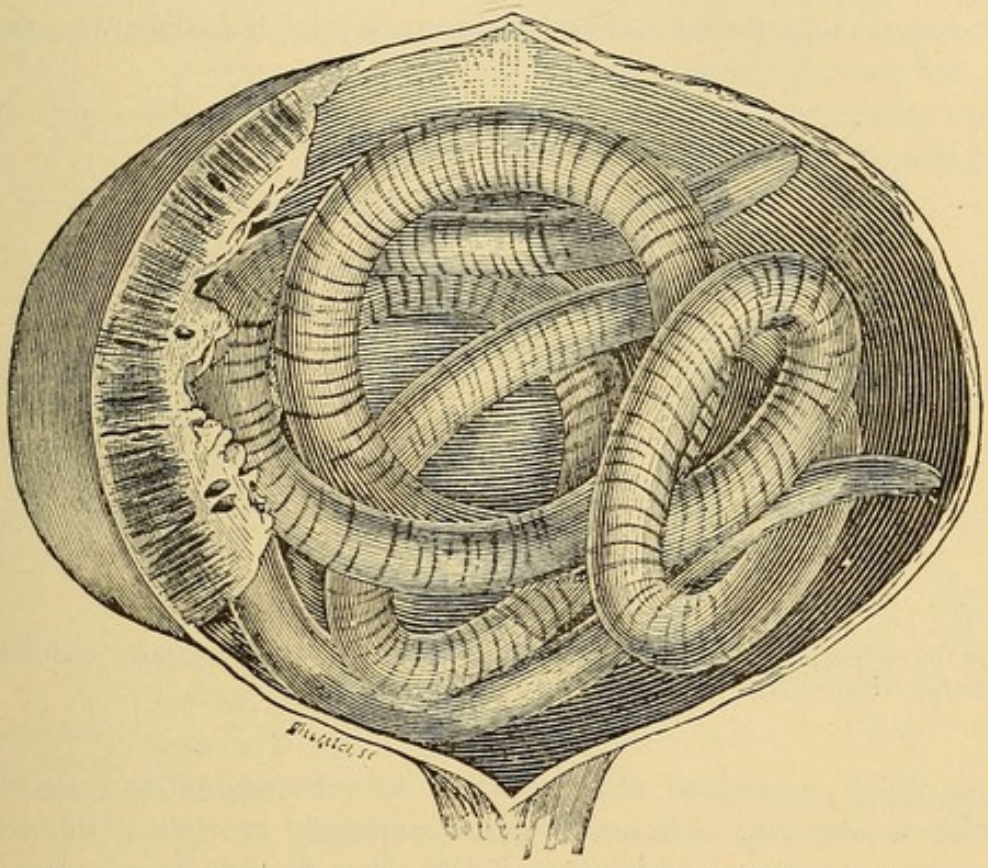

FIG. 55 —Eustrongylus gigas: female, natural size, in kidney of dog. (*Tyson after Railliet.*)

This worm, which is more common in the dog, has been reported a number of times in man. It is the largest of the nematode worms and has its habitat in the pelvis of the kidney. Little is known of its life history.

IV. PARASITES OF THE SKIN.

Arthropoda.

These are bilaterally symmetrical segmented animals whose segments do not correspond, but vary in structure, and which primitively bear upon each segment a pair of jointed appendages. The segments are often more or less fused, thus forming special body-regions which may themselves be more or less fused together as well. The covering of these animals is a comparatively

thick and strong cuticle which remains pliable between the segments of the body and of the jointed appendages, but which commonly becomes hard and shell-like from chitinous or calcareous material directly over the different body-segments and internodes of the jointed appendages. This arrangement requires that in the growth of the individual the firm external covering should from time to time be shed, such changes taking place periodically and being known as *moults*. While each segment in the primitive animal is provided with a pair of jointed appendages, these in the individual species are often lost from this or that part of the body, or may remain rudimentary and inconspicuous or may take on special features of structure from the assumption of special

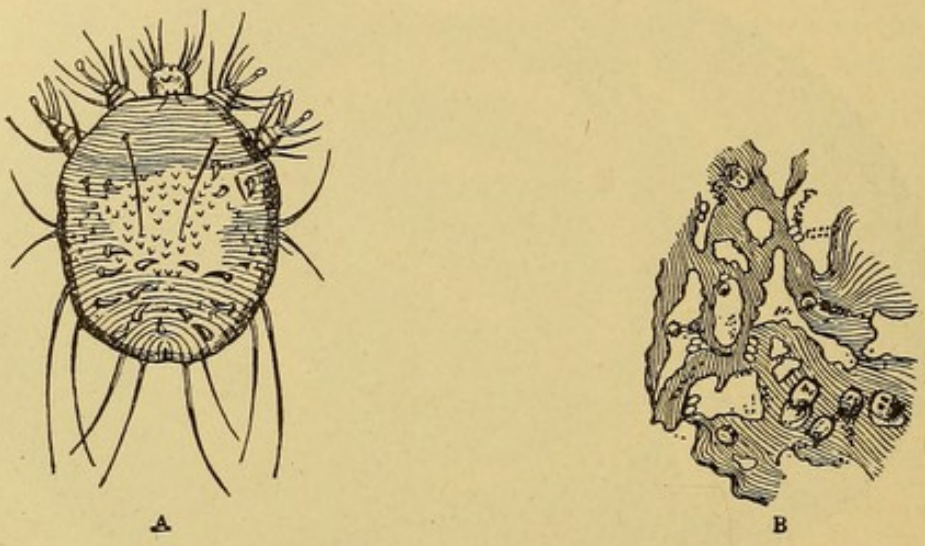

FIG. 56.—Acarus scabiei: *A*, female, dorsal view; *B*, portion of human epidermis, showing burrows with contained ova and young acarians. (*Gould*)

function which causes their wide departure from the original and common type used for locomotion. The arthropods commonly reproduce by ovulation, the development of the embryo to the adult often showing more or less complicated metamorphoses. The true parasitic forms of the arthropoda thus far met in man are limited to the Arachnoids and Insects.

(*A*). Arachnoidea.

(*a*). Sarcoptes or acarus scabiei (the Itch Parasite).

This parasite is oval in shape, is provided with horns and bristles, is barely visible to the naked eye, the male being from 0.2 to 0.3 mm. in length by 0.145 to 0.19 mm. in breadth; the female is somewhat larger, showing a length of 0.33 to 0.45 mm. and a breadth of 0.25 to 0.35 mm.

The female lies at the end of a burrow in the epidermis, in situations where the skin is most delicate, as between the fingers, at the elbows, under the knees, and in the groin. In this burrow which varies from a few millimeters to a centimeter in length the female deposits her eggs, after which she dies. The eggs hatch in from four to eight days, and in about 14 days the larvæ are sufficiently matured to make their own burrows.

The disease is communicated either by the clothing or by personal contcat. To demonstrate the parasite, the burrow is opened with a needle and the female pressed out on a slide, which is then covered and examined.

(*b*). **Demodex folliculorum.**

This parasite is very small, varying in length from 0.3 to 0.4 mm. It is somewhat cylindrical, tapering to an obtuse point at the posterior end. This parasite has its habitat in the sebaceous follicles, especially of the face and nose.

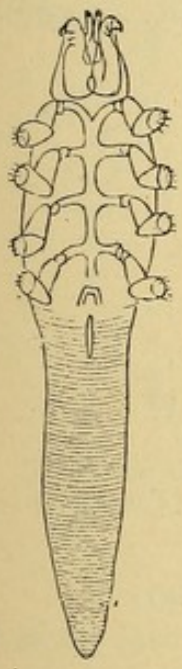

FIG 57. Demodex folliculorum: from dog, enlarged. (*Tyson after Braun.*)

(*c*). **Leptus autumnalis (Harvest-bug).**

This is a minute red parasite, from 0.3 to 0.5 mm. long, which has three pairs of legs, with rows of bristles upon its back and belly. It prevails in summer on grass and plants and attaches itself to the skin of man by its hooklets.

(*B*). **Insecta.**

(*a*). **Hemiptera.**

(1). **Pediculus capitis (Head-louse).**

The male is from 1 to 1.5 mm. long, the female 1.8 to 2 mm. long. The color of the parasite varies somewhat with the race of its host. In the Caucasian it is gray with a dark border, in the Negro and Chinamen it is much darker in color. The eggs are 0.6 mm. in length and are attached to the hairs, forming the so-called "nits." These nits are whitish oval masses which are easily visible.

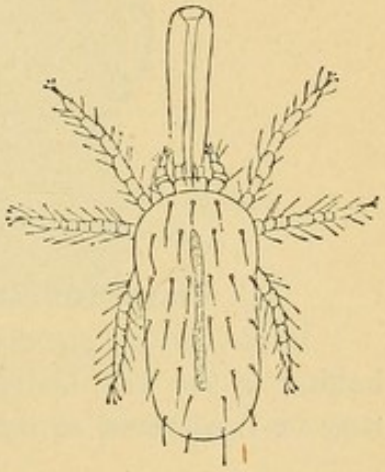

FIG. 58.—Leptus autumnalis: enlarged. (*Tyson after Braun.*)

This parasite, while usually found upon the hair of the head, may be found in other portions of the body. The symptoms may be severe or very slight.

(2). **Pediculus vestimenti (Body-louse).**

This parasite is considerably larger than the former, being from 2 to 5 mm. long and whitish-gray in color, the back part of the body being wider than the thorax. The antennæ are longer than are those of the head-louse. The eggs are from 0.7 to 0.9 mm. in length, about 70 being laid by each female.

This parasite is found upon the clothing in which it deposits its eggs, especially about the neck, back and abdomen.

(3). **Pediculus pubis (phthirius inguinalis or Crab-louse).**

This parasite is smaller than the head louse, grayish-yellow or grayish-white in color, the male being from 0.8 to 1 mm. in length, the female about

1.12 mm. in length. The eggs are pear-shaped, from 0.8 to 0.9 mm. in length and from 0.4 to 0.5 mm. in breadth.

This parasite infests the parts of the body covered by the shorter hairs, such as the pubis, axilla, eye-brows, and chest.

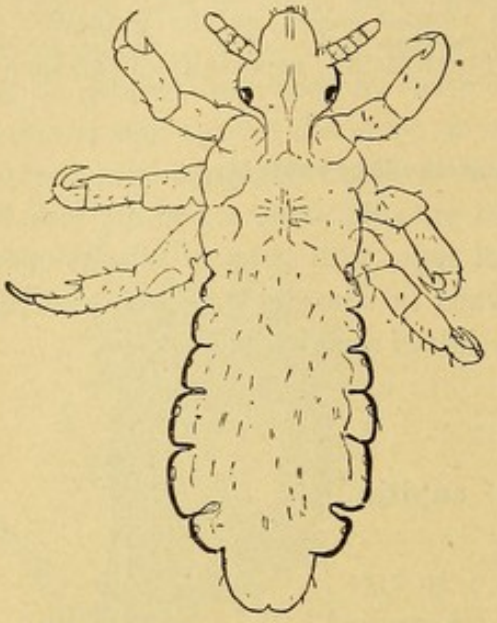

FIG. 59.—Pediculus capitis: × 15. (*Tyson after Braun.*)

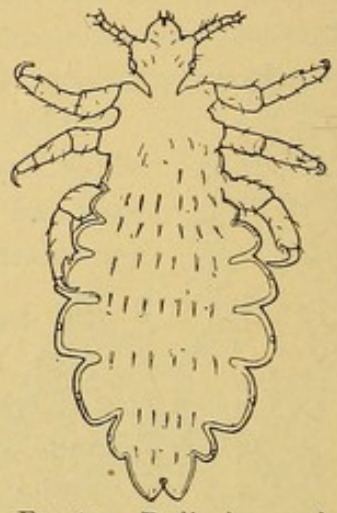

FIG. 60.—Pediculus vestimenti: × 10. (*Tyson after Braun.*)

(4). **Cimex lectularius (acanthia lectularia or Bed-bug).**

While, strictly speaking, the bed-bug is not a parasite of man, yet as its habitat is the bed, bedding, and walls of the sleeping-apartment of man, it may be considered as indirectly parasitic. It usually emerges at night from

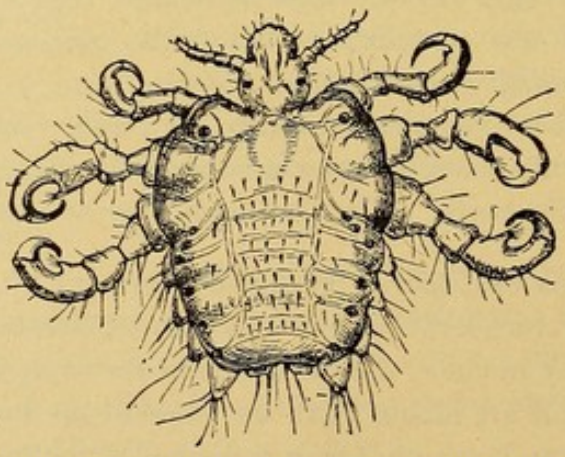

FIG. 61.—Pediculus pubis. (*Tyson after Braun.*)

its lodging for the purpose of securing its nourishment in the blood of its victims.

This parasite is reddish-brown in color, oval in shape, from 4 to 5 mm. in length and 3 mm. in breadth. These insects, if crushed between slides or as more usual between the hand and a part of the victim's body have a

characteristic odor very much resembling kerosene. The blood is drawn from the victim by means of a long proboscis. The eggs are approximately 1.12 mm. in length and require about 11 months for their development to the sexually ripe insect. These eggs are retained in the crevices of the bed, floors, furniture, wall-paper, and other parts of the dwelling so that the complete removal of these eggs and parasites is a matter of some difficulty.

That these insects have more or less importance from the standpoint of transmission of disease from one person to another must be remembered. Individuals vary in their susceptibility to the bite of the bed-bud, some being indifferent to it while others are markedly affected by it.

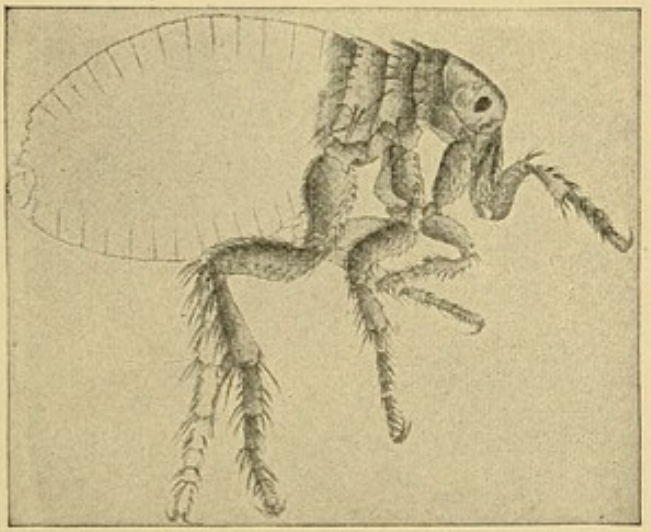

FIG. 62.—Pulex irritans: × 14. (*Tyson after Braun.*)

(*b*). **Diptera.**

(1). **Pulex irritans (Common Flea).**

The male is from 2 to 2.5 mm. in length, the female as much as 4 mm. It is a red or brownish-red insect, having a laterally compressed body, an oral haustellum, serrated soft mandibles, a tongue sheathed in an inferior labium, and a pair of labial four-jointed palpi. Each of the triple segments of the thorax bears a pair of five-jointed double-clawed legs. The female deposits her eggs, not on the human being, fortunately, but in the fissures, crevices, or holes of garments or furniture which may be accessible.

(2). **Pulex penetrans (Sand-flea or Jigger).**

This parasite is a minute, brownish-red, egg-shaped insect which penetrates the skin of man. The female is the infecting insect and produces painful irritation and even suppuration.

Vegetable Parasites.

(1). **Achorion Schönleinii.**

This organism is the cause of the disease known as *favus* or *tinea favosa*. This fungus invades the root sheaths, the bulbs, and the shafts of the hair

filaments of the scalp, but it also occurs upon the "non-hairy" portions of the skin and upon the nails. The spores gain access to the deeper layers of the skin and develop around the hair-shaft, forming a characteristic yellowish cup-shaped crust which has a peculiar mouse-like odor.

In searching for this parasite, a favus crust is softened by the addition of a few drops of water or dilute sodium hydrate solution and placed upon a slide and examined with the high-power dry lens. The hairs may also be examined in the same manner or may be stained by methods outlined in the discussion on Tinea tricophytina.



FIG. 63.—Pulex penetrans: young female, enlarged. (*Tyson after Braun.*)

The mycelial threads appear as narrow, flattened, ramifying, short or elongated, linear cells or tubes, which may be simple and empty, or be divided more or less regularly by transverse partition walls transforming the longer and simple into shorter and compound cells. The latter often contain in their cavities sporules clinging to either side, in which case the mycelial threads are termed sporophores. The conidia are encapsulated or are strung together like the beads of a necklace, and appear as round, oval, angular, or very irregularly contoured bodies. These mycelial threads branch at right angles, the spores measure from 3 to 10 microns in diameter (Hyde).

(2). **Trichophyton megalosporon endothrix.**

This organism is the cause of *tinea circinata* (herpes tonsurans, ring-worm of the body), and of *tinea sycosis* (hyphogenous sycosis, tinea barbæ, ring-worm of the beard, barber's itch).

The trichophyton is composed of spores which vary greatly in size, but which, as a rule, are somewhat larger than those of the type next to be discussed. They are frequently cuboidal, oval, or irregularly rounded, but their chief characteristic lies in their arrangement in lines or chains, extending up and

down the hair shaft. The mycelium is found without, but never within the hairs (Hyde).

These fungi may be stained by the method of Morris and Calhoun. The hair is first washed in ether to remove all fatty débris; it is then put for one or two minutes in Gram's iodin solution and is stained after drying for from one to five minutes in gentian-violet. It is again dried and treated for a minute or two with the iodin solution and for an equal length of time in aniline oil containing pure iodin, after which it is cleared with aniline oil, washed in xylol, and mounted in Canada balsam.

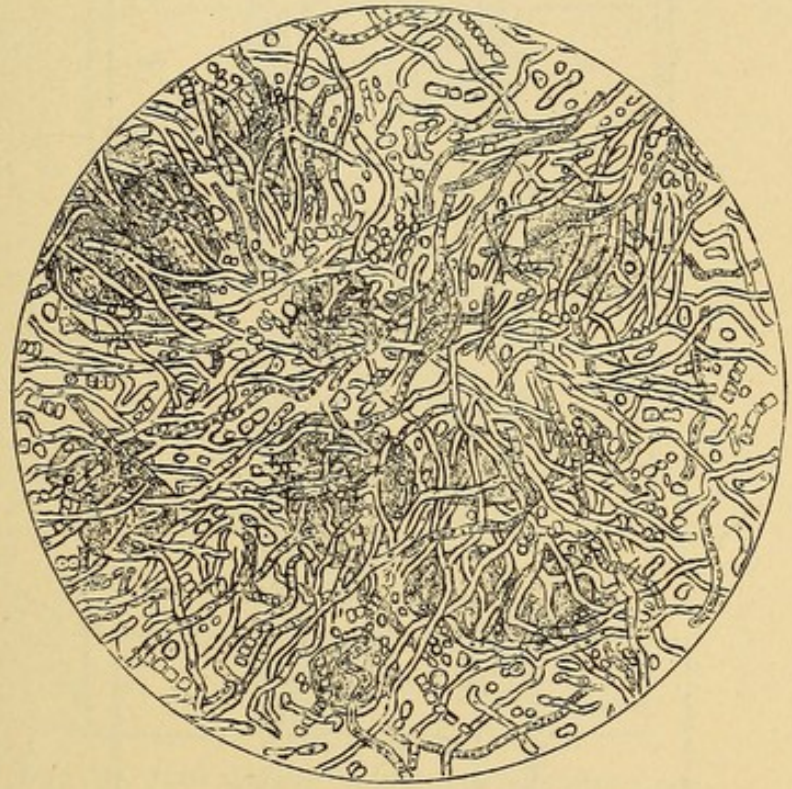

FIG. 64.—Achorion schönleinii, × 500 diameters. (*Van Harlingen.*)

(3). **Microsporon audouini (Trichophyton Microsporon).**

This parasite appears under the microscope chiefly in the form of a large number of round spores, irregularly grouped or massed about the follicular portions of the hair. Mycelial threads, large and branching, are often seen within the hair. The sheath of spores surrounding the hair is often continued upward for 1/16 to 1/8 of an inch above its exit from the follicle and may be recognized as a whitish or grayish coating of the hair. These mycelial threads are all within the hair proper, thus differing from those of the trichophyton which are never within the hair; after repeatedly dividing and subdividing they terminate on the outer surface of the shaft in fine filaments, at the extremities of which are the spores. This parasite is the cause of the disease *tinea tonsurans*, or ring-worm of the scalp.

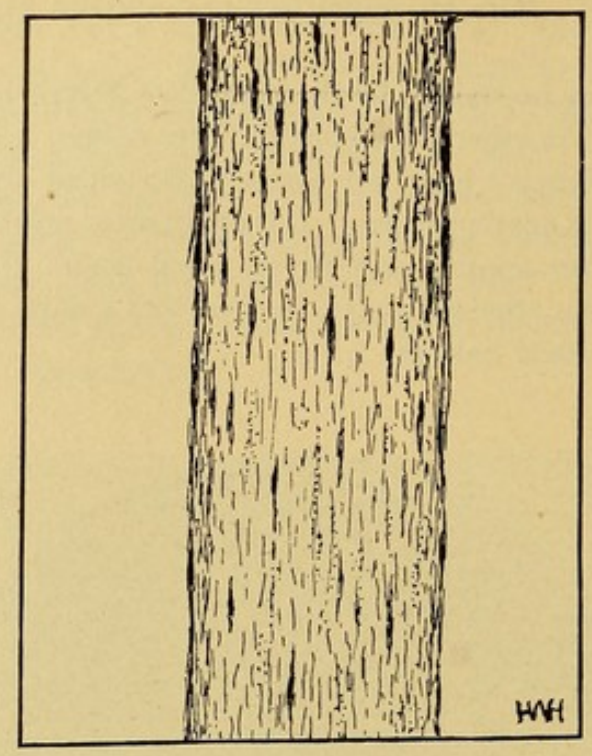

FIG. 65.—Normal hair × 900.

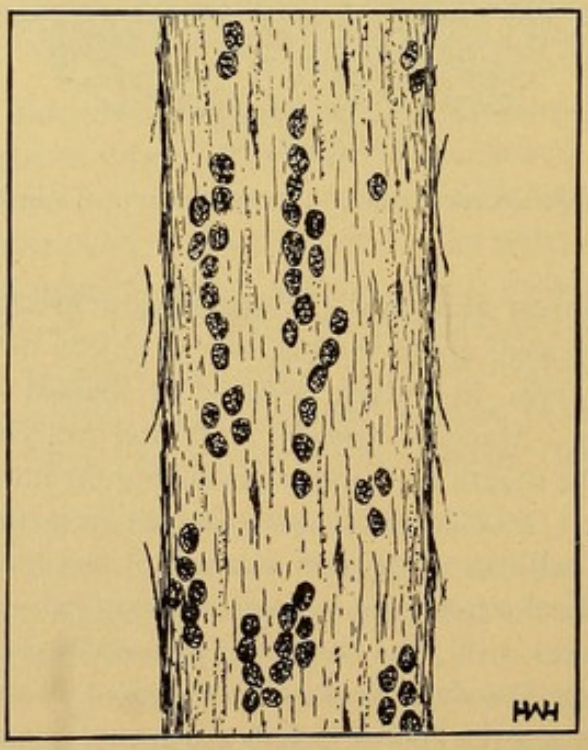

FIG. 66.—Hair showing trichophyton endo-ectothryx × 900.

(4). **Microsporon furfur.**

This parasite is readily recognized by the microscopic examination of the scales scraped from the skin. Innumerable clustered spores, highly refractive and resembling in their circular and oval contours droplets of oil, are quite characteristic. The mycelial threads are not usually branched, but lie in a close network, among which sporophores are distinguishable, with conidia and terminal elements emerging at one extremity of the spore case. Both elements of this organism are more readily stained by the aniline dyes than are those of the trichophyton or favus. This organism is the cause of the condition known as *tænia versicolor*.

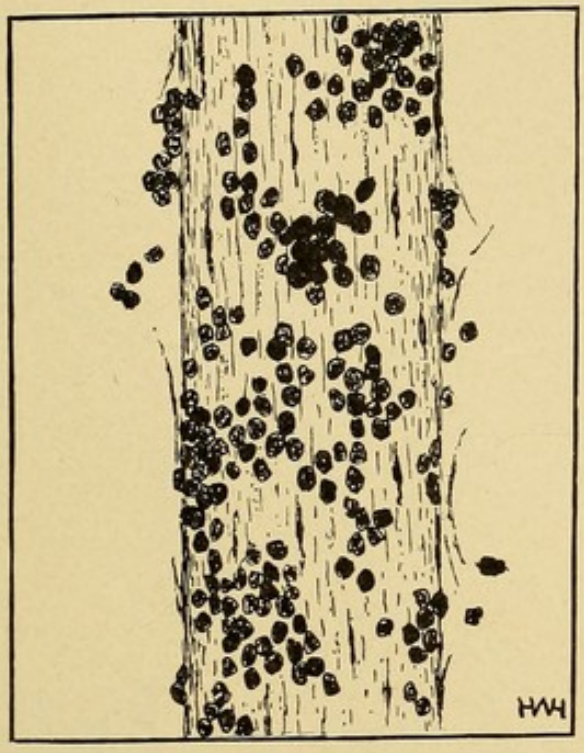

FIG. 67 —Hair showing microsporon audouini × 900.

(5). **Microsporon minutissimum.**

This organism is the etiologic factor of *erythrasma*. It is characterized by the extreme delicacy and fineness of its threads and very minute spores. The threads are either simple, cylindrical bodies of variable size or they may exhibit partition septa, may divide dichotomously, and may terminate in hooked or knobbed expansions. The largest transverse diameter is 0.6 microns, in length the mycelium presents the greatest variations.

(6). **Blastomycetes.**

These organisms may be found in the cutaneous eruptions of the skin in *blastomycosis* and may be described as follows, according to Montgomery and Ormsby. In unstained preparation the organisms appear as round or oval bodies with a double-contoured highly refractive capsule. Within the capsule, in many instances, granules or spore-like bodies can be distinguished. The

addition of a 1 to 10 per cent. solution of potassium hydrate to the specimen under examination facilitates the recognition of these bodies. In stained sections the double contoured, homogeneous capsule is usually separated from a finely or coarsely granular protoplasm by a clear space of varying width. Vacuoles of different sizes are found in some organisms. In both pus and tissue, organisms in pairs or in various stages of budding are commonly seen. The

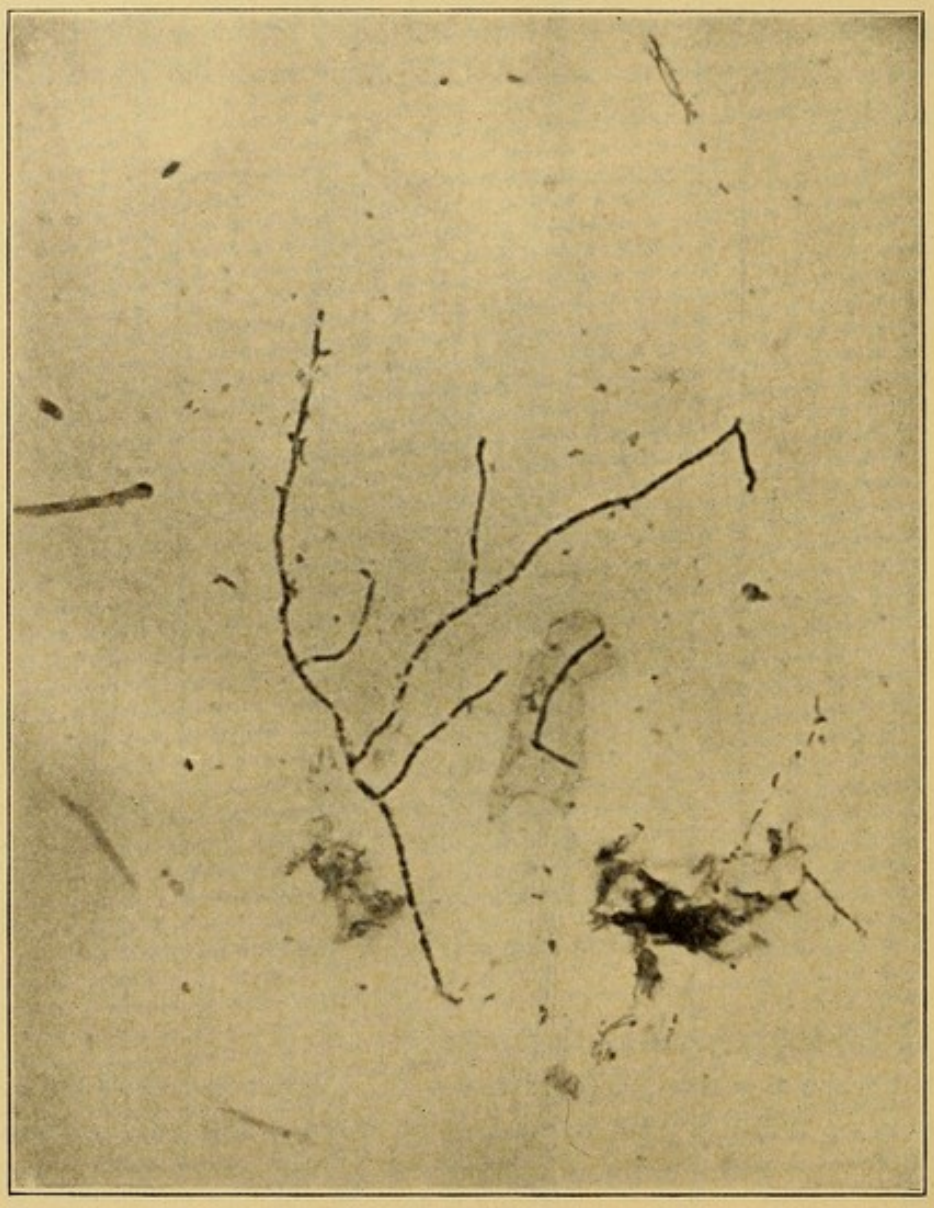

FIG. 68.—Mycelial threads of blastomyces from old agar culture. (From photograph by W. A. Pusey.)

parasite, as a rule, varies in size from 7 to 20 microns, though slightly smaller and much larger forms occur in some cases.

The organisms are readily obtained in pure culture from unbroken abscesses, from miliary abscesses in the borders of the cutaneous lesions, and from the miliary nodules and abscesses in the deep-seated organs. The peculiarities of the cultures of blastomycetes must be looked for in other works. Micro-

scropically, the organism obtained in culture appears at first as a fine, branching mycelium with a few small spore-like bodies. Later a large, segmented, often pod-like mycelium appears, together with large, round, or oval bodies with bud-like projections.

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CHAPTER VI.

THE URINE.

I. General Considerations.

The examination of the urine is one of the most important features of clinical diagnosis. So constant are the physical and chemical properties of the normal urine that any marked abnormality is easily detected. The relation between the kidneys and the blood is so close that the kidneys soon excrete any abnormal substances which have found their way into the blood-current. For this reason we find in the urine the abnormal products of perverted metabolism of the system or of special organs. It is true that the urinary findings may be, in any special case, secondary to those of the blood or of clinical examination, but in such conditions we may detect substances in the urine, which put us on our guard against making a specific diagnosis or point out the way to a correct differentiation. More or less marked changes in the character of the urine will occur whenever a pathologic condition exists anywhere in the system. These changes may not always be sufficient to be of direct diagnostic value, but in many cases may settle a differential diagnosis.

In the urine we find excreted the products arising from the metabolism of the various proximate principles, both of the tissues and the food. Knowing the intake of such material, we are able from our examination of the urine to judge of the manner in which the system is handling the material brought to it. In recent years the study of the metabolism in various conditions has been so extended that determinations which a few years ago were unusual are now matters of almost daily routine. An examination of the urine will frequently reveal the presence of irregular digestive and absorptive powers of the intestines, through the appearance of certain abnormal products of protein decomposition. Moreover, the study of the nitrogen partition of the urine is taking on increasing importance from day to day, so that the estimation of the factors determining this division should be possible by any one attempting to follow the metabolic activity of the system in any specified condition.

When the oxidative powers of the system are lessened, we find the urine showing abnormal products as an indication of such deficiency. These products are more or less characteristic and may be determined with a great degree of exactitude. Thanks to the work upon metabolism in diabetes, for instance, we now know that the glycosuria is clinically not of as great importance as is the presence of many other abnormal products associated with the sugar; in other words, a glycosuria must not be considered as identical with diabetes.

The system has a definite disintoxicating power toward certain noxious substances, whether introduced from without or formed within. While the blood is of special importance in such processes as far as bacterial products are concerned, an examination of the urine will frequently reveal much information regarding the metabolic toxins or the medicinal poisons. The estimation of the conjugated glycuronic and sulphuric acids of the urine will throw much light on the degree of this activity. In this connection it may be mentioned that indican, a product of bacterial decomposition of protein in the intestinal canal, may be taken as a direct indicator of the degree of such decomposition, but that we must not assume that all of the conjugated acids have such an origin.

Besides these general indirect points of interest, an examination of the urine will often reveal a direct anatomical lesion of the kidneys. Time was when we regarded the mere presence of albumin in the urine as indicative of a kidney lesion, but we know that a thorough clinical examination is necessary before a diagnosis is possible. Albumin may or may not mean kidney trouble and may even be purely physiologic. Too much stress can hardly be laid upon the necessity of closely associating the urinary findings in any condition with the clinical symptoms of the case. The writer will have much to say later regarding the various abnormalities of the urine, but he wishes to impress at this point the fact that no finding, no matter how abnormal it may seem, should be considered absolutely pathognomonic, without taking into consideration the clinical manifestations of the case. An albuminuria, glycosuria, cylindruria, or pyuria may mean one thing at one time and another at a second period, so that the worker is cautioned against jumping at conclusions. The urine will yield much information, providing the worker knows how to interpret his findings. A laboratory worker must be cautious in his attitude and report his findings without any attempt at interpretation, unless he is aware of the clinical history of the case in point. Frequently, however, a simple urinary examination may give much unexpected information and put the physician in a much better position to make an obscure diagnosis.

The writer must refer to works on physiology for the various theories which have arisen from time to time regarding the mechanism of secretion of the urine. Suffice it to say at this point that the urine is excreted through the activities of the kidneys, the water and salts being secreted by the glomeruli, while the majority of the excretory products are eliminated by the vital or selective activity of the epithelium of the renal tubules. It is evident, therefore, that the physical and chemical characteristics of the urine will depend both upon the blood-pressure within the capillaries and the rate of flow through these vessels as well as upon the condition of the secreting epithelium.

Collection and Preservation of the Urine.

In all urinary examinations in which quantitative relations are to be studied it is necessary that a portion of the total 24-hour specimen be examined. This should be thoroughly mixed and carefully measured. The composition of

the different voidings is so variable that no definite idea regarding the elimination can be gained from a single specimen.

If a mere qualitative examination is to be made, a single specimen may be studied. In chronic nephritis, for instance, the morning urine may show points of interest as compared with that voided in the evening after a day's activity. In diabetes it may be desired to study the effect of a carbohydrate meal upon the sugar excretion. This may best be done by an examination of the urine passed three or four hours after such a meal. The microscopic examination is best made as soon as possible after voiding, but it is to be remembered that much variation may be noted in the sediment of the different voidings.

In making the 24-hour collection, the patient is instructed to empty his bladder at a specified time, preferably at 7 A. M. This portion is thrown away and all urine passed from that time until the bladder is emptied at 7 A. M. the next day is saved. If one desires to separate the day and night urine, the voidings from 7 A. M. to 7 P. M. may be kept in one container and those from 7 P. M. to 7 A. M. in a second vessel properly labeled.

A thoroughly clean bottle of one-half to one gallon in capacity should be used as the container. This should be well corked after each addition of urine and kept in a cool place. As urine undergoes decomposition more or less readily, depending upon bacterial activity, some preservative should be added to prevent such processes. The writer is accustomed to advise the use of a slight excess of chloroform. This may be removed by heating the urine and will not then interfere with the later reactions. If not removed, its presence will lead to a pseudocarbohydrate reaction. Three or four drops of formalin may be added for each pint of urine. This is an efficient agent, but it will lead to reactions simulating those for sugar and even albumin, and, moreover, will introduce a crystalline compound of formalin and urea into the sediment as well as markedly interfering with the bile tests. Thymol may be added, but this may give a reaction similar to those for bile pigments and albumin. Camphor, chloral, and boracic acid have been used, but do not possess any virtues over the other preservatives mentioned. In any case, the worker should be on his guard in reporting abnormal findings without convincing himself that the reaction is not due to an added preservative.

II. Physical Properties.

(1). Quantity.

The amount of urine passed within 24 hours depends upon several factors and varies both for individuals and for different races of people. It is self-evident that under normal conditions, the amount of urine will vary with the quality and quantity of the substances to be excreted, the condition of the renal parenchyma, the pressure and rate of flow of the blood-current, the vasomotor disturbances, the stage of digestion, the loss of fluid in the perspiration as

influenced by the surrounding temperature, amount of exercise, and extent of fluid intake; upon the weight of the subject, the sex, and age.

As a rule, the quantity of urine excreted varies between 1200 and 1500 c.c. (40 to 50 ounces), reaching a maximum two or three hours after a large fluid intake. Women excrete somewhat less than men, while children void relatively more than do adults, although the actual amount is less. In the adult we find the amount of urine is almost directly proportional to his weight, a normal large individual excreting nearer 1,800 than 1,500 c.c., the amount being about 1 c.c. per kilo and hour, while with a child the excretion is about 4 c.c. per kilo and hour.

The physiologic limits of the urinary excretion are about 750 and 3000 c.c. In cases showing as high an output as 3,000 c.c., one is justified, perhaps, in assuming the presence of some pathologic condition. The kidneys are not easily deranged by the excess work put upon them in excreting large quantities of urine, so that we may find a secretion of many liters per day continuing for an extended period without endangering the normalty of the excreting organ.

Normally, the amount of urine excreted during the day by far exceeds that voided during the night, while the afternoon urine is usually more than that of the morning. We find, however, that in edematous conditions either of hepatic, cardiac, or renal origin, the night urine usually exceeds that of the day. This condition is known as *nycturia*. As it is, perhaps, more frequently associated with cardiac insufficiency, it may have a diagnostic importance in these cases.

Polyuria.

By this is meant an excretion of an increased amount of urine. Just what amount of urine is to be considered as indicative of polyuria will depend much upon the habits of the patient as regards daily intake of fluid. As a rule, anything above 2,500 c.c. is at least suggestive of this condition. One should, however, not be content with the examination of a single day's specimen in judging of a polyuria, but should demand that the increase extend over a period of several consecutive days.

Just what factors are to be held accountable for the polyuria, is not always easy to decide in every case. An increased intake of fluid together with an increased general blood-pressure will cause both an increased local renal pressure and an increased blood-flow through the kidney. While such a polyuria rarely exists in cases of ordinary chronic or active renal hyperemia, we find under the influence of drugs that a very decided increase in the urinary output may occur. The most important of such drugs, known as diuretics, are caffeine and digitalis.

A polyuria is observed in the convalescent stages of acute nephritis in both chronic parenchymatous and interstitial nephritis, and in amyloid degeneration of the kidney. This excretion, especially in the chronic interstitial type,

may be one in which the total solids are normal or reduced, and is then known as *hydruria*.

Diabetes mellitus is more frequently, perhaps, than other conditions associated with a polyuria. The quantity eliminated is dependent both upon the increased intake as a result of the polydipsia as well as upon the dehydrating powers of the sugar. A certain relationship exists between the amount of fluid and the sugar, the polyuria being usually diminished by measures which decrease the amount of sugar excreted. This polyuria is not necessarily continuous and may alternate with periods showing a normal or subnormal amount of urine.

In cases of diabetes insipidus we find the daily excretion of as much as 50 liters or more of urine. According to Meyer, this polyuria is due to the attempt on the part of the kidneys to secrete sufficient water to hold the solids in solution. He believes that a distinct insufficiency of the kidneys to secrete a urine of normal concentration exists, so that more water must be excreted to take care of the normal salts.

In cases associated with abnormal accumulations of fluid, such as pleuritis, ascites, and general edema, a polyuria will exist at the time of absorption of the exudates, owing to the presence of such large amounts of fluid in the blood-vessels.

The so-called "epicritic polyuria" is frequently observed during convalescence from acute febrile attacks. This is probably indicative of the elimination of toxic products which have accumulated in the system during the progress of the disease. It is supposed to be of favorable import when occurring in a febrile condition, but it is to be recalled that this polyuria may be followed by a later oliguria which is of grave significance. As a rule, however, it may be said that as the case improves the urine is increased in amount.

Polyuria may be observed in many nervous conditions, both functional and organic. The cause is probably some disturbance of the vasomotor apparatus as a result, perhaps, of irritation of the floor of the fourth ventricle, cerebellum, or cord. Hysteria, neurasthenia, epilepsy, and chorea are frequently associated with a polyuria. A paroxysmal polyuria in the course of a suspected nervous disease is more indicative of a functional derangement, while a continuous polyuria is more frequently associated with true organic disease.

Oliguria.

This is a condition characterized by the excretion of a diminished amount of urine, 800 c.c. being given as the lower normal point of the urinary output. Here, again, the absolute figure must depend upon the patient and upon his customary excretion. A single examination is not sufficient to decide whether or not an oliguria exists.

This condition is found, perhaps, most frequently in cases of broken compensation of the heart, where the blood-pressure is markedly diminished

It is present whether the cardiac incompetency be primary or secondary to hepatic, renal, or pulmonary lesions.

Oliguria is noted in practically all acute febrile disorders, especially in typhoid fever. This is due, probably, to a combination of cardiac weakness with the increased loss of water by the skin and lungs. Moreover, we may have, in such states, a retention of fluid along with a direct contraction of the renal vessels.

Acute nephritis as well as chronic parenchymatous nephritis are associated with a more or less extensive oliguria. This condition is probably referable to diminished functional activity of the glandular elements as well as to increased resistance within the tubules. A bilateral diffuse lesion is always necessary to cause much oliguria, as the sound kidney, if the trouble be unilateral, will take on vicarious activity. The more acute the condition, the greater the degree of oliguria.

Oliguria may also occur following the administration of an anesthetic, in connection with eclampsia, hysteria, or epilepsy, after the loss of large quantities of fluid by hemorrhage, diarrhea, or vomiting, in cases of portal obstruction as seen in acute yellow atrophy or hepatic cirrhosis, or in cases in which pressure is exerted upon the vascular system, especially the vena cava, by tumors.

Anuria.

This oliguria may, in almost any case, proceed to complete anuria, which may or may not be of vital significance. Cases of anuria do occur without any preceding oliguria, as shown in acute nephritis and in some cases of hysteria. Anuria, *per se*, cannot be held responsible, however, for the uremic symptoms so frequently associated with it, as it may persist for many days, 19 in a case of Adams, without any uremic signs.

Anuria may be due to obstructive, reflex, renal, and prerenal causes. We may have an occlusion of the urinary passages on one side and a reflex closure on the other. Tumors, prostatic hypertrophy, and toxic and nervous bladder disturbances may lead to a great degree of oliguria amounting, almost, to anuria.

The so-called prerenal causes of anuria include scarlet fever, which may lead to a severe nephritis, phosphorous poisoning, action of ether and chloroform, collapse, ureteral and urethral calculus, and cholera.

(2). Appearance.

Freshly voided urine should be clear and transparent. Only the faintest trace of any turbidity should be normally present, except soon after a meal rich in vegetable food, when a distinct turbidity may be noticed due to the precipitation of the phosphates in the alkaline urine.

When allowed to stand for a short time, a light cloud is noted which gradually settles to the bottom of the container in the form of the so-called "nubecula." This contains a few small granular cells and a few epithelial cells and is composed largely of mucus.

On standing for a somewhat longer period, as for instance over night, at the ordinary temperature, distinct crystals of uric acid may separate and appear in the sediment. If the temperature of the urine is allowed to fall to a considerable extent during this period, a somewhat more marked turbidity will be produced owing to the precipitation of the acid urates. This sediment is particularly noticeable if a highly acid urine becomes very cold.

If kept for a longer period at room temperature, or a shorter period during the warmer months, a diffuse cloudiness will appear, due to the precipitation of the phosphates, owing to the lessened acidity or abnormal alkalinity of the urine. This alkalinity is due to the decomposition of the urea into ammonium carbonate. The crystals in this alkaline urine will be triple phosphates, calcium phosphates, ammonium urate, and calcium carbonate.

Even before the urine becomes alkaline, a diffuse cloudiness may be present, due to the development of numerous saprophytic bacteria. This bacterial cloud is removed only with the greatest difficulty, as filtration of the urine has practically no effect upon it. Frequently, the addition of lead acetate to the urine will produce a voluminous precipitate, which may carry down the bacteria and permit of their filtration. This procedure is, however, not to be recommended, as other substances, if present in small amounts, may be carried down and thus escape detection.

If the urine is cloudy when freshly voided, the turbidity may be the result of the precipitation of phosphates through the alkalinity or it may indicate the presence of an organized sediment, such as casts, epithelial cells, blood, and pus.

The normal urine shows but very little viscosity, differing little from ordinary water in this respect. In certain conditions we find a marked degree of viscidity, which becomes especially apparent on attempting to filter the urine. In cases of chronic cystitis the excretion of a large amount of mucus may make the urine ropy and gelatinous. This increased viscosity may also be seen in cases of pyuria associated with decomposition.

(3). Color.

The color of the urine varies normally between various shades of yellow, the depth of color depending upon the concentration or specific gravity of the specimen. While the color is usually much paler in the urines of low specific gravity and very dark in those of high density, we find in diabetes mellitus a very pale urine with a high specific gravity. In cases of anemia the urine is always paler than normal, but in pernicious anemia the urine is highly colored owing to the marked destruction of the erythrocytes.

As a rule, it may be said that an acid urine is more highly colored than an alkaline one, although many exceptions to this rule occur. There seems to be some difference between the urines passed at different periods of the day; thus the urine of the day is usually a distinct amber, while that of the night may take on a greenish tinge. To what this color in the latter case is due is at present unsettled. Several color scales have been introduced, such as those

of Neubauer and Vogel and of Radde, but these are not sufficiently extensive to take in pathologic variations where they would be most important. As a rule, it is sufficient to divide the colors of the urine into those of the spectrum, making allowance for light, medium, and dark shades of each color.

The pigments causing the normal and abnormal colorations of the urine will be discussed in detail in a later section. At this point the writer would say that normally these pigments are urochrome, uroerythrin, and urobilin, while the various conjugated glycuronic and sulphuric acids, blood pigments, biliary pigments, melanin, etc., are found in pathologic conditions.

Pathologic Colorations.

Deviating from the rule that the higher the specific gravity the more intense the color, diabetes mellitus shows an extremely light color with a high specific gravity. Owing to the lack of pigment, in chlorosis we find a very pale urine. Chronic interstitial nephritis and amyloid degeneration of the kidneys are associated with an extremely pale urine.

In febrile conditions the coloration may range from an orange-red to a distinctly red tint, owing to the increase in the amount of urobilin. This deep color is especially noticeable in cases of severe pneumonia. This reddish urine may also be due to an increase in the amount of uroerythrin, which is responsible for the deep color of the urate sediment so frequent in the concentrated urines of febrile conditions, circulatory disturbance of the liver, and in cases associated with profuse perspiration.

In cases of jaundice the urine may vary from a dark yellow or green to a brown or black, depending upon the concentration of the urine, the amount of bile pigments, and upon certain chemical activity which occurs in such urine. Not only will the color of the urine be deeper, but the foam which appears on shaking the specimen will take on a distinct yellowish-brown tint. If this biliary urine be allowed to stand in the cold for some time, crystals of bilirubin may separate out and be seen in the sediment.

Urine which contains blood may have a violet shimmer, may appear smoky, blood red, brownish-black, or even deep black in color. These variations depend both upon the amount and kind of pigment present. Hemoglobin gives a more reddish tint to the urine, while methemoglobin produces a brownish shade. Such urine is always cloudy, owing to the admixture of corpuscles and other organic material. The blood found in the urine may arise from any point in the genitourinary tract or may be of systemic origin. In the latter case conditions which give rise to hemolysis will cause the appearance of hemoglobin in the urine.

The condition of *chyluria* is characterized by the presence of large numbers of highly refractile globules of fat along with many morphological constituents. This gives rise to the appearance of a milky urine and is especially characteristic of infection with the filaria. It is not infrequent to find, in certain cases of hysteria, a specimen of milky urine, owing to the fact that the patient has

added milk to the urine before sending it to be examined. The presence of a large quantity of pus will also give the urine a milky appearance.

The urine of patients suffering with melanotic tumors may be perfectly clear when freshly voided, but becomes black or dark brown on exposure to the air. This reaction is due to the transformation of the pigment melanogen to melanin, and may be hastened by the addition of oxidizing agents to the urine. This darkening of the urine extends characteristically from above downward.

The condition known as *alkaptonuria*, which is characterized by the excretion of homogentisic and uroleucic acids, gives rise to the passage of a urine which is brownish-black in color and may be syrupy in consistency. This color is not always evident in the fresh specimen, but appears soon after being voided.

In cases of peritonitis, suppuration anywhere in the system, gangrene, and marked intestinal putrefaction, the urine is frequently dark colored owing to the passage of certain aromatic products of decomposition, either indican or various derivaties of phenol. The coloration in these cases may vary from a dark brown or greenish-black to a distinct blue. These urines differ from those containing melanin in the fact that ferric chlorid does not blacken the urine as it does in the presence of melanin. This urine may contain a distinct amount of indigo, although the substance present is usually a different oxidation product. If indigo be present, a bluish-black scum will frequently rise to the surface of the specimen.

Medicinal Coloration.

After the use of carbolic acid either internally or externally, guaiacol, creosote, resorcin, naphthalin, salol, and various tar preparations the urine may vary from a dark brown to a black color. This coloration is due to the excretion of hydroquinon and of pyrocatechin, and may be evident only on allowing the urine to stand for some time. The urine containing pyrocatechin may reduce alkaline copper solutions, but will not affect such bismuth preparations. While a dark brown or black coloration of the urine may be found in cases of hemorrhages, melanosis, malaria, alkaptonuria, ochronosis, and chronic tuberculosis, one should be on his guard, as the medication of the case may be responsible for such coloration.

Methylene blue will color the urine a greenish to deep blue shade, which may last for several days. Usually within an hour after this drug is taken the urine may show a faint tinge of green which may be more clearly brought out by acidifying with acetic acid and warming.

The use of the hypnotics, trional, sulphonal, and tetronal, frequently gives rise to the voiding of a urine which is a deep red-wine color, due to the presence of hematoporphyrin. Pyramidon produces a urine of rose-red color, the pigment of which is soluble in ether, chloroform, and amyl alcohol. Antipyrin and purgatin both produce distinctly red urines. Chrysarobin,

senna, rhubarb, cascara, and santonin produce a golden-yellow urine which becomes red in the presence of alkali. This coloration is due to the excretion of chrysophanic acid. According to Gorup-Besanez, the pigment of beets, huckleberries, blackberries, etc., may under certain conditions be excreted in the urine and color it the corresponding shades.

(4). **Odor.**

The normal urine usually has a distinct aromatic odor which very much resembles that of beef broth. This odor is due to the presence of certain volatile acids and is more marked in urines of high concentration. If the urine undergoes decomposition either within the bladder or on standing, a so-called "*urinous odor*" appears which is due to the decomposition of protein material. This odor is very markedly ammoniacal. Should such an odor appear in the freshly voided specimen, it is evidence of marked cystitis.

Abnormal decomposition of the urine, as evidenced by changes in the odor, may be found in conditions associated with decomposition of pus and may be due to the presence of hydrogen sulphid along with the ammonia. This condition may be observed in cases of perforation of an abscess into the urinary tract, in which case the urine may have a distinctly fecal odor if the intestine be involved, while in carcinoma of the bladder this repulsive odor of the urine may also be noticed.

A distinct fruity odor is often present in cases of diabetes mellitus, in many febrile conditions, and in some stomach and intestinal troubles, which may be directly traceable to the presence of acetone, although Folin believes it is due to some unknown substance other than acetone.

Certain medicaments, such as oil of turpentine, give rise to a distinct odor of violets in the urine. Menthol causes an odor of peppermint, while cubebs, copaiba, sandal-wood oil, tolu, and saffron produce a peculiar spicy odor. Valerian and asafetida are excreted as such in the urine and produce their characteristic odor.

Certain foods, such as meat, bouillon, and coffee, produce a slight odor of the urine, while asparagus gives a peculiar characteristic odor due to the presence of methyl mercaptan.

(5). **Reaction.**

The normal urine has an acid reaction. According to the views recently held, this acidity was directly due to the presence of acid salts, especially to sodium dihydrogen phosphate (NaH_2PO_4), and not to the presence of any free acid. As certain procedures had shown that not all of the phosphates were in the form of the diacid type, but that some of them were present as the monacid phosphates, the variations in the acidity were supposed to represent differences, both absolute and relative, in the amounts of the diacid phosphates present.

The recent work of Folin has shown that the phosphates in the clear urine are all of the monobasic (diacid) type. His figures indicate that the acidity

of normal clear urines is ordinarily greater than the acidity of all the phosphates present and that the excess must be due to free organic acids. For this reason the methods of Freund and of Lieblein for the determination of the acidity of the urine must be given up. To quote from Folin:[1] "The current attractive, and in a measure plausible, belief that the acidity of urine is regulated by variations in the relative proportion of the two forms of 'acid phosphates' is, therefore, erroneous. If urine does at no time contain comparatively strong acids in the free form, the reason is in part the variability of the ammonia formation and in part the presence of salts of organic acids. In a mixture of salts containing an excess of acids it is the weakest which will remain uncombined and the strongest organic acids will, therefore, exist as salts; but if the total amount of acidity becomes abnormally great, the quality (the strength) of the free acids may change."

From the standpoint of physical chemistry the acidity of the urine, as of all other acid solutions, should represent the absolute number of dissociated hydrogen ions in a definite quantity of the urine. We are, therefore, face to face with the same problem confronting us in the examination of the alkalinity of the blood. In the case of the urine the question of indicator to be used in the titration test is a matter of great moment, as no two indicators will give the same degree of acidity. The one naturally to be selected would be that which will react to every possible substance of an acid nature.

If we use the methods of physical chemistry we find, according to Höber,[2] that the urine is only about 30 times as acid as is distilled water and only about one ten-thousandth as acid as the titration figures would indicate. Such being the case, we must either entirely revise our figures for the acidity of the urine or employ methods which can be more easily carried out by the general worker than can those of physical chemistry.

Folin[3] has, therefore, introduced a method which uses direct titration of the urine and employs phenolphthalein as an indicator. This indicator reacts to all bodies of an acid nature, but cannot overcome certain difficulties which are in the way of direct titration. These obstacles are (1) the occurrence of calcium in the urine in the presence of the monobasic phosphates, and (2) the presence of ammonium salts. He has found that the addition of potassium oxalate to the urine will do away with these difficulties by holding in solution both the *di-* and *tri-calcium* phosphates and by preventing the dissociation of the ammonium compounds.

Folin's Method.

Total Acidity.

Twenty-five c.c. of urine are treated with 15 to 20 grams of powdered potassium oxalate and one or two drops of a 1 per cent. alcoholic solution of phenol-phthalein. The mixture is shaken rapidly for one or two minutes

[1] Amer. Jour. of Physiol., vol. 13, 1905, p. 45.
[2] Beitr. zur chem. Physiol. u Path., Bd. 3, 1903, S. 525.
[3] *Loc. cit.*

and titrated at once with a tenth-normal sodium hydrate solution until a faint, distinct, permanent pink color is obtained. It is advisable to shake the flask during the titration so as to prolong the effects of the potassium oxalate. The acidity is expressed in terms of the amount of tenth-normal sodium hydrate solution necessary for neutralization of the 24-hour amount of urine. This is expressed as T, which is, on an average, 617.

Free Mineral and Organic Acidity.

Determine the amount of total phosphates present by titration with uranium nitrate solution as described later. Seven and one one-hundredth mg. of P_2O_5 have an acidimetric value equal to 1 c.c. of tenth-normal acid. The total acidimetric value of the phosphates of the 24-hour urine may be easily determined with the help of this factor, by converting the amount of phosphates into terms of N/10 acid.

From the total acidity (*T*) subtract the acidimetric value of the phosphates (*P*). The remainder is the acidity due to uncombined organic acids, and the difference, that obtained from calculating all the phosphoric acid as di-acid phosphate, is the free mineral acidity. For all ordinary studies of the acidity of the urine the direct titration of the total acidity and of the phosphates gives the necessary information. The excess of the total acidity above that calculated from the phosphates gives the total free acids present. If the acidity calculated from the total phosphates is greater than the titrated acidity, then there are practically no free organic acids present, and the titrated acidity represents the amount of phosphates present in the diacid form (Folin).

While the acidity of the urine is best determined and expressed as outlined above, it seems wise to the writer to retain the same style of expression for the acidity as used in stomach analysis. With this nomenclature one would state the acidity of the urine in degrees; that is, the amount of tenth-normal sodium hydrate necessary to neutralize 100 c.c. Under normal conditions this will vary from 35 to 45°. It may be increased by a diet rich in meat, while it is decreased by a vegetable diet. There are many acids produced in the oxidation of protein, among which we find sulphuric, phosphoric, uric, and the oxyaromatic acids. Ordinarily, these play an indirect part in the acidity of the urine, although this phosphoric acid may exist in part as the dihydrogen phosphate and in consequence increase the acidity of the urine. The regulation of the metabolism is such that an increase of the acids produced in the system or taken into it from without is neutralized by an increased formation of ammonia, the salts appearing in the urine as the ammonium salts which do not, of course, increase the acidity of this fluid. This is the basis upon which one estimates the amount of ammonia in following a condition of acidosis.

The reaction of the urine varies at different times of the day. The acidity appears to be highest in the morning before breakfast and is diminished after a meal, due to the secretion of hydrochloric acid into the stomach. The reaction of the urine may even be alkaline for a period of two or four hours after each

meal, in which case the urine will be turbid from the precipitation of phosphates. This reaction of the urine following meals is known as the "alkaline tide" of the urine. Between meals the acidity of the urine will gradually increase until the next meal is taken.

The reaction of the urine is modified to a great extent by the use of drugs. Thus, alkalies, such as carbonate and bicarbonate of sodium, will render the urine alkaline if taken between meals, while if taken just preceding a meal they will be neutralized by the gastric juice. All organic acids of the fatty series are oxidized in the system to carbonic acid and combine with bases forming basic salts which render the urine alkaline or less acid, providing these acids are not taken above the point of tolerance, as the writer has shown that large doses of such acids as citric acid will increase the acidity of the urine. The mineral and aromatic organic acids will, however, practically always increase the acidity.

In many pathologic conditions we find the reaction of the urine variable. Abnormal gastric activity may be either associated with an increase or a decrease in the acidity of the urine, depending upon a condition of hypo- or hyperacidity of the gastric juice. The rapid absorption of a transudate or exudate will lead to the excretion of an alkaline urine from the presence of an increased amount of alkaline salts. An alkaline urine is not infrequently seen after intestinal hemorrhage, in certain cases of pneumonia, typhoid fever, chronic nephritis, and in cases in which exudates from the urinary tracts have become mixed with the urine. In certain cases of nervous diseases and in some cases of anemia we may also find an alkaline urine. The urine in all of the above cases will show, if tested by litmus-paper, an alkaline reaction in which the bluing of the red litmus-paper is permanent. This condition is known as *fixed alkalinity* and is quite distinct from the following type.

In cases of decomposition of the urine within the urinary tract, through the influence of bacteria, the urea is decomposed into ammonium carbamate and carbonate. The alkaline reaction of the urine in such cases will be shown by a blue color of the red litmus-paper either held above it or placed in it, the blue color disappearing when the paper is dried. This condition is known as *volatile alkalinity*. If the urine shows this volatile alkalinity on being voided, the finding is significant of trouble somewhere along the urinary tract, especially within the bladder.

The acidity of the urine is increased only with difficulty and not beyond a certain point, owing to the protective metabolic influence of the increased excretion of ammonia. In diabetes mellitus we may find a very acid urine, while that of febrile conditions and some cases of the "uric acid diathesis" is highly acid.

(6). Specific Gravity.

The specific gravity of the normal urine ranges between 1,015 and 1,025, with an average of 1020. This specific gravity will depend, of course, upon

the amount of fluid intake, the quantity of the 24-hour specimen of urine, the degree of tissue activity, and the condition of the secreting organs. The intake of a large volume of water may reduce the specific gravity of the urine to a very low figure and, correspondingly, a small intake may lead to a urine of high gravity. We find, therefore, that perfectly normal urines may show specific gravities ranging from 1010 to 1030, with pathologic variations from 1,002 as high as 1,060 or more.

In general routine work it is essential that the specific gravity of the 24-hour specimen be determined. Except in unusual cases, absolutely nothing of diagnostic value may be learned by the determination of the specific gravity of a single voiding of urine. The variations at different times of the day, under the influence of food, digestion, activity of the skin and lungs, and exercise, may be so great that apparent pathologic figures may be obtained from a single specimen. Such variations are overcome for the most part in the 24-hour specimen unless pathologic conditions are present to keep up such variations. In some cases, especially in chronic diffuse nephritis, the morning specimen of urine almost invariably has a lower specific gravity than that of the other periods of the day. For this reason one may determine the specific gravity of a single specimen of such urines.

It is of especial importance that the total amount of urine in the 24-hour specimen be taken into consideration in judging of the value of a specific gravity. Thus in chronic interstitial nephritis we may find a large volume of urine with a low specific gravity, while in diabetes mellitus an even higher volume of urine may be present, showing a very high specific gravity.

Technic.

The most accurate method of determining the specific gravity is, of course, the use of the pycnometer. The principle of this method is the determination of the weight of a definite volume of urine as compared with that of the same volume of distilled water under the same conditions of temperature and atmospheric pressure. This method will be discussed in the section on Blood, to which the reader is referred.

The clinical method of estimating this factor is a distinctly areometric one. The principle of this method is that a body immersed in a fluid will displace an amount of fluid equivalent to the loss in its own weight. By the use of instruments known as hydrometers or in the case of the urine as urinometers, this displacement is measured by immersing the hydrometer in the fluid and observing the point to which this instrument sinks. The stem of the hydrometer is graduated in divisions from 1,000 to 1,060 by differences of 1°, the 1,000 point being that to which the instrument sinks when immersed in distilled water at the temperature to which the instrument is calibrated. Any variation in the density of the solution in which this hydrometer is immersed will be evident by the depth to which it sinks, the more concentrated the solution the less will the instrument sink.

The vessel in which the urine is poured should be cylindrical in shape, with parallel sides and wide base and sufficiently tall to permit of the complete sinking of the hydrometer. The forms of this cylinder with fluted sides are perhaps more desirable than the plain cylinder, as the bulb does not tend to stick to the sides of the vessel so readily.

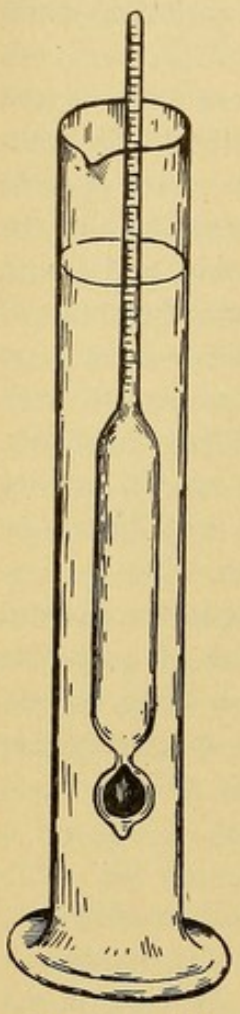


FIG. 69 — Urinometer and cylinder. (*Hawk.*)

The vessel is filled about four-fifths full of urine, any foam being removed by the use of filter-paper. The hydrometer is placed in the urine with a twisting motion and allowed to come to rest. The depth to which the stem is immersed is then read off by observing the mark which coincides with the lower meniscus of the urine as seen from below. The worker should never attempt to read the specific gravity from above as a slight meniscus interferes with the accuracy of his reading. The worker must be absolutely sure that the urinometer neither rests upon the bottom of the cylinder nor touches the sides, but should see that it floats perfectly free in the urine.

The temperature at which the reading is taken is a matter of some moment as some of these instruments are graduated at 15° C. The ordinary model, as made by Squibb, is graduated at 25° C., which is, perhaps, more nearly the working temperature of the room. A variation of 3° in temperature between that of the room and that at which the instrument is calibrated, will give a difference of 1° of specific gravity; that is, a difference in the fourth place of the specific gravity. In ordinary clinical work corrections for variations in temperature are usually unnecessary, as variations of two or three points in the fourth place of the specific gravity are of absolutely no importance, as such changes might be attributed to chemical variations on standing, even though the most accurate methods of estimating the specific gravity were used.

If the quantity of urine be very small, it may be diluted with distilled water, so that the measuring cylinder may contain enough material to permit of a density estimation. The specific gravity of this diluted urine is then determined as above and the last two figures of the specific gravity are multiplied by the degree of dilution.

Rough Estimate of Total Solids.

As the degree of specific gravity is directly proportional to the amount of solids contained in the urine, one may roughly judge of the total solids by a simple calculation as follows: If the last two figures of the specific gravity be multiplied by 2.33 (Haeser's coefficient), the result will be the approximate number of grams of total solids in every 1,000 c.c. of urine. Know-

ing the quantity of urine passed in the 24 hours, a simple calculation will yield the total 24-hour excretion of solids. Long uses the coefficient 2.6. Instead of the above figure one may multiply the last two figures of the specific gravity by 1.1 (Haines' coefficient) and obtain the number of grains of solids in each fluidounce of the urine. This figure when multiplied by the total number of ounces of the 24-hour specimen will yield the excretion of solids in grains. This latter method has some advantage for the older practitioner who has not accustomed himself to the use of the metric system.

It is to be said that neither one of the above methods can give anything but approximate results and in pathologic urines are absolutely unreliable. Highly albuminous urines invariably show a reduced specific gravity, while a high sugar content is associated with an increase in the density. In either one of these cases a calculation of the total solids by the above method will yield inexact figures. If the worker desires to know the exact amount of total solids in the 24-hour specimen, and this is sometimes advisable, recourse should be had to more exact methods of determination which will be discussed later.

The specific gravity of a specimen of urine varies, of course, with the amount of total solids. Normally, these range from 60 to 70 grams with a 24-hour excretion of 1,500 c.c. of urine. The urea usually constitutes about one-half of the total solids. As the normal percentage of urea is approximately two with a specific gravity of 1,020, the writer has been struck with the usual close relationship of the percentage of the urea to the specific gravity. In watching this point in over 2,000 examinations of urine within the last year, the writer has observed that the percentage of urea will practically parallel the last two figures of the specific gravity; in other words a specific gravity of 1,015, for instance, will normally be associated with a urea content of 1.5 per cent. This statement is true only in those cases which contain neither albumin nor sugar.

As a rule, it may be said that the specific gravity of the urine is inversely proportional to the amount of fluid eliminated. It will, therefore, be evident that conditions leading to an oliguria will produce a high specific gravity, while those causing polyuria will give a low specific gravity. This statement must be modified when considering certain pathologic conditions, as we may find a diminished amount of urine with low specific gravity as in chronic nephritis in which the salts are diminished, although the organic albuminous bodies are much increased; while in diabetes mellitus we have an abundant urine of high specific gravity.

The specific gravity of the urine is of more or less importance in judging of the activity of the kidneys. In acute nephritis we find a urine of high specific gravity, while in the chronic types of renal disease the specific gravity is low owing to the diminution of the salts. It is to be noted in this connection that in the case of so-called "functional" albuminuria, the specific gravity of the urine is above the normal figures. A marked reduction in the specific

gravity of any case of nephritis is of dangerous import. The writer will have much to say regarding the functional activity of the kidneys in a later section.

(7). Optical Activity.

According to Haas,[1] the normal urine is slightly levorotatory, ranging from 0.01 to 0.18. This optical activity is due to traces of the conjugated glycuronic acids which will be discussed later.

An increase in this levorotatory power is observed due to the presence of increased amounts of glycuronic acid, β-oxybutyric acid, albumin (in amounts over one-half part per thousand), and levulose. Dextrorotatory urines depend upon the presence of glucose, maltose, and lactose, while the presence of pentose usually gives rise to an optically inactive urine or one at least showing only a slight degree of dextrorotation.

This optical activity under the influence of pathologic products will be discussed in the section on Carbohydrates, to which the reader is referred.

III. Chemical Properties.

(A). Normal Composition.

The tables given in most text-books showing the chemical composition of the urine cannot be regarded as absolutely indicative of the excretion as shown in the every-day specimens of urine. The composition of the urine is absolutely dependent upon the diet under normal conditions, so that a table to be exact must embrace the findings under a specified diet. Perhaps the most frequently quoted table is that of Parkes, which may be found in almost any text-book dealing with the urine. This table gives the various figures for the different substances excreted, but does not take into consideration the amount of the various types of foods used in the diet leading to this excretion. The writer, therefore, feels that it is wise to omit such a table at present, as we have no method of comparison of the excretion with the intake. The recent work of Folin upon the urine of persons both under a mixed diet and a nitrogen-free diet gives an "approximately complete" determination of the urinary constituents under absolutely fixed dietary conditions. The figures for such diets will be given under the head of each individual substance discussed.

The daily urine of a healthy adult will vary between 1,200 and 1,500 c.c. in amount and will contain from 60 to 70 grams of total solids, of which the inorganic constituents form from 25 to 30 grams and the organic between 35 and 40 grams. The **inorganic** constituents consist of the phosphates of sodium, potassium, calcium, and magnesium, the chlorids and sulphates of the alkali metals, various types of ammonium salts, traces of nitrates, calcium carbonate (especially under vegetable diet), and traces of iron compounds. While these inorganic substances have not hitherto been credited with much

[1] Centralbl. f. d. Med. Wissensch., 1876.

importance, to-day we are realizing more and more that much is to be learned by a careful study of the inorganic excretion.

The **organic** substances are of especial importance both in metabolic work and in the diagnosis of pathologic conditions. While we do not by any means know everything concerning the variations in excretion of these organic products, yet we do know much which is helpful in our direct diagnostic work as well as in our study of the progress of the disease. Among the organic substances which are more or less normal (although not always in large amounts) in the urine we find the lower and higher fatty acids, oxalic acid, acetone, glycerophosphoric acid, a trace of glucose, lactose (especially in nursing mothers), carbamic acid, urea, oxaluric acid, allantoin (especially a few days after birth), creatinin, uric acid, purin bases, thiosulphuric acid, tauro-carbamic acid, cystin, chondroitin-sulphuric acid, inosite, hippuric acid, benzoic acid, phenaceturic acid, p-oxyphenyl-acetic acid, hydro-p-cumaric acid, skatol-carbonic acid, conjugated sulphuric and glycuronic acids, oxy-proteic acid, pigments, organic iron compounds, traces of protein, and ferments.

Under pathologic conditions we may find lactic acid, large amounts of acetone, aceto-acetic acid, β-oxybutyric acid, fats, large amounts of glucose, levorotatory carbohydrates, r-arabinose, lecithin, cystin, putrescin, cadaverin, ptomaines, oxymandelic acid, leucin, tyrosin, homogentisic acid, uro-leucic acid, cholesterin, cholic acid, glycocholic acid, tauro-cholic acid, various derivates of phenol, hematin, hematoporphyrin, methemoglobin, other blood pigments, bile pigments, melanin, and protein material.

(*a*). **Total Solids and Total Ash.**

The estimation of the total solids of the urine is a matter of considerable difficulty, owing to the fact that evaporation of the urine leaves a syrupy residue which is dried with great difficulty and with constant loss of ammonia. This ammonia is formed through the action of the diacid sodium phosphate upon the urea in the concentrated solution. In order to overcome this one may get very close to the accurate results by placing a weighed amount of dry clean sand in a weighed platinum dish and adding 10 to 15 c.c. of urine. This is then evaporated upon the water-bath and later in the drying oven at 105° C. The dish is then placed in the desiccator and allowed to remain until the weight becomes constant. Knowing the weight of the dish and sand, the amount of the urine added, and the weight of the dish after the urine is evaporated, a simple calculation will yield the total solids in the amount of urine taken. A much more accurate method, but one which is not clinically so available, is that of Neubauer. This method may be found in the larger works on physiological chemistry.

The estimation of the ash is as follows: Fifty c.c. of urine are evaporated to dryness over the water-bath in a weighed platinum or porcelain dish. The dish is then heated, while covered, over the free flame until gases cease to be evolved, especial care being taken not to permit sputtering of the con-

tents. In some cases it is possible to completely incinerate the urine by long-continued heat. However, a more usual procedure is to treat the carbonized residue with distilled water, thoroughly stir the mixture and filter through a filter-paper whose ash is known. The contents of the dish should be washed onto the filter several times and the material upon the filter also washed with boiling water. The filter-paper and its contents are now placed in the dish and completely incinerated. After this procedure the filtrate and washings of the original carbonized material, which contain most of the inorganic constituents, are placed in the dish and evaporated at 100° C. to dryness, and then incinerated over the free flame. The dish is now placed in a desiccator and dried to constant weight. Knowing the weight of the dish with its contents and the original weight of the dish a simple calculation will give the amount of ash in the 50 c.c. of urine taken. As originally stated the inorganic constituents will range between 25 and 30 grams under normal conditions.

(*b*). Inorganic Constituents.

(1). Chlorids.

The chlorids are one of the most important groups of inorganic solids in the urine. They are derived entirely from the food and, in consequence, the amount of excretion will depend upon the intake. The chlorids actually forming constituent parts of the food exist in combination with potassium and calcium, while those which are added as seasoning to the food are practically always in the form of sodium chlorid. As the amount contained in the food is trivial in comparison to that added, we are accustomed to regard practically all of the intake as sodium chlorid. This is a constituent of the serum of the blood and of other tissues, while the potassium salt is in more direct relation with the cellular elements.

Under normal conditions from 10 to 15 grams of sodium chlorid are eliminated in 24 hours. The administration of a diet rich in salts will increase this amount, while a salt-poor diet will diminish the amount up to a certain point. If the diet be a starvation one, or an absolutely salt-free one, the chlorids will disappear almost entirely from the urine. The regulation of the metabolic activity of the system is such that a certain amount of salt must be retained in order to preserve the osmotic equilibrium. For this reason we find that withdrawal of salt from the diet does not lead to any appreciable diminution of the normal chlorid content either of the blood or tissues. An increase in the elimination of salt is practically always followed by a retention of salt unless a sufficient supply is furnished by the food. If food containing sodium chlorid be given after a period of salt-free diet, a portion of this salt will be retained. Conversely, we find, if the body has for pathologic reasons retained sodium chlorid, that an increased elimination will follow. This metabolic activity is intimately associated with the general protein metabolism of the body. Any increase in the amount of circulating protein as compared with the living protoplasm will be followed by an increased

elimination of the chlorids, which have been previously retained by the living or active protein material of the protoplasm. This fact is shown by the relation between the elimination of the chlorids and the total nitrogen. With an ordinary diet this ratio is as one to one, but in disease it may be much disturbed owing to chlorid retention through renal insufficiency. The blood and tissues of patients with nephritis show a higher chlorid content than those of normal individuals. We should, therefore, expect, if the law of increased chlorid excretion being dependent upon increased circulating albumin were to hold, that the chlorids would be increased along with the albumin in nephritis. But we find that the kidney under these circumstances is unable to excrete the increased amount of salts circulating in the blood.

A further method of withdrawing sodium chlorid from the body consists in the administration of large quantities of alkaline carbonates or of compounds of the alkalies with vegetable acids. As soon as these are given the body becomes poor, not only in acid substances and in HCl, but also at the same time in sodium and potassium. Practically speaking, the body becomes directly impoverished in NaCl. The body may also lose chlorin when vomiting is frequent, when absorption is diminished, when the stomach is regularly washed out, and when diarrhea is marked. This loss may be especially noted in cases of hyperacidity of the gastric juice associated with vomiting. The chlorin in these conditions is withdrawn in the form of free acid, and in consequence the alkalinity of the tissues may be increased, although not to any great degree nor for any great length of time. Sodium chlorid is, therefore, seen to be of more than passing importance in the general metabolic activity of the system.

Physiologic Variations.

The amount of sodium chlorid excreted will depend directly upon the amount ingested. We may find as high as 30 grams of salt in the 24-hour specimen or it may be as low as 2 grams, salt-free diet reducing the elimination to a mere trace. The elimination may be increased by active exercise, by increasing the water intake and hence the water output, and by the intake of a large amount of vegetable food. Much more chlorid is apparently excreted during the day than during the night.

Pathologic Variations.

A marked **diminution** of the chlorids, which may in some cases be almost complete, has been supposed to be pathognomonic of pneumonia. This, however, has been shown to be fallacious, as the same condition occurs in most acute febrile states, with a possible exception of intermittent fever. In a doubtful fever a large diminution in the amount of urinary chlorids might be strongly presumptive of pneumonia, but would be conclusive only in the presence of distinct clinical signs of this disease. While the retention of these chlorids in the exudate of pneumonia may partially explain the

diminution in the urine, it cannot explain the fact that the chlorids of the food are also retained. The explanation is more likely to be found, in the writer's opinion, in an existing renal insufficiency. This same retention of chlorids will be found also in any condition in which there is a transudate or exudate of any considerable bulk, so that this factor must play a certain rôle. As crisis approaches in pneumonia, the chlorids of the urine will increase in favorable cases, while in those of bad prognosis no such increase will, as a rule, be observed. Van der Bergh believes the explanation of the diminished urinary chlorids in pneumonia to be an attempt on the part of the blood to maintain its osmotic pressure, the chlorids remaining fixed in the tissues owing to the increase of the products of metabolism in the plasma.

The chlorids are diminished in all acute and chronic renal diseases associated with albuminuria. The work of Widal upon the influence of chlorids upon the progress of a renal disease has brought out the facts that not only do we have such a chlorid retention, but that the presence of chlorids in the food will increase both the albuminuria and the edema of these conditions. While these facts are incontrovertible, we must take into consideration, as Richter has shown, the amount of water intake as well. This is such an important field to the clinician that the writer would refer to other works giving the details of the "*dechloridization*" treatment and its effects in reducing the symptoms of a nephritis.

A severe diarrhea will also diminish the amount of chlorids in the urine, as the chlorids of the food are carried off by the bowel too quickly to permit of absorption. In cases of carcinoma of the stomach, in dilatation either from hypersecretion or stricture of the pylorus, and in some cases of ulcer of the stomach a diminution or even total absence of chlorids in the urine may be observed.

In most chronic diseases, in anemic conditions, in rickets, and in marked nervous diseases, such as melancholia or mania, the amount of chlorids may be greatly reduced. If the output of chlorids be very low in a chronic disease, the prognosis becomes grave unless the diet can explain the diminution.

A diminution is observed in most febrile diseases, especially in the exanthemata, while in typhoid fever the reduction is not so marked. This slight diminution in typhoid fever may serve as a distinguishing point in the diagnosis of meningitis from typhoid fever, in the former case the diminution being much more marked than in the latter. In acute yellow atrophy of the liver the chlorids are diminished, while in cirrhosis of the liver they are somewhat increased.

The Chlorids are increased in all conditions which have previously shown a retention, according to the law which has been previously discussed. We find thus an increase in the period of convalescence from acute febrile diseases, especially pneumonia. Strangely enough, the chlorids are found markedly increased in diabetes insipidus, which is associated also with the excretion

of a large amount of urine. In epilepsy an increase may be observed following the attack.

The chlorids are increased in the urine after the use of chloroform, whether administered internally or as an anesthetic. Some of the diuretics, especially potassium acetate, produce an increase in the urinary chlorids.

In metabolic work it is frequently of advantage to study the effects of an *ash-free diet* upon the pathologic condition. Taylor[1] has recently introduced such a diet, consisting of the whites of 18 eggs, 120 grams of olive oil and 200 grams of crystallized sugar. Little work has been done under the influence of such a diet so that no definite conclusions can be drawn at present. The work of Goodall and Joslin[2] with this diet confirms the earlier views that it is practically impossible to diminish the chlorin of the body by more than 10 to 14 per cent. and that the loss of water is proportionate to this.

Estimation of the Chlorids.

For rough clinical purposes the amount of chlorids in the urine may be estimated as follows: A few c.c. of clear, filtered urine, fromwhich albumin if present is removed by heating, with acetic acid are placed in a test-tube and acidified with 10 drops of chemically pure nitric acid. This mixture is then treated with a few drops of 10 per cent. silver nitrate solution. If the chlorids are present in normal amount a distinct, curdy white precipitate will settle out. If the chlorids be increased a heavy precipitate will be observed, while if they be diminished only a cloud without any flakes will be seen.

Quantitative Determination.

The best method for such determination is, in the writer's opinion, the Arnold modification of Volhard's method. The **principle** of the test is the precipitation of the chlorids in a definite amount of urine by a standard solution of silver nitrate in the presence of an excess of free nitric acid. If the precipitate of silver chlorid be filtered from the solution, the excess of silver nitrate may be determined in the filtrate by titration with a standard solution of potassium sulphocyanate, using a strong solution of iron-ammonium-alum as an indicator. The urine should be as fresh as possible and should contain no nitrites. Albumin, unless present in very large amount, need not be removed. It is wise, however, in case the urine shows a high albumin content, to acidify the urine with acetic acid, boil, and filter off the precipitated albumin. In doing this one should take a definite volume of urine, precipitate as above, and wash the precipitate thoroughly with water in order to dissolve any chlorids which may have been retained by the albumin. The filtrate is made up to a definite volume, which represents the amount of urine originally taken. Thus, 20 c.c. of urine are treated as above and washed with sufficient water to make 50 c.c. In the test as outlined later,

[1] Univ. of Cal. Pub., Pathology, vol. 1, 1904, p. 71.
[2] Arch. of Int. Med., vol. 1, 1908, p. 615.

in which 10 c.c. of urine are used, 25 c.c. of this filtered albumin-free urine will represent 10 c.c. of original urine.

Solutions Necessary.

(1). A solution of silver nitrate of such a strength that 1 c.c. is equivalent to 0.01 gram of NaCl or 0.00606 gram of Cl. In making this solution 29.055 grams of pure anhydrous crystallized silver nitrate are dissolved in 1 liter of distilled water. The chemically pure $AgNO_3$ as found on the market is perfectly reliable and needs only an accurate chemical balance for weighing the exact amount. It is essential that this solution should contain exactly the amount specified, as the accuracy of the method depends upon the correct strength of the volumetric solutions.

(2). A solution of potassium sulphocyanate of such a strength that 20 c.c. will correspond exactly to 10 c.c. of the silver solution or, in other words, so that 2 c.c. of the cyanate solution are necessary to precipitate the silver from each c.c. of the silver nitrate solution. Other workers use solutions of potassium sulphocyanate of somewhat different strength, but the simplicity of the calculations necessary to determine the chlorids of the urine is much increased by such a relation between the two volumetric solutions. As potassium sulphocyanate is very hygroscopic, it is impossible to accurately weigh the exact amount necessary to make this solution. We, therefore, dissolve a slight excess (9 grams) of potassium sulphocyanate in approximately 1 liter of water. In order to make this solution correspond exactly to the silver solution it is necessary to find out how much water must be added to make 20 c.c. of this neutralize 10 c.c. of the silver solution. The technic is as follows: ten c.c. of the known solution of silver nitrate are measured from a buret and diluted with 50 or 60 c.c. of distilled water. Five c.c. of chemically pure nitric acid (specific gravity 1.2) and 5 c.c. of a strong solution of iron-ammonium-alum are added and thoroughly mixed. This mixture is then titrated with the potassium sulphocyanate solution whose strength is to be determined. The principle of this titration is that the KCNS first combines with the $AgNO_3$, forming a white precipitate of silver sulphocyanate. At the exact point at which this combination is complete the potassium sulphocyanate will combine with the iron of the indicator forming sulphocyanate of iron which is distinctly red in color. The titration is, therefore, carried to the point at which a permanent faintly reddish-brown color appears on shaking the mixture. The number of c.c. of the sulphocyanate solution necessary to produce this end point is then read off from the buret and we are ready for our correction. As the sulphocyanate solution was intentionally made too strong, the titration should yield fewer than 20 c.c. of this solution. Supposing 18.5 c.c. of sulphocyanate solution were used we must obviously add to every 18.5 c.c. of the remaining sulphocyanate solution 1.5 c.c. of water according to the equation $C=\frac{N\times d}{n}$ in which C represents the number of c.c. of water which must be added to the remaining solution; N the total

number of c.c. remaining after titration; *n* the number of c.c. consumed in one titration, and *d* the difference between the number of cubic centimeters theoretically required and that actually used in one titration.

The calculation would, therefore, run as follows:

$C = \frac{981.5 \times 1.5}{18.5} = 79.58$. We must, therefore, add to the remaining 981.5 c.c. of potassium sulphocyanate solution 79.58 c.c. of water to make the sulphocyanate solution of such a strength that 20 c.c. will exactly precipitate the silver from 10 c.c. of the $AgNO_3$ solution.

(3). A cold saturated solution of iron-ammonium-alum. This must be absolutely chlorin free.

(4). Chemically pure nitric acid, chlorin-free and having practically no trace of nitrous acid. Specific gravity 1.2.

Technic.

Ten c.c. of urine or 25 c.c. of the diluted urine from which the albumin has been removed are accurately measured with a pipet and placed in a 100 c.c. volumetric flask. Five c.c. of nitric acid, 50 c.c. of water, and 20 c.c. of the standard silver solution are then added and the mixture thoroughly shaken. After this mixture has stood for about 10 minutes distilled water is added up to the graduating mark of the flask, after which the whole is thoroughly mixed and the precipitated silver chlorid allowed to settle. This mixture is then filtered through a perfectly dry filter into a thoroughly dry 50 c.c. volumetric flask. This 50 c.c. of filtrate will represent, therefore, only 5 c.c. of urine, but the calculation made later will compensate for this.

This 50 c.c. is then poured into a beaker of about 250 c.c. capacity and the volumetric flask is thoroughly washed out with water, the washings being added to the solution in the beaker. Five c.c. of the alum solution are then added and the mixture titrated with the potassium sulphocyanate solution to the appearance of the first permanent reddish tinge of the solution. The number of c.c. of sulphocyanate solution, necessary to neutralize the excess of silver remaining after the chlorid of silver has been filtered off, is then read off from the buret.

Calculation.

As 20 c.c. of the sulphocyanate solution are equivalent to 10 c.c. of the silver solution, it is evident that the number of c.c. of silver solution not used in the precipitation of the chlorids corresponds to the number of c.c. of sulphocyanate solution necessary to neutralize the 50 c.c. of the filtrate. We, therefore, subtract the number of c.c. of sulphocyanate solution used from 20 (the number of c.c. of silver solution added) and obtain directly the number of c.c. of silver solution necessary to precipitate the chlorids in 10 c.c. of urine. As each c.c. of silver solution represents 0.01 gram of NaCl or 0.00606 gram of Cl, multiply these factors by the number of c.c. used, the percentage of chlorids in the urine being obtained by multiplying the amount of chlorids

in 10 c.c. by 10, and the total amount by simply multiplying this figure by the number of hundreds of c.c. in the total 24-hour specimen of urine.

If the urine is very highly colored it is advisable to add a few drops of a concentrated solution of potassium permanganate before the titration. This will usually decolorize the urine so that the end point will be much more distinct.

Purdy's Centrifugal Method.

This method, while having nothing in common with the accuracy of the preceding one, is very convenient and has the advantage of yielding quick results which are clinically available.

Ten c.c. of clear, filtered, albumin-free urine are placed in a centrifuge tube which is graduated to 15 c.c. One c.c. of strong nitric acid and 4 c.c. of a 5 per cent. solution of silver nitrate are then added. The tube is shaken by inversion and the mixture allowed to stand for a few minutes, after which it is placed in the centrifuge and whirled for three minutes at the rate of 1,200 revolutions per minute. The bulk percentage of silver chlorid is then read off, from which the percentage by weight both of sodium chlorid and of chlorin, equivalent to the precipitated silver chlorid, may be calculated. One per cent. by bulk represents 0.13 per cent. by weight of NaCl and 0.08 per cent. of Cl.

As previously stated, the amount of chlorin in the urine depends upon the amount ingested, ranging normally between 10 and 15 grams. By the use of Folin's standard diet, which contains 6.2 grams of Cl, the excretion is found to be 6.1 grams of Cl in 24 hours. On the ash-free diet of Taylor the excretion at the end of 12 days of such a diet was 0.17 gram of Cl in the 24 hours.

(2). Phosphates.

The phosphates occurring in the urine are the sodium, potassium, calcium, and magnesium salts of the tribasic orthophosphoric acid (H_3PO_4). As previously stated, in the discussion on the reaction of the urine, normal, clear, acid urine contains no dibasic monacid phosphates, but all of the phosphates under these conditions are of the monobasic diacid type. If the urine becomes less acid or amphoteric in reaction we find, however, in addition to the above, the disodium monohydrogen phosphate, the monocalcium phosphate, and the monomagnesium phosphate; while if the urine be alkaline we may find the neutral phosphates in the ascendency. It must be remembered, therefore, that the normal acidity of the urine is not strictly regulated by variations in the relative proportions of the monosodium dihydrogen phosphate and of the disodium monohydrogen phosphate as usually stated. Besides these mineral phosphates, phosphoric acid is found in the urine in combination with glycerin as glycero-phosphoric acid, which is derived largely from the hydrolytic cleavage of lecithin compounds. This organically-bound phosphoric acid does not play a large rôle in the quantitative estimation of the

phosphates, but is of some importance in the metabolic study of many nervous diseases.

The larger portion of the urinary phosphoric acid is derived from the food, the smaller portion coming from the metabolism of the tissue protein, especially the nucleins. This endogenous phosphoric acid may be of special importance as variations will be found depending upon the degree of destruction of the lecithin and nuclein compounds. It is to be remembered here that not all of the phosphoric acid ingested is excreted, as between a fourth and a third of the total quantity may remain in the feces in combination with calcium. In studying the effects of increased ingestion of phosphates, the feces must, therefore, be examined quite as closely as the urine.

Physiologic Excretion.

The amount of phosphoric acid excreted in the 24 hours is always expressed in terms of P_2O_5. The normal P_2O_5 excretion of the adult varies from 1 to 5 grams with an average of about 3.5 grams. The figures of Folin, based upon a diet containing 5.9 grams of P_2O_5, show this excretion to average 3.87 grams in 24 hours, while patients on an ash-free diet eliminate approximately 0.75 gram. In this excretion the phosphates of sodium and potassium usually exceed those of calcium and magnesium, the former being excreted in the amounts of 2 to 4 grams in the 24 hours, the latter from 1 to 1.5 grams. Little data exists regarding pathologic variations in the relation of these two types of phosphates so that no conclusion may at present be drawn. The excretion of P_2O_5 will vary with the food, especially with the amount of calcium and magnesium of the food. These bases combine in the intestine with the phosphoric radical forming phosphates which are difficultly soluble. This fact is taken advantage of by Croftan in the administration of calcium salts to precipitate the phosphates and thus diminish their activity in conditions attributable to uric acid.

The phosphates are increased on an animal diet and diminished on a vegetable diet as Zülzer has shown. During starvation an increase of the phosphates may be observed, as an indication of decomposition of the tissues. Administration of phosphates at this time will usually lead to a retention to counterbalance the previous loss. This same fact was observed in the discussion of the chlorids and may be stated as a general law, that an increased excretion is followed by a retention and a retention by an increased elimination. It must be stated, however, that an insufficient supply of phosphoric acid is not compensated for by such a great retention of phosphates as of the chlorids. The organism eliminates even more phosphoric acid in starvation than in cases of deprivation of salt, as the decomposing protein sets free the salts bound up with it. Many attempts have been made to determine where and in what form phosphoric acid is retained in the body and where and from what sources the body draws upon it for excretion. It is a difficult matter to determine what amount of the phosphoric acid retained reaches the bones,

what portion is devoted to the soft tissues, and how much of it remains organically combined in the body (Magnus-Levy). It has been found that the relation between the excretion of phosphoric acid and nitrogen is normally about one to seven, the same relations which exist between the amount of nitrogen and phosphoric acid in the human muscular tissue. It is, therefore, plausible to assume that a retention of both nitrogen and phosphoric acid will lead to a deposition of increased flesh. In starvation we find that this relation is markedly disturbed, the phosphoric acid being both relatively and absolutely increased. Such being the case the loss of P_2O_5 must be largely sustained by the bones, which are relatively poor in nitrogen. For a full discussion of this subject the writer would refer to von Noorden's work on Metabolism and Practical Medicine,[1] which gives great detail regarding all phases of metabolism.

The phosphates are increased during hard muscular exercise, while mental exercise seems to lead to a diminished excretion of the alkaline phosphates and an increased output of the earthy phosphates. The ingestion of large quantities of water is frequently associated with an increased elimination of the phosphates, although this is later followed by a slight retention.

It not infrequently happens that a freshly voided urine shows a marked turbidity and even precipitation due to the deposit of earthy phosphates. This has been supposed to be due to an increased output of the phosphates, but it is now known to be nothing but the natural consequence of a change of urinary reaction from acid to alkaline. This condition which has been called "*phosphaturia*" would, therefore, much more appropriately be styled "*alkalinuria*." This subject will be discussed in a later section to which the reader is referred.

Pathologic Variations.

A **diminished elimination** may be observed in cases of acute febrile disease, especially at the height of pneumonia. The degree of diminution is usually proportionate to the severity of the disease and usually lessens as convalescence comes on. According to Gouraud, the earthy phosphates are considerably reduced in pneumonia, while in tuberculous conditions the phosphates are increased, an interesting point in differential diagnosis. This retention in pneumonia as well as in the other acute febrile diseases is possibly due to the renal insufficiency which may be very great in such conditions. This diminished phosphatic excretion may not always obtain in the acute febrile conditions, in some cases a sudden increased output being observed. In typhoid fever Robin believes an increased elimination during the febrile rise to be an unfavorable sign, while an increase during defervescence indicates a favorable prognosis.

The phosphates appear to be diminished in most chronic diseases. In all renal diseases, whether acute or chronic, a diminished excretion is present

[1] Chicago, 1908, Keener.

due to the renal insufficiency. This diminished phosphoric acid excretion is regarded by Purdy[1] as a factor almost as constant as is the excretion of albumin. In gout the phosphoric acid excretion runs parallel to that of uric acid, decreasing immediately preceding the acute attack and rising as the attack subsides. In cases of pregnancy a diminished excretion is observed which is attributable to the withdrawal of phosphoric acid from the maternal organism for the purpose of the fetal bone formation. In certain bone diseases, such as osteomalacia, a diminished excretion is usually observed, although at times an actual increase is seen. The earthy phosphates, especially are diminished in these latter conditions while the alkaline phosphates may be increased. In cases of myositis ossificans the excretion of inorganic phosphates does not seem to be much affected as one might expect from the new bone formation.

In cases of hystero-epilepsy the phosphates are diminished, the diminution usually being proportionate to the intensity of the attack, while in true epilepsy the phosphates appear to be more or less markedly increased. It is in just the nervous diseases that one would expect to find much variation in the phosphatic excretion, but very few data are found bearing on this subject. Folin and Shaffer find that in the periods of nervous excitement the relative amount of phosphoric acid is diminished, but that the absolute amount is little changed.

In Addison's disease, hepatic cirrhosis, acute yellow atrophy, and chronic lead-poisoning we may find an extensive decrease of phosphates in the urine.

In certain cases which show most of the symptoms of diabetes mellitus without any sugar output, a **phosphatic increase** is observed in the urine. This condition has been called "*phosphatic diabetes*," and may be associated with the excretion of as high as 10 grams of P_2O_5 within 24 hours. In true diabetes mellitus the phosphates may be increased at one time and diminished at another, as there seems to be an inverse ratio between the excretion of sugar and that of the phosphates.

The phosphates seem to be increased in cases of pseudoleukemia, leukemia, hemorrhagic purpura and in cases of acute or chronic inflammatory processes of the genito-urinary tract.

As previously stated, the output of urinary nitrogen bears a relation of about seven to one to that of the phosphate excretion. This relation has been termed the "*relative value*" of phosphoric acid and represents the amount of P_2O_5 corresponding to 100 grams of N. Normally this ranges between 15 and 20.

Estimation of Phosphates.

Ten c.c. of urine are rendered alkaline with ammonia. The earthy phosphates are precipitated in the form of a flocculent precipitate and may be roughly estimated by the volume of the precipitate.

[1] Practical Urinalysis, Phila., 1900, p. 56.

If this alkalinized urine be filtered and the filtrate acidified with acetic acid, the addition of a few drops of ferric chlorid or of uranium nitrate solution will precipitate the alkaline phosphates.

These methods are purely qualitative and can have no clinical value beyond giving a general idea of the relative amounts of the earthy and alkaline phosphates. Instead of ferric chlorid or uranium solution, magnesium mixture may be used for this purpose.

Quantitative Determination.

The best method for the estimation of the urinary phosphates is that of titration with uranium nitrate or acetate solution. The *principle* of this method is that phosphoric acid compounds in acetic acid solution give, on treatment with uranium nitrate, a yellowish-white flocculent insoluble precipitate of uranium phosphate (UO_2HPO_4). As a means of recognizing the point at which an excess of uranium solution is present in the titrated fluid, one may use either a solution of ferrocyanid of potassium which gives a distinct browish color at the end point, or, preferably, a few drops of tincture of cochineal, which gives a grass-green color and has the advantage that it can be added directly to the titrated fluid, which is not the case with the ferrocyanid of potassium.

Necessary Solutions.

(1). A solution of uranium nitrate or acetate of such a strength that 20 c.c. shall correspond to 0.1 gram of P_2O_5. It is a matter of absolute indifference whether the acetate or the nitrate be used, but the writer prefers the nitrate as this is more easily obtained in the pure state. In making up a solution of uranium nitrate of the above strength, one may not rely implicitly on the weighing, as the uranium nitrate may contain impurities or excess water and thus vitiate the results. It is, therefore, necessary to have a standard phosphate solution against which the uranium solution may be titrated.

The usual solution recommended by various writers is one of disodium monohydrogen phosphate. This salt varies in its degree of hydration and its solutions do not keep well. Moreover, it is absolutely necessary when this salt be used that a definite amount of it be taken and converted into sodium pyrophosphate, after which a corresponding dilution of the solution must be made to make it of such a titer that every 50 c.c. shall be equivalent to 0.1 gram of P_2O_5. In view of these facts, the writer is accustomed to follow the suggestion of Giles[1] and use chemically pure dihydrogen monopotassium phosphate. This salt crystallizes well without any water of crystallization and does not alter on exposure to the air.

This solution is to be made such a strength that 50 c.c. corresponds to 0.1 gram of P_2O_5, in other words a liter must contain 2 grams of P_2O_5. In order to find out just how much of this salt must be dissolved in a liter of water we must have recourse to a simple calculation. The formula of dihydro-

[1] Sutton's Volumetric Analysis, Philadelphia, 1904, p. 294.

gen monopotassium phosphate is KH_2PO_4, its molecular weight being 136. Two molecules of this salt are necessary to yield one molecule of P_2O_5 according to the equation

$$2KH_2PO_4 = P_2O_5 + K_2O + 2H_2O.$$

If, therefore, one liter of the solution must contain 2 grams of P_2O_5, the amount of KH_2PO_4 which must be dissolved in a liter is easily calculated from the following proportion:

$$272 : 142 :: x : 2. \quad x = 3.83.$$

We, therefore, dissolve 3.83 grams of dihydrogen monopotassium phosphate in 1 liter of water and obtain directly a solution which contains 2 grams of P_2O_5 or one in which every 50 c.c. is equivalent to 0.1 gram of P_2O_5. It is

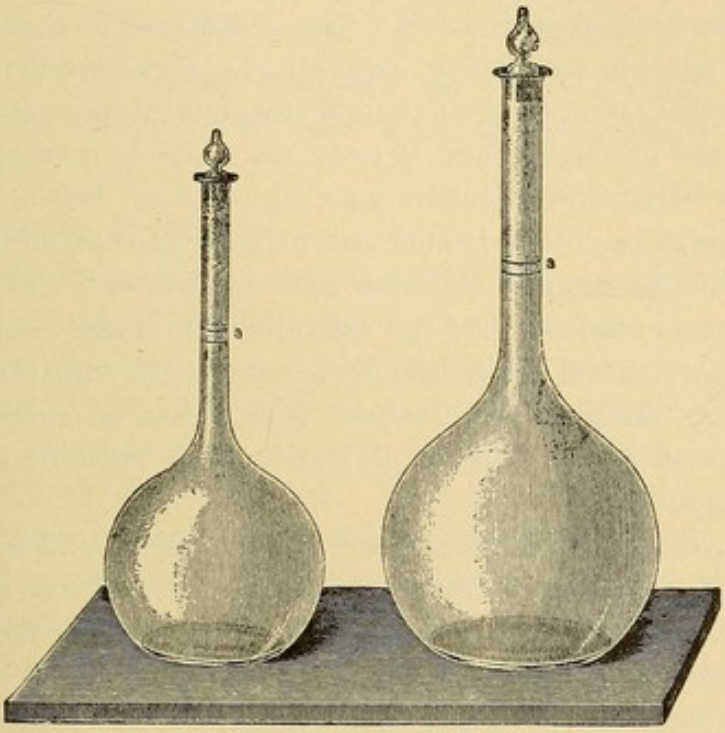


FIG. 70.—Volumetric flasks.

perhaps, needless to add that this solution should be made in an accurately standardized volumetric flask, and at the temperature at which the flask is calibrated.

Having thus obtained our standard phosphate solution, we are now in a position to make up our standard uranium nitrate solution, the titer of which must be such that 20 c.c. corresponds to 0.1 gram of P_2O_5, or, in other words, one liter of which must be equivalent to 5 grams of P_2O_5. The formula of uranium nitrate is $UO_2(NO_3)_2 6H_2O$, its molecular weight being 503.7. Uranium nitrate combines with dihydrogen potassium phosphate according to the following equation:

$$UO_2(NO_3)_2 + KH_2PO_4 = UO_2HPO_4 + HNO_3 + KNO_3.$$

As seen above, two molecules of the dihydrogen phosphate are necessary to yield one molecule of P_2O_5. We will, therefore, have, when uranium nitrate acts upon the dihydrogen phosphate, only the equivalent of 1/2 molecule of P_2O_5; that is, 71 parts. As the uranium solution must contain 5 grams of P_2O_5 to the liter we may then calculate how much uranium nitrate is necessary to form the equivalent of such a solution by the following proportion:

$$503.7 : 71 :: x : 5. \quad x=35.47.$$

Were we absolutely certain of the purity and state of hydration of our uranium nitrate, all that would be necessary would be to weigh out this exact amount. As this is not the case, we weigh out a slight excess (35.75 grams) and dissolve in one liter of distilled water. We are now ready to determine the strength of the uranium solution as follows:

Fifty c.c. of the dihydrogen monopotassium phosphate solution are placed in a beaker and treated with a few drops of tincture of cochineal and 5 c.c. of acetic acid mixture (see below, solution 3). Some workers prefer the addition of potassium ferrocyanid as an indicator, but this does not give as distinct a contrast at the end point, and if tests are made by adding a drop of the mixture to the ferrocyanid solution on a white plate, loss of substance must occur. This mixture is then heated and titrated, as soon as the boiling point is reached, with the uranium solution until a trace of a distinct green color becomes permanent on stirring the mixture. Duplicate determinations are then made, the results of which should agree exactly with the original. The number of c.c. of uranium solution used is then read off and we are prepared for the calculation of the amount of water which must be added to standardized the solution.

As 20 c.c. of this uranium solution should correspond exactly to 50 c.c. of the standard phosphate solution, we may, for the sake of example, use the same figures given for obtaining the dilution in the case of the sulphocyanate solution discussed under the heading of Chlorids. Thus if 18.5 c.c. of uranium solution were used we must add, according to the previous explanation, 79.58 c.c. of distilled water to the remaining 981.5 c.c. of uranium solution in order to make every 20 c.c. equivalent to 0.1 gram of P_2O_5.

(2). An acetic acid mixture prepared by dissolving 100 grams of sodium acetate and 30 grams of glacial acetic acid in sufficient water to make 1000 c.c. This solution must be added in the determination of the urinary phosphates in order to overcome the influence of the nitric acid liberated in the reaction and to convert any monacid phosphates into the diacid type.

(3). An indicator, preferably tincture of cochineal prepared by digesting the ground cochineal bugs in 25 per cent. alcohol and filtering. This indicator has the advantage that it may be added directly to the solution to be titrated, while potassium ferrocyanid must be used by the plate method of adding a few drops of the solution to the indicator after each addition of uranium solution.

Technic.

Fifty c.c. of clear filtered urine are placed in an Erlenmeyer flask and treated with 5 c.c. of the acetic acid mixture, for the purpose of transforming any monacid phosphates into the diacid form and of neutralizing the nitric acid formed during the titration. A few drops (5 to 10) of tincture of cochineal are added, the mixture heated to the boiling-point, and then titrated as described above. It is wise to invariably run duplicate determinations. After each addition of the uranium nitrate the precipitate is allowed to settle so that one may see more clearly the first trace of any green coloration or precipitate.

The calculation is as follows: Supposing 10 c.c. of the uranium solution were used, the corresponding amount of P_2O_5 in the 50 c.c. of urine examined would then be found from the equation:

$$20 : 0.1 : : 10 : x. \quad x = 0.05.$$

The percentage of P_2O_5 would, therefore, be 0.1 (2 x 0.05). If the total 24-hour urine were 1500 c.c., the total P_2O_5 excretion would be obviously 1.5 grams.

Total Phosphoric Acid.

The above determination gives the total P_2O_5 excretion as far as the inorganic phosphates are concerned, but it does not take into consideration the organically-bound phosphorus. This is usually very small in amount, but it is sometimes of value to know whether any variations exist.

This may be determined by incinerating 50 c.c. of urine and determining the total phosphoric acid in the ash. This, however, is not as easily done as might be imagined, owing to the fact that the urinary residue is difficultly incinerated. The writer prefers, therefore, the method of Höhnel-Glaser which will be discussed in the section on Sulphates. The mass fused by this method is dissolved in hot water, the solution filtered and washed with hot water. This solution is then slightly acidified with HCl, treated with the acetic acid mixture, and titrated as in the previous determination. The difference between the figures obtained here and those obtained by direct titration of the urine will yield the amount of organically-bound phorphorus in terms of P_2O_5. This normally varies from 0.01 to 0.1 gram in 24 hours.

Very little literature bearing upon the excretion of the organic phosphorus is found, so that conclusions may not be well founded.

Purdy's Centrifugal Method.

This method cannot be relied upon for accurate results in metabolic work, but may be of some service from the clinical standpoint. Ten c.c. of clear filtered urine are placed in a centrifuge tube graduated to 15 c.c. Two c.c. of 50 per cent. acetic acid, and 3 c.c. of 5 per cent. uranium nitrate solution are then added and thoroughly mixed with the urine by inversion of the tube. The tube is then placed in the centrifuge and operated at a speed of

1,200 revolutions for three minutes. According to Purdy, 1 per cent. by bulk of uranium phosphate equals 0.04 gram of P_2O_5 in each 100 c.c. of urine. Each succeeding percentage by bulk increases by the figure 0.01. Thus a bulk percentage of five of uranium phosphate would equal 0.04 plus 0.04, or 0.08 gram of P_2O_5 in each 100 c.c. These figures are much at variance with those of Ogden, who states that "he has found that each 1/10 of a c.c. of precipitate calculated as P_2O_5 is equivalent to 0.0225 per cent. by weight." Owing to these differences, the writer would suggest that the bulk percentage be stated as such rather than as parts by weight of P_2O_5.

(3). **Sulphur Compounds.**

The sulphur is present in the urine in three forms: (1) preformed or neutral sulphates; (2) ethereal or conjugated sulphates, sulphuric acid in combination with aromatic compounds, and (3) neutral, unoxidized, or organic sulphur. The total output of sulphur depends essentially upon the protein metabolism, both of that of the tissues and of the food. It is to be remembered that the sulphur elimination is much less accurate than that of the nitrogen as an indication of the degree of protein metabolism, owing to the fact that different protein substances vary in their sulphur-content. The daily excretion of sulphur, in terms of SO_3, varies from 1 to 3.5 grams, when the subject is upon a mixed diet. Ordinarily, the ethereal sulphates form about one-tenth of the total output. The neutral sulphur does not vary under normal conditions as far as its absolute amount is concerned, but we note, on changing the diet to one which is relatively free in protein material, that the relative amount of the neutral sulphur is markedly increased. Thus, Folin finds on a diet containing 18.9 grams of nitrogen and 3.8 grams of SO_3 the daily excretion of total SO_3 is 3.31 grams, of which the inorganic SO_3 is 2.92 (87.8 per cent. of total), the ethereal SO_3 is 0.22 (6.8 per cent.), and the neutral SO_3 0.17 (5.1 per cent.). On a nitrogen-free diet, consisting of cream and arrowroot, a total SO_3 excretion of 1.04 grams is noted, of which 0.63 gram (60.6 per cent.) is traceable to the inorganic SO_3, 0.12 (11.5 per cent.) to the ethereal SO_3, and 0.29 (27.9 per cent.) to the neutral SO_3. We are, therefore, confronted with the following fact, "the distribution of the sulphur in urine among the three chief normal representatives, inorganic sulphates, ethereal sulphates, and 'neutral sulphur,' depends on the absolute amount of sulphur present."

On the ash-free diet of Taylor we find, according to Goodall and Joslin, a total SO_3 excretion of 0.96 gram, of which the inorganic SO_3 forms 0.71 gram (74 per cent.), the ethereal sulphates 0.05 gram (5.2 per cent.), and the neutral sulphur 0.2 gram (20.8 per cent.).

It is doubtless true that practically all of the urinary sulphur is derived from protein metabolism, a definite relation being usually established between the nitrogenous and sulphur output. Normally, $N : SO_3 :: 5 : 1$, apparently regardless of whether the patient is on a nitrogen-rich or a nitrogen-free diet; the absolute amounts of each, however, differ markedly, depending upon the

diet. Folin's figures show that as the total urinary sulphur is reduced, the percentage represented by the inorganic sulphates sinks from about 90 to 60 per cent. This fact has been expressed in the above quotation from Folin.

The reduction in the inorganic sulphates must be made up by a relative increase in the other forms of sulphur. The **ethereal sulphates** have for a long time been held to be an accurate index of the degree of absorption of the products of intestinal protein decomposition. There can be no question but that increased intestinal decomposition is associated with increased output of the conjugated sulphuric acids, especially the indoxyl and skatoxyl sulphuric acids. These ethereal sulphates are diminished on a milk diet or on the cream and arrowroot diet of Folin or the vegetarian diet adopted by many of Chittenden's subjects. This is true especially as regards the urinary indican which is absolutely negative in such cases, while the total amount of ethereal sulphate is diminished only about 50 per cent. Such being the case we must have some other than intestinal origin for the large relative increase of the ethereal sulphates under a nitrogen-free diet. I quote from Folin:[1] "(1) The urinary indican is not to any extent a product of the general protein metabolism, is therefore probably, as is generally supposed, a product of intestinal putrefaction, and may consequently be assumed to indicate approximately the degree of putrefaction in the intestinal tract. (2) The ethereal sulphates can only in part be due to intestinal putrefaction, and neither their absolute nor their relative amount can be accepted as an index of the extent to which the putrefaction is taking place in the intestines. (3) The ethereal sulphates, on the contrary, represent a form of sulphur metabolism which becomes more prominent when the food contains little or no protein." Here the sulphuric acid is conjugated with aromatic bodies formed from decomposition of tissue protein.

Pathologic Variations.

The sulphates, as a whole, must be increased in any condition associated with increased protein catabolism. Thus we find in febrile conditions an increased output of sulphur corresponding to the intensity of the process, this increase being followed by a diminution as convalescence comes on. An increased elimination of sulphates has been observed in leukemia, diabetes mellitus and insipidus, progressive muscular atrophy, and following the use of such drugs as morphin, potassium bromid, sodium salicylate and acetanilid. From the clinical standpoint the elimination of sulphates has little practical value, the variation in the amounts of ethereal sulphates and neutral sulphur being the chief factors of value.

While, as shown above, the **ethereal sulphates** are subject to great variation, the indoxyl-potassium sulphate (indican) varying according to the degree of intestinal decomposition, we find some points of clinical interest in their

[1] *Loc. cit.*

study. As the putrefactive processes normally occur below the ileo-cecal valve, any condition increasing such decomposition with a consequent increase of urinary indican would indicate trouble in the lower bowel, more frequently of the chronic type. They are increased in cases showing abnormal intestinal absorption, as, for instance, in typhoid fever, intestinal tuberculosis, peritonitis, and chronic intestinal catarrh. Obstructive jaundice is usually associated with increase in the output of indican as the bile seems to have a great influence upon putrefactive processes in the intestine. They appear to be increased in cholera, while in ordinary diarrhea they are diminished in absolute amount, but may be relatively increased. In cases of gastric hypoacidity associated with bacterial decomposition in the stomach the urine may show a marked increase of indican. In acute nephritis a very intense indican reaction may be obtained, while in the chronic form the amount of indican is usually diminished. In cases of pus-formation almost anywhere within the system an increased elimination of ethereal sulphates may be observed due to the absorption of the products of decomposition of the pus. This point is of some importance in differentiating purulent from non-purulent affections of various organs. The writer has seen a persistent intense indicanuria, associated with middle-ear infection, which cleared up completely after thorough drainage.

Neutral Sulphur.

The neutral sulphur does not have a very definite relation to the amount of sulphur of the intake or to the amount formed in the decomposition of tissue protein. While it is said to vary in amounts representing from 12 to 15 per cent. of the total SO_3 of the urine, this must be true only when the absolute amount of excreted SO_3 is taken into consideration. By this is meant that although the absolute amount of neutral sulphur does not normally vary to any great extent, its percentage relation to the total sulphur varies with the amount of sulphur. Thus Folin finds on an intake of 3.8 grams of SO_3 an output of 0.17 gram of neutral SO_3, while on a nitrogen-free diet the output is 0.2.

The nature of the neutral sulphur of the urine is somewhat uncertain, as we seem to have only two well-established bodies, namely, the sulphocyanates and hydrogen sulphid. The sulphocyanates are derived largely from the absorption of material from the saliva and represent approximately one-third of the total neutral sulphur. The hydrogen sulphid may be regarded as a decomposition product. Besides these we find cysteïn, which is an intermediary product of normal protein metabolism, and tauro-carbamic acid, which is derived from the biliary material. In cases of jaundice we may find as high as 60 per cent. of the sulphur in the neutral form, which may be due to the absorption of this and other biliary material. Traces of chondroitin-sulphuric acid, oxyproteic acid, alloxyproteic acid, and uroferric acid contribute to the neutral sulphur-content of the urine.

The greatest increase of neutral sulphur is probably associated with the presence of cystin which is not normally present in the urine. This is undoubtedly derived from abnormal protein decomposition. The sulphur in the neutral form may reach as high as 30 or 40 per cent. of the total, due to the presence of cystin. The writer will refer to a later section for a discussion of the subject of cystinuria.

The variations in the neutral sulphur of the urine must be regarded as indicative of abnormal metabolic processes which are not associated with variations in the other types of urinary sulphur. As Folin has shown, "the neutral sulphur is not at all due to processes identical or similar to those which give rise to indican. The neutral sulphur represents products which in the main are independent of the total amount of sulphur eliminated or of protein catabolized."

Estimation of Total Sulphur (A).

The method followed by the writer in determining the total urinary sulphur in terms of SO_3 is that of Höhnel-Glaser as modified by Modrakowsky.[1] The technic is as follows: One or two grams of sodium peroxid are placed in a nickel crucible and 50 c.c. of urine added a few drops at a time. It is to be remembered that sodium peroxid reacts strongly in the presence of water so that the student must take great care in the addition of the urine. The mixture is evaporated to a syrup on the water-bath and two or three grams of sodium peroxid added with constant stirring of the mixture. A marked reaction may be observed at this point so that the further treatment must be delayed until the reaction somewhat abates. The crucible is now taken from the water-bath and heated directly over an alcohol flame until a thick brownish mass forms. The fusion is then cooled and dissolved in hot water. Filter the solution and wash the filter with hot distilled water, after which the filtrate is acidified with hydrochloric acid. This acidified filtrate is now heated to boiling and barium chlorid solution added drop by drop until no further precipitation is observed. After the addition of the barium chlorid the vessel is covered with a watch-glass and the contents boiled for one-half hour, after which the solution is filtered, while still hot, through an ash-free filter. If any $BaSO_4$ passes through, the filtrate must be refiltered until perfectly clear. The precipitate of barium sulphate upon the filter is washed first with boiling water and then with hot ammonium chlorid solution in such a way that, in all, five or six additions of ammonium chlorid take place in the course of the first 20 minutes of the washing (Folin). The filter-paper and precipitate are now transferred to a weighed porcelain or platinum crucible, and 3 or 4 c.c. of alcohol are poured in and ignited. This will dry and partially burn the filter paper. The residue is then heated until complete incineration of the filter-paper has occurred and the ash is colorless. It is a wise precaution at this point to add a drop of concentrated sul-

[1] Zeitsch. f. physiol. Chem., Bd. 38, 1903, S. 562.

phuric acid to the ash so that any barium sulphid formed may be reconverted into barium sulphate. The crucible is then cooled and dried in the desiccator and again weighed. The increase in weight of the crucible will represent the amount of $BaSO_4$ formed from the total sulphur of the 50 c.c. of urine. If this figure be multiplied by 0.3429 the result will be the amount of SO_3 in 50 c.c. of urine. This figure may then be multiplied by the appropriate factor to obtain the total amount of total SO_3 in the urine. Thus if the total urine was 1200 c.c., we would obviously multiply the amount of SO_3 in 50 c.c. of urine by 24.

Determination of Total Sulphates (B).

The writer has found that the variations which Folin[1] has introduced into this determination are advantageous and, therefore, adopts his method of estimating the total sulphates as well as the ethereal sulphates.

Folin's Method.

To 50 c.c. of urine in a 200 c.c. Erlenmeyer flask are added 5 c.c. of a 4 per cent. potassium chlorate solution and 5 c.c. of concentrated hydrochloric acid. This mixture is boiled for 5 or 10 minutes when it becomes colorless. Twenty-five c.c. of a 10 per cent. solution of barium chlorid are then added drop by drop to the boiling acidified urine. The addition of the barium chlorid solution should require about five minutes. After the addition of the barium chlorid the mixture is boiled for 10 minutes and the temperature is then reduced somewhat below the boiling-point and maintained for one-half to one hour at this temperature. The precipitate of barium sulphate is then placed while still hot upon a filter whose ash is known, and filtered perfectly clear, every trace of the precipitate being removed from the precipitating flask by means of a rubber-tipped rod and hot water. The washing and further treatment of the precipitate are then the same as described in the preceding determination. The total SO_3 value obtained in this estimation represents both the inorganic and ethereal sulphates. It also includes traces of hydrogen sulphid and of the sulphocyanates which have been oxidized by the potassium chlorate. If the figures obtained in this estimation (*B*) be subtracted from those of the previous determination (*A*) the remainder will represent the neutral sulphur (*C*).

Determination of Ethereal Sulphates (D).

Two hundred c.c. of urine, diluted to one liter if necessary, are measured into a beaker and 100 c.c. of 10 per cent. barium chlorid solution added. The mixture is then stirred and set aside for 24 hours, after which the clear supernatant liquid is decanted into a second dry beaker. This preliminary decantation is necessary, as otherwise the barium sulphate precipitate will pass through the filter. The decanted fluid is filtered and 150 c.c. of the clear filtrate, corresponding to 100 c.c. of urine, are measured into an Erlenmyer

[1] *Loc. cit.*

flask, 10 to 15 c.c. of concentrated hydrochloric acid and 10 to 15 c.c. of 4 per cent. potassium chlorate solution are added and the mixture heated to boiling. The remaining part of the process is exactly the same as in the preceding determination. The SO_3 determined by this method represents the ethereal sulphates together with the small amounts of sulphocyanates and hydrogen sulphid which may be present. If this figure (*D*) be subtracted from the total sulphates (*B*) the result will be the inorganic sulphates (*E*) in the 24-hour specimen of urine.

Purdy's Centrifugal Method.

Ten c.c. of clear urine are placed in a centrifuge tube and to it are added 5 c.c. of barium chlorid mixture, consisting of four parts of barium chlorid, one part of concentrated hydrochloric acid, and 16 parts of distilled water. The tube is inverted to insure mixing of the reagent and urine and is allowed to stand for a few minutes. It is then placed in a centrifuge and whirled three minutes at the rate of 1,200 revolutions per minute. Each percentage of $BaSO_4$ by bulk represents approximately 0.25 per cent. of SO_3 by weight. This result can, of course, not be as accurate as the preceding, but has some clinical advantage.

(4). **Carbonates.**

A freshly voided specimen of urine may contain small quantities of carbonates and bicarbonates and some free carbonic acid. The amount of free carbonic acid varies with the degree of acidity of the urine and the amount of carbonate-forming material in the food. It has been found that vegetable foods are almost always productive of an alkaline urine, owing to the fact that the organic acids of the vegetables may be converted into carbonates and excreted as such. The carbonate which most frequently forms as a sediment in the urine is calcium carbonate which will be treated in a later section.

If the urine be acidified and a stream of air passed through this acidified urine into a vessel containing a solution of barium hydrate, the carbon-dioxid liberated by the acids will unite with the barium forming barium carbonate which may be filtered, dried, and weighed. Such quantitative determinations are rarely made and have practically no clinical value.

(5). **Sodium and Potassium.**

These metals exist in the urine in the form of the oxids Na_2O and K_2O, the former being present in amounts of 4 to 7.5 grams, the latter varying from 2 to 4 grams. The normal relation between the excretion of these products is as 5 to 3.

The sodium is largely derived from the addition of sodium chlorid to the diet, while the potassium is a constituent of most vegetable foods. We find, therefore, that both of these substances depend largely upon the diet in normal cases, while in pathologic conditions the potassium may be excreted in larger amounts. Thus we find in fever the potassium salts predominate

over the sodium compounds up to the time of crisis, after which the sodium salts again assume their normal proportions. Increased exercise as well as increased decomposition of protein from pathologic causes will tend to increase the potassium of the urine.

From the clinical standpoint the quantitative determination of the sodium and potassium of the urine has little significance, so that the writer will refer to text-books of physiological chemistry for such procedures.

(6). **Calcium and Magnesium.**

Both of these alkali-earth metals are excreted in the urine largely in the form of the phosphates. Calculated as the oxids, the calcium excretion (CaO) varies from 0.1 to 0.3 gram and the magnesium (MgO) from 0.15 to 0.4 gram in 24 hours, the relation between the calcium and magnesium excretion being about 1 to 1.5.

The chief source of these compounds is the food, but it must be remembered that only a small portion of these substances is excreted in the urine. Calcium and, to a less extent, magnesium form compounds with phosphoric acid in the bowel and are excreted without being absorbed. Even if these compounds be injected subcutaneously the excretion is largely into the intestine. We should, therefore, study much more closely the calcium metabolism by examination of both the feces and urine.

Little is known regarding the output of either of these metals, but what work has been done is more closely connected with the calcium excretion than that of magnesium. During starvation calcium oxid is increased both relatively and absolutely, its probable source being the bones. It is increased to some extent by exercise and is diminished after the administration of alkalies. In chronic diseases we note an increase due, probably, to inanition. In tubercular conditions the calcium output is occasionally found to be diminished, while some cases of a marked increase have been noted. In diabetes mellitus, as in other conditions associated with an acidosis, the output of CaO may be greatly increased, thus running parallel to the ammonia output. In a case of myositis ossificans studied by the writer there seemed to be no great variation in the calcium output, while the magnesium content of the urine was markedly reduced. Austin has reported similar findings.

Quantitative Determination.

Calcium.

Two hundred c.c. of urine are alkalinized with ammonia and the precipitate which forms is again brought into solution by the addition of the smallest possible amount of hydrochloric acid. Sodium acetate in the form of the crystals is then added until a distinct odor of acetic acid is observed, after which a saturated solution of ammonium oxalate is added, the mixture thoroughly stirred and set aside in a warm place over night. The precipitate is then collected upon a filter and washed with boiling water until no trace of a chlorin reaction is obtained with the washings. The filtrate must be

saved for the determination of magnesium. The precipitate of calcium oxalate is then dried on the filter-paper at 100° C. and is placed in a platinum crucible and burned over the Bunsen flame until the filter-paper is completely ashed. The blast-lamp is then applied and the crucible and contents blasted for 15 to 20 minutes. The crucible is dried in a desiccator and weighed, the increase in weight representing the calcium oxid (CaO) in the 200 c.c. of urine. A simple calculation will yield the amount of CaO in the 24-hour specimen.

Magnesium.

The filtrate and washings obtained in the above determination are treated with one-third the volume of 25 per cent. ammonium hydrate. A precipitate of magnesium ammonium phosphate (NH_4MgPO_4) occurs, which is allowed to settle for several hours, is collected on an ash-free filter, is thoroughly washed with water containing one-third its volume of ammonia, and is dried in the oven at 100° C. The filter-paper and precipitate are placed in a weighed platinum crucible and burned until the filter-paper is completely destroyed, after which the crucible and contents are heated over the blast-lamp for 15 minutes. This heating converts the magnesium ammonium phosphate (NH_4MgPO_4) into Magnesium pyrophosphate ($Mg_2P_2O_7$); one part of magnesium pyrophosphate represents 0.36243 part of MgO. All that is necessary, therefore, is to multiply the amount of magnesium pyrophosphate by the above factor to obtain the amount of MgO in 200 c.c. of urine.

(7). **Iron.**

Iron is practically always present in the urine in organic combination. The amount actually present is very small, being given by Magnier as varying between 3 and 11 mg. to the liter, while Neumann and Mayer find the normal output to average 0.983 mg.

The urinary iron has little clinical significance. It is increased in fever, in malaria, diabetes mellitus, and pernicious anemia, the amounts in some cases running as high as 20 mg. during the 24 hours.

Any method for the estimation of the urinary iron must be very delicate and very accurate. The urine must be completely incinerated, the method of Neumann[1] employing a mixture of concentrated sulphuric and nitric acid being by far the best. For a discussion of the technic the writer will refer to other works.

(*c*). **Organic Constituents.**

(1). **Nitrogenous Bodies.**

(*a*). **Total Nitrogen.**

From the metabolic standpoint the estimation of the total nitrogen of the urine is one of the most important features of its chemical examination. The excretion of total nitrogen varies with the amount of nitrogen of the

[1] Arch. f. Anat. u. Physiol., Physiol. Abth., 1900, S. 159.

food and with the degree of tissue metabolism. Normally, the system so adapts itself to the nitrogenous intake that the excretion of nitrogen, in the urine, feces, perspiration, etc., is equal to the intake. In other words, a normal person is in *nitrogenous equilibrium*. This subject of nitrogenous equilibrium is of such great importance and the factors which influence it so varied that the writer feels that it will be unwise to discuss it briefly for fear of not presenting it clearly to the student's mind. As a full discussion of this subject would be too extensive for the scope of this work the writer must be content with reference to the admirable discussion by Magnus-Levy[1] in von Noorden's Hand-book of Pathology of Metabolism.

The total nitrogen of the urine may be taken as a direct index of the protein metabolism. Upon a starvation diet, or one from which the nitrogenous factors have been eliminated, we find a gradual reduction in the amount of urinary nitrogen. From about the fourth day of starvation the excretion becomes practically constant and continues until severe tissue-decomposition, as a sign of impending death, occurs. If, at this time, a diet rich in nitrogen be given, a certain amount of the intake will be retained, but not all. If this increased nitrogenous diet be continued a certain portion will be retained each day until the system again assumes a condition of nitrogenous equilibrium, the output equaling the intake. It is to be remembered in this connection that the carbohydrates and fats of the diet both have a certain direct influence in diminishing the protein metabolism; in other words, these substances act as protein-sparers.

The normal amount of total nitrogen of the urine, with the subject upon a mixed diet, varies between 10 and 16 grams per day. The work of Chittenden[2] has proven that this is much too high for individuals who desire to get the most out of their system with the least possible work. In other words "physiological economy" is much better subserved by a diet yielding from 5 to 6 grams of total nitrogen. The subjects of his experiments showed normal activities, both mental and physical, and at the end of the experiments felt much better than before them and had gained in weight. Folin[3] in his work has shown that the normal excretion of six subjects, each observed for a period of five days, was 16 grams of total nitrogen on a diet of 119 grams of protein yielding 18.9 grams of nitrogen. On a nitrogen-free diet the excretion averaged 3.6 grams. His work has led him to the following statement which has greatly changed our ideas of nitrogenous metabolism. He says: "It may, therefore, be positively stated, as a principle in the chemistry of metabolism, that the distribution of the nitrogen in urine among urea and the other nitrogenous constituents depends on the absolute amount of total nitrogen present."

The subject of the distribution of the various nitrogenous products of the urine has been much changed by this work of Folin and of Chittenden.

[1] Chicago, 1908.
[2] Physiological Economy in Nutrition, New York, 1905.
[3] *Loc. cit.*

This distribution or, as it is better called, the "nitrogen partition of the urine," varies according to the diet. The following table shows the excretion of the various nitrogenous constituents and their percentage relations to the total nitrogen under a mixed diet and under one which is nitrogen free.

	Excretion in grams.		Percentage of total N.	
	Mixed Diet.	N-Free Diet.	Mixed Diet.	N-Free Diet.
Nitrogen,	16.00	3.60	100.00	100.0
Urea N,	13.90	2.20	86.87	61.7
Ammonia N,	0.70	0.42	4.37	11.3
Creatinin N,	0.58	0.60	3.63	17.2
Uric Acid N,	0.12	0.09	0.75	2.5
Undetermined N,	0.70	0.29	4.37	7.3

It will thus be seen that the total nitrogen excretion of the urine is made up of several factors. The principal points to be gained from the above table are that the urea, on a nitrogen-free diet, is markedly reduced. This reduction must naturally be made up by increase in the other factors. We find the ammonia and especially creatinin markedly increased from the percentage standpoint, although both are absolutely diminished. We have, therefore, a distinctly endogenous nitrogen metabolism as well as an exogenous one. The urea content should, consequently, be considered as of direct importance in estimating the degree of protein tissue metabolism, although not as usually taught. This urea output cannot at the present time be considered as representing from 85 to 90 per cent. of the total endogenous nitrogen, but should be regarded more properly as representing between 60 and 65 per-cent. It is true that the urea as found under mixed diets gives this higher figure, but at least 20 per cent. of this must be placed against useless activity on the part of the system. In other words, the intake of sufficient nitrogen to yield a urea excretion amounting to 85 per cent. of the total N must be considered unnecessary. This is a somewhat enlarged expression of the fact that most people eat much more than is utilized by the system.

It is seen, therefore, that in the general run of urine examinations the urea output does not represent to us the extent of the tissue metabolism, as ordinarily we have not sufficiently controlled the diet. The above table presents a remarkable percentage increase in the amount of creatinin. The figures of Folin show that the absolute quantity of creatinin eliminated, whether upon a nitrogen-rich or on a nitrogen-free diet, is remarkably constant for the same individual. Although this is influenced to a slight extent by the diet, it is so slight that it can be disregarded (see Creatinin).

Physiologic Variations.

An increase in the total nitrogen is observed after a heavy protein meal. As previously stated, in starvation the nitrogen of the urine becomes constant

after about the fourth day. This nitrogen excretion observed in starvation is, however, less than the minimum amount that must be given in the form of protein in order to maintain a nitrogenous equilibrium. As a rule, the amount of protein taken in is much in excess of the requirement of the system, so that the amount excreted probably represents protein which has never become a part of the system. For this reason the fallacy of considering urea as a direct representative of the protein metabolism of the tissues becomes evident. The intake of protein is, in reality, readjusted to suit the actual needs of the body, so that the urea can represent only a portion, about 60 per cent., of the total metabolic activity of the tissue protein. A physiological increase in the excretion of nitrogen is observed in the infant for four or five days after birth. The nitrogen excretion is increased when the intake of water has been greater than normal. This fact should be borne in mind in metabolic experiments in which the intake of water should be quite as much regulated and as well known as the intake of other substances.

Physiologically, a diminished output of nitrogen is observed on a low nitrogen diet and also on a diet rich in carbohydrates and fat, as these latter substances provide the greater part of the necessary energy. The system must then utilize its own protein. Increased exercise is supposed to give a slight increase in the nitrogen output owing to increased muscular activity, but it is to be said that the loss of water through the increased perspiration may be such a factor in diminishing the urinary nitrogen that no increase may be observed. Certain medicaments as quinin and opium will usually diminish the output of nitrogen.

Pathologic Variations.

Increased Excretion.

Perhaps the most marked increase in the urinary nitrogen is observed in the acute febrile infections. This increase is not due to the temperature *per se* nor is there any parallelism between the urinary nitrogen and the degree of temperature. Whether or not this increase in nitrogen can be directly traced to the effects of the toxins produced by the organisms causing the disease is still unsettled. That this cannot be the only element is proven by the fact that Krehl and Matthes have shown that more protein is destroyed in the so-called aseptic fever than is the case in the normal organism under similar conditions, dietetic and physical. In some febrile conditions we find that the elimination of nitrogen may be reduced during the febrile period, while about the time of crisis a very marked output of urinary nitrogen may be observed. This is the well-known epicritical elimination of nitrogen. In the fever associated with acute nephritis the urinary nitrogen is not increased, but is rather diminished owing to the renal insufficiency as well as to the edema which occurs. A toxogenic decomposition of protein is found in cases of carcinoma, pernicious anemia, chronic tuberculosis, leukemia, scurvy, and especially in exophthalmic goiter. In cases of acute yellow atrophy and

phosphorous poisoning the total nitrogen may be increased, but the percentage of urea will be very much diminished.

In cases of diabetes mellitus the nitrogen excretion is usually much increased, due more to the effect of the increased nitrogenous diet than to increased endogenous protein metabolism. Likewise, in diabetes insipidus a large increase in urinary nitrogen may be observed. An increase is occasionally observed in cases of nephritis owing to the large albumin content of the urine. During the progress of absorption of an exudate a very high excretion may be observed, the resolution of a pneumonic exudate, for instance, being easily followed by the variations in the urinary nitrogen.

Diminished Excretion.

A diminution in the nitrogen excretion is usually observed in convalescence from acute and chronic conditions. This is probably due to the attempt on the part of the system to make up for the losses incurred during the active progress of the disease. It may be diminished in conditions in which the absorptive power of the intestine is much reduced. If the oxidative powers of the system are very much reduced as the result of chronic conditions, the urinary nitrogen will usually be much diminished. In cases of nephritis, both acute and chronic, a large diminution in the urinary nitrogen is observed. This is due primarily to the renal insufficiency and to the associated dropsy. In such conditions a marked increase may be observed in the fecal nitrogen, especially when marked diarrhea is a complicating factor. These periods of retention of nitrogen in nephritis may alternate with periods of increased elimination, so that examinations at different periods may show greatly conflicting results. When the water output of the urine is largely diminished, as a result of transudation, of exudation, or of increased perspiration, the total nitrogen may be reduced.

Estimation of Total Nitrogen (Kjeldahl).

The principle of this method is as follows: The nitrogenous constituents of the urine are oxidized by various oxidizing agents into ammonia. This ammonia is converted into ammonium sulphate by the sulphuric acid which is added at the same time as the oxidizing agents. After the preliminary decomposition and oxidation of the organic nitrogen into ammonium sulphate, free ammonia may be liberated by the action of strong sodium hydrate and distilled into a standard acid solution. Knowing the strength of the acid solution, one may then titrate the remaining acid with a standard alkali solution and determine how much ammonia has combined with the acid. One c.c. of tenth-normal sulphuric acid, used by the ammonia liberated in the distillation, represents 0.001404 gram of nitrogen.

Technic.

Five c.c. of urine are accurately measured, either with a pipet or buret, into a Kjeldahl flask of Jena glass of 800 c.c. capacity. Ten c.c. of concen-

trated sulphuric acid and approximately 1 gram of copper sulphate are added, the flask placed in a hood and heated over a low flame until white fumes of sulphuric acid are given off (Gunning's modification). Five grams of potassium sulphate are then added and the mixture heated with an increased flame to boiling for one-half to three-fourths of an hour. The solution should have lost every trace of a yellowish color and should have become by this time a clear bluish-green. The worker should be cautioned regarding the fumes given off in this process and conduct his work only in a hood with a good draft. It is frequently necessary to wash down the carbon from the sides of the vessel by shaking the fluid in such a way that the carbonized material

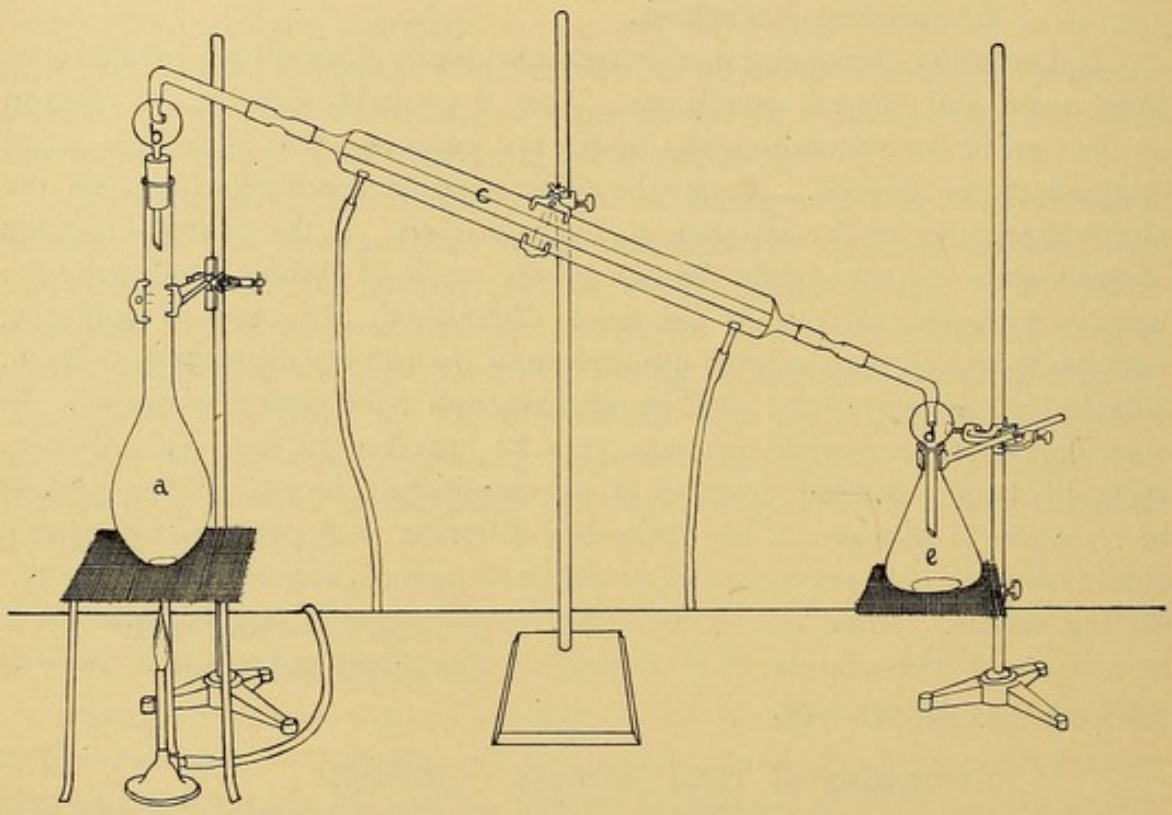


FIG. 71.—Kjeldahl's nitrogen apparatus.

is carried down to the bottom of the flask. One should be cautious lest he lose some of the liquid in this manipulation, which would not only throw out his determination but might result in a very severe burn should any of the material fall upon him. The mixture is allowed to cool completely before the further steps of the determination can be taken. Most workers advise at this juncture the transference of the material from the first flask into a second distilling flask. The writer has convinced himself that this procedure is not only unnecessary but is even unwise, as the transference may result in slight loss of material. He is, therefore, accustomed to use the same flask both for the oxidation and distillation.

After the mixture has cooled the neck of the flask is thoroughly washed with a stream of distilled water so that every trace of material may be carried

from the neck into the body of the flask. Sufficient additional water is added to bring the total up to approximately 250 c.c. A little talcum powder, a few pieces of pumice stone, or a few pieces of granulated zinc may then be added to prevent bumping of the contents when sodium sulphate separates out later in the process. Fifty c.c. of 40 per cent. sodium hydrate are then added for every 10 c.c. of sulphuric acid used in the original oxidation. Care should be taken in adding this strong alkali that none of it touches the upper portion of the neck of the flask. The alkalinized mixture is then shaken and connected with a Fresenius bulb which is attached to a Liebig condenser as shown in the accompanying cut. The outlet tube passes into an Erlenmeyer flask which should contain 50 c.c. of tenth-normal sulphuric acid. In cases with abnormally high nitrogen values it may be necessary to use a larger quantity than 50 c.c. of standard acid, but the writer has found only two instances in over 1,500 determinations in which an increased amount was necessary. The connection of the distilling flask to the bulb and condenser should be done rapidly to avoid any possible loss of ammonia.

The distilling flask is heated slowly at first and the heat increased only after boiling has become regular. If heated too quickly, spurting of the liquid may occur and traces of alkali be carried into the bulb and thence over into the standard acid. The distillation should continue until about 150 c.c. have been distilled over, which will take from 20 to 30 minutes.

The writer has observed that bumping of the mixture rarely occurs, under the conditions outlined above, before the ammonia is completely driven over. This can, however, not be taken as an absolute sign that every trace of ammonia has been distilled off. In order to see whether such is the case, one must test the outlet tube with a piece of moist red litmus-paper which will turn blue in the presence of traces of ammonia. If all ammonia has not been given off, the distillation must be continued until such is the case. If no more ammonia is being evolved, the distilling flask is disconnected from the bulb so that no suction may draw the standard acid into the condenser. The connecting bulb is removed and the tube of the condenser washed with a spray of distilled water so that any material adhering may be washed into the standard acid. The outlet tube is disconnected and washed both internally and externally into the standard acid solution.

The standard acid solution is then titrated with tenth-normal sodium hydrate solution, using cochineal, methyl-orange, alizarin-red, or rosolic acid as an indicator. The writer prefers the use of the latter. As each c.c. of tenth-normal alkali is equivalent to each c.c. of tenth-normal acid, we subtract, from the original number of c.c. of acid (50), the number of c.c. of standard alkali used to neutralize the remaining standard acid. The difference gives us the number of c.c. of standard acid neutralized by the ammonia given off in the distillation. As each c.c. of tenth-normal acid is equivalent to 0.001404 gram of nitrogen, we multiply this factor by the number of c.c. of acid neutralized by the ammonia and obtain the amount of total nitrogen

in 5 c.c. of urine. This result multiplied by 20 yields, of course, the percentage of total nitrogen, which may be changed into the actual total amount of nitrogen by multiplying it by the number of hundreds of c.c. in the 24-hour specimen. It goes, without saying, that the reagents used in this determination must be ammonia-free or, at least, that their ammonia content be known.

(*b*). Urea $(NH_2)_2CO$.

A discussion of the various factors which have to do with urea excretion cannot be taken up at this time. The recent work in the laboratory of Hofmeister[1] as well as that of Chittenden and of Folin has shown us conclusively that it is no longer possible to consider the rate of urea formation as a direct measure of protein metabolism. It is without question true, as Leathes[2] has said, that the nitrogen, or a great part of it, may be removed from the protein, converted into urea, and expelled with the urine before the oxidation of the rest of the protein molecule has been started upon; and the fact that we can trace in the urine excreted in a given time all or the greater part of the nitrogen of the protein taken at a meal, tells us nothing whatever about the fate of that part of the protein which contains, it may be, as much as 80 or 90 per cent. of the total energy of the protein. Further, urea is not a measure of the true protein catabolism, because a great part of it is formed from nitrogen that has never been beyond the liver; and it is not the measure of the protein energy because it is largely derived from protein by reactions which leave the energy value of the molecules from which it originates but little altered. The importance of the denitrifying and desamidization reactions of the tissues must be much more considered in the future than they have been in the past.

As usually stated in text-books, urea constitutes from 80 to 85 per cent. of the total nitrogen output. It has been customary to figure directly the amount of tissue protein which must have been decomposed in order to yield this amount of urea. These figures can hardly be taken as conclusive of such decomposition. Folin has shown that a definite exogenous as well as an endogenous protein metabolism occurs. With a patient upon a nitrogen-free diet, the urea constitutes only about 60 per cent. of the total nitrogen. This would represent the true endogenous urea formation. A diet which requires the patient to eliminate much higher percentages of urea is, therefore, causing increased systemic activity, but is not increasing the direct tissue decomposition, as this excess never becomes a part of the system. One of the most important laws of protein- metabolism is that the amount of nitrogen in the body is not increased by, or not in proportion to, an increase in the nitrogen intake. This field is too extensive to warrant a discussion by the writer, so that he will refer to the work of Magnus-Levy[3] in von Noorden's

[1] Lang. Beiträge zur chem. Phys. und Path., Bd. 5, 1904, S. 340.
[2] Problems in Animal Metabolism, Phila., 1906.
[3] *Loc. cit.*

Hand-book of Pathology of Metabolism and to Leathes[1] lectures on "Problems in Animal Metabolism."

The amount of urea excreted on an average diet varies from 15 to 40 grams. Folin finds this excretion to be, on a diet of 119 grams of protein yielding 18.9 grams of nitrogen, 29.8 grams. On a nitrogen-free diet the amount of urea is 2.2 grams. This excretion will, of course, vary, depending upon the diet. Von Jaksch states that the excretion of urea bears a definite relation to the total nitrogen excretion, so that for clinical purposes direct urea determinations may well be dispensed with, as the correct urea-content of the urine may be found by multiplying the simple nitrogen of the urine by the factor 2. This statement should not be regarded seriously by the practitioner as the rule, as Folin shows, would require that 93.3 per cent. of the total nitrogen in the urine be in the form of urea.

Physiologically the variations in urea will follow those of the total nitrogen so that the previous discussion applies to normal variations in the urea output.

Pathologic Variations.

The pathologic increase in the amount of urea is observed under the same conditions as those mentioned under an increase of total nitrogen. Thus in febrile conditions, in diabetes mellitus and insipidus, after the resorption of an exudate, in maliganant conditions, and in exophthalmic goiter, the urea may be markedly increased.

In conditions associated with destruction of hepatic parenchyma, or with a diminished rate of blood-flow through the liver, the urea excretion may be very considerably diminished. Thus we find in acute yellow atrophy, carcinoma, cirrhosis, and phosphorus poisoning that the normal urea of the urine is replaced by other nitrogenous constituents. The normal function of the liver in converting ammonium compounds and amino acids into urea is so markedly interfered with that the urea may completely disappear from the urine in such cases.

In acute nephritis there may or may not be a diminution in the excretion of urea, depending upon the extent of the renal insufficiency. In the chronic types of nephritis we find that the urea excretion fluctuates to a great extent, periods of increase varying with those of decrease. In the early stages, even though large amounts of albumin and casts be present, the urea may be normal, while in the later stages it is often greatly diminished.

A diminished excretion of urea is observed in melancholia and in the advanced stages of general paresis, while in epilepsy and hysteria an increase or a decrease may be observed. In some cases of diabetes mellitus Hirschfeld has shown that the urea output may be diminished as the result of delayed absorption from the intestine. In these cases of diabetes the ammonia of the urine may be markedly increased owing to its combination with acid bodies and consequent withdrawal from hepatic activity.

[1] *Loc. cit.*

While urea is a very important substance both clinically and chemically, it is very rarely tested by qualitative methods in medical work. The writer feels, therefore, that a description of the properties of this substance would best be learned by consulting works on physiologic chemistry.

Determination of Urea.

The methods for the determination of urea are numerous. Many of them are inaccurate although giving results clinically of importance. In the selection of a method for the determination of urea one must be governed entirely by the importance of the urea determination in any specific case. It should be said in advance that a urea estimation is absolutely useless unless taken in conjunction with the total nitrogen. The general practitioner usually insists on knowing both the percentage and total amount of urea excreted with utter disregard both for the nitrogen of the intake and of the output. If the urea is of any value at all it should be determined with these points in view. The prevailing idea is that a percentage output of two is approximately normal and he therefore bases his conclusion upon an increase or decrease with this as a standard. Not infrequently single voidings of urine are examined for the urea output. Such examinations are worse than useless and may even be harmful.

The methods for the determination of urea are distinctly separable into those useful for purely clinical purposes and those for the more exact metabolic work. The general practitioner, believing as he dose in the importance of urea as an indicator of systemic activity and excretion, must have a rapid and easy method for estimation of urea. It is to be said, however, that the more exact methods would better be applied if reliable conclusions are to be drawn.

Knop-Hufner Method.

The principle of this method is the decomposition of urea by means of sodium hypobromite and the measurement of the nitrogen evolved. Sodium hypobromite acts upon urea according to the following equation:

$$CO(NH_2)_2 + 3NaOBr = 3NaBr + 2N + CO_2 + 2H_2O.$$

The carbon-dioxide evolved in this reaction is absorbed by the excess of alkali used, so that all that is necessary is to measure the amount of nitrogen evolved. This can be done by direct measurement or by collection in tubes which are so calibrated that each c.c. of nitrogen represents a certain percentage of urea.

Various forms of apparatus, which are termed ureometers, have been advised for the estimation of urea by this method. The form introduced by Knop and Hüfner is probably the most accurate, but is too complicated for general clinical purposes. As this method has absolutely no claim to accuracy it is useless in scientific investigations of the nitrogen partition of the urine. For clinical purposes, however, it serves as a rough approximation of the urea output and is, for this reason, largely used by the practitioner.

This method, applied as in the following discussion, is given merely because of the fact that it is almost the only method of estimating urea which can be carried out by the general practitioner.

Doremus Ureometer.

This instrument is seen in the accompanying cut. The graduations of the tube are such that the number of mg. of urea in the 1 c.c. of urine used in the test are directly read off instead of the number of c.c. of nitrogen formed in the reaction. As 0.01 gram of urea in one c.c. represents 1 gram per 100 cc., this tube will furnish directly the percentage values of urea.

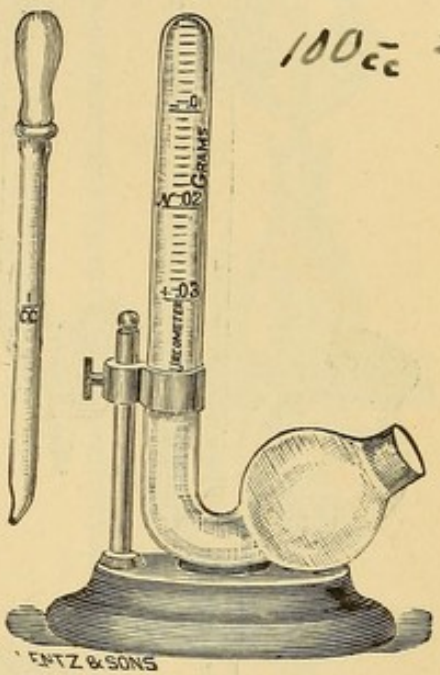


FIG. 72.—Doremus ureometer.

The tube is filled with a solution of sodium hypobromite made by adding 1 c.c. of bromin to 40 c.c. of 20 per cent. cold sodium hydrate solution. This hypobromite solution decomposes after standing for a few days so that it is never wise to attempt to keep such a solution for any length of time. In the writer's laboratory a stock solution of 20 per cent. sodium hydrate is prepared and the bromin added to it only as occasion requires for preparing fresh solutions. In this way one will always have the material at hand and need have no fear of his stock solution decomposing. After filling the tube with this hypobromite solution, 1 c.c. of urine is added by means of the curved pipet accompanying the instrument. In injecting the urine into the solution, the curved end of the pipet should be passed well under the curve of the bulb, the tube tilted slightly forward, and the urine forced into the hypobromite solution with a slow steady pressure. An evolution of gas will be observed at once and will cease in a short time. The carbon dioxid given off by the reaction of the urea upon the sodium hypobromite is absorbed by the excess of alkali and the nitrogen collects in the upper portion of the tube. As soon as the evolution of gas has ceased (5 to 10 minutes), the amount of urea is read off directly from the calibrations of the tube as previously described.

In this determination the urine should be free from both albumin and sugar and should contain preferably not more than 1 per cent. of urea. It is, therefore, wise to dilute the urine before making the test, although the writer has seldom observed variations sufficient to lead him to dilute the urine in all cases. The forms of this apparatus which substitute for the glass foot a wooden base are much to be preferred as they are not so easily broken. The modification of this instrument, as introduced by Hinds, is seen in the accompanying cut. In this form the urine is allowed to run in from the smaller

graduated tube by opening the stop-cock. This modification is an advantage, but does not yield any more accurate results than does the preceding. It is to be repeated that this apparatus is absolutely useless in scientific work, but does furnish a rough and ready method for the use of the general practitioner in his daily determination of the urea excretion.

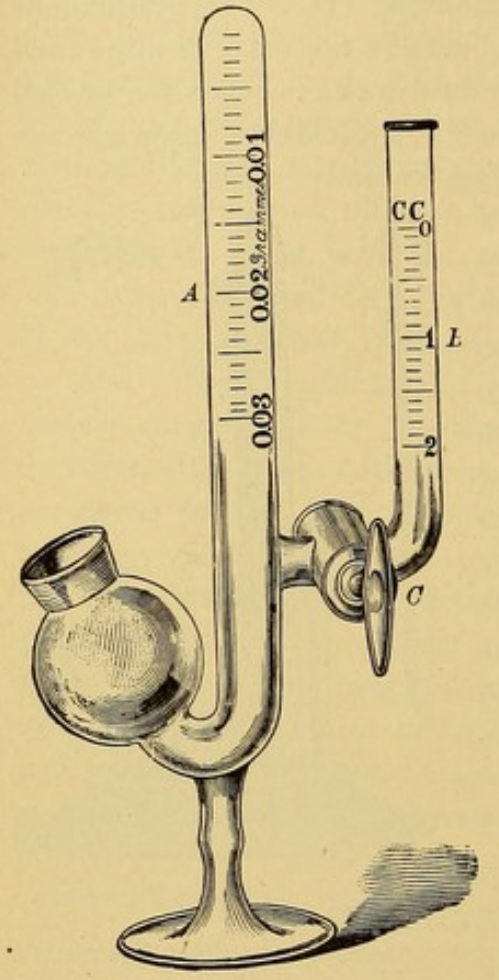


FIG. 73.—Doremus-Hinds ureometer. (*Hawk.*)

Folin's[1] Method.

The principle of this method is as follows: At a temperature of 160° C. crystallized magnesium chlorid ($MgCl_26H_2O$) boils in its water of crystallization. If urea be present it is decomposed by this boiling solution into ammonia and carbon dioxid. If the conversion be carried out in acid solution, the ammonia formed will combine with the acid and may then be liberated by alkalinizing the mixture. The ammonia is distilled into a standard acid solution and may then be determined as given under the total nitrogen. In this process the preformed ammonia as also the trace present in the magnesium chlorid will also be determined so that a separate estimation of these factors must be made and subtracted from the total amount.

Technic.

Five c.c. of urine are measured into an Erlenmeyer flask of about 200 c.c. capacity, 5 c.c. of concentrated hydrochloric acid, 20 grams of crystallized magnesium chlorid, a piece of paraffin about the size of a hazelnut and two or three drops of a 1 per cent. aqueous solution of alizarin-red are added. An especially constructed safety-tube (see cut) is then inserted and the mixture boiled until the drops flowing back from the safety-tube produce a very perceptible bump or hissing sound on coming in contact with the solution (10 to 15 minutes). The temperature is then somewhat reduced and the heating continued for one hour. It is important in this process that the reaction must not remain alkaline, and, therefore, as soon as the material in the flask turns red a very few drops of the acid distillate in the safety-tube are shaken back into the flask. At the end of the hour the contents of the flask are washed into a liter Kjeldahl flask with about 700 c.c. of water. Twenty c.c. of 10 per cent. sodium hydrate are then added and the ammonia distilled, as described under total nitrogen, into a standard tenth-normal solution of sulphuric

[1] Zeitsch. f. physiol. Chem., Bd. 32, 1901, S. 504; *Ibid.*, Bd. 37, 1903, S. 548.

acid. This distillation should be continued until the contents of the liter flask are nearly dry or till the distillate shows no trace of ammonia with litmus-paper. This will require about one hour. The distillate is then boiled to drive off the carbonic acid, is then cooled, and titrated with tenth-normal sodium hydrate to determine the amount of acid which combined with the ammonia formed. Alizarin-red or rosolic acid are used as indicators. One c.c. of tenth-normal sulphuric acid is equivalent to 0.001707 gram of ammonia (NH_3), or 0.001404 gram of nitrogen. If the nitrogen value be multiplied by 2.143 the result will be the amount of urea in the 5 c.c. of urine taken. From the total c.c. of n/10 sulphuric acid neutralized must be subtracted the n/10 sulphuric acid values for the preformed ammonia as well as for the ammonia which may be present as an impurity in the 20 grams of magnesium chlorid used.

It has been found by Schoorl that when carbohydrates and urea are heated together they form very stable condensation products (ureids). For this reason this method of Folin does not give accurate results with saccharin urine. A combination of this method with that of Mörner, which will be described later, will give absolutely accurate results. In the determination of the ammonia values of the preformed ammonia and of the magnesium chlorid, the later methods of Folin must be used.

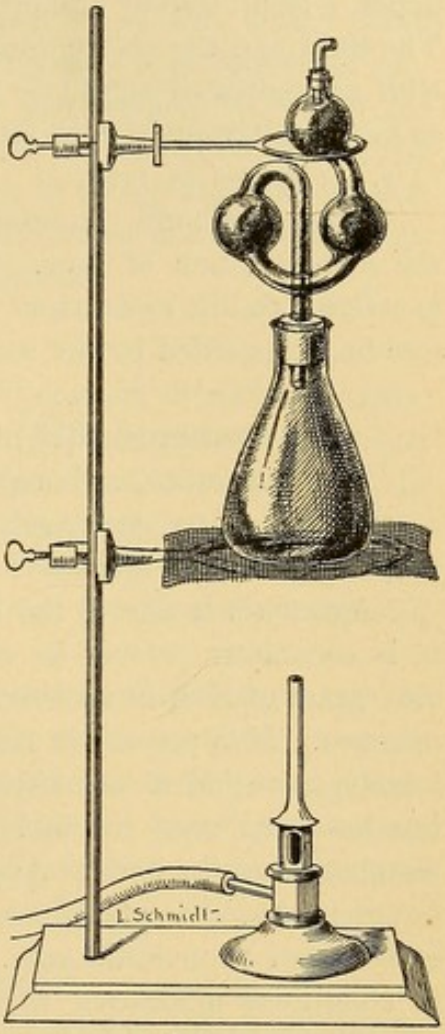


FIG. 74.—Folin's urea apparatus. (*Hawk.*)

Mörner-Sjoqvist Method.

This method[1] is an extremely accurate one, but no more so than that of Folin, except in saccharin urines. If albumin be present it must, however, be removed by heat and acetic acid and the original volume of the urine restored. If the urine contains large amounts of hippuric acid this method may not give accurate results, as Salaskin and Zaleski have shown.

Technic.

Five c.c. of urine are placed in a flask with 5 c.c. of baryta mixture consisting of a saturated barium chlorid solution containing 5 per cent. of barium hydrate. One hundred c.c. of a mixture of two parts of 97 per cent. alcohol and one part of anhydrous ether are then added and the mixture allowed to stand in a closed flask overnight. It is then filtered and the residue washed with fresh alcohol and ether mixture and the combined filtrates evaporated

[1] Skand. Arch. f. Physiol., Bd. 2, 1891, p. 438; *Ibid*, Bd. 14, 1903, S. 247.

at a low temperature (60° C). Urea will be practically the only nitrogenous body left in solution, with the exception of traces of ammonia. When the evaporated filtrate has been reduced to about 25 c.c. in volume, a few c.c. of water and a small amount of calcined magnesium oxid are then added, the mixture stirred, and heated to drive off the ammonia; or the residue may be treated by Folin's method as previously described. This heating is continued until the vapor shows no alkalinity when tested with moistened litmus paper, a result usually obtained when about 10 to 15 c.c. of the mixture remain. The fluid and the residue are then washed into a Kjeldahl flask and treated with concentrated sulphuric acid, copper sulphate, and potassium sulphate as in the determination of total nitrogen. One part of nitrogen is equivalent to 2.143 grams of urea.

Other methods, especially that of Schöndorff[1] have been advised for the determination of urea. These do not furnish, in the writer's opinion, as reliable results as do those of Folin and of Mörner-Sjoqvist, and will therefore be disregarded by the writer.

(*c*). Ammonia (NH_3).

This substance, although chemically belonging in the class of inorganic compounds, is so closely related to the nitrogen metabolism that it is more properly discussed under the heading of Nitrogenous Bodies.

Ammonia is one of the most important products of protein metabolism. It is constantly present in small amounts in normal urine averaging about 0.85 gram of NH_3 in 24 hours, representing from 4 to 5 per cent. of the total nitrogen. It is present in combination with various acids and may represent largely a portion of the nitrogen which has not been transformed into urea, but has been used to combine with acid substances formed in the protein metabolism of the body. Any increase in the production of acid in the system or any increased intake of noncarbonate-forming acids will lead to an increased excretion of ammonium salts. This is an important factor in the metabolism of conditions associated with an acidosis.

The total output of ammonia will vary under normal conditions with the diet or, in other words, with the intake of total nitrogen. While the increase of the total nitrogen of the urine on increased nitrogen intake is largely in the form of urea, yet a small increase in the absolute amount of ammonia must occur. Likewise we observe a diminished intake of nitrogen reducing the absolute value of ammonia, but largely increasing its relative value. Thus Folin finds with a total excretion of 16 grams of nitrogen, an ammonia output of 0.85 gram (4.3 per cent.); while on a nitrogen-free diet a total nitrogen output of 3.6 grams was observed with an ammonia elimination of 0.51 gram (11.3 per cent.). We therefore conclude with Folin as follows: "With pronounced diminution in the protein metabolism (as shown by the total nitrogen in the urine), there is usually, but not always, and therefore not necessarily,

[1] Archiv, f. d. ges. Physiol., Bd. 62, 1896, S. 1.

a decrease in the absolute quantity of ammonia eliminated. A pronounced reduction of the total nitrogen is, however, always accompanied by a relative increase in the ammonia nitrogen, provided that the food is not such as to yield an alkaline ash."

Although the ammonium salts of many organic acids are converted into urea in the system we find the ammonium salts of the sulphuric and phosphoric acids formed in the decomposition of protein material are excreted as such. Moreover, we observe that an increased consumption of fat, either taken in as food or derived from the tissue, is associated with a combination of ammonia with the fatty acids. This provision of metabolism, by which the system is protected against the deleterious effects of increased acidity by neutralization of acid compounds with ammonia, is of the greatest importance, as the fixed alkalies of the tissues are thereby maintained in their usual concentration unless the pathologic processes be extreme.

Pathologic Variations.

An increased output of ammonia is observed in cases of diminished oxidative powers of the system, in febrile diseases, in hepatic disturbances such as carcinoma and acute yellow atrophy, in uremia, in acid intoxication, in dyspnea from any cause, in the toxic vomiting of pregnancy, in delayed chloroform poisoning, and especially in diabetes mellitus. In this latter condition the degree of acidosis may be conveniently followed by watching the ammonia output.

A reduction in the amount of ammonia is observed in many cases of nephritis and in some cases of carcinoma of the stomach, although there is at the same time a diminished excretion of hydrochloric acid in the gastric contents. Edsall reports a reduction in cases of periodic insanity preceding the attack, while a rise is observed as the attack proceeds. Administration of large doses of the fixed alkalies will usually diminish the ammonia output.

Quantitative Determination of Ammonia.

Like the methods given under Urea, many have been advanced for the determination of the urinary ammonia. One of these, though inaccurate, has been so long used and even to-day is so relied upon in many quarters that the writer includes it with the understanding that he advocates only the accurate methods. If the ammonia is worth determining, definite results should be sought and hence the most accurate methods are the ones to be used. They are no more complicated, not as time-consuming, and give more reliable results.

Method of Schlösing.

This method is the one most commonly used, but is open to the objection that it does not yield accurate results and is time-consuming, but has the advantage of simplicity. It is argued by many that the element of time is of no importance, as clinically one would not wait for an ammonia determination

before instituting vigorous treatment. On the other hand, in metabolic work it is of a great advantage to get the work out of the way as quickly as is possible and consistent with accurate results.

Technic.

Twenty-five c.c. of urine are placed in the vessel B (preferably a Petri dish) (see cut). Above this is placed a glass triangle upon which rests a dish (C) containing 20 c.c. of tenth-normal sulphuric acid. Twenty c.c. of milk of lime are then poured into the dish containing the urine and the whole covered with a bell-jar, the borders of which have been well greased to make an air-tight union when the jar is placed upon the glass plate. This apparatus

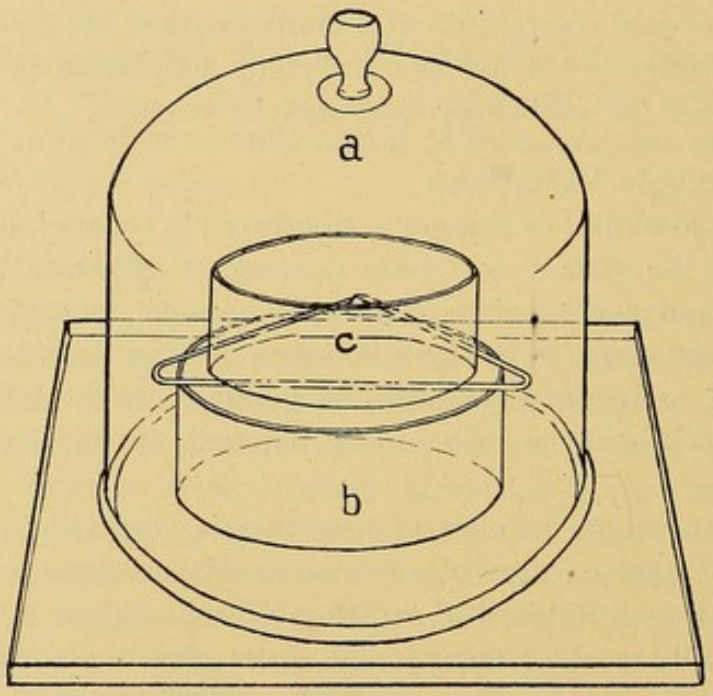


FIG. 75.—Schlösing's ammonia apparatus.

is then allowed to stand at room temperature from four to five days, during which time the ammonia, liberated by the action of the milk of lime upon the ammonium salts of the urine, will be taken up by the sulphuric acid in the vessel C. At the end of this time the bell-jar is removed, the acid titrated with tenth-normal sodium hydrate, and the number of c.c. of remaining acid determined. One c.c. of tenth-normal sulphuric acid neutralized by the evolved ammonia represents 0.001707 gram of ammonia. This figure is multiplied by 4 to obtain the percentage ammonia value. If any moisture is present on the inside of the bell-jar it should be washed into the sulphuric acid before titration.

This method, as previously stated, does not give accurate results owing to the fact that ammonia may be split off from urea and thus give figures which are somewhat high. It has been found that if the apparatus be kept at 38° C., the time necessary for this reaction may be reduced to 48 hours. If we add to the urine instead of the milk of lime, 0.5 gram of sodium carbonate

and about 10 grams of sodium chlorid, no ammonia will be split off from the urea and no decomposition of the urine will occur (Schaffer).

Folin's Method.

The ammonia in this method[1] is set free by the addition of a weak alkali (sodium carbonate), is then removed from the urine at ordinary room temperature by means of a strong air-current, is collected in tenth-normal sulphuric acid and then titrated.

Technic.

Twenty-five c.c. of urine are measured into an aerometer cylinder (30 to 40 cm. high), and about a gram of dry sodium carbonate and some crude petroleum (to prevent foaming) are added. The upper end of the cylinder is then closed by means of a doubly perforated rubber stopper, through which

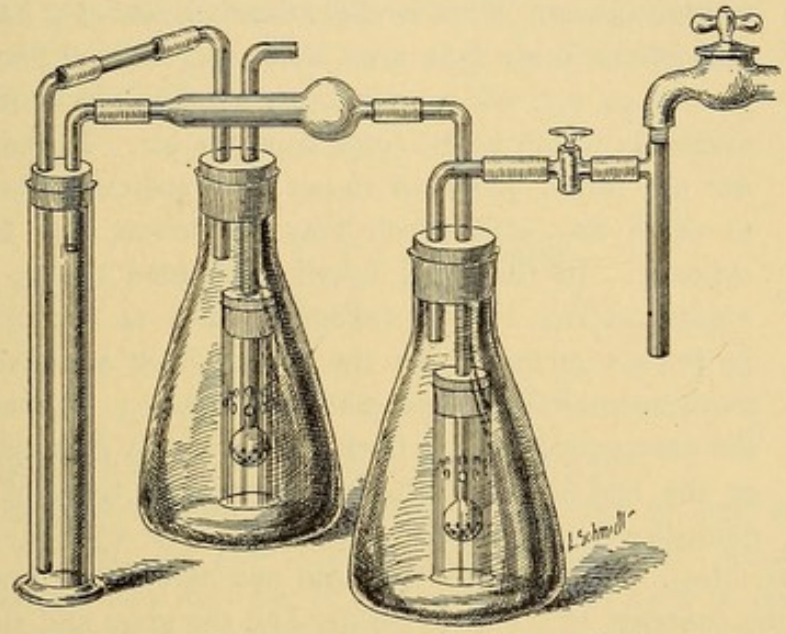


FIG. 76.—Folin's ammonia apparatus. (*Hawk.*)

pass two glass tubes, only one of which is long enough to reach below the surface of the liquid. The shorter tube (about 10 cm.) in length is connected with a calcium chlorid tube filled with cotton, which in turn is connected with a glass tube extending to the bottom of a wide-mouthed bottle (capacity about 500 c.c.) which contains 20 c.c. of tenth-normal sulphuric acid, 200 c.c. of water and a few drops of an indicator (alizarin-red). The complete absorption of the ammonia by the sulphuric acid is most easily insured by the use of a simple absorption tube which compels a very intimate contact of the air coming from the cylinder with the acid and water in the absorption bottle. This absorption bulb consists of a glass tube, measuring about 8 mm., in diameter, one extremity of which has been blown into a small bulb. By means of a heated platinum wire, 10 or 12 holes, each about 1 mm. in diameter, are made in this bulb.

[1] Zeitsch. f. physiol. Chem., Bd. 37, 1902, S. 161; *Ibid.*, Bd. 39, 1903, S. 477.

The absorption bottle is then attached by means of a glass tube and rubber connection to a filter-pump which will draw 700 liters of air per hour. The air passing through the alkaline urine will draw the ammonia into the standard acid in from one and one-half to two hours. In order to exclude any error due to the presence of ammonia in the aspirated air, a similar absorption apparatus is attached to the distal side of the evolution flask. The amount is then determined by titration of the standard acid with tenth-normal sodium hydrate, using alizarin-red as an indicator and titrating to the first red point instead of the violet color.

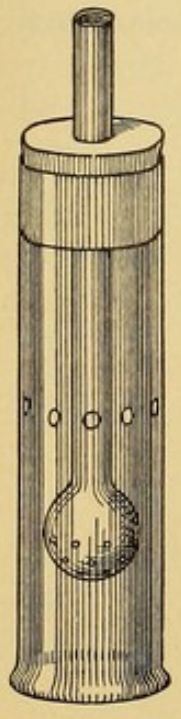


FIG. 77.—Folin's absorption bulb. (*Hawk.*)

Author's Modification.

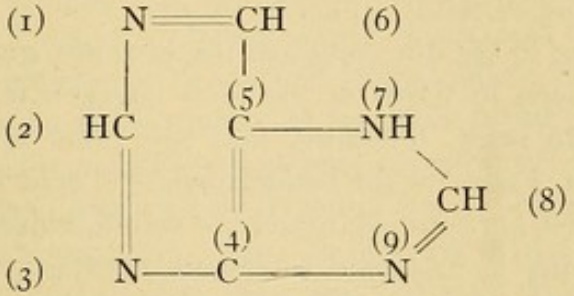
The writer has modified this method of Folin for use in his own laboratory employing compressed air instead of the suction pump. This modification consists of a battery of cylinders of the same type used by Folin. In the first cylinder are placed 50 c.c. of 10 per cent. sulphuric acid to absorb any ammonia which might come from the air. In the second cylinder are found 50 c.c. of 10 per cent. sodium carbonate solution to catch any acid which may be driven over from the first cylinder. In the third vessel are added the 25 c.c. of urine, 1 gram of dry sodium carbonate, and 10 c.c. of petroleum as in Folin's method. In the fourth flask are placed 25 c.c. of tenth-normal sulphuric acid and 200 c.c. of water to absorb the ammonia given off from the urine. A fifth cylinder is stood at the end of the battery to catch any trace of the partially neutralized acid which may have been carried over by the force of the air blast. At the end of one and one-half to two hours the air is shut off and the contents of the last cylinder and the glass and rubber connections washed out by a stream of water which passes into the acid solution. The acid is then titrated and the amount of ammonia determined. One c.c. of the standard acid neutralized by the ammonia is equivalent to 0.001707 gram of NH_3.

(*d*). Uric Acid, $C_5H_4N_4O_3$.

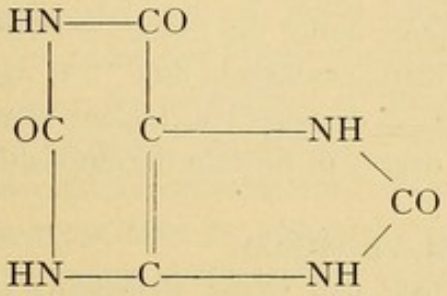
In times past uric acid has been credited with much more clinical importance than is to-day ascribed to it. It is not, as many believe, a product of protein decomposition, as such, but can be derived only by the splitting of the nucleo-proteins. These nuclein bodies are compounds of protein with nucleic acid, the latter constituent splitting up into thymic acid and derivatives of "purin," among which we find uric acid, xanthin, hypoxanthin, etc. The chemistry of these nuclein compounds is more or less complicated, so that the writer cannot take the space to present them in detail.

Uric acid, as well as the purin bases to be discussed later, are derivatives of Fischer's hypothetical purin nucleus, with the formula $C_5H_4N_4$ and the

following graphic structure. The figures in brackets represent the number of the atom or group of atoms:



Each of the purin derivatives is formed by the replacement of one or more of the hydrogen atoms in this nucleus by various atoms or groups of atoms. Thus we find that uric acid is a derivative in which 3 atoms of oxygen have been substituted for the hydrogens in positions 2, 6, and 8. The hydrogen atoms are not replaced, but shift in the direction of the double bonds, passing to the nitrogen atoms 1, 3, and 9. The structure of uric acid with the formula $C_5H_4N_4O_3$ is



Its chemical name is , therefore, 2, 6, 8, tri-oxy purin.

Uric acid is derived from the nucleins of the food, as well as from the nucleins of the tissues. We can conceive, consequently, that we may have a uric acid excretion which may be, in part, referable to each of these factors. This is the basis of the theory of endogenous and of exogenous purin metabolism, the former representing the uric acid formation from the tissue nucleins, the latter that from the food directly. It is self-evident that the exogenous purin metabolism, being directly dependent upon the diet, will vary more than will the endogenous form. This latter type is dependent not only upon the metabolism of the various cellular elements of the body, but also upon the degree of direct synthesis of uric acid in the system, as well as upon the extent of conversion of uric acid into urea. This latter statement must not be interpreted as meaning that uric acid is simply a stage in the conversion of all protein into urea. This idea, which held sway for so long, has fortunately been abandoned. However, we do know, from the work of Frerichs and Wöhler that the system does transform a certain amount of uric acid into urea.

Burian and Schur point out that the uric acid eliminated by man on a purin-free diet (endogenous uric acid metabolism) is for each individual a constant quantity and entirely independent of the total amount of nitrogen eliminated. This fact has so firmly fixed itself in the minds of the profes-

sion that the important results of Folin have been largely unnoticed. This latter worker finds that "when the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent. of the uric acid nitrogen in terms of the total nitrogen is, therefore, much increased." It would seem, therefore, that this point is still very debatable. Quoting again from Folin "if the endogenous uric acid is to be considered as derived from the cell nucleins exclusively, it would, indeed, seem highly plausible that the quantity should tend to remain constant, even with very great variations in diet. Rigid proof that the endogenous uric acid elimination is for each individual a constant quantity would be strong evidence in favor of such a theory. Burian and Schur support the view that the endogenous uric acid is derived from the cell nucleins, but they contend that in man about one-half of the uric acid so derived is destroyed inside the organism and that only the other half is eliminated. With the introduction of this important modification of the nuclein theory, there is no longer any reason why the uric acid elimination should not be a decidedly variable factor which might well be susceptible to change under the influence of many different changes in the conditions, among others, changes in diet." It will be seen, therefore, that one must be on his guard in drawing conclusions from the amount of uric acid in the urine, as to the degree of nuclein decomposition within the system.

Physiologic Variations.

The output of uric acid varies, depending upon the diet, from 0.2 to 2 grams in 24 hours. On a diet of 119 grams of protein with a total urinary nitrogen output of 16 gram the uric acid eliminated was 0.37 gram (6.8 per cent. of total N); while on a nitrogen-free diet with a urinary nitrogen of 3.6 grams the output of uric acid was 0.09 gram (2.5 per cent. of total N). It is increased physiologically by increase in the nucleins of the diet, sweet-breads, liver, kidneys, and brain yielding very large amounts of uric acid. A meat diet will lead to a larger excretion of uric acid than will a vegetable diet, the maximum output being observed about five hours after a hearty protein meal. According to Horbaczewski, this increase is dependent upon the leucolysis which occurs at the time of the disappearance of the digestive leucocytosis. The amount of exercise will also influence the output in the urine; while an intake of a large amount of water will increase the normal uric acid value. A certain relation between the amount of urea and of uric acid excreted seems to obtain. As a rule, it may be said that the nitrogen of the urea is to the nitrogen of the uric acid as 50 or 60 to 1. It is probable that variations in this relation have at present much less clinical value than in times past. It does not seem to the writer consistent to assume a special "uric acid diathesis" if this normal relation be disturbed in the sense that the uric acid is increased, as our knowledge of the factors controlling the excretion of both urea and uric acid is still more or less hazy.

Pathologic Variations.

A pathologic increase of uric acid is observed whenever we have increased protein catabolism. Thus, in fever, the output of uric acid runs parallel to that of urea. In cases associated with marked leucocytosis a large increase in the uric acid output may be noted, which is referable to the constant leucolysis. This increase is especially marked in leukemia, an output of more than 12 grams in 24 hours having been observed by Magnus Levy. In cases of pneumonia, associated with a high leucocyte count, an increase in the uric acid output is seen, being especially marked after the crisis, but may even precede the crisis.

Gout has so long been associated, in the minds of the profession, with increased uric acid formation that such a relationship is generally accepted. It is true that during the acute attack the blood may contain an increased amount of uric acid, but never in such a large amount as in cases of leukemia, for instance. The mere excess of uric acid in the blood can, therefore, not be the determining factor. As our knowledge of the true etiology of gout is so obscure the writer will not attempt a discussion of uric acid in such relations. According to Futcher the uric acid is below the normal standard preceding the acute attack, rises to much increased values during the attack, and again falls below the normal limit after the subsidence of the acute symptoms. In acute articular rheumatism an increased elimination is noted during the febrile period, a decrease being observed as convalescence approaches. In diabetes mellitus an increase or a decrease may be observed, the uric acid excretion varying inversely as that of the sugar, giving rise to the term *diabetes alternans*. In cases showing much degeneration of hepatic tissue, as in cirrhosis and acute yellow atrophy, uric acid may be largely increased.

A diminished excretion of uric acid is usually observed in the ordinary forms of anemia while in pernicious anemia an increase may be noted. Chronic interstitial nephritis, chronic lead-poisoning, purpura hemorrhagica, and some cases of epilepsy, are associated with a diminished output of uric acid. Large doses of quinin or of opium may diminish the uric acid, while salicylic acid, chinic acid, colchicin, urotropin, piperazin, nucleinic acid, etc., may increase the amount.

The chemical properties of uric acid may be found in works on physiologic chemistry. Qualitative tests are frequently desirable for the detection of the nature of certain deposits, as, for instance, renal infarcts. These will be discussed in a later section in association with the description of the various crystalline forms which uric acid may assume in a urinary deposit.

Quantitative Determination.

Folin's Method.

The principle of this method[1] is based upon the precipitation of uric acid, as ammonium urate, by the addition of ammonium sulphate. The urate is then decomposed with sulphuric acid and the liberated uric acid determined by titration with a standard solution of potassium permanganate.

[1] Zeitsch. f. physiol. Chem., Bd. 32, 1901, S. 552.

Technic.

Two hundred c.c. of urine are treated with 50 c.c. of a reagent consisting of 500 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 c.c. of 10 per cent. acetic acid, dissolved in 650 c.c. of water. The mixture is allowed to stand without stirring for about one-half hour. The precipitate of uranium phosphate has then settled and the clear supernatant liquid is removed by siphonage or by decantation. One hundred and twenty-five c.c. of this clear fluid, representing 100 c.c. of urine, are measured into a beaker, 5 c.c. of strong ammonia are added, and the mixture set aside until the following day. The precipitate of ammonium urate, which is produced by alkalinizing the solution saturated with ammonium sulphate, is then filtered off, and washed with 10 per cent. ammonium sulphate solution until the filtrate is practically free from chlorids. The filter is removed from the funnel, opened, and the precipitate rinsed back into the beaker, enough water to make about 100 c.c. being used. The precipitate is now dissolved by adding 15 c.c. of concentrated sulphuric acid. The solution is then titrated with a twentieth-normal solution of potassium permanganate, until the first pink coloration is observed extending through the entire liquid after the addition of two drops of the permanganate solution. Each c.c. of potassium permanganate used corresponds to 3.75 mg. of uric acid. Owing to the solubility of the ammonium urate, a correction of 3 mg. per 100 c.c. of urine must be made. The corrected result gives the percentage of uric acid.

A method of making a twentieth-normal potassium permanganate solution may be found in any work in general chemistry. The student is to be cautioned that a twentieth-normal solution of potassium permanganate, as used in this connection has reference to one of such a concentration that one liter would contain 0.05 gram of available oxgyen for oxidizing purposes. This solution is obtained by dissolving 1.581 grams of pure $KMnO_4$ in one liter of water. As this weighing may not be sufficient, owing to slight impurities in the permanganate, it is wise to titrate this solution against a known twentieth-normal solution of oxalic acid. If the solutions correspond, 1 c.c. of each should be the equivalents. The titration of the oxalic solution is made by measuring out 10 c.c. of oxalic acid solution, diluting to approximately 100 c.c. with distilled water, and adding 15 c.c. of concentrated sulphuric acid. The permanganate solution is then added drop by drop until a faint permanent red color is observed throughout the liquid, which does not disappear on stirring but persists for at least 30 seconds. If these solutions do not correspond the permanganate solution must be diluted according to the formula previously given under the determination of the chlorids in the urine.

Salkowski-Ludwig Method.

This method is not as convenient as that of Folin, but is inserted as it serves as a combined method of determining uric acid and the purin bases at the same time.

The principle is as follows: Uric acid is precipitated, in the form of a

double urate of silver and magnesium, by an ammoniacal silver solution in the presence of magnesium salts. The silver is removed by hydrogen sulphid and the uric acid precipitated by hydrochloric acid, after which it may be estimated by direct weighing or by determination of the nitrogen.

Technic.

Two hundred and forty c.c. of urine are treated with 60 c.c. of magnesium mixture, which is made up as follows: One hundred grams of crystallized magnesium chlorid and 200 grams of ammonium chlorid are dissolved in about 500 c.c. of water, ammonia is added until the mixture smells strongly of this substance and the whole is then made up to one liter. The above mixture is well stirred and immediately filtered through a dry filter-paper into a beaker. Two hundred and fifty c.c. of filtrate (representing 200 c.c. of urine) are then measured off and treated with 10 to 15 c.c. of an ammoniacal silver nitrate solution (one liter of which contains 26 grams of $AgNO_3$ and enough ammonia to dissolve the precipitate formed). The precipitate should be of a flocculent gelatinous nature and of a yellowish color. If the precipitate be white, too much silver chlorid is present and more ammonia must be added. The clear solution above the precipitate should contain an excess of silver chlorid which may be shown by adding a little nitric acid to a few drops of the clear supernatant liquid. This mixture is then filtered, any particles adhering to the beaker being transferred to the filter by means of water and a rubber-tipped glass rod. The residue on the filter is washed with distilled water until the wash fluid shows no trace of silver or of chlorids. The funnel is now placed in the neck of a liter flask, the tip of the filter-paper is perforated with a glass rod, and the precipitate washed into the flask and thoroughly mixed with the water. This solution is made faintly acid by the addition of two or three drops of hydrochloric acid. Three to four c.c. of 10 per cent. copper sulphate solution are then added and the mixture boiled, after which hydrogen sulphid is passed through the hot mixture to precipitate the silver salts. This gas should be passed until the solution is saturated with it, after which the solution is boiled and filtered. The precipitate is washed with hot water and the filtrate, which must be clear and colorless, is evaporated to a small bulk (10 to 15 c.c.). Ten to fifteen drops of hydrochloric acid are then added, the mixture stirred and allowed to stand for a few hours, preferably over night. This addition of hydrochloric acid precipitates the uric acid and leaves the purin bases in solution. The crystals of uric acid are filtered off on a small weighed filter, are washed with water slightly acidified with HCl to such an extent that the total wash-water and filtrate should not be more than 50 to 60 c.c. The precipitate is washed with alcohol, carbon disulphid, and ether, and is then dried and weighed. The difference between the original weight of the filter-paper and that of the paper and precipitate is the amount of uric acid in the 200 c.c. of urine. Owing to the slight solubility of uric acid in acidulated water, a correction of 0.00048 gram must be added for every 10 c.c. of the filtrate and wash-water.

Instead of weighing the uric acid, the filter-paper and contents may be placed in a Kjeldahl flask and a nitrogen determination made as previously described. The nitrogen value multiplied by 3 will give the weight of uric acid in 200 c.c. of urine.

The filtrate and wash-water from the uric acid precipitation contains the purin bases. This filtrate is alkalinized with ammonia and again precipitated with the ammoniacal silver solution. This precipitate is collected on a small filter, washed with water, dried and carefully incinerated. The ash is dissolved in nitric acid and the silver chlorid estimated by titration with potassium sulphocyanate as described under the determination of chlorids. One c.c. of the potassium sulphocyanate solution is equivalent to 0.00734 gram of silver. One part of silver, in the form of the silver compounds of the purin bases, represents 0.277 gram of nitrogen, or 0.7381 gram of the purin bases. It is evident, therefore, that 1 c.c. of the potassium sulphocyanate solution will represent 0.002 gram of nitrogen and 0.00542 gram of purin bases according to the following proportion:

$$1 : 0.00734 :: 0.277 : x$$
$$x = 0.002$$

By multiplying the number of c.c. of potassium sulphocyanate solution used to precipitate the chlorids by 0.00542 we obtain the number of grams of purin bases in the 200 c.c. of urine originally used. It is wise in determining the amount of purin bases to start with a larger quantity of urine, as, for instance, 600 to 700 c.c., as the amount present in 200 c.c. would be very small.

This method is apt to give slightly high values for uric acid, as the purin bases may not be entirely soluble in the acid solution used to separate them from the uric acid. It requires much more equipment than is usually at the disposal of the practitioner so that it can hardly be recommended for routine use in general work.

Method of Rudisch and Kleeberg.

This method[1] is quite as accurate as is the preceding and has the advantage that it can be carried out in from 20 to 30 minutes. The principle is as follows: The total purin bodies are precipitated by an excess of silver nitrate, and the excess of silver determined volumetrically by potassium iodid, using a mixture of nitrous and sulphuric acid with starch solution as an indicator. As the silver compounds of the purin bases are soluble in strong ammonia solution, it is possible to make a determination of the uric acid and then estimate the purin bases by subtraction of this value from the total purin compounds.

Technic.

One hundred and ten c.c. of urine are treated with 55 c.c. of fiftieth-normal $AgNO_3$ solution and diluted with strong ammonia to 220 c.c. (The fiftieth-normal solution of silver nitrate is made by dissolving 3.3932 grams of $AgNO_3$,

[1] Amer. Jour. of Med. Sci., vol. 128, 1904, p. 899.

which has been heated for 10 minutes with a small amount of water to 120° C., in 500 c.c. of water, adding 75 c.c. of strong ammonia and 10 grams of ammonium chlorid and making the whole up to one liter.) The addition of the ammonia dissolves the purin bases, leaving the uric acid in the precipitate. The original mixture is now filtered in such a way that two 100 c.c. portions are obtained, each portion representing 50 c.c. of urine. With the first of these portions an approximate estimation of the uric acid is made, while with the second the accurate one is carried out.

To one of these portions, fiftieth-normal potassium iodid solution is added from a buret, a few drops being removed from time to time and added to a solution of nitrous-sulphuric acid mixed with a little starch paste. (The fiftieth-normal potassium iodid solution should contain 3.32 grams of KI in one liter. The nitrous-sulphuric acid mixture is made by mixing 25 c.c. of H_2SO_4 with 75 c.c. of H_2O and 1 c.c. of fuming HNO_3.) The addition of the drops of the solution to the indicator is for the purpose of determining the point at which an excess of potassium iodid occurs. This will be shown by the appearance of a distinctly blue contact ring. When the solution shows such a reaction, on adding a few drops of the mixture to the indicator, the number of c.c. of KI is read off, and we are then prepared for the more accurate control. It is wise to keep the indicator cold by immersion in ice-water, as otherwise a violent reaction may occur.

The second 100 c.c. portion is then carefully titrated with the fiftieth-normal KI solution by running directly about 1 c.c. less of this solution into the urine than was required in the first titration. After this point is reached the KI solution should be added drop by drop and a test made after the addition of each five drops. In this way an accurate end-point may be reached. As each 100 c.c. portion represents 50 c.c. of urine it will contain 25 c.c. of fiftieth-normal $AgNO_3$ solution. Subtract from this 25 c.c. the number of c.c. of fiftieth-normal KI used and the number of c.c. of silver nitrate which combined with the uric acid of the urine is obtained. One c.c. of fiftieth-normal $AgNO_3$ solution represents 0.00336 gram of uric acid. By multiplying this factor by the number of c.c. of silver nitrate used, we obtain the amount of uric acid in 50 c.c. of urine.

The second part of this method consists in the determination of the total purins. One hundred and ten c.c. of urine are treated with 55 c.c. of fiftieth-normal $AgNO_3$ solution and diluted with water to 220 c.c. The remainder of the process is exactly as outlined above. The number of c.c. of fiftieth-normal silver nitrate used will represent the values for the total purins.

If the number of c.c. of silver nitrate solution used in the previous determination of uric acid be subtracted from the number used in the determination of total purin, the result will be the number of c.c. referable to the purin bases. One c.c. of fiftieth-normal $AgNO_3$ solution represents 0.00152 gram of purin bases calculated as xanthin. By multiplying this factor by the number of c.c. of $AgNO_3$ used we obtain the amount of xanthin in 50 c.c. of urine.

The writer has used this method frequently and has found its results very similar to those of the Salkowski-Ludwig method, although the amount of uric acid is somewhat less in this method than in the latter one.

It is very simple, is quick and accurate and may, therefore, be recommended for general work. A somewhat similar method is advocated by Bartley[1]. The writer finds this latter method fairly reliable and simple.

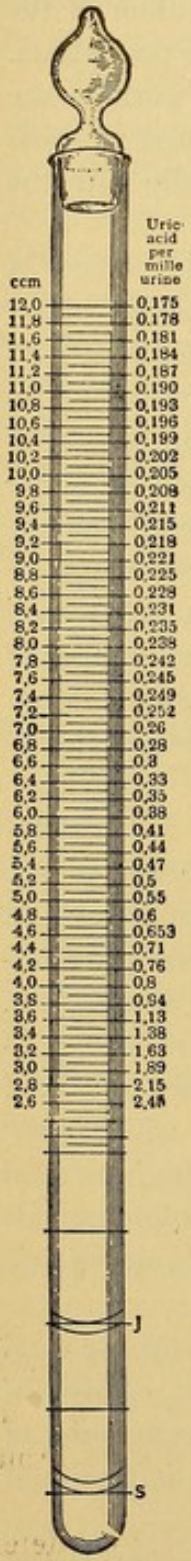


FIG. 78.—Ruhemann's uricometer.

Ruhemann's Method.

This method is a very convenient clinical one, although its results are by no means as accurate as those of the preceding methods. What the general practitioner desires, as a rule, is to know whether the uric acid is increased or diminished and does not care as to the absolute value. Such results, giving the total purins, may be obtained for clinical purposes by this method.

It consists in the use of a specially graduated tube, the uricometer, in which are placed the reagents and the urine to be tested (see cut). The calibrations of the tube are such as to represent directly the amount of uric acid in parts per 1000. The principle of the method is the decolorization of an iodin solution by the uric acid of the urine, and the measurement of the amount of urine which must be added to a definite amount of iodin solution to effect this decolorization.

Technic.

Carbon disulphid is placed in the tube up to the mark S, in such a way that the lower meniscus of this reagent rests upon the mark. A solution of iodin in potassium iodid is then added so that the upper portion of the meniscus coincides with the mark J. This iodin solution has the following composition.

Iodin,	0.50 gram
Potassium iodid,	1.25 grams
Absolute alcohol,	7.50 grams
Glycerin,	5.00 grams
Distilled water, q.s.,	100.00 grams

The urine is added slowly by means of a pipet until the lowest calibration is reached. The glass stopper is inserted and the contents of the tube mixed by repeated inversion for about 15 seconds. The carbon disulphid absorbs the iodin, taking on a distinct purple coloration. If this amount of urine does not completely decolorize the iodin, shown by the porcelain-like color of the carbon disulphid solution, more urine is added and the tube again inverted for 15 seconds. This process is continued until repeated

[1] Medical Chemistry, Philadelphia., 1904.

shaking of the tube causes the carbon disulphid to assume a pale pink color. The reaction is practically ended at this point, as by a little more shaking of the contents the indicator will assume the characteristic porcelain-white appearance. This process requires from 6 to 15 minutes. The amount of uric acid is then read off directly from the tube in parts per liter.

Should the urine contain less uric acid than can be read off from the calibrations, a second test is made adding the iodin solution to the mark midway between S and J, the amount indicated on the tube being of course divided by 2. Conversely, should the urine contain more uric acid than is represented by the lower calibration, one adds the iodin solution to the point above J and multiplies his reading by 1.5; or adds the iodin solution to the second mark above J and multiplies the reading by 2.

With this method the urine must be acid in reaction. If the urine contains a sediment of the urates, it should be thoroughly shaken before being added, so that the urates may be in suspension. Any free uric acid which may have separated in the sediment is not determined in this method. Strongly colored urines have no influence upon the decolorization. The presence of sugar does not interfere with the results, but if albumin be present in large amounts it should be removed by acidifying with dilute acetic acid, boiling, and filtering. The writer has used this method very frequently and finds it very useful for rough estimation of the uric acid outputs when the absolute amount is not of much clinical moment.

The purinometer, introduced by Hall[1], seems to be a much more reliable and useful instrument, as it employs the reagents required by the Salkowski-Ludwig method. As the writer has had no experience with this instrument, he cannot speak regarding its advantages and possible disadvantages.

(*e*). Purin Bases.

These bodies have been called purin bases, alloxur bases, xanthin bases, and nuclein bases. They are of extreme importance from the standpoint of physiologic chemistry, but are, clinically, of less value than is uric acid. The following bodies have been isolated from the urine in various conditions: adenin, guanin, epiguanin, carnin, episarkin, xanthin, hypoxanthin, heteroxanthin, paraxanthin, and methylxanthin. Certain methylated xanthin compounds are found in tea and coffee and are, therefore, introduced into the system as caffein, theobromin, and theophyllin, being excreted either as xanthin or hypoxanthin. Xanthin ($C_5H_4N_4O_2$) is 2, 6, dioxypurin.

These nuclein bases of the urine arise either from the nuclein of the food or from the increased nuclein metabolism of the system. Very little is known at the present time regarding the absolute variations in the excretion of these purin bases in the urine. Salkowski finds an excretion ranging between 0.0286 and 0.0561 gram, (calculated as xanthin), while Camerer regards an average output as 0.087 gram in 24 hours. It is interesting to note that a vegetable

[1] The Purin Bodies, Phila., 1904.

diet appears to increase this output more than a meat diet, 0.044 gram being excreted, according to Camerer, on a meat diet and 0.111 gram on a vegetable régime. This finding is the reverse of that for uric acid. As a general rule, it may be said that the output of purin bases is from 8 to 10 per cent. of that of uric acid, varying from 16 to 60 mg. per diem. It is evident that foods containing these substances should be absolutely interdicted in conditions which may be traceable to disturbances in the nuclein metabolism.

The pathologic variations in the excretion of these bodies are little understood. An increase in the amount of uric acid is usually associated with an increase of the xanthin bases, but at times no such relations obtain, a decrease in these bodies being observed. In leukemia an excretion of 0.321 gram has been reported by Magnus-Levy[1]. In certain cases of tuberculosis, nephritis, epilepsy, migraine, and pneumonia the output may be much increased. Edsall finds the urinary output increased as a result of X-ray treatment.

Xanthin is occasionally found as a constituent of the urinary sediment and may form calculi. The tests for the recognition of such excretions will be discussed in a later section.

The quantitative estimation of these bodies may be made by the tests previously outlined under Uric Acid. There can be little doubt that careful study of the urine with reference to these purin bases, using exact methods of analysis, will reveal much valuable information.

(*f*). Creatinin ($C_4H_7N_3O$).

The older ideas regarding the excretion of this nitrogenous body have suffered a severe shock from the work principally of Folin, Shaffer, Hoogenhuyze and Verploegh, Mellanby, and Klercker. Acid urines were supposed to contain creatinin and little or no creatin; while alkaline urines were thought to show creatin instead of creatinin. It has been proven, however, that "normal fresh urine, whether acid or alkaline, contains creatinin, and if the normal subject has not taken creatin in his food during the preceding days his urine will not contain creatin, whatever its reaction. There is no normal excretion of endogenous creatin, as this, when ingested, is largely retained in the body unless the food contains a large amount of protein." Thus Folin has shown that the excretion of creatinin on a diet yielding a urinary nitrogen value of 16 grams, was 1.55 grams or 3.6 per cent. of total nitrogen; while on a nitrogen-free diet with a urinary nitrogen of 3.6 grams, the creatinin output was 0.6 grams or 17.2 per cent. of total N. He showed, further, that while the actual amount excreted varied with different individuals, yet for the same person the output was practically constant, under the same conditions of health and muscular activity. He believes, therefore, that creatinin is by far the most reliable index as to the amount of a certain kind of protein metabolism occurring daily in any given individual. He bases his conclusions upon the

[1] *Loc. cit.*

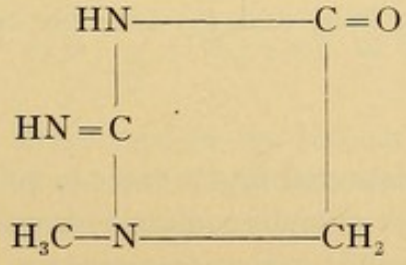
facts that "the absolute quantity of creatinin eliminated in the urine on a meat-free diet is a constant quantity, differing for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated." Shaffer[1] believes that creatinin is not an index of the total endogenous protein catabolism, as patients in whom the endogenous catabolism is much increased may excrete very little creatinin. Folin finds that the chief factor determining the amount of creatinin eliminated on any special diet is the weight of the patient. Fat or corpulent persons yield less creatinin per unit of body-weight than do lean ones. It is, therefore, necessary in metabolic work to consider not only the body-weight but, also, the excess of fat in increasing the weight.

According to Shaffer, the normal excretion of creatinin varies between 7 and 11 mg. of creatinin-nitrogen per kilo of body-weight. In pathologic subjects it is low, varying from the normal to 2 mg. per kilo of body-weight in 24 hours. He calls the creatinin-nitrogen excretion per kilo of weight the "creatinin coefficient." He thinks that creatinin is an index of some special process of normal metabolism taking place largely, if not wholly, in the muscles. Upon the intensity of this process appears to depend the muscular efficiency of the individual. In acute febrile conditions, in which an increased destruction of muscle tissue occurs, an increase is seen in the creatinin output during the active febrile period, while in the period of convalescence a diminished excretion will be observed. This excretion in fever does not run parallel to the muscular efficiency of the individual (Shaffer). Simon has shown that a diminished excretion occurs in anemia, marasmus, myositis ossificans, chlorosis, phthisis, chronic parenchymatous nephritis, progressive muscular atrophy, and pseudohypertrophic paralysis.

According to Shaffer, creatin may be excreted by subjects of acute fevers, in the acute stages of exophthalmic goiter, in other conditions in which there is a rapid loss of muscle protein, and by women during the postpartum resolution of the uterus. The source of this endogenous creatin is probably the creatin of the muscle tissues, and its appearance in urine probably indicates that muscle protein is being absorbed. For an excellent survey of this field see Mendel.[2]

Qualitative Tests for Creatinin.

Creatinin has the formula, $C_4H_7N_3O$, with the graphic structure



[1] Amer. Jour. of Physiol., vol. 23, 1908, p. 1.
[2] Science, vol. 29, 1909, p. 584.

The chemical reactions which serve for the detection of creatinin depend upon the formation of different colored compounds. It forms a distinct crystalline compound with zinc chlorid, which may be used in the quantitative estimation by Salkowski's method. For this process see works on physiologic chemistry. A point to be remembered regarding creatinin is that it reduces copper solutions and may be mistaken for sugar unless care be exercised.

Weyl's Test.

To the urine to be tested are added a few drops of a very dilute aqueous solution of freshly dissolved sodium nitroprussid and a few drops of dilute sodium hydrate solution. In the presence of creatinin a ruby-red color appears which changes, after a short time, to an intense yellow. If this solution be heated with a little glacial acetic acid the color will change to green and finally blue. Acetone gives a similar reaction, but on the addition of acetic acid changes to a purplish-red instead of green. If the urine be heated previous to the application of this test, the acetone may be driven off. This test for creatinin is sensitive to 6 parts in 10,000.

Jaffé's Test.

To the urine to be tested are added a few drops of a saturated solution of picric acid and a few drops of dilute sodium hydrate solution. If creatinin be present a red color appears immediately, which increases in intensity and remains permanent for a long time. If glacial acetic acid be added the color becomes yellow. Acetone gives a reddish-yellow color of less intensity than that produced by creatinin. Glucose, if present, may give a red color if the mixture be warmed. This test indicates one part of creatinin in 5,000.

Quantitative Determination.

Folin's Method.

The principle upon which this determinition[1] is based is the comparison of the color produced by Jaffé's reaction with that of a standard solution of potassium bichromate. A high-grade colorimeter is necessary for this comparison. Folin recommends the use of the Duboscq instrument, while the writer finds one made by Sargent & Co., of Chicago, very satisfactory. This latter has the advantage of being much less expensive.

The reagents necessary are (1) a half-normal potassium bichromate solution containing 24.55 grams per liter, (2) a saturated picric acid solution containing about 12 grams per liter, and (3) a 10 per cent. solution of sodium hydrate.

Technic.

Ten c.c. of urine are measured into a 500 c.c. volumetric flask, 15 c.c. of the picric acid solution and 5 c.c. of the sodium hydrate solution are then added, and the mixture allowed to stand for five or six minutes. This interval is used to

[1] Amer. Jour. of Physiol., vol. 13, 1905, p. 45.

pour a little of the standard bichromate solution into each of the two cylinders of the colorimeter. The depth of the solution in one of the cylinders is then accurately adjusted to the 8 mm. mark. With the solution in the other cylinder a few preliminary colorimetric readings are made simply for the sake of insuring greater accuracy in the subsequent readings of the unknown solution. The two bichromate solutions must, of course, be equal in color, and in taking their readings no two should differ more than 0.1 mm. or 0.2 mm. from the true value (8 mm.), leaving out of consideration the very first reading made, which is sometimes less accurate. Four or more readings should be made in each case, and an average taken of all but the first. After a while one becomes sure of the true point, and can take the average of the first two readings.

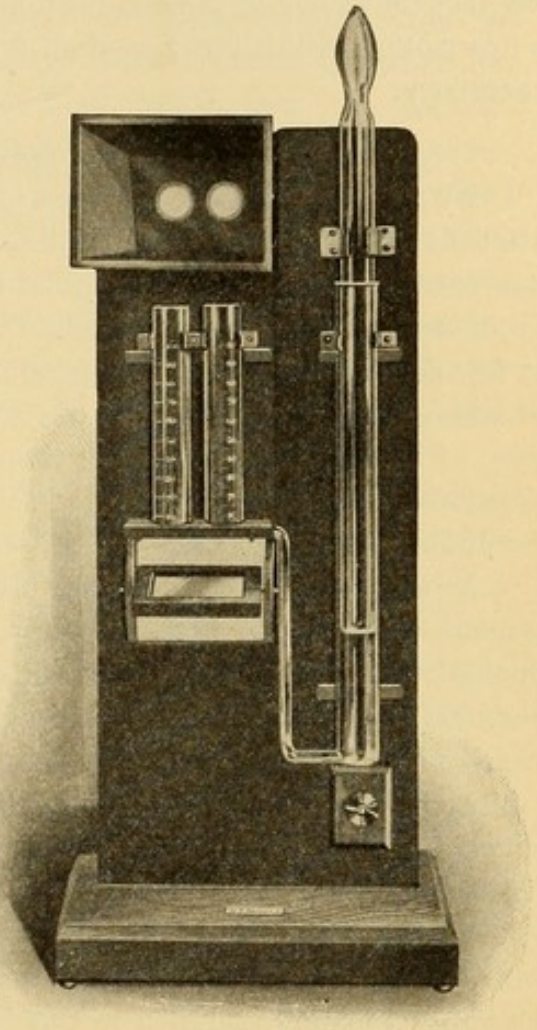


FIG. 79.—Sargent's colorimeter. (Courtesy of E. H. Sargent & Co.)

At the end of five minutes the contents in the 500 c.c. flask are diluted up to the 500 c.c. mark. The bichromate solution is thoroughly rinsed out of one of the cylinders by means of the unknown solution and several colorimetric readings are then made *at once*. The calculation of the results is very simple. It is based on the experimentally determined fact that 10 mg. of perfectly pure creatinin give, under the conditions of the determination, 500 c.c. of a solution, 8.1 mm. of which have exactly the same colorimetric value as 8 mm. of a half-normal bichromate solution.

If, for example, it is found that it takes 9.5 mm. of the unknown urine-picrate solution to equal the 8 mm. of the bichromate, then the 10 c.c. of urine contain

$$10 \times \frac{8.1}{9.5} = 8.4+\text{mg. of creatinin.}$$

If the 10 c.c. of urine used in the test are found to contain more than 15 mg. or less than 5 mg. of creatinin, the determination should be repeated with a correspondingly different amount of urine, because outside of these limits the determination is much less accurate. The color of the urine does not materially affect the results on account of the great dilution.

Sugar and albumin do not interfere with the determination, while acetone and diacetic acid do interfere and must be removed by heating.

The older quantitative method of Neubauer-Salkowski is very time-consuming, and may give results different from those of Folin. This result may be either too high or too low, depending first upon the sensitiveness of the eye of the observer in matching the color tints in Folin's method and secondly upon the fact that some of the creatinin may be lost in the Salkowski method. For a description of this latter method the writer will refer to works on physiologic chemistry.

(*g*). Undetermined Nitrogen.

By the undetermined nitrogen of the urine is meant the nitrogen remaining after that attributable to urea, uric acid, xanthin bases, ammonia, and creatinin has been subtracted from the total nitrogenous output. This factor is made up of many substances present in variable amount and determined with more or less difficulty. In this nitrogenous fraction we find various mon-amino and di-amino acids, hippuric acid, oxy-proteic and alloxy-proteic acids, and allantoin. Physiologic variations in these separate factors are not at present well understood and seem to depend upon the age of the individual, the diet, and the condition of the intestines and liver.

As the variations in the other nitrogenous constituents have been shown to depend to a great extent upon the nature of the diet, so we would expect the values for the undetermined nitrogen to show similar fluctuations. Folin finds on a diet yielding 16 grams of total nitrogen in the urine an undetermined nitrogen output of 0.6 gram or 3.75 per cent. of total nitrogen; while on a nitrogen-free diet with a urinary nitrogen value of 3.6 grams the undetermined nitrogen is 0.27 gram or 7.3 per cent. He is led, therefore, to make the following generalization: "The absolute quantity of undetermined nitrogen decreases under the influence of the starch and cream diet, but in per cent. of the total nitrogen there is always an increase." The separation of the various factors included in this undetermined nitrogen has clinically little value at the present time, as our knowledge of the importance of any or all of these constituents is extremely limited.

(1). Amino Acids.

Theoretically, we should find in the urine, whenever hepatic metabolism is disturbed, both mono- and di-amino acids, as these substances are normal products of protein hydrolysis in the intestine and are directly converted into urea in the normal liver. It has long been known that acute yellow atrophy and phosphorus poisoning were associated with a diminished urea output and the presence of the two mono-amino acids, leucin and tyrosin, in the urine. Recent work with improved methods has shown that the urine contains these and other amino acids in any condition in which hepatic activity is impaired, so that our old-time diagnostic point of acute yellow atrophy must fall by the wayside. Although these substances are found in much larger quantities

under pathologic conditions, traces of them are present in normal urine, especially when the nitrogen intake has been large. Thus von Noorden regards glycocoll (amino-acetic acid) as a normal urinary constituent, its excretion averaging 1 gram daily. These acids are also found in cases of gout, pneumonia, especially during the absorption of the exudate, in diabetes, and in leukemia.

A large number of these mon-amino acids have been isolated from the direct products of protein hydrolysis, but the number found in the urine has not been as large owing to the uncertain methods of examination. In this connection it is to be stated that the proportions of the various mon-amino acids will vary, depending upon the kind and type of protein subjected to hydrolysis or digestion. The introduction by Fischer[1] of the esterification method has added much to our knowledge of these bodies. The writer cannot take space to give this method in detail and will, therefore, refer to works on physiologic chemistry for this phase of the subject, as well as for the characteristic crystalline compounds formed by treatment of these mon-amino acids with β-naphthalin-sulpho-chlorid.

By the di-amino acids we mean the bodies lysin, arginin, and histidin, which are collectively known as the hexone bases. These bodies, together with the mon-amino acids, constitute the end-products of tryptic and to a less extent of peptic digestion. While these latter bodies have not been isolated from the urine, it is reasonable to suppose that future work with improved methods will result in the finding of both types of amino acids, especially under pathologic conditions.

(2). Hippuric Acid ($C_9H_9NO_3$).

This acid is a normal constituent of urine and varies between 0.1 and 1 gram in 24 hours. It is derived to a large extent from foods containing benzoic acid and is also formed both from the metabolism of tissue and food protein. This substance is directly synthesized in the system by the combination of benzoic acid with glycocoll, but the exact place of synthesis is uncertain. As glycocoll (aminoacetic acid) is constantly formed in the process of intestinal digestion, one may readily see that the introduction of benzoic acid or of bodies readily convertible into this acid will result in the formation of hippuric acid. Such benzoic-acid-containing substances are prunes, cranberries, green-gages, bilberries, and many other fruits.

A pathologic increase in the excretion of hippuric acid has been observed in acute febrile diseases, marked intestinal putrefaction, hepatic disturbances, and in diabetes mellitus; while in cases of acute diffuse and chronic parenchymatous nephritis as well as in amyloid degeneration of the kidneys hippuric acid is practically absent from the urine.

As little of clinical value is at present obtained by a determination of the hippuric acid of the urine the writer will refer elsewhere for a discussion of the

[1] Untersuchungen über Aminosäuren, Berlin, 1906.

quantitative methods. Occasionally this acid appears as a sediment in the urine and will be discussed in this connection in a later section.

(3). Oxyproteic and Alloxyproteic Acids.

These acids have been isolated from the urine and seem to be constant constituents, which are derived from the protein decomposition. Both of them contain sulphur, and for this reason have been credited with forming a large percentage of the neutral sulphur output of the urine. They have also been said to form from 2 to 3 per cent. of the total nitrogen excretion. The output of oxyproteic acid is about 3 grams while that of the alloxyproteic acid is approximately one gram. Bondzynski believes that the oxyproteic acid is accountable for Ehrlich's diazo-reaction, which will be discussed later.

(4). Allantoin ($C_4H_6N_4O_3$).

This substance is a product of oxidation of uric acid. It is somewhat variable in amount, usually being found only in traces, but after a large intake of meat, especially rich in nuclein, the amount may be quite perceptible. It is found in fairly large quantities in the urine of new-born children, from which it is best obtained for study. The pathologic variations in the output of this body are unknown.

The quantitative tests for this substance must be looked for in works on physiologic chemistry. A point to be remembered in connection with allantoin is that it may be present in the urine in sufficient quantities to reduce copper solutions and must, therefore, not be mistaken for sugar.

(2). Fatty Acids.

Traces of volatile fatty acids are present in all normal urines. The most important of these are formic, acetic, propionic, and butyric acids. They are doubtless formed in the intestinal tract by bacterial action upon the carbohydrates of the food, and may, therefore, be to some extent an index of the degree of carbohydrate fermentation. Their normal amount does not much exceed 0.01 gram in 24 hours, although Blumenthal[1] gives the figures under an ordinary diet as being equivalent to from 50 to 80 c.c. of tenth-normal sulphuric acid (0.25 to 0.39 gram).

These acids are increased in febrile conditions, the amount running parallel to the rise in temperature. This increase in the fatty acids of the urine is known as *lipaciduria*. According to Rosenfeld, this increase in febrile states is observed only in those cases in which absorption of decomposing albuminous material occurs, as in all suppurative processes within the system. In the convalescent stage of pneumonia these acids may be excreted in increased, amount, while a diminished output is usually observed preceding the crisis. In hyperacidity of the gastric contents the fatty acids of the urine are increased, while in hypoacidity they are diminished. In contradistinction to the increase of fatty acids in cases of fever associated with suppurative conditions, we find

[1] Pathologie des Harnes, Berlin, 1903.

a diminution in the amount of fatty acid in scarlet-fever, erysipelas, measles, and diphtheria. In cases of acute rheumatism formic acid is said to be excreted in large amounts.

A simple method of determining the fatty acids of the urine is to acidify from 250 to 500 c.c. of urine with 50 to 75 c.c. of dilute sulphuric acid and distill. The distillate is then titrated with tenth-normal sodium hydrate solution using phenol-phthalein as an indicator. The results are expressed in terms of the corresponding number of c.c. of tenth-normal acid.

(3). Oxalic Acid.

The amount of oxalic acid eliminated in the urine in 24 hours varies from 10 to 20 milligrams. A portion of this excretion is undoubtedly derived from the diet, but some of it is produced in the metabolism of the tissues, especially of those containing nuclein substances. Although the carbohydrates do not take part in the production of oxalic acid normally, we find, in conditions associated with fermentation of the carbohydrates in the stomach, a large increase in the output of oxalic acid. As oxalic acid is more or less readily formed by oxidation of uric acid, passing through the intermediate stage of *oxaluric* acid, we can readily see that increase of nuclein metabolism may increase the output of this substance. Strangely enough, it has been found that the intake either of pure nuclein or of nuclein-containing foods was not associated with a corresponding increase in the urinary oxalic acid, so that we must assume that a large portion of this intake escapes in the feces in the form of calcium oxalate. It has, moreover, been found that the administration of oxalates by the mouth is not associated with increased excretion either in the urine or feces, so that we must assume a decomposition of the oxalic acid into carbon dioxid and water somewhere in the system. This conversion appears to take place in the intestinal canal under the influence of bacterial action. Crystals of calcium oxalate are frequently found in the urine in cases showing a marked increase in the output of ethereal sulphates, so that we must either assume an increased formation from the carbohydrates of the food, an increased intake and absorption of oxalates of the food, or an increase in the nuclein metabolism as a result of absorption of the toxic products of intestinal putrefaction.

Among the foods which are known to contain relatively large amounts of oxalic acid we find spinach, rhubarb, tomatoes, carrots, celery, string-beans, green peas, potato, figs, plums, strawberries, pepper, cocoa, tea, and coffee. Those which contain very little are meat, milk, eggs, butter, cereals, rice, asparagus, cucumbers, mushrooms, lettuce, cauliflower, cabbage, pears, peaches, grapes, and melons.

The increased elimination of oxalic acid may or may not be associated with a deposition of crystals of calcium oxalate. An increased elimination is observed in cases associated with irregular activity of the gastro-intestinal tract. These cases usually show many types of nervous disorder, especially neurasthenia, and are characterized by the large deposit of oxalate crystals in the urine.

To this condition has been given the name *oxaluria*, which can hardly be dignified as a definite pathologic entity. No inference can actually be drawn regarding the degree of elimination of oxalic acid from the appearance of a deposit in the urine, as it has been shown by Fürbringer that the urine may contain a large amount of oxalic acid without a sediment of calcium oxalate crystals being formed. Such cases, however, should be watched more or less closely as separation of calcium oxalate may occur within the pelvis of the kidney and lead to the formation of a calculus. In cases of jaundice a marked oxaluria may be observed which is directly referable to the associated cholemia. In occasional cases of diabetes mellitus the elimination of oxalic acid may be much increased.

Quantitative Determination.

Baldwin's Method.[1]

Five hundred c.c. of a mixed 24-hour specimen of urine are treated with 150 c.c. of 95 per cent. alcohol and the mixture set aside for 48 hours to allow the calcium oxalate to precipitate. It is then filtered, each particle of the precipitate being transferred to the filter by means of hot water and a rubber-tipped glass rod. This precipitate is then washed with hot water and later with 1 per cent. acetic acid. The precipitate is then washed from the filter-paper by a stream of dilute hydrochloric acid from a wash-bottle until every trace of the precipitate is removed from the filter and dissolved. The filter is then washed with hot water until the washings are no longer acid in reaction. The hydrochloric acid solution and the washings are evaporated to about 20 c.c., a little calcium chlorid solution is added, the solution is neutralized with ammonia, is then rendered slightly acid with acetic acid, and 95 per cent. alcohol added in an amount equal to one-half the volume of the liquid. The mixture is then set aside for 48 hours, after which the precipitate of calcium oxalate is collected on an ash-free filter, washed with cold water and dilute acetic acid until free from chlorids, and the filter with its contents is incinerated in a weighed platinum crucible. This latter process is first carried out over a Bunsen burner and later over a blast-lamp. The crucible is then dried in a desiccator and weighed. The difference in weight represents the amount of calcium oxid obtained from 500 c.c. of urine. Each gram of this oxid represents 1.6 grams of oxalic acid.

Other organic substances are found in traces in the urine, both normal and abnormal, but do not have any clinical significance. Among these we find lactic acid, succinic acid, traces of carbohydrates, and certain little-understood organic acids. These may be found discussed in works on physiologic chemistry.

(4). Ferments.

Several ferments have been demonstrated in the normal and pathologic urine, but do not seem to have any great clinical importance at the present time.

[1] Jour. of Expr. Med., vol. 5, p. 27.

Pepsin.

This ferment is present in practically every specimen of urine. It has been found by Grober, Gehrig, Grützner, Mathes, Stadelmann, and others. It seems to be absent or very much diminished in cases of typhoid fever, gastric carcinoma, and hypoacidity. In cases of pneumonia, Lenobel and Kun and Lochbihler have observed the presence of a ferment, which seemed to be pepsin in increased amounts. Scola reports a diminution of the normal pepsin content in severe diseases of the nervous system.

Lipase.

This ferment is present normally only in minute traces. It is found, however, in cases of hemorrhagic pancreatitis, jaundice, and in diabetes mellitus. It may be detected by the method of Kastle and Loevenhart which is as follows: In each of three flasks are placed 5 c.c. of urine. One of the flasks is boiled to destroy the ferment which may be present. To a second flask are added a few drops of phenol-phthalein solution and the acidity determined by titration with tenth-normal sodium hydrate solution. The amount of alkali necessary to neutralize the 5 c.c. of urine is then added to each of the other flasks. To these flasks are then added 0.25 c.c. of ethyl-butyrate and 0.1 c.c. of toluol, the flasks being then placed in the incubator at 37° C. for 24 hours. To each of these flasks there is then added 1/2 c.c. more tenth-normal hydrochloric acid than the amount of tenth-normal alkali previously added. The mixture is then shaken out with 50 c.c. of ether and 25 c.c. of alcohol to remove the butyric acid. This is then titrated with tenth-normal sodium hydrate, each c.c. of tenth-normal sodium hydrate representing 0.0088 gram of butyric acid.

(5). **Mucin-like Substances.**

(*a*). **Mucin.**

True mucin is present in traces in practically all urine. It is found both as an insoluble portion which forms the nubecula and as a soluble portion which is much smaller in amount than the insoluble form, is precipitated by acetic acid, but is easily soluble in a slight excess of the acid. This form of protein is derived from the urinary passages and has practically no pathologic importance, although it may be much increased in catarrhal conditions of the urinary tract in which it may appear as a gelatinous ropy material, or in rare cases in the form of casts of the ureter or urethra from 1 to 10 cm. long and 3 to 4 mm. thick. These cases are rare and have been reported by von Jaksch under the name of "ureteritis membranacea" and by Frank under the name of "pyelitis productiva."

Mucin is precipitated by the ordinary reagents for albumin (to be discussed later), but is soluble in an excess of these reagents, so that it need not be mistaken for albumin. It is frequently confused with the "nucleo-albumins," but may be distinguished, chemically, by the fact that it contains no phosphorus and gives on heating with acids a substance which reduces copper solutions.

The amount of true mucin in the urine is clinically of little importance, so that the writer will refer elsewhere for quantitative methods.

(*b*). **Nucleo-albumin.**

The large majority of specimens of urine contain a substance (other than true mucin) which is precipitated on the addition of cold acetic acid. Dilute acetic acid does not redissolve the precipitate, so that true mucin is excluded, as the latter is usually dissolved even by dilutions of acetic acid which do not precipitate the other bodies. The reaction with acetic acid is somewhat intensified if the urine be diluted.

Practically every normal urine contains traces of such substances, which will give a precipitate with acetic acid, especially if the salts be removed by dialysis. In all probability this body, found in normal urines, is either euglobulin or a mixture of this protein with fibrinogen. It has been called "nucleo-albumin," but this is in all probability a misnomer. It is probably true that real nucleo-albumin is never a normal constituent of the urine. In this connection it must be stated that much confusion exists regarding the nature of true nucleo-albumin. This substance has been considered to be identical with nucleo-protein, but with absolutely no basis of chemical facts. Nucleo-protein (true nuclein) is a combination of protein with the prosthetic group, nucleinic acid, which splits up into phosphoric acid and purin bodies; while nucleo-albumin (pseudo-nuclein) is a combination of protein with paranucleinic acid, which is rich in phosphorus, but does not yield purin bases on hydrolysis. Care should, therefore, be taken in calling a substance by a specific name unless it can be shown chemically to be such a body.

Mörner[1] believes that most of the so-called "nucleo-albumin" is a compound of true serum-albumin with an albumin-precipitating body formed on addition of acetic acid. He showed that there were three such precipitating bodies present in the urine; chondroitin-sulphuric acid was practically always present, nucleinic acid occasionally present, and tauro-cholic acid, which may be normally present in traces, but in certain pathologic conditions is much increased. He believes that these precipitating bodies are normally present in excess and, therefore, any increase of a precipitate on addition of the acetic acid would mean an increased excretion of albumin. The more these precipitating bodies predominate the more the precipitate resembles "nucleo-albumin."

We see, therefore, that the true nature of this body or group of bodies is still unsettled. It is wise, in the writer's opinion, not to attempt a differentiation at present, but to regard these bodies as normal constituents, which at times may be excreted in abnormal amount.

From the pathologic standpoint what has been called true "nucleo-albumin" appears in conditions destroying the integrity of the epithelium of the uriniferous tubules or of the bladder as well as in conditions associated with the excretion of pus in the urine. Thus we would expect to find such a body

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

in acute nephritis, whether the result of bacterial or of exogenous toxins, in the acute febrile diseases, in renal hyperemia, in leukemia, in acute yellow atrophy, and in obstructive jaundice, in which case this body is derived doubtless from the bile. In cases of nephritis this body, precipitable by acetic acid in dilute solution, may precede and follow the true albuminuria. In amyloid kidney this body seems to be the chief type of protein present. In orthostatic albuminuria this substance may be the only protein present and may persist after the others have cleared up. In cases in which the urine contains a large number of epithelial cells, casts, and pus-cells, Matsumoto finds a substance precipitable by acetic acid, but only in very small amounts. This finding would seem to indicate that nucleo-albumin, if such ever occurs, is at least not an indication of cellular origin from increased epithelial desquamation. This body may be found in general catarrhal conditions of the urinary tract, as in cystitis or pyelitis, but in such cases we are more apt to obtain true mucin.

In the above discussion the writer has not attempted to differentiate these bodies, as the reports in the literature have little importance beyond the fact that a body precipitable by acetic acid was obtained. Each worker has named this body as he understood it and in many cases has had no definite basis for such conclusion. The writer feels, therefore, that this body, whatever it may be found to be, cannot at present be considered of any pathologic importance as it occurs in such widely varying conditions and has been chemically so little understood. To remove it from the urine, add a solution of lead acetate and filter; the precipitated phosphates and chlorids carry down this protein.

(6). Pigments and Chromogens.

(*a*). Urochrome.

This pigment is the chief coloring matter of normal urine, imparting a yellow, orange, or a brownish color to the urine, depending upon its concentration. It is closely related to urobilin, especially the so-called normal urobilin of MacMunn, as this latter body may be readily converted into urochrome by evaporation of its aqueous ethereal solution. This body has not been isolated, at least in the pure state, so that little is known of its chemistry. It is, in all probability, a mixture of one or more pigments, contains about 4 per cent. of nitrogen, and is free from iron. It is readily soluble in water and alcohol; sparingly soluble in acetic ether, amyl alcohol, and acetone; insoluble in ether, chloroform, and benzol. Much difference of opinion exists, regarding the spectrum of this pigment, some stating that there is no absorption spectrum, while others either describe a faint, narrow absorption band between *F* and *G*, or a broad absorption band at *F*, which extends more to the left than to the right of this line. As this substance has so little clinical importance, the writer must refer to works on physiologic chemistry for its detection.

(*b*). Uroerythrin.

This pigment is a constituent of a large majority of normal urines, although it is probable that it indicates a slight deviation from the normal. It has been

called rosacic acid by Prout and purpurin by Golding-Bird. To this pigment is due the salmon or brick-red color which the urinary sediments take in highly concentrated febrile urines. Normally, it may not be present in sufficient amount to color the urine, but under pathologic conditions it may impart a deep orange tint to this fluid. Little is known regarding the chemistry of this pigment, but it seems to be iron-free. It is soluble in amyl alcohol, slightly soluble in acetic ether and absolute alcohol and very difficultly soluble in water. For the chemical and physical properties of this pigment the writer must refer elsewhere.

This pigment appears to be increased on a meat diet, after severe exercise, profuse perspiration, or by irregular digestion. Pathologically, it is observed especially in cases of hepatic insufficiency, in chronic cardiac and pulmonary disease, in acute articular rheumatism, in malarial fever, and in general acute febrile diseases. In typhoid fever one does not find this pigment as frequently as in most other acute febrile conditions.

(*c*). Urobilin.

This substance appears in the urine not as a free pigment, but in the form of the chromogen urobilinogen, which is decomposed into urobilin through the influence of the light. It is claimed that various types of urobilin are found, as for instance the normal urobilin of MacMunn and the pathologic urobilih of Jaffé. Whether these are really different bodies is at present an unsettled question. Urobilin appears to be identical with the stercobilin of the feces and is not the same as the normal fecal hydrobilirubin. Much discussion has centered around the origin of this pigment. Passing through the stages of the hepatogenous, hematogenous, nephrogenous and histogenetic urobilinuria, the general concensus of opinion seems at present to be that most of the urinary urobilin is of enterogenous origin. According to this theory, the pathogenesis of urobilinuria may be presented as follows:

"The liver-cell, both in normal and abnormal conditions, forms only bilirubin from the blood pigment. Providing there is no marked obstruction to the passage of bile into the intestine, the bilirubin is acted upon by bacteria which reduce it so completely to urobilin that only traces of bilirubin appear in the feces. A part of the urobilin is absorbed and is excreted in the urine, while traces appear in the bile and in pathologic transudates. When bacterial action is excluded, as in the new-born, no urobilin is found in the urine. Further, when bile is not present in the intestine, as in cases of absolute occlusion of the ductus choledochus, urobilinuria does not occur. It is sparingly excreted when the production of biliary pigment is diminished, as in hunger, while the amount is small or at most normal in cases of incomplete exclusion of bile from the intestine. On the other hand, the amount excreted may reach abnormal limits if a preceding obstruction, accompanied by stasis, has been overcome, and bile flows freely into the intestine. Likewise the quantity may be abnormally large if the production of biliary pigment from the red blood-

corpuscles increases as a result of infection and intoxication, or of hepatic lesions, such as cirrhosis and cyanotic induration. In these cases the bile is tenacious and the condition may give rise to jaundice although it is seldom that the stasis is so great that the bile is completely shut off from the intestine. Indeed, in most cases, owing to the excretion of excessive pigments in the bile (pleiochromia), a more than normal amount of pigment passes into the intestine, and as a result of this there arises a marked urobilinuria with a mild degree of biliary stasis. In many cases the stagnation of bile is great enough to cause a passage of biliary pigment from the blood into the urine, leading to a marked urobilinuria, a mild degree of bilirubinuria, and a yellowing-tinting of the tissues. In other cases the absorption of bile is so slight that only yellowing of the tissues results, yet the concentration of pigment in the blood does not suffice to permit of its excretion by the kidneys, so that marked urobilinuria and yellowing of the tissues without bilirubinuria ensues" (Weintraud).

Urobilin is found in febrile conditions, chronic passive congestion, lead-poisoning, cases in which extravasation of blood into the tissues occurs, in any condition associated with marked hemolysis, hepatic cirrhosis, and in the cases of jaundice outlined above by Weintraud. It has been noticed in increased amounts in Addison's disease, extrauterine pregnancy, hemophilia, and in secondary syphilis.

The presence of an increased amount of urobilin usually causes a dark-yellow color of the urine, the foam in such cases, being colored more perhaps due to the presence of other pigments than to urobilin itself.

Urobilin is soluble in ethyl alcohol, amyl alcohol, and chloroform, and slightly soluble in ether, acetic ether, and in water. If an acid solution of urobilin be examined with a spectroscope, it shows a broad absorption band to the right of *E*, the left-border of which reaches nearly to *b*, while the right border encloses *F*. If the solution be alkaline, the spectrum shows a less broad absorption band between *E* and *F* enclosing *b*. This solution is best made as follows: Ten to 20 c.c. of the urine are acidulated with a few drops of hydrochloric acid and shaken out with from 6 to 10 c.c. of amyl alcohol, which then shows the characteristic spectrum of acid urobilin. If to a small portion of this amyl alcohol solution be added a few drops of 1 per cent. solution of zinc chlorid, which has been strongly alkalinized with ammonia, a beautiful green fluorescence appears. This solution shows the spectrum of alkaline urobilin. For the quantitative determination of this pigment text-books of physiologic chemistry should be consulted.

(*d*). Indican.

In the decomposition of protein occurring in the intestinal canal indol (C_8H_7N) and skatol (C_9H_9N) are found among the products of this bacterial cleavage. These substances are absorbed and oxidized in the blood to indoxyl (C_8H_7NO) and skatoxyl (C_9H_9NO). These bodies are then conjugated with sulphuric acid forming indoxyl and skatoxyl sulphuric acids, after which they

are excreted in the form of the potassium salt, indoxyl potassium sulphate ($C_8H_6NO—SO_2—OK$) and skatoxyl potassium sulphate ($C_9H_8NO—SO_2 - OK$). To the former of these is given the name indican. This chromogen is present in much larger amounts than is the skatoxyl compound so that our discussion will include principally the former.

The absolute amount of indican occurring in the urine depends upon the amount of decomposition occurring in the intestine. This subject has been treated of previously under the head of ethereal sulphates to which the reader is referred. The largest amounts are naturally observed in health following a meat diet, while the urine may be practically free from indican on a vegetable or a milk diet. The pathologic variations may be summed up as follows. An increased elimination of indican is observed in all diseases which are associated with an increased intestinal decomposition. This decomposition usually occurs in the large intestine, but may at times take place in the small bowel, in which cases the degree of indicanuria may be greater owing to the increased absorptive power of the small bowel. Although many writers state that indicanuria is not seen in cases of simple constipation, the writer must take exceptions to these statements as some of his most intense and persistent cases of indicanuria have been found in uncomplicated constipation. Secondly, an increased excretion of indican is observed in cases showing diminished peristalsis, as, for instance, in ileus and peritonitis. These cases frequently show an intense reaction. If the obstruction leading to ileus be in the large bowel, indican is either absent or appears much later than is the case if the small bowel be involved. Lastly, in any condition associated with protein decomposition anywhere in the system, as, for instance, in empyema, putrid bronchitis, abscess formation, etc., indican may be much increased.

The color of the urine is usually normal when voided, although a large amount of indican may be present. In some cases oxidation of this chromogen has occurred within the system and the urine appears greenish or even blue when voided. If the urine be allowed to stand until decomposition occurs, a reddish or bluish metallic-like scum may be observed, due to the conversion of the indican into indigo-blue. Most of the tests for the presence of indican in the urine depend upon the oxidation of the indican, according to the following equation (this body having been previously decomposed by concentrated HCl into indoxyl and sulphuric acid).

$$2C_8H_7NO + 2O = C_{16}H_{10}N_2O_2 + 2H_2O.$$

Tests for Indican.

Jaffé's Test.

A few c.c. of urine are treated with an equal volume of concentrated hydrochloric acid, two or three drops of a strong solution of calcium hypochlorite (bleaching powder or "chlorid of lime") are added and the contents mixed. Two c.c. of chloroform are then added and the tube inverted several times. In this process the indican is oxidized to indigo-blue which is taken up by the

chloroform. The depth of the blue coloration of the chloroform will serve as an approximate estimate of the amount of indican present. In absolutely normal urine no blue coloration or, at most, a faint bluish tinge is observed. Care must be taken in this test to avoid an excess of the hypochlorite, as this will convert the indigo-blue into isatin which is distinctly yellow, according to the following equation:

$$C_{16}H_{10}N_2O_2 + O_2 = 2C_8H_5NO_2.$$

Obermayer's Test.

A few c.c.of urine are mixed with an equal volume of Obermayer's reagent (a 0.2 per cent. solution of ferric chlorid in concentrated hydrochloric acid) and the solutions mixed by repeatedly inverting the tube. A few c.c. of chloroform are then added and the tube inverted as before. The presence of any appreciable amount of indican is indicated by a dark brown to black coloration of the urine and the later absorption of this color by the chloroform, which becomes a more or less deep shade of blue. If the urine be originally very dark in color or if bile-pigment be present, the pigments may be removed by adding a solution of lead subacetate which will precipitate the phosphates and chlorids and thus carry down the bile and other interfering pigments. If the urine contains potassium iodid or salicyluric acid a red color will be observed in the former instance, while in the latter a violet color will be noted.

Instead of using the Obermayer reagent as such, the urine may be mixed with the hydrochloric acid and one or two drops of a 10 per cent. solution of ferric chlorid may then be added to the mixture. This test is a much more reliable one than is the Jaffé test, as the latter is very prone to carry the oxidation to the isatin-stage rather than to the indigo-blue phase. Even under the best conditions a certain amount of isatin will be formed so that quantitative determinations are a matter of some difficulty.

Skatoxyl-potassium Sulphate.

A certain amount of skatoxyl-potassium sulphate is formed along with the indican. In some cases for reasons not well understood, this pigment or one closely allied to it appears in the urine in excess of the indican, so that the above test may give distinct red colorations of the choloroform instead of the usual blue. Just exactly what pigment causes these red variations is doubtful. Some of this pigment is always present along with the indican and may be extracted either with hot water or with a mixture of alcohol, ether and water. To this red pigment has been given the name indigo-red, skatoxyl-red, urorubin, urorhodin, and several others.

If the urine be heated on applying Jaffé's test, a dark red coloration is observed, while if Obermayer's test be used the coloration will be of a reddish-violet.

Rosenbach's Test.

A few c.c. of urine are boiled and concentrated nitric acid added drop by drop during the boiling. The urine takes a deep red color and the foam appears

bluish-red. If the nitric acid be much in excess the urine will assume a yellowish-red color and the foam a distinctly yellow tint. If sodium hydrate or ammonia be now added drop by drop, a bluish-red precipitate is observed which is soluble in an excess of the alkali with a brownish-red coloration. This test is due to the presence of indigo-red. Its clinical significance is the same as that of indican. This pigment may be obtained directly from the urine by neutralizing it with sodium hydrate and shaking out with ether, when the ether will take a distinct red color.

Quantitative Determination.

Wang's Method.

The principle of this method[1] is the decomposition of indican and its oxidation to indigo-blue. This compound is then transformed into indigo-sulphuric acid, which is directly determined by titration with potassium permanganate solution.

Technic.

A preliminary determination of the relative amount of indican is made with Obermayer's reagent. If a strong reaction is obtained, from 25 to 100 c.c. of urine are used, while if the reaction be slight 200 to 500 c.c. are necessary. The urine should be acidified with acetic acid, unless its reaction be already acid.

Fifty c.c. of urine, or a larger amount if the conditions mentioned above obtain, are treated with 5 c.c. of a 20 per cent. solution of lead acetate, or one-tenth the volume of urine in case a larger amount of urine be taken. The urine is then filtered and a large and accurately measured portion of the filtrate is treated, in a separatory funnel, with an equal volume of Obermayer's reagent. This mixture is then shaken out with chloroform, using 30 c.c. of this menstruum and shaking for one minute. At least four such chloroform extractions should be made, more if the chloroform still extracts indigo-blue from the mixture. The chloroform extract is placed in a small flask and the chloroform distilled. The residue in the flask is dried for a few minutes on the water-bath to remove the last traces of the chloroform, and is washed either with hot water or with a mixture of equal parts of alcohol, ether, and water. These solvents remove the red coloring matter, leaving the indigo-blue undissolved. The extract is filtered through a small filter and the indigo-blue completely transferred to the filter, after which it is thoroughly washed with hot water. This indigo-blue is dissolved on the filter with boiling chloroform, the filtrate being allowed to run into the original flask. The chloroform is again distilled and the residue dried on the water-bath. This purified indigo blue is dissolved in 10 c.c. of concentrated sulphuric acid and the solution diluted to 100 c.c. with water.

This solution of indigo-sulphuric acid is then titrated with standard potassium permanganate solution of such a strength that 1 c.c. will represent approximately 0.0062 gram of indigo-blue. This solution of potassium permanganate

[1] Zeitschr. f. Physiol. Chem., Bd. 25, p. 406.

contains about 3 grams of potassium permanganate to the liter. Its titer is determined before use by titrating against a solution of pure indigo-blue in sulphuric acid. In making this titration the concentrated solution is not used, but a dilute one made by diluting 5 c.c. of the stronger permanganate solution to 200 c.c. (each c.c. of which will represent 0.000155 gram of indigo-blue). In this titration the blue color of the indigo-sulphuric acid does not change to any extent on the addition of the first drops of the permanganate solution, but gradually turns greenish and then becomes yellowish or entirely colorless. The amount of indigo-blue in the urine used is readily ascertained by multiplying the number of c.c. of permanganate solution used by the amount of indigo-blue represented by the titer of the solution (in the writer's laboratory 1 c.c. of the diluted solution equals 0.000155 gram of indigo-blue).

It has been found by Ellinger that only about 87 per cent. of the theoretical yield is obtained by this method, owing, probably, to the simultaneous formation of isatin from the indigo-blue. The finding by the titration may, therefore, be increased by adding about one-seventh of the value.

Folin's Method.

This method has not, as yet, been extended so as to give absolutely quantitative results. Its principle is the comparison of the color given when urine is treated with Obermayer's reagent with that of Fehling's solution as a standard. To this standard has been given the arbitrary value of 100.

Exactly one one-hundredth of the 24-hour specimen of urine is taken for each determination and treated with an equal volume of Obermayer's reagent. The indigo-blue is then extracted with 5 c.c. of chloroform until all of the pigment has been dissolved. With the chloroform solutions are then made colorimetric comparisons with Fehling's solution, using either the Duboscq or Sargent instrument. Folin finds the average indican excretion, on this basis, from a diet of 119 grams of protein to be 77, while on a nitrogen-free diet no indican is found.

This method while not as yet giving absolute figures is very convenient and is useful for the comparative work of each physician.

(*e*). **Uroroseinogen.**

This chromogen is converted into the pigment urorosein, which is rose-red in color and is very soluble in amyl alcohol, but insoluble in chloroform, ether, and benzol. The coloration due to this pigment is destroyed if the urine undergoes decomposition or becomes alkaline from other causes, but is easily restored on the addition of acid. An alcoholic solution of this pigment shows a sharp narrow absorption band between *D* and *E*. This pigment appears to be the same as described by Heller under the name urophain.

The presence of this pigment is shown by the rose-red color of the urine above the chloroform in either Jaffé's or Obermayer's test for indican. If this red-colored urine be extracted with amyl alcohol the characteristic spectrum will be shown.

This pigment appears in normal urine only in traces, being increased, however, by a strict vegetable diet. Pathologically, it is associated with marked disturbance of nutrition as seen in tuberculosis, pernicious anemia, nephritis, severe chlorosis, diabetes mellitus, carcinoma, osteomalacia, typhoid fever, and in most gastric diseases. It has, therefore, no differential value.

(*B*). Abnormal Composition.

(1). Proteins.

There has been much discussion as to whether a true physiologic proteinuria occurs. Without going into a discussion of the subject the writer wishes to state his belief that a true physiologic proteinuria occurs, but that such may never be detected by the usual clinical methods of examination. For our purposes, therefore, the presence of protein of one type or another in amounts amenable to detection by clinical methods must be considered pathological.

(*a*). Serum Albumin.

From the pathologic standpoint, serum albumin is the most important protein found in the urine. The amount excreted in 24 hours is variable and does not necessarily have any relation to the severity of the kidney lesion should one really exist. From 5 to 10 grams of this protein *per diem* may be regarded as a moderate pathologic excretion, while a lesser would be of little significance and a greater would be regarded as excessive. As large amounts as 40 grams have been observed, but such findings are exceptional. Mörner considers an excretion of albumin varying from 25 to 75 mg. per liter as a normal output, which amount would, however, escape the usual clinical tests.

As some of the more delicate tests for albumin, as for instance Spiegler's reagent, will show traces of albumin in practically every specimen of urine examined, we should limit our conception of albuminuria to those cases which react with the ordinary tests rather than with the most delicate. According to Hofmeister, the standard upon which one bases a judgment as to the presence of a pathologic albuminuria is the formation of a distinct albumin ring within three minutes after the urine and nitric acid are in contact in Heller's test (see below). Moreover, the term albuminuria should be limited to those cases in which there is some disturbance of the renal epithelium, especially of the glomeruli. This does not exclude those cases of purely functional albuminuria in which no distinct lesion of the kidney exists, as these cases are all associated with some abnormality of the excreting organ, although it is not extensive enough to constitute a definite pathologic entity.

It not infrequently happens that albumin, as found in the urine, is derived from some portion of the urinary tract below the kidney, as when inflammatory exudates, blood, lymph, spermatic or prostatic fluids, pus and other extraneous material are mixed with the urine after it is excreted by the kidney. To such cases is given the name *false* or *accidental albuminuria*, in contradistinction to the true albuminuria in which the albumin is present when excreted by this

organ. Before reporting a finding as one of true albuminuria, it is absolutely imperative that extraneous albumin be excluded.

Functional Albuminuria.

Not infrequently do we find in perfectly healthy individuals a urine which is normal in every way with the exception of the presence of albumin, which is easily detected by our ordinary methods. This type of albuminuria is observed following severe muscular exercise beyond the point to which the subject is accustomed. Thus, in raw recruits under the severe forced marches during the early days of their service we find this type very prevalent. Leube states that 59 per cent. of such soldiers show a temporary albuminuria, which disappears after the subject becomes accustomed to the increased exercise. It is a common occurrence to find such a functional albuminuria in football players, bicycle riders, crew men, and general athletes after every period of increased exertion. This question may be summed up by the statement that the excretion of albumin is simply dependent, under these conditions, upon the limit of endurance of the subject, practically everyone being able to produce an albuminuria if he overdoes in any way. Moreover, cold baths, excessive mental labor, and severe emotion may lead to quite an extensive albuminuria, especially in an individual with somewhat lessened resistance. According to Rem-Picci[1] albuminuria is a constant finding after cold baths, different subjects reacting differently to the same stimuli. This albuminuria never lasts over 24 hours and may be associated with the appearance of casts and blood, as may also the type following increased exercise. The colder the bath and the longer the immersion, the more rapid the appearance of albumin. He gives as the limit of temperature necessary to produce this condition 12 to 13° C. with an immersion of not longer than three minutes.

Another type of functional albuminuria is that following the intake of a heavy protein meal. This is known as "*alimentary albuminuria*," the albumin excreted being of the same type as that of the food. Eggs seem to be more frequently the cause of this digestive albuminuria, although meat and cheese have produced it. Croftan[2] has recently shown that egg albumin may be excreted in the urine, as the precipitin tests were positive for this and negative toward serum albumin. Whether an associated serum-albuminuria may occur is questionable. If such be the case, it would seem to be due either to an increased synthesis of serum albumin from the products of the hydrolytic cleavage of egg albumin or, as Senator suggests, to direct reflex vasomotor or trophic changes affecting the kidney. In the new-born it is not infrequent to find an albuminuria when the child is first fed on cow's milk.

The albuminuria observed in the new-born for the first few days of life, aside from the influence of feeding upon foreign proteins, is probably a further example of a true functional albuminuria. Ribbert explains this by the des-

[1] Il Policlinico., Tm. 8, 1901, p. 389.

[2] Trans. of Miss. Vall. Med. Assoc., 1907, p. 163; New York Med Jour., vol. 89, 1909, p. 474.

quamation of epithelium of the glomerular capsule. Likewise, the albuminuria observed in pregnancy is usually distinctly functional. About 50 per cent. of pregnant women show this albuminuria, little difference being observed between primiparæ and multiparæ. The kidney undoubtedly shows some functional disturbance in its attempt to eliminate the toxic products absorbed from the fetus.

According to Senator[1] such albuminurias as outlined above should be considered functional when slight in degree, transitory in character, occurring after unusual strain, either physical or mental, the subjects showing a further negative history after the removal of the direct stimulus. Whether this type is to be considered physiologic rests entirely upon the conception of the normal or abnormal nature of the stimuli leading to the excretion of albumin. It would seem to the writer that the term "physiologic albuminuria" would better be given up or at least used with great caution, as it is difficult to believe that the severe strain put upon the system and kidneys, in particular, can be distinctly physiologic. He is inclined to reserve this term for the excretion of the minimal amounts of albumin, which cannot be detected by the simpler clinical tests, and to regard the types of albuminuria, as discussed above, as "*functional*" (Pavy), or "*constitutional*" (Martius).

A second group of cases showing albuminuria is observed in which the excretion of albumin may persist for a period varying from days to months, may disappear for a variable space and then return for another indefinite length of time. To this type is given the name "*intermittent albuminuria.*" This is purely functional, no lesion of the kidney being manifest. The cases usually show a history of an acute infection or of an antecedent nephritis as the result of such infection. The most frequent cause, however, is an uncompensated heart lesion which may or may not be associated with a direct renal lesion. Not infrequently we find an hereditary intermittent albuminuria in those with a distinct neurotic family history.

The functional albuminuria may, at times, follow a definite course, disappearing and reappearing with such regularity that it has been styled "*cyclic albuminuria.*" In this form the albumin usually disappears from the urine at night and when the patient is flat on his back, but reappears during the day or when the subject is erect. The terms "*orthostatic, orthotic, or postural albuminuria*" would, therefore, seem to be more appropriate than the former appellation. This type is dependent, according to Erlanger and Hooker, upon a lowering of the pulse pressure which constantly occurs when the individual changes from the recumbent to the erect position. Jehle and, more recently, Nothmann believe that this type is due to a lordosis, and style it, therefore, *lordotic albuminuria.* Certain pathologic cases, as beginning nephritis, may show a cyclic albuminuria which may extend well into the period of recovery. In true cyclic cases the negative physical findings would lead us to class the condition among the functional albuminurias. If a case of this type becomes persistent and casts are frequently found, sub-

[1] Erkrankungen der Nieren, Wien, 1902.

sequent cardiovascular changes will usually appear, changing the case into one of true nephritis. The albumin in such cases usually appears in the urine after rising and reaches a maximum from noon to 3 or 4 o'clock, then gradually declines, disappearing from 8 to 10 P. M. If the subject changes his habits of life, the cycle of albuminuria will also change. Along with the variations in the excretion of albumin, the other urinary constituents fluctuate in the same manner, the sequence being, according to Teissier, increase in pigments, albumin, uric acid, and urea. A peculiarity of this type of albuminuria is that it may be even diminished by exercise and is less, therefore, after a hard day's work, differing from the albuminuria in nephritis or in the cardiac types.

While there has been much discussion as towhe ther such cases are not truly pathological, most of them at any rate must have a certain insufficiency of the renal epithelium. This may be due to changes in the circulation following change in posture, but it is rather surprising that increased exercise would not work more strongly in this way. Krehl regards these conditions as relatively harmless as they do not usually show any subsequent history of nephritis; Broadbent does not believe such cases ever develop actual renal disease, while Senator insists that most of them are cases of nephritis, either at the onset or during a latent attack of the disease.

The patients showing this type of albuminuria are weak anemic individuals about the age of puberty, subject to fainting spells, with a heart showing intermittent attacks of dilatation and palpitation, and with a probable congenital weakness of the kidney (37.5 per cent. having a movable kidney). The adults are neurasthenics with distinct vasomotor paralysis.

A type of albuminuria has been observed in some patients with enlarged spleen, in which albumin is present when the patient is flat on his back but is absent when he is erect. This has been called "*hypostatic albuminuria*." Its pathogenesis is uncertain, but it can have only an indirect relation to the enlarged spleen, as many cases of splenic tumor do not show this albuminuria.

A still further type of functional albuminuria is known as "*albuminuria of adolescence*." This occurs between the ages of 14 and 16, and then disappears. It is different from the cyclic type, although this latter occurs in young people. The children are usually anemic, have a neurotic family history, have an unstable vasomotor system and, possibly, a congenital weakness of the kidney. In this class should be included the albuminuria shown by masturbating children or after sexual excess at this period. The kidney does not keep pace with the physical growth and activity of the system, so that the association with the unstable vasomotor system may account for the albuminuria (Emerson). According to Sutherland, movable kidney may be accountable for some of these cases, as he finds it present in one-third of his cases.

Febrile Albuminuria.

During the course of any acute fever, an albuminuria may be observed which is not associated with distinct changes in the renal parenchyma and dis-

appears with the fall of temperature. The amount of albumin excreted may be small or great, depending upon the severity of the toxic action of the bacterial products. In ordinary cases there is practically no inflammatory condition present in the kidney, the albuminuria being due to an ischemia and a later hyperemia. In some of the infectious fevers the influence of the toxins is so great that a true nephritis originates, as especially noted in scarlet fever and diphtheria. Any febrile albuminuria may pass into a true nephritis, so that the case must be closely watched for the appearance of symptoms indicating such a complication. In some cases, an increase in the albuminuria is observed during convalescence, when only traces were previously noted. This is known as "*colliquative albuminuria.*"

Traumatic Albuminuria.

A transitory albuminuria may be observed following injuries to the kidneys or even after bimanual palpation of this organ. The albumin and casts may persist for a variable period with no other signs of renal involvement. In cases of movable kidney, especially during Dietl's crises, an albuminuria may be noted due to obstruction in the renal circulation. In cases of ureteral stenosis, an albuminuria may be observed as the result of the impeded outflow of urine. This same type of albuminuria may be seen after blocking of the ureter by a calculus, pressure from a tumor, or twisting of the ureter.

Hematogenous Albuminuria.

By this type of albuminuria we have in mind one in which the albumin is excreted as a result of some alteration in the quality and quantity of the normal protein of the blood. On the other hand a distinctly hematogenous albuminuria may be the result of the excretion of an abnormal protein. This type is observed in purpura, scurvy, pernicious anemia, chronic lead or mercury poisoning, syphilis, leukemia, jaundice, cachexia, after the inhalation of anesthetics, and in diabetes.

Toxic Albuminuria.

This type is directly referable to the influence of various toxic agents upon the kidneys. The changes in the kidney may be either of a degenerative order, leading to a distinct nephritis, or may be purely circulatory. Among the substances causing such an albuminuria we find ether, chloroform, mustard, cantharides, mercury, lead, arsenic and antimony compounds, oil of turpentine, potassium nitrate and chlorate, phosphorus, carbolic acid, salicylic acid, tar compounds (aniline derivatives), and petroleum.

Neurotic Albuminuria.

A slight transitory albuminuria may be observed in epilepsy (in which condition it may not always be found but is present invariably when marked cyanosis is seen during the attack), in apoplexy, tetanus, progressive paralysis, exophthalmic goiter, mania, delirium tremens, migraine, brain tumor, injuries

to the head especially affecting the floor of the fourth ventricle, neurasthenia, and various psychoses. Not infrequently we find neurotic patients showing an albuminuria as the direct result of perverted metabolism and not as the consequence of pathologic changes in the nervous system.

Albuminuria with Definite Renal Lesions.

In acute nephritis an intense albuminuria is a constant and important symptom. The more acute the case the larger will be the amount of albumin, the elimination being generally proportionate to the severity of the disease, although some acute cases may show no albuminuria (Herringham[1]). The percentage of albumin varies inversely as the amount of urine, as a rule, so that it is much better to excrete a larger amount of urine with a low percentage of albumin than a diminished amount of urine with an increased percentage of albumin. The absolute quantity of albumin excreted varies from 0.2 to 1 per cent. It may reach as high as 5 per cent. or higher, in one case of Senator being 8 per cent., but this is rare. The total excretion in 24 hours is rarely over 25 grams. Nephritis of syphilitic origin appears to be associated with the largest outputs of albumin.

In cases of active renal congestion from exposure to cold or through the action of drugs, or in chronic passive congestion due to cardiac, pulmonary, or hepatic lesions an albuminuria may be observed without any trace of an active renal lesion. The albumin, in these cases, is small in amount and runs parallel to the quantity of urine, thus differing from the excretion in true nephritis.

In chronic parenchymatous nephritis the elimination may be relatively large, exceeding, in some cases, that of the acute form. In the chronic interstitial type the albuminuria is very slight, rarely amounting to more than 5 grams. In this type of nephritis the albumin may be absent at various examinations, so that frequent investigations of the urine must be made. In amyloid kidney, the urine closely resembles that of the interstitial type of nephritis, a total absence of albumin being, however, less frequently observed. The serum globulin in this type of kidney disease is relatively more increased than in any other type of renal disorder, so that the albumin-globulin quotient may be of some importance in diagnosis.

Tests for Serum Albumin.

This protein is soluble in distilled water and is coagulated by heat, if the solution be acid, at a temperature varying between 56 and 81° C. The temperature at which any protein coagulates on heating will depend upon the amount of salts present. This protein is precipitated by absolute alcohol and by salts of the heavy metals, as well as by the ordinary alkaloidal precipitants. It is levo-gyrate, its degree being represented by the following formula $(a)_D = -62.6°$. It is precipitated by concentrated mineral acids, but is dissolved by

[1] Trans. Clin. Soc. of London, vol. 34, p. 901.

somewhat large excess. With acetic acid the precipitate first formed is readily soluble in a slight excess of the acid. With concentrated alkali serum albumin forms an alkali-albuminate which is less soluble in water than is albumin, but which is soluble in an excess of the alkali. This fact accounts for the spontaneous precipitation of albumin in a concentrated urine which is alkaline in reaction.

Numerous tests have been given for the detection of albumin in the urine. The writer cannot attempt to describe all of these, but must select, therefore, those which he has found most useful. The qualitative tests for proteins in general may be found in any work on physiologic chemistry.

Before any test for albumin may be made, the urine must be absolutely clear. It is advisable always to use fresh specimens, but if these are not at hand methods must be adopted to clear up the urine. In the majority of cases fitration through several folds of filter-paper will usually accomplish this. If this does not succeed, as it practically never does if the urine be cloudy from the presence of bacteria, recourse must be had to precipitating agents which will carry down the suspension of bacteria. Such agents are powdered magnesium oxid or carbonate, silicic acid, or saw-dust. These substances are thoroughly mixed with the urine and the mixture then filtered through double folds of filter-paper or plugs of asbestos fiber. The addition of lead acetate or any of the salts of the heavy metals is inadvisable, as the precipitates formed will include a large part of the albumin, the other precipitants not affecting the albumin directly. Occasionally the urine may be cleared by centrifugation.

It has been found that the tests for albumin are rendered more distinct if the urine be somewhat diluted. Hallauer[1] has shown that the excess of urea and phosphates in a concentrated urine interfere to some extent with the delicacy of the reactions. As a rule, a 24-hour specimen is examined or a specimen of the urine voided in the morning and that voided at night. The variations of the voidings of the different periods of the day are occasionally quite marked, the morning specimen frequently showing no albumin while the evening specimen may show quite appreciable amounts.

Heat Test.

This test is based upon the principle that serum albumin is coagulated by heat especially in the presence of acid. One may use either acetic acid or nitric acid, but the conditions of this addition are different in each case. If acetic acid be added one must be careful lest he add an excess, as the albumin precipitate is soluble in a very slight excess of acetic acid; with nitric acid the condition is the reverse, care being taken not to add too little else the albumin will not be precipitated by the acid. A few drops of dilute acetic acid are all that is required while with nitric acid between one-twentieth and one-tenth of the volume of the urine must be added (one to two drops of 25 per cent. nitric acid per c.c. of urine).

[1] Münch med. Wochensch., Bd. 50, 1903, p. 1539.

Technic.

A test-tube is filled about three-quarters full of the clear neutral or faintly acid urine and heated by directing the flame upon the upper portion of the tube, the lower portion being held in the hand. If the fluid remains clear and the reaction is acid, no albumin is present. If a cloud is noticed it may be rendered more distinct by holding the tube against a black back-ground when the upper portion will appear more turbid than the lower. This cloud may be due to albumin or calcium phosphate, rarely to calcium carbonate. To determine which is the cause, acidulate with a few drops of 5 per cent. acetic acid. If the urine becomes clear, the precipitate first noticed is calcium phosphate; if it remains turbid and even increases in intensity of turbidity the precipitate is albumin; while if due to carbonates an effervescence will be observed. It is wise to boil the urine after the addition of each drop of acid, so that the danger of getting an excess of acid may be more easily avoided. It is to be remembered that, if the protein be very slight in amount, and especially if the urine be originally alkaline, the protein will remain in solution owing to the formation of acid albumin. If not enough acid is added, the precipitate of phosphates may not dissolve, while if too much be added the albumin will dissolve. For these reasons it is better to add the acid after boiling. In some cases the fresh urine is already too acid to permit of coagulation, so that alkali may be added to diminish the acidity.

The presence of "nucleo-albumin" may lead to a wrong interpretation in this test. This substance is precipitated in the cold by acetic acid and may thus be differentiated. The resinous acids, which are excreted in the urine after the intake of such drugs as copaiba, cubebs, and benzoin, are not so apt to interfere with this test unless a large excess of acetic acid be used, which is never admissible.

If nitric acid be used in place of acetic acid the urine is boiled as above and concentrated nitric acid added to a strongly acid reaction. The nitric acid should never be added before boiling the urine nor should the urine be boiled after the nitric acid is added, as traces of albumin will be dissolved by the hot nitric acid. A flocculent precipitate is indicative of albumin. The phosphates and carbonates do not confuse in this reaction as they are readily dissolved. The "nucleo-albumin" is also eliminated by this test as it is readily soluble in the excess of acid. In this test the urine should be set aside and allowed to cool after the boiling is complete, as albumoses, if present, will separate out on cooling as a distinct white flocculent precipitate. A precipitate of uric acid may also form on cooling, but this is more granular and is usually colored, while the albumin precipitate is white unless an admixture of blood be present.

In case the urine be poor in salts, the tests are improved by the addition of a saturated solution of sodium chlorid. The salts hinder to a great extent the solution of the albumin by the acids. In such cases, therefore, it is wise to strongly acidify the urine with acetic acid and then add one-sixth its volume of a saturated solution of sodium chlorid as recommended by Purdy. The urine

is now boiled as above when a precipitate on heating will indicate albumin. The nucleo-albumin reaction is slight, the albumoses appear only on cooling, while the resinous acids may be precipitated but are soluble in alcohol, while the albumin is rendered more compact by this reagent.

Heller's Nitric Acid Test.

This test is, perhaps, more frequently employed than any other of the tests for albumin in the urine. It has a very wide field of usefulness, although it is not as delicate as some of those to be mentioned.

A few c.c. of concentrated nitric acid are placed in a test-tube and the urine to be tested is allowed to run slowly down the side of the tube in such a way as to form a distinct layer of urine above the acid. Some workers advise the addition of the acid after the urine, but if this is done it is much better practice to allow the nitric acid to flow from a pipet introduced to the bottom of the tube. The writer is always accustomed to allow the urine to flow upon the nitric acid from a long pipet so that the urine does not perceptibly mix with the acid, the tube being held at an angle of 45 degrees. Albumin, if present, is precipitated at the zone of contact in the form of a white opaque cloud or ring. This precipitate is acid-albumin which is insoluble in a slight excess of acid. A red or reddish-violet transparent ring is always obtained with normal urine owing to the reaction of the urinary pigments with the nitric acid. If the urine contains abnormal coloring matters this colored ring may assume various tints. Thus if bile be present a play of colors from red to yellow through blue or green takes place, the green being the characteristic coloration; if indican be in excess this colored ring may be bluish or even black; while pigments due to drugs will give colors ranging from deep red to violet. This colored ring is usually below the white opaque ring due to albumin and tends to extend down into the acid instead of up into the urine. If much nitrous acid be present in the nitric acid effervescence may be observed to such an extent that the ring of albumin may be lost. This albumin ring is usually sharply defined and separated both from the urine and acid as a white opaque ring, whose breadth will depend upon the amount of albumin in the urine. If the tube be allowed to stand for some time the ring will lose its distinct outline, a more or less diffuse cloudiness rising throughout the urine.

Albumin is, however, not the only substance to be precipitated by nitric acid under the conditions of this test. Thus we find *globulin, albumoses, and resins* precipitated exactly at the line of contact of urine and acids. If the urine be heated the albumoses will dissolve while the albumin becomes more compact. If this precipitate be due to resins the precipitate will dissolve in alcohol or ether while the albumin will remain unchanged. It is sometimes wise to shake out the urine with ether before applying this test. The globulin ring may be differentiated from that of albumin only by separating these proteins by the method to be discussed under Serum Globulin. Globulin is usually associated with albumin, is practically never found by itself, and has practically the same

clinical significance, so that for clinical purposes the differentiation of these two bodies is unnecessary. Weinberger[1] has recently shown that the addition of thymol, as a preservative of the urine, leads to the formation of a grayish-white ring just at the junction of the nitric acid and urine when this test is applied. "Below the ring there is a greenish zone extending somewhat into the acid, above it a reddish somewhat smaller zone." If this substance is suspected, the urine should be extracted by agitation with an equal volume of petrolic ether. A somewhat similar reaction has been reported by Kenney[2] in cases in which several drops of formalin were added as a preservative to a small amount of urine.

Besides these rings at the zone of contact, a further white or yellowish ring may be observed at this point. This ring is found in urines which are especially rich in urea and appears as a distinctly crystalline ring due to the formation of *urea nitrate*. If the urine be previously diluted this ring does not appear. If an excess of *uric acid* be present in the urine, we observe, on allowing the tube to stand for a few minutes, a distinct white ring in the urine about 1 to 2 cm. above the point of contact of the acid and urine.

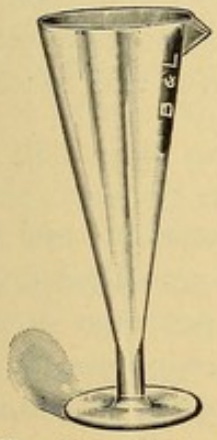


FIG. 80.—Conical test-glass.

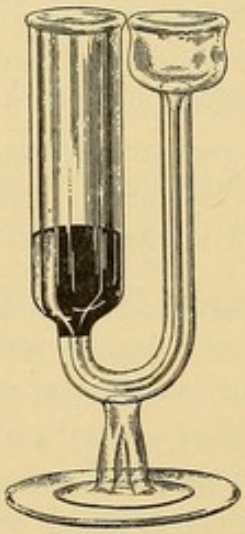


FIG. 81.—Horisma-scope.

If the *mucin-like bodies* previously discussed are present in slight excess, a diffuse cloud appears throughout the urine if the fluids have been slightly shaken or a distinct ring 1 to 2 cm. above the albumin ring may be observed if the urine is carefully added to the acid. This ring is seen in practically all urines, is never at the point of contact, and does not appear to have any clinical significance.

Instead of performing this test in a test-tube as described above, it may be done in a conical wine-glass, as recommended by Simon and Ogden, or may be employed as recommended by Boston. This latter worker uses a flat-

[1] Jour. A. M. A., vol. 52, 1909, p. 1310.
[2] New York Med. Jour., vol. 80, 1904, p. 403.

pointed pipet into which is drawn from 1 to 2 inches of the urine to be tested. The exterior of the tube is then wiped perfectly dry and the pipet, with its upper end closed with the finger, is introduced to the bottom of a bottle containing pure nitric acid. By lessening the pressure of the finger the acid gradually flows up into the pipet forming a distinct line of contact between it and the urine. The same points mentioned above obtain with this test. This modification is very simple and may be recommended for general use. A further method of performing this test is the use of the horismascope (see cut). The urine is placed in the larger tube *C* and the nitric acid allowed to flow through the capillary tube *A* so that a distinct line of contact is observed. The use of this instrument frequently brings out much more clearly the albumin ring than do the other modifications.

This test is frequently employed in combination with heat. Two methods are available, either one of which may be used, although the results are somewhat different in the two cases. Some workers advise heating the nitric acid previous to the addition of the urine. This does not seem wise to the writer, as traces of the acid albumin are undoubtedly dissolved by the hot acid and may thus escape detection. The writer is accustomed to heat only the upper portion of the tube which contains the somewhat diluted urine. In this way the urates and albumoses are thrown out of the field of action while there is much less danger of traces of albumin being dissolved. If the various points included by this test are remembered and each interfering substance removed by proper differentiation, this test is practically the most useful and reliable one for the detection of albumin in the urine.

Potassium Ferrocyanid Test.

A few c.c. of urine are strongly acidified with acetic acid and a few drops of a 10 per cent. solution of potassium ferrocyanid are added drop by drop. In the presence of albumin a faint turbidity or a flocculent precipitate will be observed, depending upon the amount of albumin present. The slow addition of the ferrocyanid is necessary, as an excess of this reagent will dissolve the precipitate first formed. In this test albumoses and nucleo-albumin are both precipitated. The former is dissolved on heating the mixture, while the latter is detected by the precipitate on the addition of the acetic acid unless the acid be added in excess. The urates do not interfere if the urine is diluted previous to making the test.

This test is recommended by many as a much more delicate one for albumin than those previously described. It reacts with a smaller amount of albumin, but it does not, in the writer's opinion, give as much general information about the urine as does Heller's test.

Sulpho-salicylic Acid Test.

This substance may be used either in the form of a 20 per cent. solution or in the solid state. If the solution be used it is added to the acidified urine in such a way that a distinct line of contact is formed. Albumin will be shown

by a distinct white ring at the point of contact. It is preferable, however, to add to the urine a small fragment of this substance, when in the presence of albumin a turbidity or a white flocculent precipitate will be observed, depending upon the amount of albumin. The albumoses are also precipitated by this reagent, but dissolve on heating. Neither uric acid nor resins react with this substance, while the mucin-like substances are not appreciably affected.

This test is, perhaps, the most convenient one for the use of the general practitioner, as the substance may be readily carried in the medicine case and added to the urine at the bedside.

Spiegler's Test.

As the original reagent of Spiegler was found to be of little value in many cases, Jolles has modified it with the following composition: 10 grams of mercuric chlorid, 20 grams of succinic acid, and 10 grams of sodium chlorid dissolved in 500 c.c. of water.

The urine is acidified with acetic acid to precipitate the nucleo-albumin if present. This substance is filtered off and the filtrate superimposed by means of a pipet upon a few c.c. of the above reagent. In the presence of albumin a distinct white ring appears at the zone of contact. This reagent precipitates the albumoses which are soluble on heating. Nucleo-albumin, if present, should be removed before applying the test. In case iodids are present, mercuric iodid will be precipitated but may be removed by alcohol.

This test is the most delicate test for albumin. It shows one part of albumin in 350,000 parts of urine. This is almost too delicate for clinical work as it will show albumin in practically every specimen of urine. The writer has found this test extremely valuable in cases which showed only a faint reaction with Heller's test. If such an urine be treated with Spiegler's reagent a much more distinct albumin ring will be observed so that all doubt is thus cleared up.

Many other tests have been advocated, but the writer does not feel that they have any advantages over those outlined above. As far as delicacy of reaction is concerned Spiegler's reagent is the most delicate, the sulphosalicylic acid, heat and acid, ferrocyanid, and Heller's test following in the order named. It will thus be seen that Heller's test is the least delicate of any of the ones spoken of above, but the writer is accustomed to use it in general work, relying upon Spiegler's reagent to settle mooted points of delicacy of reaction. As a general working rule it should be said that no one of the less delicate tests should be relied upon without being confirmed by some one of the others.

Quantitative Determination of Albumin.

Scherer's Method.

Fifty c.c. of urine are placed in a beaker and heated upon a water-bath. Two or three drops of dilute acetic acid are then added and the mixture boiled. A flocculent precipitate of albumin should separate out; if not, a drop or two more of acid is added until such a precipitate is obtained. The solution is then filtered through an ash-free filter which has been previously dried and

weighed. The filtrate should be tested by Spiegler's reagent to see if any albumin has been dissolved. If this shows no albumin, the precipitate may then be dried on the filter-paper after being washed with water, alcohol, and ether. If the filtrate shows albumin, another test with a fresh 50 c.c. of urine must be made. Much time is saved if a larger quantity of urine be originally taken, treated as above, and small portions filtered off and tested for albumin. The addition of a small amount of a saturated solution of sodium chlorid will facilitate the precipitation of the albumin. The precipitate on the filter-paper is dried and weighed, the difference in weight between the original dried paper and that with albumin representing the amount of albumin in the 50 c.c. of urine used. The total amount is determined by a simple calculation.

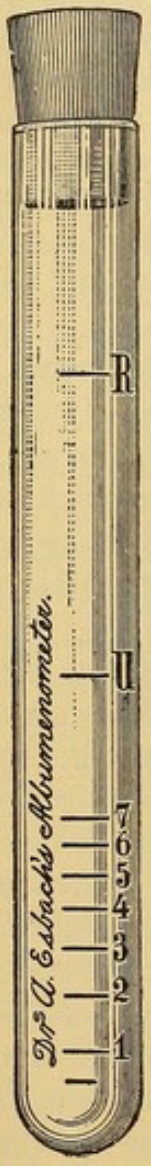


FIG. 82.—Esbach's albuminometer.

Esbach's Method.

This test is carried out in a standard graduated glass tube, known as an albuminometer (see cut). This tube is filled with acidified urine to the point *U* and the Esbach reagent added to the mark *R*. This reagent consists of a solution of 10 grams of picric acid and 20 grams of citric acid in one liter of distilled water. The tube is now closed with the rubber stopper and inverted several times in order to mix thoroughly the contents. It is then allowed to stand in a test-tube rack for 24 hours, after which the amount of albumin is read off, the graduations on the tube representing the number of grams of albumin per liter of urine.

This test can give only an approximate determination of the amount of albumin. The tube should stand in a room which does not vary much in temperature during the night, as variations in temperature may affect the depth to which the precipitate will sink. Traces of albumin will be noted in the diffuse cloudiness of the mixture, no distinct precipitate being observed. Other substances may also be precipitated. If the urine be very rich in albumin, it is wise to dilute it so that the albumin precipitate may lie somewhere between the points one and four.

Tsuchiya has introduced a modification of this method, using as the reagent a solution of phosphotungstic acid in alcohol and HCl, which gives far more accurate results than the Esbach reagent, as the figures agree closely with those of Scherer's method. It is to be remembered, however, that phosphotungstic acid precipitates all urinary nitrogenous bodies, with the exception of urea and the amino acids.

Method of Goodman and Stern.

These workers utilize the above reagent of Tsuchiya in such a way that a quick, reliable, and accurate result is obtained. They have established the fact that the acid reagent is precipitated by exactly 0.0001 gram of albumin.

A preliminary determination of the albumin is made by the Heller nitric acid test, the urine being diluted 10 times, with water acidulated with acetic acid, if much albumin be present. The reagent used is as follows:

Phosphotungstic acid,	1.5 grams
Concentrated HCl.,	5.0 c.c.
95 per cent. alcohol, q.s. ad.,	100.0 c.c.

Five c.c. of the reagent are placed in a test-tube and the filtered urine, diluted if necessary, is added drop by drop from a buret, shaking the tube after the addition of each three drops. This addition is continued until a whitish cloud appears. The number of tenths of a cubic centimeter is then read off, which represents 0.0001 gram of albumin. Thus if 1 c.c. of urine diluted ten times be used, it is obvious that 1 c.c. of the undiluted urine contains 0.001 gram of albumin, and 100 c.c. equals 0.1 gram.

It is perhaps unnecessary to state at this point that in speaking of per cent. of albumin, one should have reference only to the number of grams of albumin by weight in 100 c.c. of urine. It is not infrequent to hear of case histories in which albumin has been reported as 50 to 75 per cent. This can mean only per cent. by volume and harks back to the time when the amount of precipitate formed on boiling the urine was taken as the quantitative criterion. The urine very rarely contains more than 5 per cent. of albumin, although Salkowski has reported a case in which 8 per cent. was observed, the albumin separating out as a white amorphous precipitate on standing. This fact should be remembered, as the appearance of such a precipitate in the untreated urine might be very misleading.

Purdy's Centrifugal Method.

To 10 c.c. of the urine placed in a centrifuge tube, 3 c.c. of a 10 per cent. solution of potassium ferrocyanid and 2 c.c. of 50 per cent. acetic acid are added. The reagents and urine are then mixed by placing the thumb over the end of the tube and inverting it several times, after which the tube is allowed to stand for 10 minutes. It is then placed in a centrifuge the radius of which, with its tubes extended, must be 6 3/4 inches. The tubes are revolved for exactly three minutes at a uniform speed of 1,500 revolutions per minute. The amount of albumin is then read off in bulk percentage, each division of the tube representing 1 per cent., as only 10 c.c. of urine are used and the divisions represent tenths of a c.c. One per cent. by bulk represents 0.021 per cent. by weight of albumin.

This method is very satisfactory, although not absolutely accurate. It is difficult to keep a centrifuge running uniformly at the above rate, unless a speed indicator be watched during the entire period. The method is more exact and more expeditious than the Esbach method and is to be recommended, therefore, for clinical estimations of the amount of albumin.

Removal of Albumin.

It is advisable in many of the general quantitative tests applied to the urine that the albumin should be removed if it is present in more than traces. Usually this may be done by acidifying with acetic acid and boiling until the precipitate is flocculent. The filtrate in such cases will usually be clear and contain no albumin. As this test does not eliminate the albumoses and further as the boiling acid may hydrolyze a small amount of the albumin into albumoses, Hofmeister recommends the following method. Ten c.c. of a 40 per cent. solution of sodium acetate and the same amount of 10 per cent. ferric chlorid are added to the urine when it will be colored a bright red. The urine is neutralized or rendered very faintly acid and is then boiled. The albumin separates out along with the basic ferric acetate and is filtered off. This method is not applicable if glucose is present.

(*b*). Serum Globulin.

Serum globulin is associated in the blood with serum albumin. This protein is not a single body, but is probably a mixture of two forms known as euglobulin and pseudo-globulin. These two fractions differ in their precipitation and solubility constants and must be looked for in works on physiologic chemistry. The former of these probably occurs in most urines, constituting a large part of what has been called "nucleo-albumin" (see above).

Serum globulin, in the specific sense, is usually found in the urine in every case in which serum albumin is observed. Cases are reported in which each one of these protein bodies has appeared separately, but this is not the normal finding. Its excretion as compared with that of serum albumin varies from 10 to 75 per cent. of the total protein. The relation of the albumin to the globulin of the blood is as 1.5 is to 1. This relation, known as the "*albumin quotient*," is by no means the same in the urine. In cases of amyloid degeneration of the kidney the globulin may be very much increased beyond that observed in other chronic affections of the kidney, the albumin quotient being usually lower than one. Senator considers this an important point in the diagnosis of this condition. In the various types of nephritis we find the globulin being greater the more acute the condition, so that in acute diffuse nephritis the albumin quotient may be very low, while in chronic parenchymatous nephritis the quotient ranges from two and five-tenths to five and five-tenths. As the nephritis improves the relative amount of globulin diminishes, but increases with each acute exacerbation.

Globulin is insoluble in water, but soluble in dilute solutions of sodium chlorid, dilute acids, or alkalies, unless these be exceedingly dilute and their action not prolonged. If, therefore, urine containing globulins be highly diluted with water the globulin will be precipitated in the form of a distinct cloud. The precipitation constants with various inorganic salts must be learned from text-books on physiologic chemistry.

Qualitative Test for Globulin.

The urine is rendered alkaline by the addition of a few drops of ammonium hydrate and the precipitated phosphates filtered off. To the filtrate is then added an equal volume of a saturated solution of ammonium sulphate, the mixture is allowed to stand one hour and is then filtered. The albumoses and nucleo-albumin may also be precipitated in this way. Ammonium urate does not usually separate out in the hour. The precipitate on the filter is then washed with a half-saturated solution of ammonium sulphate until the filtrate is albumin-free. A distinct precipitate is usually evidence of the presence of globulin, as the albumin is not precipitated until the urine is completely saturated with the ammonium sulphate. In order to eliminate the other factors the precipitate is dissolved in water and heated on a water-bath to coagulate the proteins. The solution is filtered, the precipitate washed with water and heated on a water-bath with a 1 per cent. solution of sodium carbonate. It is then filtered and neutralized with acetic acid. If a precipitate occurs it is globulin, as the albumoses and nucleo-albumin would not be precipitated by such treatment.

Paton advises the use of a contact method in detecting globulin. The phosphates are removed as mentioned above and the filtered urine allowed to run down the side of a test-tube containing a few c.c. of a saturated solution of sodium sulphate. A white ring will indicate the presence of globulin.

Quantitative Determination.

The phosphates are removed as above and 100 c.c. of the clear filtered urine are treated with an equal volume of a saturated solution of ammonium sulphate or directly saturated with magnesium sulphate. The precipitated globulin is collected on a dried and weighed filter and washed with a half-saturated solution of ammonium sulphate in the former instance or with a saturated solution of magnesium sulphate in the latter case. The final washings should show no trace of a reaction for albumin. The funnel with the filter-paper and its contents are then dried at 110° C. The ammonium sulphate is washed from the precipitate with hot water and the precipitate is then dried with alcohol, ether, and finally at 110° C., until the weight becomes constant. The difference between the weight of the filter-paper and the filter-paper plus the globulin gives the amount of globulin in 100 c.c. of urine.

(*c*). Proteoses.

These are intermediate products of the digestion of protein by ferments, by acids, or by bacteria. In the normal digestion we find the products passing through the following stages: protein, acid albumin, primary proteoses (of which there are two, namely, protalbumose and heteroalbumose), secondary proteoses (the only well-established representative being deuteroalbumose), peptone, amino-acids and hexone bases. The proteoses and peptones are very soluble diffusible bodies which are not coagulated by heating. The primary proteoses are precipitated by half-saturation with ammonium sulphate,

the secondary proteoses are precipitated only after complete saturation with this salt, while the peptones are not precipitable in either one of these ways. The primary proteoses are precipitated by nitric acid, thus differing from the secondary types which are not so precipitated. In the urine we find representatives of both types of proteoses, while more or less doubt exists as to whether true peptone has ever been isolated from the urine.

Primary Proteoses.

Bence-Jones' Protein.

This body was first discovered by Bence-Jones[1] and was regarded as a heteroalbumose. Recent work by Magnus-Levy[2] has shown that it is in all probability a true albumin, as its digestion products include protalbumose which could hardly be derived from true heteroalbumose. As the exact chemical nature of this body has not been definitely settled, it will be discussed under the above heading, although it probably does not properly belong there. This body differs from all other types of protein material which occur in the urine in its property of precipitating when heated to as low a temperature as 40° C. and of practically completely dissolving on boiling, to appear again on cooling. A second characteristic of this body is the readiness with which it dissolves in dilute ammonia after it has been precipitated with alcohol. The excretion of this body in the urine has been called "heteroalbumosuria," "myelopathic albumosuria of Bradshaw," "Kahler's disease" and "Bence-Jones' albumosuria."

The amount of this protein body excreted is somewhat variable. In Bence-Jones' original case an output of 6.7 per cent. or a total amount of 70 grams in the 24 hours was observed, while Coriat[3] reports a case in which none was found in the urine, although 4 per cent. was present in the pleuritic effusion. Between these limits we find the majority of cases showing an output usually not over 1 per cent. The literature contains about 35 cases showing the excretion of this body. The output of this body appears to be constant during the day and not affected in any way by the diet. Little is known regarding the direct origin of this body. It undoubtedly has some association with the bone-marrow, but just what is not clear. "We may imagine, however, that through the agency of the cells of the abnormal tissue, that is their products of metabolism, the normal transformation of the ingested albumin into tissue-albumin is impeded, resulting in the production of the substance in question, which is then eliminated as foreign matter" (Simon).

This body is excreted in cases associated with the occurrence of multiple myelomata of the bones, especially when these affect the thoracic skeleton. In only one other case, namely, one of lymphatic leukemia, has this body been reported, so that a urinary finding may be regarded as practically pathognomonic of multiple myelomata. In some cases of this disease the urine does not

[1] Med. and Chir. Trans., vol. 33, 1850; Phil. Trans. Royal Soc., vol. 1, 1848, p. 55.
[2] Zeitsch. f. physiol. Chem., Bd. 30, 1900, S. 200.
[3] Amer. Jour. Med. Sci., vol. 126, 1903, p. 631.

show the Bence-Jones body, so that a negative finding does not necessarily preclude the condition. Ellinger has shown that this disease may take its course without the occurrence of local bone symptoms but may be associated with a marked anemia. It is, therefore, wise in cases of obscure anemia to test the urine repeatedly for this body.

Tests for Bence-Jones' Body.

The specific reaction for this protein is observed on heating the acidified urine very slowly. At a temperature varying from 50° to 60° a slight cloud changing to a marked turbidity and then into a dense cloud will be observed. This may be so intense that the urine appears distinctly milky. This turbidity may change into a heavy sticky precipitate or coagulum as the temperature approaches the boiling-point. When the boiling-point is reached the precipitate entirely or partially dissolves, especially if the boiling be continued from one to three minutes. The precipitate may not absolutely all dissolve on boiling, as variations in the acidity of the urine and the amount of mineral salts present may affect this process. If the tube be allowed to stand after being boiled the precipitate returns as the fluid cools. None of the other protein bodies give this sequence of precipitation, dissolving, and reprecipitation. Hugounenq suggests the name "*thermolytic albuminuria*" for the excretion of this body in the urine.

Upon the addition of concentrated nitric acid, drop by drop, a temporary turbidity develops which disappears on shaking, but persists if more acid be added. If the mixture be heated the precipitate will dissolve and reappear on cooling. This same reaction may be observed on applying any of the tests for serum-albumin outlined above.

This protein is precipitated from its solution by the addition of two volumes of a saturated solution of sodium chlorid to urine which has been previously acidified with acetic acid. The addition of two volumes of saturated solution of ammonium sulphate likewise causes its complete precipitation. It may be then washed with alcohol and ether and dried over sulphuric acid.

Boston[1] has proposed the following test for this body. Fifteen to twenty c.c. of filtered urine are placed in a test-tube and mixed with an equal volume of a saturated solution of sodium chlorid, the tube being shaken to insure a thorough mixing of the fluids. Two or three c.c. of a 30 per cent. solution of sodium hydrate are added and the mixture vigorously shaken. The upper one-fourth of the mixture is then gradually heated to the boiling-point and a solution of 10 per cent. lead acetate added drop by drop, the heating being continued after each addition. When the drop of lead solution comes in contact with the liquid a copious pearly or creamy cloud appears at the surface, becoming less dense as the boiling-point is neared; and when ebullition is prolonged for from one-half to one minute the upper portion of the liquid shows slight browning, which deepens to a dull black color. Standing intensifies the reaction, and if

[1] Clinical Diagnosis, Philadelphia, 1905.

this be prolonged for several hours the black precipitate falls through the clear stratum of liquid, collecting in the bottom of the tube as a coarsely granular pigment.

This reaction is based upon the fact that this body contains a large proportion of loosely-bound sulphur. Lindemann[1] finds that this body really contains no more such sulphur than does serum albumin, while Wood could obtain no more blackening than with other proteins. This test would seem to have little value as it is in no way distinctive for the Bence-Jones protein.

The quantitative determination of this body may be made by precipitation with two volumes of saturated solution of ammonium sulphate and the washing and drying of this precipitate as previously mentioned. The Esbach method is useful as an approximate estimation in the absence of albumin.

Secondary Proteoses.

Deuteroalbumose is probably the body which has been found in the urine in cases in which peptone was reported. This secondary proteose differs in its reactions from the primary proteoses and the Bence-Jones protein. It is precipitated only on complete saturation of the urine with ammonium sulphate. In performing this test the urine should be made albumin-free, preferably by the Hofmeister method previously discussed. Nucleo-albumin may be precipitated by basic lead acetate. Urine containing deuteroalbumose does not become cloudy on boiling; does not regularly give Heller's test, but does react with the ferrocyanid test when the neutral salts are present in fairly large quantities, and reacts in the cold with sulpho-salicylic acid and with Spiegler's reagent, but the precipitate dissolves on heating to reappear again on cooling. This reaction with the latter reagents in the cold may be distinguished from that of albumin by boiling the mixture and filtering while hot. The albumin remains on the filter while the albumose is present in the filtrate, appearing as a distinct precipitate as the solution cools.

Tests for Albumoses.

To a few c.c. of urine, from which albumin and nucleo-albumin have been removed as outlined above, add one-fifth its volume of concentrated acetic acid. A 10 per cent. solution of phosphotungstic acid is then added, when the urine remains clear on standing if albumoses are absent; while a milky turbidity is observed in 5 to 10 minutes if these substances are present. If this precipitate be filtered off (warming will facilitate the clumping of the precipitate), washed with distilled water and then dissolved on the filter with a very dilute solution of sodium hydrate, the solution will have a distinctly blue color. This solution is warmed until it becomes clear, more sodium hydrate being added if necessary. It is then cooled and the biuret test applied by adding a few drops of strong sodium hydrate and a few drops of dilute (2 per cent.) copper sulphate solution. On warming this mixture a beautiful violet-red color will be observed. Instead of precipitating the albumose with phosphotungstic acid, tannic acid may be

[1] Deutsch. Archiv. f. klin. Med., Bd. 81, 1904, S. 114.

used and the biuret test applied as above to the watery solution of the precipitate. It is never wise to perform the biuret test upon the urine directly unless any albumin or nucleo-albumin that may be present has been previously removed, as these substances, likewise, give this test, although the coloration is more violet than in the case of albumose.

Bang's Method.

Ten c.c. of urine are heated in a test-tube with 8 grams of finely powdered ammonium sulphate until the salt has been dissolved. The mixture is boiled for a few seconds and is then centrifugalized for one-half to one minute. The supernatant fluid is then poured off and the precipitate extracted with alcohol to remove urobilin. After pouring off the alcohol the residue is dissolved in a little water, the solution is boiled to remove albumin and filtered. The filtrate is shaken out with chloroform to remove any traces of urobilin which may have escaped previously. The watery solution is then poured off from the chloroform and tested as above for the biuret reaction.

Clinical Significance.

Deuteroalbumose may occur in the urine either alone or associated with albumin. It is observed in a great variety of conditions, so that distinct types of albumosuria may be noted.

Large accumulations of pus anywhere in the system lead to the excretion of deuteroalbumose as a result of the breaking down of the pus cells and the later absorption of the hydrolyzed material. This form, known as *pyogenic albumosuria*, is observed in pneumonia during the stage of resolution, in gangrenous processes anywhere in the system, in empyema, bronchiectasis, abscess formation, and in epidemic cerebrospinal meningitis. In this latter condition the differential diagnosis from a tubercular meningitis often rests on the appearance of albumose in the urine.

A *hepatogenous* form of albumosuria occurs in any condition associated with marked disturbance of hepatic function, as for instance in acute yellow atrophy, phosphorus poisoning, cirrhosis, carcinoma, and catarrhal jaundice. Little is known of the origin of the albumose in this condition.

An *enterogenous* type is observed in cases of gastric or intestinal ulcer, whether the latter be due to typhoid fever or dysentery, while intestinal tuberculosis is less frequently associated with the appearance of albumose in the urine. In these cases the breaking down of the tissues may be responsible for increased absorption both of the products of hydrolysis of the tissues and of the food.

An albumosuria of *hematogenous* origin has been observed in cases of scurvy, leukemia, purpura, dermatitis, poisoning with hemolytic agents, pregnancy, especially after the death of the fetus, and in various psychoses, as well as in carcinoma affecting any part of the system. The albumosuria in these cases is probably referable to the increased lysis of the cells under the influence of the exogenous or endogenous toxins.

A *febrile* type is observed in practically all fevers, more especially the infectious types, such as measles, scarlet fever, diphtheria, acute articular rheumatism, smallpox, and mumps. This is referable both to the influence of the toxins of the disease in producing increased protein disintegration as well as to the associated septic conditions.

A large number of other conditions are associated with the appearance of albumose in the urine. Such a state is due to the breaking down either of tissue or of an exudate, and may, therefore, appear in almost any type of disease. In some cases albumose may appear in the urine, following the ingestion of a large amount of albumose. This is the *digestive* or *alimentary albumosuria* and appears to be indicative of an ulcerative condition somewhere along the intestinal tract.

As previously stated, albumose may be associated in the urine with albumin, constituting the *mixed albuminuria* of Senator. In these cases the albumosuria may precede the albuminuria, may alternate with it, or continue after it has disappeared. This condition is particularly prominent in cases of nephritis, especially of the syphilitic type, and should be watched with care. In any case of albumosuria it is necessary to exclude contaminations with foreign material, especially with spermatic or prostatic secretions.

(*d*). **Peptone.**

True peptone rarely if ever appears in the urine. Peptones are the last hydrolytic products of protein which give the biuret reaction. This body is not precipitated on saturation with ammonium sulphate as are the other types of protein material. Ito reports the finding of true peptones in the urine in cases of croupous pneumonia, pulmonary tuberculosis, ulcer of the stomach and in women during the puerperal period. In these cases deuteroalbumose was also present, so that there is a possibility that the peptone isolated by Ito was derived from the urinary albumose. Many reports of peptonuria are found in the literature, but the substance dealt with in practically all of these cases was probably some type of albumose and not true peptone.

As this substance has no clinical value, the writer must refer to works on physiologic chemistry for a discussion of its properties and tests.

(*e*). **Hemoglobin.**

This body is the normal coloring matter of the blood and is to be regarded from the chemical standpoint as a chromoprotein. In the normal metabolism disintegration of red blood-corpuscles is constantly occurring, but this is not sufficient to lead to a hemoglobinemia and a resulting hemoglobinuria. These two conditions must go hand in hand, the latter being impossible without the former.

When the destruction of the red cell becomes so extensive that the accumulation of the blood pigment in the blood-current is so great that the liver is unable to convert it into bilirubin, hemoglobinemia and hemoglobinuria must result. While the distinct limit of destruction of cells necessary to produce

this condition is not definitely settled, it may in general be said to occur when approximately one-sixtieth of the hemoglobin of the corpuscles is set free. The protein really excreted in the urine is not hemoglobin, but methemoglobin, so that the term methemoglobinuria would be better used, although a direct hemoglobinemia does obtain.

From what has been said above it is evident that the excretion of this protein will inevitably occur after the use of the so-called hemolytic poisons. Among these we find ether, chloroform, snake-venom, arseniuretted hydrogen, phosphorus, hydrogen sulphid, toluylendiamin, mushrooms, anilin, lactophenin, bile salts, chlorates, pyrogallic acid, naphthol, carbolic acid, carbon monoxid, and tuberculin. Hemoglobin will appear in the urine in cases of poisoning with the above substances only when the hemoglobinemia is extensive. In mild cases, the liver will be called upon to form increased biliary pigment and the urine will, therefore, contain bile pigments instead of blood pigments. Likewise, we find a hemoglobinuria following transfusion of the blood of animals into man, after severe burns, exposure to cold, in the course of any of the specific infectious diseases, and in malaria and syphilis. The so-called "blackwater fever" is more probably a malarial hematuria than a hemoglobinuria. The use of quinin in malaria is said in some instances to lead to a hemoglobinuria, but the writer has never been fortunate enough to see such a case.

A paroxysmal type of hemoglobinuria has been occasionally reported in the literature. This occurs in typical paroxysmal forms after exposure to cold or exertion, and is often preceded by a typical "infectious" onset, such as chill, fever, and malaise, along with pain in the lumbar region. The hemoglobin may be excreted for several days and then disappear with no untoward symptoms. This condition is very rare and its cause uncertain. An epidemic hemoglobinuria occurs at times in the new-born and is associated with a distinct hemoglobinemia, jaundice, and cyanosis. Some unknown toxic agent is at the bottom of this condition.

The urine, in cases of hemoglobinuria, may be clear, but is generally turbid, and varies in color from a bright red to almost a black. The turbidity gives the appearance of a peculiar smoky or hazy urine. The urine must be examined when freshly voided, as blood-corpuscles soon disintegrate in the urine, giving it the same appearance as noted in hemoglobinuria. The clinical significance of hematuria and hemoglobinuria are much different and should not be confounded. If the urine be centrifuged the supernatant fluid will be a clear blood-colored liquid and the sediment will show none or very few red cells.

The tests for the presence of blood pigments must be applied in order to differentiate this protein from the other types. Naturally, any specimen of urine containing hemoglobin will react to the albumin tests previously given so that it may be very difficult to determine whether a true albuminuria is coexistent. As a rule, in such conditions there is an associated nephritis so that all the findings of this latter condition may obtain. The chemical tests indicate the presence of hemoglobin or of any of its derivatives and do not differentiate

a hemoglobinuria from a hematuria. Microscopic examination for the presence of red blood-cells is the only possible way of clearing up such a diagnosis. A spectroscopic examination of the urine will differentiate the types of blood pigments (see Blood).

Tests for Hemoglobin and Derivatives.

Heller's Test.

A few c.c. of urine are strongly alkalinized with sodium hydrate and heated. Either at once or on standing a brownish-red precipitate of the phosphates and the carbonates of the alkaline-earths is formed, the color being due to the hematin carried down by the phosphates if blood is present. If the urine contains a large amount of foreign pigments, this red coloration may not be easily noted. In this case filter off the precipitate and dissolve it in acetic acid, when the solution becomes red if blood pigment is present, the color gradually fading upon exposure to air. If this test be controlled by the spectroscopic tests for hematin in alkaline solution it becomes quite reliable and very delicate. It indicates one part of oxyhemoglobin in 4,000 of urine.

Donogany's Test.

Ten c.c. of urine are treated with 1 c.c. of ammonium sulphid solution and 1 c.c. of pyridin. If blood be present, the urine will assume a more or less intense orange color, which may be more evident on looking through the test-tube lengthwise. In this case the hemoglobin has been converted into hemochromogen, which may be recognized by the spectroscopic test. This test is more delicate than the previous, showing one part of blood to 8,000 of urine.

Instead of the above tests, which are more directly applied in urine, the guaiac and aloin tests as discussed under Feces may be applied. These tests have a special importance from the negative standpoint, as a positive test does not necessarily prove the presence of blood. They are more delicate than is the spectroscopic, but the latter is more reliable.

The spectrum of the various blood pigments will be discussed later, so that the writer need only refer to the section on Blood for this. If the urine contains fresh blood the spectrum is that of oxyhemoglobin, while in cases of hemoglobinuria or of hematuria of renal origin the spectrum is that of methemoglobin. The urine to be tested spectroscopically should be slightly acid and perfectly clear. If a large amount of blood pigment be present, the spectrum will be much clearer if the urine be diluted. This dilution should not be carried too far, otherwise the absorption lines will not appear. In testing for methemoglobin the spectrum of neutral as well as of alkaline methemoglobin should be looked for.

(*f*). Fibrin.

The occurrence of this protein in the urine is very rare. As fibrin is derived from fibrinogen through the action of the fibrin ferment, the presence of the

former body presupposes that the latter two substances have been present somewhere along the genitourinary tract. This substance is an elastic, grayish, stringy material insoluble in water and alcohol. Chemically, fibrin belongs to the group of globulins; it is soluble with difficulty in dilute saline solutions, is coagulated by heat, and precipitated either by great dilution with water or by saturation with magnesium sulphate.

This protein may occur in the urine either in the coagulated form or in solution. It is found in any condition in which large amounts of blood are present in the urine, whether the blood comes from the kidneys or points below. It may coagulate immediately after voiding or may occur as preformed clots which are formed either in severe inflammations of the pelvis of the kidney, of the ureter, bladder, or urethra. It occurs also in cases of chyluria and rarely in direct nephritis.

In some cases the fibrin is in solution, especially in urines containing no blood. This fibrin separates out in the form of a coagulum on standing or may change the urine into a distinctly gelatinous mass. This so-called "spontaneously coagulable urine" is seen more frequently in cases of chyluria, but may be observed in rare cases of nephritis.

Test for Fibrin.

The clotted material is filtered off from the urine, is thoroughly washed with water and boiled in a 1 per cent. solution of sodium carbonate. On cooling this solution may be tested as outlined under Serum Albumin.

Other protein bodies, such as histon and nucleo-histon, have been reported in the urine by several workers. It is also possible that protamin has been observed. As these substances are little understood, have no clinical value at present, and require more or less elaborate methods for their absolute identification, the writer must refer to other works for a discussion of them.

(2). **Carbohydrates.**

Normally the urine contains traces (0.01 to 0.03 per cent.) of carbohydrates which are incapable of detection by the ordinary clinical tests. Besides these true carbohydrates the urine contains other substances which react, especially toward copper solutions, as do the monosaccharides. These latter reducing bodies are uric acid, creatinin, conjugated glycuronic acids, and various pigments, either normal ones excreted in unusual amounts or abnormal ones excreted in usual amounts. The total output of the reducing bodies of the normal urine varies between 2 and 3 grams in 24 hours, while the normal true carbohydrates of the urine vary from 0.2 to 1 gram per diem.

(*a*). **Glucose** (d-**Glucose**) (**$CH_2OH—(CHOH)_4—CHO$**).

The normal blood contains about one part per thousand of glucose. Whether this sugar is in the free state or in combination with other molecules, as for instance as the so-called jecorin of Drechsel, is at present an unsettled question. According to Claude Bernard, sugar will appear in the urine when-

ever more than three parts per thousand are present in the circulating blood. This figure is, in view of the recent work of von Noorden, Stern, and Liefmann, much too high, as the average finding in their cases was 0.85 part per thousand. The excretion of sugar in the urine is known as glycosuria and presupposes an excess of sugar in the blood (hyperglycemia). In only one form of glycosuria do we find absence of this hyperglycemia; that is, in conditions in which the kidneys become less impervious than normally to the sugar circulating in the blood. This type of glycosuria is most frequently observed in cases of poisoning with phloridzin, and has lead to the assumption of the clinical entity "*renal diabetes mellitus*." The pathology of such a condition is little understood so that we may for the present disregard this type and limit our discussion to the glycosuria which inevitably follows a hyperglycemia.

Glycosuria.

The normal metabolism is such that any excess of carbohydrate food is converted, up to a certain point, into glycogen and stored up in the liver. Should this ingestion of carbohydrates exceed the functional power of the liver to convert it into glycogen, the excess will pass through the hepatic filter into the circulating blood, thus causing directly a hyperglycemia. Unless increased muscular activity is sufficient to utilize this excess, the kidneys will excrete sugar until the normal relations again obtain. This is the purely alimentary type of glycosuria and ceases as soon as the intake is diminished. The type of food ingested has much to do with the extent of the glycosuria. Under normal conditions it matters relatively little how much carbohydrate is ingested in the form of starch, as the products of hydrolysis are gradually absorbed and do not lead to overactivity of the liver with a resulting hyperglycemia and glycosuria. On the other hand, a certain limit, different for each individual, is observed in the amount of sugar which may be ingested without causing a glycosuria. For this reason Naunyn has regarded alimentary glycosuria as of two distinct types: (1) that following the ingestion of starch, which he styles *glycosuria ex amylo*, and (2) that following the ingestion of an excess of sugar, *glycosuria e saccharo*. The amount of starchy or of saccharine food which a person may ingest without a glycosuria is known as the *assimilation limit* or *degree of tolerance* for such food. This factor varies for each individual under normal conditions and under pathologic influences is dependent upon the state of the intestines, liver, pancreas, muscles, and kidney. A normal person may stand an intake of from 200 to 300 grams of glucose without excreting more than traces in the urine, but in the majority of persons this figure would, perhaps, be found to be more nearly 150 grams than 300. It has been found that the administration of as small amounts as 50 grams of galactose and lactose was followed by an excretion of these sugars in the urine, while maltose, dextrose, levulose, and saccharose required much larger intakes. The excreted sugar, following the increased ingestion, is in most cases similar to that taken in, although Moritz has shown that some of the polysaccharides may be partially hydrolyzed into their mono-

saccharide components. It has been found by Worm-Müller that a large intake of cane-sugar is not followed by a maximum excretion of this sugar, even though the assimilation limit has been greatly exceeded. Thus he observes, after an intake of 50 grams of cane-sugar, an excretion of 0.1 gram, while after an intake of 150 grams the excretion was only 0.85 gram.

"Alimentary glycosuria occurs in a healthy person only by saturating the organism with soluble carbohydrates. Therefore it is absent after administering starch, as in this case no more sugar will be absorbed than can be metabolized in the body. It is also scanty, or quite absent, if sugar solutions be given on a full instead of on an empty stomach. Naunyn has observed, regarding alimentary glycosuria, that there is excreted in the urine only that sugar which, according to Ginsberg, reaches the general circulation through the thoracic duct, thus avoiding the liver. Still other external influences may come into play, such as altered capacity of the tissues, especially those of the importantly concerned liver. The result of this may be that excessive doses of glucose are stored up as glycogen or fat in a given time. For saccharosuria and lactosuria the relationships are somewhat different. Here it is very obvious that these double sugars, if given in excessive quantities, are not completely split up in the intestine or during their passage through the intestinal wall, but enter the general circulation as such. The organism, like most of the yeasts, cannot decompose these sugars to any extent, so that they leave the body with the molecules unaffected" (Magnus-Levy).

In testing for the pathologic type of alimentary glycosuria, it is customary to give 100 grams of cane-sugar or glucose either in the morning on an empty stomach or two hours after a very light breakfast. The urine of healthy persons should remain free from sugar, while a pathologically lowered limit of tolerance will be observed by a more or less extensive glycosuria beginning in about one hour, reaching a maximum within two to four hours, and lasting about 8 hours. If this glycosuria follows the administration of starchy foods, the condition is probably a pure diabetic one, while a glycosuria following 100 grams of grape-sugar does not necessarily indicate a diabetes. It is probable that cases showing a somewhat low assimilation limit for sugar belong to the type of mild diabetes, although many class them in the general category of "hepatic insufficiency." That the liver is incapable in such conditions of polymerizing the sugar into glycogen cannot be disputed, but the question at issue is, as von Noorden shows, upon what does the insufficiency of the liver depend? I quote from the article in his Handbuch der Pathologie des Stoffwechsels as follows: "The cause cannot be overfilling of the glycogen reservoir, by which we explain the alimentary glycosuria of the healthy. In most cases the subjects are in ill health and their previous diet anything but excessive, so that there is little reason to suppose that their glycogen repository was already filled to overflowing. There is no more reason for supposing that they have a diminished power of utilizing sugar. In none of the affections in question are the processes of oxidation and of energy production

decreased; rather is there a great increase in oxidation and especially in the combustion of carbohydrate in certain of the diseases, such as Graves' disease and high fever, which predispose to alimentary glycosuria. Either the liver-cells are unable to polymerize all the sugar reaching them or else the glycogen formed is too quickly converted into sugar, through some increase in the diastatic process. The latter would imply that the automatic regulation between sugar combustion and sugar-formation is no longer so evenly balanced as in health. Physiologically the diastatic process is dependent only on the sugar requirements of the body; here it would also be controlled by the sugar-supply."

The idea of hepatic insufficiency as a distinct clinical entity responsible for the alimentary glycosuria can hardly hold. One should not be satisfied with a mere statement that such a condition exists, but should attempt to explain why it does obtain. In many conditions of the liver in which an undoubted insufficiency is present, no glycosuria can be caused by administration of a fairly large amount of glucose. On the other hand, administration of levulose in these conditions produces a distinct alimentary levulosuria. To the classes of cases which give this levulosuria should be, according to Strauss, applied the term "hepatic insufficiency" rather than to those showing an alimentary glycosuria.

It would lead me too far afield to discuss the various factors which influence the appearance of sugar in the urine. In many cases a restriction of the diet within the assimilation limit will rid the urine of sugar, while in others a constant production of sugar within the system occurs. This endogenous production of sugar may again be of several types. Thus we find in nervous conditions, especially in affections in the region of the fourth ventricle, an excretion of sugar which continues only so long as the glycogen of the body is not used up. If no carbohydrates be taken in the food, glycosuria will soon disappear. This condition is due to interference with the normal nervous control of the glycogenic function of the liver. This type of glycosuria, to which the name "*neurohepatogenous glycosuria*" has been applied, is found in a variety of conditions, such as progressive paralysis, multiple sclerosis, cerebral tumors, peripheral neuritis, traumatic neuroses, mania, melancholia, and hysteria. In this type we find the administration of carbohydrates in the food being followed by a glycosuria, because the diastatic processes are continually urged to increased activity, to such an extent that the hepatic artery becomes loaded with sugar. In other words, we have, in this type, an increased rate of saccharification rather than a diminished formation of glycogen.

In other cases a continuous glycosuria is observed even after complete exclusion of carbohydrates from the diet. In this type there is a constant formation of carbohydrates from the protein and fat of the food. The liver appears to be capable of storing up glycogen in the usual amount, but the system in general is practically unable to utilize the sugar brought to it. In consequence of this the liver-cells are repeatedly called upon to convert the

glycogen reserve into glucose, and as a result the blood becomes laden with sugar. Even under these circumstances the hyperglycemia would not necessarily cause a glycosuria, providing the normal ferments of the blood arising from the internal secretion of the pancreas were present. This type of glycosuria is in reality typical diabetes mellitus and is so dependent upon such a large number of factors that the writer must refer to other works for its discussion. Recently Pflüger has shown that a very close relationship exists between the nervous influences of the duodenum and pancreas. His work apparently indicates that what is generally known as pancreatic diabetes is dependent to some extent upon disturbance of the proper correlation between the duodenum and pancreas. As so little is definitely known regarding the glycolytic ferment which the pancreas furnishes to the blood it would seem at present useless to speculate upon the probable influence of the duodenum in producing a typical diabetes mellitus. A peculiarity in the true diabetic glycosuria is that other types of sugar, especially levulose, appear to be well tolerated by the diabetic individual without leading to a glycosuria. Besides, the administration of the primary hydrolytic products of glucose, as for instance gluconic and saccharic acids, apparently diminishes an already existing glycosuria. This would seem to indicate that the system is primarily unable to bring about the initial cleavage of the glucose molecule. As it has been shown that many diabetic cases are associated with sclerosis of the islands of Langerhans in the pancreas, this organ is usually regarded as the principal seat of the pathologic changes in true diabetes mellitus. On the other hand, many cases which are clinically indistinguishable from those of pancreatic diabetes show no lesion in this organ postmortem nor any characteristic lesion in other organs. We are, therefore, confronted with the possibility that this disease is a representative of a truly chemical type of pathologic perversion of metabolism rather than as one which yields gross or microscopic evidences of pathologic changes.

In conditions in which the oxygen supply of the system is markedly reduced as in suffocation, poisoning with carbon monoxid, curare, amyl nitrite, ether, and chloroform, a glycosuria of more or less transient duration is observed. It is interesting to note that for a long time diabetes was regarded as a condition in which the oxidative powers of the system were markedly reduced. It has, however, been proven beyond all doubt that in this disease the oxidative powers may be increased, at any rate are never diminished. Following the administration of many drugs, such as strychnin, morphin, cocain, and adrenalin, a glycosuria is observed in which the oxygen deprivation is supposed to be the causative factor, according to Herter.

Qualitative Tests for Glucose.

The qualitative tests for sugar depend for the most part upon the chemical structure of its molecule. The hexoses belong either to the class of aldehyds or ketones, and as such will reduce metallic oxids to lower forms. The CHO and CO groups of the aldoses and ketoses, respectively, are the reacting points

in all of the reduction tests, such as those with copper and bismuth solutions, as well as in the tests showing the formation of the characteristic osazones. Moreover, these carbohydrates show the peculiarity of fermenting, in the presence of yeast, into alcohol, carbonic acid, and other products. This fermentation test, especially with the saccharomyces cerevisiæ, is given only by the sugars having three or a multiple of three carbon atoms in the molecule. Fischer's work has shown that only those sugars may be fermented by a specific ferment in which the ferment and sugar stand to one another in such a relation that a chemical union is possible between them or as he expresses it, only when the ferment fits into the sugar molecule like a key in a lock. This explains why only certain types of the hexoses will ferment in the presence of yeast.

Before any qualitative or quantitative test may be made for the presence of sugar in the urine, albumin must be removed, especially if present in more than traces. This may be done by precipitation with lead acetate and filtering or boiling with dilute acetic acid and filtering.

Trommer's Test.

To a few c.c. of urine in a test-tube are added one-third its volume of a 10 per cent. solution of sodium hydrate and then, drop by drop, a 10 per cent. solution of copper sulphate. This copper sulphate should be added with constant shaking until a slight excess of the precipitated cupric hydrate ($Cu(OH)_2$) remains undissolved and is visible, on shaking the tube, as a distinctly greenish-blue flocculent precipitate. The upper layer of the urine is then warmed, when a yellow or red precipitate appears in the heated urine if sugar be present. As a rule, it is unnecessary and even unwise to boil the solution, as otherwise substances other than sugar may produce the reaction. It is true that the reaction is not as sensitive unless the solution be heated to boiling, but other substances do not so readily interfere with the reaction at a lower temperature. The yellow or red precipitate will gradually form throughout the mixture, from above downward, and will finally settle out, leaving a colorless or yellow fluid above.

This reaction is due to the reduction of the cupric hydrate, which is formed by the action of the sodium hydrate upon the copper sulphate, into cuprous hydrate, which becomes dehydrated, on heating, into cuprous oxid. If no sugar be present in the urine and other reducing substances are not excessive in amount, the cupric hydrate will settle out on warming as a black precipitate of cupric oxid. The equations showing these points are as follows:

$$CuSO_4 + 2NaOH = Na_2SO_4 + Cu(OH)_2$$

$$Cu(OH)_2 + \text{heat} = CuO + H_2O$$

$$2CuO + \text{glucose } (CHO-(CHOH)_4-CH_2OH) = Cu_2O + \text{ gluconic acid } (COOH-(CHOH)_4-CH_2OH).$$

Whether a yellow or a red precipitate forms will depend upon the alkalinity of the solution, the stronger the alkalinity the more pronounced is the red color due to cuprous oxid, while in less strong alkaline solutions the yellow

color of cuprous hydrate will predominate. Certain substances normally present in the urine as well as some which may be added to it have the property of holding in solution the cupric hydrate first formed. This property is shown by the deep blue color which the solution assumes. This color is very intense in the presence of sugar, but it is unwise to assume the presence of sugar from this fact alone. Among the substances which dissolve cupric hydrate and which may be present in the urine in varying amounts we find ammonium compounds, albumin, uric acid, creatinin, allantoin, mucin, glucose, lactose, maltose, pyrocatechin, hydroquinon, alkapton acids, bile pigments, and glycuronic acid. On warming the solution, which may not contain sufficient amounts of glucose to give a typical reaction, a slight reduction of the copper solution will occur leading to the formation of a dirty yellow solution. If these bodies be present in excess a distinct precipitate may occur, and as a result marked confusion may arise regarding the presence of sugar. It has been shown that uric acid and creatinin do not readily reduce at as low a temperature as does sugar, so that slight warming is much better than boiling. The presence of these bodies very frequently leads to a change in color from the bright blue to the greenish-yellow which may be due to the presence of sugar in small amounts, but should never be regarded as indicative of a pathological glycosuria.

It should be a working rule, therefore, that mere decolorization of the fluid should not be regarded as due to sugar. Glucose reduces so much more markedly than the other bodies mentioned that a distinct granular precipitate either of cuprous oxid or hydrate forms and settles out, leaving a supernatant fluid partially or completely decolorized. Normal urine or urine containing an excess of uric acid, creatinin, or ammonium compounds practically never produces an immediate precipitate unless the solution be boiled for some time. This is due to the fact that these substances hold in solution the amount of cuprous oxid which is formed by their reducing action. If such solutions be allowed to stand for a time a reddish-yellow precipitate may occur, but this should not confuse as the typical sugar reaction occurs immediately on warming unless too much copper solution be added. In case only traces of sugar be present, we may obtain little or no positive reaction because the sugar holds in solution the traces of cuprous oxid formed just as do the other substances above mentioned. As a rule, however, sugar in pathologic amounts shows a reducing action over and beyond its dissolving action, so that a red or yellow precipitate must settle out. The limit of this test for sugar is about 0.2 per cent., in which case the reduction will occur as in normal urine without the separation of the characteristic copper precipitate. Even in this case the yellow color is somewhat more intense and is clearer than the dirty yellow color produced in the presence of excessive uric acid or creatinin.

If the urine contain an excess of the conjugated glycuronates, the test may be quite as distinctive as that for sugar. To differentiate such reactions, one should resort either to the phenylhydrazin or the fermentation tests to be described later.

In the performance of Trommer's test it is essential that as much copper as possible be in solution in order that the reduced cuprous oxid may be later precipitated. This does not mean that an excess of copper should be added. If such be the case, the amount of sugar present may be insufficient to hold in solution the cupric oxid, which may, then, settle out as a granular black precipitate and thus obscure the red color of the cuprous oxid. It has been found, as a rule, that the addition of the cupric sulphate until a few flakes remain undissolved usually subserves this purpose. One part of sugar reduces about five parts of cupric hydrate, so that the aim should be to have about this relation between the sugar and copper. The sodium hydrate should be added in the proportion of approximately 11 parts to 5 of the copper solution. The excess of the hydrate has a marked effect in lowering the temperature at which sugar will reduce the copper solution. It has also been found that it is advisable to have as small amounts as possible of the normal urinary constituents, which reduce copper solutions, present. For this reason many writers advise the dilution of the urine so that these bodies may have less influence in proportion to the loss of reducing power of the glucose. The addition to the urine of such preservatives as chloral, chloroform and formalin must be borne in mind, as these substances increase the reducing power of the urine.

This test has very much the same relation to the detection of sugar in the urine as does Heller's nitric acid test toward albumin in the urine. If care be taken to add about one-tenth the volume of alkali, the copper sulphate until a few flakes of cupric hydrate remain, and to warm the solution to a point below the boiling-point, the immediate formation of a reddish or yellow precipitate will indicate sugar almost beyond the question of a doubt. The presence of the conjugated glycuronic acids, which will be discussed later, must, however, be remembered as well as a possible reduction which may occur in the presence of homogentisic and uroleucic acids. The reduction due to uric acid, creatinin, allantoin, pyrocatechin, hydroquinon, mucin, and bile pigments may usually be avoided by diluting the urine and by not heating to the boiling-point.

Fehling's Test.

In order that as much copper as possible may be in solution to be placed at the disposal of the sugar, it has been found that the addition of certain substances will greatly facilitate the test. Such additions are advisable as glucose itself cannot hold in solution as much copper as it can reduce. Fehling uses Rochelle salt (sodium and potassium tartrate) in an amount sufficient to yield 5 of $Cu(OH)_2$ to 1 of glucose. The formula for his solution is as follows:

Solution A.		Solution B.	
Copper sulphate,	34.64 gm.	Rochelle salt,	173 gm.
Distilled water, q. s., ad.,	500 c.c.	Sodium hydrate	125 gm.
		Distilled water, q. s., ad.,	500 c.c.

In performing the test with Fehling's solution equal parts of solutions A and B are taken and the mixture brought to a boil. The urine is then added, drop by drop, when a reduction of the copper solution will appear in the presence of sugar. The amount of urine added should rarely exceed 10 drops, at the outside 20, if a reduction is to be taken as typical and the solution should not be boiled for more than a few seconds after adding the urine. The addition of larger volumes of urine will usually introduce errors from the factors mentioned under Trommer's test. This test has the same points of interest as has Trommer's test, so that it is wise to dilute the urine before making the test. It is more frequently used than is Trommer's test, but it is not as convenient as two separate solutions must be measured out before the test can be applied.

Haines' Test.

Haines has introduced a modification of Trommer's test by adding glycerin, instead of Rochelle salt, to increase the amount of copper in solution. This test is the one in daily use in the writer's laboratory and has the great advantage that the mixed solution keeps almost indefinitely without the usual reduction occurring, which is so noticeable when the two parts of Fehling's solution are mixed and allowed to stand.

The composition of Haines' qualitative solution is as follows:

Copper sulphate,	12 grams.
Potassium hydrate,	45 grams
Glycerin,	90 c.c.
Water, q.s., ad.,	1000 c.c.

A perfectly clear, transparent, dark-blue liquid results which throws down a very slight reddish deposit of cuprous oxid on standing a week or more. This does not affect the value of the solution, as the clear blue solution is simply decanted as required.

One or two c.c. of this solution are placed in a test-tube and gently boiled. Six drops of the suspected urine are added and the upper portion of the mixture brought to a boil and immediately removed from the flame. If sugar be present an abundant yellow or yellowish-red precipitate is thrown down; if no such precipitate occurs sugar is absent. The precautions to be observed in using this test are never to add at the outside more than 10 drops of urine and not to boil the mixture for more than one or two seconds after the addition of urine.

This test is especially to be recommended to the general practitioner owing to its simplicity and to its fairly reliable results. Here, as in the other copper tests recommended, an excess of other reducing substances must be kept in mind. A negative test is certain evidence of the absence of sugar in pathologic amounts; while a positive test does not necessarily prove the presence of sugar unless all precautions are taken to exclude interfering substances. It is advisable in any doubtful case to resort to other tests as confirmatory evidence.

Almén-Nylander's Test.

This test is a modification of the original Böttger test and is a distinct improvement. The reagent is prepared as follows: Four grams of Rochelle salt are dissolved in 100 c.c. of 10 per cent. sodium hydrate solution with gentle heat, and as much bismuth subnitrate is added as will dissolve (about 2 grams). After the mixture is cooled the undissolved bismuth subnitrate is filtered off and the filtrate kept in a dark bottle, where it will remain permanent for a long period.

To a few c.c. of urine in a test-tube are added one-tenth of the volume of this reagent and the mixture boiled for a few minutes. If glucose is present, the fluid will darken and a black precipitate of metallic bismuth separate out. This black precipitate must occur while the solution is being warmed and not after it has cooled. If only a small amount of sugar be present the phosphates precipitated by the alkali may be slightly gray in color instead of the usual white. This test is somewhat more delicate than is the copper test, as it will show about 1/40 per cent. of sugar.

This reagent is not reduced by uric acid, creatinin, pyrocatechin, hydroquinon or homogentisic acid; but it is reduced by the conjugated glycuronic acid, excess of urinary pigment and pentoses. It is particularly necessary in this test that albumin be removed, as bismuth sulphid forms in the presence of albumin, and may precipitate either in the form of a reddish or a distinctly brownish, even black precipitate. The reduction observed after the administration of medicaments, such as rhubarb, senna, antipyrin, camphor, salicylic acid, salol, sulphonal, trional, quinin, eucalyptus, oil of turpentine, and chloral hydrate, is usually a brown rather than a black unless the solution stands for some time. The administration of saccharine usually results in a reduction of this test, while the copper tests are not affected by it. Sulphur bodies (methyl mercaptan) excreted after the patient has eaten asparagus give a distinct precipitate which may be more or less confusing. If the urine be ammoniacal the reaction may not appear in a characteristic way owing to the fact that the free ammonia is evolved and the alkalinity of the solution is reduced by the combination of the sodium ion with the acid radical formerly bound to the ammonium ion.

This test is used by many as a routine test for sugar, as it is practically always negative with normal urine. The reducing action of the glycuronates and pentoses must, however, be remembered.

Fermentation Test.

As previously stated, the reduction tests do not absolutely prove the presence of sugar. All that one can say is that a reducing substance is present and if the reaction be typical the probability is that the reducing substance is sugar. The fermentation test is, perhaps, the most certain of all the tests for glucose and depends upon the fact that only those sugars which contain three or a multiple of three carbon atoms are fermentable with yeast. Not all members

of these groups of sugars will ferment with yeast, so that for absolutely scientific purposes fermentation will not differentiate them. Fischer's work along this line should be carefully read by anyone interested in the biologic properties of the various types of sugar. As the hexoses, which occur in the urine are practically limited to glucose and levulose, we are safe in saying that any sugar fermenting with yeast is one or the other of these monosaccharides, which may be differentiated by tests to be outlined later.

The test is performed as follows: Ten c.c. of urine are placed in a test-tube and a piece of compressed yeast, which should be perfectly fresh, about the size of a pea is added and the urine gently shaken until the yeast is finely divided. This mixture is then poured into a fermentation tube, which is allowed to stand in the incubator for a few hours. Two control tests, using in one normal urine and yeast and in the other normal urine, yeast, and a trace of dextrose, are then made and placed in the incubator along with the suspected urine. The presence of sugar is indicated by gas (CO_2) in the upper portion of the fermentation tube. The rapidity of formation of this gas depends upon the amount of yeast as well as upon the age of the yeast. The test indicates from 0.1 to 0.05 per cent. of sugar, especially if the urine be sterilized by previous boiling. The compressed yeast as usually purchased develops a certain minimal amount of gas in normal urine, so that the control test is necessary both to show whether "self-fermentation" is excessive and also whether the yeast is at all active in producing CO_2 from glucose when it has been added. It is necessary, moreover, that decomposition of the urine be prevented, either by previously boiling the urine, addition of a trace of sodium fluorid, or tartaric acid.

This test has the great advantage that the other substances, which reduce copper and bismuth solutions, do not ferment. If precautions are observed to add the right amount of yeast, not to shake the yeast and urine violently enough to include much air, and to prevent bacterial decomposition, this test will positively show the presence or absence of glucose or levulose in the urine and no other substances. It is always wise to use this test either as confirmatory or decisive in conjunction with the previous reduction tests.

Phenylhydrazin Test.

This test is much more delicate than any of the previous tests mentioned. Theoretically it will show sugar in the amount present in normal urine, but practically no definite reaction is observed. The principle of the reaction is the decomposition which occurs between the aldehyd or ketone group of the sugar molecule and the amino group of the phenylhydrazin. In this reaction, characteristic bodies known as hydrazones are first formed, which are converted, in the presence of dilute acetic acid and an excess of phenylhydrazin, into crystalline bodies known as osazones. These latter bodies are characteristic of the sugar group, usually differing from one another, depending upon the original sugar from which they were formed. The osazones crystal-

lize in definite forms, the purified crystals showing rather sharp melting-points. It is, therefore, esential not only that a crystalline body be obtained in this reaction, but that the crystalline form and the melting-point of the crystal be that characteristic of the sugar suspected. The success of the test will depend largely on the relation of the sugar to the reagents, the best proportions being approximately one of sugar, two of phenylhydrazin, and three of sodium acetate. All of the members of the hexose and pentose groups show this reaction, as do many of those of the polysaccharide series. As will be seen from the reaction given below, those sugars which differ only in the space-relations of the atoms attached to the first two carbon atoms can possibly give the same osazone. For this reason we find glucose, levulose, and mannose as well as glucosamine giving exactly the same osazone (phenylglucosazone), showing a characteristic yellow needle-shaped crystalline deposit, with a melting-point of 204 to 205° C. The reactions leading to the formation of this body are shown in the following equations:

$$CH_2OH-(CHOH)_4-CHO+NH_2-NHC_6H_5=CH_2OH-(CHOH)_4-CH=N-NHC_6H_5+H_2O$$

$$CH_2OH-(CHOH)_3-CHOH-CH=N-NHC_6H_5+NH_2-NHC_6H_5=CH_2OH-(CHOH)_3-C-CH+2H_2O$$

$$\begin{array}{c} \\ \\ \\

PLATE VIII



OSAZONS. (*Hawk.*)

Upper form, dextrosazon; central form, maltosazon; lower form, lactosazon.

hydrazin. This mixture is then evaporated by boiling to 3 c.c., after which it is cooled quickly and is then rewarmed and allowed to cool slowly. If glucose be present to the amount of 0.02 per cent., pure crystals of phenylglucosazone separate out in 5 to 10 minutes. If the urine has a high specific gravity and a low sugar content the crystals do not form so quickly. This test is much to be preferred in well-equipped laboratories, but is not as convenient as the use of the crystalline phenylhydrazin hydrochlorate for the general worker, as the fluid phenylhydrazin is quite irritating and is not as easy to work with.

This test cannot be used for quantitative purposes as the yield is never complete. For qualitative purposes, however, it is to be especially recommended. When properly applied it is the most delicate and one of the most reliable tests at our disposal. If the melting-point of the crystals be determined the only confusing substances will be levulose, glucosamin, and mannose. The osazones formed from the other carbohydrates and the glycuronic acid compounds crystallize in somewhat similar form, but do not show the characteristic melting-point of 204° to 205° C. of the phenylglucosazone. It is true that the impure crystals melt at somewhat lower temperature, but if carefully purified from 60 per cent. alcohol they will melt at approximately 204°.

Quantitative Methods.

Fehling's Method.

The solutions to be used in this test have been given under the qualitative test for sugar. The principle of the test is the determination of the exact amount of sugar necessary to decolorize a mixture of 5 c.c. each of solutions A and B. Providing chemically pure copper sulphate has been used in preparing solution A, the mixture is reduced by an amount of urine which contains 0.05 gram of glucose. If there is any question regarding the purity of the copper sulphate, the strength of the solution must be determined by titrating against a known solution of chemically pure glucose, or of cane-sugar which has been inverted by heating with dilute acid (see Sutton's Volumetric Analysis).

Technic.

Five c.c. each of solutions A and B (p. 276) are carefully measured from a burette or pipette into an Erlenmeyer flask of about 250 c.c. capacity. Fifty c.c. of distilled water are then added and the mixture boiled. The urine is now added in small quantities from a buret to this boiling copper solution until the blue color has entirely disappeared. It is advisable to add the urine rather rapidly as a preliminary test, it being practically impossible with a single titration to obtain accurate results. In doing this one adds a c.c. of urine at a time and observes whether the color still remains. If so, the titration is continued, adding from 1/2 to 1 c.c. at each period, until the color has disappeared. The number of c.c. used is then observed and a second test made as follows: To the boiling copper solution is added directly 1 c.c. less of

urine than was used for complete decolorization in the preliminary test. The urine is then added two drops at a time, noting after each addition whether the color remains. In this way an accurate end-point may be reached.

This test is not as simple as it would seem. The final disappearance of every trace of blue from the liquid is best observed by placing the eyes on a level with the meniscus of the fluid, traces of blue being more easily observed in this way than by looking at the liquid from above. The final color of the liquid will vary between a perfectly colorless fluid and one showing a faint tinge of yellow. If too much urine has been added the color will be a deep yellow to orange. As this test is not so accurate with high concentrations of the urine, it is necessary that the sugar content should range between 1/2 and 1 per cent. We are, therefore, forced, as a rule, to dilute the urine so that the sugar may come within these limits. In deciding as to the proper degree of dilution, the specific gravity of the specimen may be of great value. The following table, taken from Naunyn, will show approximately the percentage of sugar under different conditions:

2 liters of urine of specific gravity 1028 to 1030 = 2 to 3 per cent.
3 liters of urine of specific gravity 1028 to 1032 = 3 to 5 per cent.
5 liters of urine of specific gravity 1030 to 1035 = 5 to 7 per cent.
6 to 10 liters of urine of specific gravity 1030 to 1042 = 6 to 10 per cent.

A good working rule is to dilute a urine of a specific gravity of 1030 five times (by adding four volumes of water), and 10 times if the specific gravity be above 1035. As this test shows the presence of sugar when present to the amount of 0.08 per cent., there is little danger in this way of getting the dilution too low to show the characteristic reaction for sugar. It not infrequently happens that the red precipitate of cuprous oxid obscures the final end-point. It is, therefore, wise to remove the flask from the flame when the color has apparently disappeared from the fluid. Caution must be taken, however, at this point not to allow the solution to remain for more than a few seconds in contact with the air, as rapid oxidation may occur and a solution which was colorless may again become slightly blue, thus making one believe that the final end-point had not been reached. Some workers advise the removal of a drop of the fluid from the flask and the placing of it upon a sheet of filter-paper. If this wet spot be touched with a drop of dilute acetic acid and one of potassium ferrocyanid, a brown coloration, due to the formation of copper ferrocyanid, will appear unless the copper has been entirely precipitated from the solution. In this way one may definitely prove that the end-point has been reached. The writer, however, has rarely found this precaution necessary if ordinary care be taken to watch the meniscus of the fluid for faint traces of blue coloration.

As the amount of urine used to decolorize the 10 c.c. of copper solution is equivalent to 0.05 gram of glucose a simple calculation will yield the percentage of glucose in the urine. Thus if 5 c.c. of urine diluted five times were

necessary to decolorize the copper solution it is evident that the undiluted urine contains 5 per cent. of glucose according to the following calculation:

$$5 : .05 : : 100 : x = 1.$$

As the dilution was 5, $5 \times 1 = 5$ per cent.

Knowing the quantity of the 24-hour specimen, the total sugar excretion is obtained by a simple multiplication.

In the use of this method as well as in the other methods to be described, albumin must first be removed if present in more than traces. As this test reacts also to the various reducing bodies previously mentioned, a marked increase in any of these substances will introduce a slight error into the result. This is overcome to a large extent, however, by the dilution of the urine which is always advisable. It occasionally happens that the reduction of a copper solution does not occur so typically, but instead a yellowish green fluid forms from which the cuprous oxid does not separate. In such cases the recognition of the end-point is almost an impossibility. For this reason there have been introduced various modifications of Fehling's solution, which are much to be preferred as routine methods.

Purdy's Method.

It has been found that the addition of strong ammonia to the mixed copper and tartrate solutions makes the end-point much more distinct. Pavy, Sahli, Kumagawa and Suto, Kinoshita, and others have modified the original Fehling's solution in this way. It was shown, however, by Löwe that the substitution of glycerin for the Rochelle salt made a much more stable solution and one which could be originally mixed and kept for indefinite lengths of time. Purdy has succeeded in obtaining a copper solution which, in the writer's experience, is much to be preferred for general laboratory purposes over most of the other modifications of Fehling's solution. His formula is as follows:

Chemically pure copper sulphate,	4.752 grams
Potassium hydrate,	23.500 grams
Strong C. P. ammonia (sp. g. 0.88),	350.000 c.c.
Glycerin,	38.000 c.c.
Distilled water, q.s. ad.,	1,000.000 c.c.

This solution is prepared by dissolving the copper sulphate and glycerin in 200 c.c. of distilled water, heating gently if necessary. The potassium hydrate is dissolved in a second 200 c.c. of water and mixed with the copper solution. When the mixture has cooled add the ammonia and bring the total volume up to 1 liter with distilled water. Thirty-five c.c. of this solution are decolorized by 0.02 gram of glucose.

Haines has slightly modified this original solution of Purdy so that 10 c.c. of the solution are decolorized by 0.01 gram of glucose. This titer is somewhat more convenient than that of Purdy as the calculation is distinctly simplified. The formula for his modification is as follows:

Pure copper sulphate,	8.314 grams
Pure potassium hydrate,	25.000 grams
Glycerin,	40.000 c.c.
Ammonia,	350.000 c.c.
Distilled water, q. s. ad.,	1,000.000 c.c.

The principle of this test depends upon the fact that, in the reduction of cupric oxid in solutions of definite strength by glucose, the blue coloration disappears on the addition of a definite amount of glucose without any attendant precipitate, the reduced solution remaining transparent and colorless.

Technic.

Thirty-five c.c. of Purdy's test solution or 10 c.c. of Haines' solution are measured into an Erlenmeyer flask and 50 c.c. of water added. The flask is then closed with a doubly perforated rubber stopper, through one hole of which passes the stem of a buret containing the suspected urine and through the other a bent glass tube to conduct the fumes of ammonia away from the observer. The object of closing the flask with the stopper is to exclude the air and thus prevent reoxidation of the cuprous oxid. The contents of the flask are now brought to a gentle boil and the urine added, 1 c.c. at a time, shaking the flask after each addition until the fluid is completely decolorized. The number of c.c. used is then noted and a second test made, adding at once 1 c.c. less of urine than the total number of c.c. used in the first experiment. The last portions of the urine are added two drops at a time, allowing from three to five seconds to elapse between the addition of these separate portions. When the urine is completely decolorized the number of c.c. used is noted. As the amount of urine used is equivalent to 0.02 gram of glucose with Purdy's solution or 0.01 gram with Haines' solution, the percentage may be obtained as in the previous method. It is necessary with this method that the urine be diluted for the same reasons as previously mentioned.

In all of the copper tests for glucose the influence of preservative agents must be remembered. Thus chloroform, chloral hydrate, and formalin will all reduce copper solutions so that an error may be introduced unless these substances are removed.

This method of Purdy is used daily in the writer's laboratory and the results are eminently satisfactory. He has frequently checked his solution and the method by titration against known glucose solutions so that he is convinced of the accuracy of the method.

Many other modifications of the copper tests have been advocated, but the writer does not feel that they have any advantage from the clinical standpoint over those discussed. The more scientific method of Allihn, in which a copper solution is reduced and the precipitated cuprous oxid either weighed or further reduced to metallic copper in a stream of hydrogen is not clinically available. The modification of Rudisch, Rudisch and Celler, Gerrard and

Allan, and the very recent extremely accurate method of Benedict[1] are not as simple, do not give more accurate results, and would, therefore, find a place more in the larger laboratories than in the equipment of the general practitioner.

Polariscopic Method.

In this test the urine must be absolutely clear and must contain no albumin. Perhaps the best clearing method is to mix the urine with a solution of lead acetate, which will precipitate the albumin and remove any excess of pigment. The mixture is then filtered and the clear filtrate used for the test. If the urine contains no albumin, magnesium oxid or silicic acid may be used as clearing agents. It must be remembered that in clearing the urine with a solution of lead acetate, a correction must be made in the percentage of sugar in the filtrate. This may be done by precipitating 75 c.c. of urine with 25 c.c. of 10 per cent. solution of lead acetate and filtering. The sugar in the filtrate represents, therefore, only three-fourths that of the original urine. All that

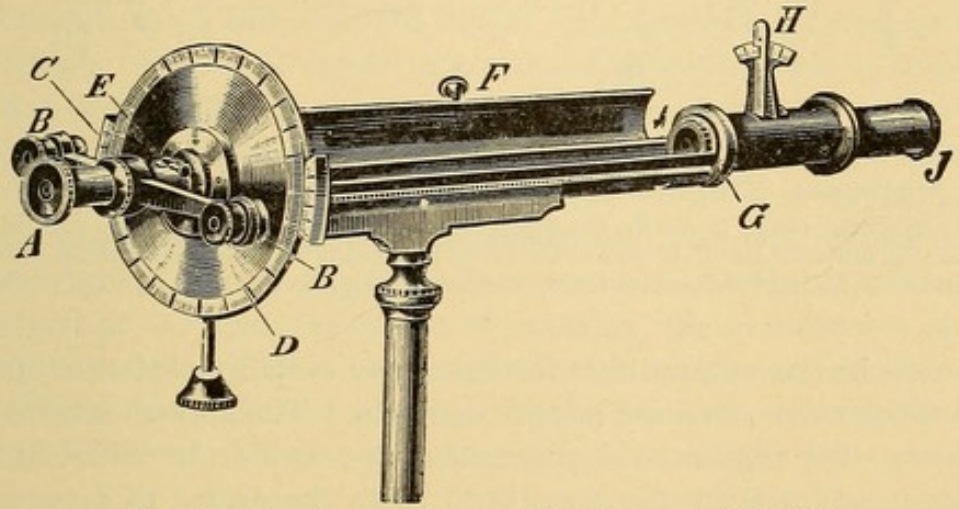


FIG. 83.—One form of Laurent Polariscope. (*Hawk.*)

B, Microscope for reading the scale; *C*, a vernier; *E*, position of the analyzing Nicol prism; *H*, polarizing Nicol prism in the tube below this point.

is necessary is to correct as follows: If the filtrate contains 4.8 per cent. of sugar it is obvious that the total percentage of the original sugar is 6.4, according to the following calculation: If 4.8 per cent. represents three-fourths of the total sugar, one-fourth is equivalent to one-third of 4.8 or 1.6, and four-fourths represents 4 x 1.6, or 6.4 per cent.

Various types of polarimeters have been suggested and most of them are quite satisfactory for the determination of sugar. One of these with its description is seen in the accompanying cut and legend. The principle upon which their use depends is the fact that optically active substances when in solution have the power of turning the plane of polarized light either to the right or the left. The zero point of the instrument is determined by observing the point at which the halves of the optical field have exactly the same degree

[1] New York Med. Jour., vol. 86, 1907, p 497.

of illumination, when the light passes through a tube either empty or containing an optically negative fluid. The point at which the graduated scale of the instrument and the vernier correspond is regarded as the zero-point. In the use of the polariscope any deviation of the plane of light passing through the polarizing Nicol prism will be noticed by a darkening of one portion of the field, so that the compensating or analyzing Nicol must be rotated until both parts of the field are equally illuminated. In this way one readily determines how much the plane of polarized light has been deviated, by observing the degree of rotation necessary to bring the two portions of the field into equal illumination when the light passes through an optically active fluid. The most reliable instruments for general work are known as the "half-shadow" types, but they rarely find a place in the equipment of the general worker. The examination is usually made in a dark room, the light passing through the tube of the polariscope from a sodium flame.

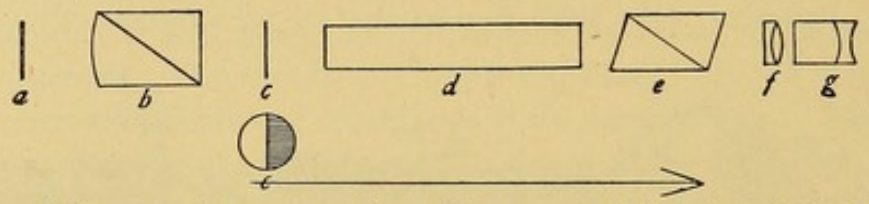


FIG. 84.—Diagrammatic representation of the course of light through the Laurent polariscope. (Direction reversed from that of previous figure.) (*Hawk.*)

a, Bichromate plate to purify the light; *b*, the polarizing Nicol prism; *c*, a thin quartz plate covering one-half the field and essential in producing a second polarized plane; *d*, tube to contain the liquid under examination; *e*, the analyzing Nicol prism; *f* and *g*, ocular lenses.

It must be remembered that the urine may contain substances other than glucose which rotate the plane of polarized light. The normal urine is slightly levorotatory, the degree varying between 0.05 and 0.18. Albumin is also levorotatory and will interfere markedly with the degree of rotation referable to glucose, unless it be very slight in amount or be entirely removed. Levulose, β-oxybutyric acid, and the conjugated glycuronates are also levorotatory, the second being especially prone to interfere with the glucose rotation as it so frequently is associated with glucose in diabetic conditions. If the urine has been previously heated with acid before being examined in the polari scope, glycuronic acid will also interfere, as it shows distinct dextrorotation.- Cane-sugar as well as lactose are occasionally found in the urine and may lead to confusing results as both are dextrorotatory substances. It is, therefore, necessary before any reliable results may be obtained with the polariscope to remove the interfering substances, either as preliminary to the determination of glucose or subsequently, a correction being then made for the rotation of the interfering substance. It is to be said that the polariscope is more fitted for a clinical laboratory than for the general practitioner.

The rotation of light when passing through optically active solutions is dependent upon several factors, among which we find the temperature at which the observation is made, the length of tube through which the light passes and

the concentration of the active solution. By specific rotation of a fluid is meant the rotation observed when light passes through a solution containing 1 gram of the active substance per c.c. of fluid and placed in a tube one dcm. in length. Thus we find the specific rotation of glucose in such a tube being $(a)_D = +52.74$. It is, therefore, evident that the percentage of sugar in an unknown solution contained in a tube 1 dcm. in length may be obtained by dividing the degree of rotation by 0.5274. Some of the tubes for clinical purpose are constructed of such a length (188.6 mm.) that one degree of rotation equals 1 per cent. of glucose. For a full discussion of the subject of optical activity of fluids the writer would refer to the work of Landolt.[1]

Technic.

Having determined the zero-point of the instrument, the tube, which should be thoroughly cleaned and dry, is filled with the fluid to be examined. In filling this tube precautions must be taken not to include any bubbles of air, which is best done by filling the tube until a convex meniscus is observed and then sliding the glass disk over the end in such a way that the excess of fluid is shoved off. The metal cap is then screwed on tightly, but in such a way that undue compression is not exerted upon the glass disk. The tube is placed in position, the field distinctly focussed and the degree of rotation determined by revolving the analyzing Nicol until the two portions of the optical field have exactly the same intensity of illumination. This will require considerable experience, as the accuracy of the determination will depend not only upon the clearness of the fluid, the degree of focussing, the sensitiveness of the instrument, and the brightness of the light, but also upon the sensitiveness of the observer's eyes. Several readings should be made, turning the prisms from both directions and observing the degree at which the fields are isochromatic, the average being taken as the final result. As the eyes soon become fatigued, they should be used for only a few seconds at a time.

Having determined the degree of rotation, the percentage of sugar is then calculated as previously mentioned. This method, if carefully used and interfering substances avoided, is the most accurate method for the determination of glucose. It is, however, difficult, requires much experience, and is not as sensitive as the other methods, rarely detecting the presence of less than 0.2 per cent. of glucose. Theoretically, the percentage of sugar as determined by the polariscope should exactly agree with that obtained by titration of copper solutions, but in general work such is never the case, as interfering substances may be present or have not been completely removed.

The writer must refer elsewhere for methods of correcting the readings of the polariscope when interfering substances are present, as he does not think it wise to recommend the general worker to waste his time with this instrument when sufficiently accurate clinical results may be obtained by methods which are for him much easier and less liable to error.

[1] Das optische Drehungs-Vermögen, Braunschweig, 1898.

Fermentation Methods.

The principle of this method has been previously outlined. It has been found that, if the precautions mentioned are observed, carbon dioxid is evolved quantitatively from glucose by the action of yeast. The most convenient method of applying the test is to use an Einhorn *fermentation tube* which is so graduated that the amount of CO_2 evolved is directly read off in terms of per cent. of glucose. For this purpose the urine must contain less than 1 per cent. of glucose, the urine being diluted as previously described to bring the amount within this figure, multiplying the result, of course, by the degree of dilution. The urine, which should be acid in reaction, is shaken with a piece of compressed yeast about the size of a small pea, all precautions being observed as mentioned above. The mixture is then poured into the fermentation tube in such a way that no air-bubbles collect at the upper end of the tube. Controls as previously described are then made and the three tubes placed in the incubator at 37° C. In a few hours bubbles of gas (CO_2) will collect at the top of the tube, the fermentation being practically complete overnight. The percentage of sugar is then read direct from the calibration (1/4–1 per cent.) on the tube (the figures from one to five representing c.c. of gas and not per cent. of sugar).

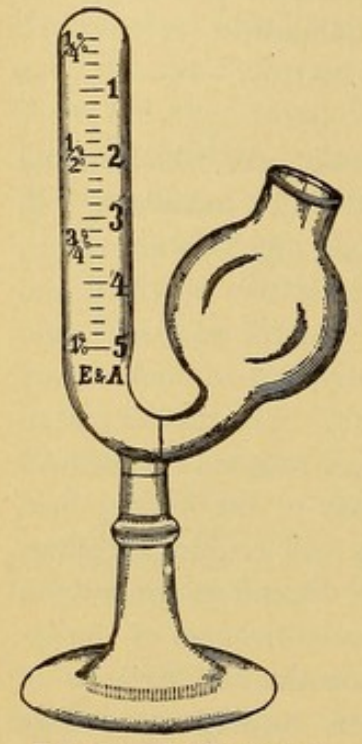


FIG. 85.—Einhorn saccharometer.

Lohnstein's Saccharometer.

This apparatus is seen in the accompanying cut. Twelve c.c. of mercury are placed in the bulb of the apparatus. One-half c.c. of the urine to be tested is then floated upon the mercury and treated with a thick paste of compressed yeast diluted two or three times with water. The stopper is then carefully greased with vaseline and inserted so that the two apertures correspond. By tipping the apparatus a trifle the column of mercury in the long tube is then adjusted to the zero point of the scale. When this is done the stopper is turned so that the holes no longer correspond and the weight is placed on the stopper to prevent leakage from the increased pressure of the gas liberated in the fermentation. The apparatus is then placed in the incubator and the extent of fermentation read off by noticing the height to which the column of mercury rises in the long arm of the instrument. The fermentation is usually complete within six hours. After removing the apparatus from the incubator it is allowed to stand in the air for a few minutes to adjust itself to room temperature, as the scale is graduated in this way.

This method gives results which correspond very closely to those of titration and is to be recommended for the quantitative determination by fermentation methods.

Lohnstein has also introduced a saccharometer which may be used with diluted urine. It is seen in the accompanying cut. It does not, in the writer's opinion, have any advantage over the above-mentioned apparatus as the principle is the same, although the urine must be diluted.

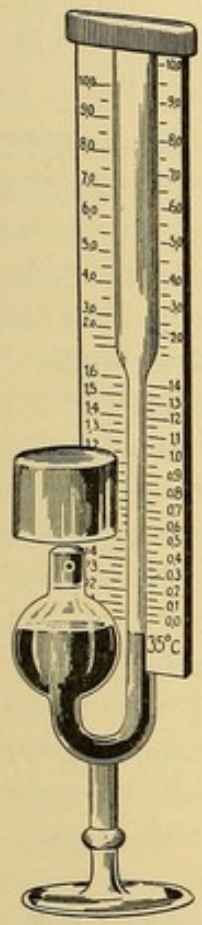


FIG. 86.—Lohnstein's fermentation tube for undiluted urine.

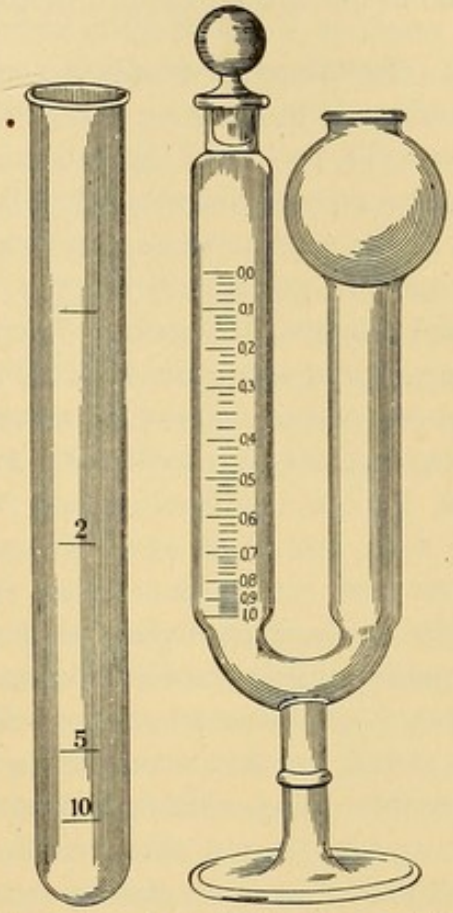


FIG. 87.—Lohnstein's fermentation tube for diluted urine.

Robert's Method.

This method has been recommended for the quantitative determination of sugar and is based upon the fact that the specific gravity of the urine is changed in a quantitative way when the sugar of the urine is fermented.

The urine must be acid before applying this test. A piece of yeast about the size of a bean is added to the urine which is allowed to ferment at incubator temperature until no further qualitative test for sugar is obtained. This will usually require from 24 to 48 hours, so that a trace of sodium fluorid should be added to the urine to prevent bacterial action. The specific gravity of the urine before it is subjected to fermentation is very carefully taken either with a very accurately standardized hydrometer or, preferably, with the pycnometer. After fermentation is complete the specific gravity of the fermented urine is determined in the same way. The difference in the specific gravity of the two specimens is then multiplied by 234 to obtain the percentage of sugar. Or,

according to Purdy, each degree of specific gravity lost in fermentation corresponds to 1 grain of sugar per fluidounce.

If the specific gravity be accurately determined the results are correct within 0.1 per cent. As this method requires, however, the use of a pycnometer and a very accurate chemical balance, it can hardly be recommended to the general practitioner for his work, although some writers state that it should be preferred to the more uncertain titration with Fehling's solution.[1]

(*b*). **Levulose (d-fructose). $CH_2OH—(CHOH)_3—CO—CH_2OH$.**

Levulose is found very widely distributed throughout the vegetable kingdom, especially in fruits. Honey is almost a pure levulose. It may be found in the urine, transudates, or exudates, after a large intake of levulose-containing food or may occur spontaneously, when the subject has taken little such food. The *levulosuria*, reported by Zimmer, Ventzke, Czapek, Worm-Müller, Seegen, Mauthner, Cotton, Roehmann, Personne, Henninger, Marie, and Robinson, must be accepted with reserve, as the incompleteness of the methods then in use afforded no certain means of recognizing levulose (Neuberg[2]). The most authentic cases of true levulosuria are those of May, Schlesinger, Rosin and Laband, Lépine and Boulud, and Neubauer. This pure levulosuria occurs in both sexes and at all ages, the amount of sugar excreted being subject to variations from 2.7 grams per diem (Schlesinger) to 24 grams (Lépine).

More common than pure levulosuria is its association with a glycosuria. This combination appears in all forms of diabetes, in the severe types levulose being practically never missed, according to Umber, especially when no restriction is placed on the carbohydrate intake. Neubauer's observations on this point are interesting. He finds that withdrawal of carbohydrates causes both levulose and dextrose to disappear from the urine. When levulose was given it was utilized, but when glucose was taken it was less completely assimilated, being excreted in part as levulose.

The tolerance for levulose is, as a rule, less than that for glucose, so that we are not surprised to find that the administration of 100 grams of levulose to normal individuals is followed by a levulosuria in about 10 per cent. of cases, while no such effect may be observed in diabetes mellitus. Just why diabetics should tolerate levulose and not glucose is not clear, but we must remember that not all diabetics do. Here the question of individual tolerance must be considered, as Umber finds 25 grams of levulose excreted following an intake of 100 grams, while the writer has observed an excretion of 75 grams on the same intake. Strauss[3] finds that an alimentary levulosuria occurs, after an intake of 100 grams of levulose, in 90 per cent. of cases of functional hepatic disturbance. This test would seem, therefore, to be a valuable indication of hepatic insufficiency, as Sachs, Chajes, Lépine, Baylack and Arnaud, and Bruining assume, although Landsberg doubts its value. In a case of spon-

[1] Simon. Clinical Diagnosis, Phila., 1907.
[2] Handbuch der Pathologie des Stoffwechsels, Berlin, 1907 S. 716.
[3] Deutsch med. Wochensch., Bd. 27, 1901, S. 757 and 786.

taneous levulosuria in which the urine showed about 0.9 per cent. of levulose, Rosin and Laband could not cause an increase in the degree of excretion by administering 100 grams of levulose. It is not necessary, as Neuberg and Strauss have shown, that a previous ingestion of levulose take place for a levulosuria to occur.

The chemical reactions of levulose are very similar to those of dextrose. Owing to the presence of the ketone group in its molecule, it shows the same reducing actions as does the aldehyd group of the glucose. Like glucose it ferments, but not quite so readily. It is levorotatory, its specific rotation being $(a)_D = -91$ degrees. Leo has reported the finding of a levogyrate carbohydrate in diabetic urines, which he believes to be laiose. This differs from levulose in being unfermentable with yeast. Levulose forms exactly the same phenylosazon as does glucose, so that it is a matter of great difficulty to differentiate these bodies by the tests given above. Lobry de Bruin and Alberda von Eckenstein have shown that glucose and levulose may change, the one into the other, by the action of traces of alkali, acids, and neutral salts, such as sodium acetate. This point must be borne in mind, as it may explain why levulose is excreted in certain cases.

Seliwanoff's Test.

This test has been advanced as one characteristic for the ketoses in distinction from the aldoses. Ten c.c. of urine are treated with a few crystals of resorcin and 10 c.c. of concentrated HCl. If the mixture be warmed a brilliant red color appears in the presence of a ketone (levulose) while no coloration is observed with an aldehyd (glucose). Müller has shown that glucosamin gives this test, while R. and O. Adler find a reaction in the presence of nitrous acid. If the mixture be heated too strongly or too long, mannose and maltose may also give a positive test. Adler finds that the use of acetic acid with a trace of HCl gives better results than HCl alone. It will be seen, therefore, that this test is not so characteristic as was believed, but it serves to distinguish levulose from glucose, which is the important point.

If the red solution formed in this reaction be neutralized with sodium carbonate and extracted with amyl alcohol or, preferably, with acetic ether (Borchardt), the extract will have a yellow color with a faint green fluorescence and becomes rose-red on the addition of alcohol. The spectrum of this solution shows a sharp line in the green between *E* and *b*, while if the solution be quite concentrated a second weaker line will be seen in the blue at *F*.

Neuberg[1] has recently shown that fructose forms a characteristic osazone with phenylmethylhydrazin, while no such compound is obtained with glucose, mannose, or glucosamine. The test is, therefore, the most reliable and scientific one for the presence of levulose, although it is not clinically so acceptable as the Seliwanoff reaction.

[1] Zeitsch. f. physiol. Chem., Bd. 36, 1902, S. 227; Ber. d. d. chem. Ges., Bd. 35, 1902, S. 959; *Ibid.*, Bd. 37, 1904, S. 4616.

The formation of this osazone occurs according to the following equation.

$$CH_2OH-(CHOH)_3-CO-CH_2OH+2NH_2-N(CH_3)(C_6H_5)=CH_2OH-(CHOH)_3-C-CH+2H_2O+2H$$

$$(CH_3)(C_6H_5)N-N \qquad N-N(CH_3)(C_6H_5)$$

Technic.

The urine is acidified with acetic acid and boiled to remove albumin if present. The mixture is then filtered and the clear filtrate, which must be acid, is evaporated in a vacuum, at a temperature not over 40 °, to a thin syrup. The reaction must remain acid during the evaporation so that a drop of acetic acid may be added if necessary. The residue is thoroughly extracted with 98 per cent. alcohol, using an amount of alcohol equal to one-half the original volume of urine. Filter and re-extract the residue with alcohol should any reducing action be observed in it. The alcoholic extracts are mixed and decolorized with animal charcoal. A portion of the extract is examined for its sugar-content by Fehling's test, all of the reduction being attributed to levulose. Methylphenylhydrazin is then added to the alcoholic solution, which should not measure over 30 c.c. The amount of the hydrazin to be added is in the proportion of 3 molecules for 1 of levulose or, in other words, for each gram of sugar a trifle over 2 grams of methylphenylhydrazin. The mixture is allowed to stand for a few hours in the cold and filtered if a precipitate forms. The filtrate is treated with 50 per cent. acetic acid, using the same amount of acid as of the methylphenylhydrazin, and sufficient alcohol is added to give a clear solution. The mixture is heated from three to five minutes or, preferably, allowed to stand at 40° C. for 24 hours in an incubator. Crystals will usually separate out at the end of this time, but if not they will appear on the addition of a few drops of water. The crude product is purified by recrystallization either from a mixture of chloroform and petroleum ether or from hot water to which pyridin has been added. The yield by this method is 81 per cent. of the total sugar if pure solutions are used, while from urine it is but 50 per cent.

The crystals of *methylphenylosazone* are delicate yellow, long, fine, needles, melting from 158 to 160° C. This method can hardly find application in the hands of the general practitioner.

Pure levulosuria is recognized by the levorotation of the urine, which possesses reducing properties and is capable of fermenting with yeast. After fermentation, the urine loses its reducing and optical properties. The presence of a levulosuria is indicated by a considerable difference between the results obtained by titration and polarization, providing a glycosuria be coexistent. The Seliwanoff reaction should be used as a routine in every case which shows both fermentation and reduction. The general practitioner will rarely have access to a polariscope and even then may be misled if other interfering substances, such as albumin, glycuronic acid, and β-oxybutyric acid be present.

Do not presuppose that glucose is the only fermentable and reducing sugar of the urine, as the recognition of the true condition may make much difference in the treatment as well as in the prognosis.

(*c*). Pentose.

The pentose[1] group of carbohydrates comprises eight possible stereo-isomers with the general molecular formula of $C_5H_{10}O_5$. Three members of this group, rhamnose, fucose, and chinovose, are substituted pentoses, viz., methyl pentoses having the formula $C_6H_{12}O_5$. These latter are the exceptions to the general rule that carbohydrates contain the same number of carbon atoms as of oxygen atoms, and have led to the more scientific method of classifying the carbohydrates according to the number of oxygen atoms rather than of carbon atoms. The pentoses are widely spread throughout the vegetable kingdom in the form of their anhydrids or in combination with other groups of atoms. They are also found as constituents of the nucleoproteins of animal tissues, being especially abundant in the pancreas.

It has been found that the ingestion of large amounts of pentose-containing food, such as apples, cherries, plums, beets, and the leguminous vegetables, leads to the excretion of pentose in the urine. This *alimentary type of pentosuria* is characterized by the presence of optically active xylose or arabinose and appears after the ingestion of small amounts, in some cases following an intake of as low as 50 mg. of the pure carbohydrate. In true diabetes mellitus the urine frequently contains l-xylose which probably arises from the breaking down of the pancreatic nucleoprotein. Alfthan states that the pentoses are constantly present in diabetic urine, so that it is highly probable that these sugars might be found whenever search was made for them in diabetic conditions. It is not strange that these carbohydrates are not more frequently reported, as their reducing action leads to confusion unless controlled by fermentation methods. The excretion of pentoses in diabetic conditions is not necessarily increased in direct proportion to the intake, as their absorption may be so slow that accumulation is not possible before oxidation has occurred.

Salkowski and Jastrowitz[2] reported in 1892 the finding of a pentosuria which was not associated with intake of pentose food nor with diabetes mellitus. This type is known as *idiopathic*, *essential*, or *intrinsic pentosuria*, of which 24 cases were found by Janeway[3] up to 1906. The peculiar thing of this type of pentosuria is that the sugar excreted is r-arabinose, an optically inactive pentose. This is the single exception in which an optically inactive pentose is found in all nature. This fact characterizes this type of pentosuria as an anomaly of metabolism *sui generis*. The origin of this urinary pentose is still unsettled. The source must be within the organism, as no inactive arabinose is taken as food, and if it be given in experimental cases it appears in the urine as d-arabinose. Moreover, it cannot be derived from the nucleoprotein as the

[1] See Bendix, Die Pentosuria, Stuttgart, 1903.
[2] Centralbl. f. d. med. Wissensch., 1892, p. 337.
[3] Amer. Jour. of Med. Sci., vol. 132, 1906, p. 423.

pentose in these cases is l-xylose. Neuberg suggests that galactose might be considered the source of this pentose, but no proof of this has been forthcoming.

In the true idiopathic pentosuria the assimilation of other carbohydrates is unchanged and does not influence in any way the excretion of r-arabinose, although the active types of this pentose may be excreted at the same time in the urine. It is interesting to find that the pentoses taken in as food are excreted in different proportions by the diabetic and nondiabetic subjects. Thus von Jaksch observes that diabetics excrete from 49 to 82 per cent. of arabinose of the food and nondiabetics 1 to 47 per cent., while nondiabetics excrete from 19 to 55 per cent. of xylose and diabetics only a trace. The amount of pentose excreted in essential pentosuria has been reported as varying between 0.08 and 1 per cent. Neuberg has recently shown that a certain amount of the r-arabinose is combined with urea in the form of a ureid, which does not reduce Fehling's solution until it undergoes hydrolysis with acid. For this reason he believes that the amount of pentose reported is in practically all cases 100 per cent. too low.

Recently Luzzatto[1] has reported the excretion of the optically active l-arabinose entirely independent of the food intake. It is questionable whether this case is to be classified with the idiopathic pentosurias.

The pentoses reduce copper solutions as do other carbohydrates, but the reduction is much slower, appearing during the cooling of the fluid. Ten c.c. of Fehling's solution are reduced by 0.0542 gram of pentose. They do not ferment with yeast and do not give a typical reduction with the Almén-Nylander test, the color being a gray rather than a black. In the true iodiopathic pentosuria no reaction is observed with the polariscope, while in the alimentary type a slight dextrorotation is usually noted, although von Jaksch reports the excretion of an inactive arabinose after the ingestion of active pentoses. These pentoses form more or less typical osazones which melt between 157 and 160°, but the reaction is not so easily produced. These pentosazones are readily soluble in warm water and show dextrorotation. The most characteristic chemical property of these types of carbohydrate is the formation of furfurol ($C_5H_4O_2$) when they are distilled in the presence of acids. The color reactions given below are based upon the production of furfurol and the formation of distinct colorations on treatment with various reagents.

Tollen's Test.

A few c.c. of concentrated hydrochloric acid are saturated with phloroglucin, care being taken to leave a small amount undissolved. This solution is then divided into two equal parts, to one of which is added 1/2 c.c. of the suspected urine and to the other 1/2 c.c. of normal urine. Both tubes are then placed in a boiling water-bath for a few minutes, when an intense red zone will appear in the upper portion of the tube if pentose be present. After a few moments this red color will gradually spread throughout the fluid, while the

[1] Beitr. z. chem. Physiol. u. Path., Bd. 6, 1905, S. 87.

control urine shows no marked change in color. It is advisable to remove the tubes from the water-bath as soon as the color appears, as the clearness of the reaction is interfered with by prolonged heating. The coloring matter is then extracted by shaking with amyl alcohol, when spectroscopic examination will show an absorption band between *D* and *E*.

This test reacts in the same way with glycuronic acid so that it has little value in differentiating pentose from the former substance. As a rule, the free glycuronic acid is not so easily split from its conjugated compound as is furfurol from pentose, so that the test is at least suggestive of pentose.

Orcin Test.

For this test the urine should be decolorized by heating with animal charcoal and filtering. Five c.c. of urine are treated with an equal volume of concentrated hydrochloric acid and a few crystals of orcin are added. The mixture is then warmed approximately to the boiling-point, when a dark green color appears in the presence of pentose or glycuronic acid. The formation of a greenish-blue precipitate is very strong evidence of pentose rather than glycuronic acid. The pigment is then extracted with amyl alcohol, when spectroscopic examination shows a characteristic absorption band between *C* and *D*. The presence of glucose may interfere with the reaction, so that it may be necessary to remove it by fermentation.

Bial has modified this test in such a way that glycuronic acid is less apt to be a disturbing factor. His reagent consists of 500 c.c. of 30 per cent. HCl to which are added 1 gram of orcin and 25 drops of 10 per cent. ferric chlorid solution. Four to five c.c. of this reagent are heated to boiling and removed from the flame. The suspected urine is then added drop by drop, not exceeding 1 c.c. in all, when a green color should appear almost immediately if pentose be present. The heat employed is hardly sufficient to split off glycuronic acid. Here also glucose, if present, should be removed by fermentation with a pure culture of yeast rather than with compressed yeast, as the bacteria possibly present in yeast may break up the pentoses at the same time.

The osazone may be formed as previously given under Glucose. The glucosazone is separated from the pentosazone by digesting with water not over 60° in temperature, the pentosazones being dissolved. If the pentosazone be treated with 20 c.c. of water and 5 c.c. of concentrated hydrochloric acid and distilled, the distillate will give a beautiful test with Bial's reagent, which absolutely eliminates glycuronic acid and other interfering substances possibly present in diabetic urine.

Quantitative Determination.

Neuberg and Wohlgemuth[1] have recently introduced a method by which the arabinose of the urine may be accurately determined. A preliminary determination of the sugar present is made by Purdy's solution, eliminating glucose by previous fermentation. If less than 1 per cent. of reducing sugar,

[1] Zeitsch. f. physiol. Chem., Bd., 35, 1902, S. 31 and 41.

which is assumed to be arabinose, is present, the urine must be concentrated in a vacuum so that the sugar content is slightly over 1 per cent.

Technic.

One hundred c.c. of urine are acidified with two drops of 30 per cent. acetic acid and evaporated on a water-bath to approximately 40 c.c. It is then treated with 40 c.c. of 96 per cent. alcohol, the mixture is allowed to stand for two hours, and is then filtered from the separated urates and inorganic salts. The residue is carefully washed with 40 c.c. of 50 per cent. alcohol. To the filtrate 1.4 grams of pure diphenylhydrazin are added and the mixture heated on a boiling water-bath for one-half hour, the alcohol being replaced as it evaporates. The mixture is allowed to stand for 24 hours and is filtered through a Gooch filter, using the mother liquor to transfer the precipitate. The crystals are then washed with 30 c.c. of 30 per cent. alcohol, and the Gooch with its contents dried at 80° C. to constant weight. The amount of arabinose is obtained by multiplying the weight of the diphenylhydrazone by 0.4747 or by dividing by 2.107.

Cammidge's Reaction.

Recently Cammidge[1] has found that the urine in cases of pancreatic disease contains a substance which gives an osazone when treated with phenylhydrazin. In his earlier work he was led to believe that this substance was possibly glycerin or a derivative. He advanced two reactions, the first of which he found to be due to a mixture of glycuronic acid and a true carbohydrate, while the second was apparently due only to the glycuronic acid. He has recently improved his method in such a way that glycuronic acid has been eliminated and believes that the mother substance of the osazone is a pentose, probably derived from the nucleoprotein of the pancreas, although he has not as yet succeeded in isolating the carbohydrate itself. The writer has used this test in several cases of pancreatic disease and has found it present in all of them. Whether this test is to be regarded as pathognomonic of pancreatic disease must be left for the future to determine, but at present it is not generally so considered.

Technic.

The writer gives only the improved method known as "Reaction C" referring to the original work of Cammidge for reactions A and B. The urine to be tested should be a portion of the 24-hour specimen and must be freed from glucose and albumin by methods previously outlined.

Forty c.c. of clear, filtered acid urine are acidified with 2 c.c. of concentrated HCl and boiled for 10 minutes. The mixture is then cooled and made up to 40 c.c. with distilled water. The excess of acid is then neutralized by the addition of 8 grams of lead carbonate and the mixture cooled if necessary. Filter off the resulting precipitate and treat the filtrate with 8 grams of powdered

[1] Lancet, vol. 1, 1904, p. 782; *Ibid.*, vol. 2, 1905, p. 14; Robson and Cammidge, Surgery of the Pancreas, London, 1907.

tribasic lead acetate to remove the glycuronic acid. Filter, treat filtrate with 4 grams of powdered sodium sulphate, heat to the boiling-point, and allow to cool. The lead sulphate is removed by filtration. Ten c.c. of the clear filtrate are made up to 17 c.c. with distilled water, 0.8 gram of phenylhydrazin, 2 grams of sodium acetate, and 1 c.c. of 50 per cent. acetic acid are added and the mixture boiled for 10 minutes. Filter while hot and make the filtrate up to 15 c.c. with warm water. The mixture is allowed to cool, when yellow crystals arranged in sheaves and rosettes may be observed under the high-power lens.

(*d*). Lactose ($C_{12}H_{22}O_{11}$).

Lactose is found in the urine of women during the period of lactation and may be found in patients who have been on an exclusive milk diet for a long period. A distinct type of alimentary lactosuria is observed on account of the low assimilation limit for milk-sugar. In breast-fed children with gastrointestinal disturbance lactose associated with galactose may be found in the urine. In this case Langstein and Steinitz[1] have shown that the excretion is not due to failure of the normal enzyme, but to an unknown derangement of the activity of lactase, which renders it incapable of splitting up the whole of the lactose, the remainder being absorbed unchanged. From the portion which is split up in the bowel the resulting easily assimilable glucose is utilized by the organism, while galactose partly escapes by the kidneys, on account of the much lower assimilation limit for this latter carbohydrate. Lactose in these cases is usually associated, therefore, with galactose (Neuberg). According to the work of Voit, an increase of lactose in the diet of a diabetic is associated with an increased output of glucose.

The usual form of *lactosuria* is that observed in the parturient female. It is ordinarily first seen a few days after delivery of the child, but occasionally appears during the latter days of gestation, as Ney, Lemaire, and Porcher have shown. The amount of lactose excreted by the nursing mother equals 2 to 3 per cent., according to Naunyn, while McCann places the average at 0.35 per cent. for the first few days of the puerperium. Lactose may continue in the urine for some time, the actual amount depending upon the quantity of milk as well as its quality. If nursing is interrupted for any reason, more lactose will be found than when nursing is regular.

Lactose reduces copper solutions, although somewhat less actively than does glucose. It also shows a positive Almén-Nylander reaction. It has a strong rotatory power, its specific rotation being practically the same as that of glucose ($+52.5°$). It does not ferment with yeast, although bacteria if present may hydrolyze it into its constituents, glucose and galactose, the former of which will show fermentation. It is, therefore, advisable when applying the fermentation test not to judge of a reaction which has progressed longer than a few hours. With phenylhydrazin it forms a lactosazon which appears in the form

[1] Beitr. z. chem. Physiol. u. Path., Bd. 7, 1906, S. 575.

of sheaves of delicate curved needles much resembling bunches of yellow thread. These crystals melt at 200° C. The test is not easily obtained unless the urine be concentrated to a small bulk and the residue extracted with alcohol, when the alcohol is evaporated and this residue taken up with water and the phenylhydrazin test then applied.

Rubner's Test.

Ten c.c. of urine are treated with an excess (3 grams) of lead acetate and boiled for a few minutes. The yellowish or brown solution is then filtered and ammonia added to the filtrate until a slight permanent precipitate remains. An intense brick-red fluid is obtained which later shows the deposition of a cherry-red precipitate with a colorless supernatant fluid. This test is not very delicate as it shows lactose only when present in amounts varying from 0.3 to 0.5 per cent. Glucose gives with this test a red solution, but a more distinctly yellow precipitate.

Lactosuria is to be assumed when the urine possesses reducing properties and dextrorotation, but is incapable of fermenting with ordinary yeast within 12 hours. If the urine be boiled with 2 per cent. sulphuric acid and then neutralized, its optical activity will be increased and it will be capable of undergoing fermentation. It is to be remembered in testing for the amount of lactose by the use of Fehling's solution that 10 c.c. of this solution are reduced by 0.0678 gram of lactose instead of by 0.05 as in the case of glucose or levulose.

(*e*). **Maltose** ($C_{12}H_{22}O_{11}$).

Maltose has occasionally been reported in the urine, although many of the cases are questionable as the proper identification of the sugar was not thoroughly carried out. The most reliable cases appear to be those of Noble, von Ackeren, Rosenheim and Flatow, and especially that of Magnus-Levy. In this latter case the urine showed a considerable excess of rotation when compared with its reducing power. After inversion with dilute acid, by which each molecule of maltose was converted into two molecules of glucose, the rotation diminished and the reduction increased, so that the polarimetric and titration methods gave concordant results. The urine underwent complete fermentation with synchronous loss of optical activity and of reducing power. Calculations founded on these determinations showed that 1.5 per cent. of maltose and 2 per cent. of glucose were present. This seems to be a case in which the amount of maltose excreted exceeds all records (Neuberg).

The cases in which maltose appears in the urine seem to be those of disease of the pancreas, especially those with interstitial lesions. It is possible that the Cammidge reaction previously mentioned is due to maltose rather than pentose, but at present the question is unsettled.

Maltose reduces copper solutions, but not as strongly as does glucose. Ten c.c. of Fehling's solution are completely reduced by 0.0807 gram of maltose. It is much more strongly dextrorotatory than glucose and forms an osazone which crystallizes in large prism-like needles arranged in sheaves, and melts

at 207° C. This osazone is soluble in water and shows a dextrorotation, it being more distinctly identified by determination of the nitrogen content, which should equal 10.6 per cent. Maltose ferments with yeast only after inversion by heating with acid, the splitting products being two molecules of glucose.

Other carbohydrates, such as dextrin, isomaltose, and saccharose, have been reported in the urine. These are extremely rare and need little comment in this place. In the case of cane-sugar the assimilation limit is so high that an alimentary saccharosuria could occur only after an enormous intake. Spontaneous excretion of cane-sugar has never been actually proven, but this sugar may be found in the urine of hysterical patients who have added it to deceive the physician. The so-called *animal gum*, first isolated by Landwehr, seems to be a normal constituent of urine. Alfthan[1] finds it is present in practically every case of diabetes to the extent of 1 to 37 grams per diem. This substance is probably not a definite chemical body, but a mixture of several. Little is known of its chemistry.

Inosite was regarded for a long time as a carbohydrate, but it is now known to be a hexaoxyhexahydrobenzol with the formula $C_6H_6(OH)_6$. This substance, has, therefore, nothing to do with true carbohydrate metabolism, but is discussed at this point as it has so long been regarded in this connection. Inosite enters into the composition of almost all animal tissues and occurs both in the optically active and inactive forms. A physiologic excretion of inosite is not infrequent, according to Hoppe-Seyler. It may occur in the urine in nephritis, diabetes mellitus and insipidus, and after a large intake of animal food. For its detection the writer must refer to works on physiologic chemistry.

(*f*). Glycuronic Acid ($CHO-(CHOH)_4-COOH$).

Glycuronic acid is an intermediate product of the oxidation of carbohydrate, the CH_2OH group being converted into CHO while the original CHO group is oxidized into COOH. This acid still retains the aldehyd group, in consequence of which it shows the same reducing action as does glucose. It seems to be characteristic of glycuronic acid that, when produced naturally, it is never found in the free state, but only in the combined form as the conjugated glycuronic acid. It seems to be especially capable of combining with substances showing alcoholic or phenolic characteristics. The free glycuronic acid may be split off from its conjugated compounds by heating with acid and other hydrolyzing agents. The conjugated glycuronates are levorotatory while the free acid shows dextrorotation. Among the substances with which glycuronic acid combines we find chloralhydrate, butyl chloral hydrate, chloralamid, camphor, menthol, carbolic acid, resorcin, acetanilid, antipyrin, phenacetin, pyramidon, sandal-oil, morphin and cocain. The normal metabolism following intake of any of these substances is such that excretion of conjugated glycuronic acids will follow and may, therefore, lead to the assumption of sugar in the urine unless precautions are

[1] Ueber dextrinartige Substanzen im diabetischen Harn, Helsingfors, 1904.

taken to properly differentiate these compounds. Besides conjugated glycuronic acid of the above type we find a combination of urea with glycuronic acid as well as a certain amount of indoxyl, skatoxyl, phenol, and cresol in combination with this acid. Most of the products of bacterial decomposition in the intestine are excreted in combination with sulphuric acid, but some is invariably present as a conjugated glycuronate.

The origin and formation of glycuronic acid within the system is not entirely understood. It has been supposed to be derived from protein as especially advocated by Loewi, but Mayer has rather disproven Loewi's work, and shows that probably glycuronic acid is a direct derivative of glucose and that all carbohydrate oxidation must pass through the intermediate stage of glycuronic acid.

The status of this question is very well summed up by Neuberg as follows: The formation of glycuronic acid out of protein is by no means excluded, nor yet from fat; but as it is difficult to eliminate the direct formation of glycuronic acid or its secondary development from previously existing grape sugar, Mayer justly contends that the question of glycuronic acid formation from these substances is practically included in the broader question of the formation of sugar from fat and protein.

The exact point of conjugation of glycuronic acid is unsettled. It has been assumed by some to occur in the liver, while others find that the liver plays no part. It is probable that the synthesis takes place in various parts of the organism.

It has been shown that the output of glycuronic acid may be increased in diabetes mellitus, in mild cases the unoxidized sugar being present largely in this form. Mayer[1] advances the hypothesis of incomplete oxidation of sugar to explain its appearance in these cases. He shows that after the administration of glucose in amounts beyond the assimilation limit, an occasional excretion of glycuronic acid occurs with an equivalent diminution of the ethereal sulphates. It is possible that the substance conjugating with glycuronic acid is unknown and that we have the same results as though similar substances were introduced by mouth. As Mayer has advanced no direct proof of the correctness of his theory, the decision must be left for the future. Clinically, the question of the highest importance is whether the excretion of glycuronic acid is of any diagnostic value and whether it is of prognostic significance in diabetes. It does not seem wise to assume that a patient showing an occasional increase in the glycuronic acid excretion, which cannot be accounted for by intake or increased production of conjugating substances, will in the future show typical diabetes. Edsall does not believe in the value of glycuronic acid in the diagnosis of a latent diabetes, nor does Neuberg regard an increased excretion of glycuronic acid as the original derangement which may determine other deviations from health.

[1] Zeitsch. f. physiol. Chem., Bd. 32, 1901, S. 518; Berl. klin. Wochensch., Bd. 40, 1903, S. 292 and 514.

Very few of the conjugated glycuronates show reducing action when treated with copper solutions using the precautions previously laid down. The chloral and camphor compounds are much more apt to produce typical reduction, but even these require heating for somewhat longer periods than does glucose. A diagnosis of a glycuronic acid excretion is based upon the following points: The fresh urine is levorotatory, but shows little or no reducing properties and does not ferment. This same finding will be observed if β-oxybutyric acid be present so that a diagnosis may not rest on these findings alone. After being boiled with dilute acid for a period varying from one-quarter to three-quarters of an hour, the levorotation is changed to dextrorotation and the urine shows strong reducing powers. Such tests will not be given by β-oxybutyric acid. In some cases after heating the urine with acid the action upon light may remain levorotatory or the solution may be optically inactive, on account of the fact that the conjugating substance may be levorotatory or that complete hydrolysis has not been effected. On heating the urine for some time with Bial's modification of the orcin test a positive reaction appears with the liberated glycuronic acid. This acid crystallizes with phenylhydrazin forming distinct yellow needles which melt at 114 to 115° C. This test is, however, not readily obtained so that it is difficult to identify glycuronic acid by its osazone. It will be seen, therefore, that glycuronic acid is differentiated from the pentoses largely by its levorotation when in the conjugated state or dextrorotation when free.

Neuberg's Test.

Owing to the fact that glycuronic acid is both an aldehyde and an acid, several possibilities of union with phenylhydrazin exist. Thus we find reports of compounds with melting-points of 114° to 155° C. as well as osazones with melting-point from 200° to 205°. For this reason Neuberg[1] has introduced the following test for absolute identification of glycuronic acid.

Five hundred c.c. of urine are treated with sufficient sulphuric or phosphoric acid to make the acidity from 1 to 2 per cent. This acidified urine is then heated in an autoclave for two hours at a temperature of 115° C. The mixture is then cooled, neutralized with sodium carbonate, acidulated with acetic acid, and filtered. Two hundred and fifty c.c. of the filtrate are mixed with a hot aqueous solution of 5 grams of parabromphenylhydrazin hydrochlorate and 6 grams of sodium acetate. The mixture becomes cloudy at once, but on heating the cloudiness will disappear. As the mixture cools needle-shaped crystals will separate out and may be filtered off, the filtrate being again heated and cooled to obtain more crystals. This process may be repeated until no more crystals form. These crystals are then washed with distilled water followed by absolute alcohol, and are then recrystallized by dissolving in 60 per cent. alcohol and gradually evaporating. They are clear yellow in color,

[1] Ber. d. d. chem. Ges., Bd. 32, 1900, S. 2395; Zeitsch. f. physiol. chem., Bd. 44, 1905, S. 127

melt at 236° C., and show marked levorotation when dissolved in a mixture of pyridin (4) and absolute alcohol (6).

The above test is quite characteristic for glycuronic acid and may be confirmed by the formation of the cinchonin salt, which crystallizes in needles showing a melting-point of 204° C.

(3). **Acetone Bodies.**

By the acetone bodies[1] we mean acetone, diacetic acid and β-oxybutyric acid. The latter of these is the mother substance so that this group would better be called the β-oxybutyric bodies. The chemical relation between these bodies is very close, the β-oxybutyric acid being oxidized to diacetic acid, which then splits up into acetone and carbon-dioxid. This may be seen from the following formulæ:

$CH_3 - CHOH - CH_2 - COOH$	β-oxybutyric acid.
$CH_3 - CO - CH_2 - COOH$	Diacetic acid.
$CH_3 - CO - CH_3$	Acetone.

Formerly these substances were supposed to be derived from protein material, the β-oxybutyric acid being formed from the β-amino acids by desamidization and oxidation in the β position. This theory is, however, not generally held at present, being replaced by the more modern idea that the fats are the chief source of the acetone bodies. It has been found that in perfectly sound, well-nourished individuals the addition of fat causes only a very slight increase in the output of acetone bodies and, strangely enough, that butyric acid itself causes no acetonuria. On the other hand in normal individuals from whom the dietary carbohydrate has been removed or in a diabetic who is not utilizing what carbohydrate he may be allowed, a marked excretion of acetone bodies may occur. While a portion of this acetone may possibly be derived from the carbohydrate groups of the protein molecules, it can hardly explain the enormous excretion in diabetes, as the amount of protein catabolism, as shown by the urinary nitrogen, is greatly insufficient to yield any such amount of acetone bodies. The fats are, therefore, the more probable source of these bodies. As long as the body is supplied sufficient carbohydrate or is able to oxidize a sufficient amount the acetone bodies of the urine remain low; but when the system is no longer capable of oxidizing the carbohydrates, the amount of acetone bodies increases to a marked extent. We see, therefore, that the older method of allowing diabetics practically no carbohydrate food may directly lead to aggravation of the symptoms which the withdrawal was supposed to remedy. In other words, the normal or the diabetic individual must have a certain amount of carbohydrate food in order that proper metabolism may be maintained. This is not the time or place to discuss the therapy or dietetic treatment of diabetes, but it must be remembered that the most successful diet is one which contains carbohydrates up to the point of tolerance. Even

[1] See Waldvogel, Die Acetonkörper, Stuttgart, 1903; Magnus-Levy, Ergebnisse der klin Med., Jena, 1908.

here we find that certain types of carbohydrates may be given to diabetics without increasing the glycosuria, while at the same time leading to a diminution in the excretion of acetone bodies. Such diets are the oatmeal diet of von Noorden and the potato diet of Mosse.

The condition arising from a surcharging of the blood with these acetone bodies is known as *acidosis*. For a long time it was supposed that the carbohydrates were not only accountable for glycosuria, but also for the acetonuria and acidosis noted in diabetes. In the advance of pathologic chemistry it has been shown that, instead of causing these latter symptoms and conditions, the carbohydrates in reality lessened them. This may be shown by the administration of a definite amount of sugar, especially in the milder types of diabetes, to patients from whose diet sugars have been previously excluded. The omission of sugars from the diet forces the organism to utilize its protein and fat and thus gives rise to an accumulation of nitrogenous and fatty metabolic products as well as to an increase in the acids of the body fluids. In this way an acidosis already present would be increased in intensity. If the carbohydrate-free diet be continued for some time, a readjustment takes place and the acetonuria may gradually diminish, as is instanced by the fact that certain races show no acetonuria even though on an absolutely carbohydrate-free diet.

With regard to the proteins as the mother substances of these bodies, we must admit that their influence is to some extent a double one. In the first place protein tends to diminish the acetonuria on account of its carbohydrate content, those proteins containing the greatest number of carbohydrate groups not necessarily exerting the greatest effect either on this condition or on the glycosuria. With a diet excessive in protein the influence is, however, not of this sort. The sulphuric and phosphoric acids as well as the small amount of acetone bodies formed by the hydrolysis of the protein tend to increase an existing acidosis, while the carbohydrates formed in the splitting of these proteins may greatly increase an existing glycosuria. These points, together with the fact that the products of nitrogenous metabolism may greatly increase the osmotic tension of the blood and thus lead to disordered cell function, show us that proteins cannot be advantageous as an exclusive diet in diabetes. As is well known, the nitrogenous excretion is much more marked in a diabetic than in a nondiabetic owing to several factors. In the first place, the diabetic consumes more protein than the normal individual because his diet is limited as regards carbohydrates and must be made up to a requisite caloric value by protein and fat. Secondly, owing to the lack of the protein-sparing function of the carbohydrates, excessive protein is broken down and elaborated in order to furnish a portion of the energy necessary to maintain the body function. It must, however, be said that the diabetic protects himself for a time from the unusual loss of protein by the utilization of fat.

Concerning the fats, it is to be recalled that, although formally accredited with no power of influencing acetonuria, to-day they are regarded as directly

affecting this condition to a great extent. This is true of the fatty acids, especially of the lower members, and not of the neutral fats. If the contention of Castle and Loevenhart be true, that a reversible action of lipase converts the fatty acids and glycerin formed by a previous hydrolysis again into neutral fats, then the influence of fats on the acidosis is variable, or else we must assume a lack of lipase in the cells of the diabetic. We know that the fatty acids belong to the ketoplastic group (substances increasing excretion of acetone bodies), yet as Borchardt has recently shown this ketoplastic action is, doubtless, due to the union of the fatty acids with glycerin, thus withdrawing from the system the antiketoplastic body, glycerin, and enabling the remaining fatty acids to exert their influence on the formation of the acetone bodies. Fats do not increase an existing glycosuria as many experiments have shown, yet we must grant that a formation of sugar from fat does take place. Von Noorden speaks of a "facultative formation of sugar from fat," referring to the fact that the demand for sugar may become so great that this source is called upon to furnish its quota of carbohydrates. We must also remember that the synthesis of fat from disintegrated carbohydrate is much affected in diabetes. Were this not the case, a large part of the sugar, reaching the blood as such, would be synthesized by the fat-forming cells and glycosuria would be diminished. Conceiving this latter function to be normal while the former is abnormal, we may readily see the close relationship between obesity and later diabetes. With an excessive diet of fat no more fat is oxidized than when the diet is low in fat. In the latter case, the body-fat is utilized to furnish the difference, while in the former the excess is deposited in the usual fat depositories.

Besides the excretion of acetone bodies observed in diabetes, we find fever, carcinoma, inanition, lesions of the central nervous system, digestive disturbances, delayed chloroform poisoning, cases of pregnancy in which death of the fetus has occurred or in which persistent toxic vomiting is noted, and other conditions associated with an increased output of the acetone bodies. According to Mohr, most of the cases may be traceable either to limitation of carbohydrates in the food or to diminished power of utilizing them. Increased protein catabolism may play a rôle in the pathogenesis of this condition, but in the writer's opinion only a secondary one. A general statement should be that the excretion of the acetone bodies is little influenced by the amount of fat in the food of the normal individual, providing the carbohydrate content of the diet is good; but in pathologic conditions the factors influencing carbohydrate utilization are so numerous that fats play a much greater rôle than do proteins in bringing about an acetonuria.

As β-oxybutyric acid is the mother substance from which the other acetone bodies are formed by oxidation, we should expect to find, as we actually do, the severest cases showing large amounts of β-oxybutyric acid and small or even no excretion of the other members of this group. As a rule, it may be said that the more acetone the less β-oxybutyric acid but this is not always

the case. In the diabetic coma we usually find large amounts of the first two members of this group while acetone may be absolutely lacking, and, on the other hand, we find in some of the milder types of diabetes acetone and no diacetic or oxybutyric acids. As diacetic and oxybutyric acid combine with ammonia and are excreted as ammonium compounds, a very accurate and simple method of following the excretion of these bodies is by the determination of the ammonia output. Whenever the ammonia excretion equals or exceeds 10 per cent. of the total nitrogen, a probable approach of coma is indicated.

(*a*). **Acetone** ($CH_3-CO-CH_3$).

Chemically, acetone is dimethyl ketone. It shows, therefore, the reactions for this group of chemical compounds but is easily confused, both with the aldehyds and alcohols. The urine rarely shows typical reactions for acetone if the older tests are applied directly to the urine, so that it is necessary to distill and examine the distillate. In this process diacetic acid is split up into acetone and carbon dioxid, so that it is impossible to tell whether acetone was preformed or was produced by heating. From the clinical standpoint it is a matter of indifference, as acetone and diacetic acid are so closely related that their clinical significance is the same, acetone representing merely a further stage in the oxidation of this group of bodies. In these tests the urine must be perfectly fresh. If it is desired to eliminate the influence of the diacetic acid upon the acetone reaction, the urine may be alkalinized with sodium hydrate and extracted with pure ether. The ether removes the diacetic acid salt, enabling us to make a separate determination of the diacetic acid and acetone.

Legal's Test (Le Noble's Test).

To a few c.c. of the urine are added a few drops of a fairly concentrated solution of sodium nitroprussid and then sodium or potassium hydrate until the mixture is strongly alkaline. A ruby-red color, later changing to yellow, appears in the presence of acetone. It will be remembered that this same test is given by creatinin, so that further modifications are necessary to permit of differentiation. If the ruby-red solution be treated with an excess of glacial acetic acid, the first red color will change into a carmine or reddish-purple color in the presence of acetone, while the same treatment with creatinin solutions yields a yellow, changing to green and finally to a blue coloration. As Le Noble has found, ammonium hydrate does not give this reaction with creatinin, but with acetone, although the reaction is much slower in appearing. This test is given by diacetic acid, by alcohol, and by acetic aldehyd, so that is it not especially distinctive for acetone. It is perhaps better if this test is to be used at all that the urine be previously acidified and distilled, the distillate yielding a reaction which is more sensitive, according to Studer, although diacetic acid will thus be converted into acetone. Recently Folin has shown that most of the qualitative tests for acetone are really tests for diacetic acid. He says: "The qualitative chemical tests for acetone in urine as usually carried out may likewise be regarded as pure fiction, for I

have never yet seen a fresh diabetic urine the acetone concentration of which was sufficient to give a positive test with the nitroprussid reaction."

Lieben's Test.

To a few c.c. of urine or, preferably, of the distillate are added a few drops of concentrated sodium or potassium hydrate and a few drops of a solution of iodin in potassium iodid. On slightly warming the mixture yellow crystals of iodoform will separate, which may be recognized by their characteristic odor as well as by their hexagonal shape when examined under the microscope. This test is given by alcohol as well as by aldehyds, and will show amounts of acetone varying between 1/100 and 1/1000 of a mg.

Gunning's Test.

This is a modification of the previous test and is much more specific, being given only by acetone. To the distillate from the urine are added a few drops of an alcoholic solution of iodin and the mixture treated with ammonia until a black precipitate of nitrogen iodid forms. On allowing the tube to stand for periods varying between 12 and 24 hours, this black precipitate disappears, leaving a yellow sediment of iodoform, which may be recognized as mentioned above. This test is less delicate than the original one of Lieben, detecting acetone when present in amounts of 1/100 of a mg. per c.c. of urine.

Frommer's Test.

Recently Frommer[1] has introduced a test which seems to be distinctive for acetone and at the same time very delicate. It is based upon the fact that acetone reacts with salicyl aldehyd to form dioxydibenzoylacetone, according to the following equations. The alkali salt is distinctly red.

$$C_6H_4OHCHO + (CH_3)_2CO = C_6H_4OHCH=CHCH_3CO + H_2O$$

$$C_6H_4OHCH=CH \begin{matrix} \diagdown \\ \diagup \end{matrix} \begin{matrix} CO \\ CH_3 \end{matrix} + CHOOHC_6H_4 = C_6H_4OHCH=CH \begin{matrix} \diagdown \\ \diagup \end{matrix} \begin{matrix} CO \\ CH=CHOHC_6H_4 \end{matrix} + H_2O.$$

The test may be performed as follows: Ten c.c. of urine are strongly alkalinized with potassium hydrate and 10 to 12 drops of a 10 per cent. solution of salicyl aldehyd in absolute alcohol are added and the mixture warmed to about 70° C. In the presence of acetone the fluid becomes yellow, then red, later purplish-red, and, on long standing, dark red. In the absence of acetone the color of the urine is practically unchanged. This test is said by Frommer to indicate the presence of one one-millionth of a gram in 8 c.c. of urine.

Instead of applying the test as above, we may add about 1 gram of potassium hydrate (in the solid state) to 10 c.c. of urine and, without waiting for complete solution to occur, treat the mixture with 10 to 12 drops of the alcoholic solution

[1] Berl. klin. Woch., Bd. 42, 1905, S. 1008.

of salicyl aldehyd and warm to 70°. At the zone of contact of the alkali and the salicyl aldehyd an intense purplish-red ring is observed only in the presence of acetone.

The writer has frequently used this test and finds it very satisfactory, as it does not react with diacetic acid unless the heating be long continued. It is quite as simple as the preceding tests and should find wide application.

Quantitative Determination of Acetone.

Many of the quantitative methods given for acetone are more or less complicated and at the same time do not yield absolutely accurate results. The writer selects, therefore, those proving most satisfactory in his hands. All of these methods, with the exception of Folin's, give the amount of preformed acetone as well as that derived, by distillation, from the diacetic acid.

Huppert-Messinger Method.

The principle of this method is the determination of the amount of iodin necessary to transform into iodoform the acetone derived in the distillation of the urine. Knowing this factor, a simple calculation yields the amount of acetone present.

For this determination certain solutions are necessary:

(1). A 50 per cent. solution of acetic acid.

(2). A tenth-normal solution of sodium thiosulphate. In preparing this solution 24.8 grams of crystallized sodium thiosulphate ($Na_2S_2O_35H_2O$) are carefully weighed out and dissolved in distilled water, the solution being made in a volumetric flask and diluted exactly to the liter mark.

(3). A tenth-normal solution of iodin. This solution requires exactly 12.685 grams of iodin in one liter. As iodin is difficultly weighable on account of its volatility, it is advisable to weigh out approximately 13 grams of iodin and dissolve in approximately 1 liter of water to which has been added 25 grams of potassium iodid. This solution is then standardized by titrating against the previously made tenth-normal thiosulphate solution, using thin starch paste as an indicator and adding the iodin solution from a buret until the blue color of iodid of starch just appears. This determination is then confirmed by duplicate estimations. Twenty c.c. of the iodin solution should be the equivalent of 20 c.c. of the thiosulphate solution, so that the necessary dilution of the iodin solution may be determined by the formula previously given under Determination of Chlorids in the Urine (page 184). One c.c. of the standard tenth-normal iodin solution equals 0.012685 gram of iodin and represents 0.967 mg. of acetone.

Technic.

Five hundred c.c. of normal acid urine or 100 c.c. of acetone-rich urine are treated with 2 c.c. of 50 per cent. acetic acid for every 100 c.c. of urine and distilled until 9/10 of the volume has passed over. The distillate is collected in a receiving flask, which is cooled with ice and which contains water to absorb the

acetone. The flask is tightly closed with a doubly perforated stopper, through one hole of which passes the tube of the condenser reaching below the surface of the water and through the second opening a bulb filled with water to act as a safety bulb. The tube is then washed with distilled water and the fluid in the safety bulb is emptied into the receiving flask. It is important that the distilling flask be disconnected before the heat is shut off, as otherwise the fluid might suck back. This distillate is treated with calcium carbonate to remove any nitrous or formic acid which may have distilled over, and the mixture thoroughly shaken.

This distillate is then acidified by the addition of 1 c. c. of dilute sulphuric acid (diluted eight times) and redistilled until one-tenth of the volume remains in the distilling flask. This second distillate is received in a flask arranged as in the previous distillation. It is then poured into a flask which can be closed with a tight-fitting glass stopper. Distillate and wash-water must not fill the vessel more than one-third full. A large excess of carefully measured N/10 iodin solution is added, the mixture well shaken, and strong sodium hydrate solution added drop by drop. The flask is then stoppered, shaken for one-fourth minute, and allowed to stand for five minutes. The stopper is then removed, the fluid clinging to it washed into the flask, and the fluid acidified with strong HCl. The excess of iodin is then determined by allowing N/10 thiosulphate solution to flow from a buret until the mixture is but slightly yellow, when a few c.c. of starch paste are added and the titration continued until the blue color just disappears. One c.c. of iodin solution used by the fluid corresponds to 0.967 mg. acetone, so that all that is necessary is to subtract the number of c.c. of thiosulphate used from the number of c.c. of iodine solution added and multiply by the above factor. The result is the acetone in the amount of urine taken.

This method yields results which are from 5 to 10 per cent. low, so that slight variations in the acetone excretion, as determined by this method, have little significance.

Folin's Method.

This method[1] yields only the preformed acetone occurring in the urine and does not regard the acetone which may be derived from the diacetic acid. If the urine be distilled and this method employed, the total possible acetone will result. It is somewhat more accurate than the previous method and is much more simple and less time-consuming. The writer finds it extremely serviceable and adopts it in all acetone determinations.

Twenty-five c.c. of urine are measured into an aerometer cylinder, similar to that used in Folin's ammonia apparatus, a few drops of 10 per cent. phosphoric acid, 10 grams of sodium chlorid, and a little petroleum are added. In the absorbing bottle which is fitted with an absorption bulb are placed 300 c.c. of water, 20 c.c. of 40 per cent. potassium hydrate, and an excess of tenth-normal iodin solution. The apparatus is connected with the filter-pump in the same

[1] Jour. of Biol Chem., vol. 3, 1907, p. 177.

manner as described under Ammonia and an air-current drawn through for one-half hour. The air should not be passed as rapidly as in the ammonia determination. Each worker should check his air blast by control estimations, using known solutions of acetone. In this way the acetone will be removed from the urine and converted, in the receiving flask, into iodoform. The contents of the receiving flask are acidified with concentrated HCl, using 10 c.c. of acid for every 10 c.c. of alkali previously used, and the excess of iodin titrated with tenth-normal sodium thiosulphate as in the previous method.

If this method be employed with the distilled urine, subtract from the total acetone, obtained in the distillate, the performed acetone, derived from the fresh urine, and obtain the acetone referable to diacetic acid. If this value be multiplied by 1.758 the result will be the amount of diacetic acid, as such, present in the urine taken.

Shaffer has recently introduced a method for the determination of the total acetone, which is closly related to his method for estimating β-oxybutyric acid and will be discussed in a later section.

(*b*). Diacetic acid ($CH_3-CO-CH_2-COOH$).

This substance is derived from β-oxybutyric acid and is the precursor of acetone. As a rule, if acetone be present in large amounts, diacetic acid is also present and indicates a much graver condition than does the mere presence of acetone. If Folin's contentions are true, most of what we now call acetone is, in realty, diacetic acid, so that this latter subtance should be tested for in routine urinary work. The remarks previously made regarding the appearance of acetone in the urine also hold for diacetic acid. This substance is very volatile and disappears from the urine in a relatively short time so that the tests should be applied to perfectly fresh specimens.

Gerhardt's Test.

To 10 c.c. of urine are added a few drops of 10 per cent. ferric chlorid solution. This is best added as long as a precipitate of phosphates occurs, these latter bodies being then filtered off. To the filtrate are added a few drops more of ferric chlorid solution when the urine shows a Bordeaux-red color in the presence of diacetic acid. This color appears cherry-red by transmitted light and purplish-red by reflected light.

Unfortunately, however, this test is not specific for diacetic acid. A red color is observed in the presence of cyantes, formates, acetates, phenol compounds, salicylates, the conjugated glycuronates of phenacetin, antipyrin, thallin, kryofin, and kairin, as well as meconic acid which may be excreted after intake of opium. It is necessary, therefore, to differentiate the red color produced by diacetic acid. If the urine be heated diacetic acid decomposes more or less completely into acetone and carbon dioxid, so that the red color due to diacetic acid will either disappear or become much weaker, while that with the other substances mentioned is not affected by heat. This same disappearance of color is noted if the mixture be allowed to stand from 24 to

48 hours. Schreiber recommends filtration of the urine through animal charcoal, using about 10 grams of charcoal to 100 c.c. of urine. Antipyrin, phenacetin, and kryofin are retained while sufficient of the diacetic acid passes through to permit of detection. The urine may even be acidified with sulphuric acid and extracted with ether. This ethereal solution, which contains the diacetic acid, is then shaken with water and ferric chlorid solution added. A deep red color will be seen in the watery layer in the presence of diacetic acid. If the red color, on addition of ferric chlorid, be due to the presence of meconic acid, it will disappear on the further addition of stannous chlorid or of alkali hypochlorites, while that due to diacetic acid is unaffected. This test is, perhaps, more frequently used than any other test for diacetic acid in the urine, but is by no means as serviceable as the following.

Arnold's Test.

For the performance of this test two reagents are employed: (1) a solution consisting of 1 gram of paraamidoacetophenon, 100 c.c. of distilled water, and 2 c.c. of concentrated hydrochloric acid; (2) 1 per cent. sodium nitrite solution. Fifteen c.c. of urine are treated with a mixture consisting of 10 c.c. of solution 1 and 5 c.c. of solution 2 and one drop of concentrated ammonia added. In practically all urine, whether it contains diacetic acid or not, a brownish-red coloration is observed which changes in the absence of diacetic acid into yellow if the mixture be treated with an excess of concentrated hydrochloric acid, while if diacetic acid be present the color changes to a beautiful purple on the addition of acid. If the mixture be shaken the foam also shows a distinct violet coloration.

As this test is somewhat difficult in the presence of small amounts of diacetic acid, *Lipliawsky*[1] has modified it as follows: Six c.c. of solution 1 and 3 c.c. of solution 2 are treated with the same volume of urine, a drop of ammonia is added and the mixture shaken, when it assumes a brick-red color. According to the probable contents of the urine in diacetic acid 10 drops to 2 c.c. of this mixture are treated with 15 to 20 c.c. of concentrated hydrochloric acid, 3 c.c. of chloroform and two to four drops of ferric chlorid solution. The test-tube is then closed with a cork and gently shaken for one-half to one minute. In the presence of traces of diacetic acid the chloroform assumes a characteristic violet coloration, while in the absence of this acid the color is yellow or light red. This test is positive for one part in 400,000 of water. Acetone and β-oxybutyric acid do not react with this test nor do the drugs previously mentioned under the Gerhardt Test interfere. If the urine is highly colored it is advisable to filter through animal charcoal.

(*c*). β-Oxybutyric Acid[2] ($CH_3-CHOH-CH_2-COOH$).

This acid, the mother substance of the acetone bodies, is found in the urine in extreme cases of the conditions described under Acetone. The amount

[1] Deutsch. med. Wochensch., Bd. 27, 1901, S. 151.

[2] The more scientific name for this body is β-hydroxybutyric acid.

excreted may vary from traces to as high as 100 grams. Külz has reported an excretion of 225 grams in 24 hours. The free acid is practically never found in the urine, being excreted either as the ammonium, sodium, or potassium salt. These salts as well as the free acid are levorotatory and may be detected by the polariscope after previous fermentation of the carbohydrates.

In the condition known as diabetic coma a specific intoxication with β-oxybutyric acid is assumed as the causative factor. While this is undoubtedly true to a large extent it cannot be regarded as the only factor in diabetic coma, as administration of β-oxybutyric acid in large quantities will not necessarily lead to such a syndrome unless largely retained. We do undoubtedly have an acidosis which acts by ultimately depriving the tissues of fixed alkalies and may be regarded, therefore, as of great importance in following a diabetic case. Were this acid, *per se*, accountable for the entire symptomatology, we should be able, by administering alkalies, to overcome the effect of the acid intoxication. In some cases such therapy is extremely beneficial, while in others it is practically useless, as it cannot influence the formation of the toxic bodies and may not increase their elimination.

As previously stated, the ammonia output of the urine is an invaluable guide in following the course of an acidosis, especially in diabetes. One gram of ammonia (NH_3) is equivalent to 6.12 grams of β-oxybutyric acid, especially when in excess of the amount directly due to the food. In any case, if the ammonia output exceeds 10 per cent. of the total nitrogen, coma is probably imminent.

The urine is very rarely tested qualitatively for the presence of β-oxybutyric acid, as such tests are rarely satisfactory and not clinically available. If the urine be fermented and then extracted with ether followed by acidifying with phosphoric acid and reextraction with ether, it will show distinct levorotation which is due to β-oxybutyric acid. The determination of the presence of β-oxybutyric acid by the formation of crystals of crotonic acid may be found in works on physiologic chemistry.

Quantitative Determination.

A large number of methods have been advanced for the determination of β-oxybutyric acid in the urine. Among these we find the methods of Külz, Tollens, Wolpe, Magnus-Levy, Bergell, Stadelmann, and Darmstädter. While some of these are accurate under certain conditions, so many precautions must be taken that widely varying results may be obtained. On the other hand, the method of Magnus-Levy, which is undoubtedly very exact, requires 24 hours, while that of Bergell depends to a large extent upon the condition of the powder which is extracted with ether. For these reasons the writer feels that certain newer methods are much to be preferred to the older ones.

Black's Method.

This method[1] is a modification of those of Magnus-Levy and of Bergell,

[1] Jour. of Biol. Chem., vol. 5, 1908, p. 207.

but in the writer's hands has given much more satisfactory results. One hundred c.c. of urine are faintly alkalinized with sodium carbonate and evaporated in a porcelain dish to one-third or one-fourth of the original volume. The residue is then concentrated to about 10 c.c. on a water bath in order to completely remove the diacetic acid. This is then cooled, acidified with a few drops of hydrochloric acid, and made into a thick paste with plaster of Paris. This mixture soon begins to set, when it is stirred and broken up with a glass rod. This porous mass is then transferred to a Soxhlet apparatus and extracted with pure ether for two hours. The ether extract is evaporated, the residue taken up with water, decolorized with bone-black and filtered perfectly clear. The filtrate is then made up to 25 c.c. and its rotation determined with a polariscope. In determining the amount of β-oxybutyric acid from its rotation we must make use of the following calculation. The specific rotation of the free acid is —24.12 in a decimeter tube. One division of the scale in the case of glucose equals 2 per cent., so that we may find the percentage of β-oxybutyric acid by the following proportion:

$$2 : X :: 24.12 : 52.7. \quad X = 4.37 \text{ per cent.}$$

By a similar proportion it may be found that one division of the scale corresponds to 7.1 per cent. of sodium β-oxybutyrate, whose specific rotation is —14.35.

This method is just as exact as those of Magnus-Levy and Bergell and has the advantages that it is simpler, more reliable, and may be performed in shorter time. The greatest difficulty with this method arises in the determination of the exact point of the scale at which the two portions of the polarimetric field are equally illuminated. Magnus-Levy has shown that a difference of 1/10 of 1° in reading the polariscope amounts to about three grams per liter of β-oxybutyric acid, so that great care must be used with the polariscope in this method as well as in all others in which it is applied.

Shaffer's Method.

This method[1] seems to the writer to be the most desirable one that has been advanced. It is so recent that criticism has not as yet arisen. In the few determinations which the writer has made by this method he has found it very reliable and less apt to give erroneous results if Shaffer's precautions are followed.

The principle of the method is the oxidation of β-oxybutyric acid to acetone and carbon dioxid and the determination of the amount of acetone thus evolved. The acetone and diacetic acid already existing as such in the urine are previously determined by this method, so that it serves as a general one for the acetone bodies.

From 25 to 250 c.c. of urine, depending upon the amount of β-oxybutyric acid expected, are measured into a 500 c.c. volumetric flask and an excess of

[1] Jour. of Biol. Chem., vol. 5, 1908, p. 211

basic lead acetate and 10 c.c. of concentrated ammonia are added. In selecting the amount of urine to be taken one should use sufficient to yield from 25 to 50 mg. of acetone derived from β-oxybutyric acid. The solution in the flask is then diluted to the graduating mark, is thoroughly shaken, and filtered. Two hundred c.c. of the filtrate (representing 2/5 of the original volume of urine taken) are diluted with water to 500 or 600 c.c. 15 c.c. of concentrated sulphuric acid and a few grams of talcum are added, and the mixture distilled until 200 to 250 c.c. of distillate collects (distillate A).

In this distillation, the distilling flask, which may be an 800 c.c. Kjeldahl flask, should be fitted with a dropping funnel and water run in to prevent the volume of fluid in the flask from becoming less than 400 c.c. This distillate (A) contains the preformed acetone and that from the diacetic acid as well as volatile fatty acids which may be present in the urine. To remove the fatty acids, especially formic acid, the distillate A is redistilled after adding 5 c.c. of 10 per cent. sodium hydrate solution. This distillate (A_2) is then titrated with standard tenth-normal iodin and thiosulphate solutions as described in the Huppert-Messinger method for determination of acetone.

The residue of urine and sulphuric acid from which A was obtained is again distilled, dropping in 400 to 600 c.c. of 0.1 per cent. to 0.5 per cent potassium brichromate solution. This bichromate must not be added faster than the distillate collects unless the boiling liquid turns a pure green color, indicating that the bichromate is being used up more rapidly. When about 500 c.c. of distillate (B) have collected, 20 c.c. of 3 per cent. H_2O_2 are added to the distillate together with a few c.c. of sodium hydrate solution and this redistilled. This second distillate (B_2) is then titrated with tenth-normal iodin and thiosulphate solution. One mg. of acetone represents 1.794 mg. of β-oxybutyric acid.

(4). Abnormal Pigments.

(*a*). Blood Pigments.

The principal blood pigment appearing in the urine is hemoglobin, which has been previously discussed under the heading of Protein in the Urine. Certain derivatives of this pigment are found, however, in conditions in which hemoglobin does not appear (see hemoglobinuria).

Hematoporphyrin.

This is an iron-free derivative of hemoglobin and appears to be present in minute traces in normal urine. Pathologically, it has been found in cases of rheumatism, phthisis, Addison's disease, pericarditis, paroxysmal hemoglobinuria, cirrhosis of the liver, exophthalmic goiter, croupous pneumonia, lead poisoning, syphilis, and many acute infectious diseases. Long-continued use of certain hypnotics, such as sulphonal, trional, and tetronal, is frequently associated with the appearance of hematoporphyrinuria. It is rather uncertain what is the causative factor in this condition.

Urine containing hematoporphyrin is usually dark-red in color, but may

vary from a brownish-red or port-wine color to a distinct Bordeaux-red. Hammarsten has shown that this color is not entirely due to the hematoporphyrin, but partially to other abnormal pigments whose identity is not certain.

In examining urine for the presence of hematoporphyrin the spectroscopic method is practically the only one available. If this pigment be present in large amounts the urine may be directly examined with the spectroscope, showing the four bands of alkali hematoporphyrin discussed in the section on Blood. As this method is not always certain and not always easy of application, it would seem preferable to treat 50 c.c. of urine with 10 c.c. of 10 per cent. sodium hydrate solution. The precipitated phosphates carry down the pigments. This precipitate is then treated with 10 drops of concentrated hydrochloric acid and 15 c.c. of absolute alcohol. The solution is filtered if necessary and examined with the spectroscope when the two absorption, bands of acid hematoporphyrin will be observed.

(*b*). Biliary Pigments.

Normally, bile pigments do not occur in the human urine. As was previously discussed in the section on Urobilin, biliary pigments may appear in the urine in conditions interfering with the passage of the bile into the intestine or when increased formation of biliary pigments from blood pigments has occurred and an associated obstruction of the bile-ducts is present.

We see, therefore, that choluria occurs in every case in which there is obstruction to the outflow of bile into the intestine. Thus in catarrhal jaundice, biliary calculi in the common duct, carcinoma of the liver, and cirrhosis, bilirubinuria is frequent. Moreover, we may also find biliary pigments arising from purely hematogenous conditions, such as pernicious anemia, malaria, typhoid fever, arsenical poisoning, and yellow fever. Whether this latter type is not really hepatogenous in origin, as Stadelmann believes, is still unsettled, but it would seem more plausible to assume a primary breaking down of the red cells and a secondary insufficiency of the liver.

The chief biliary pigment found in the urine is bilirubin, which is intermediate between hemoglobin and urobilin. On oxidation of this pigment, either in the system or in the methods of examination, various other pigments may arise. Thus we may find biliverdin, bilicyanin, bilifuscin, biliprasin, cholecyanin, and choletelin. In the fresh urine bilirubin is the only pigment noted, while any one of the others mentioned, especially biliverdin, may be found in older specimens.

A bile-containing urine may show various shadings of color, ranging from a greenish-yellow, through yellowish-brown, deep brown, or greenish-brown, to a pure green. If the urine be shaken, a yellowish or greenish-yellow foam is observed, while in normal urines the foam is practically colorless. The presence of an excess of urobilin may also give a brownish foam. Urine containing bile always shows the presence of nucleoalbumin along with slight traces of serum albumin, so that bile should be regarded as a source

of extraneous albumin. The sediment will usually be more or less colored by the biliary pigment, the casts in yellow fever, for instance, being usually distinctly bile-stained.

Qualitative Tests.

A large number of tests have been advanced for the detection of biliary pigments in the urine and many of them are distinctly unsatisfactory. If large amounts of bile be present, the urine, acidified with HCl, may be shaken out with chloroform which dissolves the bilirubin. If the chloroform be evaporated, rhombic crystals with rounded edges or distinct needles of a brownish-red color will be observed. These crystals will give the color tests mentioned below.

Smith's Test.

This test has been described under other names, as those of Trousseau Kathrein, Rosin, and Marechalt. A few c.c. of urine, acidified if necessary with acetic acid, are treated with a 1 per cent. alcoholic solution of iodin in such a way that the latter solution is superimposed upon the urine, forming a distinct line of contact. If bilirubin or other biliary pigments be present, a beautiful emerald-green color is observed at the point of contact. This test is especially recommended, but it is not very sensitive, indicating only one part of biliary pigment in 10,000 of urine. Certain drugs, especially antipyrin, may lead to the formation of a green color with this test. Thymol, if used as a preservative, may give rise to confusion with this and other bile tests.

Gmelin's Test.

One or two c.c. of nitric acid are placed in a test-tube and the same amount of urine is allowed to flow from a pipet in such a way that a distinct contact line is formed. If the nitric acid contains a trace of nitrous acid and the urine biliary pigment, a distinct green ring will be observed at the line of contact. In some cases, as the nitric acid oxidizes the pigment, a play of colors may be seen from green, through a blue, violet, and red, to a yellow. The primary green is the characteristic color, other colorations being occasionally due to pigments other than biliary. This test is supposed to indicate 1 part of bilirubin in 80,000 of urine.

If the urine contains an excess of indican a deep-blue ring may be observed or the combination of this blue with the yellow of the urine may give a green. In the presence of skatoxyl a violet-red ring may be noted, while various medicaments may give colorations ranging through the entire spectrum. In such cases it is always advisable to extract the acidified urine with chloroform and apply the test either to the evaporated residue or to its aqueous solution.

This test is, perhaps, more frequently used than any of the biliary tests, but requires considerable experience for its proper interpretation. If the urine be diluted the test is somewhat more distinctive. Many modifications of this test have been advocated, the most serviceable being the following:

Rosenbach's Test.

A large quantity of urine, which has been acidified with HCl, is filtered several times through a thick filter-paper, which will hold back the bile-stained elements of the urine. It is sometimes advisable to add a little milk of lime to the urine before filtering, instead of the HCl, as this will throw down the phosphates which will carry with them the biliary pigment. If the filter-paper and contents be dried by pressing with a second dry filter-paper and a drop of yellow nitric acid allowed to fall upon it, distinct rings will be seen, which will be colored as in the previous test, the green one being external.

Nakayama's Test.

This is a modification of the older Huppert test. Five c.c. of acid urine are treated with an equal volume of 10 per cent. barium chlorid solution and the mixture centrifuged. The barium chlorid precipitates the phosphates and sulphates and carries down the biliary pigments. The supernatant fluid is then poured off and 2 c.c. of the following reagent are added to the precipitate. The reagent consists of 99 c.c. of 95 per cent. alcohol, 1 c.c. of concentrated HCl, and 0.4 gram of ferric chlorid. If this mixture of precipitate and reagent be heated to boiling, a bluish-green or a brilliant green solution is obtained, which becomes violet or red on the addition of nitric acid. This test is said to indicate one part of bilirubin in 1,200,000 parts of urine.

Hammarsten's Test.

As the reagent in this test we use a mixture of one part of 25 per cent. HNO_3 and 19 parts of 25 per cent. HCl. Before use one part of this reagent is mixed with four parts of alcohol. A few drops of the urine are added to this mixture, when it assumes a green color in the presence of biliary pigments. If the urine be treated as in Nakayama's test and the precipitate mixed with 1 to 2 c.c. of the acid alcohol reagent and the whole centrifuged for a short time, a green solution is obtained if one part of bilirubin in 1,000,000 of urine be present.

Bile Acids.

The bile acids, taurocholic and glycocholic acids, are found in the urine in the form of their sodium salts. They may be found in small amounts in the same conditions in which the biliary pigments are present, but their amount is usually so small that they cannot be detected, as a rule, without being previously isolated. It seems to be fairly well established that the bile acids must be present to the extent of $\frac{1}{2}$ per cent. before detection in the urine is possible.

As the clinical significance of these acids is the same as that of the pigment and as their amount is so small that the methods of isolation require a fairly large volume of urine, the writer will refer to works on physiologic chemistry for such procedures.

No absolutely reliable test is known for the detection of the bile acids in the untreated urine. There are, however, a few tests which are occasionally given providing the bile acids be present in sufficient amount. It need not be surprising if these tests usually fail, but occasionally one may be rewarded with a positive reaction.

Hay's Test.

This test depends upon the reduction of the surface tension of the urine in the presence of the bile acids. As advocated by Beddard and Pembrey, a pinch of powdered sulphur is sprinkled upon the surface of urine which should be preferably at a temperature not over 17° C. In normal urines the sulphur will float upon the surface, while if the urine contains bile acids the sulphur may sink at once indicating one part in 10,000 or may sink only after a few seconds to one minute, in this latter case indicating one part in 50,000. According to Sahli this test does not discriminate between biliary acids and biliary pigments, but clinically it is a matter of indifference which one is present. Phenol or aniline compounds lower the surface tension of the urine so that their presence may lead to wrong conclusions.

Oliver's Test.

This test is based upon the well-known property possessed by the bile acids of precipitating peptone when it acid solution. The reagent is as follows:

Powdered peptone,	8.33 grams
Salicylic acid,	1.12 grams
Acetic acid,	2 drops
Distilled water,	1 liter

One to two c.c. of clear filtered urine are placed in a test-tube and treated with 5 c.c. of the reagent. In the presence of bile acids a decided milkiness appears at once, being the more intense the larger the amount of bile acids.

Other tests, such as those of Pettenkofer and Udránzky, are only serviceable in the testing of the isolated bile acids, so that they must be looked for elsewhere.

(*c*). **Melanin.**

In cases of melanotic tumors the urine not infrequently contains a chromogen (melanogen), which is converted into melanin on allowing the urine to stand or after adding alkalies or oxidizing agents. The urine when freshly voided is normal in color, but on exposure to air gradually darkens until it becomes distinctly black. This coloration is first noticed in the upper portion of the urine and gradually extends toward the bottom. If ferric chlorid be added to the urine the coloration may be somewhat intensified so that one may assume an excess of phenol derivatives. It is to be remembered that indican must first be split up with acid before giving the coloration with ferric chlorid; moreover, melanin is insoluble in chloroform while the indigo is readily soluble.

The addition of the ferric chlorid may produce a black precipitate which is soluble in sodium carbonate solution, from which it may be reprecipitated by mineral acids. This pigment may also be found in some cases of chronic malaria so that it is not absolutely pathognomonic of melanotic tumors.

(*d*). **Phenol Derivatives.**

As previously mentioned in the discussion of the variations in color of the urine, it may be dark colored either on voiding or after standing. While such colorations may be due to melanin, they are much more frequently traceable to the presence of sulphuric acid in conjugation with phenol, paracresol, pyrocatechin, and hydroquinone. These substances are excreted especially in conditions associated either with increased intestinal putrefaction or with putrefactive processes elsewhere in the system. Aside from direct poisoning with these substances, they may be regarded as having much the same clinical significance as indican.

Qualitative and quantitative tests for these substances are rarely of clinical importance. They may be roughly determined by estimating the amount of ethereal sulphates in the urine, when a large increase may be assumed to be referable to these bodies unless indican is greatly in excess.

(*e*). **Alkapton.**

In certain conditions of disturbed protein metabolism, the urine contains pigments which cause it to turn dark on the addition of alkali or on standing. These urines are normal in color when voided and become black almost immediately on the addition of alkali, whence the name alkapton bodies and *alkaptonuria* for their excretion in the urine. The substances causing this change of color are hydroquinon-acetic acid (as it has been prepared synthetically from gentisic aldehyd, it has been called *homogentisic acid* with the formula $C_6H_3(OH)_2(CH_2COOH)$) and uroleucic acid, whose structure has not been absolutely settled. The former of these acids is present in all cases of alkaptonuria, while the latter may be present in all cases, but in such small amounts that it remains undetected. Urines containing these bodies strongly reduce copper and ammoniacal silver solutions, while bismuth solutions are little affected. The so-called *glycosuric acid* of Marshall is probably identical with homogentisic acid and is not a definite chemical entity.

This condition is observed at various periods of life and seems to be of theoretical more than practical interest, as of the 45 cases reported none appeared to be much disturbed in health, the condition usually being detected accidentally.

The source of these substances is still uncertain. Baumann and Wolkow believe that the influence of specific bacteria in the intestinal canal upon the tyrosin, formed in the hydrolytic cleavage of the proteins, leads to direct formation of homogentisic acid. While this accounts for some of the abnormal substance, it cannot account for the entire amount of 3 to 7 grams of homogentisic acid excreted in the 24 hours. From the work of Meyer, Falta,

Langstein, Wohlgemuth, and others it has been definitely established that this condition depends not on an abnormal formation of homogentisic acid, but on an incapacity for further oxidizing it when formed. In other words, alkaptonuria is simply a perversion of protein metabolism which has very few ill effects, although transitory symptoms have been observed in occasional cases of diabetes, cirrhosis of the liver, tuberculosis, pyonephrosis and gastritis. The browning of the cartilages occurring in ochronosis is supposed by Albrecht and Zdarek to have some relation to the alkapton bodies, although Langstein could not show the alkapton acids in the urine in such cases.

Qualitative and quantitative tests for these acids are rarely necessary. The characteristic change of color on alkalinizing the urine is, at least, suggestive of homogentisic acid. Like all other hydroxy derivatives of benzol, this substance shows the Schiff reaction with ferric chlorid, producing a transitory blue coloration when present in amounts of 1 to 4,000. The reduction of copper solutions should not be mistaken for sugar, as homogentisic acid neither ferments nor shows any optical activity. The urine will usually show a relatively high acidity. As this substance is practically harmless in the system we should expect to find, as we actually do, no increase in the conjugated sulphates or glycuronates nor any increase in the ammonia content of the urine (Neuberg).

(*f*). Ehrlich's Diazo Reaction.

Under certain pathologic conditions the urine has been found to contain a chromogen which gives a deep red color to the urine when treated with diazo compounds and ammonia. This is known as the diazo reaction, the urinary substance causing it being somewhat uncertain. According to Bondzynski, the alloxyproteic acid is the causative factor, but this needs confirmation, as Clemens has apparently shown that the body producing the reaction is sulphur-free.

In the performance of this test two reagents are used: (1) sulphanilic acid 5 grams, 50 c.c. of concentrated hydrochloric acid, and 1,000 c.c. of water; (2) 1/2 per cent. aqueous sodium nitrite solution.

To a mixture consisting of 50 parts of solution 1 and one part of solution 2 is added an equal volume of the urine. The mixture is then shaken and about one-tenth of the bulk of ammonia added quickly, and the mixture thoroughly shaken. The ammonia may be added in such a way that a line of contact forms, this latter method frequently bringing out a much more beautiful reaction.

If the test be positive the entire urine will assume an intense red coloration or a colored ring will be observed at the point of contact between the ammonia and the mixture. With normal urine a distinct orange color may be observed. On shaking the mixture the foam will be more or less brilliant red in color, which is more characteristic than the red coloration of the mixture. On allowing the mixture to stand for 24 hours a green precipitate may be observed

at the bottom of the tube, which Ehrlich regards as especially characteristic of the true diazo reaction. This green precipitate is not always present and is not necessary for a positive reaction, the red coloration of the mixture and of the foam being more frequent.

According to Greene, a mixture consisting of 100 parts of solution 1 to one of solution 2 renders the test more delicate. Instead of sulphanilic acid, paraamidoacetophenon may be used, as suggested by Friedenwald.

The administration of certain drugs may markedly affect this test. Thus we find that naphthalin, chrysarobin, opium, and phenol derivatives give a reaction which is very similar to the true diazo reaction, but may be distinguished, according to Wood, by the fact that color is more permanent in alkaline solutions, does not fade to any extent on the addition of a strong mineral acid, the foam is more yellow than in the typhoid reaction, and the green precipitate does not appear on standing. Burghart has found that, following the administration of tannic acid, gallic acid, tannigen, and tannalbin, the diazo reaction disappears from the urine, the inhibiting effect being perhaps exerted upon the reagents used rather than upon the unknown factor which usually causes the coloration.

According to Ehrlich, if the urine contains an excess of biliary pigment, a dark cloudy discoloration may occur, which is changed on boiling to a distinct reddish-violet. He also finds on applying the diazo test in some cases that the urine and foam become yellow before the addition of ammonia. After ammonia is added the color changes to a lighter yellow. This reaction is known as Ehrlich's "*egg-yellow*" *reaction*, and is supposed to be due to the presence of urobilinogen. He regards this latter test as especially important in predicting the crisis of pneumonia.

This diazo reaction is never positive in health. It was formerly regarded as pathognomonic of typhoid fever, but it has been shown to occur in many other conditions. It is true that in typhoid fever it may be present as early as the third or fourth day and may persist for some time, reappearing if a relapse occurs, while the Widal test, as will be remembered, does not vary with a relapse. Moreover, the intensity of this reaction is somewhat parallel with the severity of the case, while the Widal reaction may not be present in the severest types of typhoid fever. A positive diazo reaction occurs frequently in measles, somewhat less frequently in pneumonia, miliary tuberculosis, scarlet fever, diphtheria, and erysipelas, while in rheumatism and meningitis it is rarely obtained. Michaelis believes that the presence of a positive diazo reaction in pulmonary tuberculosis indicates a progressive condition with a grave prognosis.

This reaction is, therefore, a valuable aid in diagnostic work. As it is present in about 80 per cent. of cases of typhoid fever, a negative reaction would not necessarily exclude typhoid nor would a positive reaction prove its presence. Its appearance in a relapse is of value, as the Widal test would give no information under such conditions. It is to be said, however, that

the diagnosis should by no means rest upon this test any more than it should upon the Widal test.

(*g*). **Russo's Test.**

This test[1] has been recently advanced and seems to have somewhat more diagnostic importance than has the diazo reaction. The technic is very simple and is as follows: Four drops of a 1 to 1,000 aqueous solution of methylene blue are added to 4 or 5 c.c. of suspected urine. If the reaction be positive the mixture turns to an emerald or mint-green hue. A light green or bluish-green tint shows a negative reaction. The positive reaction is not affected by boiling the urine or by the previous ingestion of such compounds as phenacetine, salol, quinin, and calomel. The difficulty in the application of the test comes in the ability to recognize the various tints of green which may be present. With a little practice, however, a positive reaction may be readily detected, especially if a control test be made with normal urine.

This test is shown as early as the second day of typhoid fever and persists throughout its course. The mint-green hue is first observed, the emerald-green tint appearing as the disease progresses. If the course is favorable the color tone becomes more and more bluish, while if unfavorable the emerald tint persists. This test is also given in measles, smallpox, chronic and suppurative tuberculosis, but is negative in varioloid, varicella, scarlet fever, miliary tuberculosis, appendicitis, and malaria.

The chief point in favor of this test is its simplicity as compared with the diazo reaction. It is quite as reliable as the diazo and would seem to be much more valuable in differentiating typhoid fever from acute miliary tuberculosis than is Ehrlich's reaction.

(*h*). **Dimethylaminobenzaldehyd Reaction.**

This test has also been advanced by Ehrlich and is applied as follows: A 2 per cent. solution of dimethylaminobenzaldehyd in equal parts of concentrated hydrochloric acid and water is prepared. If a few drops of this solution be added to a few c.c. of urine a cherry-red color will be observed within a few minutes. This coloration may be extracted with chloroform or epichlorhydrin. Heating slightly facilitates the reaction, so that the normal urine may even give a similar coloration under these conditions. If this test be performed in the cold a normal urine usually gives a greenish-yellow coloration, although a reddish tone may sometimes obtain, while pathologic urines give a distinct cherry-red color. A positive reaction is more commonly observed in tuberculosis, although typhoid fever, pneumonia, appendicitis, and many other conditions frequently show positive results. The clinical value of this test is, therefore, uncertain.

(*i*). **Drug Reactions.**

After the administration of a large number of drugs, conjugated glycuronates or sulphates may appear in the urine which give rise to various colora-

[1] Riforma Medica, Tm. 21, 1905; abstract Jour. A. M. A., vol. 45, 1905, p. 363.

tions. The more important of these have been previously discussed so that the writer will refer thence for the treatment of this subject. The methods of detecting these various drugs, as such, must be found in works on pharmaceutical chemistry.

IV. Microscopic Examination of the Urine.

The microscopic examination of the urine is important in every case. So varied are the elements which appear in the microscopic field that considerable experience is necessary before absolute interpretation can be made. Not infrequently the character of the sediment will change a diagnosis, as, for instance, when pus- or blood-cells are present in sufficiently large amounts to account for an albuminuria previously determined by chemical methods. It is, therefore, essential that the microscopic examination of urine form a part of the ordinary routine.

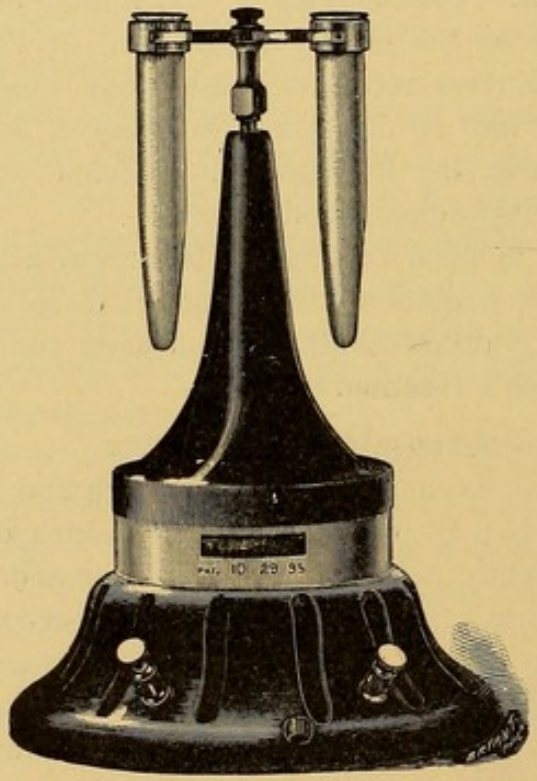


Fig. 88.—Purdy electric centrifuge.

In obtaining the sediment of the urine for microscopic examination, two methods are possible. In the first place, the urine may be allowed to stand in a conical glass for periods ranging from 12 to 24 hours. The sediment originally present in the urine as well as that formed by chemical changes taking place during the standing will collect in the lowest portion of the glass and may be removed by a glass-tube drawn out to a somewhat small point. It is to be remembered that a sediment at the end of 12 to 24 hours may be entirely different from that originally present in the freshly voided specimen.

The changes in the reaction of the urine will, necessarily, lead to the dissolving of certain types of crystals and to the formation of other varieties. Moreover, casts, if originally present, may dissolve or disappear as a result of the reaction of the urine becoming alkaline. For these reasons it is absolutely essential, in the use of this gravity method, that preservatives be added to the urine. Among the preservatives which may be used to prevent bacterial action during the sedimentation, we find a small piece of camphor or a rather large crystal of thymol serving the purpose. Some workers add one-fifth the volume of a 4 per cent. solution of borax which is equally useful, but the addition of chloroform or formalin, does not serve as well in these cases, as the former does not completely preserve the casts and the latter introduces a crystalline compound of formalin and urea which may be confusing, as it is not unlike impure leucin. This method is not to be recommended for routine work, as it requires too long a period and as changes may occur which make it absolutely impossible to differentiate between a primary and a secondary sediment.

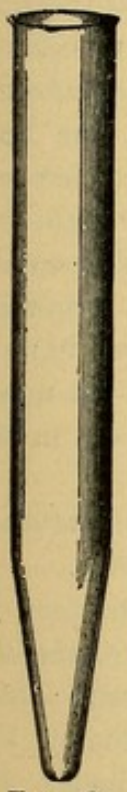


FIG. 89.—Sediment tube for Purdy centrifuge.

Secondly, the sediment may be thrown down by the use of the centrifuge. This apparatus is seen in the accompanying cut. By the use of this method no preservative is needed, a deposit is obtained within three minutes in a much more concentrated form, and changes in the sediment do not take place. The writer would recommend, therefore, the use preferably of the electric centrifuge or, at least, of the type run by hand.

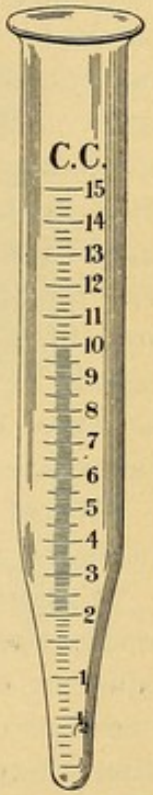


FIG. 90.—Percentage centrifuge tube.

Whatever method may be adopted for obtaining the urinary sediment, the next steps in the process are the same. A pipet, consisting of a glass tube drawn out to a point about one-half the diameter of the tube, is introduced to the bottom of the vessel containing the sediment, a finger being placed over the upper end to prevent fluid passing into the tube as it is introduced. When the tip of the pipet comes in contact with the deposit, the pressure of the finger on the upper end of the pipet is removed and the deposit allowed to flow up into the pipet. The finger is then placed tightly over the tube as it is withdrawn from the fluid. By placing the tip of the pipet in contact with a perfectly clean slide which is absolutely free from scratches and by gradually rotating the pipet, a small portion of the sediment is obtained. A cover-glass is then placed upon this drop avoiding any undue pressure, which might distort the organized elements of the sediment. Some workers dispense with the cover-glass and use somewhat larger amounts

of sediment, but the writer does not find this method as acceptable owing to the fact that the lens of the objective may dip into the fluid and thus give indefinite microscopic pictures. Moreover, the focus cannot be as accurately adjusted without the use of the cover-glass.

In the examination of the microscopic specimen, prepared as above, the point of special importance to be observed is the proper adjustment of the light. The writer is accustomed to use the low-power objective in the preliminary examination. In this case it is essential that the light be shut off to a large extent, as the recognition of casts cannot be made in a brilliantly illuminated field. This examination with the low power has the advantages that larger visual fields are subject to inspection, casts are easily recognized and crystalline deposits, as well as morphological elements, are usually differentiated. After the preliminary examination with the low power, the final examination is made with the high-power dry lens. In this way elements which appear suspicious under the low power are more clearly brought out and differentiations made possible between various types of cellular elements. With the high-power lens it is, of course, essential that the field be somewhat more illuminated than when the low-power is used. While a mechanical stage is, at times, advantageous in the microscopic examination of the urine, the writer has found that the fingers serve practically every purpose in the manipulation of the slide under examination.

Where the urine is to be examined for the presence of bacteria, stained specimens must be made and examined with the oil-immersion lens.

Urinary sediments are classified into two divisions: (a) chemical or nonorganized and (b) anatomical or organized sediments. The nonorganized sediments exist in solution in normal urine and appear as deposits under conditions of excessive formation, excessive excretion, or of alterations in the urine affecting its solvent properties. The chief chemical sediments are uric acid and its salts, calcium oxalate, phosphates, sulphates, cystin, leucin, tyrosin, xanthin, fat, and fatty acids. The organized sediments are usually foreign substances and are not met with in normal urine. They consist of epithelial cells, pus corpuscles, blood-cells, renal casts, spermatozoa, infusoria, bacteria, and tissue fragments.

(*A*). Unorganized Sediments.

(*a*). Those Appearing in Acid Urine.

(1). Uric Acid ($C_5H_4N_4O_3$).

This occurs as a sediment in the urine under 3 conditions: (1) great concentration, (2) high acidity, and (3) low temperature. The deposit differs from others in possessing a deep yellow or orange-red color, although some of the smaller crystals are occasionally colorless. The primary form of the uric acid crystal is that of the rhombic prism. Modifications of this, in the form of square plates, cubes, ovoids, dumb-bells, or whetstone crystals are sometimes noticed. A rare type, especially of the colorless crystals, is a per-

fect hexagon which resembles cystin so closely that chemical means of differentiation must be used. The crystals may be single or grouped in rosettes or fan-shaped masses. Occasionally typical needle-shaped crystals may be seen which are arranged in sheaves.

The microscopic peculiarities of uric acid are usually such as to permit of its easy recognition. In some cases, however, it is wise to confirm the microscopic finding by the *murexid test* as follows: Place a small quantity of the sediment in an evaporating dish and add a few drops of concentrated nitric acid. Evaporate on the water-bath to dryness, when a yellowish or reddish residue will remain. Allow the residue to cool and add a few drops of

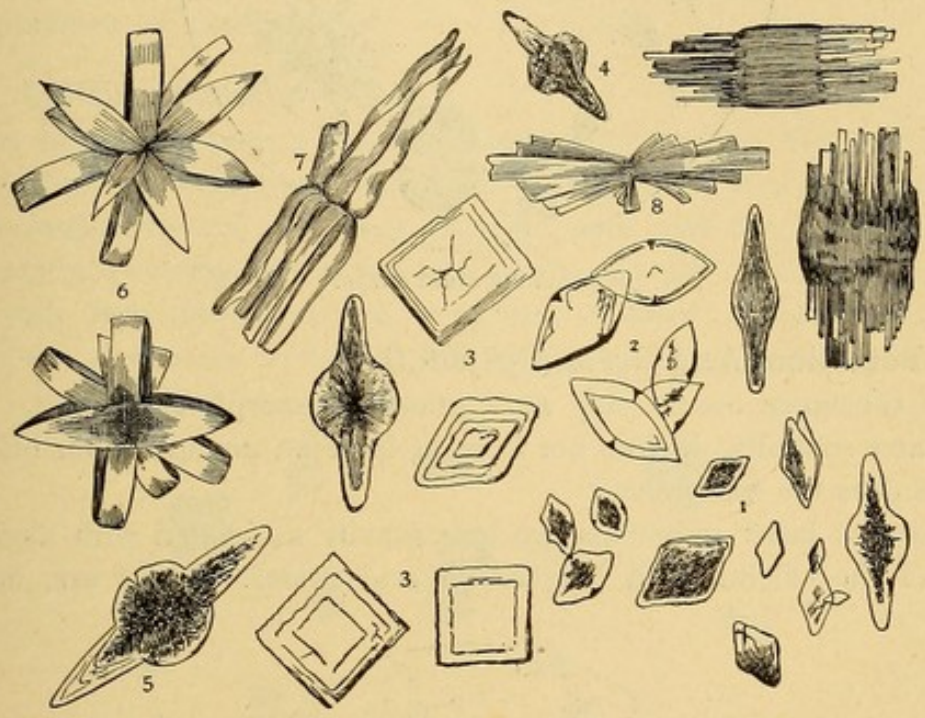


FIG. 91 —Various forms of uric acid. 1, Rhombic plates; 2, whetstone forms; 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosettes; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine. (*Hawk.*)

ammonium hydrate solution. In the presence of uric acid a distinct reddish purple color will appear. If water be added to this purple solution and the mixture evaporated to dryness the color disappears. This latter point is of importance as xanthin, which may resemble unusual types of uric acid in microscopic appearance, also gives the murexid test, but the color does not disappear on heating with water.

(2). Sodium Acid Urate ($C_5H_3NaN_4O_3$).

This salt of uric acid forms the bulk of the "brick-dust deposit" or "sedimentum lateritium" found when urine has cooled. In such cases the urine first shows a milky appearance and the sediment soon settles on the sides and bottom of the container. This deposit is usually in the form of irregular amorphous granules of a brownish or pink color. Occasionally the sediment

may be distinctly crystalline, occurring as prismatic needle-like crystals which are grouped in star-shaped, fan-shaped or dumb-bell-like clusters.

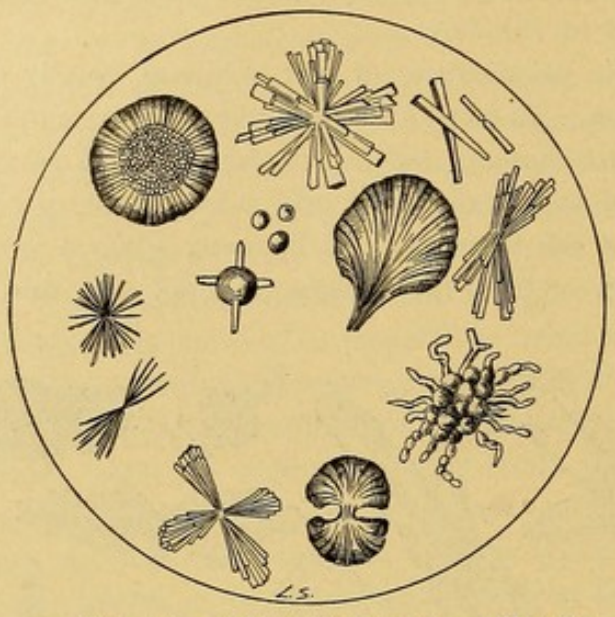


FIG. 92.—Acid sodium urate. (*Hawk.*)

(3). **Potassium Acid Urate ($C_5H_3KN_4O_3$).**

This substance occurs only as a granular amorphous deposit. Owing to its greater solubility, it does not form as large an amount of the brick-dust deposit as does the sodium salt.

These two latter sediments are occasionally associated with amorphous deposits of the calcium and magnesium acid urates. These are, however,



FIG. 93.—Xanthin. (*Hawk.*)

rare and need not be separately considered. In detecting the presence of the urates in a deposit, a small portion of the turbid urine is poured into a test-tube and gently heated. If urates are present the sediment will completely dissolve. These salts also give the murexid test.

(4). **Xanthin** ($C_5H_4N_4O_2$).

This substance which is chemically closely related to uric acid is rarely found as a sediment in the urine. Its chief clinical importance is found in its appearance as a urinary calculus. It crystallizes in whetstone-shaped colorless crystals which resemble those of uric acid, from which it is differentiated by its solubility on heating and in hydrochloric acid as well as ammonia. It may be chemically recognized by *Weidel's reaction*. Place a portion of the suspected crystalline deposit in an evaporating dish and dissolve by warming with a few drops of bromine water. Evaporate to dryness and place the dish containing the residue under a large beaker, allow the fumes of ammonia to fill the inverted beaker when a red or purplish-violet color will be produced in the presence of xanthin.

(5). **Calcium Oxalate** (CaC_2O_4).

This substance appears most frequently in acid urine, but may be found after the urine has undergone alkaline fermentation. If it occurs in acid urine it is associated with uric acid; if in alkaline urine, with the triple phosphates. The deposit is a colorless crystalline one having two distinct forms: (1) octahedral crystals (four-sided pyramids lying base to base); viewed from the side, these appear as squares crossed by two sharp lines, giving the so-called "en-

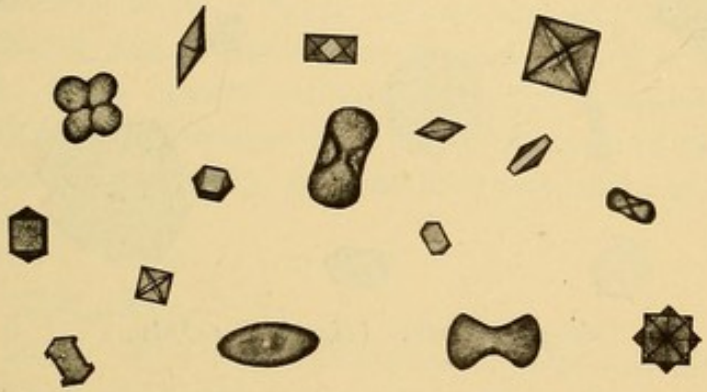


FIG. 94.—Calcium oxalate. (*Hawk after Ogden.*)

velope" crystal. (2) Dumb-bell crystals in the form of ovoid or circular disks with round margins depressed at the centers. These latter often present radial striations. Emerson has called attention to a rare type of calcium oxalate crystal which appears in the form of flat plates with parallel sides and rounded ends, looking like superimposed sheets of mica. It is characteristic of the crystallization of this substance, as of most crystalline urinary deposits, that the crystals are practically always of the same type, variations rarely appearing in the same specimen of urine.

These crystals are insoluble in acetic acid, but soluble in hydrochloric acid. This is a point of some importance, as it is occasionally difficult to distinguish microscopically between calcium oxalate and some crystals of triple phosphate. This latter crystal is soluble in acetic acid. These crystals

may be chemically identified by dissolving them in hydrochloric acid, alkalinizing with ammonium hydrate, and precipitating with ammonium oxalate.

(6). **Cystin** $(C_3H_6NSO_2)_2$.

The appearance of cystin in the urine is known as *cystinuria*. This is a condition of perverted protein metabolism which is not well understood. It may make its appearance at any period of life and in either sex, perhaps somewhat more frequently in the male. It shows a remarkably frequent hereditary character, being observed in some cases through several generations and in several children of the same parents. Many of these cases do not show any clinical characteristics, being present during their entire life without any apparent symptoms. In other cases, owing to the formation of calculi, frequent manifestations are noted and surgical intervention interposed. This condition is not very frequent, being reported only 132 times in the literature.

"Prior to the time when cystin was found to be a product of the disintegration of protein substances it had been conjectured, on the ground of its content in sulphur and nitrogen, that it might be a product of the intermediate protein metabolism. The explanation which von Udránzki and Baumann gave

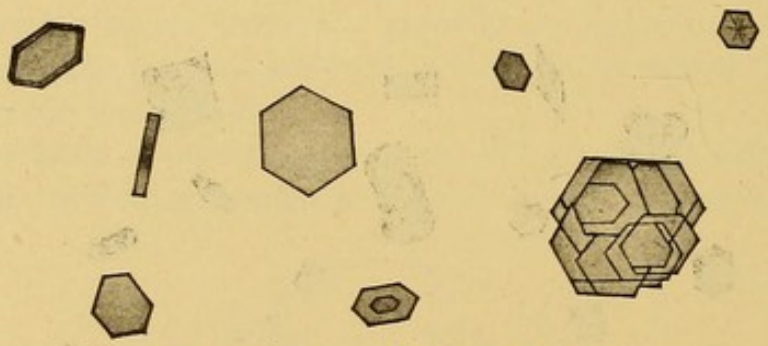


FIG. 95.—Cystin. (*Hawk after Ogden.*)

of its excretion corresponds in principle to that which is familiar to-day for the appearance of conjugation products of glycuronic acid and of glycocoll in urine. Like the latter, it should be normally further oxidized, and only in the presence of definite bodies should it be intercepted. These authors considered the binding substances to be the previously mentioned diamins, especially putrescin and cadaverin, which they had found in the urine and in the feces of a cystinuric patient. The formation of the diamins was supposed to be brought about by specific bacteria in the digestive tract by an extraordinary chronic intestinal mycosis. The resorbed part of the cadaverin and putrescin was supposed to protect the cystin from combustion, just as benzoic acid does glycocoll, by entering into a loose combination which decomposes again after passing through the kidneys. Serious difficulties, however, have opposed themselves to this interpretation of cystinuria. First, numerous cases have been described without simultaneous *diaminuria*, and,

conversely, diaminuria occurs in malaria, and in the conditions brought about by the cholera vibrions and the Finckler-Prior bacillus, without cystinuria having ever set in, any more than it does when diamins themselves are administered."

"Further investigations have shown that cystinuria is really a disturbance of the amino-acid metabolism. Of the end-products of protein hydrolysis which arise in the system, the cystinuric cannot avail himself in the normal way of the cystin, and in part excretes it; the remaining products of protein hydrolysis undergo their ordinary fate. If free monomolecular α-amino acids appear in places which are at present not well known, or if they occur there even in unusual amounts, then, unlike the normal individual, the cystinuric is unable to burn them and they leave the organism unchanged just as cystin itself does. The basic diamino acids behave in practically the same way, except that the CO_2 group is split off from them and we arrive at diaminuria" (Neuberg).

Crystals of cystin are rare in the urinary sediment. In some of the cases reported the cystin did not separate from the urine until this was acidified with acetic acid and allowed to stand for 12 hours. It crystallizes in two forms: (1) six-sided tablets having an opalescent luster and sometimes traced with fine lines of secondary crystallization; (2) four-sided square prisms lying separately or in stellate forms. These crystals are soluble in hydrochloric acid, alkaline hydrates, and insoluble in acetic acid. These tests differentiate it from uric acid. If the urinary sediment suspected of containing cystin be treated with strong sodium hydrate solution and a few drops of benzoyl chlorid, and the mixture shaken vigorously, a voluminous precipitate of benzoyl cystin is obtained.

(7). Leucin ($C_6H_{13}NO_2$).

Chemically leucin is α-aminoisobutylacetic acid. It occurs in the urine in conditions associated with more or less marked derangement of hepatic function (see amino acids). As found in the urine, leucin appears in the form of yellowish, highly refractile spherules, with alternating light and dark concentric layers and with radial striations. In the pure state it crystallizes either in thin, white, hexagonal plates or as scales or rosettes of irregular shapes.

Leucin is soluble in water, acids, and alkalies, and insoluble, to a more or less extent, in alcohol. Not always do we find crystals of leucin in the sediment when the urine contains this substance. If it be suspected, the urine should be evaporated to a small bulk and alcohol added to the residue, which may then be examined for the characteristic crystals. This leucin may be identified by *Scherer's test* as follows: Some of the solid residue obtained by concentrating the urine to a small bulk is evaporated with concentrated nitric acid on a platinum crucible cover. With pure leucin the residue remains colorless, but as usually applied to the urine a yellowish residue obtains. This

is heated with a few drops of sodium hydrate solution, when a yellowish or brown color will be observed. If further heating be applied the leucin will collect into an oily drop which rolls around on the heated surface. As leucin does not stain with Sudan-III it should not be confused with fat.

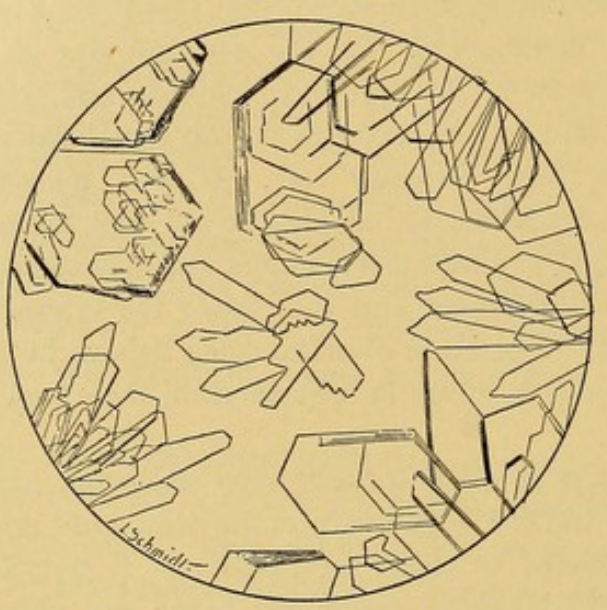


FIG. 96.—Pure leucin. (*Hawk.*)

(8). **Tyrosin ($C_9H_{11}NO_3$).**

Chemically, tyrosin is p-oxyphenyl-α-amino-propionic acid. As found in the urine tyrosin crystallizes in the form of fine colorless needles, which may appear black and are arranged in sheaf-like collections or rosettes. Like leucin, it may not crystallize out unless the urine be concentrated. Tyrosin



FIG. 97 —Impure leucin. (*Hawk after Ogden.*)

is soluble in water, acids, and alkalies, while it is slightly soluble in alcohol and insoluble in ether. As other crystals, which may appear in the urine closely resemble the tyrosin needles, it is advisable to confirm the microscopic findings by chemical tests. This may be done by evaporating the urine to a small bulk, removing the fluid and dissolving the residue in water. *Mörner's test* may then be applied as follows: To this aqueous solution is added 1 c.c.

of a reagent consisting of 1 c.c. of formalin, 55 c.c. of concentrated sulphuric acid, and 45 c.c. of water. If the mixture be heated to boiling a beautiful green color will be observed in the presence of tyrosin.

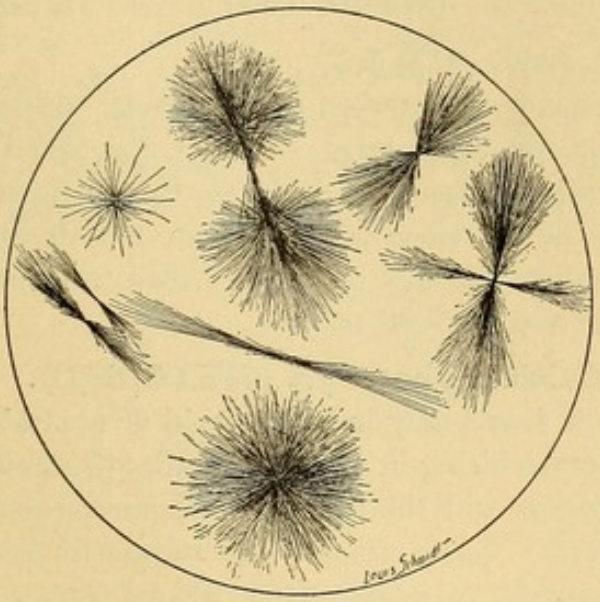


FIG. 98.—Tyrosin. (*Hawk.*)

(9). **Calcium Sulphate ($CaSO_4 2H_2O$).**

This is a very rare sediment, appearing only when the urine is extremely acid. The crystals appear in the form of long, thin, rhombic plates or needles which may be single, but are more frequently observed in clusters. If the sediment be boiled with hydrochloric acid and barium chlorid added, a precipitate of barium sulphate will point to the presence of calcium sulphate in the sediment.



FIG. 99.—Calcium sulphate. (*Hawk after Hensel and Weil.*)



FIG. 100.—Bilirubin (Hæmatoidin). (*Hawk after Ogden.*)

(10). **Bilirubin ($C_{16}H_{18}N_2O_3$).**

Bilirubin or its isomer hematoidin may appear in the urine in conditions previously discussed. The type of crystal is either a brilliant yellow or ruby-red rhomb or a yellow needle. Rarely the deposit may be in the form of a

yellow granular sediment. Not infrequently small curved needle-like spines are observed projecting from the angles of the rhombic crystals. These crystals may be identified by extracting the acid urine with chloroform and applying the tests previously discussed under Biliary Pigments.

(11). **Hippuric Acid** ($C_9H_9NO_3$).

This substance has been observed as a sediment, although rarely. It appears in the form of semitransparent, colorless, four-sided prisms, or in long pointed rods or needles, occasionally in forms closely resembling those of the triple phosphates to be described later. These crystals are soluble in warm water, alcohol and ether and may be distinguished from uric acid by the fact that they do not give the murexid test.

(12). **Neutral Calcium Phosphate** ($CaHPO_4 2H_2O$).

This substance is found only in faintly acid or neutral urine. It is quite rare as a sediment, crystallizing in colorless needles or slender pyramids which group themselves together with their points in a common center to form rosettes or cross-shaped figures. These crystals are soluble in acetic acid and may be converted into calcium carbonate when treated with a strong solution of ammonium carbonate.

(13). **Fat.**

Under normal conditions the urine contains no free fat, but amounts varying from traces to rather large excretions may be found under pathologic conditions. The excretion of fat in the urine is known as *lipuria*. This is characterized by the presence of small or large strongly refractile globules which may be stained black with osmic acid or red with Sudan-III. These globules are soluble in ether and may, therefore, be extracted from the urine by shaking out with this solvent. It not infrequently happens that the urine is contaminated with fat which may have been used in obtaining catheterized specimens or with fat coming from the bottle containing the urine. This may lead to a diagnosis of lipuria unless care be taken to exclude such a source. True lipuria has been observed in various conditions. Thus we may find after a large intake of fat in the diet or as a therapeutic agent the so-called "*alimentary lipuria*." Pathologically, it has been observed in various cachectic conditions, in crushing injuries, especially of the bones, in eclampsia, in chronic heart disease, fatty tumors, diabetes mellitus, tuberculosis, various affections of the pancreas and liver, nephritis, and after the use of various general protoplasmic poisons. In these cases the blood may also contain an excess of fat, although this has not been observed in all cases. In fatty degeneration along the genitourinary tract, fat droplets may be seen in the epithelial cells and in the casts. Free fat is rarely found in such conditions, but occasionally it may collect in droplets which float on the surface of the urine and then constitutes true lipuria. Occasionally flat superimposed plates with notched corners (cholesterin) may be seen and may be so numerous as to justify the term "cholesterinuria."

In conditions associated with infection by the filaria, large amounts of fat may be present, giving rise to the appearance of an emulsion. To this condition has been given the name *chyluria*. In this form the fat may be present occasionally in large masses resembling tallow, but more frequently is seen in finer clumps of globules. The appearance of the urine is much like that of skimmed milk, but may have a reddish tinge due to the presence of blood. On allowing the urine to stand, a cream-like mass of fat will rise to the surface. It is not unusual in such cases to find the ova or the parasite in the masses of coagulated material. The excretion of the fatty material

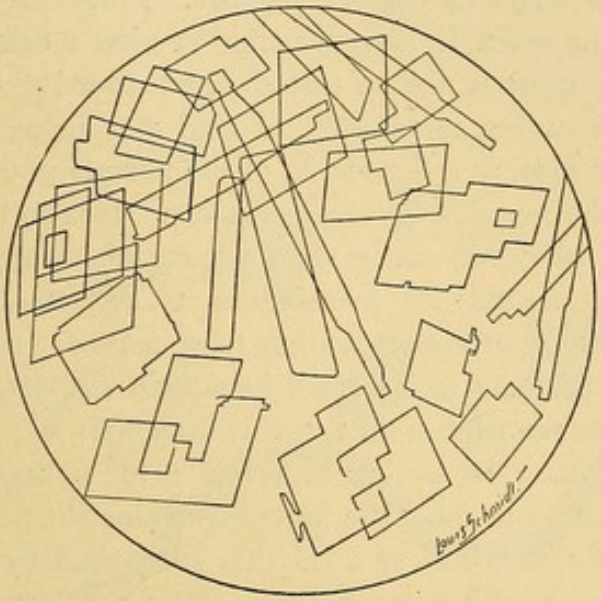


FIG. 101.—Cholesterin. (*Hawk.*)

at times runs a somewhat cyclic course, being present during the day and absent at night or *vice versa*. Occasionally the excretion varies with the position of the patient, being somewhat more frequent when he is erect, and may be markedly increased after severe exercise. This condition should be taken simply as a symptom of filariasis.

A nonparasitic type of chyluria has been observed, but its etiology is somewhat uncertain. It probably is closely related to the conditions above mentioned as causing true lipuria. It does have some relation to an increased fat diet and apparently is associated with exudation from the lymphatic vessels, as the cellular elements are largely lymphocytes.

(*b*). Those Occurring in Alkaline Urine.

(1). Ammonium Urate ($C_5H_3(NH_4)N_4O_3$).

This sediment occurs most frequently in combination with amorphous calcium phosphate and triple phosphate crystals. It is the only urate deposit found in alkaline urine, but may occur in neutral urine. It appears as a crystalline deposit of dark brown spherical masses studded with fine spiculæ, from which fact the name of "thorn-apple" crystals has been given to them.

Occasionally these spheres may show concentric or radial striations. Not infrequently one observes crystals having irregular shapes, such as those of a dumb-bell or a pear.

Chemically, these crystals may be identified by dissolving in hydrochloric acid, when uric acid, which may be identified by the murexid test, will separate. If sodium hydrate be added to the dry sediment and heat applied, vapors of ammonia are given off.

(2). **Calcium Triphosphate** ($Ca_3(PO_4)_2$).

This compound is frequently found in alkaline urine, as a white amorphous flocculent deposit arranged in irregular patches. This is the usual deposit which appears in the urine when it becomes alkaline after meals. In the so-called "*phosphaturia*" the urine is always turbid when voided so that the assumption was made that an excess of phosphoric acid was being excreted. Such is found not to be the case, as a deposition of the normal phosphates of the urine must occur when the reaction becomes alkaline. It is to be said in this place that no conclusion whatever can be drawn from the separation of a substance in the sediment as regards an increase in its excretion. So many factors influence the separation or nonseparation of a sediment that a finding should not be regarded as evidence of increased formation and excretion unless quantitative chemical examination points in this direction.

Calcium phosphate is soluble in acetic acid without evolution of gas, which test may be used to show the presence of this substance in the deposits. It may be absolutely identified as a calcium compound by dissolving in acetic acid and precipitating with ammonium oxalate; the phosphoric acid radical may be proven by dissolving the sediment in nitric acid and precipitating with ammonium molybdate.

(3). **Magnesium Phosphate** ($Mg_3(PO_4)_2$).

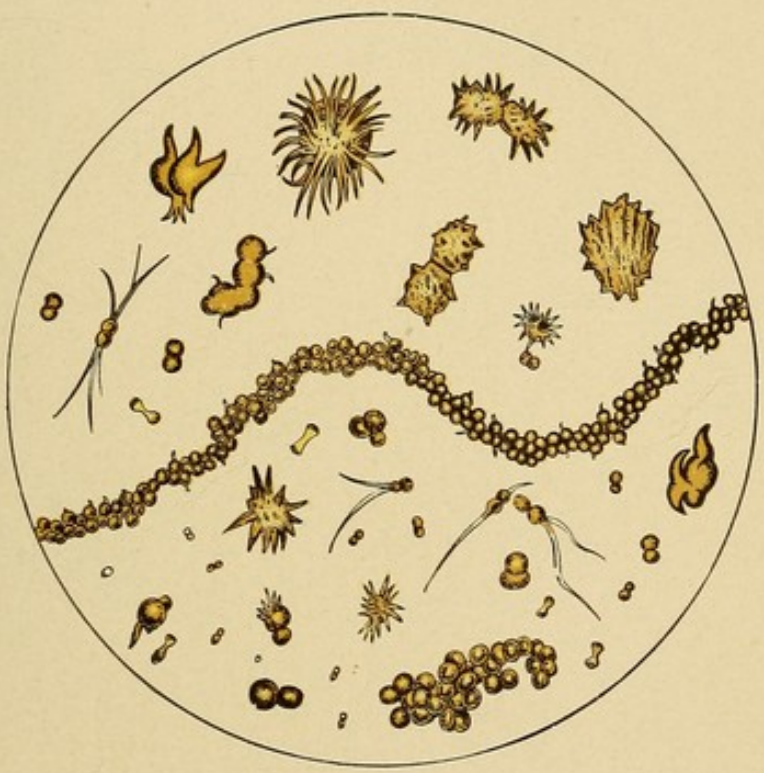
Theoretically this compound appears along with calcium phosphate as an amorphous deposit in alkaline urine. Its amount is, however, usually less than the latter compound.

It is observed in rare cases as large, long, rhombic plates with beveled edges which closely resemble the crystals of triple phosphates. These crystals are found in cases in which not sufficient ammonia is present to form the true triple phosphate, and may be considered as transition crystals.

(4). **Magnesium-ammonium Phosphate** ($Mg(NH_4)PO_4$).

The appearance of this substance in the urine is essentially characteristic of ammoniacal urine. It may very rarely be seen in amphoteric urine when ammonium salts are present in large amounts. The crystals belong to the rhombic system, appearing most frequently as triangular prisms or "coffin-lid" crystals. These may be shortened in the form of squares or one or more corners may be rounded or beveled. By refracted light a greenish tone is observed when these crystals are present. A second type of the "triple phos-

PLATE IX



Ammonium Urate, showing Spherules and Thorn-apple-shaped Crystals.
(From *Ogden*, after *Peyer*.)

phate" is that of a star-shaped feathery crystal with points not unlike fern-leaves. These crystals are easily soluble in acetic acid and may be identified by treating with sodium hydrate and warming when ammonia is evolved.

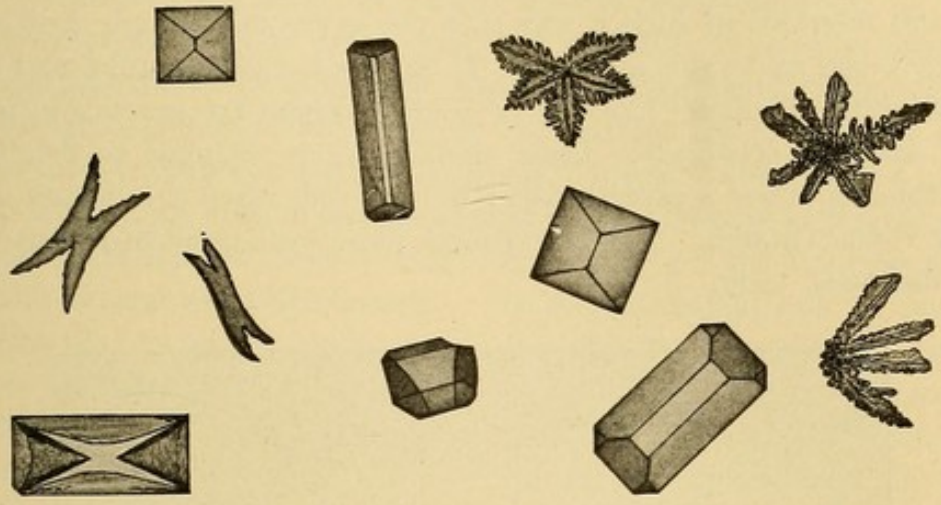


FIG. 102.—Magnesium-ammonium phosphates. (*Hawk after Ogden.*)

(5). **Calcium Carbonate ($CaCO_3$).**

This substance frequently occurs in alkaline urine in association with the amorphous phosphates. It may appear as groups of amorphous material or may form large spheroidal masses with concentric radiations. Occasionally

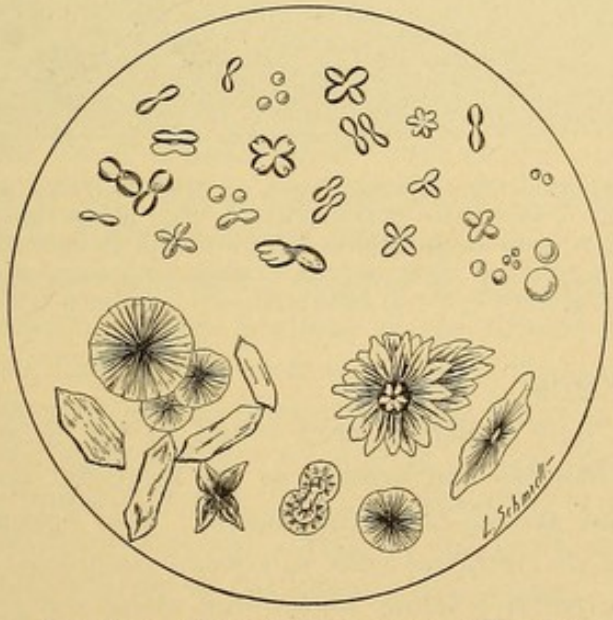


FIG. 103.—Calcium carbonate. (*Hawk.*)

it may be observed in dumb bell-like masses which resemble somewhat the same type of calcium oxalate crystal, from which it may be differentiated by the fact that it is soluble in acetic acid with production of CO_2, while calcium oxalate remains undissolved.

(B). Organized Sediments.

(1). Mucoid material.

Mucus is a constituent of practically every specimen of urine, in the form of the "nubecula." This appears in the form of small threads which branch and interlace in such a way that the entire microscopic field may be practically taken up by this material. In the meshes of the nubecular threads are observed the so-called "mucous corpuscles," which are practically identical with the ordinary leucocyte. Little significance is attached to this form of mucous threads unless a great increase is observed, when it indicates, as does mucin, a vesicle catarrh. The larger more pathologic types of mucus threads will be discussed later.

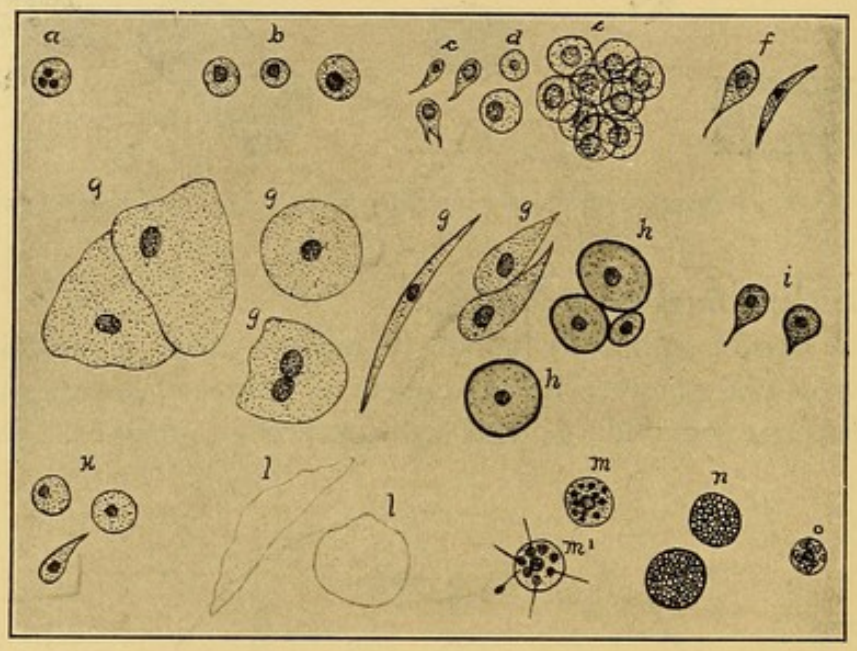


FIG. 104.—Epithelium from different areas of urinary tract. *a*, leucocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, squamous epithelium from bladder; *h*, neck of bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, scaly epithelium; *m*, *m*¹, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell. (*Hawk after Ogden.*)

(2). Epithelial Cells.

Normally, the only epithelial cells found in the urine are the irregular flat cells from the bladder and urethra or the large flat epithelia seen in the urine of women and arising from the vagina. The presence of large numbers of other types of epithelial cells is always pathological and denotes an inflammatory or destructive lesion somewhere along the genito-urinary tract. It is a matter of more or less difficulty to absolutely identify, in all cases, the source of the epithelium found in the urine. According to Heitzmann, the positive recognition is based largely upon the size of the cell, as the shape may vary from pathologic conditions as well as from the portion from which they are derived. As stratified epithelium is found in the pelvis of the kidney, the ureters, bladder, and urethra, it is to be expected that large flat cells,

cuboidal cells, or columnar cells will appear depending upon the layer from which the cell is derived. As the simple epithelium exists in the uriniferous tubules, the prostrate gland, seminal vesicles, and ejaculatory ducts, the recognition of such cells will limit their origin, the size being important in determining the exact point from which they are derived. It is to be remembered, therefore, that the shape of the cell is of far less importance than is its size.

The epithelial cells derived from the bladder are usually the large flat irregular cells commonly seen in all normal urine. They have a clear protoplasm and usually a small distinct central nucleus and are extremely granular. These flat epithelial cells may be single, in groups, or if the irritation is marked may occur in large sheet-like masses. The large cuboidal cells of the bladder epithelium may be seen in acute cystitis in which they are associated with large numbers of the flat cells previously mentioned. If the conditions become chronic the flat cells may entirely disappear and be replaced by cuboidal and by a few columnar epithelial cells. These latter cells are especially observed in the severe inflammatory processes in the bladder.

The large, flat, squamous epithelial cells derived from the vagina are more frequently arranged in stratified groups so that their recognition is usually simple. As these types of cells denote simple desquamation, being pathologic only when present in enormous numbers, an absolute differentiation is of little consequence clinically and, if it be so, the clinical symptoms of the case will usually clear up the decision.

The urethral epithelium very closely resembles that above described. The cells are large and irregular, being partly flat, partly cuboidal and partly columnar. The cylindrical types of urethral epithelium may occur in the form of longer, irregular, smaller types, than those of the bladder or vagina. This type constitutes the so-called "tailed" cells, which may be derived from the pelvis of the kidney and were at one time held to be indicative of a pyelitis. Sahli regards a preponderance of such tailed cells over the flatter and more regular types as distinct evidence of trouble in the renal pelves. As these cells may be derived from other portions of the urinary tracts it is unwise to make an absolute diagnosis on such a finding. The small polygonal cells as well as tailed cells may be derived also from the ureter so that our diagnosis would necessarily rest upon findings other than such epithelium.

In the writer's opinion, it is a practical impossibility to make a positive diagnosis of a lesion in any specific portion of the genitourinary tract based entirely upon the appearance of the urinary epithelium. The points to be remembered are that we may have any type of epithelium and may have many variations in shape as well as in size. Such variations may be present in any portion of the urinary system, although distinctly renal epithelial cells are more frequently in the form of round or cubical cells somewhat larger than the leucocyte and containing a large vesicular nucleus. These latter renal cells are the only ones which seem to the writer distinctly diagnostic. They are differentiated from the similar cells arising from the ureters and prostate

gland by the fact that the latter cells are about twice the size of the pus-cell, being consequently larger than the true renal cell.

Degenerative changes are frequently observed in these epithelial cells, even when examined immediately after voiding. The usual type of this degeneration is the presence of fat granules or globules, especially in the small renal cells. If the sediment be treated with Sudan-III these granules will appear distinctly red.

(3). **Pus-cells.**

A few leucocytes may be observed in practically every specimen of urine, especially in those from women, in which case they may be in large numbers and derived from the vagina. A marked increase, as recognized by numerous, indistinct, small, circular or irregular, granular cells, should be regarded as pathologic. To this condition the name *pyuria* has been given. The simple finding of a pyuria does not necessarily indicate the point from which these cells were derived. Severe inflammatory processes anywhere along the

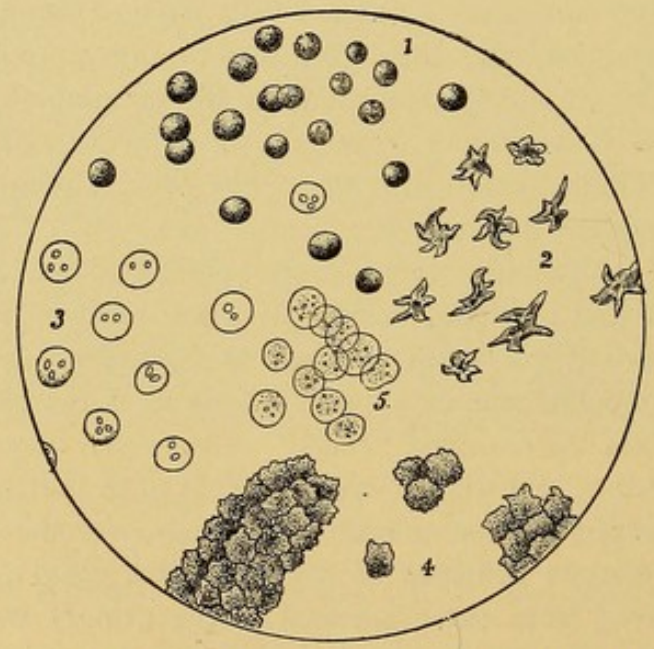


FIG. 105.—Pus corpuscles. 1, Normal; 2, showing ameboid movements; 3, nuclei rendered distinct by acetic acid; 4, as observed in chronic pyelitis; 5, swollen by ammonium carbonate. (*Hawk after Ultzmann.*)

genitourinary tract or the rupture of an abscess into the urinary tract will be associated with a pyuria, so that other features must be relied upon in deciding as to the source. As a rule, it may be said that the amount of pus is small in direct affections of the renal cortex, while disease of the urinary passages is associated with a larger number. If an abscess has ruptured into the pelvis of the kidney the number of cells may be enormous. If the pyuria be of renal origin, it will be associated with the presence of the small, round, renal epithelial cells as well as with tubular casts. Frequently leucocytes in small numbers are found adherent to the casts or they may even be

so closely grouped as to give the name pus cast to such formations. If large numbers of pus-cells appear in the course of a chronic nephritis, they indicate either an acute exacerbation of the condition or a complicating process in some other portion of the urinary tract. The sudden appearance of very large numbers of pus-cells is especially indicative of a ruptured abscess. In inflammatory processes in the pelvis of the kidney the amount of pus may vary within wide limits. In some cases the urine may be perfectly clear when voided, showing the presence of only a few pus-cells, while in others enormous numbers may appear. This paradoxical condition may be accounted for by the possibility of obstruction of the ureter on the affected side and the later forcing out of the large numbers of pus-cells. In pyelitis the urine is usually acid, which may serve as a distinguishing point from cystitis in which the urine is almost always alkaline.

In tuberculosis of the renal parenchyma pus-cells appear very early and vary in number from a few to many thousands. This pyuria is usually constant, and is frequently associated with hematuria. The pus-cells in tuberculosis are usually of the mononuclear type instead of the ordinary polymorphonuclear form. This is not easily determined, as the degenerative processes make it somewhat difficult to distinguish the nuclear form. In such conditions the sediment should be frequently examined for the presence of tubercle bacilli and a portion inoculated into a guinea-pig. This is the only certain method of making a diagnosis of tuberculosis of the kidney. It is, perhaps, needless to add that for absolute differentiation a specimen obtained by ureteral catheterization must be examined.

In cystitis the number of pus-cells appearing in the urine will vary according to the severity of the condition, the more severe the more pus-cells. In this condition the urine is alkaline and may be, when voided, glairy and ropy. In chronic cases of cystitis the pus-cells, although present in the bladder in large numbers, may be so degenerated by the alkalinity of the contents that practically no cells are recognizable. Here we find the appearance of a large amount of mucus, the urine being in some cases distinctly jelly-like.

In inflammatory processes of the urethra pus may be present in varying amounts. In the acute conditions the number of cells is much more numerous that in the chronic types. The recognition of the causative factor, in most cases the gonococcus, will be treated of in a later section. As the acute condition becomes subacute or chronic, the urine contains large numbers of the so-called *gonorrheal threads* which enclose numerous pus-cells. These will be treated in detail later. It is sometimes a matter of clinical importance to distinguish between an anterior and a posterior urethritis. This is best done by the so-called "two-glass" test. If the first portions of the urine be collected in a receiving vessel and the later portions in a second vessel, the urine in the first vessel will be cloudy while that in the second vessel is clear in the case of anterior urethritis; while in posterior urethritis associated with the anterior type the first portion will be cloudy and the second usually so, although at

times it may be clear. The reaction of the urine in both vessels will be acid unless a complicating cystitis has arisen, when the urine in the second vessel will usually be alkaline.

The appearance of the pus-cells will vary depending upon the reaction of the urine. In acid urine their structure is very well preserved, the addition of acetic acid rendering the nucleus somewhat more distinct. Their usual form is that of the polymorphonuclear neutrophile, their size varying from 7 to 12 microns. If stained the vesicular character of the nucleus of the renal epithelial cell will absolutely differentiate it from the irregular type of pus-cell. In alkaline urine the cells swell up, lose their shape and become opaque. The addition of acetic acid usually clears them in such a way that the nucleus becomes visible, but occasionally does not. If the urine remains long in contact with the alkaline material in the bladder it becomes slimy, stringy, and gelatinuous, owing to its large content in mucus. Albumin is always present so that it may be difficult to decide whether or not a true albuminuria exists.

As the pus-cells may undergo such marked change when in alkaline urine and be converted entirely into a gelatinous mass in which corpuscles cannot be detected, certain tests must be applied for positive recognition of pus in such cases.

Vitali's Test.

Acidify the urine with acetic acid and filter. Treat the material on the filter with a few drops of tincture of guaiac, when a deep blue color will appear in the presence of pus. If the material is not filterable, as happens when the purulent material is extremely gelatinous, place a portion of this slimy urine in a test-tube and allow a few drops of tincture of guaiac to flow upon the surface. If pus be present a distinct blue line of contact will be observed.

Donne's Test.

A portion of the urinary sediment in a centrifuge tube is treated with a few drops of concentrated solution of sodium hydrate. If pus be present an extremely viscid gelatinous mass will be obtained. If this mixture be heated, it will dissolve, according to Müller, with the formation of β-nucleinic acid.

If the pus-cells be treated under the microscope with a few drops of Lugōl's solution, they will take a mahogany-brown color owing to the presence of glycogen.

Enumeration of Pus-cells.

Such a procedure is not a part of the ordinary routine examination of urine. It is, however, sometimes advisable, as it permits of a decision regarding the presence or absence of a true albuminuria. If the latter exists, casts and renal epithelial cells will usually be present so that a diagnosis is often possible without a count of the cells.

Technic.

A portion of the 24-hour specimen of urine is thoroughly shaken to bring the corpuscles into suspension. This turbid fluid is then drawn up to the upper (11) mark of the leucocytometer, a drop placed upon the glass slide and the cells counted as described under Blood. If more than 30,000 per cmm. are present, it is advisable to dilute five times with 3 per cent. sodium chlorid solution. For each 100,000 leucocytes per cmm. of urine 0.1 per cent. of albumin is assumed to be present, according to Wunderlich.

(4). **Red Blood-cells.**

The presence of red blood-cells in the urine is known as *hematuria*. This condition should be sharply differentiated from hemoglobinuria as the clinical significance is entirely distinct. Blood may be found in the urine in a variety of conditions. Thus in the more malignant types of the acute infectious fevers hematuria is frequently observed. Likewise, in scurvy, hemophilia, purpura, leukemia, and Werlhof's disease, the kidney may be so markedly affected that hematuria obtains.

In the hematuria of purely renal origin we find both acute and chronic congestions as well as inflammatory processes in the kidney associated with this condition. In the more acute types of nephritis, hematuria is so common that the name "hemorrhagic nephritis" is frequently applied. Such cases are especially observed after poisoning with cantharides and phenol derivatives. The chronic parenchymatous type of nephritis is, according to Weigert, always hemorrhagic in type, the number of red corpuscles being an indication of the intensity of the process. In malignant growths of the kidney, tuberculosis, renal calculus, and cystic degeneration of the kidney, hematuria is especially common; while in infection with certain parasites, such as the filaria, echinococcus, and the distoma hematobium, hematuria is relatively frequent although few cases of these conditions are seen.

Hematuria may also be observed as a result of lesions or disease of any portion of the urinary tract. Thus stone in the ureter or urethra, tumors, ulcers, and parasites of the bladder, urethritis, prostatitis or injury during catheterization may also be associated with the appearance of red cells in the urine.

A further type of cases in which hematuria occurs is known as the *functional or idiopathic hematuria*. In this class of cases no definite lesion has been found to account for the condition. It has been called "Gull's renal epistaxis," "essential renal hematuria," "angioneurotic hematuria," "renal hemophilia" and "renal aneurysm." The lesion, whatever it may be, is usually unilateral and the attacks appear at variable intervals. Some of the cases recover without any treatment after one or two profuse hemorrhages, while others require extensive treatment of the nervous system.

In the diagnosis of a hematuria it is important to observe the appearance of the urine both with the naked eye and with the microscope. The urine

is turbid, and varies from a light, hazy, "smoky" appearance to a bright-red or deep-brown color. The red cells appear in various stages of preservation. In some cases the normal yellow color of the cell will be quite distinct while in others the color will be entirely washed out. If the urine be particularly concentrated many crenated forms will also be observed.

The blood-cells may exist singly and scattered, or may be grouped in large masses forming distinct clots or adherent to tube casts forming the so-called blood casts. In true renal hematuria the blood is intimately mixed with the urine, the individual corpuscles usually appearing as pale shadows or "ghosts." In hemorrhage from the bladder the urine may show the presence of blood-clots of irregular form and size. If the two-glass test be applied, the second glass will contain the more blood, while in hematuria of renal origin both glasses will show equal amounts. In some cases clots of blood in distinct casts are seen. In the chronic parenchymatous nephritis clots are rarely present, while in malignant disease of the kidney clots are relatively common.

It is important in making a diagnosis from the presence of blood that extraneous sources of blood-cells be excluded. If the blood be of renal origin it will be associated with the presence of casts and epithelial cells while no such elements will be present from a hemorrhage lower down in the genito-urinary tract. Albumin will also be present in more or less amount. It has been stated that if the blood be derived from other than renal sources, the clear supernatant fluid in the centrifuge tube will be albumin-free. The writer has convinced himself that this is an error as he has practically always been able to obtain faint albumin reactions in cases of hemorrhage other than renal.

(5). Casts.

True casts are moulds of the uriniferous tubules. Their mode of formation is not entirely clear. Undoubtedly a colloid substance is thrown into the lumen of the tubule and later solidifies forming a distinct cast of that particular tubule. In this process of hardening the material may enclose cells of different types which are, also, present in the tubule. Whether this coagulable material is derived from the blood as a transudate, whether it be a secretion of the epithelial cells which have become pathologic, or whether it be material arising from degeneration of renal cells is not at present settled, the latter source being the more probable. In the urine we find true casts of the renal tubules, as well as pseudo casts which have nothing in common with the true type of these pathologic formations.

True Casts.

Hyaline Casts.

The true hyaline casts are pale, transparent, homogeneous cylinders with rounded ends. Their size may vary from a very small fragment to one several mm. in length. In diameter they may be narrow or broad. As a rule, little

difference is clinically made between these various types of hyaline casts, but the broader types seem to the writer to be somewhat more significant than do the narrower ones. The characteristics of the true cast are their cylindrical appearance, their sides being parallel and usually straight, although they may at times be observed in typical tortuous forms. They are never tapering at the ends, but may show an irregular outline at one or both ends, but the length and the parallel sides will usually differentiate them.

The pure hyaline casts are perfectly homogeneous and free from granules. Such types are, however, not frequently observed as very fine granules may almost always be detected embedded in the surrounding homogeneous material.

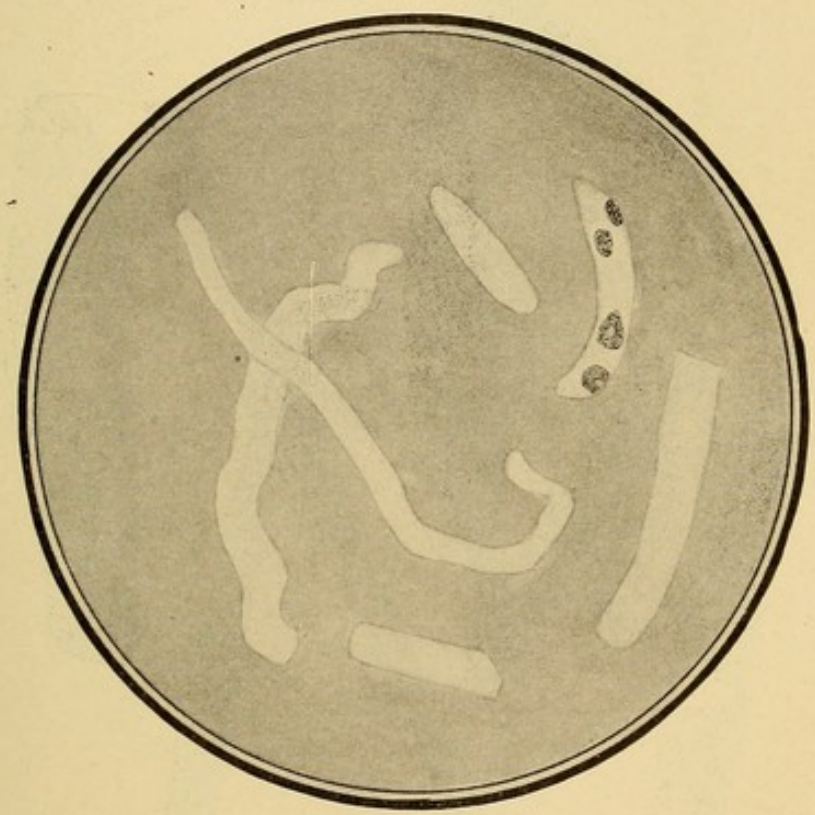


FIG. 106.—Hyaline casts. One cast is impregnated with four renal cells. (*Hawk.*)

There may be even inclusions of epithelial, renal, blood-, or pus-cells, so that the gradations between the pure type of hyaline casts and many of the other varieties are outlined with difficulty. It should be stated at this point that a distinction exists between hyaline casts with enclosures of cells to such an extent that the cast is named from the variety of cell present and the type of pseudo cast in which groups of such cells are massed so as to form an apparent cast, but which do not have any definite matrix. The true hyaline cast is soluble in acetic acid and may be stained yellow with Lugol's solution.

These hyaline casts are not always easy to find in the sediment. In examining the urine for the presence of casts the light should be shut off as much as possible and a low-power lens used. With the use of the high power the field is much limited and one is not so apt to observe the cast as with the

low power. It is, however, always advisable to examine a cast, first seen with the low power, under the high power, so that the decision may be much more definite as to whether the cellular elements are real inclusions or simply material resting upon the true cast. The same is to be said regarding the presence of granules in the hyaline cast.

Significance.

Regarding the significance of hyaline casts in the urine, it is to be said that they occur in any condition in which the kidney is altered by circulatory,

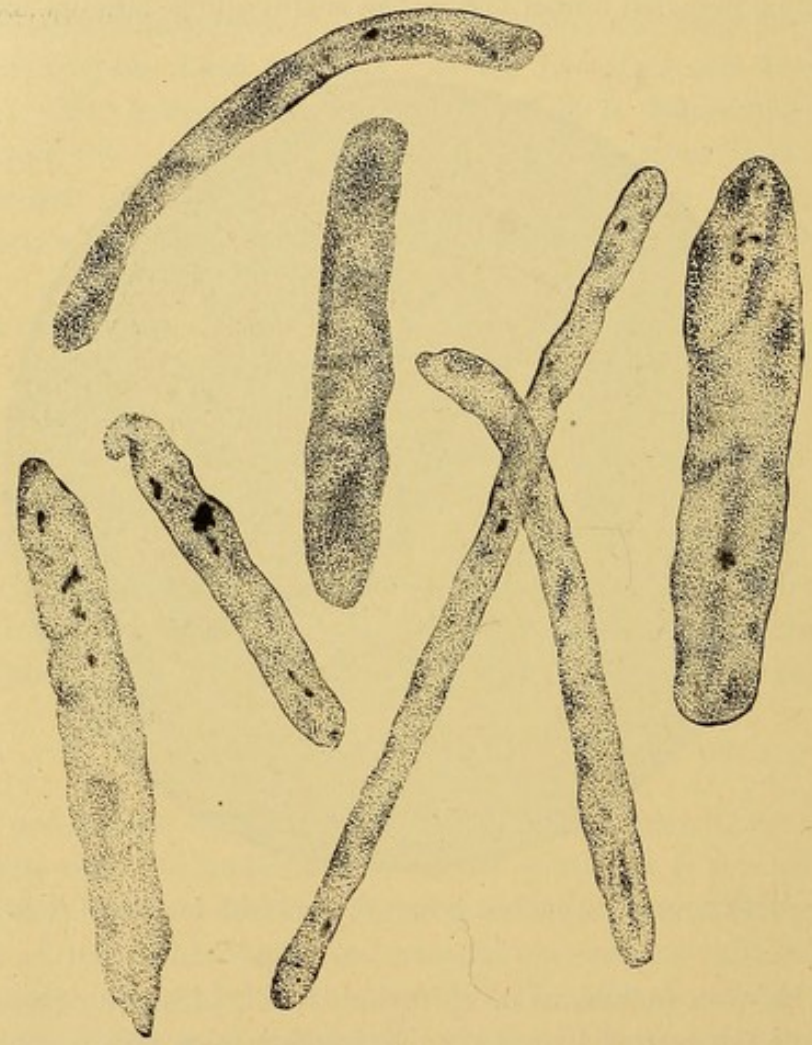


FIG. 107.—Granular casts, × 225. (*Tyson*)

toxic, or inflammatory disturbances. They are not pathognomonic of any one condition and may be found as a result of simple functional disturbance. A few hyaline casts may be found in practically every urine, providing sufficient search is made. Any undue strain, such as running for a car in one who is not used to such exertion, may be sufficient to add quite a number of hyaline casts to the urine. In many thousand urine examinations made in the writer's laboratory, it has been rather the unusual thing not to find an occasional hyaline cast. It would seem, therefore, that no significance what-

PLATE X



Waxy Casts Treated with Iodine. (*Tyson.*)

ever should be attached to the presence of an occasional hyaline cast. When, however, these casts become very numerous they should then be interpreted as meaning a disturbance of the kidney, although the absence of other types would rather speak against a marked pathologic change. In diabetes mellitus, "showers" of casts are especially observed preceding the appearance of coma (Külz).

Granular Casts.

These are modifications of the true hyaline cast in the sense that fine or coarse granules are found in the matrix of the hyaline cast. Several types of granular casts are observed. The granules may be very fine, very coarse, or may be distinctly composed of degenerated epithelial cells. The fine as well as the coarse granules are undoubtedly derived from the renal epithelium, which has degenerated completely. The coarser the granules the more severe the inflammatory process.

These granular casts vary in shape and in size, but are usually shorter than the hyaline type. To these granular casts may be attached various cells so that it is difficult to tell whether the cast is really a true granular or a cellular one. In some cases these cellular inclusions may undergo fatty degeneration giving a much higher refractility to the specimen. Not infrequently one observes hyaline casts which are distinctly granular in one portion while the other is perfectly homogeneous.

The so-called *brown granular casts* appear to be almost entirely degenerated epithelial cells, although the coloring matter is probably hemoglobin. The fact that the hyaline matrix cannot be distinctly made out does not argue against this type being truly hyaline in character, although the matrix is completely saturated with the pigment.

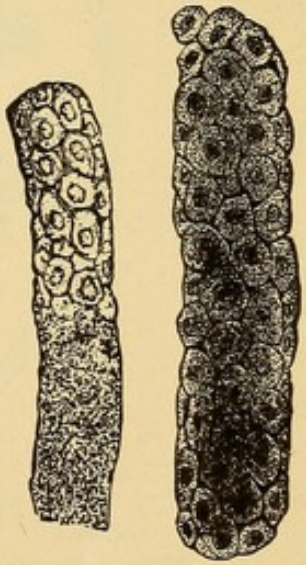


FIG. 108.—Epithelial casts. (*Hawk.*)

Waxy Casts.

This type is very refractile, transparent, and either perfectly colorless or showing a slight shade of yellow. Usually they are very long and broad and may be either straight or curved. The ends show a very distinct fracture while the cast itself may show a tendency to split transversely. Their appearance is, therefore, that of ordinary wax. They may have any type of cellular element attached and may show marked fatty degeneration. Some of these casts show the amyloid reaction while many of them do not.

Waxy casts were at one time believed to be pathognomonic of amyloid degeneration of the kidney. It is true that they do appear earlier in this type of kidney lesion, but it is to be remembered that they occur in all varieties of chronic kidney disease. They are usually of bad prognostic omen as they indicate a very advanced process.

Fibrinous Casts.

These are very highly refractile, transparent, and always of a yellowish or brown color. They may be granular and have various cellular inclusions. Their shapes vary as do those of the hyaline types and they show a tendency to become fractured, the fracture usually being ragged, while in the waxy cast it is sharp-cut.

Fibrinous casts usually appear in the acute renal conditions and disappear when this condition clears up. They do not have, therefore, the grave significance of the waxy cast and should be sharply differentiated. These casts are not composed of fibrin as their name would indicate, but are so called on account of their brownish color, which resembles fibrin.

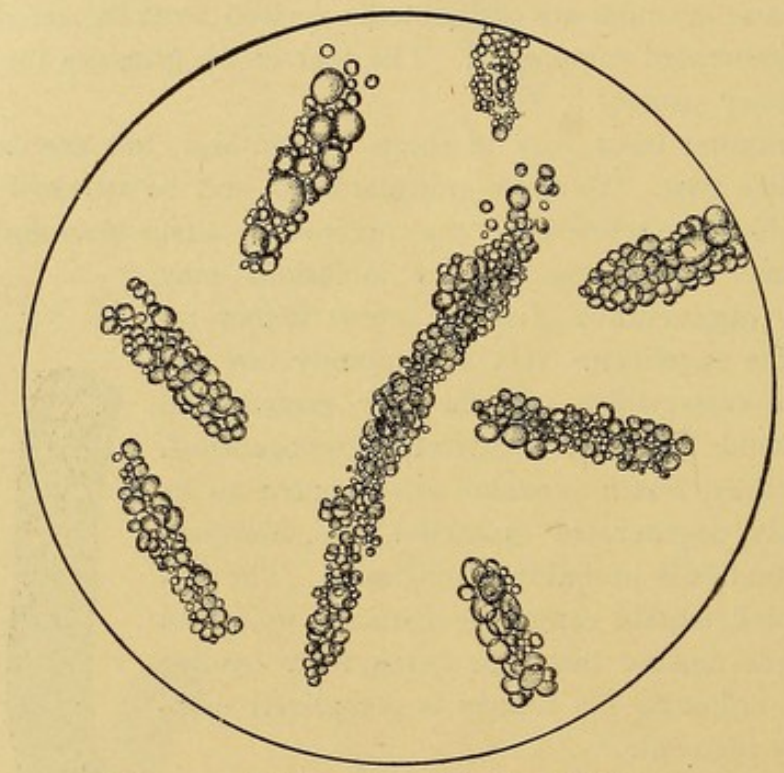


FIG. 109.—Fatty casts. (*Hawk after Peyer.*)

Epithelial Casts.

These casts are true hyaline casts which include so many renal epithelial cells that the hyaline matrix may be lost. For the name epithelial cast to be accurate it is not necessary that more than a few cells be present. The cells may be well preserved or show marked fatty or granular degeneration. The nuclei of these cells are round and vesicular so that they may be easily recognized.

Distinct gradations exist between the true epithelial cast and the coarsely granular and fatty cast. This type of cast is indicative of a severe destructive lesion of the kidney epithelium.

Fatty Casts.

These casts are masses of epithelial cells which have so markedly degenerated that little is recognized beyond the original outline of the cell and the

numerous fatty globules contained therein. They may be yellowish or black in color, the globules being soluble in ether and staining black with osmic acid or red with Sudan III.

Blood Casts.

These are casts including large numbers of blood-cells. The casts are formed within the tubules of the kidney, the cells occasionally being very pale. These casts indicate a serious advanced lesion of the renal parenchyma.

Pus Casts.

These like the other types of casts are true hyaline casts with enclosures of pus-cells. They are formed within the tubules of the kidney and usually



FIG. 110.—Blood, Pus, Hyaline and Epithelial Casts. (*Greene.*)
a Blood casts; *b*, pus cast; *c*, hyaline cast impregnated with renal cells; *d*, epithelial casts.

indicate an acute pyelonephritis. For the differentiation of these casts from epithelial casts it is advisable to add acetic acid to the sediment when the typical polymorphous character of the nucleus will distinguish the pus-cell from the epithelial cell with its vesicular nucleus. Moreover, the pus-cell is much more spherical than is the epithelial cell.

Cylindroids.

It is not infrequent to find in the urine formations which resemble the true hyaline casts to a marked degree. They, however, differ in the fact that at one or at both ends they taper off into a point which may be prolonged into a distinct thread. If, however, these ends are broken off, as may occur in

the centrifugation, it is a practical impossibility to distinguish them from a hyaline cast. They are both found in the urine under the same conditions and their significance is practically the same. From the chemical standpoint they appear similar to the hyaline casts, their origin, therefore, being presumably in the renal parenchyma. If these bodies are true mucin and are insoluble in acetic acid, their origin is more probably in the bladder.

A second type of cylindroid appears in the urine in the form of long tapering transparent shreds. They very much resemble ribbon which varies in diameter and may show under high power a distinctly fibrillar structure. These threads largely compose the nubecula. They are much longer than the hyaline cast

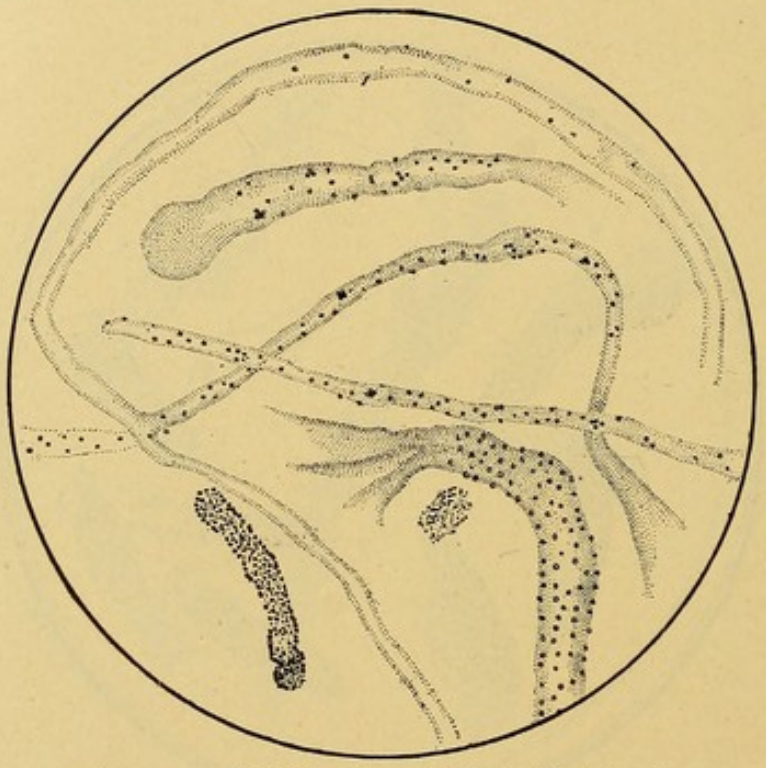


FIG. 111.—Cylindroids. (*Hawk after Peyer.*)

and considerably narrower so that confusion should not arise. In cases of gonorrhea one finds mucus shreds which may vary from a few mm. to 1 cm. in length and yellowish or pure white in color. In the meshes of these shreds one finds embedded large numbers of pus and epithelial cells. These should be sharply differentiated from the true cast by their larger size and typical mucoid character. Frequently they may be observed by the naked eye in large numbers.

Pseudocasts.

Not infrequently do we find in urine crystalline material arranged in masses much resembling casts. The most important of these are uric acid and the urates. It is true that any cast in a concentrated urine may become covered with urates so that the true nature of the cast becomes indefinite.



MUCOUS THREADS IN URINE. (UNSTAINED SPECIMEN.)

If the slide be warmed these pseudourate casts will disappear, while the true casts will remain. Masses of bacteria, pus-cells, epithelial cells and blood-cells may so group themselves as to closely resemble true casts. As a rule such masses will show irregular outlines and no evidence of a distinct matrix. Moreover, the use of an old slide upon which there may be many scratches should be avoided as the writer has seen several instances in which supposed casts were found to be due to such scratches.

Cylindruria.

This is the name given to the appearance of casts in the urine. As a rule, it should be said that the presence of a few hyaline casts is not of particular moment unless associated with other evidences of marked renal disturbances. While albuminuria and cylindruria usually go hand in hand, yet we do find cases in which one exists without the other.

It is undoubtedly true that casts indicate a disturbance of the renal epithelium. This, however, need not be anything more than disturbed nutritional or circulatory conditions. However, when the true hyaline casts are present in large numbers and when many other types of casts also exist, then a distinct pathologic lesion of the kidney must be assumed. As a rule, the granular types of cast are observed in the chronic processes, while the cellular forms are more usually present in the acute conditions. This rule, however, is not invariable, so that it may generally be stated that no type of cast is pathognomonic of any single condition. In this connection we should remember that recent work, especially that of Cabot, has shown that it is unwise to base a diagnosis of a kidney lesion upon the finding even of both albuminuria and cylindruria. So much discrepancy was shown to exist between the urinary and autopsy findings that one must remain in doubt as to whether it is possible to make a definite diagnosis unless clinical symptoms other than urinary are made the basis of a diagnosis. On the other hand, some of the most typical cases of nephritis, as shown post-mortem, gave absolutely no indication in the urine that such condition existed. We are, therefore, face to face with the proposition that urinary examination must be in any case simply one of the diagnostic links. This fact is of special importance in life insurance examinations, as most companies absolutely refuse insurance to one who has ever shown albumin or casts in the urine. This would seem to the writer not only very short sighted, but based upon an absolutely erroneous idea of the importance of albumin and casts in the urine of one who showed absolutely no clinical signs of renal involvement. Remembering that albumin and casts may not appear, even though the kidney be seriously affected, it would seem just as plausible to refuse life insurance because these substances were not present. In this connection the writer would say that only when the urine is considered as a whole may definite conclusions be made regarding any type of renal disease. The clinician, who is thoroughly familiar with the course of the case, is the only one capable of interpreting the findings of the laboratory, so that it should be an unvarying rule for a laboratory

worker to avoid diagnostic remarks unless he is thoroughly *en rapport* with the patient. The writer does not wish to be interpreted as stating that a diagnosis of renal disease may never be made from an examination of the urine, but he wishes to impress upon his readers that both albuminuria and cylindruria may occur without direct kidney disease or may not appear when such is present.

(6). **Spermatozoa.**

Spermatozoa are frequently observed in the urine of healthy adults, especially after intercourse or nocturnal emissions. In females they may also be observed as an evidence of intercourse, which fact is of some importance in cases of suspected rape.

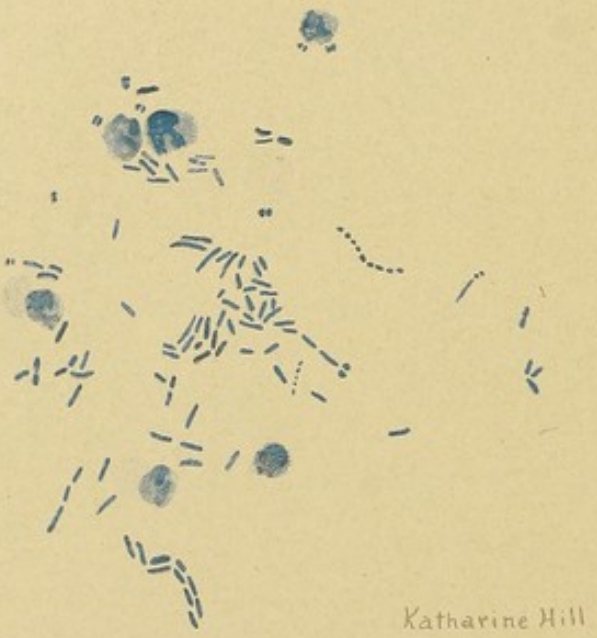
Pathologically, they may be found in cases of marked constipation, when the pressure of the impacted feces upon the seminal vesicles may induce an emission. In occasional cases of cystitis, associated with stricture, these bodies may be observed as reported by Simon. In cases of epilepsy and hysteroepilepsy, as well as in spinal disease following vertebral fractures and dislocations, spermatozoa are not infrequent. Masturbation and venereal excess frequently lead to almost constant *spermatorrhea*. Their occurrence in cases of prostatitis will be discussed in the section on Semen.

(7). **Tissue Fragments.**

It is not infrequent to find shreds of tissue in the urine, which may throw some light upon a pathologic condition. In cases of carcinoma of the bladder, more rarely of the kidney, true malignant tissue may be obtained, which may permit of a tentative diagnosis, although the material is usually too necrotic to make an absolute diagnosis possible, the principal finding being that of elastic tissue.

(8). **Bacteria.**

It should be stated in the beginning of this discussion that an examination of the urine for bacteria should be made only upon specimens obtained with the greatest possible precaution and preserved in absolutely sterile vessels. Soon after the urine is voided, especially if it remains in contact with the air, large numbers of saprophytic organisms may be found which, of course, did not exist in the original urine. In obtaining a specimen from the male it is not always necessary to catheterize the patient. If the surface of the glans and the orifice of the meatus be carefully washed with bichlorid solution followed by sterile water and the first portion of the urine voided be thrown away, the last portion may be collected in a sterile vessel and later put in work. With female patients, however, it is absolutely essential that catheterization be performed. The external genitalia and especially the orifice of the urethra are well washed with green soap and water. The opening of the urethra is then dried with sterilized cotton pads which are soaked in boracic acid. A sterilized glass catheter, whose external end is covered with a rubber tube about four inches long and large enough to fit loosely over the catheter is



CYSTITIS DUE TO COLON BACILLUS. (METHYLENE BLUE STAIN.)

then inserted, care being taken that it touches only the orifice of the urethra. The urine is allowed to flow freely for a short time when the last portion is collected in a sterile vessel, the rubber tube being previously removed. (Kelly). Cultures are then made from the urine and the remainder centrifuged in a sterile closed tube in order to throw down any bacteria which may be present. It is sometimes advisable, in order to diminish the specific gravity of the specimen, to add an equal volume of 95 per cent. alcohol and centrifuge the mixture. Practically all of the bacteria present will then be found in the sediment.

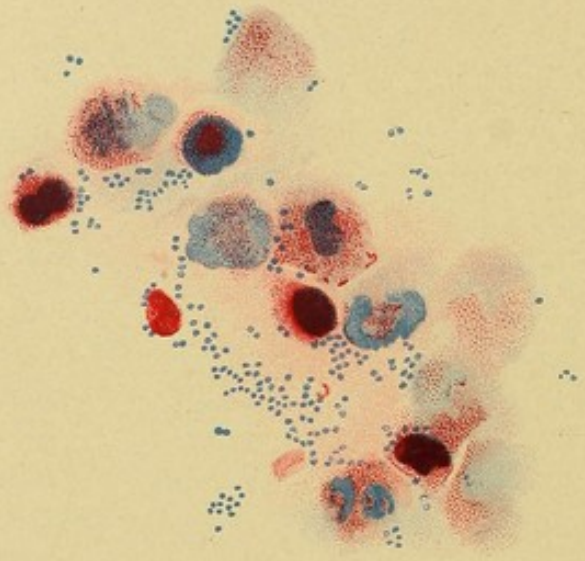
The supernatant fluid is then removed by quickly inverting the tube and allowing the fluid to run out. The sediment, by this manipulation, will usually remain in the smaller portion of the tube. Smears are then made upon a glass slide and dried first in the air and then over the flame. It is not always the simplest matter to prepare smears which will remain after treatment with the staining solution, as the urea and salts of the sediment may be removed by the washing and carry with them the bacteria. If pus-cells are present, satisfactory smears are usually obtained; but if such conditions do not exist it is advisable to add a solution of egg albumin to the sediment before drying over the flame.

The methods of staining the sediment for the various bacteria will depend entirely upon the organism supposed to be present. As a rule, a preliminary examination is very satisfactorily made by treatment with Löffler's methylene-blue solution, which stains practically all organisms. If the tubercle bacillus is suspected it may be detected in exactly the same way as outlined under Sputum by staining with carbol-fuchsin solution. Should one suspect the presence of the gonococcus this may be stained, as described in the next section, by Gram's method. Outside of these two types of organisms, it is almost impossible to differentiate the bacteria of the urine by staining methods. The peculiarities of the various bacteria upon culture media may be learned from works on bacteriology.

If the urine be collected with the precautions mentioned above, any organisms found must be attributed to their presence in the urine as voided. In this connection we must remember that the presence of the tubercle bacillus *does not necessarily indicate* tuberculosis along the genitourinary tract. Tubercle bacilli are found in the urine in cases of miliary tuberculosis and have been reported in pulmonary tuberculosis, although it is more frequent to find them as evidences of local tubercular conditions. Of course, if large numbers of pus- and blood-cells be present along with tubercle bacilli, the diagnosis is usually certain. A word of caution is, however, necessary at this point. The smegma bacillus grows in abundance on the external genital organs and its morphological and staining characteristics may closely resemble those of the tubercle bacillus. If the proper precautions be observed, as they should be, no differentiation is necessary, bacilli showing the true morphological and staining character of the tubercle bacillus can only be this organism, as extraneous bacteria have

been avoided. In the clinical laboratory, however, one may never be sure whether the proper precautions have been taken, so that absolute methods of differentiation (see sputum) should be part of the technic unless the worker absolutely knows that contamination was avoided. A second point regarding the tubercle bacillus is that it may not be found by microscopic examination even after repeated attempts. Under such conditions the wisest course to pursue is the inoculation of a guinea-pig with the washed urinary sediment. The obtaining of the urine must in this case be absolutely accurately done by observing every precaution to prevent contamination. The reason for this is not because contaminating organisms will cause lesions similar to those of the tubercle bacillus, but because such secondary invaders may so infect the animal that death results from causes other than those for which we are looking. The washed sediment is injected intraperitoneally and the animal kept under observation for three weeks to one month unless death results previously. At the end of this time the animal is killed and a postmortem examination made for evidences of tuberculosis. The retroperitoneal glands, spleen, and liver are the especial organs to show such lesions. These organs should be sectioned, portions run through the regular pathologic routine, and sections examined microscopically. A finding of tuberculosis in this way is unequivocal and is the quickest way in the long run of making a positive diagnosis, although a single examination may show the presence of tubercle bacilli in the urine, but rarely such is the case.

Having found the tubercle bacilli in the urine, we are confronted with the question of the part affected. As a primary tuberculosis of the bladder is rare we may usually assume the seat of the difficulty to be the kidney, although if evidences of cystitis be present a combination may exist. As the symptoms of genitourinary tuberculosis are frequently vesicle in origin, a kidney lesion may not be suspected, but should be assumed until the contrary is proven. Thanks to the introduction of methods of cystoscopic examination and especially ureteral catheterization, we are in a better position to make a positive diagnosis of renal tuberculosis and exclude that of bladder origin. An interesting point regarding tuberculous cystitis is that the urine, although frequently containing large amounts of pus, is practically always acid in reaction. Moreover, this pus is frequently sterile in tubercular cystitis. It is not the province of the writer, at the present time, to outline methods of differential diagnosis of various conditions; he will refer, therefore, to works on genitourinary diseases for the various types of cystitis and their clinical differentiation. Suffice it to say at this point that cystitis may be an ascending or a descending one and should always be correlated with the associated condition. It is usually a simple matter to determine the presence of the gonococcus in the urethral discharge, but it is far from easy to demonstrate this organism in the case of gonorrheal cystitis. Under such conditions the symptoms and the association with an existing gonorrhea would furnish the decisive clue.



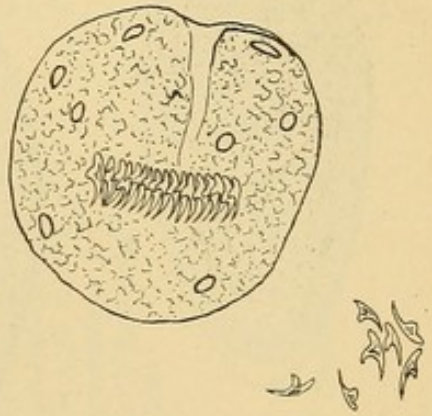
Katharine Hill

STAPHYLOCOCCUS CYSTITIS. (LEISHMAN STAIN.)

Bacilluria.

By this condition is meant the presence in the freshly voided urine of so many organisms that the urine is distinctly cloudy. These organisms are usually those associated with a mild cystitis or may be those of an existing general infection. In cases of persistent bacilluria, we may find a true renal origin, which is largely associated with the presence of the typhoid and colon bacillus. As has been well established, the typhoid organism may be excreted for months after the patient is convalescenent so that it becomes necessary to use strict measures to disinfect all urine of typhoid patients. The colon bacillus is at present assuming so much importance in clinical work that cases are being almost daily recognized which may be directly traceable to the colon bacillus and not to the typhoid as usually assumed. It is necessary, therefore, for the laboratory worker especially, and, where possible, for the general practitioner to be able to recognize each of these organisms when present either in the feces, urine, milk, or water-supply (see Feces). A second general class of cases associated with bacilluria are those of urethritis and prostatitis. Usually there is a secondary cystitis arising from the same organism or, at least, the resistance of the bladder has been so far reduced that these organisms which find their way into the bladder develop profusely therein.

Regarding the presence of the various bacteria which may be found in the urine after it has been exposed to the air, the writer cannot take up space for their description. Their number is extensive and their type protean. Reference must be made to general works.



Katharine Hill

FIG. 112.—Scolex and hooklets of tænia echinococcus in urine.

(9). **Parasites.**

Various types of parasites are observed in the urine. Thus the trichomonas vaginalis has been found by Künstler, Miura, and Dock. Amebæ have been found by Bälz, Jürgens, Wijchoff, and by Musgrave and Clegg. Various portions of hydatid cysts are frequently observed, among which we find the echinococcus hooklets and fragments of membrane. Nematode worms, especially the filaria sanguinis hominis, are present in cases of chyluria, while the anguillula aceti or "vinegar-eel" has been reported, especially by Stiles, while Billings and Miller report its presence as a possible contamination from the bottle in which the urine was collected.

Eggs of the schistosomum hæmatobium are not infrequently observed together with large numbers of red cells in cases of bilharziasis. This worm as well as its ova will be discussed in the section on Blood. Stuertz has reported the findings of the egg of eustrongylus gigas in the urine in a case of chyluria.

V. Calculi.

Concretions of a more or less hard and dense character are prone to form in the urinary passages. These bodies are termed, according to their size and location, sand, gravel, stone, and calculi. Theoretically, these formations may consist of accretions of any of the various crystalline or amorphous sediments previously mentioned, the type of stone depending upon the reaction of the urine to a large extent.

These calculi are formed by the deposition of the crystalline material around a definite nucleus, which usually consists of organic material, such as fibrin, blood, desquamated epithelial cells, mucus, or even a crystal of uric acid or calcium oxalate. It is very difficult to decide as to the reason for the

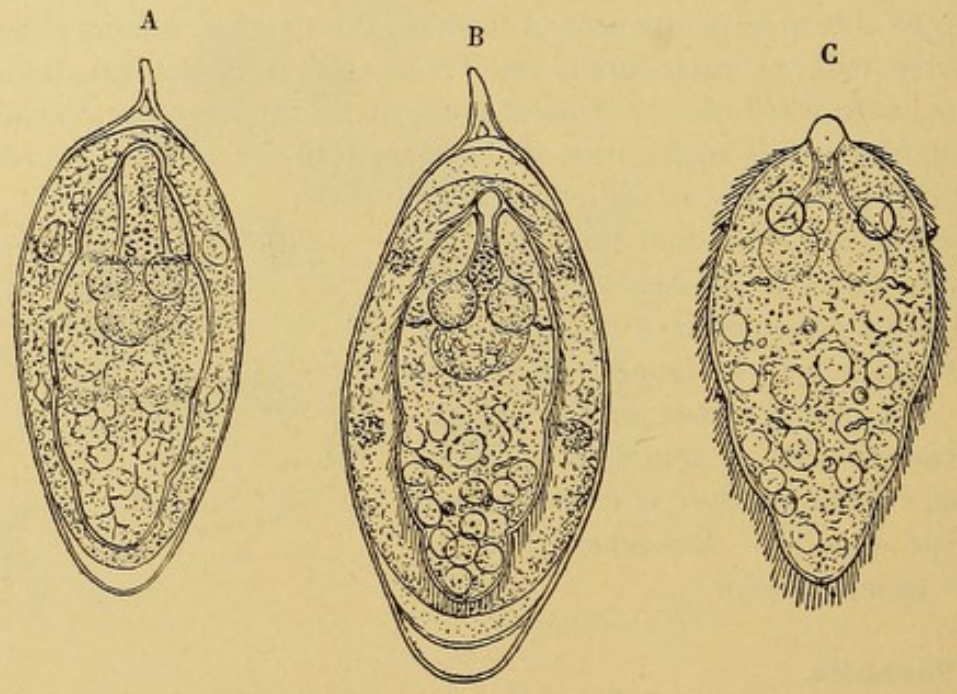


Fig. 113.—Ova and miracidium of schistosomum hematobium, × 300: *A*, Ovum as seen in urine; *B*, the same after addition of water; *C*, miracidium. (*Tyson after Railliet*)

deposition of this material in the form of a renal stone. The growth of the calculus takes place by accretion, the deposition of successive layers of material occurring around the original nucleus. The material of which the stone consists will usually be of one kind, so that we speak of uric acid or phosphate calculi, for instance, while, occasionally, mixed calculi may be formed by the deposition of two or more chemical combinations.

The classification of urinary concretions is based on the chemical constituents of which they are composed. Before examining a calculus chemically a thorough optical examination should be made, as this may give a definite clue as to its composition. After this preliminary examination, the calculus is ground to a fine powder and examined according to the following table of Heller:

HELLER'S TABLE FOR EXAMINATION OF URINARY CALCULI.

On Heating the Powder on Platinum Foil it

DOES NOT BURN.				DOES BURN.				
The powder when treated with hydrochloric acid				With flame.			Without flame.	
Does not effervesce.			Effervesces.				Does not give the murexid test. The powder dissolves in nitric acid without effervescence. The dried yellow residue becomes orange on addition of alkali, red if heat be applied.	The powder gives the murexid test.
The gently heated powder with hydrochloric acid		Effervesces.						The powder when treated with potassium hydrate gives
The powder when moistened with a little potassium hydrate								
Abundant ammonia. The powder dissolves in acetic or hydrochloric acid. This solution gives a crystalline precipitate with ammonia.	No ammonia, or, at least, only traces of it. Powder dissolves in acetic or hydrochloric acid. This solution gives an amorphous precipitate with ammonia			Flame yellow and continuous. Odor of burned feathers. Insoluble in alcohol and ether. Soluble in potassium hydrate on heating. Precipitated from alkaline solution by acetic acid with generation of hydrogen sulphid.	Flame pale yellow, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether.	Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, from which six-sided plates separate on the spontaneous evaporation of the ammonia.		A strong ammonia reaction. / No noticeable reaction for ammonia.
Triple phosphate mixed with an unknown amount of earthy phosphates.	Magnesium and calcium phosphates.	Calcium oxalate.	Calcium carbonate.	Fibrin.	Urostealith.	Cystin.	Xanthin.	Ammonium urate. / Uric acid.

Uric Acid Calculi.

These are, perhaps, the most common renal stones. They are not always composed of pure uric acid, but are made up of a mixture of this substance with the urates. They are always colored, usually yellowish or brownish, but may at times appear distinctly red. They are fairly hard and usually show a rough irregular nodular surface, although at times this may be smooth. They fracture very easily and show, on cross section, a distinctly laminated structure, the layers frequently being of different colors, in some cases even being composed of deposits other than uric acid. If heated on a platinum foil they are combustible, burning without a flame. They give the murexid test and do not liberate appreciable amounts of ammonia on treatment with sodium hydrate.

Ammonium Urate Stones.

The ammonium urate calculi occur rarely in the adult. They are small, yellow and very soft, being distinctly clay-like and easily powdered when dry. These stones give the murexid test and also give a strong reaction for ammonia on treatment with sodium hydrate.

Calcium Oxalate Stones.

Next to the uric acid calculus the oxalate stone is most frequently met. The smaller types of these calculi are practically colorless and have a smooth surface, while the larger ones are grayish, brownish, or even black in color and have a rough nodular surface with sharp projecting angles. These stones frequently cause severe hemorrhage and much irritation when passing through the ureter and urethra. From their appearance they have been called the mulberry calculi. They are, perhaps, the hardest of the urinary stones.

These calculi are insoluble in acetic acid, but soluble in hydrochloric acid without effervescence unless the powder is previously heated. It is rare to find these stones perfectly pure, admixtures with various other sediments leading to distinct concentric arrangement as shown on fracture.

Phosphatic Calculi.

Stones composed of pure alkaline phosphates or triple phosphates are exceedingly rare. Usually the phosphatic calculi contain admixtures of ammonium urate, calcium carbonate, and calcium oxalate. The color of such stones may range from a white through yellow to some with distinct reddish tones. They are frequently of very large size, especially when formed in the bladder, are of a chalky consistency, and show a rough surface.

These calculi are soluble in hydrochloric or acetic acid, such solutions giving reactions both for phosphoric acid and the alkaline earths.

Calcium Carbonate Calculi.

Such stones are exceedingly rare. They are small in size, are distinctly chalk-like in consistency and color, and have a smooth surface. If treated with acid, carbon dioxid is evolved.

Cystin Calculi.

These stones are white or pale yellow in color, have either a smooth or irregular surface, and are soft and waxy in consistency. They vary in size occasionally being found as large as a hen's egg, although those of true renal origin are about the size of a pea. The formation of such calculi and their passage through the ureter and urethra constitute practically all of the untoward symptoms shown by subjects affected with cystinuria.

Such stones burn readily if heated on a platinum foil, giving off a peculiar sharp odor. The powder is soluble in ammonia from which the characteristic hexagonal plates separate on allowing the ammonia to evaporate.

Xanthin Calculi.

These stones occur especially in children, although even here they are very rare. They are usually light brown in color, moderately hard, and vary in size from that of a pea to a tennis-ball. On cross section they appear amorphous and if rubbed take a polish much resembling that of wax. The powder shows the typical reaction for xanthin previously outlined.

Urostealith Calculi.

These masses consists of fat, calcium and magnesium soaps, and cholesterin. They are usually soft and may be somewhat irregular in shape. This material burns with a pale yellow flame giving an odor of resin. The dry powder is soluble in alcohol and ether, from which rhombic notched plates of cholesterin separate on evaporation.

VI. Functional Diagnosis.

It is usually of great importance, especially in cases in which surgical intervention is contemplated, to know just exactly what the functional capabilities of the kidney are. If one kidney is to be removed, the question arises as to whether the remaining kidney can sufficiently accomodate itself to the increased work which must be put upon it.

As such a variation has been occasionally found between the results of the chemical and microscopical examination of the urine on the one hand and the pathologic condition shown in the kidneys postmortem on the other, an attempt has been made to find delicate tests by which a true renal lesion might be indubitably determined and thus permit of an absolute diagnosis even though the urinary findings were or were not conclusive. It is granted that a more or less severe lesion of the kidney may exist and yet its functional capacity be almost normal. If this functional capacity can be determined by tests which are more or less simple, it is evident that such methods should form part of the daily routine of the practitioner.

Before discussing some of these tests, which have been advocated for estimating the functional activity of the kidney, the writer would say that none

of them has proven entirely satisfactory. If we are to regard nephritis as a constitutional condition with secondary renal manifestations, it is self-evident that such tests can show only the manner in which a normal or pathologic kidney reacts toward certain substances and can have little reference to the primary etiologic factors.

Cryoscopy.

The method of determining the freezing-point of a solution is one of the most delicate of those of physical chemistry. As it is based upon the principle that substances in solution lower the freezing-point of the solvent in direct proportion to the molecular or ionic concentration of the solution, this method serves as a ready means of determining the molecular weight of a substance as well as the molecular concentration of a solution. For a successful outcome of a cryoscopic determination, the most assiduous attention to detail must be paid, so that this method certainly can find no place in the hands of the general practitioner or even in those of the laboratory worker, who has not been especially trained along these lines. Such being the case, it is absurd to expect that slight variations in the freezing-point ($\triangle$) of such a complex mixture as the urine can yield any valuable information, especially when one remembers that fluctuations wider than those shown under pathological conditions may be noted as a result of not observing such slight details as that of constantly agitating the urine during the cooling and freezing. The normal freezing-point of the urine varies between —0.9 and —2° C., an increase being known as hypersthenuria and a decrease as hyposthenuria.

As will be seen in the discussion of this subject in the section on Blood, the results obtained by this method have been far from satisfactory, as nothing of distinct diagnostic value has as yet been derived from comparative studies by various workers. It would seem to the writer, therefore, that for the present this method would better be left to the research worker than to be adopted by the general or special student, who should make use of methods which will yield results of more immediate value to him. For these reasons the writer must refer to other works for a detailed description of the method.

Electric Conductivity.

The remarks made later in the section on Blood regarding electric conductivity are especially applicable to the urine. This test is altogether too delicate to be applied to such complex fluids as the urine with the hope that slight variations in the conductivity will show anything of importance. As the electric current is conducted only by dissociable compounds, this method can show absolutely nothing regarding the excretion of the nondissociable organic substances. Such being the case the writer can see no reason for resorting to such delicate procedures as the determination of the conductivity of the urine when no attempt is made, as may be observed in many of the experiments reported, to control the intake of the inorganic substances which would affect the conductivity of the urine. A little more attention to ordinary methods

of chemical examination, with especial regard to ascertaining the intake and output of the patient, would, in the writer's opinion, yield much more valuable information than could be obtained by the rather uncertain urinary manipulations with the method of Kohlrausch.

Chlorid Excretion.

By the determination of the chlorid excretion in a given case under the influence of a specific intake of sodium chlorid it was hoped that some information might be derived as to the "glomerular sufficiency" of the kidney. This test, known as *alimentary chloruria*, advanced by Claude and Mantè[1], has like the above yielded little of value. The exceptions to the general rule of pathologic retention are too numerous to permit of absolute differentiation. While it has been shown by Widal that a retention of sodium chlorid does occur in nephritis and that a restriction of the salt in the diet frequently does alleviate the edema and albuminuria, yet many factors not well understood frequently give rise to opposite results (see Chlorids in Urine). The general consensus of opinion regarding this test may be summed up by the statement that the chlorid excretion in cases of health and disease is of no distinct value in diagnosing the functional activity of the kidney.

Methylene Blue Test.

It was hoped by the introduction of such tests that the capability of the kidney to excrete substances present in the blood could be determined by the ease with which artificially introduced substances were eliminated. Achard and Castaigne administer by the mouth 0.1 gram of methylene blue in a capsule or, preferably, 0.05 gram subcutaneously. This substance is excreted by the kidneys in the form of a colorless chromogen in 15 to 30 minutes after injection. This chromogen may be converted into the colored pigment by heating with acetic acid. In about five minutes after the excretion of the chromogen, the urine becomes greenish-blue from the excretion of the pigment itself. Under normal conditions the excretion reaches a maximum in from three to four hours and may last two to three days. If the pigment is not excreted within one hour after subcutaneous injection, pathologic conditions are supposed to exist.

In some cases of chronic interstitial nephritis it was found that such injections were not followed by an excretion of the pigment until one to three hours had elapsed. This would be a valuable diagnostic method had it not also been shown conclusively that many cases of advanced interstitial nephritis show the same excretion of this pigment as normal individuals. Such being the case the test is of no more use than many clinical and routine urine methods. Herter grants that a delay may mean disease, but also shows that some patients have periods of normal output. The variations do not appear to be sufficiently marked, so that it is unreasonable to judge, of the permeability of the kidney to normal or abnormal constituents, from the excretion of such an abnormal substance as methylene blue. On the other hand, methylene blue, for reasons

[1] Arch. gén. de méd., Tm. 8, 1902, p. 129.

little understood, may be entirely destroyed in the body and none of it reach the urine. In such cases it would be rational to suppose that a person, although really perfectly normal, was in a most deplorable condition, if reliance were placed upon such a test. The writer has, therefore, no hesitancy in branding this test as impracticable under the present conditions.

Phloridzin Test.

This test is based on the assumption that phloridzin normally gives rise to a glycosuria through distinct alterations in the renal cells. In other words a so-called "renal diabetes" is set up. The technic is as follows: One c.c. of a 1:200 solution of phloridzin is injected subcutaneously. The urine is tested at intervals of 15 minutes for the appearance of sugar. Normally, sugar may be detected in from one-half to one hour and may be present for as long as five hours. The quantity eliminated may vary from 0.5 to 3 grams of glucose. In nephritis the sugar is usually absent or below 0.5 gram.

This test does not distinguish, of course, the various types of nephritis, yet it does usually indicate that renal activity is disturbed. It would seem to the writer to be the only one advanced which can in any way aid a diagnosis, but it is to be remembered that the important principle of differentiation is absent. Other clinical evidences are usually present pointing to a nephritis so that this test becomes merely confirmatory.

For the medical man these tests are rarely of value. "For surgical conditions the case is very different, as Emerson puts it, the question is the justification of removing a diseased kidney. In such a case the first question is the presence of another kidney; the second is, can this second kidney do the work of both? To decide these questions, if by means of ureteral catheterization we can separate the urines excreted at the same time, a comparison of these is of value in determining, first, the presence of the second kidney; second, the relative values of their activity; while the freezing-point of the blood will determine their united insufficiency. The chief difference between these two points of view may be that surgical cases are chiefly of renal disease which destroys all renal function, while among the medical cases there are so many in which all the functions which can be tested are normal, but that unknown but all important one, failure of which means uremia, escapes detection."

In deciding as to the value of the above tests of the functional diagnosis of the kidney, it is to be said that little of value is, at present, determined by such methods. At any rate, no one of the tests alone is sufficient, and when attempts are made to confirm one by another method, the results are frequently diametrically opposed. We are , therefore, in no better position than before, as it is impossible to decide which of the two tests is reliable. The above statements of Emerson seem to the writer to fit the point exactly. For medical men these tests are of no value, while for surgeons who undoubtedly will operate, utterly regardless of the results of any of these tests, providing the clinical symptoms seem to them sufficient, the results are undoubtedly of

value, as they indicate what has already been determined upon in their own mind, namely, operation.

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CHAPTER VII.

SECRETIONS OF THE GENITAL ORGANS.

I. Male Secretions.

General Considerations.

The normal secretion of the male generative organs is known as semen and is a mixture of the secretions of the prostate gland, the glands of Cowper, the testicles, and the seminal vesicles. It is a practical impossibility from the clinical standpoint to separate the different elements of the semen, so that this must be discussed as a whole.

The semen is a white or slightly yellowish, somewhat thick and viscid liquid with a peculiar odor, somewhat resembling fresh glue, and showing a neutral or faintly alkaline reaction, a nonhomogeneous milky appearance, and a specific gravity greater than that of water. It is composed of semisolid material in the form of white masses floating in a limpid liquid and holding in suspension specific elements, derived from the secretory glands of the genital apparatus and from the desquamation of the various canals through which the semen passes.

Semen contains about 6 per cent. of organic and 4 per cent. of inorganic matter. Its chief chemical characteristic is the presence of spermin $(C_2H_5N)_2$, which is at least isomeric, if not identical, with diethylen-diamin, according to Ladenburg and Abel. This spermin, which is derived largely from the prostate gland, combines with the phosphoric acid radical to form spermin phosphate, which crystallizes in the form of four-sided spindles or prisms which may appear as flattened needles. In some cases these crystals resemble very closely the diamond-shaped double pyramids known as Charcot-Leyden crystals, which are found in the sputum. They are, however, of a different crystalline group and are soluble in formalin, while those found in the sputum are insoluble in this menstruum. These spermin crystals are known as Böttcher's crystals. Miescher has studied the composition of the heads of the spermatozoa and has been able to isolate certain bodies, known as protamins, which are the simplest type of protein material.

Microscopic Examination.

The most important and characteristic constituent of semen are the spermatozoa. These sexual elements consist of an anterior broader portion or head and a narrow thread-like tail. The former is oval or egg-shaped and measures about 5 microns in length, 4 in breadth, and 2 in thickness. Just behind this pyriform head is a short cylindrical portion measuring 6 microns in length,

which is known as the middle piece. This tapers somewhat to the point of union with the tail. This so-called tail is a thread-like posterior portion and is approximately 45 microns in length. In the freshly voided semen these tail portions show active undulatory whip-like motions, which persist for 24 to 48 hours, and even longer under proper conditions, and enable the spermatozoa to progress from point to point. Alkalies seem to favor this movement, while dilute acids inhibit it very rapidly. This movement of the spermatozoa is closely associated with their sexual activity, as the cells showing no movement

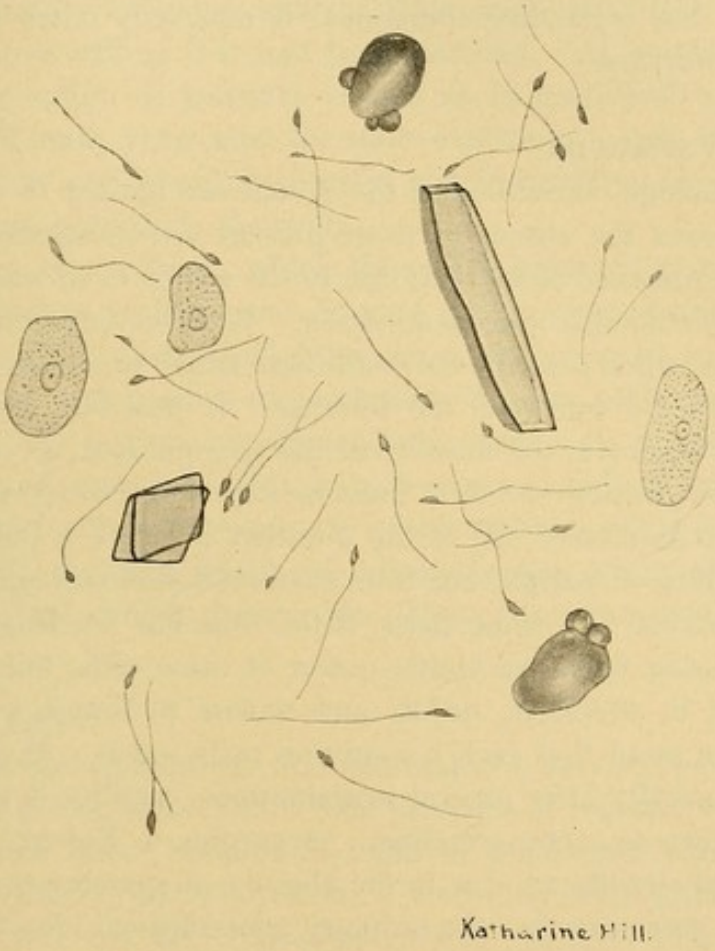


FIG. 114.—Normal semen.

when freshly voided may be regarded as possessing no functional power. For a discussion of spermatogenesis as well as of fertilization of the ovum the writer would refer to the admirable description of McMurrich.[1]

Besides these characteristic portions of the semen, large numbers of lecithin globules are seen, which give the milky appearance to the fluid. Especially to be noted among the cellular elements are the so-called corpora amylacea which resemble very closely starch granules, having concentric striations, a finely granular center and occasionally a nucleus. These cells take a distinct blue color on treatment with iodin solution. Moreover, various epithelial

[1] The Development of the Human Body, Philadelphia, 1907.

cells are observed, which are derived from the several glands contributing to the composition of the semen. Some of these cells are distinctly granular, some contain fat globules and very closely resemble the colostrum corpuscles of the lacteal secretion, while some of the granules resemble myelin. In rare cases cylindrical casts are seen which simulate the hyaline casts of the urine, but as a rule they are larger and longer. These are supposed to be derived from the prostate gland and seminal vesicles. If the semen be allowed to stand for a few minutes, several types of crystal may be observed, especially the phosphate of spermin, ammonium-magnesium phosphate, fatty acids, and oxalate of calcium. This last crystalline component is especially noted in the urine in cases of spermatorrhea.

Pathologic Variations.

Direct pathologic variations in the semen are limited to two conditions. Either spermatozoa are absent or those present are nonmotile. The determination of the presence of spermatozoa in the semen or in suspected stains is a matter of simple microscopic examination. With the question of the motility of such elements, when present, the conditions under which the examination is made may markedly influence the findings. If possible, semen should be examined, with regard to the motility of the spermatozoa, as soon as ejected, but if such is not possible the fluid must be kept warm until examination may be made. It is absolutely out of the question to make a positive diagnosis of true nonmotility of spermatozoa from examination of specimens, which have been allowed to cool. In some cases, if the time has not been too extended, warming may bring back the motile power of these cells, but in many cases it does not. It is, therefore, unjust and unwise to brand a man as sterile without absolute proof that such a condition really exists. If no spermatozoa are present, especially after several examinations, sterility is absolute. This condition is known as azoöspermatism. According to Kehrer, 40 per cent. of cases of conjugal sterility are due to the absence of spermatozoa in the semen. It is, therefore, necessary that the ordinary gynecological idea that women are the responsible factors in the family sterility should be, at least, partly borne by the man, as it is unjust to the woman to blame her for faults existing in the husband. The writer does not wish to be interpreted as stating that sterility does not frequently exist in women, but he does desire to emphasize the point that many more men are sterile than is generally supposed and that the sterile women are in this condition largely through the results of gonorrheal infection through their husbands.

Spermatozoa may be absent from the semen during convalescence from acute febrile conditions, valvular heart disease, and in general conditions associated with lowered nutrition. On the other hand, the constant presence of spermatozoa in the urine, as well as in the semen, may be noted as a result of various pathological conditions as well as of venereal excesses or masturbation. To this condition is given the name of spermatorrhea.

Medicolegal Aspects.

Not infrequently the physician is called upon to decide whether certain stains are due to spermatic fluid or whether assault has been committed. If the question is one of suspected rape, an examination of a drop of the vaginal fluid or of scrapings from the vulva or vagina will usually reveal the spermatozoa. Of course other signs, which are important from the medicolegal point of view, will be observed in the examination of the external organs.

The stains usually subjected to medicolegal examinations for the presence of spermatic fluid have a grayish-yellow color, their size is somewhat variable, their contour usually irregular, and the linen upon which the stain is usually found is almost as stiff as if it had been starched. As spermatozoa are very resistant to the action of reagents as well as to putrefactive processes, they may be detected many years after the stain was made. It is, therefore, almost an impossibility to say by examination of a stain anything about the length of time the stain has been upon the cloth.

A fragment of the linen, which shows the stain, is placed in a watch-glass and allowed to soak for one hour in 30 per cent. alcohol or in faintly alkaline water. It is then lifted from the solvent, placed in another watch-glass, and teased with needles in a solution of 1 per cent. eosin in glycerin. A few drops of this mixture are then placed upon a glass slide, covered with a cover-glass, and examined with a high-power dry lens. Spermatozoa, if present, will practically always be seen by this method. The heads are stained a deep red while the tails, which are usually broken off by the teasing, show a light reddish tint, which distinguishes them from the unstained vegetable fibers.

Florence's Test.

It not infrequently happens that spermatozoa may not be found, although the stain be due to spermatic fluid. The principle of this test is that spermatic fluid when treated with a solution of iodin in potassium iodid gives crystals which were supposed to be iodospermin. According to Bocarius, this substance is not iodospermin, but an iodin compound of cholin. This test would be given, therefore, by any substance containing cholin and cannot, for this reason, be distinctive for spermatic fluid. Such being the case, a negative result is of far greater importance than is a positive one.

Technic.

The reagent used consists of 1.65 grams of iodin and 2.54 grams of potassium iodid dissolved in 30 c.c. of distilled water. If a drop of spermatic fluid or of an aqueous extract of a suspected stain be treated with a drop of this solution and immediately examined under the low-power lens, long rhombic platelets of a dark brown color, fine needles, or lance-shaped bodies often grouped in rosettes may be observed. A positive reaction is seen many years after the formation of the stain so that a positive result is of value when other sources of cholin are excluded.

Barberio's Test.

Barberio has found that the treatment of a drop of spermatic fluid or an aqueous extract of a suspected stain with a saturated aqueous solution of picric acid gives immediately a precipitate of sharply refractile, yellow, ovoid or needle-shaped crystals which gradually increase in size. This test was supposed to be of much greater diagnostic importance than that of Florence, but the recent work of Fraenkel and Müller has shown that the crystals are not sufficiently characteristic to permit of an absolute diagnosis. They call attention to the fact that substances other than spermatic and prostatic fluids may give similar crystals, but that in such cases these crystals are isolated and form usually on the border of the drop, while with spermatic fluids the crystals are numerous and are formed throughout the specimen. These workers recommend this test for the recognition of prostatic secretions or for the condition of azoöspermatism, but caution the worker against making an absolute diagnosis from its presence in medicolegal cases. A negative result does not necessarily exclude the presence of semen.

It will be seen, therefore, that neither one of the microchemical tests given above should be regarded as absolutely indicative of the presence of semen. It is much better practice to make repeated search for spermatozoa than to absolutely identify a stain as semen by the microchemical method.

II. Female Secretions.

(1). Vaginal Secretions.

The normal secretion of the vagina is scanty, usually just sufficient to moisten the mucous membrane. It is clear or occasionally opalescent, semi-liquid in character, and is composed largely of mucus and epithelial masses. Its reaction appears variable. As a rule, it should be considered acid in the case of virgins, while in those who have borne children it is usually alkaline.

Little is known regarding the chemical properties of this secretion. From the clinical standpoint the normal vaginal secretion is of importance owing to the fact that it possesses marked bactericidal properties. According to Krönig, pus organisms introduced into the vagina of pregnant women disappear in from four to thirty-six hours. Whether this bactericidal power is due to the reaction of the secretion or to some unknown agent is unsettled. A remarkable fact seems to be that frequent irrigation of the vagina with water or antiseptic solutions decreases the bactericidal power. If this be true, it is questionable whether frequent douching is advisable.

Microscopic Examination.

Outside of the great number of large, irregular, stratified, squamous epithelial cells which are constantly found in the vaginal secretion, one observes mucous corpuscles, a few large mononuclear leucocytes, cellular débris, and numerous bacteria. The bacterial flora of the vagina is very extensive.

These organisms are normally saprophytic and rarely take on pathologic functions, but they may occasionally do so. Among these bacteria we find the colon bacilli, streptococci, staphylococci, and bacilli which are not unlike true diphtheria bacilli. None of these organisms are particularly important from the clinical standpoint and will be passed with mere mention. We do find, however, certain organisms which give rise to no particular clinical symptoms, but which are extremely confusing in the examination for the presence of the gonococcus. As will be learned from the later discussion of this organism of Neisser, it appears in the form of biscuit or coffee-berry shaped diplococci, which are both intra- and extracellular and do not stain by Gram's method. The chief of these confusing organisms has been called the *orchiococcus of Eraud and Hugounenq*. This has the same morphology as the gonococcus, but is slightly larger, is negative to Gram's stain, and is rarely intracellular. It is, however, differentiated by the fact that it grows easily upon ordinary media, while the gonococcus requires special media. The more or less normal presence of this orchiococcus should be constantly in mind and a diagnosis of gonorrhea made only when clinical symptoms are present to point to the gonococcus. It is wise in all doubtful cases to resort to cultivation, as one may very much regret having made a diagnosis of gonorrhea when such did not really exist. The practitioner should be cautioned to take his smear high up, in the vagina, preferably from the region of the cervix uteri. If this be done as a routine fewer specimens will be found showing these confusing orchiococci. Smears are frequently sent to laboratories for examination which will not show the gonococcus even though present in large numbers in the vagina. It is not sufficient to take a specimen from simple purulent material which may be present in the lower portion of the vagina as the organisms are frequently absent in these locations.

Pathology.

Blennorrhea.

Physiologically, an increased vaginal secretion (blennorrhea) is seen during sexual excitement, preceding menstruation, and during pregnancy, when a very profuse secretion may be observed. If this secretion contains a large number of epithelial cells and leucocytes, as seen in nonspecific inflammations, it becomes more or less creamy in color and is then called leucorrhea. This type of leucorrhea should be sharply differentiated from the true pus secretions observed in the blennorrhagia of gonorrhea, as the former is not necessarily associated with pus formation. In pregnancy a slight catarrhal vaginitis is not infrequent, so that leucorrhea is more apt to appear at such times. If the inflammatory process becomes intense, large shreds of epithelium may be found and ulceration followed by vaginovesicle or vaginorectal fistulæ may be observed. Such pathologic findings are usually the result of gonorrhea. In slight catarrhal conditions of the vagina yellowish-gray patches may be seen, which are due to infection with mycotic fungi.

It is not infrequent to find the trichomonas vaginalis in the vaginal preparations. This organism has been previously discussed in the section on Feces, to which the reader is referred. The oxyuris vermicularis as well as its ova have been reported in the vaginal discharge, but do not seem to have excited any pathologic changes.

Purulent Secretions.

True purulent secretions arising from the vagina are almost always due to the presence of the gonococcus. This organism is accountable for a large number of gynecological conditions, so that it is wise for the practitioner to be able to identify it both from its clinical manifestations as well as by its laboratory detection.

In doubtful cases cultures should be made and a portion of the pus dropped into the eye of a rabbit. The gonococcus itself may later be recovered from the conjunctival exudate.

It is not to be assumed that the finding of the gonococcus in the vaginal discharge is necessarily evidence of a gonorrheal vaginitis or vulvovaginitis. It has been shown that the semen of the male as well as the urethral discharge may contain gonococci and that these may be introduced into a perfectly normal vagina without necessarily setting up gonorrhea. However, this is unusual. The gonococcus may arise from the urethra, the Bartholin glands, acute inflammatory processes of the uterus, or from a ruptured pyosalpinx. In any of these cases the gonococcus may be found in the vaginal discharge, so that the laboratory worker should be guarded in his diagnosis of a vaginitis. Further, suppurative processes which were originally due to the gonococcus may later take on a type of mixed infection or even become of the sterile type. This latter condition is especially observed in old Bartholinitis, metritis, and cystic salpingitis. The organisms usually associated with the gonococcus in the mixed infection are the streptococcus, staphylococcus, colon bacillus and pseudodiphtheria bacillus. In chronic metritis or salpingitis it is not infrequent to find the tubercle bacillus as the causative agent.

Fetid Secretions.

In these conditions the pus is usually chocolate colored, has a fatty appearance, is extremely repulsive, is frequently sanguinolent and contains numerous degenerated cells as a result of marked leucolysis. This condition is especially observed in puerperal infection and may be extremely severe.

(2). Uterine Secretions.

Normally, the uterus has no secretion beyond a slight mucoid one which is recognizable clinically. In inflammatory conditions, during normal menstruation, or following abortion or parturition, certain types of discharges are observed which have some clinical importance.

Menstruation.

Under normal conditions the menstrual fluid is at first mucoid in character, but within a short time red cells appear and later the discharge takes on almost the character of pure blood. This menstrual fluid should be bright red in color, should contain no clots and should be discharged without causing active pain. This fluid contains red cells, leucocytes, and prismatic epithelial cells showing large areas of fatty degeneration. The duration of the menstrual period is variable, running between two and five days. The amount of blood lost under normal conditions averages about 200 grams, but may be much larger under pathologic conditions. Not infrequently do we find cases in which menstruation is associated with marked pain during more or less of the period of flow. This condition is known as dysmenorrhea and may be associated with the exfoliation of large shreds of mucous membrane, in some cases reported these shreds constituting almost a cast of the uterine cavity. To this latter condition is given the name membranous dysmenorrhea. For the pathologic significance of these abnormal types of menstruation as well as for a discussion of the condition associated with failure of menstruation (amenorrhea) the writer must refer to works on gynecology.

The Lochia.

By this term we have reference to the discharges from the uterine cavity during the puerperium. At first such discharges consist of blood, which may be in the form of clots, and decidual shreds along with epithelial cells which are probably of vaginal origin. This type is known as the *lochia rubra or cruenta*. During the next two or three days the discharges become paler and thinner, the red cells diminish and the leucocytes increase, while the decidual shreds may continue approximately the same. This type is known as the *lochia serosa*. After about a week the discharge assumes a grayish or yellowish color and a creamy consistency, the red cells diminishing rapidly and the white cells increasing markedly. Microscopic examination shows, besides the leucocytes and epithelial cells, numerous fat globules and cholesterin crystals. This discharge may continue during the remainder of the period of uterine involution and is known as the *lochia alba*. Under normal conditions the lochial discharge has a faint odor, but is never fetid. If a portion of the placenta or membranes have been retained, the lochia may assume a dirty brownish color and become extremely fetid. After the first two or three days numerous bacteria, such as staphylococci, streptococci and colon bacilli, may be present, but no untoward symptoms exist unless these, along with other saprophytes, give rise to a distinct puerperal infection or sapremia.

Amniotic Fluid.

This is a thin, whitish or pale-yellow fluid containing the constituents of ordinary transudates. The reaction is neutral or faintly alkaline, the specific gravity varies between 1002 and 1008, and the amount of solids rarely reaches 2 per cent. The albuminous bodies are principally vitellin, serum

albumin, and traces of mucin, while glucuse is absent. Urea and allantoin are present in traces, while creatinin has occasionally been reported.

The amount of amniotic fluid varies between 700 and 1,000 c.c. Under pathologic conditions, however, this amount may be increased or decreased, giving rise on the one hand to polyhydramnios or dropsy of the amnion and on the other to oligohydramnios. For a discussion of these pathologic variations as well as for a treatment of the subject of pathologic changes in the membranes, the writer will refer to works on obstetrics.

Abortion.

The recognition of abortion is usually made by examination of the material discharged from the uterine cavity. Usually one finds blood-clots in which the villi of the chorion are present. These usually appear as club-shaped

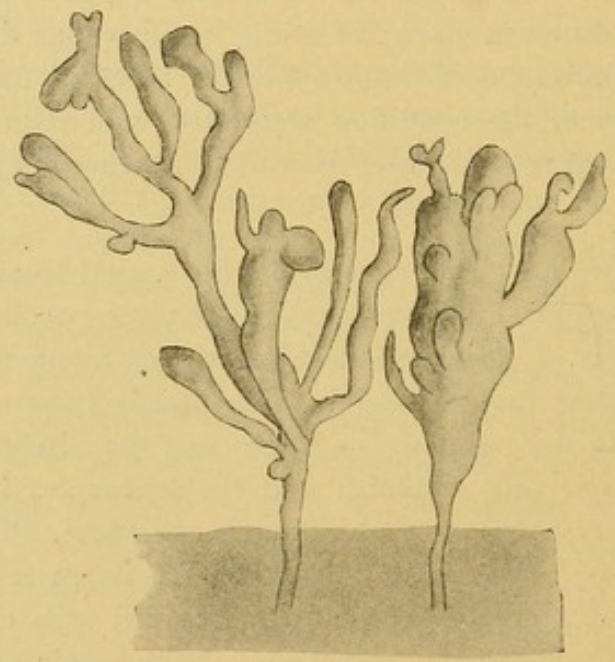


FIG. 115.—Chorionic villi. (*McMurrich.*)

masses with epithelial coverings, showing the characteristic capillary network. Moreover, decidual cells are usually present, and may be recognized by their large size, their round, polygonal, or spindle-shaped form, and their irregular and large nuclei with nucleoli.

Vesicular Mole.

This condition has been called dropsy of the villi of the chorion, hydatidiform degeneration of the chorionic villi, cystic mole, and myxoma of the placenta. One of its most important symptoms is the expulsion through the vagina of the vesicles forming the degenerated mass. The mole is a mass of pedunculated vesicles which closely resemble a bunch of grapes or gooseberries. Each vesicle may vary in size from a millet seed to a large hazelnut and contains a fluid which is usually colorless and limpid, but may be reddish and some-

what dense. Microscopic examination of the tissue shows the peculiar myxomatous degeneration of the chorionic villi.

Carcinoma.

It is not infrequent to observe, in cases of severe hemorrhage through the vagina, the appearance of occasional shreds, which on microscopic examination show the characteristic appearances of carcinoma of the cervix or body of the uterus. The diagnosis of carcinoma, however, would better be made upon sections removed by the surgeon rather than upon shreds found in the hemorrhagic fluids. Although a diagnosis may be at times possible, it should be somewhat guarded unless the clinical symptoms are distinctive.

CHAPTER VIII.

THE BLOOD.

I. General Considerations.

The blood is perhaps the most important tissue in the body, inasmuch as it is at once the purifier and the nutritive source of the cell. Any normal or abnormal product of cellular activity finds its way ultimately into the blood, either to be taken up by the assimilatory organs or to be thrown out by the excretory ones. While but relatively few disease processes are associated with diagnostic findings in this tissue, yet many are characterized by definite manifestations which are invaluable aids to the clinician.

It is, therefore, of the utmost importance that we should have a proper knowledge of the normal blood in order better to understand the various phases which characterize abnormal blood and which give to hematology such an interesting and important position in diagnosis. While it is true that some of the methods involved in hematological examinations require definite apparatus and a large experience for their proper interpretation, yet the results obtainable from the ordinary routine blood examinations are so invaluable, being in some cases pathognomonic, that no practitioner should consider himself fitted to give his patients the proper service without being equipped with a clear working knowledge of the methods of examination and the findings of normal and of abnormal blood.

It is essential to remember that certain physiologic as well as pathologic conditions influence the quantity and quality of the blood. So great are the effects of digestion, exercise, nervous factors, massage, cold, heat, sweats, dysentery, constitutional and specific diseases, that one does not wonder at the many conflicting reports of cases showing widely varying hematological findings. As Grawitz has pointed out, no conclusion should be drawn from an examination of the blood without taking into consideration the physiologic and pathologic condition of the patient.

It is a pleasure to observe in these days the tendency toward a mere rational and thorough study of the plasma, the so-called "intracellular fluid" of the blood. We have forgotten, in our enthusiasm over the many valuable findings obtained from histological investigations, that the relations of the fluid portions of the blood are, in some cases, of quite as much importance as are the variations in the cellular elements. It is necessary only to cite the work on lysins, precipitins, agglutinins, opsonins, etc., to show the value of a more extended study of the plasma or serum.

Regarding the technic of blood examinations, the writer will have much

to say later, but he wishes to impress upon his readers one point which has been well expressed by Türk, namely, an indispensable basis for the proper utilization of any diagnostic, prognostic, or therapeutic method of clinical examination is a knowledge of the absolute limitations of the method. Reliable results can, however, be obtained only by those who are thoroughly familiar with the principles as well as with the technic and the little "knacks" of the method used. It will be found, when the attempts are made to apply the methods outlined, that quite as much depends on the exactitude with which the separate details are carried out as upon the selection of the method itself. It is not to be expected that a first trial will yield exact results or that a few determinations will perfect one in the methods of examination. Experience is the only teacher that can equip one with the skill and power of interpretation necessary to cope with the many difficulties to be overcome in the hematological investigations.

II. Physiology and Chemistry.

(1). Blood Formation and Blood-forming Organs.

While it is impossible in a general work of this character to go into great detail regarding the formation of the blood, yet it seems to the writer that a brief discussion of this subject is extremely valuable both to the student and practitioner. The following section, taken largely from Ewing and McMurrich, will outline the generally accepted views on these points.

Red Corpuscles.

According to Kolliker, the first blood-corpuscles have their origin, in embryonal life, in the embryonic heart and blood-vessels. They appear as nucleated colorless cells, which later develop into colored corpuscles by the appearance of hemoglobin in some of the cells of the mesodermal cord, which cells go to form the first capillaries. Upon the formation of these vessels the cells lie within them as nucleated reds. At this time there are no true leucocytes and none appear until after the complete formation of the red cells, which is advanced as an argument against Pappenheim's theory of single origin of red and white cells. It will thus be noted that the vessel wall and the primitive erythrocyte have a common origin in the mesodermal cord, the peripheral cells going to form the endothelium of the vessel and the internal cells the corpuscles. Up to the end of the fourth or fifth week of embryonal life all of the red cells are nucleated; while from that time on the relation of the nonnucleated to the nucleated forms gradually increases until at birth few if any nucleated cells obtain.

In later embryonal life (about the third month), the liver becomes the chief seat of blood formation. During the fifth month, the spleen and lymph-glands take up this work, and finally the bone-marrow becomes the seat of such activity.

In extrauterine life, the bone-marrow is the chief point of formation of the red cells, but under pathological conditions the spleen and liver may assume their embryonic functions. It appears that the formation of nucleated reds in the adult is practically the same as in the embryo and that, at all periods of life, the red cell is the product of several series of mitoses of a colorless mesoblastic cell. The difficulty of tracing this series, from the large nucleated red cell to the colorless mesoblastic "mother-cell" in the marrow, has given rise to the diverse opinions now held regarding the ultimate development of the red corpuscles.

Leucocytes.

The earliest indications of the formation of leucocytes are seen in the presence of primary wandering cells, of mesodermal origin, which are found principally in the loose connective tissues of the early embryo. Though of mesodermal origin they are, from the first, quite distinct in morphology and, apparently, in function from the capillary endothelium and fixed connective-tissue cells. Their development has been traced by Ziegler to masses of mesodermal cells surrounding the cords from which the capillaries are formed. It thus seems that originally the parent leucocytes lie outside the vessels, into which they make their way by virture of ameboid powers.

Most observers find that the primary wandering cells produce, by mitotic division, one or more generations of colorless cells which gradually approach, in morphology, the early basophilic leucocytes of the circulation. Denys, Löwit, Ziegler, von der Stricht, and others claim that red cells and white cells develop from separate series of cells, which have become differentiated from the primary mesodermal cells with the first appearance of blood and blood-vessels. Kostianecki, Müller, Schmidt, Saxer, Pappenheim, and others believe that the primary wandering cell persists in the blood-forming organs as the parent of both red and white cells.

Before the leucocytes begin to appear in the circulation, mitotic figures are abundantly seen in the primary wandering cells in various situations. These are gathered in groups, first in the loose connective tissues of various regions, where lymph nodes subsequently develop; but the chief seat of the production of the leucocytes is found in the embryonal liver. In both situations the wandering cells are found in the lymph and blood capillaries, in the interstices of the connective tissues, and between the liver cells. In later embryonal life the process is gradually transferred from the liver to the lymphoid and adenoid tissues, as indicated by the development of lymph nodes, spleen, marrow, and thymus. Under normal conditions, the reproduction of leucocytes, in the adult, is limited to the lymphoid structures both of the lymph-glands and bone-marrow.

(2). Total Volume of Blood.

The various methods which have been advanced for the estimation of the total quantity of blood in the body are subject to such wide variations

that they have yielded little exact information regarding this subject. The procedures advocated by Valentine, Vierordt, Buntzen, and Thibault have an error sufficiently great to exceed the physiological and pathological variations of the blood. By these methods, the quantity of blood in the body has been estimated as equal to one-thirteenth of the body-weight. The so-called clinical methods of Quincke or of Tarchanoff are of purely theoretical interest, because certain factors, such as the appearance of the patient or the volume of the pulse, are taken into consideration in making a rather unreliable guess as to the total quantity of blood in the body (Buckmaster).

By the use of a method, recently introduced by Haldane and Smith,[1] the total volume of blood may be fairly accurately estimated. This method is based on the following points. The capacity of hemoglobin for oxygen and for carbon monoxid is identical. On the assumption that none of this latter gas is oxidized in the body, and that no substance in the blood, other than hemoglobin, unites with it, the experimenter is able to determine the CO capacity and hence the O capacity of the blood. This method has, however, little clinical application and will be left with reference to the original work. In the cases studied by Haldane and Smith by this method, the average value was 3,240 c.c. or, on the basis of a specific gravity of 1,060, about 3,434 grams. This yields, according to Smith, a figure ranging between one-sixteenth and one-thirtieth of the body-weight. In obese persons the volume of blood is less, proportionately, than in the more normal specimens of mankind.

The question of the volume of blood in the body is of great importance in the study of the changes taking place in this tissue. It must be remembered that the number of red or of white cells in a cmm. of blood will depend upon the total amount of blood present. If for any reason the volume is diminished or increased, corresponding changes, in the inverse sense, will be observed in the number of the cellular elements per cmm. It seems to the writer, therefore, that certain factors, not ordinarily taken into account in blood examinations, should be known before any definite report is made upon a blood count. It may be readily seen that a concentration of the blood, due to hemorrhage, diarrhea, sweating, etc., will lead to an apparent increase in the number of corpuscles. Certain abnormal blood counts, known to the writer, have undoubtedly been due to variations in these points.

Certain physiologic and pathologic conditions lead to definite changes in the volume of the blood, as such, or of some of its constituents. As Plehn[2] has recently shown, the volume remains quite constant or is adjusted through the activity of the capillary endothelium and through the influence of the nervous system. However, definite changes of a more or less transitory nature do occur and exert marked influences on the results of blood examinations as well as upon many pathologic conditions.

[1] Jour. of Physiol., vol. 20, 1896, p. 497; *Ibid.*, vol. 25, 1900, p. 333.
[2] Deut. Archiv. f. klin. Med., Bd. 91, 1908, S. 1.

Oligema.

By this term is meant a reduction in the total volume of blood, both as regards the liquid and the cellular portions. This condition is most frequently noticed after profuse hemorrhage and may be so marked that death results. In other cases, in which the hemorrhage is less extensive, the loss of blood is made up by osmosis from the lymph spaces into the capillaries and, later, by an increase of the cellular elements due to compensatory activity of the hematopoietic organs.

Plethora.

The opposite of the preceding condition is known as plethora, a state characterized by an increase in the total volume of blood. There has been much discussion as to whether a true plethora exists, but there can be little doubt that a transitory *plethora vera* may occur as a result of direct transfusion of blood, and also, according to Bergmann and Heissler, who have established the fact that there is a direct ratio between the volume of blood and the size of the heart, on the one hand, and the muscular development of the subject, on the other, as the result of increased muscular activity, provided the loss of fluid by perspiration is not excessive. Such a plethora disappears, of course, in a very short time.

In this discussion we must distinguish between a serous and a cellular plethora. By the former is meant an increase in the volume of blood due to excessive quantities of its liquid and soluble constituents; while by the latter we understand an increase in the number of corpuscular elements, that is a polycythemia. Serous plethora is most frequently observed in organic lesions of the kidneys and of the heart, in which a diminished elimination of water and inorganic constituents is noted. This condition is usually of a transient duration, as the volume of blood is soon restored to normal by intra-capillary transudation and by diffusion. Osmotic effects must also be taken into consideration here, inasmuch as the salts will tend to diffuse out from the blood and will consequently draw water after them, giving rise, under certain conditions, to dropsical effusions of a more or less transient duration.

Hydremia.

Another condition of the blood is frequently observed, in which an increase in the quantity of the liquid constituents is observed. This is known as hydremia and is different from serous plethora, as the latter carries with it an increase in the saline as well as in the watery portion of the blood. In hydremia the specific gravity of the blood is reduced, while in serous plethora it is increased. As Engel[1] has shown, the estimation of the coefficient of refraction of the blood serum is a reliable method for the clinical study of the subject of the water content of the blood.

Hydremia may be produced by any factor which changes the normal relationship of the blood constituents in such a way that the watery portion

[1] Magyar Orvosi Arch., Bd. 7, 1906, S. 555.

is relatively increased. It is in these more or less physiologic states that we are apt to observe the greatest variation in the blood counts. As the cellular elements are not simultaneously increased, the drop of blood under examination contains relatively fewer cells than normally. The most common physiologic causes of hydremia are the ingestion of large quantities of fluids, saline transfusions, and vasomotor dilatations as a result of exercise or nervous influences. In severe anemias we find the watery portion of the blood relatively increased. In dropsical states, following cardiac or renal lesions, we often observe such a condition, whose duration will depend, of course, upon the etiological factors of the trouble.

Anhydremia.

This is a condition characterized by a diminution in the liquid constituents of the blood. There is no change in the cellular elements and hence a blood count will show an erroneous increase in the number of cells. In this condition the specific gravity of the blood is naturally increased.

Anhydremia follows any condition which results in the excessive loss of fluid from the body, as, for instance, that following profuse diarrhea, polyuria, sweating, vomiting, and effusions into the various serous cavities of the body. According to Oliver, this state may be caused by influences which increase the arterial tension and hence bring about an increase in the passage of water from the vessels into the tissues. Thus, for instance, we may observe anhydremia following local and general exercise, massage, bathing, etc.

(3). **Volume Relations of Cells to Plasma.**

The study of the relationship between the cellular and the intracellular portions of the blood is a comparatively recent addition to the technic of blood examinations. This determination is based on the principle that the corpuscles may be thrown by centrifugal force to the distal end of a calibrated tube, while the plasma will collect in the proximal portion. If the tube be properly calibrated, the percentage relations of the cells and plasma may be readily ascertained. These ideas were used by Hedin in elaborating the earlier methods of Blix.

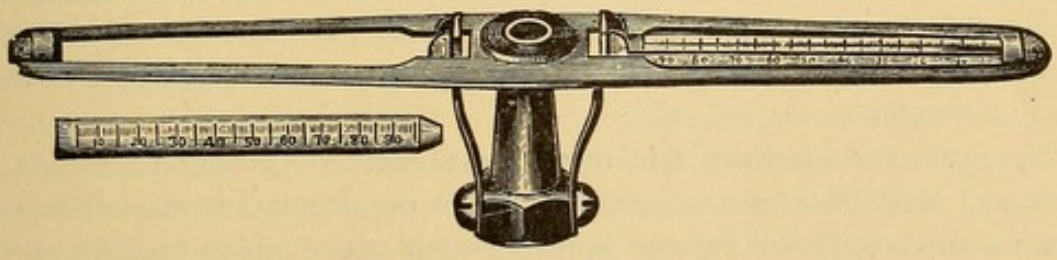


FIG. 116.—Daland's hematocrit.

Daland's Hematocrit.

Daland has introduced a modification of the clumsy model of Hedin and has succeeded in simplifying the technic to such an extent that this method is directly applicable to clinical use. His instrument is shown in the accompanying cut.

One of the calibrated glass tubes is fitted with the rubber tubing and is filled with blood from the ear or finger. The forefinger, smeared with a little vaselin, is then placed over the beveled end of the tube and the rubber tubing withdrawn. Insert the tube into one arm of the frame, the other arm of which should carry the second tube filled in the same manner, in order to balance the instrument and to control the reading. Rotate the spindle for three minutes at such a rate of speed as will insure 10,000 revolutions per minute (80 revolutions of handle). In this way the corpuscles are separated

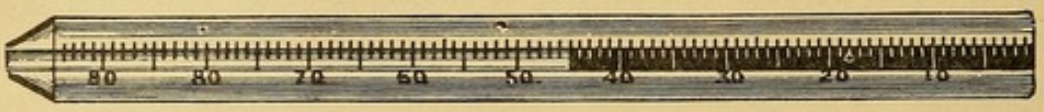


FIG. 117.—Hematocrit tube.

from the plasma and are distinguishable as a distinct column, which may be read off directly from the graduations of the tube. These divisions will give the percentage relations of the cells and plasma as the tube is divided into 100 equal portions, each division of the scale representing approximately 100,000 cells. This latter makes it possible to make a rather rough blood count with this instrument, but it is to be remembered that accurate results cannot follow, as we find such variations both in the size and elasticity of the cells in the different conditions in which the number of cells is most sought.

Volume Index.

Recently Capps[1] has introduced the conception of volume index, that is the quotient of the volume per cent. as obtained with the hematocrit, and the blood count in terms of per cent. Sahli advises the use of the expression volume quotient or volume value for this factor.

By means of the hematocrit the volume of the red cells, as compared with that of the whole blood, is taken. In normal cases this is about 50 per cent. which is reckoned as one. Hence the volume of the red cells may be obtained directly in percentage value. The red cells are then counted by the method to be later outlined, and the result is expressed in percentage by comparing this count with a so-called normal one of 5,000,000 red cells. By dividing the volume of red cells (in per cent.) by the per cent. of red cells, Capps obtains his volume index of the red cells.

In normal conditions this quotient is one. According to Capps, an increase of this index is a constant factor in pernicious anemia. The color index never exceeds the volume index in such cases, which fact shows that there is no supersaturation of the corpuscles with hemoglobin. In primary and also in secondary anemia this factor is diminished. Here we find the color index often falling below the volume index.

This method may be used in detecting various pathological conditions of the blood. According to Emerson, it is used in the Johns Hopkins Hospital

[1] Jour. Med. Res., Vol. 10, 1903, p. 367.

in ascertaining the presence of lipemia, cholemia, or hemoglobinemia. It would seem to the writer that it could be employed with advantage as a routine procedure, especially in hospital practice. The osmotic pressure of the plasma plays a great rôle in this determination, as the concentration of the blood may be such as to cause swelling or shrinkage of the cells. As Capps has found, normal red cells with a volume index of one have their discoplasm saturated with hemoglobin. Hence, if the hemoglobin index becomes greater than one, an enlargement of the red cells is indicated. Upon the other hand, the color index may fall, regardless of a corresponding lowering of the volume index. It follows, therefore, that, if the color index is above normal, the volume index must also be increased; while, if the hemoglobin index is below normal, the volume index is not necessarily diminished (Sahli).

(4). Methods of Obtaining Blood.

The method of obtaining blood for examination depends upon the amount desired and on the examination which is to be made. For ordinary routine work only a few drops are necessary, while for bacteriological investigations 2 to 20 c.c. may be required.

In obtaining the blood no set rule can be laid down as to the proper place from which to take the specimen. We should select the part which promises the best results, avoiding naturally the points which are cyanosed, eczematous, edematous, hyperemic, cold, unduly heated, or, in other words, any part which is not normal. The ear usually furnishes the best results, in the writer's opinion, because its puncture is relatively painless, it is easily accessible (which point is often of importance in attempting to obtain blood from those who are comatose or who refuse to submit their hand for puncture), and because the patient, who may be easily affected by the sight of blood, can not see the drop. If the lobe of the ear is thick it is usually pricked on the flat side, but if it be thin it is well to make the puncture on the edge and parallel to the surface. Some workers prefer the palmar surface of the ball of the middle or second finger of the left hand and others advise pricking the arm over a small superficial vein. In cases in which repeated examination of the blood are to be made the parts should be varied in order to prevent soreness and also to avoid anesthesias which occasionally follow repeated use of the same site of puncture.

Quite a number of special forms of blood needles, as, for instance, those of Francke and of Daland, are on the market, and each of them has its advocates. Many of them are in the form of special holders, which permit a puncture of a desired depth to be made without any danger of going too deep. These are to be recommended to those only who seem unable to control their stab. The writer does not find that the results obtained by the student are any better with such instruments than are those following the use of the ordinary Hagedorn surgical needle. In lieu of any better article, a clean steel pen with one nib broken will yield admirable results. The one thing to bear in mind

in selecting a needle for blood work is that the point must have a cutting-edge and should not be round or sharp nor should it be too long or slender.

Having decided upon the part from which the blood is to be taken, this surface is washed carefully with water and alcohol or ether and is then thoroughly dried. Avoid any undue rubbing as this will cause hyperemia and will introduce an error into the work. It is, as a rule, unnecessary to sterilize the needle but if it seems advisable to do so on account of the patient's attitude, the sterilization is best done by heat, hydrogen peroxid or alcohol, allowing the needle to cool before making the puncture. In this latter part of the technic, much depends on the amount of blood desired as regards the use of a short quick stab or a slow steady puncture. The latter procedure usually yields more blood but is more painful. It is much better to prick the patient too deeply, going even through the lobe of the ear, than it is to subject him to repeated punctures. The part pricked should not be squeezed nor held in a position which will cause an abnormal circulation. If the puncture is successful, the blood will come out in good-sized drops. The first of these are wiped away and subsequent ones used for the examination. As often happens, the blood coagulates fairly quickly, so that the coagulum should be wiped off with a little alcohol followed by a dry cloth. It is important to remember that a patient with hemophilic tendencies or history may bleed very easily from a very slight puncture. Care should, therefore, be taken to question the patient regarding the ease with which blood flows from a wound and also regarding the history of "bleeders" in the family.

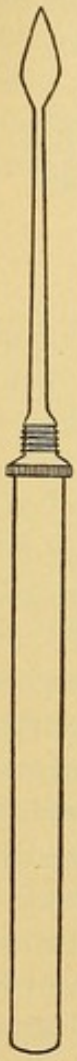


FIG. 118.—Blood needle.

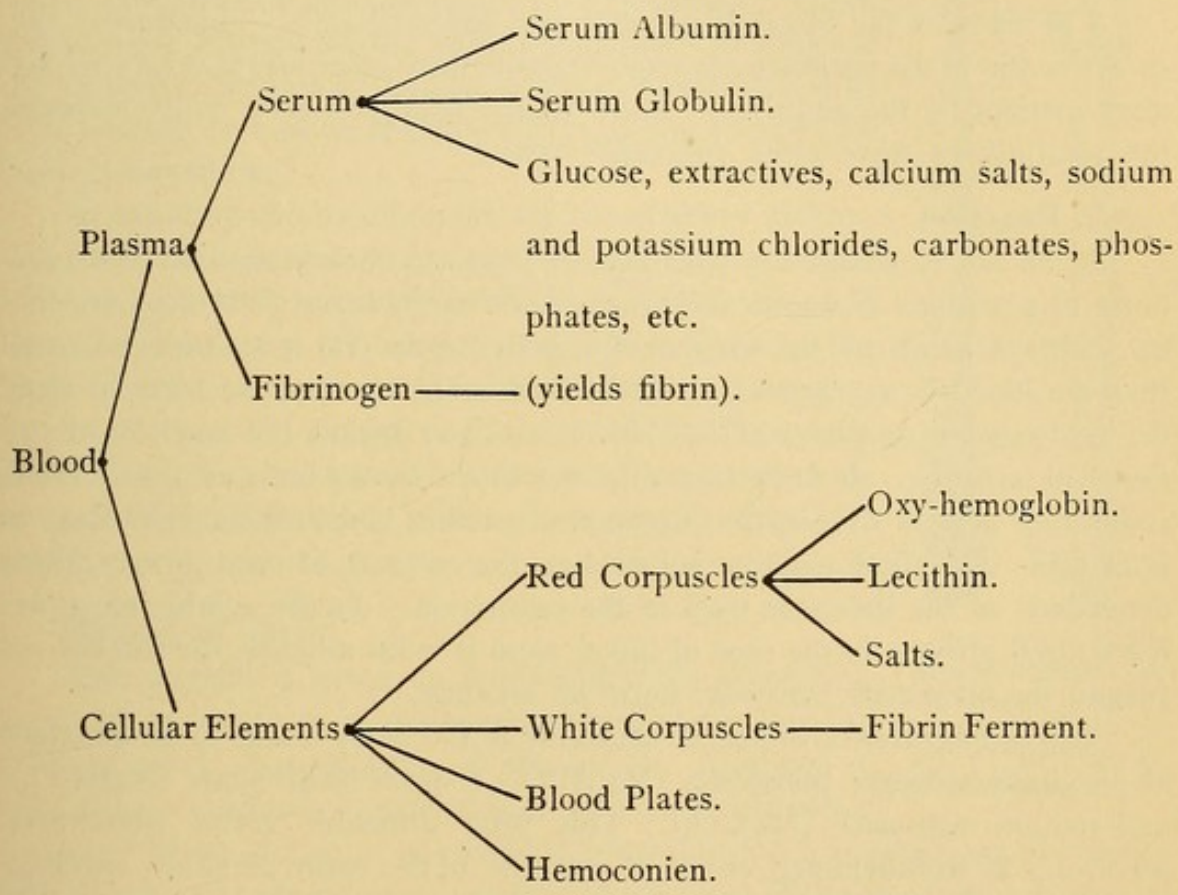
If considerable blood is desired, resort must be made to venous puncture. The site of this operation is usually the median basilic vein at the bend of the elbow. This vein may be made more prominent by tying a tight bandage around the arm, but the bandage should be removed before the blood is withdrawn, except when serum reactions are to be studied. The site of puncture must be thoroughly cleansed, using the precautions observed before any surgical operation. Likewise, the needle and the aspirating instrument must be absolutely sterile before puncture is made. The instrument best adapted for this purpose is, in the writer's experience, the Lüer syringe, which is made of glass with a tightly-fitting glass plunger and adjustable platinum needle. From 2 to 20 c.c. of blood are withdrawn and immediately placed in work. The question of the bacteriological examination and of serum reactions will be discussed in a later section.

(5). Physical Properties.

The blood must be regarded as a fluid tissue, consisting of a transparent liquid, the plasma or *liquor sanguinis*, in which are suspended the corpuscular

elements, erythrocytes, and leucocytes. Besides these latter cells we find two constituents, the blood plates of Bizzozero and the hemoconien (blood dust), which are hardly to be considered as true corpuscular entities.

As it flows from the vessels, blood is a thick, viscid, red liquid, having a peculiar odor, a salty taste, and an alkaline reaction to litmus. If allowed to stand it shows, unless certain precautions are observed, the peculiar phenomenon of coagulation. In this process the blood is separated into two portions, the cellular elements and the plasma, the latter changing, as the process goes on, into serum and the clot (fibrin), which holds the corpuscles in its meshes. In the diagram given below, adapted by Webster and Koch, may be seen the composition of the blood.



(*A*). **Color.**

The color of the blood is due to the presence in the erythrocytes of an iron-containing albuminous substance, hemoglobin, which has remarkable affinity for oxygen and other gases. This latter property enables this pigment to play one of the most important rôles in the body economy. Arterial blood is bright red in color, while venous blood shows a purplish-blue tint. These variations are due entirely to the relative proportions of oxygen and of carbon dioxid in the two types of blood. Many different shadings are observed, physiologically and pathologically, in the coloration of the blood, and each is due to some combination of hemoglobin with normal or abnormal substances.

The presence of large numbers of red corpuscles in the blood gives rise

to a characteristic opacity of this tissue. If, for any reason, such as admixture of blood with water, dilute salt solutions, urea, ether, snake venom, extract of mushrooms, etc., the blood loses its opacity, the change is due to the dissolving out of the hemoglobin from the red cells. This is the well-known phenomenon of "laking" or, better, of hemolysis, about which we will have something to say later.

The normal color of the blood is often changed in pathological conditions. Thus, in anemia the blood is pale and watery; in leukemia it may be milky; in diabetes buff-colored; while in poisoning with potassium chlorate it is chocolate-colored and in that with carbon monoxid it is bright red.

(*B*). **Odor.**

The odor of the blood is peculiar and indescribable. This *halitus sanguinis* is due to the presence of certain volatile fatty acids and may be rendered more distinct by the addition of concentrated sulphuric acid, which increases the volatility of these acids (Barruel's test).

(*C*). **Reaction.**

If we are to accept the teachings of physical chemistry, that the alkalinity of a solution is due to the presence of free hydroxyl (OH) ions and that its acidity depends on the surplus of free hydrogen (H) ions, we must grant that the blood is a practically neutral fluid. If, however, we have in mind the acid-combining power of the blood, we must regard the reaction of this tissue as alkaline. It is certain that the blood shows both acid and alkali combining powers due to the presence of protein constituents as well as to both acid- and alkali-reacting substances, the measure of such powers being dependent on the indicator used in the estimation. As the combining power for acids is greater, in the case of blood, than it is for alkalies, the reaction, as judged by titrimetric methods, must be alkaline.

The normal free or diffusible alkalinity of the blood is due to the presence of disodium hydrogen phosphate (Na_2HPO_4), sodium bicarbonate ($NaHCO_3$), and sodium carbonate (Na_2CO_3). This total diffusible alkali constitutes, according to Brandenburg, about 20 per cent. of the entire alkalinity and may be measured by dialyzing against known alkaline solutions and observing the concentration at which the strength of the known solution does not change. This factor represents the so-called alkaline tension of the blood and remains fairly constant, in normal cases, at about 60 mg. of NaOH per 100 c.c. of blood, while in pathologic conditions, such as uremia, diabetes, etc., it is somewhat reduced.

Besides this diffusible alkali, the blood contains nondiffusible alkali bound to the proteins. This portion represents normally about 80 per cent. of the total alkalinity, and is dependent largely on the cellular content of the blood, as the soluble protein constituents are not generally subject to wide variations. The fluctuations in this nondiffusible alkali are no doubt accountable for the great differences in the figures given for the alkalinity of the blood.

The subject of the reaction of the blood is one which should furnish, if properly studied by reliable methods, much valuable data upon subjects which are now very obscure. Unfortunately, however, most of the methods at our command are so unreliable and so inexact that the results have little more than a comparative value. As Moore and Wilson[1] have pointed out, the titration methods do not give us the true neutrality of the blood, but rather the amount of alkali or of acid which may be added to it without raising the hydroxyl or hydrogen concentration above certain low limiting values. The reaction of the blood depends on the indicator used for the determination of the neutral point and cannot be definitely measured by any method which employs an indicator for such purposes. These writers have introduced the term "reactivity" to indicate the property, possessed by the blood, of combining with both alkalies and acids in such a way as not to raise its ionic composition.

In selecting the methods for the quantitative estimation of the so-called alkalinity of the blood, I limit myself to those of Dare[2] and of Salkowski,[3] as neither depends upon the titration of the blood. The figures given by the older titration methods have such a wide range, being as low as 162 mg. and as high as 800 mg. of NaOH per 100 c.c. of blood, that the writer feels compelled to give them up for methods which seem to yield more exact and acceptable results.

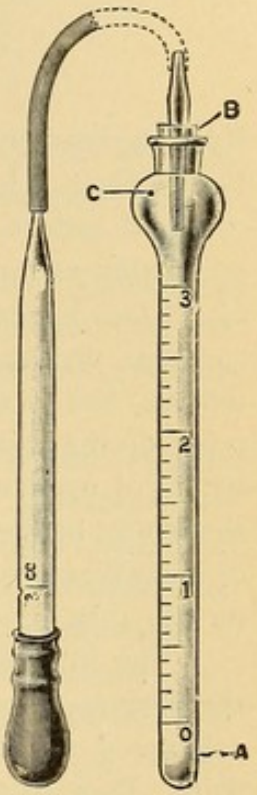


FIG. 119.—Dare's hemoalkalimeter: *A*, alkalimeter tube; *B*, automatic blood pipet; *C*, air hole; *D*, reagent pipet.

Method of Dare.

This method is based on the fact that the absorption bands of the spectrum of oxyhemoglobin disappear at the point of exact neutralization of the blood. Although it has not been conclusively proven that oxyhemoglobin is not destroyed before the point of complete neutralization is reached, yet the results with this method are rarely so variable as are those with the titration methods.

The apparatus (hemoalkalimeter) may be seen in the accompanying illustration. It consists of a special graduated tube (A), a blood pipet (B) which holds 20 cmm. (15 mg. by weight) of blood, a reagent pipet (D), and a hand spectroscope (E).

The method of using this instrument is as follows: The tube (A), into which fits the pipet (B), is held horizontally in such a manner that the pipet may be filled by capillary attraction with blood from the finger or ear. With a medicine dropper, containing distilled water, the pipet is washed free of blood, using sufficient water to bring the diluted blood to the zero-point of the

[1] Biochem. Jour. vol. 1, 1906, p. 297.
[2] Phila. Med. Jour., vol. 11. 1903, p. 137.
[3] Centralbl. f. d. med. Wissensch., Bd. 36, 1898, S. 913.

tube A. This diluted blood is then thoroughly mixed by shaking and inverting the tube, after which agitation it is allowed to settle. In this process care should be taken to close the opening (C) with the thumb to prevent loss of fluid. The reagent pipet (D) is then filled with the following test solution (N/200 tartaric acid, each c.c. of which is equivalent to .0002 gram of NaOH) and is connected with the end of the blood pipet by means of rubber tubing.

Tartaric acid,	0.375 gram.
Alcohol (95 per cent.),	100.000 c.c.
Distilled water, q. s. ad.,	1,000 000 c.c.

By compression of the bulb of the pipet the reagent is forced through the blood pipet into the tube A. Mix the fluids by inversion, care being taken to avoid loss of fluid. Adjust the spectroscope to the tube below the zero point after each addition of reagent and observe the spectrum for the presence of the two bands of oxyhemoglobin. This is best done by holding the tube a constant distance from an artificial light. This part of the technic is very tedious, but is necessary until the observer learns to associate the change in color of the mixture with the point of neutralization. If the spectroscopic bands of oxyhemoglobin still persist, more reagent is added and the mixture shaken as before, until these bands can no longer be seen. This is the point of neutralization and the end-point of the test. Read off, from the graduations on the tube, the number of c.c. of reagent used.

The following table, representing the graduations on the tube, gives the equivalent of NaOH for every fraction of a c.c. of reagent used. It is calculated on the basis of 15 mg. of blood for every 2 c.c. of the N/200 tartaric acid. Dare considers the normal figure to be 2 c.c. of acid, which represents 266 mg. of NaOH per 100 c.c. of blood.

C.C. of reagent.	Milligrams of NaOH per 100 c.c. of blood.
3.0	376.0
2.8	360.0
2.6	345.0
2.4	319.0
2.2	292.0
2.0	266.0
1.8	239.0
1.6	212.0
1.2	176.0
1.0	133.0
0.8	96.0
0.6	79.0
0.4	53.0
0.2	26.6

The writer has found that this method is capable of easy application and, with a little practice, will give very reliable and constant clinical results. Naturally the personal equation arises in the reading of the spectroscope, but this is easily corrected by experience. As the spectroscopic changes are sometimes slow in appearing, one should not be in too great haste to finish his determination. The figures obtained with this method are somewhat lower than those with the Salkowski technic and are probably more nearly repre-presentative of the nondiffusible than of the total alkalinity.

Method of Salkowski.

This method, like the preceding one, has the advantage of avoiding direct titration of the blood, but has the objection that a considerable time is necessary to obtain certain results.

The method is very simple and is based on the principle that ammonium salts are decomposed, in the presence of alkalies, with the liberation of free ammonia, which latter substance may be absorbed by a standard acid solution and determined by titration of the excess of acid.

Twenty grams of finely powdered chemically pure ammonium sulphate $(NH_4)_2SO_4$ are placed in the larger lower dish of Schlösing's apparatus (see Urine) and are dissolved in 20 c.c. of distilled water. Ten c.c. of N/10 sulphuric acid are placed in the upper dish. Pour into the lower dish, which contains the ammonium sulphate solution, 10 c.c. of blood, which has been measured in a cylinder previously washed with 1 per cent. solution of sodium oxalate to prevent coagulation of the blood. Mix the blood and ammonium sulphate solution thoroughly and cover the dishes as quickly as possible with a bell jar, which should be tightly fitted to the glass plate with vaseline. Allow the apparatus to stand at room temperature for five to six days, when the ammonia, liberated by the action of the alkali of the blood upon the ammonium sulphate, will have been taken up by the sulphuric acid. Titrate this acid solution with N/10 NaOH, using methyl orange or rosolic acid as an indicator, until the point of neutralization is reached. Subtract the number of c.c. of NaOH used in the titration from the original number of c.c. of H_2SO_4 taken and multiply the result by 0.004006, which will give the amount of sodium hydrate equivalent to the ammonia liberated from the 10 c.c. of blood. To obtain the amount of NaOH per 100 c.c., multiply the above result by 10.

This method is appealed to on account of its simplicity and its reliability, being certainly more accurate than the methods of direct titration. According to Waldvogel, the normal values obtained by this method are 350 to 400 mg. of NaOH per 100 c.c. of blood, being somewhat higher than those obtained with Dare's method owing to the fact that all of the alkali present, whether diffusible or nondiffusible, will react by this method.

The reaction of the blood varies in certain physiologic states, as, for instance, during digestion. The alkalinity or the "reactivity to acids" is higher in man than in woman and the child; is at its minimum in the early morning,

rises during the afternoon, and falls in the evening; is increased during digestion and falls after digestion is complete; is decreased by excessive exercise and also by a diet deficient in nitrogenous constituents. Many of the beneficial results of massage and baths are unquestionably due to an increase in this factor.

It must be remembered that the normal balance between the acid and alkali constituents of the blood is easily maintained, otherwise the system would be in a state of constant disorder, subject as it is to the influence of acids, either produced within itself or taken into it from without. As Moore and Wilson have shown, the susceptibility of the living cell to increase in concentration of either hydrogen or hydroxyl ions is due to the fact that the protein of the cells possesses strong affinity for both these ions, forming feebly dissociated salts with them. Increased alkalinity of the blood is supposed to denote increased antibactericidal power of this tissue. For this reason we must assume some direct relationship between immunity and alkalinity. Moreover, the further study of the opsonic, lytic, and other properties of the plasma may reveal definite influences of the alkalinity upon such processes.

It has been found that pathologic changes are very often evident in the reaction of the blood. Thus, for instance, we find in severe secondary anemia and in pernicious anemia a marked decrease in the alkalinity, while in chlorosis we observe little or no change. The degree of alkalinity is reduced in practically all infectious diseases, in uremia, in diabetes, and organic diseases of the liver, etc., while in chronic diseases it may, at times, show an increase. The study of the conditions associated with an "acidosis" would be, undoubtedly, made much clearer by the application of reliable methods of estimating the reaction of the blood.

(*D*). Specific Gravity.

The specific gravity of normal blood varies between, 1,055 and 1,065, the average being 1,060. Certain variations in this figure are observed depending on the sex or age of the patient or upon the time and temperature at which the determinations are made.

The most accurate method of determining the specific gravity is, of course, the use of the pycnometer. This method is open to the objection that it requires much more blood than can usually be obtained in routine work. In cases in which bleeding can be resorted to without detriment to the patient this method is the one to use, as it gives the most reliable and accurate results. The writer has used it in many cases of pneumonia, where the withdrawal of a certain amount of blood is often beneficial, and finds the results all that could be desired. Besides the quantity of blood (5 to 50 c.c.) which is required, there is also necessary a very accurate chemical balance, else the results will be influenced by the inaccuracies in the weighings.

The technic is as follows: weigh the pycnometer empty, filled with distilled water, and then filled with blood. Care should be taken to have the

vessel absolutely dry and clean before weighing it empty and before filling with either water or blood. Subtract the weight of the empty bottle from that of the bottle filled with blood and divide this figure by the difference in weight between the bottle filled with water and the empty bottle. The result will be the specific gravity of the blood, water being taken as unity. We should be careful in this determination to have the temperature of the water the same as that of the blood in order to insure accurate results.

Method of Schmaltz.

This method is a modification of the above and does not give quite as accurate results. It consists in the use of small tubes, which hold about 1/10 c.c. These tubes are constricted at the end to prevent loss of blood and are filled by capillary attraction. The determination is made in the same way as with the pycnometer. More or less manipulative skill is necessary in the handling of these tubes, but the results are sufficiently accurate for most purposes.

The more frequently employed methods of determining the specific gravity are the so-called areometric ones. The principle of these procedures is the determination, by the use of accurate hydrometers, of the specific gravity of a liquid mixture, in the center of which a drop of blood will remain suspended.

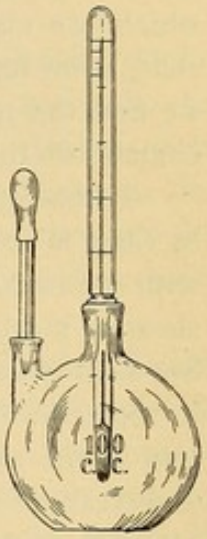


FIG. 120.—Pycnometer.

Method of Hammerschlag.

This method is a strictly areometric one and consists in the use of a mixture of benzol and chloroform into which a drop of blood is introduced through a capillary tube. If the drop rises in the mixture benzol is added and if it sinks chloroform is used. The point at which the drop of blood remains suspended in the center of the perpendicular axis of the mixture is taken as the one representing the specific gravity of the blood. As the means of estimating the density of this mixture, we employ an accurately graduated hydrometer. It must be remembered that the fluid mixture should be well stirred after the addition of either benzol or of chloroform in order to insure uniform density throughout. As the mixture evaporates rapidly we must work quickly and should confirm our results by a duplicate determination. A further precaution should be to allow no bubbles of air to adhere to the drop. This is fairly well accomplished by the use of the capillary blood pipet. It is also essential that the temperature of the mixture should not vary to any appreciable extent. This method is simple and, with the precautions mentioned, will yield good clinical results.

The specific gravity of the serum may be tested in this same way, first allowing the blood to coagulate in sealed tubes and then drawing off a drop or two of the separated serum.

Naturally, the specific gravity of the blood is a measure of its concentration

and, hence, of its water content. We are, therefore, certain to find variations in this factor under the influence of any physiologic or pathologic changes, which are associated with fluctuations in the volume of blood.

Physiologically, the specific gravity is higher in men than in women and children; is higher in venous than in arterial blood, and is lower after ingestion of large quantities of fluid in the food or after infusions. Clinically, we find that the specific gravity of the blood runs parallel to the number of corpuscles and to the amount of hemoglobin in the red cells. So striking is this ratio that it was formerly used to determine the percentage of hemoglobin in the blood. Any marked alteration in these constituents gives rise to a variation in the specific gravity. Thus, there is observed in anemia in which there is a lowered percentage of hemoglobin, and in those forms of secondary anemia which are characterized more particularly by diminution in the number of cells, a low figure for the specific gravity. In polycythemia, on the other hand, we find the specific gravity increased as a result of the increased corpuscular content of the blood.

Pathologically, the specific gravity may run between 1,026 and 1,068. An increase is noted in practically all febrile diseases, in those conditions associated with cyanosis, and in disorder leading to obstructive jaundice. In conditions showing marked diuresis, diarrhea, or sweating an increase is likewise observed, but such changes are usually of slight duration, as the blood soon adapts itself to the condition by withdrawing liquid from the tissues to compensate for the loss in the above processes. In nephritis we may find either an increase or a decrease in the specific gravity, depending on the osmotic changes which take place in this disease.

(*E*). Viscosity of the Blood.

Freshly drawn blood has a greasy feeling, which is replaced by a stickiness as coagulation proceeds. This viscosity or internal resistance of the blood depends, to a large extent, upon the cellular content of the tissue and is distinct from the phenomenon of coagulation.

Hirsch and Beck have elaborated a clinical method for the determination of this viscosity. It consists in the measurement of the time necessary for a known volume of blood to flow through a capillary tube under certain conditions of temperature and pressure. The results obtained by this method are not at present sufficiently numerous or definite to warrant the incorporation of such estimations in the routine blood examination. Determann[1] has devised a simple viscosimeter which may be used at the bedside. As the writer has had no experience with this instrument, he cannot speak definitely concerning it, but he is inclined to believe that the determination at room temperature is inexact.

It has been shown that the degree of viscosity (n) is influenced by cold, withdrawal and application of heat, the former factors causing an increase, while

[1] Münch. med. Woch., Bd. 54, 1907, S. 1,130.

the latter lowers it. Hirsch and Beck demonstrate that the lower the specific gravity of the blood the less marked is its viscosity. These results agree with those that indicate that the lower the specific gravity the lower the number of cellular elements. We may readily see, therefore, why the blood in anemia and leukemia shows some a slight tendency to become sticky or to form rouleaux. The researches of Rotky[1] show that n varies between 5.02 and 5.52 under normal conditions (water being 1), is 1.69 in anemias, 3.34 to 5.58 in nephritis, 13.56 in febrile states and 16.93 in cyanotic conditions. According to Hess[2] the normal viscosity of male blood ranges between 4.3 and 5.3, while that of female blood varies from 3.9 to 4.9. The normal relation

$$\frac{\text{Hemoglobin}}{\text{Viscosity}} = 17 \text{ to } 21.$$

(*F*). Coagulation of the Blood.

It is impossible in this place to discuss the physics and chemistry of the process of coagulation, more than to say that this phenomenon is due to the conversion of the fibrinogen of the plasma into fibrin. This change takes place under the influence of a ferment, thrombase, which is present in the leucocytes in the form of prothrombase. This latter zymogen, through the influence of calcium compounds, is changed into thrombase, the active agent in bringing about coagulation.

It is occasionally of clinical importance to know the time of coagulation of the blood under certain conditions, as one of the normal processes of this tissue, when outside the vessels, is coagulation. This process takes place normally in from two to eight minutes, depending on several factors, among which are the length of time the blood is in contact with the tissues, the depth of the incision, the pressure with which the blood is expelled, the nature of the vessel into which the blood flows, and the temperature at which coagulation takes place.

It has been found that certain variations in the normal coagulability are present at different hours of the day and are observed when blood is drawn from different parts of the body. The whole question of coagulation of the blood being so intimately associated with its chemical composition and extravascular coagulation being only significant of the changes which take place when blood coagulates intravascularly, I must leave undiscussed this phase of the question, referring the reader to works on pathology for a treatment of coagulation in its relation to thrombosis.

In a general way the time necessary for blood to coagulate may be determined by taking a drop either from the finger or the ear and allowing it to fall upon a glass slide. Several drops are collected in this way and tested, at intervals of one minute, by drawing a broom straw lightly through each drop until a thread of fibrin is seen clinging to the straw. The time which elapses between the taking of the drop and the appearance of the fibrin rep-

[1] Zeitsch. f. Heilkde., Bd. 28, 1907, S. 106.
[2] Deutsch Archiv. f. klin. Med., Bd. 94, 1908, S. 404.

resents roughly the coagulation time of the blood. Instead of the straw, a white horse hair, which has been previously boiled in alcohol and ether, may be used, as advised by Vierordt.

Wright's Method.[1]

Wright has devised an instrument called the coagulometer (see cut), by means of which more accurate results may be obtained, although the method is more time consuming and complicated. Tbe apparatus consists of a reservoir containing a removable rack holding a thermometer and 12 cali-

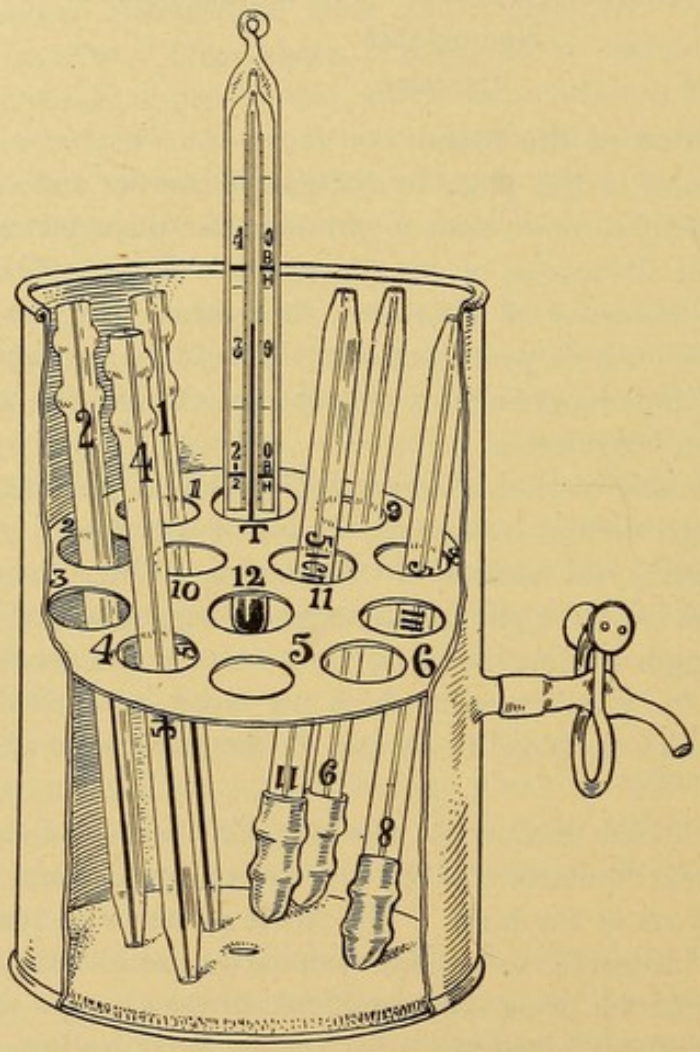


FIG 121.—Wright's coagulometer. (*Da Costa.*)

brated capillary tubes, which have an internal diameter of 0.25 mm., a length of about 10 cm. and a calibration at 5 cm. They are provided with rubber caps to seal their blunt ends when first immersed. Each tube is numbered so that it may be placed in its proper place in the rack.

The vessel is partially filled with water and heated slowly to 37° C. (blood heat) or preferably to 18.5° C. (half blood heat). While this heating is taking place the tubes are carefully cleaned with water, alcohol

[1] Lancet, vol. 2, 1902, p. 15.

and ether, and are then dried. When the temperature of the water has reached the desired point, immerse the tubes, with the blunt end sealed with the rubber caps downward, and allow them to come to the temperature of the water. Remove the tubes, dry them, and take off the rubber caps. Fill tube 1 up to the 5 cm. mark with blood and note the time of filling. It is usually necessary to draw the blood into the tube by suction of a rubber bulb, as the capillarity is not always sufficient to permit of filling the tube to the mark. Immerse the tube with point down leaving off the rubber cap. At one-minute intervals fill the other tubes in the same manner and immerse them in their proper positions. It is necessary that the temperature of the water should remain constant in order to insure accurate results. After an interval of a minute or less, the tube first filled is removed and tested by blowing into the tube and observing the readiness with which the drop is forced onto a piece of white paper. The tubes must be inspected in definite order until one is found from which the blood cannot be expelled. The time between the filling of the tube and the point at which the blood is completely clotted is taken as the coagulation time. The tubes may be easily cleaned after use by first dislodging the clot with a fine wire and then thoroughly washing them with distilled water, alcohol, and ether.

Method of Russell and Brodie.[1]

This method, as modified by Boggs, gives much more accurate results than that of Wright and is based on the fact that the corpuscles, set in motion by a current of air in a moist chamber, move freely and independently of one another at first but, as coagulation proceeds, clumping of the cells occurs, and finally an elastic radial motion of these cells obtains, which is to be observed under the low power of the microscope. The writer must refer the reader to the original articles for a description of the apparatus and the method of application (see cut).

Normally, the coagulation time, as evidenced by the formation of fibrin, is between two and eight minutes. Anything above nine minutes means delayed coagulation. The importance of testing this function of the blood is observed more particularly in cases of suspected hemorrhagic diathesis, in which the period of coagulation is remarkably increased. In some cases of hemophilia it requires fifty minutes, while in certain of the purpuras from 10 to 15 or more (Emerson). Likewise, it is customary to test this factor in cases of long-standing jaundice, in which surgical intervention for obstructive lesions of the biliary passages is to be undertaken, as here also the time is much increased. Moreover, we find the coagulation time delayed or imperfect in cases of hemoglobinemia, asphyxia, and general dropsy. Poisoning from the bite of certain serpents, such as the cobra, is characterized by a greatly delayed coagulation of the blood as well as by hemolysis. On the other hand, we notice a quicker coagulation in conditions associated with stasis, repeated

[1] Jour. of Physiol., vol. 21, 1897, p. 403.

hemorrhages, transfusion, hunger, and also under the therapeutic use of calcium chlorid and of gelatin.

The formation of fibrin, as the end-product of coagulation, is increased in some cases and diminished in others. An increase in the amount of fibrin in the blood (hyperinosis) is observed in acute inflammatory processes and in most infectious diseases. Hayem states that the density of the fibrin network, observed when blood is allowed to dry in thick smears on a glass slide, indicates the degree of resisting power of the individual against disease. The largest amounts of fibrin are observed in such conditions as pneumonia and acute

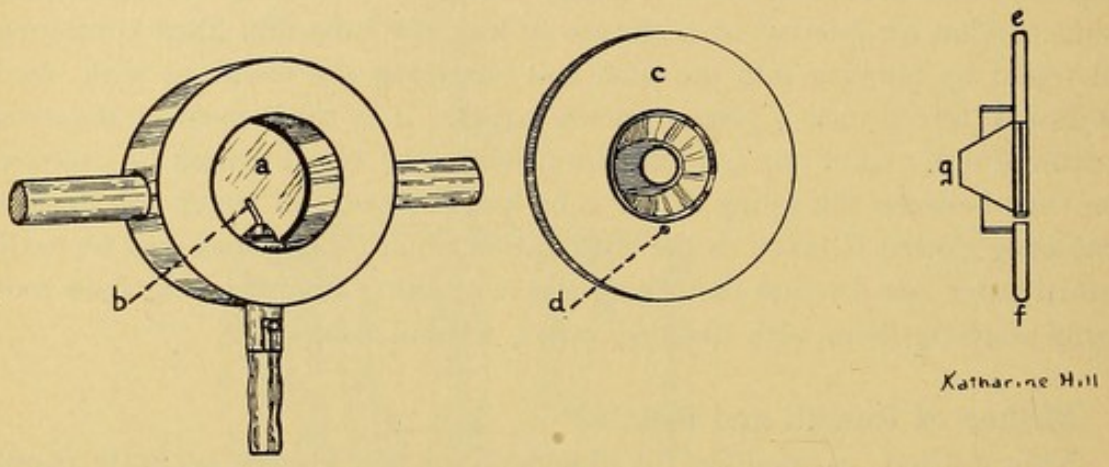


FIG. 122.—Boggs' coagulometer: *a*, moist chamber; *b*, tip of tube through which air passes; *c*, cover which fits over moist chamber and which holds glass cone; *d*, pin-hole for escape of air; *e f*, cross section of cover c; *g*, tip of glass cone upon which is placed the drop of blood.

articular rheumatism, but are seen to a lesser extent in parenchymatous inflammation, in inflammations of the mucous membranes and the skin, in the febrile stages of chronic suppurations, in hepatitis, influenza, diphtheria, acute gout, and erysipelas. A decrease in the amount of fibrin (hypinosis) may be observed in malignant growths, malarial fever, pernicious anemia, leukemia, and purpura. In parenchymatous nephritis the amount of fibrin is but slightly if at all increased, while in interstitial nephritis the increase may be notable (Da Costa).

(*G*). Osmotic Pressure and Cryoscopy.

The cryoscopic examination of the blood may be of some importance in the diagnosis of certain conditions, particularly in reference to the sufficiency or insufficiency of the kidneys as regards the elimination of the urinary solids. While this subject has not yielded as much information as was expected of it, a brief discussion seems essential. For a theoretical discussion of osmotic pressure and cryoscopy, I must refer the reader to works on physical chemistry.

By cryoscopy from the Greek *kryos*, frost, and *skopeo*, to see, is meant the

determination of the freezing-point of a solution and the referring of this figure to the freezing-point of the solvent, which is regarded as 0. Substances in solution lower the freezing-point of the solvent in direct proportion to their molecular (dissociated or nondissociated) concentration. In determining the osmotic pressure of a solution, and cryoscopy is one of such methods, it is important to remember that the proteins have practically no osmotic value. We have, therefore, in this cryoscopic method a means of ascertaining directly the molecular concentration of the body fluids.

The determination of the freezing-point of the blood is best made by means of the Beckmann apparatus, which may be found in works on physical chemistry. As the osmotic pressure of the serum is equal to that of the plasma or of the whole blood, the serum is generally used for this determination. Withdraw from a vein, preferably the median basilic, about 30 c.c. of blood and allow it to coagulate in a clean closed vessel. Place the serum in the freezing tube of the Beckmann apparatus, adjust the thermometer and stirring rods, and proceed as directed elsewhere.

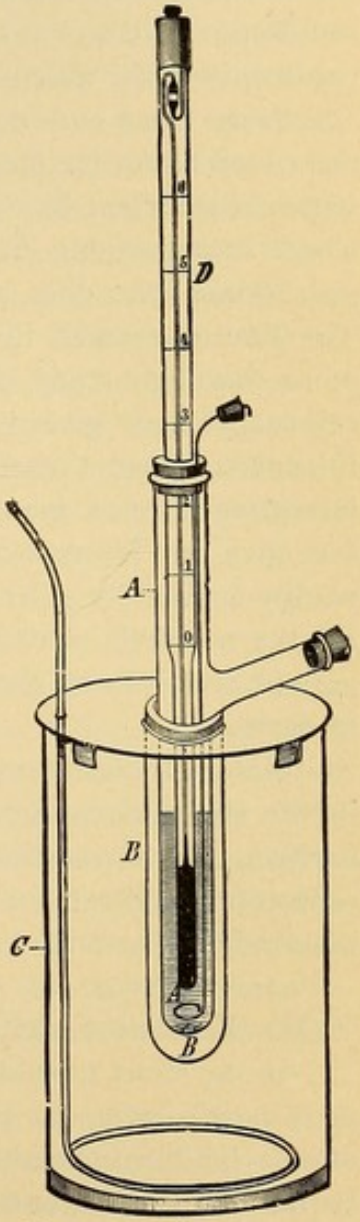


FIG. 123.—Beckmann apparatus. (*Long.*)

The freezing-point of the blood is usually designated as the small Greek delta (δ), while that of the urine is given the sign $\triangle$. Normally, blood freezes at—0.56° C., distilled water freezing at 0. This point is subject to more or less physiologic variation depending upon the changes which occur in the blood after meals, exercise, baths, etc. This physiologic variation is transient and slight because the kidneys soon regulate the osmotic pressure (molecular concentration) of the blood by withdrawal of the constituents which have caused the abnormal tension. This close relationship between the kidneys and the blood is of great importance in the study of renal insufficiency, as many products of metabolic activity remain in the blood in cases of renal disturbance. This is more particularly true of the inorganic constitutents of the blood, as the organic elements do not materially affect its osmotic proportions. It is in those cases of renal insufficiency with a tendency to uremia that the most is to be expected from the cryoscopic examination of the blood, yet we find that Schoenborn,[1] in the study of 88 cases, observed practically normal figures for the cryoscopic point of the blood. Engelmann,[2] on the other hand, reported

[1] Wiesbaden, 1904.
[2] Mitteil, a. d. Grenzg. d. Med. u. Chir., Bd. 12, 1903, p. 396.

a series of 36 cases in which the freezing-point averaged—0.664 C. It is true that in certain surgical conditions of the kidneys a parallelism is noticed between the lowering of the freezing-point and the development of the uremic symptoms. In cyanotic conditions of any origin whatever we find the cryoscopic point lower than the normal, owing to the fact that the CO_2 is increased in amount in such cases. If the CO_2 be driven off, the freezing-point becomes normal. Hence we see why, in certain cases of uncompensated cardiac disease, the freezing-point has frequently been reported much lower than the normal standard. There is practically no disease of the blood itself about which a cryoscopic study will furnish any information.

From these considerations, as well as from many others, which I have not enumerated, the study of the cryoscopy of the blood does not seem to the writer of sufficient importance to warrant its adoption as a routine method of blood examination. It is, certainly, of no value whatever to the general practitioner who does not have access to a thoroughly equipped laboratory. The time consumed in such examination would be much better spent in a more thorough study of the fresh and of the stained specimens. While cryoscopy may give some idea of the osmotic activity of the kidneys, it adds to blood examinations a slightly enlarged laboratory record, whose interpretation is a matter of more or less difficulty and whose results rarely ever give any information, beyond that which may be more easily and more readily learned by other methods. It is a purely theoretical method of following the metabolic activity of the system and reveals no signs of an oncoming uremia nor does it show us anything regarding the results of therapeutic measures.

Naturally, variations in the osmotic pressure of the blood are of importance in the study of conditions associated with effusions into the various serous cavities, as such exudations are markedly influenced by the molecular concentration of the blood. For a discussion of such phases of the subject I must refer the reader to works on pathology.

(*H*). Electric Conductivity.

In the effort to enlarge the scope of blood examinations, the clinician and, more particularly, the laboratory worker have taken advantage of everything offered for the furtherance of their aim. In so doing they have often overstepped themselves and complicated the examinations by useless additions to their technic.

By electric conductivity is meant the reciprocal of the resistance, which a certain amount of solution offers to the passage of an electric current of known strength between two platinum electrodes of given size and a given distance apart. This method is merely a measure of the number of electrolytes (both dissociated and nondissociated) in solution and is not affected by the nonelectrolytic organic substances of the blood, although, according to Hardy,[1]

[1] Proc. Royal Soc., vol. 66, 1900, p. 95.

the proteins move to one or the other pole of the battery depending on the reaction of the fluid. The method usually adopted for estimating this factor is that of Kohlrausch, the resistance being balanced on a Wheatstone bridge against a rheostat, the point of equilibrium being determined by means of a telephone attachment.

As this determination can give us no information regarding the retention of organic products in the system, it is of comparatively little value in the study of metabolic or of blood diseases. It is in those conditions which are associated with retention of inorganic constituents that this method should be of value, and yet we find, according to the work of various authors, that in advanced nephritis, in which the retention of chlorids has been assumed, the conductivity of the serum is not increased to any extent, although the freezing-point is lower than the normal standards. This proves, its seems to the writer, that in such cases the substances capable of increasing the conductivity—that is, the chlorids—are either not retained in the blood or are in such a combination as not to influence the conductivity of this tissue. We must, therefore, assume that the lowering of the freezing-point observed in such conditions is due more to the organic than to the inorganic constituents of the blood. As this determination is, in the writer's opinion, of so little practical value in blood work, a detailed description of the methods and results of observations will not be taken up.

(6). Chemical Properties.

The chemical examination of the blood resolves itself into a consideration of the composition of the whole blood, of the plasma, of the serum, and of the various cellular elements. It is evident that the composition of the whole blood will depend upon the relations in which the single constituents of the blood stand to one another. Naturally, physiologic influences are of great importance in the consideration of the composition of the fluid portion, while the cellular elements are less affected by these changes. In view of the recent work on opsonins and immunity, we must conclude that the chemical examination of the fluid elements of the blood may yield much important information as soon as proper methods of research are evolved.

Normally, the composition of the cellular elements remains almost constant and is but slightly affected by the varying composition of the fluid portions. As is well known, the corpuscles act more or less like semipermeable membranes, although Kahlenberg would have us believe that no such a condition is possible. At any rate, the membrane surrounding the erythrocyte allows the passage of certain inorganic and organic constituents of the plasma into the cells and permits the back-passage of certain constituents of the cells into the plasma. This question is intimately related to the subject of hemolysis, which will be discussed in detail later. It is true that a many-sided exchange exists between the red cells and the hypertonic plasma in which they float, such an exchange accounting very often for the alterations in the volume

of the corpuscles and frequently being the cause of abnormally developed cells. This exchange is subject to certain restrictions. Thus we find that the corpuscles show a very high content in potassium salts and a very low sodium content, while the reverse conditions obtain in the serum. If free exchange took place between the cells and serum no such conditions could exist. It is known that potassium salts are closely associated with phenomena of growth and hence it might be possible to prove that cells, which are morphologically the older, show a less potassium content than do the newly developing ones.

It is evident that a quantitative analysis of the blood can be of only comparative value as far as the blood as a whole is concerned. We should ascertain on one side the relationship of the plasma and blood-corpuscles to each other, and on the other side the composition of each of these two chief constituents. As there are many difficulties in the way of such determinations, we will not go into detail regarding the chemical composition of the different portions of the blood, but will discuss the blood as a whole. According to C. Schmidt, the composition of the blood is as outlined in the following table. This table, while giving the composition of the whole blood, may readily serve as one from which the composition of the corpuscles and serum may be obtained by reducing the constituents to parts per 1,000 of either corpuscles or serum. It is beyond the scope of this work to discuss the chemical properties of the blood or of its constituents in detail, but certain points which have clinical interest must be taken up.

The large excess of chlorin in the serum of man as compared with that of women and the excess of phosphoric acid in the serum of woman as compared with that of man is noteworthy. These variations may later be shown to have a great influence upon sexual differences as regards metabolism.

	Man's.		Woman's.	
Corpuscles	513.02		396.24	
Water		349.690		272.560
Solids		163.330		123.680
Hemoglobin, proteins, and other organic bodies		159.590		120.130
Inorganic bodies		3.740		3.550
K_2O		1.586		1.412
Na_2O		0.241		0.648
CaO, MgO, Fe_2O_3		0.320		0.485
Cl		0.898		0.362
P_2O_5		0.695		0.643

	Man's	Woman's.
Serum	486.98	603.76
Water	439.020	551.990
Solids	47.960	51.770
Protein and other organic bodies	43.820	46.700
Inorganic bodies.	4.140	5.070
K_2O	0.153	0.200
Na_2O	1.661	1.916
CaO, MgO	0.533	0.608
Cl	1.722	0.144
P_2O_5	0.071	2.202

(*A*). Total Solids.

The determination of the total solids and, hence, of the water content of the blood is often of importance, especially in cases of anemia. The method is as follows: Allow about 1 c.c. of blood to flow onto one of two previously weighed matched watch-glasses. Cover this with its mate and weigh them while in the moist condition. Separate the glasses and place them in a desiccator over $CaCl_2$ or H_2SO_4 for twenty-four hours, at the end of which time weigh them as before. The blood should have dried to a hard glassy mass in this time. The loss of weight will represent the water of the blood taken, from which amount the percentage may be calculated. Gumprecht and Stintzing and Biernacki advise the use of higher than room temperature, the former using 67° C. in an incubator, while the latter employs a heat of 100 to 120° C. Drying by these latter methods is often associated with loss; hence the writer is accustomed to use room temperature (about 20° C.).

Working in this way, many have found the dry residue (total solids) of the blood to be from 21 to 22.5 per cent., the water content being 77.5 to 79 per cent. This figure shows marked variation under the influence of such processes as diarrhea, excessive sweating, and exudation into serous cavities. In anemia the solids are much reduced, while in leukemia they are increased.

(*B*). Blood Pigments.

Hemoglobin.

The normal color of the blood is due to the presence of the pigments, hemoglobin and oxyhemoglobin. Usually the former is not large in amount, while the latter is the predominant factor in the color. In blood after asphyxia we find a mixture of hemoglobin, pseudohemoglobin, and parhemoglobin; in arterial blood large amounts of oxyhemoglobin, and in venous blood a mixture of hemoglobin and oxyhemoglobin.

Hemoglobin belongs to the class of bodies known as "chromoproteins" and, owing to its power of combining with various gases and thus aiding in the gaseous exchange of the body, may be styled a "respiratory protein." It is easily decomposed into a protein, globin (about 96 per cent.), and a pig-

ment, hemochromogen (4 per cent.), which contains iron and is easily changed in the presence of oxygen into hematin. The iron content of hemoglobin is the portion which enables this pigment to exert its peculiar vital power of oxygen transference.

The amount of hemoglobin in normal blood is variable, depending on the age of the subject examined. Normally, 100 c.c. of adult human blood contain from 13 to 14 grams of hemoglobin (the average being about 13.77), which amount is not a constant factor at all ages. From the following table of Leichtenstern it will be seen that a definite age curve exists for this substance.

Age.	*Grams per 100 c.c. of blood.*
1 to 4 days,	19.329 to 21.160
8 to 14 days,	17.869 to 16.124
8 to 20 weeks,	15.362 to 12.928
6 months to 5 years,	10.971 to 11.373
5 to 15 years,	11.151 to 11.796
15 to 25 years,	13.034 to 13.870
25 to 45 years,	14.727 to 15.013
45 to 60 years,	12.484 to 13.150
Over 60 years,	14.790

Under certain conditions the hemoglobin is dissolved out from the red cells, leaving only their stromata behind. This gives rise to the condition known as hemoglobinemia which will be discussed in a later section. According as hemoglobin is free or combined with certain gases, we have in the blood various derivatives of this pigment, each one of which has certain characteristics and a definite clinical importance. These derivatives are recognized by



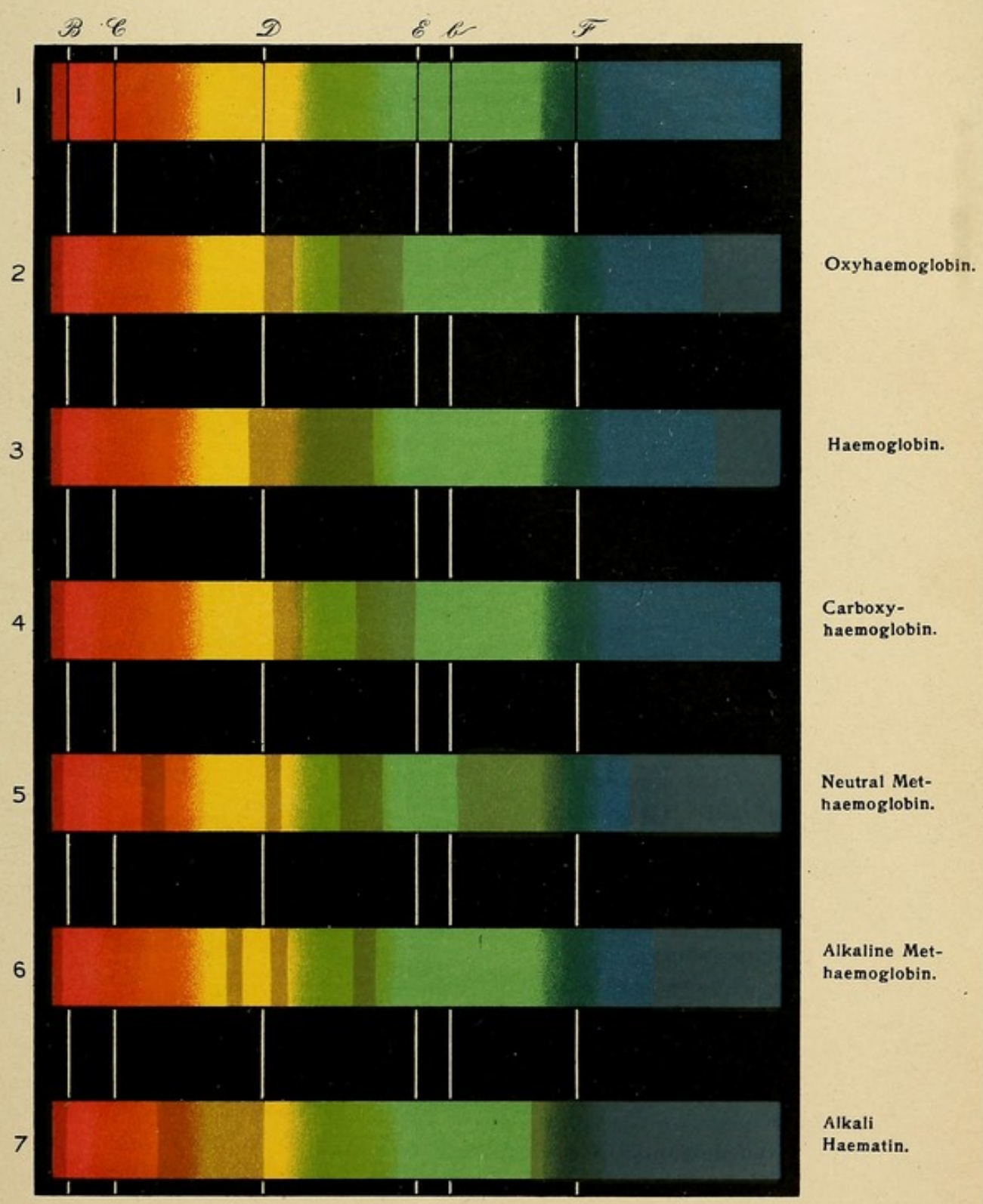
FIG. 124.—Direct-vision spectroscope.

qualitative tests, especially by spectroscopic methods, and will be presented here; while the important tests used for the recognition of hemoglobin, either in the fresh state or as dried stains, will be taken up under the medicolegal discussion.

Hemoglobin, also called reduced hemoglobin, is much more soluble than oxyhemoglobin, its solution in water being more violet or purplish than one of oxyhemoglobin of the same concentration. Such solutions of hemoglobin absorb the blue and violet rays of the spectrum to a less marked degree than do those of oxyhemoglobin, but they strongly absorb the rays lying between *C* and *D*. In proper dilution a solution of hemoglobin shows a spectrum with one broad not clearly defined band between *D* and *E*, lying toward the red end of the spectrum a little over the Fraunhofer line *D* (See plate).

Absorption Spectra.

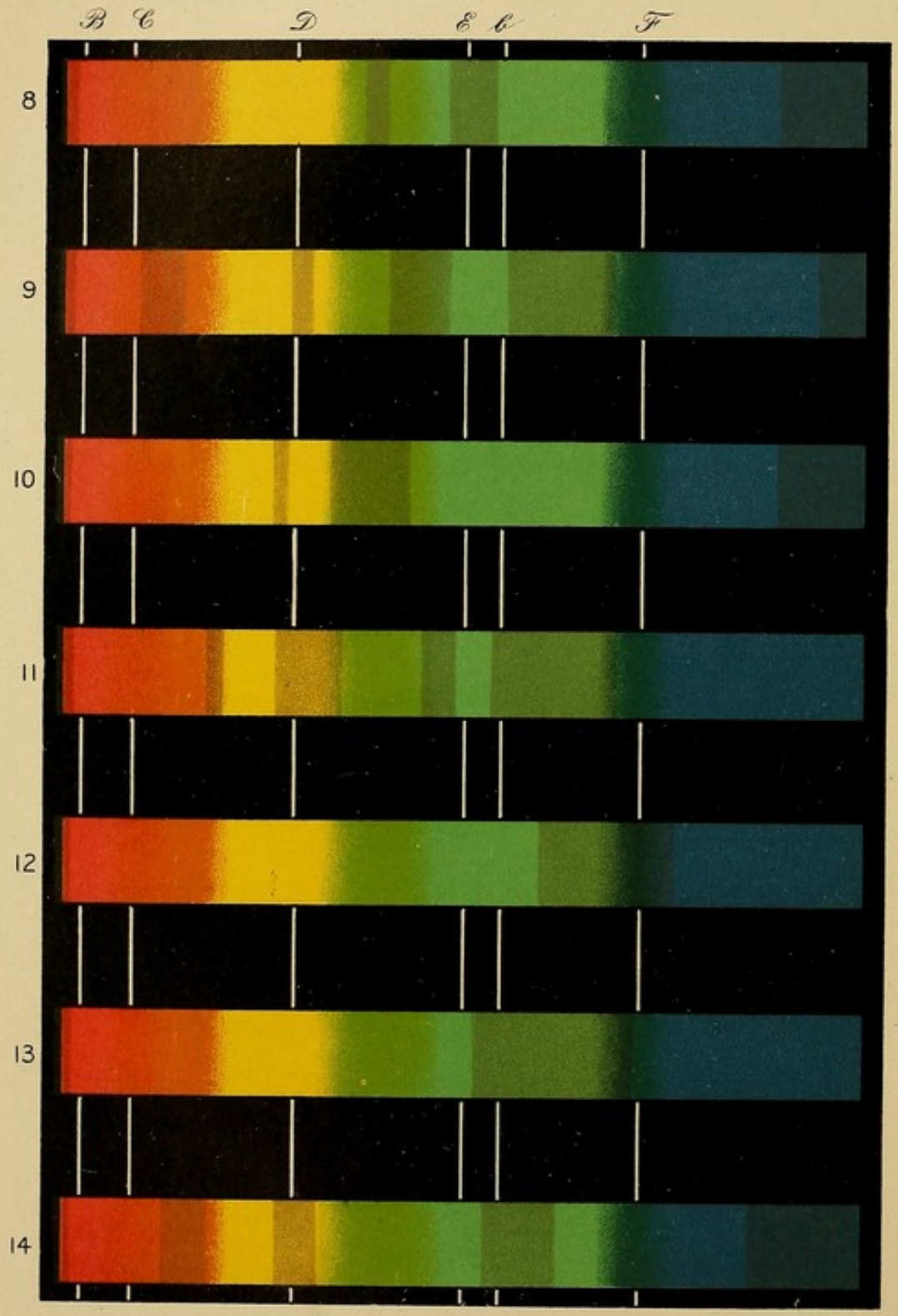
PLATE XIV.



(FROM HAWK'S "PHYSIOLOGICAL CHEMISTRY")

Absorption Spectra.

PLATE XV.



8 Reduced Alkali Haematin or Haemochromogen.

9 Acid Haematin in ethereal solution.

10 Acid Haematoporphyrin.

11 Alkaline Haematoporphyrin.

12 Urobilin or Hydrobilirubin in acid solution.

13 Urobilin or Hydrobilirubin in alkaline solution after the addition of zinc chloride solution.

14 Bilicyanin or Cholecyanin in alkaline solution.

(FROM HAWK'S "PHYSIOLOGICAL CHEMISTRY")

Pseudohemoglobin.

According to Ludwig and Siegfried, blood, reduced by hyposulphites or by a stream of hydrogen to such an extent that the spectrum of oxyhemoglobin disappears, yields large amounts of oxygen when exposed to a vacuum. This loose combination of hemoglobin and oxygen, which gives the spectrum of hemoglobin, is called pseudohemoglobin. Hammarsten considers it an intermediate body between hemoglobin and oxyhemoglobin.

Oxyhemoglobin.

Oxyhemoglobin, also called hematoglobulin, is a molecular combination of hemoglobin and oxygen. The ability of hemoglobin to take up oxygen is a function of its iron content. When this factor is calculated as 0.33–0.40 per cent., 1 atom of iron in the hemoglobin molecule corresponds to two atoms (one molecule) of oxygen. This combination is a loose one and hence the quantity taken up will depend upon the partial pressure of the oxygen. This oxygen is set free when the oxygen pressure is reduced, thus giving rise to the characteristic property of oxygen transference. As Pflüger has shown, oxyhemoglobin may, when it is gradually oxidized, act as an "ozone exciter" by the decomposition of neutral oxygen into the atomic form. It may also act as an "ozone transmitter" as in certain tests to be outlined later.

A dilute solution of oxyhemoglobin or of arterial blood shows a spectrum with two absorption bands between the Fraunhofer lines *D* and *E*. One band, α, is narrower, but darker and sharper and lies on the line *D*, the other, β, is broader, less defined and less dark and lies at *E*. As the dilution becomes weaker the band β first disappears. By increased concentration the two bands become broader, the space between them smaller, and the blue and violet parts of the spectrum darkened. Other substances may give this same absorption spectrum, but oxyhemoglobin may be differentiated by its behavior toward reducing agents, such as ammonium sulphid or Stokes' solution of ammoniacal ferrotartrate (see plate).

Methemoglobin.

This pigment is closely related to oxyhemoglobin, as it contains the same amount of oxygen and is isomeric with it. The oxygen is, however, not in loose combination and cannot, therefore, be utilized by the system. This coloring matter is formed by the spontaneous decomposition of blood, as observed in hemorrhagic transudates and cystic fluids, and occurs also in cases of poisoning with potassium permanganate, potassium ferricyanid, chlorates, nitrites, nitrobenzol, acetanilid, antipyrin, turpentine, sulphonal, and arsenic.

According to Jäderholm and Bertin-Sans, the absorption spectrum of methemoglobin, in aqueous or slightly acidified solution, is similar to that of acid hematin (see below), but is easily distinguished from the latter by the readiness with which it turns into that of hemoglobin on treatment with alkali and a reducing substance. Hematin, under the same conditions, gives the

spectrum of an alkaline solution of hemochromogen (Hammarsten). Methemoglobin, in alkaline solution, shows two absorption bands, which are like those of oxyhemoglobin, but differ from them in that the band nearer *E* is stronger than the one at *D*. A third fainter band may be observed, according to Hammarsten, lying between *C* and *D*.

Carbon-monoxid Hemoglobin.

This pigment is a molecular combination of one molecule of hemoglobin with one molecule of CO. This combination is stronger than those of hemoglobin and oxygen. The oxygen of hemoglobin is easily replaced, therefore, by CO and, in consequence, the tissues suffer for want of oxygen. This pigment imparts to the blood a bright cherry-red color both in the venous and arterial circulation.

The most common cause of formation of this pigment is the inhalation of coal gas or of illuminating gas. The characteristic color in these cases may disappear after a few hours or it may persist for days, depending on the severity of the cause, being frequently found in the blood after death. Such blood shows an absorption spectrum similar to that of oxyhemoglobin, but the two bands lie more toward the violet end of the spectrum than in the case of oxyhemoglobin. On the addition of a reducing agent to blood showing this spectrum, the absorption bands of carbon-monoxid hemoglobin are unaffected, while those of oxyhemoglobin are changed to those of hemoglobin. This spectroscopic test is not delicate as less than 20 per cent. of carbon monoxid hemoglobin in the blood is difficultily, if at all, detected.

Carbon-dioxid Hemoglobin.

According to Bohr, hemoglobin forms three molecular combinations with CO_2, in which products, α, β, and γ, one gram of hemoglobin combines with 1.5, 3, and 6 c.c. of CO_2. The spectrum of these compounds is similar to that of reduced hemoglobin. If a large excess of CO_2 be present the hemoglobin is decomposed and globin is precipitated. The absorption band under these conditions is probably referable to the presence of free hemochromogen.

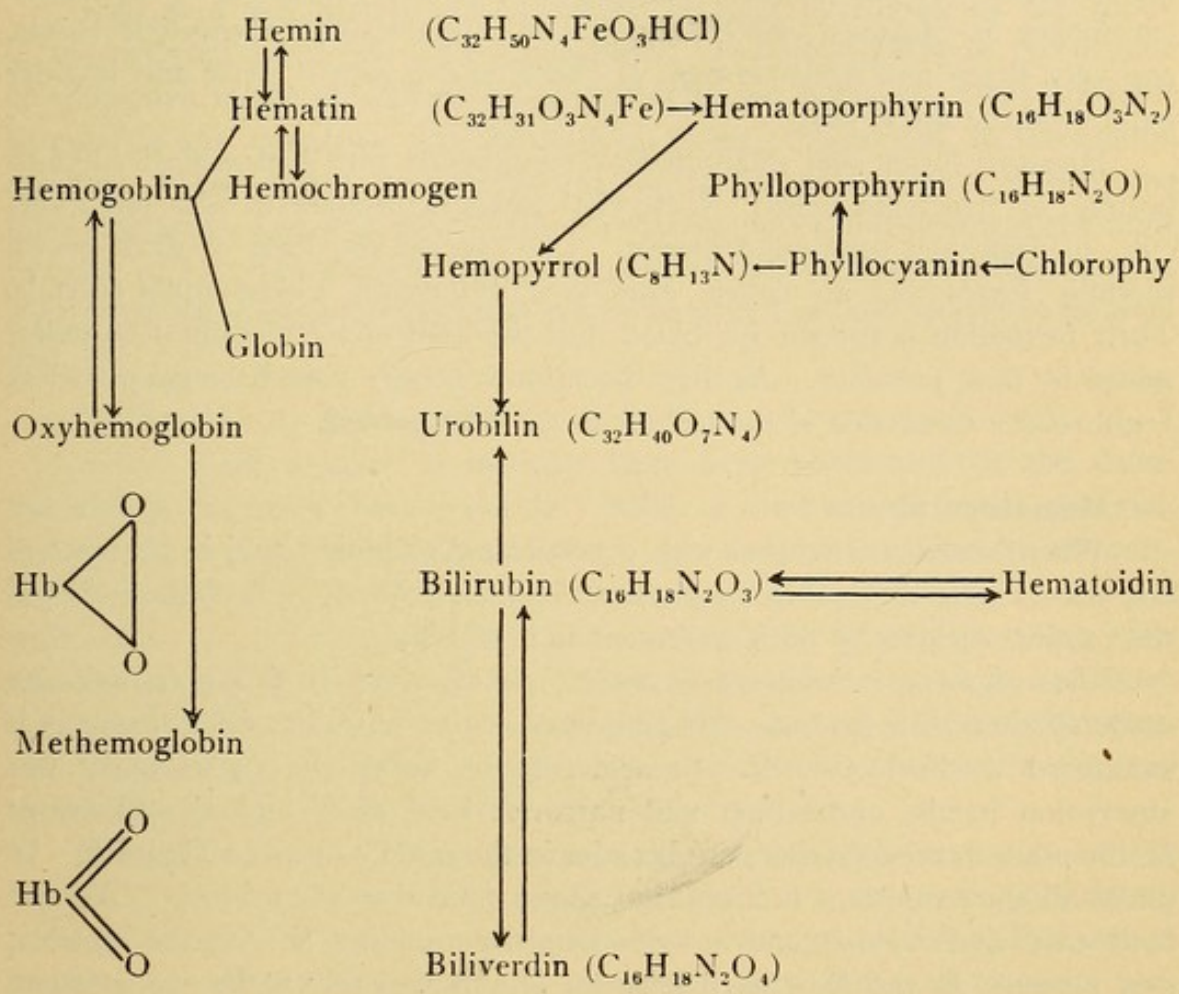
Nitric-oxid Hemoglobin.

Hemoglobin forms with nitric oxid, NO, a molecular combination which is even stronger than that with CO. The absorption spectrum is similar to that of CO-hemoglobin, but the two absorption bands are paler and less distinct than are those of the latter pigment and do not disappear on addition of a reducing agent.

Other compounds of hemoglobin with various gases are known, but little of clinical value is forthcoming from their study. Thus hydrogen sulphid, hydrocyanic acid, and acetylene form combinations which interfere with the proper oxygen exchange and cause death by asphyxiation or, more accurately speaking, from oxygen hunger.

Decomposition Products of Blood Pigments.

As stated previously, hemoglobin and oxyhemoglobin are proteins, which are converted under the action of different physical and chemical means into a globulin-like protein, globin, and an iron-containing pigment, hemochromogen. From the table below, adapted from Webster and Koch,[1] may be seen the relations of hemoglobin to its various derivatives and also to the bile pigments and to chlorophyl.



Hematin.

This decomposition product is found in any situation in which oxyhemoglobin is destroyed; thus, in the digestive tract where it is formed by the action of gastric or pancreatic juice on oxyhemoglobin, in old extravasations, in the stools after hemorrhage, in urine after poisoning with arsenical compounds, and in the blood of persons poisoned with nitrobenzol and acetanilid. Hematin is a dark brown or blue-black amorphous powder, insoluble in water, dilute acids, alcohol, ether, and chloroform, but readily soluble in acidified alcohol or ether and in dilute alkalies.

Arnold has shown that there are three modifications of this pigment, a neutral, an alkaline, and an acid hematin, each showing spectroscopic differences. The neutral hematin has little importance clinically and will be passed

[1] Laboratory Manual of Physiological Chemistry, Chicago, 1903.

in this discussion. Acid-hematin solutions show four absorption bands, one between *C* and *D*, a second broad but not clearly defined band between *D* and *F*, which divides under certain conditions into two narrower bands, a fourth band between *D* and *E* which is nearer *D* and is very weak. Usually only the band between *C* and *D* and the broad band between *D* and *F* can be seen (see plate).

Solutions of alkaline hematin show one absorption band between *C* and *D* which reaches out to some extent between *D* and *E*. If the alkaline hematin solution be reduced with ammonium sulphid, the spectrum of hemochromogen is observed, which shows two characteristic absorption bands, one very sharp and dark between *D* and *E* and a second paler and broader band covering the *E* line.

Hematin forms very characteristic compounds with hydrochloric, hydrobromic, or hydriodic acids. With HCl hematin crystallizes with one molecule to form the compound hemin. The crystals are light or dark brown rhombic forms and are called, after their discoverer, Teichmann's crystals. Their formation is specific for blood, but the kind of blood cannot be determined by their presence. As their detection is largely a medicolegal question, I will refer a discussion of the technic to the later section.

Hematoporphyrin.

When hematin is treated with concentrated sulphuric acid in presence of air, iron is split off leaving the pigment hematoporphyrin. If air be excluded the product yielded by such treatment is hematolin.

This pigment is insoluble in water, but dissolves in alcohol, strong and weak alkalies, and in acids. It is isomeric with bilirubin, with which it is associated in the liver cells. In acid solution hematoporphyrin shows two absorption bands, one fainter and narrower between *C* and *D* and nearer *D*, the other darker, sharper, and broader in the middle between *D* and *E*. In dilute alkaline solutions this pigment shows four absorption bands. A band between *C* and *D*, a second broader band surrounding *D* with the broadest part between *D* and *E*, a third between *D* and *E* nearly at *E*, and a fourth broad and dark band between *E* and *F*. Hematoporphyrin is of great importance from the medicolegal standpoint as certain suspected stains may be identified only by its spectrum. For this phase see the later sections.

Hematoidin.

This ruby-red or reddish-yellow pigment is derived from blood coloring matter and like hematoporphyrin is iron free. It is found in old blood-clots, in hemorrhagic exudates, in sputum, and in feces. It is more abundant when the blood pigment is not much exposed to the action of living cells, as in the center of large extravasations and in hemorrhages into preformed cavities of the body (Ziegler). Hematoidin is identical with bilirubin and shows no absorption bands, but only a strong absorption of the violet to the green portion of the spectrum.

Hemosiderin.

This yellow, orange, or brown pigment is a derivative of hemoglobin and contains iron. Unlike hematoidin, it is found more particularly in extravasated blood which has been subjected to the action of living cells. After a time this pigment changes into one (probably hematoidin) which contains no iron.

Melanin.

This is a yellowish-brown or black pigment produced by the action of the malarial parasites upon hemoglobin. It is found free in the plasma or embedded in the protoplasm of the red cells and parasites. It is insoluble in most of the menstrua, but dissolves in strong alkalies and in ammonium sulphid. Pigment has been found in the leucocytes in cases of relapsing fever, melanotic sarcoma, and Addison's disease, but whether this is the same melanin as found in malaria is questionable, as the malarial melanin contains iron, while the so-called melanin of relapsing fever has been shown to be iron-free.

Estimation of Hemoglobin.

A very large number of methods have been introduced for the determination of the blood coloring matter. Some of these are extremely accurate, but are too complicated for general clinical purposes; others are less accurate, but are more applicable to our work. It may be said that, as a rule, the clinical instruments at our command will give comparative figures, providing their construction and their standardization is accurate. However, we find so much inaccuracy or even lack of proper standardization in some of these instruments, that no absolute comparison may be attempted between the results obtained by various workers using the same or different methods.

Normal adult blood contains about 13.77 grams of hemoglobin per 100 c.c. of blood, this figure being subject, however, to variations at different periods of life, so that those instruments which are standardized against normal adult blood and which furnish the amount of hemoglobin in terms of percentage of such blood cannot be absolutely accurate no matter how perfectly constructed or how accurately standardized, when they are used for the estimation of hemoglobin in the blood of a child or of an elderly patient. It is, therefore, much better practice to obtain the values in terms of the actual amount per 100 c.c. of blood. It is well to recognize, as Türk points out, that we are working with possible errors and can obtain comparative results only when we use the same exactitude and care in each of our estimations. The errors are essentially constant, but we must remember that the arbitrary color standards change as time goes on, necessitating a restandardization of our instruments if accurate results are to follow. The writer has seen errors of 50 per cent. arise from the use of an old von Fleischl instrument and one of 25 per cent. from a faulty standardization of Sahli's solution.

It must be insisted upon that we must not assume that a patient, who is

pale and "anemic looking," is in reality anemic. Pallor depends not only upon the amount of pigment in the blood but also upon the delicacy and transparency of the skin and upon the superficial distribution and size of the blood-vessels. By remembering these points we may often save ourselves unnecessary chagrin on finding that the results of our determinations do not accord with the "anemic" expression of the patient. A simple puncture of the ear, allowing the blood to drop upon a clean linen towel (so-called "towel test"), will many times set us right and prevent a diagnosis of anemia without further examination of the blood. Such examinationless diagnoses are unwarranted and inexcusable.

Direct Methods of Estimation.

These methods are, owing to their complexity, not applicable to clinical work. The spectrophotometer of Hüfner is undoubtedly the very best and most accurate method, but I must refer to the original article for its description. Likewise the colorimetric double pipet of Hoppe-Seyler and the methods of Nebelthau and of Zangemeister must be neglected.

Indirect Colorimetric Methods.

These indirect methods employ a comparison of the blood solution with a second medium which approximates the blood in color. Two principles are possible in such methods. We may either have a fixed unchangeable medium of comparison, with which the blood is matched by constant dilution, or we may use a fixed blood solution of known strength and compare therewith the standard graduated color scale. Both of these principles have been used in such determinations, the variations being shown in the fact that instruments for this purpose have been introduced by Gowers, Sahli, Hayem, Malassez, Henocque, Bizzozero, von Fleischl, Miescher, Haldane, Grützner, Gärtner, Dare, Oliver, and Tallqvist.

It is evident, *a priori*, that these indirect methods must have greater errors than the direct. Two things are essential for any exactitude whatever. First, the medium of comparison must agree in the most complete manner with the various color tones which the blood shows at different percentage values of hemoglobin. Second, the standardization of the medium of comparison must be extremely accurate. We must have no illusions regarding the exactness of the hemoglobin values obtained by these methods, as it is very difficult to construct two instruments of the same kind that will agree exactly. Certainly no two instruments of different make will agree, but comparative results sufficiently accurate for clinical purposes are obtained by the use of the more reliable instruments. The personal equation in reading the color comparisons must be remembered, as some individuals show abnormal sensitiveness or lack of sensitiveness to shadings of red. It is an impossibility in this book to give in detail all of the methods advanced for the estimation of hemoglobin. I select, therefore, those that have proven most reliable in my hands.

Hemometer of Fleischl-Miescher.

Up to recent times the most frequently used of the instruments for the estimation of hemoglobin was the old von Fleischl instrument. With the introduction of the Miescher modification, this original form has been or should be less often employed. We avoid, therefore, a discussion of the older instrument, referring to other works which have included it.

This new apparatus, made by Reichert, under the direction of Miescher, is similar in general appearance to the old von Fleischl. It has the same stand and the same scale principle, although this latter is standardized differently and graduated on a different basis. It differs, materially, in the method of measuring and diluting the blood, in the form of the comparison chamber, and in the meaning of the graduation of the scale (see cut and legend for its description).

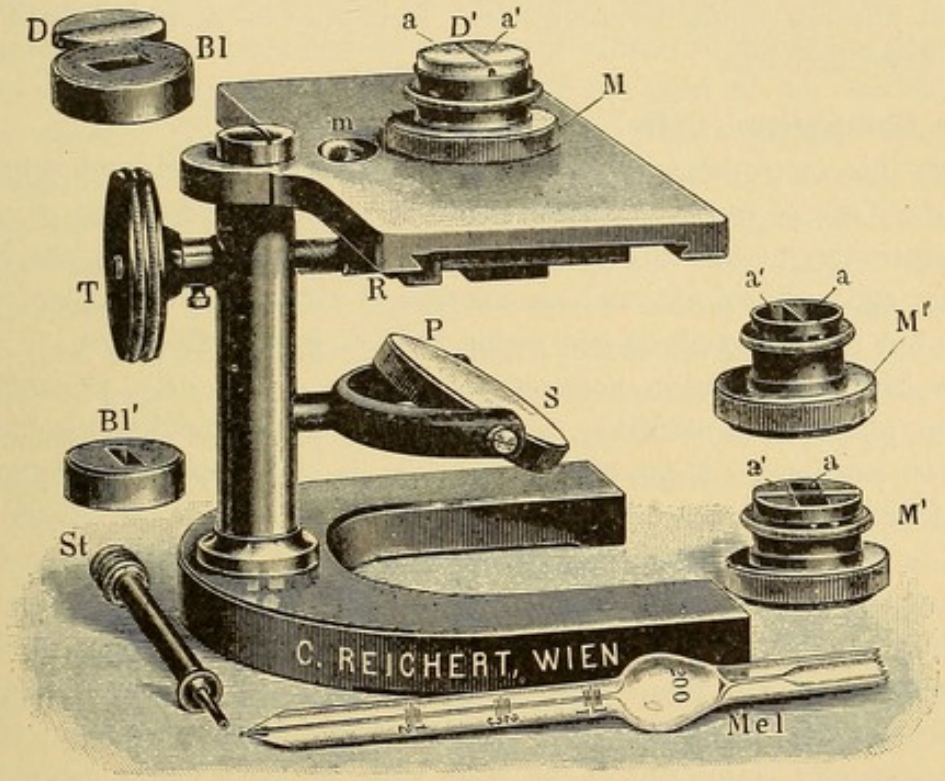


FIG. 125.—Hemometer of Fleischl-Miescher: *R*, Stage; T, milled head, which moves the color scale; *m*, opening in stage through which the instrument is read; *M*, mixing cell; *D'*, cover glass; *D*, cap; *PS*, gypsum mirror from which light is reflected; *mel*, diluting pipet.

The Diluting Pipet.

This is similar in construction to the pipet of the Thoma-Zeiss hemocytometer, its calibrations, however, being different. The marks are 1/2, 2/3, and 1. Above and below each of these main divisions are two marks each corresponding to 1/100 of the contents of the capillary tube. This device enables the worker to measure accurately the column of blood taken, in case he gets too little or too much blood in the tube. The relation of the capillary to the ampulla is such that blood, drawn to the mark 1 and diluted to the mark above the ampulla, receives a dilution of 200; if drawn

to the mark 2/3 the dilution is 300; while the line 1/2 furnishes a dilution of 400. The diluent used is 1/10 per cent. sodium carbonate solution. This dissolves the stromata of the red cells furnishing a clear solution. Occasionally the diluent becomes turbid after standing some time, and should be freshly made and should contain no bicarbonate.

The taking of the blood and the mixing with a diluent is done with the same precautions mentioned under the method of making the blood count. In choosing the proper dilution, one should select that which will enable him to use the central portions of the graduated scale. A dilution of 400 is usually applicable except in cases in which marked anemia is suspected when dilutions of 300, 200, or even 100 should be made. In making this last dilution the erythrocytometer may be used. In doing accurate work, it is well to make a preliminary determination of hemoglobin in order to tell, the better, just what dilution would be advisable. This may be done by the Tallqvist method described later.

The Comparison Cells.

With this instrument two cells for holding the blood and diluent are furnished. One of these has a depth of 15 mm., the other one of 12 mm. the former is the standard cell, the latter the control, giving 4/5 the value of the larger cell. Their external appearance is similar to the cell of the old Fleischl, but their capacity is less owing to their greater thickness.

The dividing partition between the halves of the cells projects about 1/2 mm. above the borders, thus preventing any mixing of the fluids in the two portions. A grooved cover-glass is slid over the compartments without fear of mixing the fluids. If fluid is lost no error is introduced, as the dilution is uniform in the pipet and the depth of the chamber is definite. A diaphragm, with an opening 4 mm. wide, is placed over the cover-glass after this latter has been adjusted, the opening being so placed that its long axis is perpendicular to that of the vertical partition. The field of comparison is thus limited to 4 mm., which corresponds to about 3° of the scale, affording a comparison of a single tint of the scale with the color of the diluted blood.

Graduation of the Scale.

The slide for comparison of color is tinted with Cassius' golden-purple, as in the old Fleischl instrument. The graduations of this color scale are made by comparison with standard solution of hemoglobin and not with the arbitrary standard of blood of so-called normal individuals. The scale shows the same divisions as that of the Fleischl instrument, but their significance is different in the Miescher modification. Here one does not read directly the percentage of hemoglobin, but must obtain the corresponding value by reference to a "table of calibrations," which accompanies each instrument. While the figure thus obtained is in terms of actual percentage (grams per 100 c.c.), it is quite different from the percentage figure given by the Fleischl instrument.

Method.

After testing the chamber by placing in one compartment the diluting fluid in order to see that none runs into the other, the blood is drawn into the pipet to the desired point and diluted to the mark, all precautions mentioned under Blood Counting being observed. Thoroughly mix the blood and diluent by shaking and blow out the unmixed contents of the capillary tube. Fill one compartment of the 15 mm. cell with the diluted blood so that a convex meniscus appears above the border of the chamber, the other compartment being filled with the diluent or with distilled water. Adjust the grooved slide and cap, place the cell in the central opening of the stand, and adjust the light. This latter portion of the technic is of considerable moment, as this instrument gives the best results when used in a dark room illuminated only by a small candle flame which is placed about 18 inches from the stand and to the side. In the absence of a dark room, a light-proof shield for the eyes may serve. This may readily be obtained by the use of a tube of stiff, dark paper, which fits over the comparison cell and shuts off the light of the candle from the field of vision. The observer should stand in such a position that he looks into the cell from the side and not from the front or back, the eyes being about one foot above the cell. In the comparison of the color tones the variations are much better seen by quick movements of the slide rather than by slow gradual changes. The eyes should be rested at short intervals to prevent fatigue and lack of sensitiveness to the different shadings of color. When the color is matched take the reading of the instrument, by observing what mark of the scale coincides with the notch on the edge of the opening above the scale, and control this reading by several duplicates.

Remove the blood from the 15 mm. cell by means of the pipet and transfer it to one compartment of the 12 mm. chamber, tested as was the larger one. Adjust this chamber and make readings in the same way. These values should be only four-fifths of those obtained with the 15 mm. cell. This modification has the advantage of using different portions of the color scale and should give comparative figures. If there should be any variation, which should never be over 1 per cent., a correction may be made.

Calculation of Results.

This is possible only with the use of the "table of calibrations," which contains the series of scale divisions and the absolute amount of hemoglobin in mg. per 1,000 c.c. of blood, corresponding to each division of the scale when the 15 mm. chamber is used. Thus, if the scale shows 56, we find the value corresponding to this to be 447 mg. of hemoglobin in 1,000 c.c. of diluted blood. As the dilution may have been 400, we would have, in 1,000 c.c. of undiluted blood, 400 x 447 = 178.8 grams. As we wish to know the amount in 100 c.c. we have merely to divide by 10, thus obtaining 17.88 grams of hemoglobin. If we wish to get the percentage figures, as read on the old Fleischl, we divide the figure obtained with the Miescher, in this case 17.88,

by the amount of hemoglobin corresponding to the 100 division of the scale of the instrument used, in this case 14, and we obtain 127.7 per cent.

This Miescher modification is our very best clinical apparatus for estimation of hemoglobin, giving results which are accurate within 0.2 to 0.5 per cent. It is open to the objections that it is bulky, expensive, and requires more time and practice for its use than is at the control of the busy practitioner. For hospital use, however, it is the instrument *par excellence* and should never be substituted by others. It has one disadvantage that each instrument is standardized, not against a known hemoglobin solution, but against a so-called "normal" instrument, which has been properly standardized. It is to be hoped that each instrument will, in the future, be properly calibrated and thus insure us against the possibility of errors arising from any change in the standardization of the "normal" instrument.

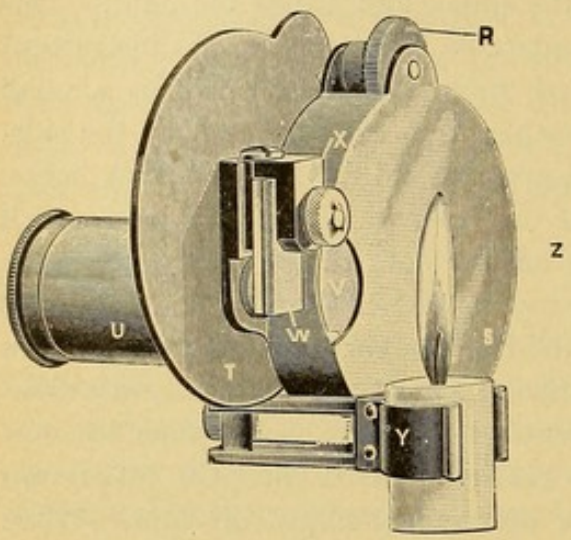


FIG. 126.—Hemoglobinometer of Dare; R, milled wheel; S, case inclosing the color disc; T, movable wing, which is swung outward; U, telescoping camera; V, aperture admitting light; W, capillary blood pipet; Y, detachable candle holder; Z, slot through which the percentage of hemoglobin is read.

Hemoglobinometer of Dare.

The instrument, introduced by Dare, has the advantage of using undiluted blood, and avoids any error consequent upon dilution. The principle of this instrument is as follows: The color of undiluted blood is compared by artificial light with that of a graduated glass scale colored with golden-purple, the 100-point of which is standardized against a solution of 13.77 grams of hemoglobin in 100 c.c. of serum. For a description of the apparatus see cut.

Method.

Swing outward the movable screen, which serves as a cover for the case, adjust the camera tube, and fit the candle attachment in its place opposite the camera tube. The candle should be so adjusted that its upper end is flush with the top of the clips which hold it. If the wick be curved, it should be so turned that the intensity of the light is midway between the two apertures. See that the pipet, composed of the rectangular glass plates, is thoroughly cleansed and dry. The space between the plates is filled by applying the edge of the pipet to the side of a fairly large drop of blood. Adjust this pipet in its place and rotate the colored scale, by means of the milled screw, until the colors match. Hold the instrument steady to prevent the flickering of the flame as much as possible. No dark room is necessary, but it is advisable to point the instrument at some dark object and to avoid direct sunlight, as the shadings of color are not so easily matched by direct daylight. As soon as

the colors are matched make the readings and check the results with several duplicates. This reading is observed on the left side of the case in the small open space, the line which coincides with the beveled edge of the opening representing the percentage of hemoglobin, on the basis of a value of 13.77 grams of hemoglobin per 100 c.c. as 100 per cent. It is, therefore, easy to calculate the direct amount of hemoglobin in the blood examined.

This instrument has the advantages that undiluted blood is used, that the scale of comparison is usually very accurately standardized, that it is convenient, easy of manipulation, and rapid in giving results. Coagulation of the blood does not occur sufficiently soon to introduce an error, providing the reading is taken within a reasonable time. It is more convenient for general

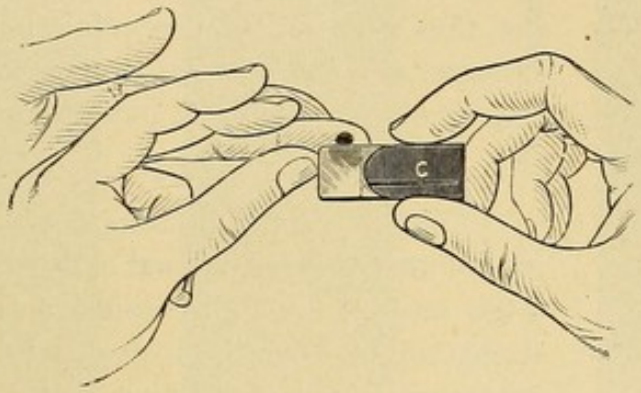


FIG. 127.—Method of filling the Dare blood-pipet. (*Da Costa.*)

use than is the Miescher, is less expensive, can be used in a light room, and gives results second only to those of the Miescher. The disadvantages of this instrument are that an occasional faulty standardization may introduce errors, it costs much more than some of the instruments to be described, and it is not a long-lived instrument unless care is taken in handling it. In the writer's laboratory this instrument has given great satisfaction and can be recommended for general use on the ground of its convenience and ready application to clinical work.

Hemometer of Sahli.

This instrument is a new modification of the older hemoglobinometer of Gowers, and has so many advantages over the older instrument that this latter will be passed over. A modification of the Gowers instrument introduced by Haldane is simple and reliable, but has the disadvantage that coal gas is used in converting the hemoglobin into CO hemoglobin, and consequently, is not of easy application for bedside work.

As Sahli has pointed out, a colored fluid under examination should not be compared with a different substance similar in color, but with a solution of known strength of the same coloring matter. His modification of the Gowers method employs an instrument constructed on exactly the same lines, but using a different standard of comparison. This standard of comparison

is an acid hematin solution in a concentration corresponding to a 1 per cent. solution of normal blood. This solution is somewhat dark, as it is standardized against blood showing high hemoglobin value. As Sahli states, the standard fluid, as furnished with his hemometer, corresponds to a blood which shows with the Miescher instrument at a dilution of 200 a reading of 109, or an absolute quantity of 17.2 grams of hemoglobin per 100 c.c. of blood. The fine particles of hemin, which are in suspension, may adhere to the glass, especially if the instrument lies unused for some time. This process changes the color of the standard to some extent, so that it is supposed that deterioration has occurred. This may be remedied by completely inverting the tube, without violent shaking, thus allowing the precipitate to diffuse uniformly. Occasionally one of these instruments is found to be improperly standardized, but this is rare.

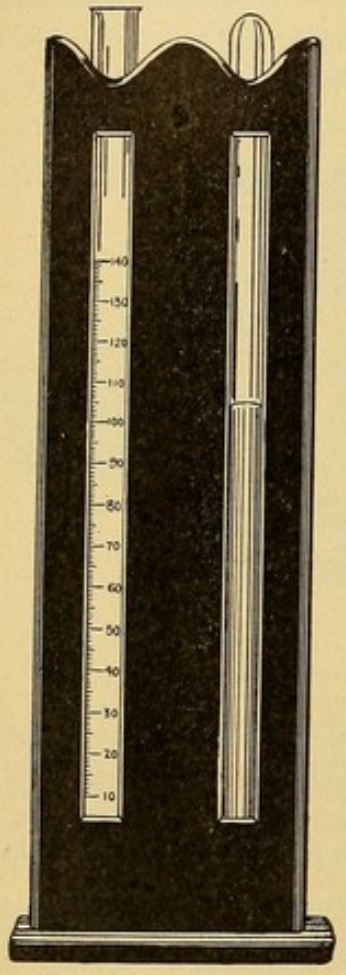


FIG. 128.—Hemometer of Sahli. (*Greene*)

Method.

This consists in diluting the blood with 10 times its volume of N/10 normal hydrochloric acid. After a few seconds the fluid becomes dark brown from the formation of hematin hydrochlorid (hemin), which substance is not in solution, but in fine suspension. The blood is taken with the 20 cmm. pipet and blown into the graduated tube, which contains N/10 normal HCl up to the mark 10. This acid may be accurately enough made by diluting 15 c.c. of concentrated chemically pure HCl to 1,000 c.c. Shake the mixture of blood and acid and dilute with ordinary water as soon as a clear dark brown color is visible. Add water until the shade of the mixture corresponds exactly with that of the standard solution, when the percentage of hemoglobin may be read off. The water should be added very carefully from a dropping pipet, as the accuracy of the method depends upon adding the exact amount of fluid necessary. The comparison of colors may be made in any light, as the two solutions, being the same, will be similarly affected.

This instrument is very conveniently gotten up, being mounted in a case carrying a white-glass plate to reflect the light to better advantage. It is simple, inexpensive, and is accurate within 2 per cent. The author would recommend this instrument to the general worker above all others, with the possible exception of the Dare, whose advantages have been mentioned.

Hemoglobinometer of Oliver.

In this method the color of the blood in a definite dilution is compared, by light reflected from a white surface, with a series of tinted glass standards.

Such a method has the advantage that the color of the diluted blood is compared with one single tint at one time. The standard glass disks correspond accurately, as determined by the tintometer, to the specific color curve of

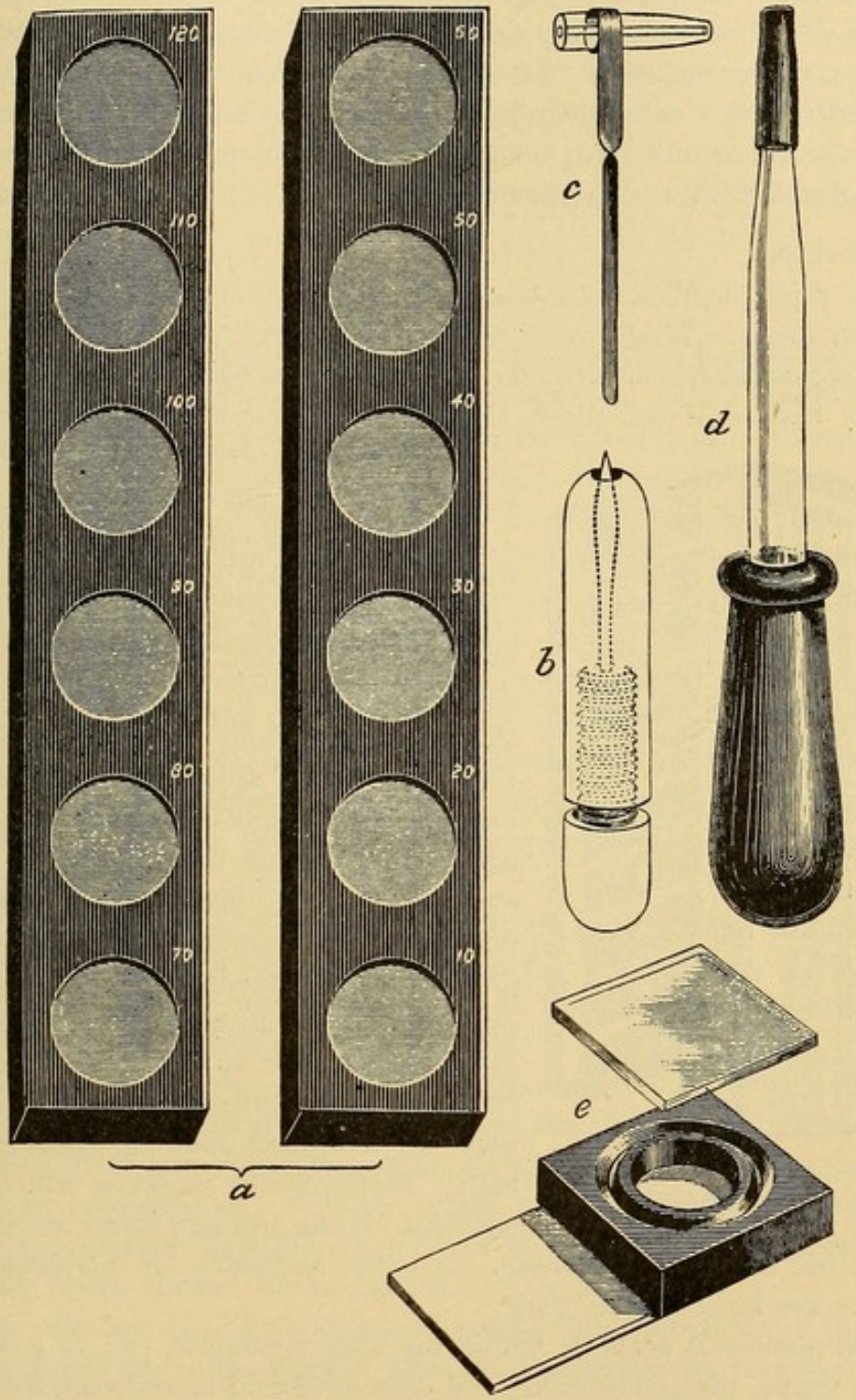


FIG. 129.—Hemoglobinometer of Oliver. (*Coplin.*)

progressive dilutions of normal blood. Two sets of standards are furnished, one for use in daylight, the other by candlelight, the latter being preferable. Each set of 12 disks is mounted on plaster of Paris and enclosed in two wooden frames, six disks in each frame. They represent the color of solu-

tions of hemoglobin with percentages from 10 to 120. To obtain intermediate values, colored riders are used, each representing 2.5 and 5 per cent., of hemoglobin when used with the disks from 70 to 120, but twice these values if used with the disks from 10 to 60.

The blood is taken in a capillary pipet similar to that of von Fleischl, holding 5 cmm. of blood. The diluting chamber has a white background of plaster of Paris and, when filled with distilled water in which one pipetful of blood has been dissolved, yields a solution of 1 per cent. When filled the cell is covered with a blue glass cover.

Method.

The pipet is filled with blood drawn by capillarity from a puncture of the ear or finger. Wash the blood from the pipet into the diluting chamber by means of water from a medicine dropper, fill the chamber with water, stir well with the handle of the pipet, and adjust the cover-glass. A small bubble of air should be included as an evidence that the chamber was not overfilled.

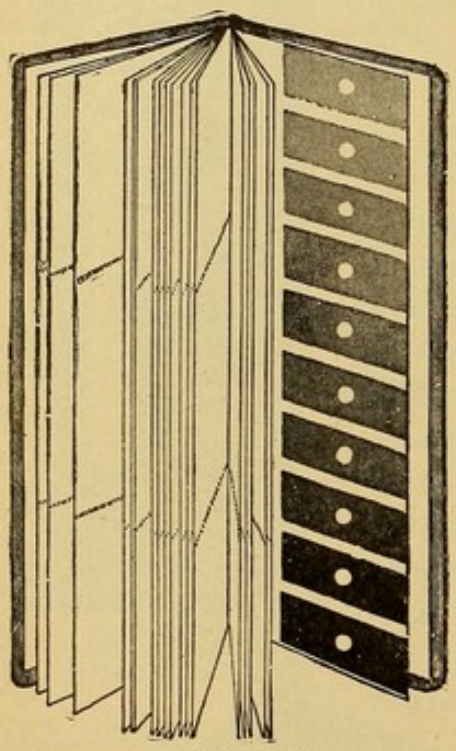


FIG. 130.—Tallqvist's hemoglobinometer.

Compare the color of this solution with that of the standard disks, using the light of a candle placed 3 or 4 inches away in such a manner that the light strikes the two chambers alike. It is well to use the camera tube to shield the eyes and enable one to compare the tints more accurately. If the color matches that of any of the disks, the percentage is read directly from the case. If, however, the color is intermediate between two disks, the riders must be superimposed on the disk of lower percentage, and a second comparison made by using a corresponding plate of unstained glass as a rider on the diluting chamber. The adjustment of tints and riders must be continued until the colors match exactly. Naturally the variation will equal two per cent. as the ordinary riders are equivalent to 2 1/2 per cent. of hemoglobin. It is not necessary for this determination that the room be absolutely dark.

This method is accurate within the limit mentioned (2.5 to 5 per cent.), but it is trying, time consuming, and does not equal in accuracy the methods of Dare and of Sahli. The instrument is expensive and the disks are prone to deteriorate.

Tallqvist's Hemoglobinometer.

Tallqvist has introduced a method for the estimation of hemoglobin which is so simple that certain writers have been led to recommend it above

other methods. The principle of this method is essentially the same as Oliver's method, although the application is entirely different. Tallqvist compares the color of the undiluted blood with that of a series of lithographed standard tints, which range by differences of 10° from 10 to 100 per cent. These standard tints were prepared by matching in water colors the tint of the blood of various patients (whose hemoglobin values had been determined with the von Fleischl instrument) when soaked into standard filter-paper. These tints were then lithographed and bound up with sheets of filter-paper, the combination making a very simple and convenient book which may be easily carried in the pocket.

Method.

Allow a fairly good-sized drop of blood to soak into a portion of the filter-paper by holding the edge of the sheet against the drop. Care should be taken to allow this to take place very slowly so that the color may be uniform throughout. If carefully done it will not be necessary to blot the stain, but sometimes this is essential. As soon as the stain has lost its humid gloss compare its color with that of the lithographed scale. Do not allow the stain to become dry, as the color comparisons are disturbed by the coagulation. Hold the scale and the stained paper in such a way that the light (daylight only) is well reflected from the color scale. The percentage of hemoglobin is then read off directly by noticing the point of the scale with which the blood stain exactly corresponds. As this scale does not read closer than 10 per cent., the intermediate percentages must be judged by difference. Here personal factors are of great importance, some workers being so skilled as to detect small variations.

This method can furnish, at best, only an approximate result and has nothing in common with the other methods discussed. It is to be advised only when some more reliable method is not at hand or when a rough estimate only is wanted. It is recommended by some writers as being as generally useful and accurate as any of the other methods, but the writer can see no advantage whatever in its use, as the results obtainable are, in his opinion, not reliable and are not as satisfactory to one who is not especially accustomed to such color comparisons. This test would better be limited to rough, preliminary, approximate determinations than to be used in the more careful estimations which clinical work requires.

In the selection of a method for estimating the hemoglobin of the blood, the writer would advise the Fleischl-Miescher instrument for those who are in close touch with hospital or clinical laboratory facilities. For the general practitioner who must make his own determinations under varied circumstances, the Dare or the Sahli instrument may be unequivocally recommended, the latter having the advantage of cheapness along with accuracy and ease of manipulation.

Variations in the Amount of Hemoglobin.

The percentage values are misleading, as the hemoglobin varies with age and, to some extent, with sex. For this reason it is preferable to obtain the absolute amount of hemoglobin, which is done directly by the Miescher and, by a very simple calculation, with the Dare or Sahli instruments. In those methods which read in direct percentages one may readily calculate the absolute values per 100 c.c. of blood by multiplying the percentages obtained by 0.1377. In estimating the true figures for the hemoglobin in the blood of women, it is necessary to add one-eighth to the percentage values as read, as female blood runs from 12 1/2 to 20 per cent. less in hemoglobin than does the blood of man; likewise for a child we should add one-seventh to the percentage. In this way we correct the readings of the instrument which is calibrated against adult male blood. It has been found by comparative tests, that the blood of rural residents reaches the normal standards much more frequently than does that of their city brothers, this being due, no doubt, to the purer fresh air which the country dweller enjoys.

A relative diminution in the amount of hemoglobin, as determined by the above methods, is known as oligochromemia or as achroiocythemia. This condition is usually associated with a decrease in the number of erythrocytes (oligocythemia), but in cases of chlorosis the diminution of hemoglobin is an absolute reduction, each cell showing less hemoglobin than normally and no oligocythemia being, as a rule, present. In pernicious anemia, on the other hand, each cell may show an absolute increase in hemoglobin, although the percentage value is reduced owing to the marked oliogocythemia present at the same time. Factors bringing about conditions of hydremia lead to a transient oligochromemia, while factors acting as etiologic units in the production of anhydremia lead to a reverse condition of polychromemia.

Oligochromemia is observed in chlorosis, pernicious anemia, leukemia, and secondary anemias, following chronic infections, hemorrhage, malignant growths, and some constitutional diseases. It is noted in chronic nephritis, chronic enteritis, and mineral poisonings, especially those with lead and mercury compounds. It has been stated that low hemoglobin values sometimes observed in cases which are to have surgical intervention are contraindications to operative treatment as the anesthetics themselves may bring on a condition of oligochromemia. While it is wise to watch with extreme care cases showing less than 50 per cent. hemoglobin, yet surgical operations have been successfully performed on cases with a more marked diminution of the hemoglobin.

Color-index.

This term is used to indicate the amount of hemoglobin contained in each cell, as compared with the amount present when a normal number of erythrocytes obtains. In other words, it is the quotient of the hemoglobin percentage divided by the percentage of red cells. This latter factor is obtained by dividing

the number of red cells, as found by the count, by the number reckoned as normal, namely, 5,000,000. A very simple method of getting this percentage is to multiply the number of hundreds of thousands of red cells by two; thus if 2,650,000 reds were counted we obtain 53 as the percentage of red cells. Sahli recommends the use of the term "hemoglobin quotient" or "hemoglobin value" for this factor, but the expression color-index has become so general that it will be hard to replace. Moreover, the latter term seems to convey a more definite idea to most of us than would the substitutes suggested.

The color-index is normally one; that is, a hemoglobin value of 100 per cent. is associated with a blood count of 5,000,000 red cells. We find, however, in the different anemias that this figure varies markedly. In those anemias, in which the reds are diminished to the same relative extent as is the hemoglobin, the index remains normal; while in those cases in which the hemoglobin is markedly reduced without a coincident decrease in the reds, the value is necessarily less than one. This latter condition is observed especially in chlorosis and in splenic anemia, while in the pernicious types of anemia we find the diminution of the reds much greater, proportionately, than that of the hemoglobin, a high color index consequently obtaining. In such cases the index may run from 1.02 to 1.9 as in one case observed by the writer.

Such variations are the rule, but are not invariable. We may find the various anemias showing, at times, very unusual color indices. As this factor is intended to show only the relations of the hemoglobin to the cells it must not be taken as absolutely diagnostic, but rather as merely significant. It must be remembered, moreover, that this figure cannot be absolutely exact as it is based on a purely arbitrary number of red cells as the normal value and as the instruments for estimating hemoglobin are often improperly standardized. The results are, however, comparable and often yield valuable clinical information, if individual, racial, and seasonal variations in both the amount of hemoglobin and the number of red cells are taken into consideration.

(*C*). Proteins of the Blood.

From the point of view of physiological chemistry a discussion of the various protein bodies found in the blood embraces, necessarily, those of the intracellular fluid as well as those of the cellular elements. From the clinical stand-point, however, the discussion of this field is limited more or less to the proteins of the serum. I can, therefore, do little more than refer to the fact that the red cells contain, besides the hemoglobin which constitutes about 90 per cent. of their organic matter, a nucleoprotein which shows properties resembling those of both the globulins and albumins. The proteins of the white cells are still little differentiated. Miescher found five different forms of protein, showing various solubility and coagulation relations. Besides these the leucocytes contain, as characteristic proteins, nucleins, which are compounds of the phosphoric acid-containing nucleinic acid with simple

albumins. The metabolism of the nucleins is an important factor in various clinical conditions, but I must refer elsewhere for such discussions. Little is known regarding the chemical composition of the blood-plates. Löwit affirms that they are composed principally of globulin, while Lilienfeld asserts that their substance belongs to the class of nucleo-albumins. For the differentiation of the subdivisions of the proteins mentioned above, works on physiological chemistry must be consulted.

In the process of coagulation, fibrinogen, a protein of the plasma, is converted into fibrin through the influence of thrombin (fibrin ferment), whose chemical nature is not absolutely settled; it belongs probably to the class of nucleo-proteins, possessing, however, many of the characteristics of the globulins. Blood serum, of both physiologic and pathologic types, contains two protein bodies, serum globulin and serum albumin. A third body, called by Chabrie albumon, has been assumed, but the researches of Drechsel and of Brunner have shown that this body is not preformed in the serum, but arises from serum albumin and serum globulin during the process of coagulation.

According to Hammarsten, normal human blood serum contains 7.62 per cent. of total protein, while Schmidt gives this figure as 8.26 per cent. The researches of Reiss, Strauss and Chajes, and more recently of Engel have shown that the refraction coefficient of serum, in health, is between 1.3487 and 1.3517, corresponding to a percentage of 7.74 to 9.13 of protein. The introduction of the refraction coefficient into the study of the serum and plasma has furnished a method of clinically studying hydremia as well as one by which the water content and serum proteins may be easily estimated. These factors are of great importance in the study of the various anemias, as we know that the serum or plasma is of much more importance, from the pathologic standpoint, than are the variations in the cellular structures, which are simply manifestations of profound changes in the liquid portions of the blood.

In this connection we must distinguish between an increase in the proteins of the blood (hyperalbuminosis) and a decrease in their amount (hypalbuminosis). The former is observed whenever water is more rapidly withdrawn from the system, and so from the blood, than it can be supplied. Such conditions are furnished by marked diarrhea, cholera, profuse perspiration, and polyuria without extra intake of fluid. This increase in protein content is only transient and is a result of mere concentration of the blood, the proteins passing out in relatively less amount than does the water. If the above conditions are associated with true exudation, then, of course, a hypalbuminosis of transient duration will replace the hyperalbuminosis. This decrease in the amount of total proteins is observed whenever direct loss of protein from the blood occurs. Thus Becquerel and Rodier observed a diminution of the proteins in Bright's disease, cardiac edema, and puerperal fever. Hoppe-Seyler noted a loss in melanosarcoma, while Schmidt, von Jaksch, Panum and Limbeck, and Pick have reported such a condition in severe anemias and leukemias. In most severe infections, as Ewing states, the proteins are but

slightly reduced. Along with hypalbuminosis we often observe a hydremia which may be referable to a direct absorption of fluid from the tissue under the influence of a hypertonic state of the blood.

In contrast to the constancy of the total protein values of the serum, we find the relationship between the albumin and globulin markedly disturbed at times. These two bodies are in reality not definite chemical compounds, but are separable, each into two distinct substances with different solubilities and precipitation constants. This fact may have great importance as further study is made, inasmuch as Pick has shown that pseudoglobulin has associated with it the antitoxins of diphtheria and of tetanus. The normal amount of total protein being taken as 7.62 per cent., Hammarsten has shown that this percentage is made up of 3.10 per cent. of serum globulin and 4.52 per cent. of serum albumin, the relationship of globulin to albumin beings as 1:1.5. This ratio is variable, running, according to Limbeck and Pick, globulin 16.9 to 38.3 per cent. of total protein, albumin 61.7 to 83.1 per cent. of the total albuminous content. Such a wide variation makes it difficult to establish any absolute relations in disease. Erben has shown that the albumin remains about normal in pernicious anemia, while the globulin is markedly reduced. The researches of Estelle, Hoffmann, Halliburton, and Mya and Viglezio show marked pathological variations not only in the total protein content, but in the so-called "protein quotient," $\frac{\text{albumin}}{\text{globulin}}$. The latter authors conclude among other deductions that the relation of the proteins is greatly changed in disease, especially in conditions associated with transudation and exudation, in the sense that the globulins are increased while the albumins are diminished. In such states globulin is seen to be relatively more resistant and less diffusible than is albumin (Gottschalk). That this view is not uniformly applicable is noted from the work of Freund who observed in the serum of nephritis a relationship of 1:11.3 between the globulin and albumin, instead of the normal 1:1.5. Ducceschi has reported an interesting observation on this point. He found that, during the period preceding the convulsions which follow thyroidectomy, a percentage increase of albumin as regards globulins obtains, while during the convulsions the reverse conditions are present.

In this discussion I have taken no account of the total protein of the whole blood. In its determination we include not only the albumin and globulin of the serum, but also the hemoglobin of the red cells, the nucleoproteins of the white cells, and the fibrinogen of the plasma. Traces of albumose and peptone (?) have been found in pathological conditions, the former possibly in normal states, while still other nitrogenous constituents are commonly determined with the proteins. The total protein of the blood has been given by von Jaksch as 22.62 per cent., while Limbeck states a percentage of 25. So much depends upon the method adopted for its estimation and so much on the physiological state of the blood that comparative figures are difficultly obtainable from the literature. Regarding the determination of the total protein and of the globulin and albumin, the reader is referred to the section on Urine.

Regarding the question of the presence of peptone in the blood, as reported by von Jaksch, Freund and Obermayer, and Ludwig, much depends on the proper differentiation of the substance found. So much has been called peptone that is, in reality, albumose, that we are uncertain whether peptone was present or, if it were, whether it was not formed in the processes used or was not a postmortem product. Devoto and Wagner could not confirm the finding of true peptone when blood from the living subject was examined. It is rather strange that peptone, which reduces the coagulability of blood when added in small amounts, should not exert this power in the vessels during life were it really present. The fact, however, that peptone and albumose have both been repeatedly found in the urine in various conditions by competent observers points to the probability of the existence of these products in the blood, although they may be combined in such a way as not to be capable of easy detection. Further elaboration of our methods of detection and differentiation may clear up a much disputed field. Bywaters[1] believes the so-called albumose of the blood to be identical with sero-mucoid.

(*D*). Other Nitrogenous Constituents.

Other nitrogenous bodies than those already discussed are found in the blood both in normal and in abnormal conditions. While these remaining azotized bodies are not as important as are the proteins, yet in some cases they have great clinical and experimental value, especially in metabolic studies.

Total Nitrogen.

There is no question but that the variation in the total nitrogen of the blood is worthy of a more extended study, especially in the various constitutional diseases. Metabolism in febrile diseases is so intimately associated with increased protein disintegration that a proper study of the changes taking place under the influence of abnormal body temperatures should include an estimation of the total nitrogen as well as of the nitrogen-partition of the blood. The methods to be used in such study have been discussed in detail under The Urine to which section the reader is referred. Slight changes in the technic of certain determinations are necessarily adopted in blood work, owing to the preponderance of the proteins over the other nitrogenous constituents.

Von Jaksch in his studies found the total nitrogen of normal blood serum to be 1.37 per cent., while that of the whole blood showed a value of 3.62 per cent. Variations in these figures are no doubt frequent, but have been rarely reported owing to lack of work along these lines. General conclusions can, therefore, not be drawn from the meager literature.

Urea.

This is the most important nitrogenous constituent of the blood aside from the proteins already discussed. Its importance lies not so much in its pathological significance as in its relatively large amount. Traces of urea are normally present in the blood in amounts of 0.011 to 0.02 per cent., accord-

[1] Biochem. Zeitsch., Bd. 15, 1909, S. 344.

ing to Gottlieb and Schröder, while Schöndorf has shown that during digestion this value may reach 0.61 per cent. and in pathological conditions, especially in uremia and other states complicated with a marked nephritis, it may reach even higher figures. This points to the accumulation of urea in the system whenever its elimination through the kidneys is interfered with. No further pathological significance can attach to such accumulations of urea as they are not sufficient to bring out the hemolytic effects noticed when blood is treated with relatively strong solutions of urea or to cause the marked diuresis which is observed following the administration of urea. The retention of urea in the blood has been held responsible by various workers for the condition of uremia, but more recent research has shown that such a view is untenable.

In febrile conditions, associated with increased protein decomposition, urea is found in increased amounts in the blood. As the liver is the chief seat of formation of urea, we find in cases of marked insufficiency of this organ the urea content of the blood and also of the urine markedly reduced; instead of urea, its precursors, the amino acids and ammonium salts, being found in increased amount. For the methods of detection and determination of urea in the blood, I must refer to the section on Urine or to works on physiological chemistry.

Uric Acid.

Like many other substances formed outside the kidneys but excreted by them, uric acid occurs in the blood in traces too small to yield reactions with the ordinary tests. According to Magnus-Levy,[1] uric acid may be detected in the blood only when it is present in the amount of 1 mg. in 100 c.c. of blood, as a minimum. The presence in the blood of an increased amount of uric acid is known as uricacidemia and is associated with the so-called uric acid or lithemic diathesis. Weintraud found 5 mg. of uric acid in 100 c.c. of blood following the ingestion of a large amount of sweetbreads. This points doubtless to the fact that the kidneys have a limited power of excreting uric acid when a surplus is suddenly poured into the blood from the digestive tract. Further, we observe in cases of severe nephritis, in which the eliminatory power of the kidneys is reduced, quite an accumulation of uric acid in the blood (3 to 6 mg. and over). Uricacidemia is likewise observed in conditions associated with insufficient aeration of the blood, as found in organic heart disease, emphysema, and exudative pleurisy. Febrile states have, *per se*, nothing in common with uricacidemia, although in some conditions associated with fever uric acid may accumulate in the blood, but not as a direct result of the increased temperature of the body.

For some time, following the ideas of Garrod, an accumulation of uric acid in the blood was held to be pathognomonic of gout. He based his theory on the fact that uric acid was detected in the blood by the so-called "thread test" which is now known to be imperfect and unreliable. Although his con-

[1] Berlin, 1906.

clusions regarding the direct relation of gout to accumulation of uric acid has been proven by later writers to be ill-founded, yet we do find a lithemia in gout. This condition arises, however, from the increased nuclein metabolism in this disease, which is of pure endogenous origin as Schittenhelm has shown. This endogenous uric acid metabolism is not peculiar to gout, as accumulations arising from the same source are observed to as great, or even greater, extent in pneumonia, leukemia, and other severe anemias. The conclusions of Haig regarding the influence of excess of uric acid in the blood in various conditions seem to the writer absolutely unwarranted and unscientific. Klemperer[1] and especially Magnus-Levy[2] have reported the most careful researches on such accumulations of uric acid in gout, showing that no increase occurs preceding or during the attack, but that a marked accumulation obtains following the seizure. The method of detection as well as the chemical and physical properties of uric acid must be found elsewhere.

Xanthin Bases.

The xanthin, alloxur, or purin bases do not occur in normal human blood in appreciable amounts. Their close relationship to uric acid makes it decidedly probable that they are present, along with uric acid, in cases of gout, leukemia, emphysema, pleurisy, and nephritis, although the literature does not yield any extensive researches covering this point. This subject is discussed in considerable detail under The Urine, to which the reader is referred.

Ammonia.

It is highly probable that this substance is a normal blood constituent, being present in venous blood, according to Winterberg, to the amount of 1 mg. per 100 c.c. of blood. The presence of an increased amount of ammonia or of ammonium compounds in pathological blood is certain. The normal metabolism is so regulated that any excess of acids, introduced into the system from without or produced within the body by increased decomposition of tissue, is neutralized up to a certain point by a corresponding increase in the ammonia produced in the breaking-down processes. While this subject has not been so carefully worked out in the case of the blood as it has in the urine, yet we are justified in assuming that a preceding increase of ammonium compounds must obtain in the blood in order to allow an excretion of such an excess in the urine. An increase in the acids of the system must, therefore, carry with it an increase in the ammonium compounds. If the acid intoxication (acidosis) is not too severe, the increase of ammonia is sufficient to neutralize the effects of the acid. However, in most cases showing this symptom-complex, the fixed alkalies are called upon to make up the deficit. This is the basis of the alkali-therapy in febrile states (Walter), in diabetic conditions (Naunyn), in cases of cyclic vomiting (Czerny and Keller), and in pneumonic attacks (Billings).

[1] Deut. med. Woch., Bd. 21, 1895, S. 655.
[2] Zeitsch. f. klin. Med., Bd. 36, 1899, S. 353.

It has been shown by various workers that uremia is associated with an increased excretion of ammonia in the urine and probably with an accumulation in the blood. This ammoniemia is not to be confounded with the narrow idea of Treitz and of von Jaksch, that uremia is due to the increase of ammonia in the blood, as this view has been shown to be untenable. The increased amount of ammonia, both in the blood and urine, in uremic conditions is probably due to a direct insufficiency on the part of the liver to elaborate urea from its precursors. Moreover, a contributory cause may be found in an abnormal production of acids calling for an increase in ammonia as a neutralizing substance (Senator).

Regarding the presence in the blood of other nitrogenous bodies, such as carbamic acid, creatin, hippuric acid, and amino-acids, the writer has something to say in the section on urine.

(*E*). Carbohydrates.

The presence of sugar in the blood, both of normal and of abnormal types, is a well-demonstrated fact. While this sugar is probably not in the free molecular state and is not dialyzable, it is uncertain in just what combination it does exist. The discovery of jecorin (a combination of glucose with lecithin) by Drechsel shows us that such combinations occur in the blood; yet it has not shown that this is the usual blood sugar. The sugar usually present in the combined state is glucose, but maltose is occasionally found in the blood of nursing mothers, while levulose or pentose may occur both in the blood and urine after intake of large amounts of food containing them.

Glucose.

The occurrence of glucose in the blood is termed glycemia. This hexose is found normally in quantities of 1 to 1.5 parts per 1,000. Under pathological conditions this amount may increase to as high as nine parts per 1,000, as Hoppe-Seyler reports in a case of diabetes. According to Claude-Bernard, whenever the sugar of the blood reaches three parts per 1,000, diuresis and glycosuria occur as evidences of the systemic effort to control the hyperglycemia. This figure is probably too high, as the later methods of study have given a lower value for the normal sugar content and hence for the point at which excretion occurs. Indeed, Pavy believes that traces of free glucose in the blood will be immediately excreted. While a hyperglycemia may be produced by purely alimentary excesses, the most common condition associated with it is diabetes mellitus. Here of course many factors, such as the pancreatic, duodenal, hepatic, and myogenic influences come into action as causative or contributory agents in such production.

Freund reports that the blood of patients suffering with carcinoma shows a strong reducing action due to the presence of sugar. As he was able to find no such condition in the blood of patients with sarcoma he considers hyperglycemia a differentiating point between the two malignant states. Trinkler confirms these findings and adds that the blood of nephritics shows a very

small amount of sugar, while that of febrile cases shows an increase. Such conditions as the preceding are prone to be associated with the appearance of reducing substances in the blood, but there is some doubt as to whether sugar is accountable for such reactions, although increased protein disintegration occurs, which in itself might give rise to a transient hyperglycemia.

A hyperglycemia is observed following the removal of the pancreas or after perversion of its function, as von Mering and Minkowski have shown. Recently Pflüger[1] has been able to demonstrate a marked glycosuria and hence an hypothetical hyperglycemia, following removal of the duodenum. Both the preceding conditions give rise to diabetes and cannot be discussed in this place. Several blood tests advocated as diagnostic of diabetes have been advanced, especially by Williamson and Bremer, but these will be discussed in a later section.

No doubt can be raised as to the presence in the blood of reducing substances other than glucose. According to Paul Mayer, we must assume that both normal and abnormal metabolism are associated with the conjugation of various aromatic substances with glycuronic acid. These glycuronates easily split up into their constitutent elements and show reducing properties. Whether sugar, in its normal metabolism, must invariably pass through the intermediate stage of glycuronic acid is debatable, yet the theory is a fascinating one and fits into many obscure points.

Glycogen.

This polysaccharid undoubtedly appears in the blood singly or in combination with albuminous bodies. Salomon, Frerichs, Lepine, Ehrlich, and Gabritschewsky have reported it, while Caminer was unable to find it. Huppert obtained it in quantities ranging from 0.114 to 1.56 grams per 100 parts of blood. Much depends on the method used to isolate and determine this substance, as it is easily lost by careless manipulation. Certain properties of the granules found in the blood, both extra- and intracellularly, have led many to believe that glycogen is present as a characteristic in many conditions. There is much reason, however, to assume that these granules, which stain brownish with iodin, are not glycogen, but rather albuminous bodies of indefinite composition. For a discussion of this subject see the treatment of iodophilia.

Cellulose.

This carbohydate has not as far as I am aware, been found in normal blood, but has been reported by Freund in that of tubercular patients.

(*F*). Fats and Fatty Acids.

The presence of free fats (palmitin, stearin, and olein) in the blood has been frequently observed both in health and disease. The relative quantities of these fats vary in different animals and are subject to wide variation in the

[1] Pflüger's Archiv., Bd. 118, 1907, S. 267.

same animal under influence of diet. Bonninger gives the fat content of normal human blood as 0.75 to 0.85 per cent., while Engelhardt finds 0.101 to 0.273 per cent., these latter figures being practically confirmed by Becquerel and Rodier.

The presence of an excess of free fat in the blood is referred to as lipemia. The physiologic variations are more notable than are the pathological, being observed after ingestion of a meal rich in fats, in breast-fed children, in pregnant women, and in the obese. Pathologically lipemia more or less permanent may be observed in various conditions, as in acute and chronic alcoholism, diabetes, arteriosclerosis, chronic nephritis, phthisis, carbon monoxide and phosphorus poisoning, gout, typhoid fever, fat embolism following injuries of the long bones, pneumonia, leukemia, acute infections, cachexia from inanition or malignant disease, hepatic diseases, and malaria. It has, therefore, little differential diagnostic value.

The extent of the lipemia may vary from the presence of isolated fat droplets to the overloading of the blood to such a degree that it becomes salmon-colored, turbid, and milky. This fat may be either the normal fat which has been transported from different parts of the body or may be that abnormal to the body arising from excess of fat in the diet. The fat is soluble in ether and stains black with osmic acid and red with Sudan-III. Regarding the quantitative estimations of fat, I must refer to other sections.

Concerning the presence of fatty acids in the blood little is known. Traces of volatile acids are sometimes present, but probably not as normal constituents. Gaglio, Spiro, Irisawa, and Berlinerblau report sarcolactic acid as a normal finding, while Zweifel finds an excess of this acid in the blood and in the urine in cases of toxemia of pregnancy. An excess of fatty acids in the blood is known as lipacidemia. Von Jaksch found fatty acids in the blood in cases of diabetic coma, leukemia, acute yellow atrophy of the liver, while Hougounenq reports the presence of β-oxybutyric acid in the cadaveric blood of a diabetic. It is rational to assume that in all those conditions associated with acidosis, fatty acids are present in the blood, as these may be detected in large amount in the urine in these states.

(*G*). Acetone.

The occurrence in the blood of demonstrable amounts of acetone is known as acetonemia. Deichmüller and von Jaksch have found a substance giving the reaction of acetone in various conditions, especially in fevers, which finding has been confirmed by Reale. Whether acetone is a product of normal intermediary metabolism and, as such, is found in many physiologic and pathologic states, must be found in the section on Urine.

(*H*). Biliary Constituents.

The conditions in which the biliary constituents, especially the pigments and acids, are found in the blood is termed cholemia. It is usually stated that these elements are not found in normal blood, but Croftan has shown

that the bile acids are observed in the blood of healthy subjects. This is not unexpected as they are completely absorbed from the intestines and are re-excreted in the bile (Weintraud).

Pathologically, both the acids and the pigments are found in the blood in any condition associated with their appearance in the urine. Oftentimes they may be found in the blood when no reaction for them is obtainable in the urine. While the bile acids exert marked toxic effects, such as hemolysis, the biliary pigments show, as Bouchard, de Bruin, Lugli, and Colosanti have demonstrated, certain harmful influences. Flint ascribes the toxic effects observed in cholemia to cholesterin, but this idea needs confirmation. The most usual condition showing cholemia is jaundice. Whether the blood changes observed in the various types of jaundice are due, primarily, to the cholemia or to a primary hemolysis or to a combination of these effects as a result of intoxication is an unsettled question. These phases of the blood variations in jaundice will be discussed later.

(*I*). Inorganic Constituents.

The inorganic composition of the blood shows quite a marked variation both under physiologic and pathologic influences. This variation applies both to the cellular and intracellular constituents of the blood. Changes in the molecular concentration as well as changes in the concentration of specific inorganic combinations are observed in the blood of the two sexes, as may be seen by consulting the table on page 396.

Regarding the special significance of the different inorganic constituents little is known, but chemical analyses of the blood ash in health and disease have shown that the pathological variations are more important as regards the chlorids, phosphates, and the iron compounds.

Chlorids.

Physiologically, a certain (about six parts per mille) concentration of sodium chlorid is necessary to hold the proteins in solution, as well as to maintain the proper osmotic tension of the serum. The larger the proportion of plasma, the greater the percentage of chlorids in the blood. This is true only within certain limits as the NaCl-content remains practically constant, no matter how large an amount is ingested. This constant value is regulated by the increased or diminished renal excretion.

In anemias the chlorids of the blood are usually high, yet, according to Limbeck, cases are occasionally found in which normal amounts of sodium chlorid obtain. In pneumonia, in which a diminished urinary excretion of chlorids is observed, the blood does not show an excess of chlorids but may even show a decrease owing to the effects of the exudative process. Diminished ingestion of food, vomiting, diarrhea, and general exudative processes may be associated with a decrease in the chlorids of the blood, but this is merely temporary. In cases of marked nephritis, associated with retention of chlorids, the blood may show an excess of sodium chlorid, but this condition soon

disappears through the influence of the increased edema. This phase of the question, originally advanced by Widal and Javal, has been previously discussed.

Phosphates.

These compounds exist in the blood as neutral or alkaline salts of sodium, potassium, calcium, and magnesium, as well as in organic combinations in the red and white cells in the form of lecithin and nuclein. The inorganic phosphates are concerned, at least in part, with the solubility of the proteins. Just what variations in the amounts of the organic and inorganic phosphorus compounds occur in health and disease is unknown. It is certain that a definite phosphorus metabolism exists and that this is characterized, to a great extent, by variations in the normal relationship between the compounds of the alkali and alkali-earth groups. Just what these relations are in physiologic and pathologic states experiment must determine. That the system retains compounds of phosphorus more energetically than it does any other mineral constituents is proven. As the phosphates of the blood and of the urine come both from the food and from the breaking down of the nuclein-containing protein material of the system, large variations are possible under the influence of many factors. The subject of the metabolism of phosphorus is taken up under The Urine.

Iron.

The iron of the blood is found principally in the hemoglobin which contains about 0.42 per cent. of Fe. It is also found in traces in the plasma, and, according to Hammarsten, in the nuclein compounds. The attempt to estimate the amount of hemoglobin by determinations of the blood iron have proven failures, as no definite relations exist between them. All of the blood iron is not in the form of colored compounds and, moreover, some of the derivatives of hemoglobin are iron-free. Biernacki has shown that the direct quantitative estimation of iron yields higher results than could be obtained by computation from the percentage of hemoglobin, this finding being confirmed by Jolles and Jellinek.

According to Schmidt and Becquerel and Rodier, the amount of iron in the blood ranges between 0.056 and 0.058 per cent. Female blood shows a somewhat lower value, just as it does for hemoglobin. For the estimation of the iron of the blood, I must refer to other works for the details, as the method is too cumbersome for general clinical use. The principle of the method is based on the comparison of the colors of a known solution of iron treated with ammonium sulphocyanate solution, with that of a solution of blood iron, obtained by incinerating blood, fusing the ash with potassium bisulphate, dissolving the fused mass in water, and treating it with sulphocyanate solution. The instrument used for the colorimetric tests is called Jolles' ferrometer, the amount of iron in 1,000 c.c. of blood being obtained by reference to a table accompanying the instrument. If it is desired to obtain the actual percentage by weight, the calculation must include the determination of the specific gravity of

the blood. Having this latter factor, we may use the equation G : V :: 100 : X, in which G represents the specific gravity and V the percentage by volume of iron which is obtained from the table. The hemoglobin value may be found by use of the formula of von Jaksch, $Hb = \frac{100 \times M}{0.42}$, in which M equals the percentage of iron by weight.

(*J*). **Blood Gases.**

The gases existing in the blood are oxygen, carbon dioxide, and nitrogen, the latter having little importance in the body functions, its amount corresponding to that which would be absorbed by an equal volume of distilled water, namely, 1.8 volumes per cent.

The amount of oxygen and of CO_2 varies widely, depending on the arterial or venous character of the blood, upon the velocity of the blood flow, upon the temperature, amount of exercise, etc. Oxygen occurs principally in chemical combination with hemoglobin, but a small amount, about one-fourth per cent., is present in solution in the plasma. About one-tenth of the CO_2 is held in solution in the blood, while the remaining nine-tenth is apportioned as follows, one-third loosely bound to the alkalies and hemoglobin of the corpuscles, two-thirds held in chemical combination with the alkalies and proteins of the plasma.

The following table made up from the figures of Setschenow, Ludwig and Sczelkow, and Hammarsten, will show the relation between these gases.

	Arterial.	*Venous.*
Oxygen,	21.6% by volume.	6.8% by volume.
Carbon dioxid,	40.3% by volume.	48.0% by volume.
Nitrogen,	1.8% by volume.	1.8% by volume.

(*K*). **Ferments of the Blood.**

The fact has been well established by Jacobi that the various organs and tissues of the body contain ferments which are proteolytic as far as the corresponding tissues are concerned, but are usually inactive when applied to the proteins of other organs. In other words, these ferments are autolytic, but not, as a rule, heterolytic. Much experimental work of former and latter years has shown that many metabolic processes, associated with the building up and breaking down of various tissue elements, are influenced to a great extent by the presence of ferments arising from many sources.

It is not unreasonable to assume that the blood, like other tissues, contains various ferments which have to do with general and special metabolism. The work of Schönbein on the oxidases of the blood, of Hanriot and, indirectly, of Castle and Loevenhart on the lipolytic ferment, of Lepine, Seegen, and Spitzer on the glycolytic ferment shows that such properties are resident in the blood. The work of Ascoli and Moreschi, Jochmann and Müller, and of Stern and Eppenstein has opened up an entirely new field of work on the proteolytic properties of the leucocytes. This proteolytic ferment action is

both autolytic and heterolytic and is influenced to a great extent by the checking action of the antiferments, which have been so well studied by Opie. Further, it has been shown that differences exist between the proteolytic properties of the many varieties of leucocytes and that the ferments are not always heterolytic (Mosse).

It would lead me too far afield to discuss this subject in detail; hence, I must be content with reference to the presence of these substances. Much benefit will be forthcoming from a further study of such properties of the blood and of certain constituents of the blood. It is to be recalled that ferment action may be accountable for the influences of the toxophore and other groups of Ehrlich's complement, but this phase must be discussed later.

(7). Enumeration of Red and White Cells.

This section of hematological technic is, perhaps, the most perfected and most frequently employed. The red and white cells are usually counted, as the enumeration of the platelets has little practical or scientific value at the present time and the latter technic is not sufficiently perfected to admit of conclusions.

Various instruments have been introduced for the purpose of counting the corpuscles in a given volume of blood. Most of them are based on the principle that a layer of diluted blood, of a certain depth, covering a certain known space, shows a definite number of corpuscles for every drop of blood used. The general method of making the count consists in diluting the fresh blood in definite proportions with some indifferent fluid and counting, under the microscope, the number of cells in a drop of this diluted blood, which is contained in a small glass cell on the floor of which is ruled a series of micrometer squares of known dimensions. The cubic contents of the cell and the degree of dilution of the blood being known, the number of corpuscles counted in any given number of squares of the ruled area may be taken as a basis for calculating the total number of cells in a cmm. of blood. Strong and Seligmann dispense with a special counting chamber and enumerate the cells in a definite quantity of blood diluted in exact proportions with a diluent stain and mounted as a permanent specimen. Einhorn and Laporte use a somewhat similar method and arrive at very good comparative results.

The normal number of red cells in the adult male is, approximately, 5,000,000 per cmm. of blood, while in the female it is somewhat lower, namely, 4,500,000. Marked variations in this figure are observed in pathological conditions and will be discussed in a later section along with the treatment of the physiological factors which influence the number and appearance of these erythrocytes. In the normal adult the number of white cells varies between 5,000 and 10,000, the average being 7,500. This figure is subject to both physiologic and pathologic influences to a greater extent than are the red cells.

Many instruments, such as those of Hayem, Gowers, Malassez, Thoma-Zeiss, Alferow, and Durham, have been introduced, but the most universally

used and the best adapted for such investigation is, in the writer's opinion, that of Thoma-Zeiss. This combines certain modifications of the mixing pipet of Malassez, the counting chamber of Hayem, and the micrometer rulings of Gowers. It gives us, therefore, a most complete instrument for such work.

Hemocytometer of Thoma-Zeiss.

The blood counter, originated by Thoma and constructed by Zeiss, is all that could be desired. Leitz, Reichert, and other makers of optical goods have introduced similar types of counters, but our experience with them has not been so good. So much depends upon the accuracy with which pipets are graduated and upon the trueness of the rulings of the counting chamber that we recommend the general use of the Zeiss instrument.

This instrument consists of a pipet for mixing the blood to a certain dilution, a counting chamber by means of which a layer of known depth and area is obtained, and a special cover-glass for the chamber.

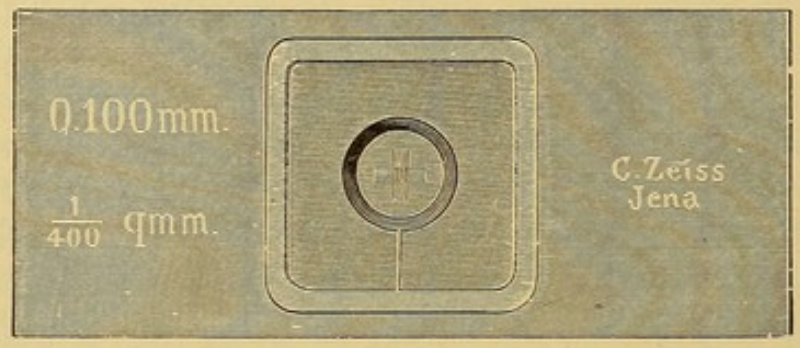


Fig. 131.—Thoma-Zeiss counting chamber.

Pipets.

The original form of the Thoma apparatus included but one pipet this being used for the dilution when both red and white cells were to be counted. Experience has shown, however, that the dilution given by this pipet is, in most cases, too great to permit of accurate counting of both types of cell. A modification has, therefore, been introduced to allow of greater accuracy by giving a lower dilution and by furnishing a larger number of cells in the counting chamber.

Erythrocytometer.

This is the original pipet of Thoma. It consists of a graduated capillary tube (A) opening into a dilatation (B), at the opposite end of which is a shorter glass tube (C), graduated with a line marked 101 and to which is attached a rubber tube with an ivory mouth-piece. This pipet is so graduated that the capacity of the ampulla (B) is exactly 100 times that of the capillary tube from its point to the line marked 1 and 200 times that from the point to the line marked 0.5. Other lines, both below and above this latter point, are calibrated on the tube, each line representing one-tenth of the capacity of the capillary. In some

of the pipets, especially that of the Miescher hemoglobinometer, two smaller marks each representing one one-hundredth of the length of the tube are calibrated on each side of the major divisions. By this means the dilution of the blood may be definitely known if the tube be not filled exactly to the point described. Those pipets with obtuse ends are much to be preferred to those with the more acute angles. In the ampulla is a small glass bead which is of service in properly mixing the blood with the diluting fluid. This pipet may be used in counting both the red and white cells and is all that is absolutely necessary when one uses the Zappert or Türk counting chamber. In general work, however, it is used only in the count of the red cells as the extreme dilution given is better adapted to this estimation than it is to the counting of the white cells.

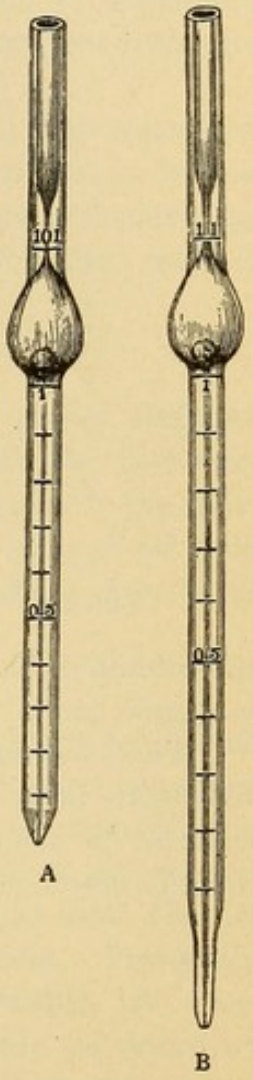


FIG. 132.—Diluting pipets; A, Erythrocytometer; B, leucocytometer.

Leucocytometer.

This is a graduated capillary tube similar to the erythrocytometer in construction but having a larger lumen, which will permit of lower dilutions. It is graduated into ten divisions, with the marks 0.5 and 1 representing these measures of the total capacity of the capillary. Above the ampulla is a graduation, 11, which is so calibrated that blood, drawn to the mark 1 and diluted to 11, gives a dilution of 10, while if drawn to the point 0.5 the dilution is 20. Those forms of this pipet which have the lower end tapering to a fine point giving a gradually decreasing lumen, are much to be preferred to the older models. As the caliber of this instrument is relatively large, the student is cautioned against using too great suction in making the dilution, and also against placing too large a drop on the counting surface.

The Counting Chamber.

"This consists of a heavy glass slide, A, on which is cemented a thick glass ring, B, the surface of which is highly polished. This ring surrounds a circular table of glass, D, the height of which is just 0.1 mm. less than that of the surrounding ring. Between this glass table and the inner edge of the ring is a small ditch, C, to catch the drop which may run off from the table and to prevent its flowing up between the ring and the cover-glass on the other side of the ditch. On the central glass table cross at right angles 21 parallel lines, equidistant, and between the extremes of which is exactly 1 mm. Hence we have an area of 1 square millimeter divided into four hundred small equal squares. Through each fifth row of squares is ruled an extra line, which is not a boundary but is merely an aid in keeping one's position in the ruled area. Indicated, not

bounded, by these extra lines, the square millimeter is divided into 16 units of 25 small squares each" (Emerson).

As the ruled surface of one square millimeter is divided into 400 small squares, each small square has an area of 1/400 square mm. The height of

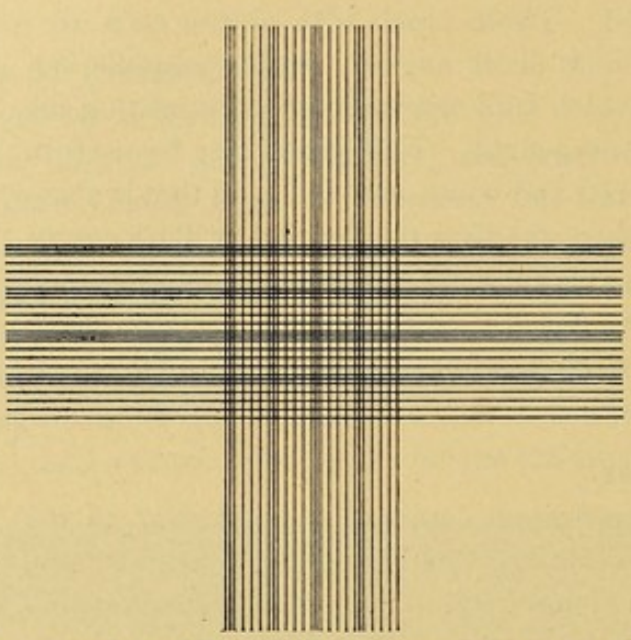


FIG. 133.—Ruled surface of Thoma-Zeiss counting chamber. (*Da Costa.*)

the column of blood being 1/10 mm., the cubic contents of each small square is 1/4000 cmm.

This counting chamber of Thoma does not permit of the counting of a sufficiently large number of leucocytes, especially when the dilution has been made in the same degree as is used for the enumeration of the red cells. In order to overcome this difficulty and also to give a larger ruled area in which

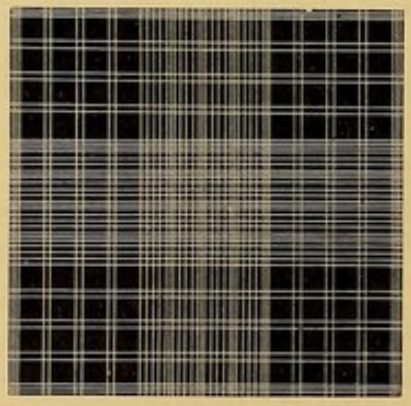


FIG. 134.—Türk's ruling of the counting chamber.

the leucocytes may be counted, Zappert has modified the original ruling in such a way that a counting surface of 9 sq. mm. is afforded. This modification has been improved by Ewing and by Türk in such a way that the four large corner squares, each of 1 sq. mm., are subdivided into 16 smaller squares, each of which is equal in area to the total 25 smallest squares of the Thoma chamber. This latter modification is much the best and is used

exclusively by the writer. Its advantage in counting both red and white cells will be appreciated when the student compares it with the older chamber. The sixteen central squares are used in counting the erythrocytes, while the entire area may be used in the enumeration of the leucocytes. Simon has recently introduced a different modification of the Thoma ruling, which is extremely simple and should prove very satisfactory. Lack of experience with it prevents the writer, however, from making comments upon its advantages and possible disadvantages.

Before use the counting chamber should be well washed with water and carefully dried. Precautions should be taken to see that no lint is left on the surface of the glass ring and that no alcohol or ether are used in the cleaning process, as these substances loosen the cement with which the glass table is fastened to the slide.

The Cover-glass.

This is made of heavy polished glass with accurately planed surfaces. The ordinary cover-glasses should never be employed with the counting-chamber as they are often uneven in surface and do not fit tightly to the slide. Moreover, these ordinary cover slips are so thin that the capillarity of the drop of blood may bend them down to some extent. The cover-glass must be as carefully cleaned and dried as is the chamber.

Diluting Fluids.

In order that the blood may be properly examined, it must be diluted with a solution which will at the same time prevent coagulation and hemolysis and will preserve the corpuscles intact. There are numerous formulæ for such solutions and the choice is largely a matter of experience. Much will depend on whether the red and white cells are both to be counted at the same dilution or whether two different pipets are used in making the dilutions. Personally the writer prefers the use of two pipets using different diluents, but other workers use one pipet and one diluent. The red cells may be destroyed by certain fluids leaving the white cells intact or the white cells may be colored by the same diluent used in counting the reds.

Hayem's Solution.

This solution preserves the red-cells permanently and permits the corpuscles to settle slowly, thus furnishing an even distribution of the cells. Moreover, it will keep almost indefinitely and does not permit of the development of yeast spores which so readily multiply in many of the other diluents. The writer can recommend this diluent as the most generally useful of the preparations advised. It is made up as follows:

Mercuric chloride,	0.500 gram.
Sodium sulphate,	5.000 grams.
Sodium chloride,	1.000 gram.
Distilled water,	200.000 c.c.

Owing to the presence of mercuric chloride, this fluid cannot be mixed with an aniline coloring substance to stain the leucocytes and is, therefore, not applicable to the combined counting of the red and white cells.

Toisson's Fluid.

Sodium chloride,	1.000 gram.
Sodium sulphate,	8.000 grams.
Neutral glycerin,	30.000 c.c.
Distilled water,	160.000 c.c.
Methyl violet 5 B.,	0.025 gram.

The addition of methyl violet serves to color the leucocytes and permits of their recognition along with the erythrocytes. Occasionally this fluid hemolyzes the red cells and thus invalidates the count. Moreover, it easily becomes infected with yeast spores which develop profusely in it. For this reason it is advisable to filter the fluid before use, each filtration, however, weakening it, so that it becomes after a time useless.

Other diluents, such as the solutions of Pacini, Löwit, Petrone, Acquisto, Marcano, and Edington, have been used, but the more generally applicable ones are those mentioned above. Toisson's fluid is particularly useful when the count of reds and whites is to be made in the same specimen. The coloring of the leucocytes is not necessary for their recognition, but it is a convenience to one who is not making blood counts frequently. Hayem's solution is the very best diluent at our command for general purposes.

If it is desired to count the white cells alone, and this is always wise, a one per cent. solution of acetic acid, to which is added gentian violet to bring out the white cells a little more clearly, may be used. This solution destroys the red cells and thus gives only the white cells in the preparation. For this reason the addition of the gentian violet is unnecessary. Yeast cells develop in this solution with more or less readiness, hence one should employ only freshly made solutions, as these yeast cells resemble, to some extent, mononuclear leucocytes and may introduce an error into the count.

Method of Counting the Corpuscles.

With this process of counting the cells, whether red or white, there are five steps to be taken:

1. **Obtaining the blood.**
2. **Diluting and mixing the blood.**
3. **Filling the counting chamber.**
4. **Counting the cells.**
5. **Cleaning the apparatus.**

Erythrocytes.

(1). **Obtaining the Blood.**

As previously stated, the blood may be drawn from a puncture of the ear or finger. Personally, the writer always uses the ear, unless some valid

reason exists for not doing so. As soon as a good-sized drop appears, which is obtained without pressure or constriction, the tip of the pipet is placed in the drop and is supported by a finger of the left hand, which holds the ear in position. The blood is drawn by suction to the mark 0.5, in cases in which anemia is not suspected, or to the mark 1 in such cases, as a routine the former mark being preferable. As the student will find, some practice is needed to stop the column of blood exactly at the point desired. If the blood be drawn a little too far, the tip of the pipet may be rubbed with the finger or the excess may be shaken down by tapping the tip against a towel or, preferably, the blood may be drawn to the next mark and the necessary correction made in the dilution. Unless this error can be corrected, the pipet must be cleaned or a second one used. In those pipets which have extra marks, indicating 1/100 of the length of the capillary, we have a measure which may prevent the necessity of using a second test, as the correction may be made in the final calculation. In any event, if the blood be not accurately measured, we must reject the determination. Moreover, the work should be done rapidly to prevent coagulation of the blood. Hence, too much time should not be spent in adjusting the column of blood.

(2). Diluting and Mixing the Blood.

As soon as the column of blood is adjusted at the desired height of the capillary, the tip of the pipet is carefully wiped with the fingers to remove any adherent blood and is immediately dipped into the diluting fluid in such a way that no portion of the blood is lost. Türk recommends closing the end of the pipet with the finger and exerting slight suction on the closed tube in order to prevent any loss on immersion of the pipet. I have never seen it necessary to use this measure, providing ordinary care is employed in applying the suction as soon as the pipet touches the diluent. The diluting fluid should stand ready in a small dish or bottle and should be carefully examined before use to see that no flocculi or spores are present.

The diluent should be at once drawn into the capillary by suction. The fluid rises slowly in the tube, the pipet which is held vertically being rotated between the finger and thumb of the left hand as the fluid rises. By this rotation, the diluting fluid is mixed with the blood at once and bubbles of air, which often cling to the inside of the tube, are avoided. The glass bead in the ampulla serves the purpose of thoroughly mixing the blood and diluent. As the column approaches the mark 101 on the upper end of the pipet, care should be taken that the aspiration is not too strong. While the error, introduced by drawing the diluted blood beyond this mark, is not as great as it is in drawing the undiluted blood to the mark 0.5, yet both should be avoided. When this upper mark is reached, withdraw the pipet from the diluent, close the tip with the finger, bend the rubber tubing down over the other end, and close this with the thumb. Some prefer to remove the rubber tubing at this juncture, but this is not at all necessary. Shake the pipet vigorously for at

least one minute to insure a thoroughly uniform mixing of the contents. If for any reason the count is not to be made at once or if it be desired to carry the blood to the laboratory for examination, the rubber tubing may be removed and the ends of the pipet closed by means of a rubber band. Naturally, before the mixture can be used it must be again thoroughly shaken. If the examination is to be done at once, proceed as follows. Blow three or four drops from the pipet in order to remove the column of fluid which has remained in the capillary and has not mixed with the blood. If Toisson's fluid has been used as the diluent, it is better, before blowing out the drops, to allow the pipet to lie horizontally for ten to fifteen minutes in order to permit of the staining of the leucocytes. Shake the tube well, blow out the drops as stated, and adjust a small drop on the counting table.

(3). **Filling the Counting Chamber.**

As a rule, it is better, unless Toisson's fluids has been the diluent, to fill the counting-chamber at once as errors may creep in by allowing the pipet to stand, even though the later mixing may be thorough. A small drop, the size of which can be learned only by experience, is blown onto the center of the ruled area of the counting chamber. This drop should not be so large as to run over into the moat, but should be large enough to practically cover the glass table. A drop which is either too large or too small will introduce an error into the count.

Adjust the cover-glass at once. This is a point in the technic which requires considerable practice and must be mastered before accurate results can be obtained. Emerson's advice on this point is admirable, "grasp the cover-glass by two diagonal corners, place a third corner against the slide with the edge of the glass ring as a fulcrum, and hold it in that position by a finger of the left hand. By now raising the finger the cover is rotated onto the drop radidly and also in such a way that no air-bubble is left." Breathing upon the cover-glass before it is adjusted is often servicable in making this preparation. If there has been no dust on the slide or cover-glass and they are perfectly clean, the student will observe, providing the adjustment has been properly made, a beautiful band of colors known as Newton's rings, which are due to a phenomenon of interference of light. If these rings do not appear, they may often be brought out by firm pressure on the edges of the cover-glass. If they are not persistent, after the pressure has been removed, the adjustment of the two polished surfaces must be assumed to be imperfect and the preparation rejected. It happens at times that these rings are difficult to obtain and some workers state that they are not necessary for an accurate count. The writer prefers, however, to reject those slides which do not show these diffraction rings than to run the risk of including a possible error. These rings may best be seen by holding the slide on a level with the eyes in such a way that the light is totally reflected from the surface of the cover-glass. After the slide is properly adjusted allow it to stand for three or four minutes before proceeding with

the next step of the determination, in order to insure the proper settling of the corpuscles upon the counting table.

(4). **Counting the Cells.**

Before any count of the cells is attempted, the entire surface covered by the blood must be examined with the low-power lens to ascertain whether the distribution of the cells is uniform throughout. If not, the slide should be rejected, even though the points mentioned previously have obtained. It is much better to stop proceedings at this point than to attempt to equalize an uneven distribution by a larger counting area. If the specimen proves satisfactory the count may then be undertaken. The lenses, best suited to this purpose, are Bausch & Lomb $\frac{1}{6}$, Zeiss D, Leitz 6 or 7, and Reichert $\frac{1}{6}$. As the student becomes accustomed to cell-counting, he may use a lower objective. This has the advantage of bringing the entire field of one sq. mm. into focus. A mechanical stage is of some convenience in making the count, but the fingers answer quite as well after some practice in manipulating the slide.

The unit of counting surface is a matter of individual preference. Sahli recommends a unit of four small squares, Grawitz and Simon use sixteen of these squares, while Cabot advises the use of 36. In common with Türk, Ewing, Da Costa, Wood, and Emerson, the writer prefers the unit of 25 small squares, as this is the unit of the ruling and the calculation is much simpler than with the other units.

In order to simplify the process of counting, some routine method must be used. These methods depend on the worker, but the usual procedure in the writer's laboratory is as follows: Adjust the slide upon the table of the microscope so that the upper left-hand corner of the central ruled area of 16 large squares of the Türk chamber is brought into the field in such a manner that one may count the cells in the small squares from left to right. If the low-power objective is used, the complete group of 16 units will lie within the field of observation and may be easily examined. With the higher power, which the student may be forced to use in his earlier work, the optical field is necessarily limited. Count the total number of cells lying within the 25 small squares of the single unit. In doing this count the upper row of the unit from left to right, drop down a row, and count the cells from right to left and so on until the cells in the whole unit have been counted. The accompanying cut will indicate the method to be followed. In making this count, cells which touch the right hand or lower boundaries of the unit are disregarded, while those which touch the upper and left-hand line are included in the count of that square. After counting the cells in the upper left-hand unit, count those in the remaining fifteen units, thus covering a field of 16 units of 25 small squares each, making a total of 400 small squares counted. Türk recommends the counting of eight units as a minimum, Emerson advises the counting of the four corner units in each of two separate preparations, while Da Costa and others count the cells in four groups of units from above downward and repeat with four units not

adjacent. It seems to the writer that the error is less the larger the area covered, and he, therefore, advises the beginner to make the total count of the sixteen units, although it is not supposed that one will try to cover up defects in technic by this larger counting area. A variation of more than 25 cells in the count of the varions units should be taken as evidence that the distribution is not perfect. In such cases the count would better be rejected on the ground of inaccuracy. After the technic is mastered and the worker has discovered just exactly wherein his error lies, a count of 8 or 4 units will suffice. It is advisable, where accurate scientific results are indispensable, to clean the slide and make a second count with a fresh drop of blood so that one may have a check on his work. For clinical purposes, however, the count of four units will ordinarily be sufficient.

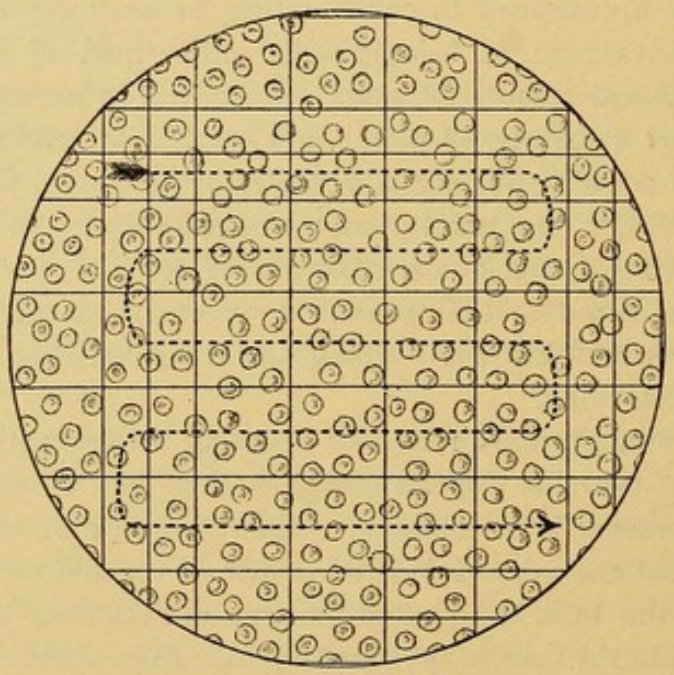


FIG. 135.—Plan of Counting the Cells. (*Da Costa.*)
The small squares are examined in the order indicated by the arrow

In ordinary counting it is not necessary to differentiate the red cells from the leucocytes, as the error thus introduced is small and may be disregarded. In cases, however, which show large leucocyte values, this error will be quite appreciable and must be overcome by the following method. All of the cells observed may be counted as erythrocytes and the reduction made for the number of leucocytes as obtained by the special leucocyte count to be described later. In the use of Hayem's diluting fluid, the leucocytes are not colored, while the erythrocyctes retain their normal yellow color. The leucocytes appear, by good illumination of the field, of a bluish tone, are somewhat larger than the red cells, and are characterized by a sharper border. These facts will enable one after some experience to distinguish the white from the red cells. Toisson's fluid stains the leucocytes blue, and may, therefore, be used to outline these cells. As a rule, it is better to learn to recognize the leucocytes by differences in refraction than to rely on the staining qualities of these cells. In some cases

the methyl violet of the diluent colors some of the red cells so that they cannot be easily distinguished from the white ones.

The number of cells in the blood is invariably reported as the number contained in a cubic millimeter of blood. In making the calculation of this number it is necessary to know the number of units counted, the number of cells in these units, the area of each small square of the unit, and the degree of dilution of the blood. Thus, if 16 units, each of 25 squares, have been counted, the total number of small cells is 400, each having a cubic area of 1/4000 cmm. The total volume of the units counted is, therefore, 1/10 cmm. It is evident that the number of cells in 1 cmm. of blood is 10 times that in the area counted over if the blood were undiluted. But the count is always made with diluted blood and we must, therefore, take this factor into account. With a dilution of 100 multiply the number of cells in 1 cmm. by 100, and with a dilution of 200 multiply by this factor. Thus, if 2,500 cells were counted in the 400 small squares and the cubic contents of the units gone over was 1/10 cmm. then 1 cmm. of diluted blood would contain 25,000 cells. As the blood was diluted 200 times, the total number of cells in one cmm. of undiluted blood is 5,000,000. A very simple way of remembering this calculation is to multiply the number of cells counted in the total area of 400 small squares by 1,000 if the dilution was 100, and by 2,000 if the dilution was 200.

If the total area of 16 units be not counted, the method of calculation is the same but the factors are variable. This method goes as follows: Multiply the number of cells counted by the degree of dilution and this result by the cubic contents of each small square (4,000). Divide this result by the number of small squares counted. Thus, to calculate the number of cells in a cmm. of blood when 100 small squares (4 units) were counted at a dilution of 200, the count being 625 cells, the equation is as follows: $\frac{625 \times 200 \times 4000}{100} =$ 5,000,000.

Leucocytes.

In counting the leucocytes much depends on the sort of ruled slide at the disposal of the worker. With the old Thoma chamber at least five different drops must be examined in order that a sufficiently large number of leucocytes may be counted, while with the Türk cell a counting area of 9 sq. mm. is afforded for each drop. The more leucocytes counted so much the less is the error. It is usually sufficient to count the white cells in a single drop, using the Türk chamber, but for scientific purposes three or even four drops would better be examined.

If it is desired to count the leucocytes in the same specimen as the red cells the procedure is as follows: Prepare the drop of blood exactly as described for counting the red cells, using Toisson's fluid as the diluent and the erythrocytometer as the diluting pipet. After the reds have been counted, enumerate the whites in the entire ruled area of this chamber. In this process the leucocytes will be found to be stained a faint blue. It is often advisable, in case of low

leucocyte counts, to repeat this process with a second drop of blood. The calculation by this method is very simple. As the entire ruled area of the Türk chamber covers a surface of 9 sq. mm., each equal to the central area used in counting the red cells, we have the equivalent of 3,600 small squares in the ruled surface. Multiply this figure by the number of drops used to obtain the total number of small squares covered by the count. Thus, if 54 leucocytes were observed in two drops (7,200 small squares) and the dilution was 200, then we have the equation, $\frac{54 \times 200 \times 4000}{7200} = 6{,}000$ cells.

A second method of calculating the number is to consider each sq. mm. of the surface of the Türk chamber as a unit. If, then, the number of cells counted in two drops (18 units) be 54, we divide this number by the number of units counted, 18, and multiply the result by 10 (the cubic contents of each unit) and then by the dilution. Thus, $\frac{54 \times 10 \times 200}{18} = 6{,}000$.

It is usually preferable in counting the leucocytes to use the special leucocytometer previously described, as this gives a smaller dilution, and consequently a larger number of leucocytes to the counting surface. In using this pipet, the blood is drawn to the mark 1 and the diluent, 1 per cent. acetic acid, added to the mark 11. This diluent destroys the red cells and brings out the leucocytes clearly. The dilution of the blood will thus be 10. If a large increase in the number of leucocytes is anticipated, it is better to use a dilution of 20, drawing the blood to the mark 0.5, as a routine the dilution of 10 being, however, preferred.

In counting the white cells with a dilution of 10, using the Türk chamber, it is not as a rule necessary to go over the entire counting field, but more accurate results will obtain if this be done. A count of at least 250 cells is advisable, while one of 1,000 is more to be preferred in scientific work. The method of calculation is the same as given above. Thus if 540 cells were counted in the 9 sq. mm. of the ruled surface, the dilution being 10, we have $\frac{540 \times 10 \times 10}{9} = 6{,}000$. If we have not used the total 9 sq. mm., but have used 4 or any other number which may be considered sufficient by the worker, the divisor in this division will be the number of units counted.

If only a Thoma chamber be at hand for counting the cells, two methods are available. By the first, several drops may be gone over counting the total number of cells in the ruled sq. mm. of the cell. In the second method we find the cubical contents of each visual field and then count the leucocytes in many of such fields. The method of computing these factors is given by Stengel[1] to whose work the author refers the reader who may not have access to a Zappert or a Türk chamber.

In making this leucocyte count great care must be exercised to have the diluent fresh and free from yeast spores, which so freely develop in such mixtures. If this factor be observed, all the cells seen may be counted as

[1] Twentieth Century Practice of Medicine. New York, vol. 7, 1896, p. 271.

leucocytes, but occasionally nucleated red cells may be confusing, especially if these be present in large numbers. The physiological condition of the patient should always be considered in making a report on a leucocyte count, as such factors as digestion and exercise influence this count to a great extent.

The normal error in making a leucocyte count, with a count of 200 and more leucocytes, is about 5 per cent., while in the case of a red count it should not be over 3 per cent,. Careful work with special attention to all the details mentioned will often reduce this error to a lower figure. Naturally, the error in counting the leucocytes will be much reduced by using the Türk chamber and giving the blood a dilution of 10. There are certain errors due to faulty construction both of the pipette and of the counting chamber which remain constant in the same apparatus. Hence it is wise to procure the very best equipment possible and to test the different portions of the pipet for such errors. In this way a very appreciable difference may be obviated. Not all of these blood counters show such variations, but some do, and it is, therefore, a matter of moment to know your tools.

(5). **Cleaning the Apparatus.**

This is the last step in the technic of making a blood count. While perhaps not as important as some of the other steps, yet if not properly carried out it will introduce errors which may prove very annoying. If is readily seen that an unclean pipet or counting chamber will interfere with the proper manipulations as described above.

After the cover-glass has been removed from the slide, wash out the chamber with distilled water and dry thoroughly by means of a clean piece of linen. Place the slide in its proper receptacle, so that it may be conveniently found when desired. This may seem a small point, but the author has seen too many slides lost by carelessness in putting them away. The cover-glass is rubbed clean and dry and put in the case with the slide.

Wash out the pipet with water until all of the blood is gone. In some cases fine clots will be observed sticking to the side of the tube. Under such conditions remove the adherent blood by means of a fine wire or, if this is not effective, draw a little strong potassium hydrate solution into the capillary and allow it to act on the clot. After the tube is apparently clean, wash it out with alcohol and ether. Blow a stream of air through the tube by means of compressed air or the suction pump. Be certain that the apparatus is perfectly dry and clean, and that the glass bead in the ampulla is freely movable as the tube is shaken, before putting the pipet in its case.

Durham's Hemocytometer.

Recently Durham has introduced a modification of the older instruments for blood-counting. This embodies the principles of the various methods, but substitutes a self-measuring capillary pipet for the suction pipet of the Thoma apparatus, and special mixing vessels for the dilution of the blood. This device makes it possible for one inexperienced in blood-counting to accu-

rately measure the blood and diluting fluid and thus eliminates the error possible with the older pipet. The direct count is made in the counting chamber of Thoma. This capillary of Durham is more easily cleaned than that of Thoma, thus giving an advantage in cases where several blood examinations are to be made in a limited time. While this method of diluting the blood has the advantages above mentioned, the writer has never been able to convince himself that the use of this apparatus gives the student any better results than

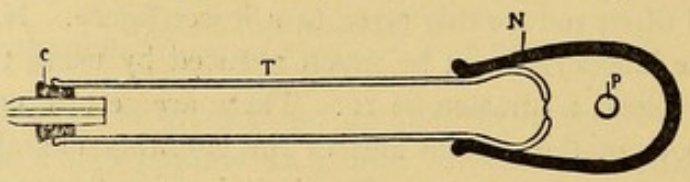


FIG. 136.—Cross-section of Durham's Blood Pipet. (*Da Costa.*)
T, Glass tube; N, rubber nipple; p, lateral perforation in nipple; c, cork in which a capillary pipet is fitted.

he obtains with the Thoma instrument. After some experience, and this is needed in any method, the dilution of the blood with the Thoma pipet is quite as easily made as with the Durham modification, and the results of the count give quite as close checks as do those with the newer method.

Oliver's Hemocytometer.

This instrument was intended to furnish a more accurate method of counting the red cells than was given by the older instruments. The method is based on a principle entirely different from that of the older instruments and does not afford an actual count of the cells. If blood be diluted with a fluid, which preserves the corpuscles, in a rectangular test vessel composed of longitudinally striated glass, each striation of the glass will act as a lens projecting an image of a candle flame viewed through the suspension of opaque particles of the blood, providing the suspension is of a sufficient dilution to permit of the almost unobstructed passage of the rays of light. At the proper dilution, these images of the candle flame will form a bright streak horizontally across the tube. Experiments have shown that the development of this bright line, on dilution of the blood with Hayem's solution, is an accurate measure of the percentage of red cells in the specimen examined. The dilution of the blood is made in a rectangular glass cylinder by means of a capillary pipet, which is washed out with Hayem's solution. The cylinder is graduated into divisions from 10 to 120, each division representing 50,000 red corpuscles.

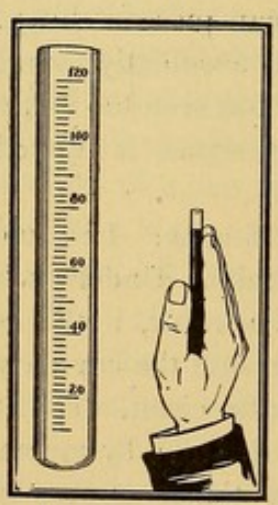


FIG. 137.—Oliver's Hemocytometer. (*Greene.*)

As this instrument has little clinical value, owing to the fact that the error is very great in cases in which the blood is diseased, I refer the reader to other works for a description of the method in detail. In the study of the physiologic variations of the red cells, this method affords very accurate results

giving figures which would be lost with the Thoma instrument. It must be remembered that, in this method, as in many others the personal equation plays a large rôle and may account for serious error which is not found with the Thoma instrument. Emerson has shown that the variations in the results may be as much as 2,000,000 cells when this instrument is compared with the Thoma in counting the blood in primary anemia. Baumgarten has proven that variations in the size of the cell as well as deformities of the cell will introduce a serious error into this method. It, therefore, should be used only to examine normal blood and also to separate those cases with anemia from those without, and should never be used to the exclusion of the Thoma apparatus in studying abnormal blood, as it cannot give an accurate figure for the number of red cells and does not give us any idea of the number of white cells.

Counting the Blood-platelets.

The technic of counting these cellular elements is imperfect and the results inexact and unimportant from the clinical or scientific standpoint. The methods employed are both direct and indirect. The technic of Determann and of Brodie and Russell belong to the latter class and are the more frequently employed, while the method of Helber is a direct one. Determann determines the ratio of blood-plates to the red cells by puncturing the finger through a drop of reagent (9 per cent. aqueous solution of sodium chlorid to which a little methyl violet has been added), mixing thoroughly and examining in a Thoma chamber. A red count is then made in the usual manner and the number of plates calculated. Brodie and Russell use a reagent consisting of equal parts of a 2 per cent. solution of sodium chlorid and a saturated solution of dahlia in glycerin. The technic is the same as in Determann's method, rapid work being necessary as the solutions soon attack the red cells and the staining differences between the plates and the reds are lost. Helber makes a direct count, mixing the blood with a 10 per cent. solution of sodium metaphosphate in a pipet giving a dilution of 1 : 30. The count is made on a ruled slide similar to that of the Thoma chamber, but differing in the thickness of the layer of blood, this being in Helber's slide 0.02 mm.

According to Determann, the ratio between the red cells and the blood plates is, on the average, 22:1. The normal number of plates is about 250,000, this number varying in the same person at different times of day and under certain physiological influences. These variations, as well as those due to pathological causes, will be discussed in a later section.

III. Morphology of the Blood.

Before any examination which is concerned with the study of the morphological characteristics of the blood can be made, it is essential that all of the glassware which comes in contact with the blood should be absolutely clean and dry. The glass slides as they come from the shops, are often coated with substances which are removable with difficulty. Moreover, these slides

are not in all cases perfectly level on both surfaces. It will need but one experience with an uneven slide to convince the worker that it is a loss of time to attempt the use of such slightly convex or concave slides. The cover-glasses should be of the very best quality of glass, should be as thin as possible (number o), and three-fourths inch square. The seven-eighths inch square covers as also the larger rectangular ones are not as desirable for blood work, especially in the examination of fresh specimens.

The slides and covers should be cleaned with soap and water followed by water and alcohol. In some cases it is necessary to soak them in concentrated hydrochloric acid for some hours and then wash with water, alcohol, and ether, or the ordinary acid-alcohol may be used. After being cleaned they should be kept either in 95 per cent. alcohol or, preferably, polished with a clean linen cloth or a piece of tissue-paper and kept in dust-proof receptacles. It is a wise precaution invariably to polish the slides and covers before use, as dust particles are prone to collect even under the best conditions. As a rule, it is better to use only new cover-glasses and not attempt to clean them after use. The slides may, however, be cleaned by boiling with a strong alkali solution, washing with hot water acidified with hydrochloric acid, then with hot water, alcohol, and ether.

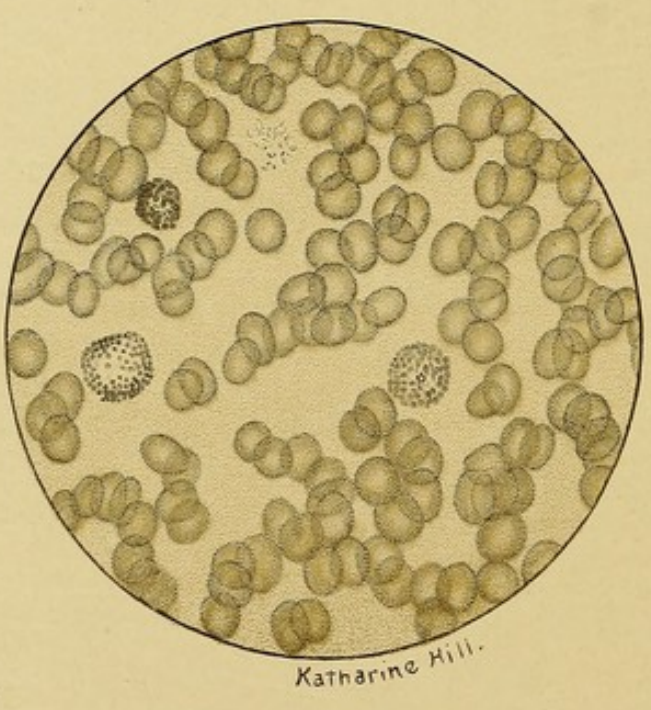
After the cover-glasses have been polished it is the best practice to handle them only with forceps, as moisture is almost certain to collect on them if the fingers be used. This is not only better technic, but more rapid work may be done with their use. Two kinds of forceps are necessary in such work. The first is one for holding the cover firmly, being found as the locking forceps of Ehrlich or the cross-point forceps, while the second is the ordinary pinch forceps with which the second cover-glass is handled in making smears.

(1). Examination of Fresh Blood.

The examination of fresh blood is a very important part of hematological work and should be a routine procedure in every case possible. If the blood cannot be examined for several hours after being taken, it is wise not to attempt the study of a fresh specimen, as so many changes will occur in such slides that no certain findings obtain. The information obtainable from such examination of fresh blood often supplements that which one may derive from a study of the stained specimens, as some peculiarities, such as the ameboid movement of the leucocytes or the motility of the malarial parasite, may be studied only in this way.

Technic.

Assuming that the slides and cover-glasses are clean and dry, the ear is punctured as previously described. Wipe away the first few drops of blood and touch the center of a cover-glass, held with the pinch forceps, to the top of the next drop, which should be about the size of a small black-headed pin. If this drop be too large the layer of blood will be too thick to permit of proper examination. Care should be taken that the cover-glass does not touch the



FRESH NORMAL BLOOD. (ZEISS OCULAR 4, OBJECTIVE DD.)

skin. Drop this cover onto a slide, which has been warmed by rubbing or by passing through a flame. If the glassware be clean, the drop will spread evenly in a thin circular layer, not quite to the edge of the cover-glass. Under no circumstances should pressure be used to thin the layer or to readjust the cover after it has settled on the slide, as artefacts may be easily introduced in this way. The slides thus prepared are examined first with a low-power lens to obtain an idea of the even distribution of the cells over the entire area. The detailed study is carried out with the 1/12 oil immersion lens, but it should be remembered that a smaller magnification may give a better general survey. These preparations will keep long enough for the purposes of examination, but if one wishes to preserve the blood fresh and uncoagulated for a longer period it is well to enclose the cover-glass with vaselin or paraffin or to use the ordinary hanging-drop chamber as suggested by Rosin and Bibergeil. It is sometimes desirable, especially in the study of malarial parasites, to use a warm stage or a warm chamber. If, however, the specimen is examined soon after its preparation, no such precaution is necessary provided, the room is not cold.

In order to judge of the changes which abnormal blood may show in the fresh state, one must be thoroughly familiar with the appearance of normal blood. This latter knowledge can be obtained only by frequent study of fresh normal specimens and not from any text-book description. To attempt to learn without microscopic study the size, shape, color, and refraction of the red and white cells, the relation of the blood-plates to fibrin formation, the number of the various cells and their ratio to one another, would be absolute idiocy.

An examination of the fresh blood as described above gives information regarding the presence or absence of the malarial parasite, the spirochete of relapsing fever, the filaria, and trypanosomes. It affords evidence of increased or decreased rouleaux formation, number, deformities, and degenerations, as well as of the amount of hemoglobin of the red cells; the presence of a leucocytosis or of a leucopenia and of ameboid movement of the leucocytes. However, care must be taken to avoid premature conclusions from such study and to institute further examinations of the stained specimen to clear up doubtful points. The observer must be on his guard lest he mistake the normal Brownian movement in the protoplasm of the cells for ameboid or parasitic movement. Curious phenomena are observed in the fresh specimen as the blood dries and should not be misinterpreted. The various characteristics of fresh blood will be taken up in detail later. The introduction of the ultra-condenser or dark-field illuminator has opened up a field of great possibilities in the examination of specimens of fresh blood, especially when malarial parasites or spirochætæ pallidæ are suspected.

(2). Preparation of Smears.

To prepare blood smears, which are to be later examined in the stained condition, one may spread the blood in capillary layers on slides or between

cover-glasses. The former method is the one used in the writer's laboratory and has given excellent and satisfactory results. A fair-sized drop of blood is collected on one end of a clean dry slide, held between the thumb and second and third finger of the left hand. A second slide is held in the same manner by the right hand, but at an angle of 45 degrees to the first one and touching the drop of blood. Allow the blood to spread out by capillarity along the edge of the second slide. As soon as this occurs, draw the drop of blood along the first slide with a clean sweep, exerting little pressure with the second slide and maintaining the angle of 45° between the two slides, allowing the second slide to rest rather upon the blood than upon the slide (see cut). In the

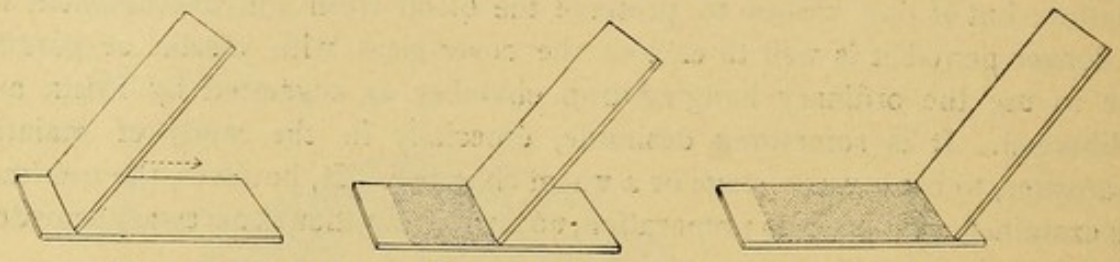


FIG. 138.—Preparation of smears with two glass slides. (*Da Costa.*)

process, as recommended by some writers, the second slide is gradually drawn into a position perpendicular to the first one. This procedure does not yield, in the writer's hands, as good results as the former method, as it is more difficult to maintain equal pressure, the smear being as a result too thick or too thin in places. Instead of a slide, a cigarette paper may be used as a spreader and gives good results. This method of making blood smears has the advantage of offering a large surface for examination, of making smears which are fairly uniform after some practice, of dispensing with the necessity of mounting the specimen, and of permitting the fixation of the smear in the free flame. It is less expensive than the method to be described later and permits of the

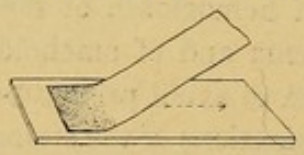


FIG. 139.—Preparation of blood smear with cigarette paper. (*Da Costa.*)

cleaning and later use of the slides. The beginner may find, on examining his early specimens made by this method, that the leucocytes collect at the distal end of the smear and that the general surface contains few white cells. This is due to the use of undue pressure in making the smear, and may be avoided by proper attention to this detail.

A second method which has many advocates is the use of two cover-glasses. One clean, dry cover-glass, which should not be too large (preferably three-fourths inch square or the larger rectangular slips), is held by the Ehrlich or cross-bladed forceps or, as some advise, between the thumb and first finger of the left hand. The other cover, held in the pinch forceps or between the

thumb and first finger of the right hand, is touched to the drop of blood as it wells from the puncture in the ear. This second cover is then dropped at once upon the first in such a way that the corners of the two glasses do not coincide. If the glasses are clean the blood spreads out evenly in a thin capillary layer between them. As soon as the spreading is complete, the two covers are drawn apart, in a line parallel to the plane of their surfaces, by a steady, quick motion, being sure to avoid lifting them apart. This manipulation can be learned only by practice and never from any description. If the fingers are dry and if care be taken to touch only the corners of the covers the forceps need not be used in separating the covers, but it must be remembered that moisture will

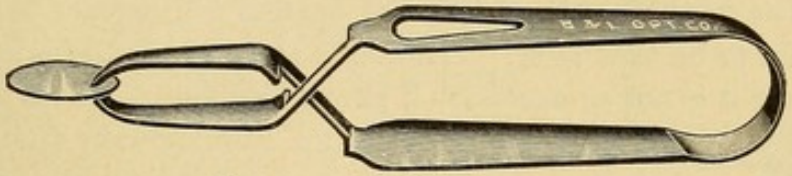


FIG. 140.—Ehrlich forceps.

cause changes in the specimen. It is, therefore, advisable to use the forceps in this part of the technic unless the rectangular slips be used. As soon as the covers are separated, they are allowed to dry in the air or by waving them two or three times to and fro. They should be at once placed in a clean, closed receptacle, such as a Petri dish, until ready for the later complete fixation and staining, as dust will collect upon them or flies may attack them if left in the open air. It is rarely necessary to fix these smears at once, but with some stains such treatment is advisable. This second method of making the blood smears is more difficult than the first, is not so reliable, does not give as great a surface for examination, and always shows the lower cover-glass better and more uniformly spread than the upper.

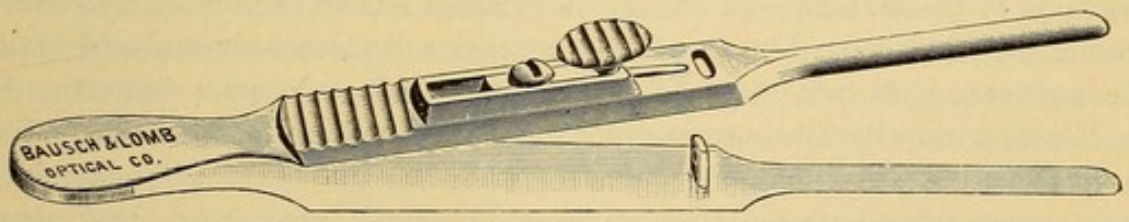


FIG. 141 —Pinch forceps.

Smears made by either of these methods should be uniform throughout with the exception of the edges, which should never be used as they are too thick for allowing definite conclusions to be drawn. The red cells should lie on their broad surface, should not be in rouleaux except at the edges, and should not show deformities due to errors in technic. The leucocytes frequently collect at the edges of the specimen if too great pressure be used in making the smear, while the platelets always collect at the point first touched by the second slide or cover-glass. The preparation of thin even smears is necessary for the proper carrying out of the later technic. Those specimens which are irregular or are too thick would better be discarded, as the time

consumed in studying such specimens will not be compensated for by the results obtained. It is much better to make several smears than to be content with a few bad ones. A little experience with poor smears will convince the worker that it is advisable to use great care in preparing them, especially if a differential count is to be made or if a study of the degenerations and deformities of the cells is to be undertaken.

(3). Fixation of Smears.

Before any staining of the cellular elements of the smear takes place, the protein constituents of the blood must be coagulated by exposing the air-dried film to the action of a high degree of heat or to that of various chemical reagents. The selection of the method of fixation will depend to a great extent upon the stain to be used later. Fixation is always essential if aqueous stains are used, while it is not so necessary if strong alcoholic solutions are employed. In the use of the different modifications of the Romanowsky stain, the fixation is done by the methyl alcohol employed as a solvent for the various stains. As a general rule, fixation by heat is preferable to that by chemicals, as artefacts are less prone to appear, providing the degree of heat is carefully regulated.

Fixation by Heat.

This method, which is the most difficult to use and which is at the same time the best, is the only one which is reliable when Ehrlich's triple stain is to be employed. The principle is as follows: The air-dired specimen is subjected to the action of a temperature of 110° to 150°, for a more or less varying length of time, depending on the experience of the special worker. The lower the temperature the longer must its action be exerted.

The apparatus most frequently employed is the copper plate introduced by Ehrlich. This is an unpolished triangular plate of copper about 3 mm. thick, 30 to 50 cm. long, and 10 cm. wide, which is held in position by vertical standards. It is heated by an alcohol lamp or a Bunsen burner placed under the narrower end until the temperature of the plate becomes constant, the parts nearer the flame being naturally warmer than those more remote. The temperature of the different portions of the plate may be readily found by determining the points at which water (100), toluol (110), xylol (140), or of turpentine (150) boil. The slides or cover-glasses are then placed, smeared side up, at the desired point (the outer margin of the glass being three-fourths inch from the boiling-point and toward the flame). Just how long a period is necessary, at the temperature selected, to give a perfect fixation, will depend upon the age of the specimen and upon the condition to be studied. It is good practice to place several slides at the point desired for a period of one hour and then remove a slide at intervals of 15 minutes thereafter. One of the specimens is sure to be good by this method and the remaining ones may, therefore, be properly heated. Freshly made specimens require longer heating than the old ones, while normal blood requires a longer exposure than abnormal specimens. As a rule, the specimens require, when the triple stain is to be

used (and this is the one most frequently employed with heat fixation), from one to one and one-half hours at a temperature of 110, although some workers use only a few minutes' (one to three) exposure to such temperatures. The higher the temperature the less time is essential. Rubinstein uses a point at which a drop of water does not boil, but assumes the spheroidal state (so-called Leidenfrost phenomenon) and places the slides, with the smeared side down, upon the plate at this point for one-half to three-fourths minute. Some workers, as Pappenheim, use this point, but place the smeared side upward.

Instead of the copper plate, one may use the ordinary drying oven or a Victor Meyer heater. It is the writer's custom to use a copper drying oven heated by a gas flame regulated by a thermostat. The slides, with the smeared side downward, are placed on a glass plate whose temperature is measured by an accurate thermometer. The temperature is allowed to increase gradually to about 80°, from which point it is more quickly raised until the desired stage is approximated, when the heating must continue slowly, the final temperature being maintained for 15 minutes. The best temperature for fixation, in the writer's experience, is 110° to 120° for 15 minutes when the staining is to be done with eosin-methylene blue or eosin-hemotoxylin, while for the tri-acid stain a temperature of 120° to 125° for one and one-half hours should be maintained. Some workers heat to 160° rapidly and then allow the films to cool to 30°, when fixation is complete in about 15 minutes. Engel and Cabot recommend, in the absence of other equipment, the passing of the smear through the flame several times. Such treatment often yields excellent results, but is uncertain and requires much experience. It is to be remembered that too rapid changes of temperature are to be avoided, as shrinking or splitting of the cells will occur under such conditions. So much depends upon proper fixation that a little more time spent in obtaining good specimens will shorten the time necessary for the future examination. Proper staining depends on proper fixation, especially with the tri-acid stain, in which cases the over- or under-heating is evident in the color tone of the erythrocytes.

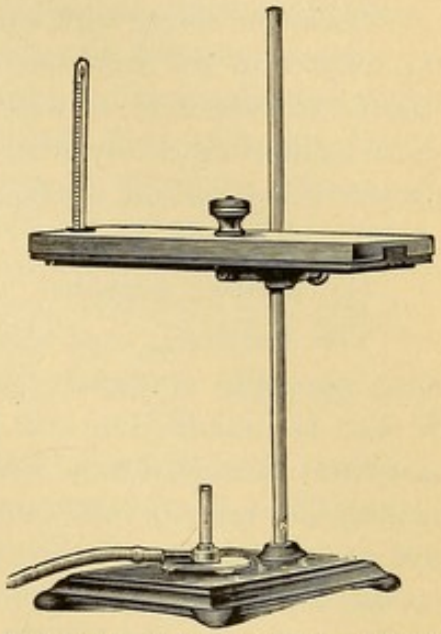


FIG. 142.—Oven for fixing blood films. (*Da Costa.*)

Chemical Fixation.

(1). Absolute Alcohol.

Allow the alcohol to act for five minutes to one hour, or boiling alcohol for one minute. The slide is simply covered with the fixative and left for the desired period. Much depends upon the stain to be used as to the time necessary for good fixation. If an alcoholic stain, five minutes is sufficient; if a

watery or alkaline one, a longer time is essential. After fixation drain off the alcohol and allow the residue to evaporate in the air or wash with water and dry between sheets of filter-paper. If alcoholic stains are to be used the moist specimen may be directly passed through the flame. This fixative is unreliable if a study of the neutrophile granules is to be made, but it brings out the nuclear structures fairly well.

(2). **Nikiforoff's Method.**

Cover the smear with equal parts of absolute alcohol and ether and allow the fixative to act from one-half to two hours. After the fixation allow the fixative to evaporate or wash with water and dry. Some workers advocate short fixation especially where the malarial organism is to be studied. This fixative brings out the degenerations of the red cells in good shape.

(3). **Methyl Alcohol.**

This fixative is used absolutely pure for three to five minutes. It is the most generally applicable chemical fixative and gives beautiful specimens. If used in combination with stains, as in the Romanowsky methods, it gives as perfect preparations as absolute alcohol in one-half hour and brings out the neutrophile granulations in better outline. A longer fixation than three to five minutes does no harm; and a shorter one, especially if the fixative is the solvent for the stain, will give fair results, the outline of the cells being sharper the longer the fixative is allowed to act.

(4). **Formalin.**

This fixative may be used as a 1 per cent. solution in 95 per cent. alcohol and allowed to act for one minute, as Benario advocates, but the writer has had much better success with the 0.25 per cent. solution in 95 per cent. alcohol for one minute, as Futcher and Lazear suggest. Allow the fixative to act for one minute, wash in water, and dry between filter-paper. Some writers advocate the direct treatment of the fixed smear with the stain without an intermediate washing and drying, but I have never found the results as good by this method. Instead of the formalin solutions, the vapors may be used by placing the specimen under a bell jar with a few drops of 40 per cent. formalin and allowing the fixative to act for one to five minutes. The normal staining of the cells is not always as clear as could be desired after the use of such a fixative.

A large number of inorganic fixatives have been advised, among them being mercuric chlorid, chromic acid and osmic acid, but these fixatives are much more apt to produce artefacts than are the others mentioned above. While these latter fixatives give good demonstrations of the nuclear structures and of mitotic figures, the granules are very imperfect; but chromic acid has many advantages as a fixative in the study of the chromatin elements.

(4). Staining Methods.

(*A*). General Considerations.

Since the work of Witt, we have recognized that the color of an organic substance is due to the presence of two definite atomic complexes in the molecule of the colored substance. The first of these, the chromophore group, is responsible for the chromogenic properties of the substance, while its influence as a dye is increased by the presence of the second or auxochromic group. The color of the compound is the more intense the more of these groups are present. Groups or atoms which intensify the color of the substance are called, by Schütze, bathochromes, while those which reduce the color are called hypsochromes.

While the dyes, the so-called anilin dyes, used in hematological work are all aromatic derivatives, it must not be assumed that such are alone characterized by staining qualities, as many simple aliphatic derivatives show a definite color and exert some staining property, depending on the presence of the two groups above mentioned. The chief chromophore groups are the CO (carbonyl) group, the CS group, CN, HCN, the $-N=N-$(azo group), the $\begin{matrix} -N \\ | \\ -N \end{matrix}\!\!>\!O$ (azoxy group), NO (nitroso group), $-N\!<\!\begin{matrix} O \\ | \\ O \end{matrix}$ (nitro group), and the $-N=SO$ group. The most important auxochrome groups are the NH_2 and the OH groups, the former being a much more powerful one than the latter. These auxochrome or haptophore groups make possible the union of the stain with the tissue as a direct salt formation. Substances, which possess a chromophore group and are colored or intensified by the presence of the auxochrome group, are called chromogens. It is, therefore, evident that the effect of an auxochrome depends not only upon its own nature, but also upon that of the chromogen. Through the introduction of H by means of hydrocarbon radicals, new auxochromes are produced and the color becomes intensified, the effect being so much the greater the higher the molecular weight of the substituting hydrocarbon radical.

As a large number of colored substances have a tendency to form tautomeric compounds, the salt formed by combination with the auxochrome groups may have a different constitution from that of the free base or acid. Many chromophore groups are also capable of forming salts, but only with strong acids or bases. In this formation of salts, by union with the auxochrome or chromophore groups, we must remember that halochromia may be seen. By this is meant that uncolored or weakly colored substances may combine with acids to form salts without the color being due to the presence of a chromophore group. It is essential that the chromophore group possess a double bond of union, as oscillation in one portion of the molecule is thus possible. As the chromophoric as well as the auxochromic radicals may have acid or basic tendencies, it is manifest that the reaction of the substitution product will depend upon the interrelations of the acid and the basic radicals. The best dyes are, therefore, obtained by substitution in one direction, that is, by accumulating either basic or acid groups separately.

It is thus seen that we have two general classes of dyes, the acid and basic, depending upon the preponderance of the total acid or basic groups in the molecule of the dye. It has been shown by Ehrlich that these acid and basic dyes, may be so combined that a third one results, showing neither acid nor basic properties. This class of dyes, known as neutral dyes, is of the greatest importance in hematological work. In them we have not only the staining properties due to the original chromophore and auxochrome groups of both the acid and basic dyes, but also those due to the union of the component groups in the neutral dye. Such dyes are hence called polychrome dyes and are usually soluble in an excess of one of the component mother dyes, generally the acid one. It must be understood that in speaking of a dye as acid, basic, or neutral, we do not refer so much in our staining work to the chemical reaction of the dye, but to the portion of the dye to which the staining is due—that is, to the specific auxochrome and chromophore groups.

Among the basic stains we find methyl green, methylene blue, amethyst violet, neutral red, dahlia, pyronin, thionin, fuchsin, methyl violet, Bismarck brown, alum hematoxylin, and safranin. Most of these stains, depending on the strength of their component groups, color the nuclear substance of both red and white cells, as also the cytoplasm and certain granules of abnormal red and of some normal white cells, the staining being influenced, as Matthews has shown, by the alkaline reaction of the tissues. The most important acid stains are eosin, acid fuchsin, orange-G, indulin, nigrosin, aurantia, and salts of picric acid. These dyes color the red cells and the eosinophile (oxyphile) granulations of the leucocytes. The neutral portion of the dyes color the so-called neutrophile granules of the leucocytes.

In the process of staining, it is a question whether we have to do with purely chemical processes as Knecht's theory assumes or whether the solid-solution theory of Witt or the mechanical theory of surface attraction are accountable for the phenomena observed. It is probable that the salts of the dyes are decomposed by the cells and that new compounds result from the union of the acid and basic stains and the various chemical entities of the cells. Yet we have instances in which the dye is simply stored in the cell without any chemical union taking place. We must, however, account for the elective character of certain stains by a purely chemical activity, as, for instance, chromatin, which undoubtedly consists of nucleinic acid, always takes a basic stain, even though a neutral compound is used as the staining agent. It is to be remarked that a neutral stain does not color all of the acidophile or basophile substances of the cells of the same tint. Thus, eosinophile granules are differently colored from the oxyphile hemoglobin of the red cells. The neutral portion of the stain has nothing to do with the staining process beyond the coloring of the neutrophile substances in the protoplasm of certain leucocytes.

In the selection of a stain for general hematological work, it is necessary to use a compound stain or, at least, two simple stains, one after the other. A single simple stain colors only a few of the elements and affords no general

idea of the blood picture. Indeed, to obtain definite conceptions of the finer structure of the blood, it is necessary to study several slides stained by different methods. It is usual to select for routine work a stain which will reveal the greatest amount of information regarding the histological characteristics of the blood. This is the so-called panoptic staining and is to-day generally accomplished by the use of the various forms of neutral stains. In making the stains for one's self or in buying them in the market, one must be certain that only chemically pure pigments are used and that the solution is made according to the formula with the purest solvent obtainable. It will need only one experience with a poorly made stain to convince the worker of the importance of this detail.

(B). Methods of Staining.

In his work the author uses, for routine purposes, the Wright's stain, the eosin-methylene blue, and eosin-hematoxylin stains. These stains have the advantage of giving clear-cut pictures of practically all of the important blood elements along with simplicity of technic. As it is not always possible to secure or even to make a Wright or an Ehrlich triple stain which will give reliable results under all circumstances, the writer feels that the general worker would better use the eosin-methylene blue stain for his daily work. The many modifications of the Romanowsky stain have their advocates, and it must be said that they yield reliable results when they are working properly, but no one can say when they will go wrong or how to make them work right when once they do give poor results.

Eosin-Methylene Blue.

A number of methods of using these two simple stains have been advocated by such workers as Chenzinsky, Ehrlich and Lazarus, von Willebrand, Plehn, Aldehoff, and Gabritschewsky, but the writer has found the method advocated by Müllern[1] to be the most generally applicable and, if properly applied, the most reliable modification. By it we are able to stain all of the blood elements, including the neutrophile granules and obtain, thus, a panoptic picture whose findings are not excelled by those of the various modifications of the Romanowsky stain.

Technic.

(*a*) Fixation of the smear in pure methyl alcohol for three minutes. (*b*) Preliminary staining in 1/2 per cent. alcoholic (70 per cent.) solution of Grübler's "french pure" eosin from three to five minutes. (*c*) Wash in distilled water and dry between filter-paper. (*d*) Lay the slide in a carefully measured and well-mixed solution of 20 drops of 1/4 per cent. aqueous solution of methylene blue (B. pat.) and ten drops of the above eosin solution for one-half to one minute. (*e*) Wash quickly and briefly with distilled water and dry at once between filter-paper or over the flame. (*f*) Mount if desired in Canada balsam or examine directly with a high-power lens.

[1] Türk's Vorlesungen, Wien, 1904.

This stain shows the red cells and the eosinophile granules of the leucocytes of a bright red tone, the neutrophile granules pink to bright red (distinguished from the eosinophiles by their smaller size), the nuclei, mast-cell granules, bodies of the lymphocytes, platelets, malarial organisms, trypanosomes, and filaria varying shades of blue. The preliminary staining with eosin serves the purpose of bringing out the neutrophile granules more clearly while the basophile granulations are of course unaffected by such treatment. It is probable that the later staining with the eosin-methylene blue mixture has the same characteristics as the neutral stains to be mentioned later. While this stain has the advantage of simplicity, reliability, and panoptic power of staining, it is somewhat inferior to other stains in bringing out some forms of the malarial organism, owing to its lack of chromatin staining qualities. The writer has found that the eosin and methylene blue above mentioned are the best to use in this process and that Türk's advice, regarding the use of fairly fresh eosin solutions and of old methylene-blue solutions, is warranted. The blood preparations should not be over a few days old to show the best pictures, as those over a week old may show a diffuse plasma staining and the neutrophile granules not clearly differentiated. If the nuclei do not stain well with the methylene blue as many writers claim, make a second preparation fixing somewhat longer in methyl alcohol. By following out these precautions the worker will be rewarded with beautiful specimens. This method is to be recommended for all routine examinations.

Eosin Hematoxylin.

This stain is especially important in cases in which the nuclear structures are to be studied. It stains the nuclei beautifully, showing their finer structure, karyokinetic figures, and pycnotic qualities, as well as the basophile granules of both red and white cells. The solutions required are: (1) 1/2 per cent. Grübler's blood eosin in 70 per cent. alcohol. We may use, with equally good results, the eosin mentioned in the previous method. (2) Delafield's hematoxylin, the formula of which is

Hematoxylin crystals,	4 grams
Alcohol (absolute),	25 c.c.
Ammonium-alum crystals C.P.,	52 grams.
Distilled water,	400 c.c.
Glycerin, C. P.,	100 c.c.
Methyl alcohol, C. P.,	100 c.c.

Rub up the hematoxylin crystals with the alcohol until they are dissolved and place the solution in a loosely-corked glass bottle, allowing it to stand exposed to the light for four days. Dissolve the ammonium-alum in the water and allow it to stand exposed in the same way for four days. At the end of this time mix the two solutions, shake thoroughly, and filter at the end of three hours. Add the glycerin and methyl alcohol to the filtrate and allow this to

stand overnight. Filter the mixture, place it in a clear bottle, and allow it to ripen, exposed to the light for six weeks, when it is ready for use.

Technic.

Stain the specimen with the eosin solution for one-half minute, and wash in water. Without drying place the slide in the hematoxylin solution for one to three minutes, the time varying with the particular stain and with the experience of the worker. Wash with water, dry, and mount. This stain does not give as good results as does the former method, but is to be especially recommended when the nuclear structures are to be studied.

Ehrlich's Triple Stain.

In the literature of hematology we find the expressions "triacid" and "triple stain" used synonymously. The triacid stain, as originated by Ehrlich, was a mixture of equal parts of saturated solutions of indulin, nigrosin, and aurantia, and was used to differentiate the eosinophile granules. When used synonymously in these days, the triple stain is always meant. The composition of this latter stain is as follows, made up of two acid and one basic stain:

Saturated watery solution of orange-G,	13–14	c.c.
Saturated watery solution of acid fuschin,	6–7	c.c.
Distilled water,	15	c.c.
Alcohol (absolute),	15	c.c.
Saturated watery solution of methyl green oo,	12.5	c.c.
Alcohol (absolute),	10	c.c.
Glycerin,	10	c.c.

The pigments must be chemically pure and the solutions must be added in the order named, the methyl-green solution being added drop by drop with constant stirring.

This stain is difficultly prepared, being usually a failure. The writer would, therefore, advise the worker to obtain it already made; and even then it may not prove satisfactory. The correct stain should have a russet-brown color and should not show any trace of a precipitate. It improves up to a certain point with age but, after a time, precipitates invariably occur, making it useless. It should never be filtered or shaken, the solution to be used being taken from the center of the bottle with a dropping pipet.

Technic.

The smear should be fixed by heat as previously described. After proper fixation, cover the smear with the stain and allow it to act for one to ten minutes as the experience of the worker with the particular stain may indicate. Wash with distilled water, dry, and mount. One advantage of this stain is that it cannot overstain, those films appearing so being underheated, while those understained are overheated.

This stain shows the red cells of a buff or orange color, without the slightest shade of red (a reddish tint is given with underfixed specimens while a yellow tone is shown by those over-heated), the nuclei of the leucocytes a dark green, those of the normoblasts black, the neutrophile granules a lilac color (though some occasionally show a reddish tinge), and the eosinophile granules of a crimson tone.

This stain was introduced as specific for the neutrophile granules, but its disadvantages are too numerous to warrant its recommendation as a routine stain. It is a poor nuclear stain, does not show the structure of the normal mononuclear leucocytes, does not stain the basophile granules, nor the malarial or other parasites. For a reliable preparation, showing those features for which it is especially adapted, a proper fixation is an absolute essential.

On account of the lack of nuclear staining with the triple stain, Pappenheim has substituted methylene blue or methylene azure for the methyl green, and eosin for the acid fuchsin. The writer has had no experience with this modification and cannot, therefore, speak regarding its value.

Polychrome Methylene-blue-eosin Stains.

These stains are very numerous, each having its advocates. They are easy to use, contain a reliable fixative, and give satisfactory results, but are not always obtainable or easily prepared. They are the stains which give, perhaps, the best panoptic results and are especially serviceable in the study of the malarial organism and other parasites as many of them contain chromatin-staining elements. The granulations of the leucocytes are not as well marked in all cases as they should be, so that for a complete study of the various types of granules in the cells several stains would better be used.

Romanowsky had found that the addition of a watery eosin solution to an aqueous methylene-blue solution, until an insoluble precipitate began to form, gave rise to new staining properties of the solutions, in the sense that the chromatin substance of malarial organisms was stained a beautiful red. The specific staining properties of this mixture were later found to be due not to a combination of eosin and methylene blue, *per se*, but rather to the formation of a new compound between eosin and an impurity or decomposition product in the methylene blue, namely, methylene azure. Jenner in his stain makes use of a methyl-alcohol solution of the isolated precipitate, the eosinate of methylene blue, which lacks the red chromatin staining element. The same may be said of the May-Grünwald stain.

In making up these polychrome stains it is not general to use the pure methylene azure and eosin, but rather solutions of methylene blue containing a variable amount of the methylene azure to which eosin is added. Recently Wilson,[1] in a careful study of the methylene-blue-eosin combinations, has shown that very little methylene azure and methylene violet exist in the stains as commonly employed. He finds evidences of at least four staining bodies in

[1] Jour. of Exp. Med., vol. 9, 1907, p. 645.

such mixtures, namely, the eosinate of methylene blue, eosinate of methylene violet, eosinate of methylene azure, and eosinate of thionin.

Wright's Stain.

Preparation.

To a 1/2 per cent. solution of sodium bicarbonate in distilled water is added 1 per cent. by weight of Grübler's medicinal methylene blue (any of the varieties of the dye may be used). Place the alkaline mixture in an Erlenmeyer flask and steam in an Arnold sterilizer for one hour, counting from the time steam begins to be evolved. This process develops the polychromatic powers of the alkaline solution of methylene blue and increases its virtues as a nuclear and granular stain. On cooling, the steamed solution is poured into a large evaporating-dish and to it is added, with constant stirring, enough of a 1/10 per cent. aqueous solution of Grübler's yellow water-soluble eosin to change the color from blue to purple and to form a metallic luster on the surface. This will require about 500 c.c. of the eosin solution for every 100 c.c. of the methylene blue solution. The resulting granular black precipitate contains the active principles of the stain. Collect this precipitate on a filter and allow it to dry, being careful not to wash it. When thoroughly dry, make up a 3/10 per cent. solution of this precipitate in pure methyl alcohol. This will require some time and some stirring of the mixture as the precipitate does not go into solution easily. Filter the solution and add to the filtrate 25 per cent. of its volume of methyl alcohol to dilute the stain and lessen its tendency to become precipitated during the staining process.

Technic.

The air dried films are covered with the stain for one minute; without washing off the stain add to it, drop by drop, water until the mixture becomes semitranslucent and a reddish tinge becomes visible at the edges of the slide or cover-glass. Eight or ten drops of water will usually suffice for this purpose. This diluted stain is then allowed to act for two to five minutes, after which it is washed off with distilled water, the blood film now appearing of a deep blue or purple color. Immerse the slide, stained side downward, in a dish of distilled water, to develop the differential staining properties of the various elements by decolorizing the overstained specimen. This process is complete, as a rule, in three to five minutes, but the experience of the worker, with any particular specimen, may require longer decolorization. The films will now appear reddish in color. When the desired degree of decolorization has occurred, dry the specimen quickly between filter-paper, mount if desired, and examine first with the low-power lens to observe the staining effects, and then with the high-power lens for the more minute study. When searching for malarial parasites, the decolorization would better be of short duration, as the chromatin suffers to a great extent in this process.

This stain shows the red cells colored either orange or pink (depending on the time of decolorization), nuclei of leucocytes blue or dark lilac, neutro-

phile granules lilac, eosinophile granules red or pink, fine basophile granules deep blue, large mast-cell granules purple, protoplasm of the lymphocytes robin's-egg blue, blood-plates deep blue or purple, bacteria blue, malarial and other parasites blue, the chromatin element varying from lilac to ruby-red to black. Polychromatophilia and granular degenerations are well shown, the granules being blue. This stain is very useful in studying lymphocytes, mast cells, blood-plaques, and the finer structure of the malarial organism; but the leucocytic granules, at times, are not sufficiently differentiated. The writer has, however, found this stain very well adapted for making a differential count as it brings out, in clear relief, the most important characteristics of the various types of leucocytes. When a good Wright stain is at hand it can be recommended as a most serviceable one for routine work.

Giemsa Stain.

Giemsa has shown that the complicated methods of preparing the dyes may be dispensed with by the use of the pure staining substance extracted from the polychrome methylene blue, namely, the methylene azure. If this pure pigment be used the stain becomes a pure chromatin one. It is advisable, therefore, to use Grübler's azure II, a combination of equal parts of methylene azure and medicinal methylene blue. The formula of the Giemsa stain is as follows :

Azure II,	3.0 grams.
Eosin B. A.,	0.8 gram.
Glycerin (C .P.),	250.0 c.c.
Methyl alcohol (C. P.),	250.0 c.c.

Grind up the dyes in the alcohol and then add the glycerin.

Technic.

Fix the films in methyl alcohol, and stain for five minutes in a mixture of 14 drops of the stain to 10 c.c. of distilled water. If necessary, a trace of sodium carbonate may be added to the water to intensify the basic stains. Wash in water, dry, and mount. It is to be remembered that oil of cedar will bleach these specimens quite rapidly, so that it is advisable, if the films are to be kept, to stain them upon the slides so that mounting becomes unnecessary. Strong light should not be allowed to act on these stained films for any length of time as the chromogen stain fades rather quickly under these conditions. The various elements are stained as with the Wright stain, but the neutrophile granules are often not well defined. This modification of the Romanowsky stain is especially serviceable in the study of the malarial organism and of smears which have been kept for months before being stained, the diffuse plasma staining which so often occurs with the other modifications being largely avoided by the use of this stain.

Specific Stains for Malarial Organisms.

While the above stains all give good results with the malarial organisms, enabling one to make a diagnosis from an examination of the smear, yet certain other stains are often of advantage in that little else is stained and thus the confusion arising from indefinite staining is obviated.

Thionin Stain (Futcher and Lazear).

Add to 100 c.c. of 2 per cent. carbolic acid 20 c.c. of a saturated solution of thionin (Lauth's violet) in 50 per cent. alcohol, and allow this mixture to ripen for a few days. Fix the specimen by the formalin method given above and stain the smear for 10 to 15 seconds. Wash in water, dry, and mount. These preparations do not keep indefinitely, usually fading within a year.

The plasmodia are shown as deep purple, irregular masses enclosed in the faint green red cells. The hyaline forms show as reddish-violet ring-like bodies. The basophile granules and the nuclei are the only other elements showing any particular staining qualities.

Nocht's Stain.

This is essentially a Romanowsky stain and is made as follows: Add two or three drops of 1 per cent. aqueous eosin solution to 2 c.c. of water. To this diluted eosin solution polychrome methylene blue is added, drop by drop, until the red color of the eosin is only faintly visible (the polychrome methylene blue is a 1 per cent. solution of methylene blue chemically changed by heating with 1/2 per cent. sodium carbonate solution for two days at 55° C.).

Technic.

Fix the film in methyl or ethyl alcohol and lay it face downward in the above mixture for five to ten minutes. Wash with water, dry, and examine. The picture is the same given by Wright's stain, except that the nuclear transformations and the chromatin substance are better differentiated, being stained a bright red color.

The many other stains for the examination of fixed specimens will be passed over, as the writer finds the above stains applicable to practically all routine work. Many writers prefer different stains from those mentioned, but these stains do not seem to have any advantages which would warrant their use to the exclusion of those given above. Special stains used for bringing out certain granules and deformities will be discussed under later headings.

Vital Staining.

Although the examination of stained specimens of the blood is usually made with the dried and fixed smear, excellent results obtain when the fresh blood is stained without previous fixation. It is true that a "vital" staining of the blood-cells does not actually take place, as the dyes are decolorized by the reducing and oxidizing action of the living cells. However, a "post vital" staining, that is the staining of whole cells or portions of the cells, after

their removal from the circulation and before the death of the cell results, may be accomplished in several ways.

We may either add to the fresh drop of blood a few crystals of the stain, as advised by Arnold, and note the staining of certain leucocytic granules and nuclei and recticular structure of many erythrocytes; or we may first dry a staining solution upon the slide, cover this dry stain with a drop of fresh blood, adjust the cover-glass, and seal this to the slide with wax. Some workers prefer to use the hanging-drop slide in the preparation of these fresh specimens.

The stains which may be used for vital staining of the nuclei, granules, and plates are methylene blue, toluidin blue, thionin, neutral violet, Capri blue, Nile blue, brilliant-cresyl blue, Janus green, and paraphenyl blue. Of the protoplasmic stains we have fuschsin, acridin red, pyronin, safranin, and neutral red.

While the results derived from this method of staining are not as numerous as those of the more usual method, yet it affords much valuable information regarding the vital properties of the cells and regarding the normal structure and the circulatory changes of the cells. This method has been used extensively by Ito, Rosin and Bibergeil, Levaditi, Cesaris-Demel, and Pappenheim. Their results lead us to assume that further study will give us much valuable information concerning details of structure both of the cell and of the nucleus. It may be possible by this method to differentiate between certain forms of degeneration of the cell which are now known only indefinitely under the names of metachromatic and polychromatic staining.

(5). Erythrocytes.

(*A*). Appearance and Structure.

In fresh, normal blood the red cells, or erythrocytes, appear as thin, flattened, homogeneous, biconcave, nonnucleated, discoid bodies with a sharply defined regular outline and a clear semitransparent center. In some cases they may appear distinctly cup or bell-shaped. The cells show, when examined singly, a pale greenish-yellow color but, when more thickly grouped, exhibit a reddish tint. The degree of color in these cells depends upon their hemoglobin content, the clear central area becoming larger and the entire cell becoming paler as the hemoglobin decreases. The loss of hemoglobin may be so great as to lead to the formation of the so-called "pessary" form, in which only the periphery of the cell is apparent. This central pale area varies much in individual cells and is not at all evident in those which are flattened out. In dry specimens, if thinly spread, the cells are circular, the normal biconcavity is obliterated, and a uniform stain is observed. If the smear be thick the central pale area is observed.

In chlorosis and secondary anemias, the color of the cells is usually uniformly paler than normally, while in pernicious anemias the color may be even deeper than normal. These are the usual but not invariable pathologic

findings. In malarial conditions, discolored cells are often observed in the fresh specimens, the bronzed or "brassy" tone often drawing attention to the presence of a parasite of the estivoautumnal type or of the quartan form.

The red cells show a marked tendency to cohere to one another in more or less regularly arranged piles, forming long rows (rouleaux), like rolls of coin piled up face to face. The exact cause of this phenomenon is unknown, but it may be dependent on the presence of the fatty membrane surrounding the cell, as Peskind's findings show that the red cells are enveloped by a layer composed of lecithin, cholesterin, and a nucleoprotein. In certain pathologic conditions this normal rouleaux formation is increased and in certain ones is de-

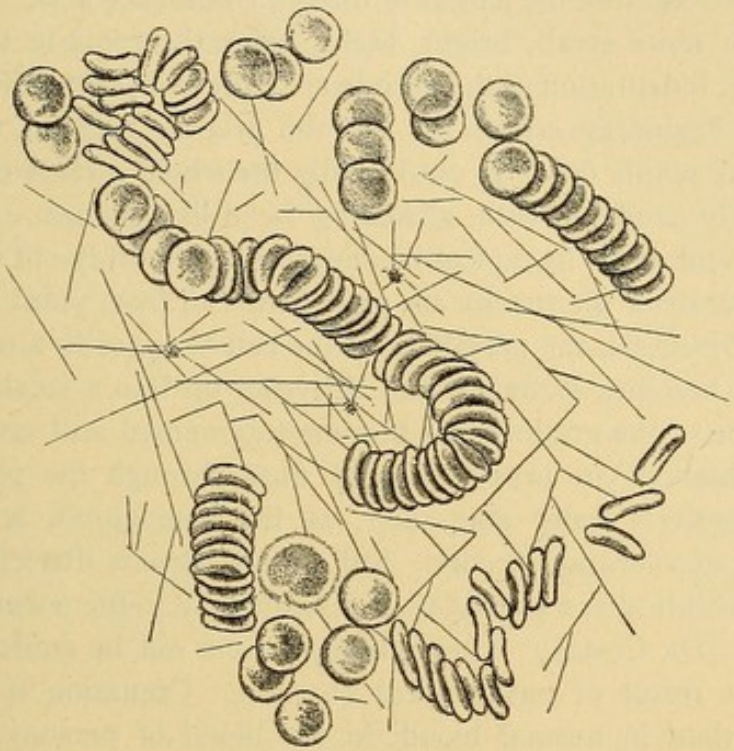


FIG. 143.—Normal blood showing rouleaux formation and fibrin network. (*Da Costa.*)

creased. The diminished rouleaux formation is observed in conditions associated with increased viscosity of the cells, as observed in most inflammatory diseases and in the anemias due to malignant disease. This hyperviscosity of the cells has not at present much clinical significance, but may be shown to be of importance when our knowledge concerning the normal viscosity of the blood becomes more extensive.

The structure of the erythrocytes is still an unsettled point in hematology. Neither membrane nor stroma have been fully demonstrated yet after the hemoglobin has been removed from the cells by hemolysis, a stroma may be definitely seen which could hardly be called an artefact. Schäfer assumes that the hemoglobin is held in firm combination by chemical union with other albuminous constituents of the cell and supported by a stroma similar to that of Peskind's outer membrane. That some sort of an outer membrane does exist would seem to be proven by the experiments on hemolysis which have

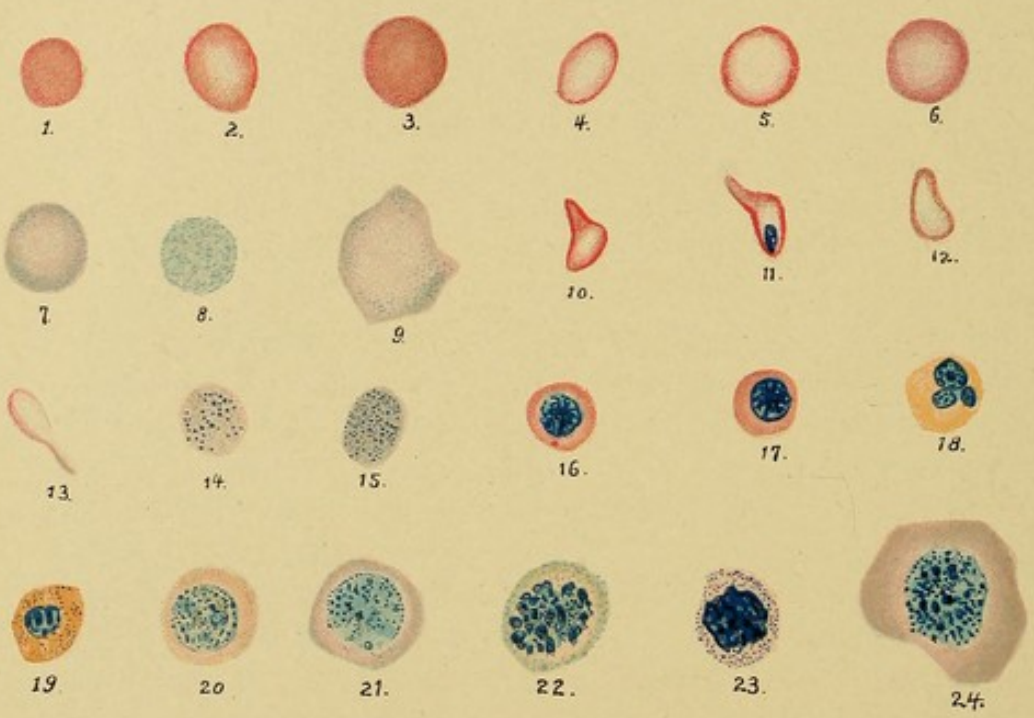
shown that many substances penetrate the cell producing hemolysis, while others in the same concentration have no such effect. It is hard to believe that a selective vital activity is at work here, as the results follow too closely the laws of physical chemistry as applied to diffusion and osmosis. The corpuscles are very elastic and contractile so that rapid and marked temporary distortions of shape are possible under the influence of variations in the composition of the circulating plasma.

As the blood dries various changes in the appearance of the red cells are observed. These changes, known as crenation, are due to the evaporation of water and depend upon the quantity of air which comes in contact with the specimen as well as upon the length of time this influence acts. "The development of one or more small, bright, highly refractile spots in the body of the cell and a slight indentation of the periphery of the cell are the most conspicuous indications of beginning crenation. As the process goes on, more and more of these hyaline points develop, until finally the whole surface of the corpuscle becomes thickly studded with glistening bead-like spines. As the stroma becomes drier and drier, its typical biconcavity and sharply-cut outline are lost, contracting strands of the stroma are seen to extend from point to point among the beaded projections, the periphery of the cell changes to a cogged rim, and finally the cell becomes shrunken and shriveled up into a small, many-starred asterisk. Some of the erythrocytes become fragmented and small bits of their stroma are observed to break off and float through the plasma. Others become progressively paler and paler, as the hemoglobin is dissolved out, until complete decoloration occurs. Still others become distorted into designs of every conceivable shape so that their resemblance to the normal cell becomes more remote" (Da Costa). These changes must not be confused with those occurring as a result of pathological changes. Crenation is often induced more rapidly than in normal blood, in the blood of persons suffering from acute infection and from chronic diseases. True ameboid movement of the red cells is sometimes seen as a result of a high-grade anemia.

(*B*). Size and Shape.

The average diameter of these normal human erythrocytes (normocytes) is 7.5 μ, the normal variations being between 6 and 9 μ (a micromillimeter, 1/1000 of a millimeter). The size varies depending on the method of preparing the specimen and also upon the osmotic tension of the plasma. Although dwarf and giant cells may occur to a slight degree at all ages, the normal infant blood shows these variations more markedly (3.3 to 10.3 μ, according to Hayem). According to Hamburger, the cells are slightly larger in the venous than in arterial blood. Gram states that the size of these cells varies with climatic conditions, being greater in those of the northern cooler countries than in those of the southern warmer climates. The variations in the size of the red cells is very slight in the different sexes.

Pathologically, variation in the size of these cells is a common and



Katharine Hill

TYPES OF RED CELLS.
(WRIGHT'S STAIN. ZEISS OCULAR 4, OIL IMMERSION OBJECTIVE.)

1—NORMAL CELL.
2-3-4—NORMAL CELLS AS SEEN WITH DIFFERENT FOCUS.
5—SO-CALLED "PESSARY FORM."
6-7-8—POLYCHROMATOPHILIC CELLS.
9—MACROCYTE.
10-12-13—POIKILOCYTES.
11—POIKILOBLAST.
14-15—PUNCTATE BASOPHILIA IN RED CELLS.
16-17—NORMOBLASTS.
18—NORMOBLAST WITH PYCNOTIC NUCLEUS.
19—PUNCTATED NORMOBLAST.
20-23—MEGALOBLASTS.
24—GIGANTOBLAST.

important feature. Generally speaking, variations in the cellular size indicate a severe and chronic anemia, while in the more mild acute forms of anemia such variations are unusual. The average size is said to be increased in jaundice, cholera, lead-poisoning, leukemia, congenital heart disease, and cretinism.

Types of Pathological Erythrocytes.

Variation in the normal size of the red cells is indicated by the term anisocytosis. This term does not include the misshappen red cells which appear in the blood as a result of degeneration or of mechanical injury and to which are given the name of poikilocytes.

Microcytes.

These are cells under the normal size, the variations being between 1 μ and 6 μ, the usual representatives being about 3.5 μ. It cannot be stated at present whether these cells are mere schistocytes (fragments of larger cells) or are perfect cells of degenerative origin. They occur normally in the blood of embryos and infants, but are rare in that of the adult except in pathological conditions. It is certainly true that these undersized cells may arise from purely physical causes, as a result of increased osmotic pressure of the plasma as well as from division of undersized mother cells. This latter phase is made possible by the appearance, especially in pernicious anemia, of nucleated reds of corresponding size.

Pathologically, these smaller cells are observed in all severe anemias. As a rule, they stain deeply and evenly, but in some cases of pernicious anemia these cells are deficient in hemoglobin and show irregular staining, yet many of them may have an increased hemoglobin content. Occasionally, but not invariably, we find these undersized cells in chlorosis, in which the hemoglobin is deficient. At times these cells may show polychromatophilia, but this is not the rule. According to Tallqvist, an increase in the number of these microcytes (microcytosis) is an indication of rapid destruction of blood.

Macrocytes.

These are cells above the normal size, the variations being between 10 and 20 μ,. Those cells from 9 to 12 μ are called macrocytes; those between 12 and 16 μ are known as megalocytes; while those above 16 μ are termed gigantocytes.

These cells are of regular shape, of even staining qualities, and generally without a well-defined central clear area. The larger size of these cells may be partly due to the swelling incident to a lowered osmotic tension of the blood, but more probably this increase in size is traceable to the origin of the macrocytes from the large nucleated reds of the bone-marrow. These cells may show an excess or deficiency in hemoglobin, the former characteristic being observed in the primary pernicious anemias, while the latter is evident in the secondary forms.

Pathologically, the presence of these various forms of macrocytes, giving

rise to the condition of macrocytosis, indicates a severe and unusually chronic anemia. They are most frequently seen in pernicious anemia, in which the largest cells are sometimes the darkest and some of the microcytes are exceedingly pale. These large cells do occur, however, in leukemia, cholemia, and chlorosis, being frequently pale or "chlorotic" and "dropsical." These "dropsical" cells are not sufficiently numerous, however, to change the volume index, as Capps has shown, of a secondary anemia to one shown in true pernicious anemia.

Poikilocytes.

These are misshapen red cells of large or of small size, the varieties of such deformities being numerous. The presence of poikilocytes in the blood is known as poikilocytosis (first described by Damon) and is closely related to crenation as in both cases the cells may be similarly misshapen. The former is a pathologic process demonstrable the moment the blood is taken, while the latter is a physiological process appearing only after the blood has been in contact with air for some time.

Poikilocytes arise in several ways. First, faulty technic, especially pressure on the cover-glass of the fresh specimen, will give rise to fragmentation of some of the corpuscles into small spherical masses, dagger-shaped bodies, and small elongated rods (pseudo bacilli of Hayem). Such fragmentation is indicative of lowered vitality and feeble powers of resistance of the cells. If the cover-glass be moved after the cells have spread, a large number of them will be distorted into oval or pear-shaped forms, the long axes of which usually point in the same direction. Secondly, true poikilocytes, or cells misshapen while in the circulation, are probably due to ameboid motion of a portion or the whole of a cell or to alterations in the plasma. These misshapen cells are usually pear-shaped with a budded projection at one or more poles. Cells may be seen which resemble a tennis-racket, a kidney, tomahawk-blade, dumb-bell, or anvil, while oval forms are especially observed in pernicious anemia and are considered by Cabot of diagnostic importance. Poikilocytosis is an indication of severe anemia with degenerative changes in the red cells; although it is not characteristic of any single disease, it is found more frequently in pernicious anemia and leukemia.

(*C*). **Nucleation.**

Nucleated red cells may be considered pathological at any period of extrauterine life, although they are usually found in the blood of the child during the first few days of life. These nucleated reds are always found in the bone-marrow, the normal and large forms being quite distinctive. The large form is the oldest and gives rise by cell division to the smaller cell or the normoblast. It is probably true that the nonnucleated red cells are derived from the nucleated form, but the denucleation takes place before the normal cells reach the blood. Just how we are to explain the disappearance of all trace of the nucleus from the normal erythrocyte is a question, but the general

concensus of opinion seems to be that the nuclear material gradually fades within the cell, although some slight evidence of extrusion of the nucleus can be advanced. While there is abundant evidence to show that the normal red cell is not entirely devoid of nuclear material, our ordinary methods of examination do not show such indications.

Normoblasts (Trachyochromatic Erythroblasts).

These are nucleated red cells similar in size, shape, and color to the normocytes. They do not usually show a biconcave form and do not unite in rouleaux. The protoplasm of the cell is usually regular in outline, stains more intensely than does that of the normocyte and frequently shows evidence of polychromatophilia, although it is normally orthochromatic. In myeloid leukemia, cells are frequently observed in which the protoplasm presents a ragged outline and may even, in some cases, be so degenerated as to show only a small fragment attached to the nucleus. These latter cells are practically always polychromatophilic. This type of small cell might be called a microblast, which is the rarest form of erythroblast and corresponds in size to the microcyte. Moreover, we may find such cells or fragments of cells attached to a nucleus, such cells corresponding in size and shape to the poikilocytes and being termed poikiloblasts.

The nucleus of the mature normoblast (Howell's mature nucleated red) has a diameter of about one-third that of the cell, is densely stained, homogeneous, sharply defined, spheroidal in shape, and without any decided chromatin network (the so-called pycnotic nucleus). It is situated rather toward the periphery than in the central portion of the cell and is surrounded by a clear zone shading off into the cellular protoplasm. Occasionally the nucleus is observed resting upon a margin of the red cell or even extruded entirely from it probably as a result of degeneration of the surrounding protoplasm (Pappenheim). These nuclei often show amitotic figures, being subdivided into two or more lobes or fragments which may be connected by strands of chromatin. These mature forms may show all gradations in which the chromatin network becomes more and more evident, until we reach the very immature forms (Howell's immature nucleated reds). These latter cells are somewhat larger than the mature forms and are somewhat lighter in color; the nucleus is relatively larger and is composed of delicate faintly basic chromatin fibers radially arranged, and frequently showing mitotic figures.

These two types of cell are the forerunners of the normocyte and their appearance in the adult blood is indicative of increased activity of the hematopoietic organs, especially of the bone-marrow, either as a result of a poor condition of the blood itself or as a direct disease of the blood-forming organ. These cells are most commonly seen in the milder forms of anemia, chlorosis, and acute anemia from hemorrhage, inanition, or organic disease. In the severe types of anemia they are constantly met with and are usually associated with the larger megaloblasts. During the course of severe anemia, especially in chlorosis, there

obtains a periodic increase of normoblasts and of leucocytes lasting several days. This is followed by a marked increase in the normal number of red cells, giving rise to the condition described by von Noorden as a "blood crisis." The normoblasts disappear, the blood count falls off, and a second crisis may obtain. This condition is considered as a transitory attempt on the part of the bone-marrow to regenerate the blood. Such a crisis is not always a sign of improvement, but it does demonstrate that the type of blood formation is becoming physiological and that recovery may follow.

Megaloblasts (Amblyochromatic Erythroblasts).

These are cells of larger than normal dimensions, corresponding in size to the macrocytes and varying from 9 to 20 microns in diameter, if exceeding 20 microns they are known as the gigantoblasts of Ehrlich. Occasionally some cells are seen which are no larger than a normoblast, but in which the nucleus shows definite characteristics which should enable one to classify the cell as a megaloblast (Pappenheim).

The protoplasm of the megaloblast often appears swollen and enlarged (dropsical). The cell is usually circular or oval, but it is easily deformed, giving rise to irregular-shaped bodies. Although it usually contains an excess of hemoglobin, it may show a deficiency. It is usually polychromatophilic, the shade of cellular staining varying from yellow to purple. These various color tones may not be regular, but may be varied by tintings of almost any shade in the same cell.

The nucleus of the megaloblast is very large, varying between 6 and 10 microns. This may be situated either centrally or somewhat peripherally. It shows a great variety of forms appearing as a vesicular body with intranuclear network and nodal thickening, but without nucleoli. It may rarely be pycnotic, may show mitoses or many stages of karyorrhexis with fragmentation, vacuolation, fading of the segments of the nuclei, as well as minute subdivisions into fine basic staining particles widely scattered in the cell (Ewing). It is frequently poorly defined and shows feeble basic staining qualities. It may be sharply differentiated from the body of the cell by a distinct white margin which is thrown into relief by the deeper staining of the nuclear and cell-substances. Occasionally the nucleus is overlooked owing to the polychromatophilic properties of the cell, which do not always permit of clear differentiation of nucleus and cell substance. Careful examination of the nuclear structure, with its wide-open meshwork, should, however, prevent the mistake of classifying this cell as a large lymphocyte.

The clinical significance of the megaloblast is more or less in doubt. As such cells are foreign to the blood of an adult and as they are not present beyond 15 per cent. of the total nucleated reds in the marrow, according to Emerson, it must be assumed that their presence in considerable number is indicative of a reversion to an embryonal type of blood formation, or at least denotes an arrested development of normal cells and in consequence an increased

production of these abnormal types. Megaloblasts are, therefore, evidences of degeneration of blood-forming organs, while normoblasts are significant of regeneration of blood. A few of these cells, along with a larger number of normoblasts, has no special significance in cases of severe anemia, being found in small numbers in any variety of anemia. If, however, the majority of the nucleated red cells be megaloblasts, especially if gigantoblasts be present and unequal mitoses be observed, a diagnosis of primary pernicious anemia seems justified. It may be considered that the number of erythroblasts has no special significance as regards the severity of a particular case, but that it indicates merely the effort on the part of the bone-marrow to overcome the effects of blood destruction. The appearance of megaloblasts may, therefore, be regarded as an evidence of incomplete formation of the younger elements and, not necessarily, as an unfavorable sign. In the anemia following infection with bothriocephalus latus, the specific toxins produce a megaloblastic degeneration of the bone-marrow, so that the blood picture may assume the characteristics of a severe pernicious anemia. Ehrlich and Lindenthal have reported a case of nitrobenzol poisoning, in the later stages of which the megaloblasts out-numbered the erythroblasts. In other severe anemias these large nucleated cells, although often present, are never so numerous as are the normoblasts.

(*D*). Number of Red Cells.

The normal number of red cells is generally regarded as 5,000,000 per cmm. in the blood of the adult male, while the normal value for the female is 4,500,000. These figures are purely arbitrary, but they serve as an approximate basis upon which one may form his opinion as to the probable normality of a specific specimen of blood. We frequently observe marked variations in the red count under the influence of both physiologic and pathologic conditions. These variations may be due to an actual increase or decrease in the number of cells or to a change in the volume of the plasma as discussed under Total Volume of Blood on page 374. It is not uncommon to find much higher counts than these normal ones in healthy individuals, especially in those living the "simple life," so that any special count must be a law unto itself and must be considered normal or abnormal only after considering all the factors which may influence the number of cells.

Physiologic Variations.

(1). Sex.

The variations due to sex are not so marked as those from other causes. While the adult woman almost invariably shows a lower blood count than does the adult man, yet we occasionally find the girl showing, before puberty, a somewhat higher count than the boy of corresponding age and also the woman, after the menopause, showing somewhat higher values than does her brother of similar years. It can hardly be doubted that menstruation, as well as pregnancy and lactation, have some influence in lowering a count in woman at certain periods of life, yet these conditions are simply transient and would not seem to

have as much bearing on the question as does the somewhat more hydremic state of the normal plasma of woman.

(2). **Age.**

The number of the red cells varies more or less with the age of the subject examined. The highest values are usually observed at birth, when the count may run as high as 7,500,000, as in a case observed by the writer, the hemoglobin being practically always over 100 per cent. at this time; but, as a rule, a lower count is noted, averaging about 6,000,000. These high figures are due, to some extent, to the concentration of the plasma at birth through the loss of body fluid before a compensatory intake. The count gradually falls during the first few days and becomes fairly constant about the tenth day, when it is rare to find nucleated red cells. The number of cells is then stated to be somewhat reduced until the age of puberty, when a gradual increase occurs until about 40 years of age, after which a slight decrease may be observed in man. These variations, observed at different ages, are to be regarded as slight and influenced, to some extent, by the many modifications of the plasma as the result of growth and development as well as to the gradual decrease of functional activity after the middle periods of life.

(3). **Altitude.**

An increase in the number of cells has been observed under the influence of a higher altitude. The count has heen shown to increase at the rate of approximately 50,000 cells per 1,000 feet of ascent and to diminish, within 36 hours, at a corresponding rate, this increase or decrease being more marked the more sudden the ascent or descent. Just what factors are at work in causing these changes is undecided. The rise is too rapid to be entirely accounted for by new formation of blood-cells under the influence of diminished oxygen tension, and the fall is not accompanied by signs of destruction of the reds. Weinzirl considers the increased count of high altitudes due to the lowered temperature at these elevations. This factor would seem to have some influence, as counts are frequently observed in which a variation is noted at places showing the same elevation. Change of residence from warm to a cold or from cold to a warm climate may lead to an increase or decrease, as the case may be, in the number of cells varying from 500,000 to 2,500,000.

(4). **Nutrition.**

The general nutritive condition of the subject has more or less influence upon the number of red cells per cmm. This statement must not be interpreted to mean that the obese person shows a higher blood count than does his sparer brother. It is well known that obesity is an indication of poor assimilation of food; hence we should expect to find, as we really do, that the muscular person has a somewhat higher count than the obese subject and well-developed robust individuals a larger value than do the poorly nourished patients. Diet has, of course, much to do with the general nutrition, so we find the meat eaters averag-

ing somewhat higher in their percentage of red cells than do vegetarians. Immediately following a hearty meal we may observe a temporary decrease in the number of red cells, but this soon returns to normal, owing to the rapid adjustment of the water content of the blood.

(5). **Exercise.**

Active muscular exercise produces a transient increase in the red count due both to the increased blood-pressure and to the concentration of the blood through the loss of water by perspiration. Physical exercise, taken to the point of producing fatigue, may produce a marked diminution in the number of red cells, due probably to the fact that regeneration of new cells cannot keep up with the destruction of the old cells. Passive exercise, in the form of massage, has a transient influence in producing an increase in the number of the red cells, owing to its increasing the general circulatory tone.

(6). **Baths.**

Careful investigation of the influences of both cold and hot baths has shown that an increase in the number of reds occurs under the action of these two conditions. The increase as a result of a cold bath may be as great as 2,000,000 cells, due to capillary stasis as a result of vasomotor constriction. Likewise, a hot bath will increase the number of cells by causing dilatation of the peripheral vessels and a consequent increase in the amount of blood at the point from which the specimen is taken. When marked perspiration follows either a hot or cold bath the blood becomes concentrated and, as a result, an increase in the number of red cells will obtain.

(7). **Therapeutic Measures.**

Any drugs which cause rapid loss of fluid from the body, as for instance emetics, purgatives, diuretics, and diaphoretics, will cause concentration of the blood and hence a coincident increase in the number of cells, providing the change is sufficiently rapid and tbe blood examination is made before compensation occurs. Among the drugs which increase the number of red cells we find iron and arsenic, both of which are particularly valuable in anemic conditions, the former in chlorosis and the latter in pernicious anemia. The compounds of mercury and of lead, on the other hand, have a destructive action upon the red cells, so that we find these drugs causing a diminution in the number of cells.

Pathologic Variations.

(1). **Oligocythemia.**

This is a condition characterized by a diminution in the number of red cells. It is usually associated with a decrease in the percentage of hemoglobin and with a slight reduction in the total volume of blood, although this latter factor is not invariably present. This condition is found, to a more or less degree, in all forms of anemia and may be temporary or permanent. The extent of the decrease in the number of red cells varies from 500,000 cells to

a reduction of 4,000,000. This diminution in the number of cells is usually an indication of the severity of the anemia, the most marked decrease being observed in the pernicious types, Osler reporting probably the lowest count recorded, namely, 100,000 red cells.

While this oligocythemia is usually associated with oligochromemia, yet we find in chlorosis that the diminution of red cells is not as marked as is the reduction in the amount of hemoglobin, the color index in this condition being usually low. In some cases of chlorosis, however, the oligochromemia may keep pace with the oligocythemia. In pernicious anemia, on the other hand, we have a marked reduction in the number of cells and coincidently a large decrease in the amount of hemoglobin, the result being that we have a very high color index as the usual sign of this condition. Cases showing the loss of a large amount of blood, as the result of hemorrhage have naturally an oligocythemia, but the percentage of hemoglobin may not necessarily be reduced to any great extent. This gives rise to the condition of secondary anemia, in which the color index may be high. In these cases of hemorrhage a sudden reduction to the point of 1,000,000 or less cells is usually followed by a fatal result, although even here recovery has heen made possible by rapid infusion of salt solution and compensatory activity of the blood-forming organs. In leukemia we find that the red cells are not usually diminished to a very great extent, the oligocythemia being generally more marked in the lymphatic than in the myeloid variety. Occasionally, however, we do find very low counts, in both varieties of leukemia. These same statements apply to splenic anemia, although the count here very rarely reaches a lower point than 2,500,000 cells.

A large number of conditions, aside from direct blood diseases, cause a diminution in the number of these red cells. Thus we find that the toxins of certain of the specific fevers, such as typhoid and pneumonia, may cause a marked anemia, but this is not necessarily the rule. Acute infections with pus organisms is frequently observed to cause an extreme and rapidly progressive anemia, the destruction of the red cells being in some cases very extensive. In malignant disease we usually find, especially if cachexia is present, a very extensive anemia which may lead to a diagnosis of the pernicious type. It is to be remembered, however, that a secondary anemia may assume all of the characteristics of the primary pernicious type, so that we should be on our guard as to the cause of an anemia and not be content with the simple findings of such a condition.

(2). Polycythemia.

This is a condition characterized by an increase in the number of red cells and is sometimes called polyglobulia. Whether this increase is actual and permanent or whether it be simply apparent, due to concentration of the blood or to unequal distribution of blood in the peripheral vessels, is still an unsettled question. It is true that many physiological causes previously

enumerated do bring on a polycythemia, so that these should be remembered whenever a blood count is to be made. From the pathologic standpoint we find an increase in the number of red cells occasionally following active blood regeneration after hemorrhage. Likewise, we observe an increase in the number of cells in phosphorus poisoning, acute yellow atrophy of the liver, and in certain cases of general hepatic insufficiency. Why such an increase of cells occurs in these conditions is very hard to say. It has been found that the solids of the plasma are not increased, so that we are not warranted in assuming a concentration of the fluid elements. A polycythemia of uncertain origin is also seen in cases of poisoning with carbon monoxid and illuminating gas.

In a condition called by Osler autotoxic enterogenous cyanosis, which is characterized by a marked increase in the number of red cells and also by enlargement of the spleen, we have reason to assume that a direct real increase in the number of red cells occurs, but the pathogenesis of the condition is uncertain. It has been traced to improper aeration of the blood and occurs also in congenital heart disease, mitral lesions in the adult, and in pneumonia and acute miliary tuberculosis. While this high blood count must be definitely admitted, its importance from a clinical standpoint is still unsettled. It is uncertain whether the rapid increase in the number of reds is due to an attempt on the part of the blood-forming organs to overcome the influences of the toxic substances upon proper oxidation in the system; yet it is certain that relief very frequently follows such an increase in the number of red cells, although in Osler's disease or, as it is called by some, Vaquez's disease, death frequently follows without any relief from the condition.

(*E*). Staining Properties of the Red Cells.

The normal red cell, like all living cells, is incapable of being stained with anilin dyes; that is, it is achromatophilic. Previous to staining, fixation of the cell must take place, the protoplasm being killed in this process. The so-called vital staining has been considered previously. The normal fixed red cell has a marked affinity for various dyes of the acid type, such as eosin, orange-G, acid fuchsin, and indulin, and is therefore called acidophilic or oxyphilic. This cell normally takes up but one color from a mixture of dyes, and is called, therefore, monochromatophilic.

Polychromatophilia.

Under various pathologic conditions we find red cells which show a tendency to take up the basic stains, and for this reason are called basophilic or polychromatophilic cells. The tint of such abnormal red cells varies from that of a light indefinite shade of the basic stain to a dark distinct tone. Just what factors are at the bottom of this change, which is called polychromatophilia or polychromasia, is difficult to say. The normal acidophilic tendencies of the cell are due to the presence of hemoglobin and, in consequence, the normal staining properties will depend upon the relative richness of the cell in this

pigment. As hemoglobin is always acidophilic, we cannot assume that the polychromatophilic properties of the abnormal red cells are due to variations in the hemoglobin, but must content ourselves with the belief that these changes have something to do with the protoplasm of the cell.

This condition, which has been termed by Ehrlich anemic degeneration, is characterized by a diffuse basic staining property of the red cells. These basophilic cells are somewhat larger than the normal ones, show less biconcavity, and often are abnormal in shape. The megaloblasts are practically always polychromatophilic and the normal erythroblasts of the bone-marrow usually show this degeneration. This fact would seem to point to the probability of this condition being a sign of regeneration of the blood as the erythroblasts are more prone to be polychromatophilic the younger they are.

Two distinct forms of polychromasia have been found. The first of the these, known as the *polychromatophilic degeneration of Gabritschewsky*, is the diffuse basophilic staining of the cells which is found in various forms of anemia and extensively in the cells of the normal bone-marrow. The second of these, *the polychromasia of Maragliano*, is shown in severe anemias and in toxemias and appears as a more punctate basophilia, being closely related to the basophilic degeneration of Grawitz, which will be discussed later. While the polychromatophilia of the diffuse type is considered by Ehrlich as evidence of a degenerative process, that is as a coagulation necrosis of the discoplasm in consequence of which this takes up the albuminous principles of the plasma while it loses its power of retaining hemoglobin, yet there are evidences showing that this may be a regenerative process. Polychromatophilia is often seen in cells which are undergoing degeneration, especially in myelogenous leukemia, pernicious anemia, severe secondary anemia, especially those following malignant disease, eruptive fevers, malaria, and after various poisons, such as that of snake venom.

The forms of partial polychromatophilia, which have been described under various names, such as vacuolization, pseudonucleation, globular decolorization, and more commonly Maragliano's endoglobular degeneration, are seen in normal blood in from 30 to 70 minutes after the specimen is made. They are usually found in the center of the cell, but may be near the periphery. There may be several such areas in a single cell, but the more common form is the single area of degeneration, which is usually round, may be elliptical, and may resemble a vacuole. These are shown quite distinctly in the unstained specimen and in this condition very frequently are seen in rapid motion. This motion is not of the true ameboid type, but is due more to the gradually progressing coagulation and consequent constriction of the protoplasm. These areas, whether in the fresh or stained specimens, may be mistaken for malarial parasites, so that the worker should be on his guard lest he make a wrong diagnosis without sufficient evidence. They differ in size from the parasites, and on focusing the specimen are characterized, usually, by a change from a smaller to a larger form, which variation is not evident with the malarial organism.

The longer one searches in the unstained specimen for malarial organisms the more apt a mistake in diagnosis is to be made, as these "Mariaglianos" develop quite rapidly. In the stained specimen these areas of coagulation necrosis will show a basophilic staining quality, while the chromatin element of the malarial organism will differentiate this body from the more usual degenerative area.

(*F*). Degenerations.

While the preceding conditions of polychromatophilia are to a certain extent true degenerations, yet they are more properly grouped under the heading of atypical staining reactions of the red cell. Other forms of degeneration, which are likewise characterized by abnormal staining qualities, are nevertheless different in many ways.

Basophilic Degeneration of the Red Cells.

This condition is distinct from the polychromatophilia above described and is known as the *punctate basophilia of Grawitz.* It is characterized by the appearance, in the body of the red cell, of granules of varying size, which stain with the basic dyes. The cell may be dotted throughout with these granules or may show this degeneration only in parts. These granules are not observed in the fresh unstained specimen and are not increased, as are the Maragliano areas, by allowing the blood to stand. These cells are observed in pernicious anemias, leukemia, in the toxemia of malignant states and especially in cases of lead-poisoning.

The size of the granules may vary from small dots to large granules, showing a diameter of one or more microns. Their origin is much in doubt, but they are generally considered to be areas of coagulation necrosis associated with either degeneration or regeneration of the red cells. They are quite distinct from the granular basic "stippling" observed in malarial conditions, but are probably evidences of direct toxic effects upon the protoplasm of the red cells. Their greatest clinical importance is probably in lead poisoning, where they may be the only signs of abnormality in the blood picture. They may vary in number from day to day, showing in some cases five or six in one microscopic field, but, as a rule, they are present in much fewer numbers, as one observes them only after examining several visual fields. As a rule, they appear very early in cases of lead-poisoning, in one case observed by the writer within three days, and they may be present in the blood of a lead worker for several years after his exposure to the effects of the lead. They are usually the first sign of anemic change and usually persist longer than do the other abnormalities of the blood.

Ring Bodies.

Occasionally one observes in the red cells curious ring-like bodies which are in shape very much like the hyaline malarial ring with a circular refractive center. They change their shape in a peculiar way, much resembling the

undulatory movements of the hyaline body. They do not increase in number or grow larger on standing as do the Maraglianos and they are observed in a large number of conditions, such as measles, pernicious anemia, and severe secondary anemia. Two types of these ring bodies must be distinguished, the first is usually more peripherally situated, occasionally has a definite crescentic shape or has an appearance much resembling that of a Maragliano, and is found especially in cases of measles. This finding has lead to the assumption that these bodies were the organisms causing measles. The second form has been described by Cabot[1] and more recently by Schleip[2] in cases of pernicious anemia, leukemia, and secondary anemias. These latter bodies are larger than the former, more distinct, irregular in shape, frequently forming figure-eight bodies and are usually stained bright red with Wright's stain, but may occasionally show a blue shading. Just what is the pathologic significance of these ring-like bodies it is impossible to state at the present time.

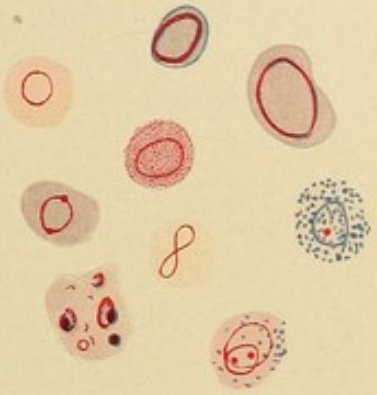
Various other forms of degeneration of the red cells occur, as for instance the appearance of rod-like areas resembling bacilli, which may keep up a constant vibratory motion carrying them through the entire substance of the cell. This finding should not confuse one in making a diagnosis of the presence of bacteria. Another form of degeneration, known as Ehrlich's hemoglobinemic degeneration, has the appearance of a small dark cell lying upon a larger paler one. These are probably areas of condensed protoplasm with the hemoglobin distinctly separated from the stroma. Such cells appear, occasionally, in certain types of malaria and are shown as corpuscles in which the hemoglobin is apparently condensed around the parasite. This degeneration is occasionally seen in nucleated red cells and may give the appearance of a microblast lying upon a macrocyte. It is best seen in cases of pernicious anemia and may explain some of the "acidophilic granules" of the red cells which have been described (Emerson).

(*G*). Isotonicity and Resistance of the Red Cells.

Normally, the relations of the cellular to the fluid portions of the blood are such that the hemoglobin and other constituents of the red cells are held intact within the limiting membrane of the cell. Any change in the osmotic pressure of the plasma is certain to be manifested by variations both in size and in the composition of the cellular elements. We find, therefore, as previously discussed under the heading of Osmotic Pressure, that the inorganic constituents of the plasma are the factors upon which the volume of the red cell depends to a large extent.

By an isotonic solution, as applied to the blood, we mean a solution of such a strength as to preserve the corpuscles and to prevent passage of water, salts, and organic bodies from the plasma into the corpuscle or from the corpuscle into the plasma. In other words, an isotonic solution is one whose osmotic

[1] Jour. Med. Res., vol. 9, 1903, p. 15.
[2] Deut. Archiv. f. klin. Med., Bd. 91, 1907, S. 449.



Katharine Hill

RING BODIES IN RED CELLS. (AFTER SCHLEIP.)

pressure is equal to that of the contents of the red cell. While the limiting structure of the red cell is indefinitely known, it seems to have certain properties which would lead us to assume that it is a semipermeable membrane, that is one which will permit the passage of organic compounds or of salts or their ions in both directions. This apparent selective activity is dependent both upon the laws of osmosis and diffusion through a semipermeable membrane and upon the basic ideas of Ehrlich's side-chain theory. While other figures have been used at various times to represent the strength of a solution isotonic with the red blood-corpuscle, the one which seems to more nearly duplicate the actual condition is a 0.9 per cent. solution of sodium chlorid. This is or should be the normal salt solution which is used in physiological work and in transfusion. Loeb has shown that a properly balanced solution containing the chlorids of sodium, potassium, and calcium more nearly represents a proper "physiological salt solution" than does the simple 0.9 per cent. sodium chlorid solution. Such an isotonic solution will preserve the corpuscles, will prevent the passage of hemoglobin from the cells into the plasma, and will not permit of shrinkage or swelling of the cells. Solutions which are stronger than the one above mentioned are called hypertonic solutions, while those of a lower concentration are termed hypotonic solutions. In the first of these shrinkage of the cells will occur, while in the latter phenomena of swelling will be observed. These facts are of importance in the study of the volume of the corpuscle and should not be forgotten in reporting variations in size of the cells.

The erythrocyte normally shows a certain amount of resistance to variations in the osmotic pressure of the blood and to the hemolyzing effects of various substances. It is true that the osmotic pressure of the plasma may be raised by the introduction of certain substances into the blood without causing phenomena of shrinking, swelling, or hemolysis to occur, yet we must not assume that the cell is not influenced by such changes in osmotic pressure. Simple variations in osmotic pressure come more nearly to the foreground in the explanation of these changes, especially of those occurring in most disease processes of a chronic type, while in the more acute infectious types associated with toxemia the biologic theory must be invoked. As yet little of value has been forthcoming from the study of the resistance of the blood-cells to variation in osmotic pressure, as the scope of the examination has not been sufficiently widened by application of accurate experimental methods. The writer is forced to say, from the results of his own experiments, as well as those of others with the more usual method of Hamburger,[1] that no reliable data are at hand. It is highly probable that the little understood biologic influences are clinically much more important than are those due to variations in osmotic pressure, as this latter process cannot explain the hemolytic results of the toxins or of certain definite chemical compounds which bring about these results in concentrations which have little influence upon the tonicity of the blood.

[1] Wiesbaden, 1902.

(*H*). Variations of the Red Cells in Childhood and in Old Age.

As previously stated, the average number of red cells is much higher than the normal figure at birth, gradually decreasing until the age of puberty when a gradual increase occurs again until middle life, after which a steady decrease is observed. It is to be expected that variations in the color tones (hemoglobin content) of these cells will vary normally with the age of the subject, being very high in the early days of life, gradually diminishing as puberty approaches, increasing until the middle periods of life, and steadily declining as the age increases. Aside from the number of cells, the blood in childhood shows little variation in the characteristics of the red cells, but the variations in the plasma are more or less marked, as the synthetic and retrograde products of tissue metabolism are much more evident in the period of development than during the middle periods of life.

As the subject becomes older the blood is laden with products of retrograde tissue change and the blood-forming organs become less active. It must be remembered that the various organs of the body contribute certain factors to the blood and may, therefore, all be considered as blood-forming organs in a definite sense. The ductless glands, in particular, pour into the blood certain substances which markedly influence the proper correlation of these various organs. Just what these substances are is not well established, so that the introduction of the word "hormone" by Starling does not at present have a specific meaning. As old age approaches variations become evident in the plasma, and through such influences changes in the morphology of the red cells are more or less frequent. Besides these plasma changes, gradual atrophic degeneration of the blood-forming organs takes place, so that we may find red cells showing the various types of anemic degeneration and may even see nucleated red cells as an evidence of the attempt on the part of the bone-marrow to overcome the gradual destruction of cells. The more notable variations in the red cells are observed in their size, owing to the hydremic state which is almost always characteristic of the plasma in advanced periods of life, but associated diseased conditions will, of course, give their characteristic changes which have no place in our discussion at this point.

(*I*). Functions of the Red Blood-cells.

The only well-recognized functions of these cells is the transportation of oxygen to the different parts of the body by means of the hemoglobin which they contain. While this function is necessary for the proper maintenance of life, yet it should not be considered as the most important one of the blood. As is well known, the fluid portions of the blood are much more influenced by the various organs of the body than are the cellular elements, although the red cells will naturally be affected by variations in the hematopoietic organs, such as the bone-marrow, liver, and spleen. Our knowledge regarding the variation in the plasma is so uncertain that we have been led to interpret the changes in the histological appearance of the blood as significant of certain

DESCRIPTION OF PLATE XIX

(Triacid Stain.)

1, 2, 3, 4. Small Lymphocytes.
Contrast the faintly colored protoplasm of these cells in the triple stained specimen with their intensely basic protoplasm in the film stained with eosin and methylene-blue, 17 and 18. The cell body of 1 is invisible. Note the kidney-shaped nucleus in 4.

5, 6. Large Lymphocytes.
With this stain the nucleus reacts more strongly than the protoplasm; with eosin and methylene-blue (19, 20), on the contrary, the protoplasm is so deeply stained that the nucleus appears pale by contrast. This peculiarity is also observed in the smaller forms of lymphocytes.

7, 8. Transitional Forms.
Note the moderately basic and indented nucleus, and the almost hyaline non-granular protoplasm. Compare 8 with the myelocyte, 7, Plate IV, these cells differing chiefly in that the myelocyte contains neutrophile granules.

9, 10, 11. Polynuclear Neutrophiles.
These cells are characterized by a polymorphous or polynuclear nucleus, surrounded by a cell body filled with fine neutrophile granules. In 11 the nuclear structure is obviously separated into four parts; in 9 it is moderately, and in 10 markedly, polymorphous.

12, 13. Eosinophiles.
The nuclei are not unlike those of the polynuclear neutrophile, except that they are somewhat less convoluted, and poorer in chromatin, staining less intensely. The protoplasm is filled with coarse eosinophile granules, the characteristics of which are clearly illustrated by 13, a " fractured " eosinophile.

14. Eosinophilic Myelocyte.
Compare with 15

15, 16. Myelocytes. *(Neutrophilic.)*
These cells are morphologically similar to 14, except that they contain neutrophile instead of eosinophile granules. Note that the granules of the myelocyte are identical with those of the polynuclear neutrophile. A dwarf form of myelocyte is represented by 16.

(Eosin and Methylene-blue.)

17, 18. Small Lymphocytes.
Note the narrow rim of pseudo-granular basic protoplasm surrounding the nucleus, and the pale appearance of the latter.

19, 20. Large Lymphocytes.
Budding of the basic zone of protoplasm is represented by 20. Both of these cells belong to the same type as 5 and 6.

21, 22. Large Mononuclear Leucocytes.
Compared with 19 and 20, these cells have a decidedly less basic protoplasm, but a somewhat more basic nucleus. In the triple stained film these differences cannot be detected, so that they must be classed as large lymphocytes.

23. Transitional Form.
The distinction between this cell and 24 is not marked; the nucleus of the latter simply being somewhat more basic and convoluted.

24, 25, 26, 27. Polynuclear Neutrophiles.
With this stain these cells show a feebly acid protoplasm, and lack granules. Note that the more twisted the nucleus the deeper it is stained. Compare with 9, 10, and 11.

28, 29. Eosinophiles.
Compare with 12 and 13.

30. Eosinophilic Myelocyte.
Compare with 14.

31. Basophile. *(Finely granular.)*
This cell is characterized by the presence of exceedingly fine δ-granules, staining the *pure* color of the basic dye. The nucleus is markedly convoluted and deficient in chromatin The cell here shown was found in normal blood

32, 33 34, 35, 36. Mast Cells
The granules take a *modified* basic color, as shown by their royal-purple tint in this illustration. Note their unusually large size and ovoid shade in 35, their peculiar distribution in 35 and 36, and their irregularity in size in 32 and 36. With the triacid mixture these granules, as well as those of the finely granular basophile, 31, remain unstained, showing as dull-white stippled areas in the cell body. The nuclear chromatin of the mast cell is so delicate and so freely stained that it is barely visible. These cells were found in the blood of a case of spleno-medullary leukemia.

PLATE XIX



THE LEUCOCYTES.

(1–16, *Triacid Stain*; 17–36, *Eosin and Methylene-blue.*)

(E. F. FABER, *fec.*)

(From *Da Costa's "Clinical Hematology."*)

pathological disease conditions. It is true that in some cases we do have direct disease of the blood-forming organs and, in consequence, variations in the cellular structures are of diagnostic importance, yet in the large majority of cases the preliminary changes must be resident in the plasma, those in the cellular structure being simply incidental. It may be, therefore, necessary to assume a functional activity on the part of the red cells to overcome biologic changes in the plasma and if so we must attempt to discover just what plasma changes we are to regard as significant of the various conditions shown by the red cells in each true blood disease.

(6). Leucocytes.

(*A*). Appearance.

In the fresh specimen the leucocytes appear as colorless, highly refractive bodies, somewhat larger than the red cells, and showing a definite nucleus. These white cells show distinct ameboid movement in the fresh specimen by virtue of which they are able to surround a foreign body and enclose it within their own protoplasm. This is the well-known property of phagocytosis which is such an important factor in Metschnikoff's theory of immunity. In contradistinction to the red cells, these white cells show many variations both in size and in shape. As the relations between the nucleus and the cellular protoplasm is quite distinct in many of these forms, some writers have been led to classify the white cells according to the peculiarities of the nucleus. Other writers classify them into granular and nongranular forms, as many of these cells show distinct granulations which are more clearly differentiated in the stained specimens. As more or less confusion exists regarding these different variations, it seems more rational at present to still classify these white cells with Ehrlich, who combines both the nuclear and granular characteristics in his classification.

(*B*). Leucocytes in Normal Blood.

(1). Lymphocytes.

The cells of this class which occur normally in the blood are of two kinds, (*a*) the small cell, about 5 to 8 microns in diameter and (*b*) large cells showing a diameter of from 8 to 10 microns. Both of these cells have a very large nucleus which is usually centrally located but may have an eccentric position. It may be compact or coarsely reticulated and is not always as strongly stained as is the surrounding cytoplasm. Nor is it as deeply stained as is the nucleus of the normoblast, although it is very rich in chromatin. The nucleus of the larger cell may show very irregular staining properties. The nuclei of both of these cells is usually circular in outline, but may show an oval or a kidney-shaped structure, in which at times may be seen a distinct nucleolus. The protoplasm of these cells is usually in the form of a narrow rim surrounding the nucleus and showing a strongly basophilic homogeneous cytoplasm. The older cells may at times show a difficult staining property, may even be acido-

philic, and occasionally exhibit a net-like structure in which are observed a few granules scattered throughout the protoplasm of the cell. This granulation, which occurs in about one-third of the cells seems to have little clinical significance, except as a probable sign of age of the cell, this granular stage being regarded as the end-point of their development. These granules are probably not representatives of true granulation, but seem to be more referable to nodal points of the reticular structure of the protoplasm.

These cells usually constitute from 20 to 25 per cent. of the leucocytes, their absolute numbers being from 1,200 to 2,000 per cmm., but the relative proportion of the small and large form being unsettled. The larger types of these cells are more closely associated with increased functional activity of the lymphoid tissues and should probably be considered the older cell of the two. According to Pappenheim, the large lymphocyte represents the mother cell from which all other leucocytes as well as erythrocytes are indirectly developed as a result of heteroplastic differentiation. Variations in technic of preparing the slides will often lead to differences in size of these cells, the thinner preparations showing these cells as larger forms, while the thicker preparations present the smaller type of these cells.

These cells are beautifully colored by the hematoxylin-eosin stain and by the various modifications of the Romanowsky stain, although the boundary between the nucleus and the protoplasm is not always clearly outlined by these latter stains. The granules, which are occasionally found in these cells and which are in size between the α and the ϵ granules of Ehrlich, show most clearly in preparations stained by the Giemsa stain and appear distinctly azurophilic. These granules do not appear at all with the triple stain.

These cells are frequently increased and frequently diminished, the former condition being spoken of as lymphocytosis, the latter as lymphopenia. A relative increase of the number of these lymphocytes is regarded as more or less characteristic of typhoid fever, especially when associated with a diminution in the total number of white cells. In lymphatic leukemia these cells are present in large numbers and are indicative of marked disturbance in the lymphatic structures.

(2). Large Mononuclear Leucocytes.

These cells, which are supposed to be derived from the spleen and are called, therefore, splenocytes, are as a rule two or three times as large as a red cell (12 to 15 microns), and show a nucleus which may be large and round, but is more frequently oval in shape or may be indented, forming the so-called kidney type of nucleus. It is to be remembered that we may have both large and small types of this large mononuclear leucocyte, the smaller forms being distinguished from the lymphocyte by the relation of the protoplasm to the nucleus. These nuclei are usually eccentric in position and are not always sharply outlined, they are poor in chromatin, but are strongly basophilic, although less so than are the nuclei of the lymphocytes. The protoplasm

of these cells is very abundant in relation to the size of the nucleus and is very clear, hyaline, and nongranular in appearance. This protoplasm is much less basophilic than is the nucleus and shows a very fine reticulum with nodal thickenings which are somewhat more strongly basophilic and may give the appearance of granulation. These cells should not, however, be regarded as granular cells.

Occasionally we find cells, derived from the above, which have been called by Ehrlich "transition forms," which are the largest of all the white cells. The nucleus is pale, often deeply notched giving it usually the appearance of a saddle-bag, and shows the characteristics of the large mononuclear type, being distinguished from the polymorphous nucleus of the neutrophile cells by its greater thickness and by its diminished intensity of staining. The extensive granulation of the polymorphonuclear leucocyte should make any mistake in diagnosis impossible. The protoplasm of these transitional cells is very abundant and shows faint basophilic properties and may even have a few fine granules, which are neutrophilic in character, in the neighborhood of the nucleus. These two forms of cell constitute between 3 and 5 per cent. of the leucocytes, their absolute number varying between 200 and 400 per cmm. Pappenheim considers that these large mononuclear cells develop directly from the large lymphocytes and then pass into the transition forms, which are the final developmental types.

(3). Polymorphonuclear Cells.

(*a*). Polymorphonuclear Neutrophiles.

These cells, sometimes called the finely granular cells of Schultze, are from two to three times the size of the red cell (10 to 12 microns) in diameter, the size depending upon the extent to which the cell is flattened by pressure. These cells are always smaller than the large mononuclear type and quite small specimens are frequently found in cases of myeloid leukemia. They are the most sharply characterized cells of the blood, their protoplasm being relatively great and showing slightly acidophilic staining properties. The reticular portion of the protoplasm is very slightly basophilic, showing occasionally nodal thickening or granules when stained with the methylene blue dyes. Throughout this protoplasm are scattered fine dust-like granules, the ϵ granules of Ehrlich, which are not all of the same size and which stain with the neutral principle of the dyes. In some cases these granules may gradually diminish so that they may be apparently absent or at least undetected. The triple stain is the characteristic stain for these granules, showing them of a distinct lilac color; but various other acid dyes, such as eosin, give various tints to them, so that they are recognizable as distinctly reddish granules in specimens stained by any of the Romanowsky modifications. The nuclei are elongated and constricted and may appear in the form of a bent rod or a mass of interwoven fine fibers, showing a thin chromatin-rich structure, or a distinctly S-shaped formation with oval thickenings. Frequently these nuclear masses appear

as if separated into two or more distinct nuclei, but as a rule these masses are connected by fine bands and should, therefore, be considered rather as polymorphous than as polynuclear. The nucleus shows a reticular structure with nodal thickenings and is very basophilic. These forms constitute from 65 to 75 per cent. of the total number of cells, their absolute number averaging 5,000 per cmm., the percentage ranging from 20 to 40 per cent. in the first years of life when the lymphocyctes are increased. When outside of the blood-vessels they form the usual pus-cells and are extremely active phagocytes. These cells are derived from the mononuclear neutrophilic myelocytes which are normal habitants of the bone-marrow. They run their course as such and are not transformed into other cells, their granules diminishing in pathological conditions or with age, such change being associated with degeneration in the nucleus.

Perinuclear Granulation.

Sometime ago Neusser reported a finding of basophilic granules in certain leucocytes, especially the mononuclear and polynuclear type. These granules surrounded the nucleus and even appeared attached to it, showing a variable size and being more or less refractive. He regarded these perinuclear granulations as characteristic of the uric acid diathesis, but the work of Futcher and of Simon show that these granules are undoubtedly artefacts which can be produced by heating and by variation in the staining and appear in both health and disease, having no relation whatever to the output of purin bodies in the urine.

Arneth's Classification.

Arneth[1] in his study of the blood both in health and disease has been led to classify the polymorphonuclear neutrophiles into five classes depending upon the number of nuclear lobes. His divisions seem to be fairly constant in health, but show great variation, especially in infectious conditions. His first subdivision, known as Class 1, is divided into (*a*) M cells, which are mononuclear forms identical with Ehrlich's myelocyte, (*b*) W cells, representing forms which show only a slightly indented nucleus, the indentation never extending beyond the middle of the nucleus, this cell forms what is called the metamyelocyte, (*c*) T cells, in which the indentation of the nucleus is deeper than in the W cell, but there is no distinct separation into isolated loops, this form constituting the true polymorphonuclear type. The first two variaties of Class 1 are usually seen only under abnormal conditions, although the W cell may be found to the extent of 0.2 per cent in healthy condition. The cells of Class 1 are usually present, according to Arneth, to the extent of 5 per cent., Simon giving this percentage as from 4 to 9. The second class of Arneth embraces cells with two distinct nuclear fragmentations and shows three subdivisions: (*a*) 2 K cells, neutrophiles whose nucleus consists of two round

[1] Jena, 1904.

nuclear portions; (*b*) 2 S cells, neutrophiles whose nucleus consists of two distinct S-shaped forms; (*c*) 1 K 1 S cell, neutrophiles whose nucleus consists of one round nuclear portion and one S-shaped division. These cells of Class 2 constitute, according to Arneth, 35 per cent. of the total neutrophiles, while Simon's figures are 21 to 47 per cent. In Arneth's figures we find the 2 S cells forming about 23 per cent. of the total of this class. The third class has three nuclear divisions and is subdivided into four parts, as follows: (*a*) 3 K cells, (*b*) 3 S cells, (*c*) 2 K 1 S cells, (*d*) 2 S 1 K cells. Arneth gives the percentage of Class 3 as 41, the 2 K 1 S and the 2 S 1 K subdivisions, each representing approximately 16 per cent. of the total of this class. Simon's figures for this class range from 33 to 48 per cent. The fourth class comprises cells with four nuclear divisions showing five subgroups, as follows: (*a*) 4 K cells, (*b*) 4 S cells, (*c*) 3 K 1 S cells, (*d*) 3 S 1 K and (*e*) 2 K 2 S cells. This class, from Arneth's figures, shows a percentage of 17, the 4 K, 3 K 1 S, and 2 K 2 S types being largely in excess of the other forms. The fifth class comprises cells with five or more nuclear subdivisions and may be arranged into five groups, this class representing about 2 per cent. of the total neutrophiles. It is probable that these various classes represent the gradual development of the polymorphonuclear neutrophile, the older the cell the greater the tendency to reach Class 5, while in conditions associated with new formation of cells, as in infectious conditions, we find the percentages of the earlier classes being increased, that of the later ones diminished. Arneth has also shown much variation in this karyolobism in the various types of anemia.

(*b*). Polymorphonuclear Eosinophiles.

These cells, sometimes called the coarsely granular cells of Schultze, are somewhat smaller than the preceding, varying in size from that of a lymphocyte to that of the neutrophile. Their protoplasm is usually somewhat less in amount than is that of the neutrophile and may not be distinct. It is filled with coarse, round, or slightly oval granules about one micron in diameter, which are very refractile and appear in the fresh specimen distinctly black, while in the stained smear they take up the acid portion of the dye and are called, therefore, acidophilic, oxyphilic, or eosinophilic cells. These granules are the α granules of Ehrlich, and are sometimes associated with the β granules of Ehrlich which are about the same size or a little smaller than the α granules and take both the acid and the basic stains, although with the ordinary staining solutions they appear, like the α forms, stained with eosin. The nuclei of these cells are coarsely reticulated, are larger and thicker than those of the neutrophiles, are usually bilobed, and more frequently show distinct separation of these lobes. These nuclei do not stain very deeply with the nuclear dyes, so that they may be rather indistinct. These cells are probably derived from the mononuclear eosinophile myelocytes of the bone-marrow and constitute from two to four per cent. of the total number of leucocytes, their average number being from 100 to 200 cells.

(*c*). **Polymorphonuclear Basophiles.**

These cells, frequentiy called mast cells, resemble the neutrophile cells in the fresh specimen, but show quite distinct characteristics in the stained form. Their protoplasm is much the same as that of the neutrophile and shows the same relation toward the nucleus which does not, however, so frequently form distinct lobes as does the nucleus of the neutrophile. The size of these cells averages about 10 microns, but shows a marked variation, being very small in myeloid leukemia. These cells are characterized by their granules, the γ granulation of Ehrlich, which are more irregular in size than are the neutrophile granules and are not so extensively scattered through the protoplasm. These granules show the peculiar property of metachromasia, being colored red with violet dyes and with the blue dyes violet, although with absolutely pure methylene blue they take a blue shading. In cases of myeloid leukemia these granules are particularly soluble in water and may, therefore, not be seen if aqueous stains are used, while in normal blood the granules are usually water-fast. These cells stain best with Ehrlich's dahlia stain, or Türk's iodin solution, and take a tone which is not strictly basophilic, but resembles more that of mucin, on which account they have been called mucinophiles. Whether these granules are all of the same significance is questionable. The true mast cells granules are known as the γ granules, while other basophilic granulations have been found by Ehrlich and are known as the delta (δ) granules. These latter granules are found in the large mononuclear cells, especially in the lymphocytes, and do not stain with Ehrlich's dahlia stain. Whether these latter bodies are true granules or nodal thickenings is at present uncertain. True mast cells probably originate in bone-marrow from a granular mononuclear type corresponding to other types of myelocytes (Pappenheim). These cells constitute about 1/2 per cent. of the total leucocytes, averaging between 0 and 50 per cmm.

Ehrlich's dahlia stain for these mast cells is made as follows:

Distilled water,	100 c.c.
Saturated absolute alcohol solution of dahlia,	50 c.c.
Glacial acetic acid,	10 c.c.

The specimens are heated or fixed by alcohol and are then stained in the above stain for 5 to 10 minutes, the mast cells appearing of a distinctly violet tone.

Turk's Iodin Method.

The specimens are fixed with heat and are then first stained in a 1 per cent. alcoholic methylene blue solution, warming the slide very carefully until it steams. It is then allowed to cool, washed quickly in water, and dried between filter-paper. The slide is then covered with a solution of iodin in potassium iodid of the strength 1:300. Allow this solution to act not longer than 1/2 minute, pour off and mount in the following syrup:

Iodin,	1 gram.
Potassium iodid,	3 grams.
Distilled water,	100 grams.
Gum arabic q.s. to make a syrup.	

The mast-cell granules appear, when treated in this manner, very distinctly outlined and colored black. The nuclei are brownish in color, the erythrocytes yellowish-green, and the polychromatophilic erythrocytes dark green, the neutrophile and eosinophile granules faintly yellow.

(*C*). Leucocytes in Pathological Blood.

(1). Myelocytes.

Under this heading we must regard any cell of the bone-marrow as a myelocyte, but for diagnostic purposes we have reference more to mononuclear cells which are distinctly granular. While the granulations of these cells are usually either neutrophilic or eosinophilic, we may rarely find, especially in myelogenous leukemia, cells which show basophile granulations. The size of these myelocytes varies from that of the red blood-corpuscle to that of a large mononuclear cell.

(*a*). Neutrophile Myelocytes.

This myelocyte may be either large or small, in the first case being known as Cornil's myelocyte (amblyochromatic type), a cell which is much larger than the polymorphonuclear leucocyte (at least 15 microns in diameter), and showing a round, pale, eccentric nucleus which stains feebly but not diffusely. This cell shows many distinct neutrophile granulations and has a narrow zone of basophilic protoplasm surrounding the nucleus. It is found almost entirely in myelogenous leukemia and has been called by Pappenheim the heteroplastic promyelocyte. The second type of neutrophile myelocyte is known as the Ehrlich myelocyte (trachyochromatic type of Pappenheim) which is a medium-sized cell with a pale central nucleus which stains deeply but not diffusely. This cell shows extensive neutrophilic granulation of the protoplasm, which is faintly oxyphilic, and has a nucleus which is either perfectly round, oval, or indented, but is never lobed nor pycnotic. A distinction between the myelocyte and the polymorphonuclear leucocyte should be based entirely upon the structure of the nucleus, all those cells with round, oval, or kidney-shaped nuclei which occupy at least one-half of the cell and show neutrophile granulations but no diffuse staining of the nucleus must be called myelocytes. It is to be remembered, however, that this type develops into the polymorphonuclear neutrophile, so that in abnormal blood we may have all gradations between these two types.

(*b*). Eosinophile Myelocytes.

These cells are exactly analogous to the preceding, with the exception that the granules of the more mature form show distinct eosinophilic tendencies. The younger forms of these granules may show a purplish-violet or even blue color, owing, as Simon states, to the fact that the young eosinophilic granule is

physically cyanophilic and chemically amphophilic, whereas the mature granule is physically erythrophilic, but chemically absolutely oxyphilic. The size of these cells is more or less variable, so that it is probable that we have the two types of eosinophile myelocytes, corresponding to the large and small neutrophile myelocytes. These cells occur more frequently in leukemia, in association with tumors of the bone-marrow and in the pseudoleukemic anemia of children.

(*c*). **Basophile Myelocytes.**

These myelocytes may be of variable size, but are characterized by the large centrally-located nucleus, which is not clearly defined from that of the surrounding slightly basophilic protoplasm. The granules are distinctly basophilic and in some cases are very numerous, while in others they may be widely scattered through the protoplasm. These cells are practically never seen except in cases of severe splenomyelogenous leukemia, in which they may reach as high as 47 per cent. with an absolute count of 140,000 cells (Taylor).

(2). **Irritation Forms.**

These cells vary in size from a lymphocyte to a large mononuclear cell, resembling more nearly the former. They are mononuclear, nongranular cells, thus differing from the myelocyte which is always granular. The nucleus is round and eccentrically placed, showing a very slight chromatin network and staining with the triple stain of a bluish-green color while with the Romanowsky dyes the color is a pale blue. The protoplasm is stained a deep brown with the triple stain and is thus differentiated from other forms of cells. With the methylene blue dyes the protoplasm appears more deeply stained than does the nucleus. These cells were first described by Türk and would seem to have the same significance as do the myelocytes, namely, an indication of marked activity of the bone-marrow. Pappenheim regards them as plasma cells and largely derived from the lymphocytes.

(3). **Degenerated Forms.**

Occasionally we find in normal blood degenerated leucocytes which stain poorly and show no granules. These may be even so much degenerated that they show as the so-called basket cells or "shadows." This condition is very frequently seen in severe infectious diseases, while in the acute leucocytoses which occur under many influences diminution in the number of neutrophilic granules as well as swelling and fragmentation of the bodies of the leucocytes is very common. The changes in the staining qualities of the nucleus seem to be the most significant of the lesions in the acute type of degeneration of the leucocytes. In chronic degeneration of the leucocytes we find hydropic degeneration, which is frequent in the blood of chlorosis and, when the nuclei is involved in this degeneration, seems to be limited to certain cases of leukemia. Besides such changes we find fatty degeneration as well as glycogenic degeneration. This fatty degeneration is characterized by the appearance of fat globules in the leucocytes which stain with osmic acid and with Sudan-III. For this



IODOPHILIA.

latter reason they have been styled sudanophiles, and have been very carefully studied by Buttini and Comesatti as well at by Cesaris-Demel.

Iodophilia.

In many pathologic conditions, especially in acute infectious diseases and in those associated with all types of sepsis, a so-called glycogen reaction or iodophilia may be demonstrated in the bodies of the leucocytes, as well as in certain extracellular granules. The technic of this method is as follows: An unfixed dry blood smear is exposed to the vapor of solid iodin until it is stained a brownish color. After the specimen is stained it is mounted in the syrup described on page 481 and is examined with an oil-immersion lens. The blood of normal individuals, stained by this method, shows the protoplasm of the leucocytes of a bright yellow, while the nucleus takes on a much lighter tint. In pathologic blood two types of reaction can be noted. The intracellular one, which is of greater clinical importance, shows a more or less marked diffuse brown color of the entire protoplasm of the leucocyte, or the protoplasm contains reddish-brown granules which may be more or less distinct. The extracellular reaction is evident in the blood plates, while the intracellular type is more particularly confined to the neutrophiles, although the mononuclear leucocytes may occasionally be tinged brown. Much difference of opinion exists as to the nature of this brown-staining substance, Ehrlich regarding it as glycogen, while Czerny considers it as an antecedent of amyloid, and Goldberger and Weiss regard it as peptone. Kaminer regards this reaction as a degenerative change and not as an evidence of regeneration. While this reaction has little value in differentiating infectious or septic conditions one from the other, it is sometimes of importance in making a diagnosis between purulent and nonpurulent affections, being present in the former and absent in the latter. These granules have been found by Hofbauer in pernicious anemia, secondary anemia, and leukemia, but not in chlorosis or pseudoleukemia. It would seem, therefore, to be not necessarily dependent upon the presence of infectious material, although it is of frequent value as an evidence of pus formation somewhere in the system.

(*D*). Differential Counting of the Leucocytes.

By a differential counting of the leucocytes is meant the counting of the different varieties of the leucocytes found in the stained smear and the calculation of each type in terms of percentage. The technic of this method is that previously outlined and consists in making an even smear upon a glass slide and staining it with any of the stains previously mentioned, noting that the triple stain does not bring out the granulations of the leucocytes with the exception of those of the neutrophiles. It is self-evident that the larger the number of leucocytes counted the greater will be the possibility of arriving at true percentage relations. It is wise, therefore, to count at least 250 of these cells, and in many cases, to extend this to 500. If the smear is even and the leucocytes well distributed throughout, 100 cells will frequently suffice.

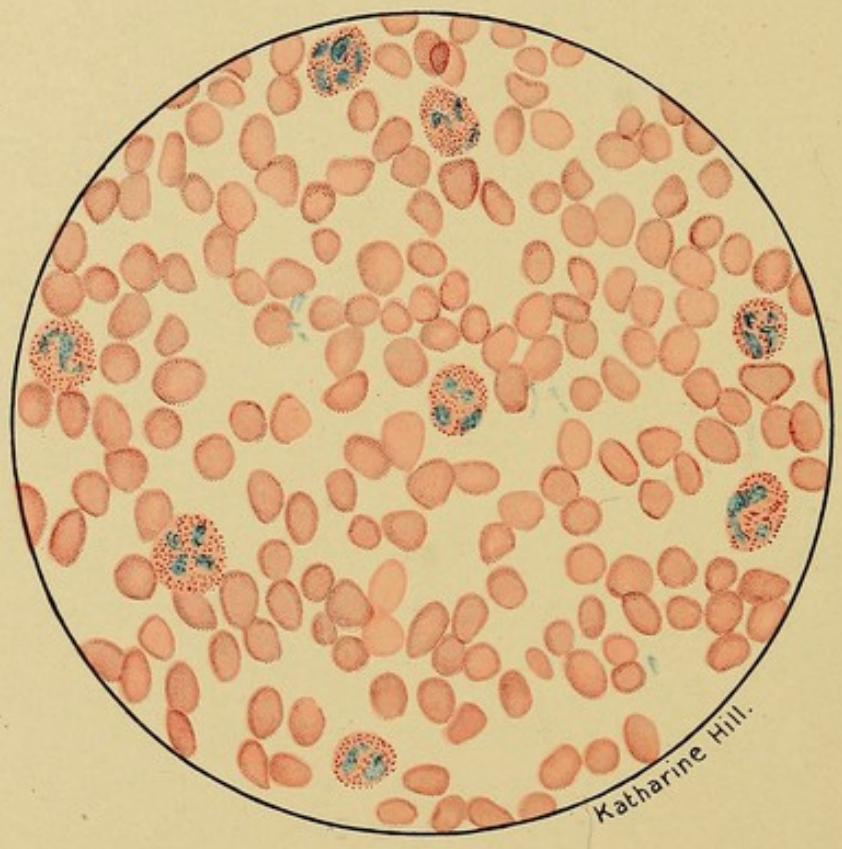
For a differential count a satisfactory classification is an absolute essential. As none of the systems at present advanced are entirely adequate, we still use the classification of Ehrlich, which is as follows: Small mononuclears, large mononuclears (including the transitional), polymorphonuclear neutrophiles, eosinophiles, basophiles (mast cells), and myelocytes. The characteristics of these cells have been previously given, but may be summed up in this connection. By a small mononuclear is meant any nongranular mononucleated cell smaller than a polymorphonuclear neutrophile. A large mononuclear is any nongranular cell with a round or oval nucleus and larger than a polymorphonuclear neutrophile. A cell of the same description and size but with an indented nucleus is a transitional form. The polymorphonuclears are cells which are about 10 microns in size and show a diffuse granulation, which may be either neutrophile, eosinophile, or basophile. It should be remembered that the mast cells appear as nongranular forms when the triple stain is used, so that the characteristics of the nucleus in its relation to the protoplasm must be borne in mind. The percentage relations of these cells in normal blood are as follows:

	Percentage.	*Number per cmm.*
Small mononuclears,	20–25	1200–2000
Large mononuclears,	3–5	200–400
Polymorphonuclear neutrophiles,	65–75	5000
Polymorphonuclear eosinophiles,	2–4	100–200
Polymorphonuclear basophiles,	0–1/2	0–50

In the writer's laboratory differential staining is usually carried out with the use of the Wright or Giemsa stain as he has found the usual triple stain quite unreliable. Although the granular differentiation is not as distinct as could be desired, yet one soon becomes accustomed to the staining of various cells so that it is not a matter of great difficulty to distinguish the various types. In many cases it is not the easiest matter to distinguish myelocytes from the small mononuclear types, but careful study will usually clear up any obscurities which exist. The writer cannot go into the variations which these leucocytes suffer in pathological conditions, but must leave the percentage relations in disease to later subdivisions.

(*E*). Number of Leucocytes.

The normal number of leucocytes in a cmm. of blood has been given various figures. As a rule, it may be said that anything above 10,000 leucocytes per cmm. should be considered pathological, the normal variation running from 5,000 to 9,000 cells. In estimating the normal number of white cells, both in health and in disease, a large number of factors which influence these cells must be taken into consideration. Thus vasomotor phenomena, variations in the volume of the plasma, inflammatory processes, state of digestion, age, variations in different parts of the circulatory system, and many different



POLYNUCLEAR LEUCOCYTOSIS. (WRIGHT'S STAIN.)

disease processes usually bring about an increase in this number, while many pathological conditions are associated with a reduction. An increase in the number of white cells is usually spoken of as a leucocytosis, but it must be remembered that such an increase may be purely physiological and should be sharply differentiated from a pathological increase which is clinically the more important. This increase is usually referable to the increase in the number of the polymorphonuclear neutrophiles, while an increase in the other varieties of cells is spoken of as a lymphocytosis, myelocytosis, esoinophilia or an eosinophilocytosis, or a mixed leucocytosis.

A diminution of the number of polynuclear neutrophiles is designated as a leucopenia, which is the more usual form in which reduction of the cells occurs. It is to be remembered that these conditions may be transitory and symptomatic pointing to a purely physiological process, while a more permanent and more marked increase or decrease in the number should be considered pathological. It is rare that we find the increase limited absolutely to one variety of cell, the increase in the others being less marked. The absolute number of these cells is much more to be regarded than their percentage relation, as with an increased number of leucocytes the actual number of some of these varieties may be increased, although the percentage may be diminished; while with a low leucocyte count the percentage may be increased and the absolute number diminished. It is wise, therefore, to report not only the percentage relations of the leucocytes, but also the actual number of these cells per cmm. This will correct mistaken ideas as to an apparent increase or decrease in any particular variety of cell. Thus a differential leucocyte count may show a percentage of 50 for the neutrophiles and at the same time an actual number of 10,000 per cmm., giving rise to confusion as to the actual relation of these important leucocytes.

Leucocytosis. (Polymorphonuclear Neutrophiliosis).

As stated above, anything above 10,000 leucocytes per cmm. should be regarded as a leucocytosis. This is, however, relative and should not be considered pathological without taking into consideration all of the physiological and pathological influences. Regarding the theoretical cause of leucocytosis, little is definitely known. The influence of infectious processes is such as to usually increase the number of leucocytes in the blood, as an attempt on the part of the system to overcome by phagocytosis the action of the bacteria of the various diseases. Yet we find in some of these infectious processes, notably in typhoid fever, a marked reduction in the number of the white cells, although the bacillus typhosus is present in large numbers in the blood at the same time. We must, therefore, assume some specific influence upon phagocytosis and chemotaxis, as a general infection is not necessarily associated with an increase in the number of white cells, but is dependent more upon the specific nature of the infection. The work upon opsonins and vaccine therapy may open up an entirely new field in our study of this subject. The leucocytosis shown in

noninfectious conditions is still a matter of much dispute and has probably more to do with variations in the plasma than with direct increase in the number of white cells.

Classification.

According to Limbeck,[1] the following classification of the various leucocytoses is the most comprehensive:

(1). Physiological leucocytosis.
  (a). Leucocytosis of digestion.
  (b). Leucocytosis of pregnancy.
  (c). Leucocytosis of the new-born.
(2). Pathological leucocytosis.
  (a). Inflammatory leucocytosis.
  (b). Leucocytosis associated with malignant tumors (cachectic leucocytosis).
  (c). Posthemorrhagic leucocytosis.
  (d). Agonal or antemortem leucocytosis.
(3). Leucocytosis following medicinal and therapeutic measures.
(4). Leucocytosis from other causes.

(1). Physiological Leucocytosis.

(*a*). Leucocytosis of Digestion.

Normally a leucocytosis of the polynuclear type will be observed beginning about one hour after a meal rich in proteins and will reach its maximum in from three to five hours. The actual figure reached varies in different persons, being usually an increase of about one-third, the maximum often reaching 15,000 cells, but more usually not much over 10,000. In this increase the small mononuclear cells may be absolutely as well as relatively increased. In some persons this leucocytosis of digestion does not appear, which fact may be referable to marked torpidity of the intestines, to a prolongation of the process of digestion, or to a large absorption of fluids. It has been found that a highly albuminous diet has a much more marked influence upon this leucocytosis than does a diet of vegetables and fat. The rapidity of absorption and of digestion must be taken into consideration as factors which influence the appearance or nonappearance of the leucocytosis. In children the increase in cells is much more marked than in the adult, due probably to the increased digestive and absorptive powers, providing the food taken is, as it should be, easily digestible and absorbable.

Various pathologic conditions influence this digestion leucocytosis. Thus Müller has found that in cases of carcinoma of the stomach a digestion leucocytosis is rarely observed after heavy meals. There are, however, a few cases of gastric cancer in which a slight increase has been observed. This failure of leucocytosis is probably due, as Schneyer shows, to lessened absorption as

[1] Jena, 1896.

a result of involvement of the lymphatics rather than to a malignant stenosis of the pylorus, as the benign stenoses are usually associated with a leucocytosis. That the lack of leucocytosis is due more to diminished absorption than to lack of digestive power is shown by the usual occurrence of a digestion leucocytosis in ulcer of the stomach, chronic gastritis, and in dyspeptic conditions. Just why carcinoma, with or without stenosis, should be associated with a normal or subnormal number of leucocytes after digestion is hard to explain, in view of the fact that these other conditions are associated with a leucocytosis. Practically all absorption of food material occurs from the bowel and we would naturally expect to find disorders of the intestinal canal leading more frequently to a normal leucocyte count than to a leucocytosis. Very little detailed work has been done upon the influence of enteric troubles upon the leucocyte count, but the writer has seen several cases in which a digestion leucocytosis did not occur and in which the findings both ante- and postmortem were entirely related to the bowels. The examination of the blood may, however, clear up a diagnosis of carcinoma, but will not always permit of a differential diagnosis between this condition and pernicious anemia. It is to be remembered that a leucocyte count must be frequently made in order that this test may be of any value whatever and that a considerable rise only is to be taken as evidence of a leucocytosis. Patients with cancer either of the stomach or of other viscera frequently show a leucocytosis as the result of cachexia and this should be remembered in the interpretation of a leucocyte count following digestion.

(*b*). Leucocytosis of Pregnancy.

It has been shown that from 50 to 75 per cent. of cases of pregnancy are associated with a leucocytosis, averaging about 15,000 cells per cmm. This is especially true of primiparæ, but is often shown in the multiparæ. Just what cause can be given for this rise is uncertain. It is more than likely that a condition of slight intoxication is present due to the overloading of the blood of the patient with the products of metabolism of the fetus. These substances are not normal to the blood of the woman and in consequence act as foreign bodies which may attract, by their chemotactic influence, the white cells and bring about an increase. As these cases of pregnancy show an absence of a digestion leucocytosis it has been assumed that the increase in leucocytes is due to a prolonged digestion leucocytosis, but this does not seem probable. The changes in the breasts and in the uterus during this period have suggested these organs as the factors influencing this leucocytosis, but such inferences do not seem to be directly warrantable. The leucocytosis of pregnancy is a mixed leucocytosis, all of the various types of leucocytes, with the exception of the eosinophiles being increased. After the birth of the child the leucocytes gradually diminish in number and normally reach their usual values in from four to fourteen days after delivery. Should complication, such as postpartum hemorrhage or septic fever arise, the leucocyte

count may remain high until these complications have subsided. In multiparæ these changes in the number of leucocytes are not so marked as in the case of the primiparæ, but a slight rise always occurs. This fact has been attributed to the lessened reactivity of the organisms to the influence of the toxic substances thrown into the blood from the cells of the fetus.

(*c*). Leucocytosis of the New-born.

As Askanazy has found, the blood of the fetus shows a diminution in the number of leucocytes, owing to the fact that there is no function as yet established for these cells *in utero*. In the blood of the new-born, however, a leucocytosis running from 15,000 to 20,000 cells may be observed, these figures going as high as 40,000 under the influence of the first feeding. As the weight of the child begins to diminish these cells are markedly reduced in number to about 8,000, and are subsequently increased to 10,000 as the child begins to gain in weight. The high leucocyte count at birth is more probably due to the rapid blood formation than to a concentration of the blood, although this latter factor as well as that of the influence of digestion must be taken into consideration. The increase in the number of cells is chiefly limited to that of the small mononuclear cell, the differential count of the leucocytes showing during the early periods of life from 40 to 60 per cent. of the total number. This leucocyte count, if taken at the moment of birth, will not, however, vary much from that of the adult, the change becoming more marked in the early periods of life.

(2). Pathological Leucocytosis.

(*a*). Inflammatory Leucocytosis.

In most cases of inflammatory nature, of acute infections and general febrile diseases, there is observed an absolute increase of the polymorphonuclear neutrophiles, which runs more or less parallel to the temperature. This increase may run from 10,000 to 50,000 cells and diminishes as the influence of the inflammatory process is diminished. In general it may be said that leucocytosis represents the reaction of the individual to the disease. A high count may mean a vigorous reaction to the infection; a low count may mean either a poor reaction and hence an unfavorable condition of the patient, or it may indicate a very mild degree of infection with a normal reactivity of the patient. It must be said that all diseases of an infectious nature are not necessarily associated with a leucocytosis. For instance, pneumonia shows a leucocytosis which runs parallel to the degree of virulence; measles, influenza, malaria, and tuberculosis are rarely if ever associated with a leucocytosis unless complications arise or the conditions becomes very severe. In typhoid fever we usually find a leucopenia which, however, is associated with a relative lymphocytosis; if complications such as perforation arise a leucocytosis may appear. Among the conditions causing leucocytosis are acute lobar pneumonia, the count running between 20,000 and 100,000, depending upon the severity of the infection and the degree of resistance against the infection.

Acute articular rheumatism, diphtheria, acute cerebrospinal meningitis, follicular and suppurative tonsillitis, scarlet fever, mumps, rabies, erysipelas, ulcerative endocarditis, small-pox, cholera, general pus infections of the serous membranes and of the mucous membrane, acute bronchitis, and many other conditions cause a leucocytosis of the polymorphonuclear type. The variations in these many conditions will be discussed under the Pathology of the Blood.

The exact cause of this leucocytosis following inflammatory processes is still a matter of much discussion. The bone-marrow has been shown to be markedly increased as regards cellular proliferation in the early stages of the inflammation and may be so markedly changed as to cause permanent derangement in the functions of this blood-forming organ. That leucocytosis has much to do with immunity both from the standpoint of phagocytosis and from that of Ehrlich's side-chain theory cannot be questioned. A leucocytosis must represent the attempt on the part of nature to rid the blood and the system of the bacterial and toxic products of the disease. Whether phagocytosis as such or under the influence of various opsonins is the direct cause of immunity and of recovery from any specific infection must be left to a later chapter. Suffice it to say that the work upon Antitoxins in the acute infections and of the vaccines in the chronic suppurating types of disease is bearing remarkable results.

(*b*). Cachectic Leucocytosis.

This variety of leucocytosis is the least uncertain. The cases of carcinoma and of sarcoma show a leucocytosis which is not definite for the particular kind of cancer, but is more usual with the latter than with the former type. The leucocytosis is usually one of the polymorphonuclear type, but is frequently associated with an increase in the number of mononuclear cells. This leucocytosis has no direct relation with the situation of the tumor and is not always present in all cases of cancer. Whether this leucocytosis be due to an intercurrent infection is a question which must be left for more detailed work, but it would seem wise to accept Ewing's statement that a marked leucocytosis in the course of a cachexia, from tertiary syphilis, tuberculosis, nephritis, and in the majority of cases of carcinoma, should suggest a search for a complicating infection, while in the sarcomata a leucocytosis is much more common as a direct result of the disease.

(*c*). Posthemorrhagic Leucocytosis.

A well-marked leucocytosis, which may begin in from ten to fifteen minutes and may reach as high as 20,000 cells within an hour, has been often observed following extensive acute hemorrhages. The leucocytosis in these cases bears a general relation to the extent and rapidity of the loss of blood and usually disappears or diminishes long before regeneration of the blood has occurred. This leucocytosis is of the polymorphonuclear type and is referable rather to the sudden outflow of lymph which occurs as a compensation for the

loss of fluid than to a new production of cells, as this latter process does not take place for some time. In hemorrhages which are slight and long-continued as in cases of gastric or intestinal ulcer, the duration of the leucocytosis as well as its extent is very brief. Stassano and Billou assume from their work that a hypoleucocytosis follows a severe hemorrhage while a true leucocytosis is observed after the loss of small quantities of blood. These findings seem to be rather doubtful, except where the hemorrhage has been so extensive as to cause death.

(*d*). Antemortem Leucocytosis.

This form of leucocytosis has been questioned, especially by Arneth, but there seems to be little doubt that such a leucocytosis may occur if death does not take place too rapidly. In some diseases the leucocytes do not fall in number, but in some a distinct rise is noted which has been attributed by Ehrlich to the accumulation of white cells along the periphery of the blood-vessels as a result of slowing or stasis of the circulation. This type of leucocytosis is usually of the polymorphonuclear variety and may support the view of Limbeck that antemortem leucocytosis, when it does occur, is the result of a terminal infection, although it cannot explain those cases which show a lymphocytosis rather than the ordinary leucocytosis. The character of the antemortem leucocytosis must depend largely upon the precedent condition and will be associated with antemortem dissemination of bacteria, antemortem hyperpyrexia, vasomotor paralysis, serous exudation, and many other causes whose influences are not well established.

(3). Leucocytosis Following Therapeutic Measures.

(*a*). Drugs.

It has been found following administration of tonic drugs, ethereal oils, myrrh, turpentine, camphor, peppermint, quinin, and other drugs, that a leucocytosis of a more or less extent occurs, which is probably referable to the same cause as is digestion leucocytosis. Many of these drugs if applied locally for the purpose of counterirritation were shown to have the same effect. Extracts of tissues, especially those containing large amounts of nucleinic acid and of purin substances, produce an extensive leucocytosis, which fact has been taken advantage of in the administration of the nuclein substances as therapeutic remedies. In the case of those drugs which destroy the red cells, such as the coal-tar antipyretics, chlorates, and illuminating gas, a normal number of white cells is usually observed, although Simon wrongly states that a hyperleucocytosis follows the use of such drugs. After the prolonged use of chloroform or ether a polynuclear leucocytosis is generally observed which is usually of short duration. In this connection it is well to remember that an increase in the number of leucocytes of 10,000 to 15,000 above the normal value of the individual should be regarded as evidence of infection if this increase is sustained for more than a few hours.

(*b*). **Baths.**

After a cold bath the leucocytes of the polynuclear variety have been shown to be increased from 100 to 300 per cent. This is true only if the bath is of moderate duration, a prolonged cold bath taken to the point of exhaustion will diminish rather than raise the number of white cells. Experiments on the result of hot baths have shown just the reverse condition, namely, a hot bath of short duration produces a decrease, while one of long duration causes an increase in the number of white cells. Massage in itself following either a hot or a cold bath tends to increase the number of leucocytes.

(*c*). **Exercise.**

Prolonged muscular exercise, as taken in the form of gymnasium work, as applied in the various therapeutic movement treatments, and also as given in the more violent athletic contests produces a rise in the number of leucocytes which is temporary and is characterized more by an increase in the number of polynuclear cells than of any other variety of the leucocytes.

(4). **Leucocytosis from Other Causes.**

Cyanosis, passive hyperemia obtained by the method of Bier, shock whether physical or mental, injection of various toxins, such as Koch's tuberculin, the various autovaccines, and injection of various organic principles, such as peptone, pus, organ extracts, etc., have all been shown to produce a polynuclear hyperleucocytosis, either by causing stasis of the circulation or by increasing the chemotactic power of the serum. The exact explanation of chemotaxis is still in doubt and we find that some substances are positively and others negatively chemotactic, thus accounting for the lack of phagocytosis in certain conditions, such as pneumonia, although in these same conditions we may have a leucocytosis as a direct result of an infection. From the work of Rosenow we learn that the phagocytic action of the leucocytes in pneumonia is increased by the addition of an extract of the active pneumococci; whether this extract increases the chemotactic power of the bacilli in the blood or whether certain restraining influences are overcome is still unsettled. Future work upon phagocytosis and upon the factors governing increased opsonic power in the various infections may show us what causes are effective in such conditions.

In the previous discussion of leucocytosis we have had reference more to an increase in the number of polynuclear cells, this type being more properly called polynuclear hyperleucocytosis. It is very rare that this condition exists in the absolutely pure state, being frequently associated with an absolute or relative increase in the number of lymphocytes.

Mixed Leucocytosis.

By this is meant an increase of both granular and of nongranular cells of various types, its more common interpretation, however, being a leucocytosis characterized by an increase in the number of neutrophile myelocytes. This neutrophilic myelocytosis is best seen in leukemia in which the absolute number

of myelocytes may reach 150,000, the increase in no other condition, according to Ehrlich, rising above 1,000 cells. The increase in the myelocytes in leukemia is not limited to those of the neutrophile variety, but is commonly associated with a marked increase in the number of eosinophile types as well as with an increase in the number of mast cells. In pernicious anemia we may also find a large increase in the number of these myelocytes. These cells when increased in number are clinically significant of exhaustion of the bone-marrow, but should never be interpreted in this way unless they remain while the leucocyte count is falling; in other words, they are significant only when constituting a large percentage of the leucocytes.

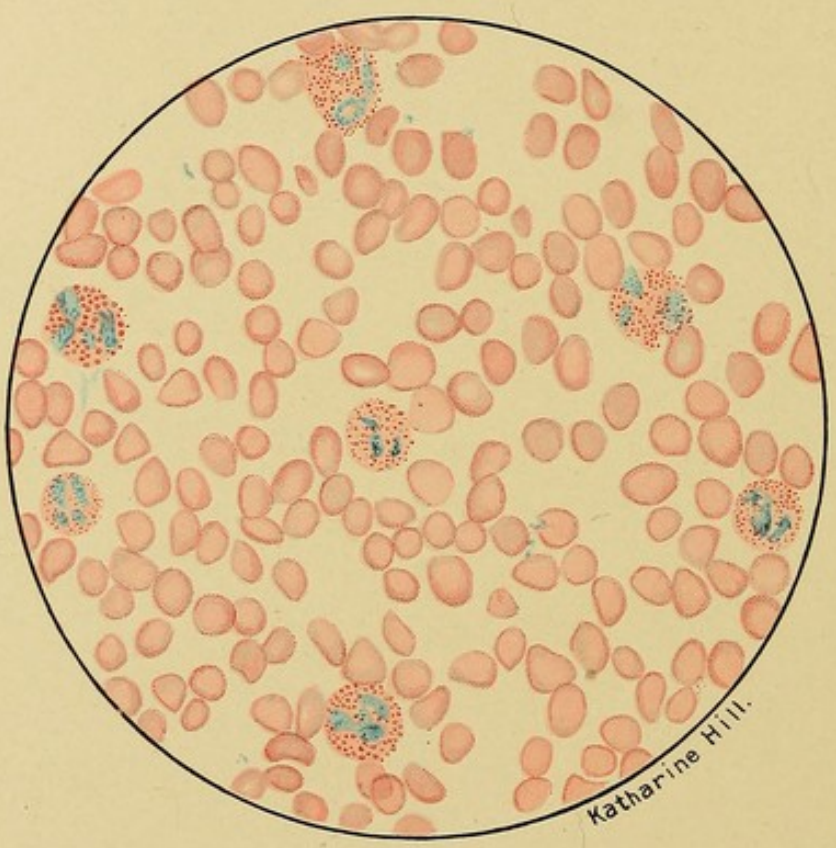
Lymphocytosis.

A relative or absolute increase in the number of lymphocytes is of frequent occurrence and has occasionally some significance. In judging of this condition the actual number of cells present as well as their numerical relations to the other varieties of leucocytes must be considered. The term lymphocytosis must always be applied to an absolute increase in the number of these cells, the normal number being between 1,200 and 1,500 per cmm. Physiologically we find this condition in infants and during a digestive leucocytosis in the adult. The child shows at birth from 50 to 65 per cent. of these cells which percentage gradually diminishes, reaching the normal percentage (20–25) about the age of puberty.

Pathologically this condition is observed in poorly nourished children, those showing the "constitutio lymphatica," rachitis, whooping-cough, gastro-intestinal disturbances in the child, cervical adenitis, splenic tumors, in most infectious diseases of children, and especially in lymphatic leukemia, in which as high as 90 per cent. of a largely increased leucocyte count is referable to the small lymphocytes. In the splenomyelogenous form of leukemia and following removal of the spleen we observe a steady increase in the number of lymphocytes continuing during the first year. It must be remembered that a leucocyte count may be low and yet a lymphocytosis exist. This is shown in typhoid fever, amebic dysentery, chlorosis, pernicious anemia, scurvy, and other conditions in which the leucocyte count is low, the granular cells being diminished in number, but the lymphocytes being increased. In the congenital and also secondary acquired syphilis we find a lymphocytosis which must be referred to involvement of the lymph-glands. The worker must not be led into a mistaken interpretation of his blood-findings, as enlarged lymph-glands may give the same picture of lymphocytosis as is seen in Hodgkin's disease, chronic and acute lymphatic leukemia.

Eosinophilia.

By this is meant an absolute increase in the number of eosinophilic cells. The average number of these cells is between one and two hundred per cmm., hence the term eosinophilia should be limited to those cases showing counts something above 250. We find a physiological eosinophilia during childhood,



EOSINOPHILIA. (WRIGHT'S STAIN.)

the average increase being about 1 to 2 per cent. above the normal adult finding. No physiological relations have been established between eosinophilia and sex, pregnancy, menstruation, digestion, or old age; however racial distinctions are sometimes shown by eosinophilia, the natives of southern China showing between 15 and 20 per cent. of the leucocytes as eosinophiles.

Pathological Eosinophilia.

We observe variations in the number of eosinophiles in various affections of the bone-marrow. Thus in splenomyelogenous leukemia, we may find these cells increased as high as 30,000 per cmm. According to Ehrlich, a diagnosis of this form of leukemia is warranted only when we have an increase in the number of eosinophiles, but it cannot be doubted that cases are relatively frequent in which these cells are not increased. In sarcoma of the bone-marrow as well as in osteomyelitis and osteomalacia, we find these cells usually increased in number. In chlorotic conditions we may find the eosinophiles moderately increased, while in secondary anemia especially those following infection with parasites a marked eosinophilia may be observed. After extirpation of the spleen as well as in cases of chronic splenic tumor we find an eosinophilia which may run from 10 to 40 per cent. higher than the normal, constituting as high as 40 per cent. of the total number of leucocytes. Whether or not a pure disease of the lymph-glands is associated with the eosinophilia is unsettled, but if metastases have extended to the bone, an eosinophilia of marked degree is usual.

True bronchial asthma is always associated with an increase in the number of eosinophiles, one case of Billings showing 54 per cent. of the total leucocytes as eosinophiles. This relationship in asthma is of importance from a diagnostic standpoint as asthmatic attacks from other causes are not associated with an eosinophilia. It is true that in emphysema a marked eosinophilia does occur so that we may find, in cases in which this condition complicates a tuberculosis, that an eosinophilia obtains. Tuberculosis of the lungs or of other tissues does not show an eosinophilia unless complicated by emphysema, cachexia, or secondary infection.

A large number of skin diseases is associated with eosinophilia, the highest count being reported by Zappert in a case of pemphigus showing 4,800 cells per cmm. The occurrence of this condition in skin diseases depends not so much upon the nature of the lesion as upon its extent, intensity and lack of healing tendency. Many skin lesions are known to be produced by toxic agents which have special chemotactic influences over the eosinophile cells; this fact may account for the eosinophilia in such conditions. The most usual skin diseases showing this increase are pemphigus, eczema, psoriasis, urticaria, purpura, scleroderma, lupus, leprosy, herpes zoster, and general gouty affections.

It is a general rule that the infectious fevers are not associated with eosinophilia, but with the acute polynuclear leucocytosis. In scarlet fever, however, we find that eosinophiles are frequently markedly increased, while in acute

rheumatism and in malaria these cells are usually present in more or less increased numbers. In gonorrheal infections the eosinophile leucocytes are very frequently marked in the discharge in the early days of infection, diminishing in number as the number of neutrophiles increases, and increasing again as the discharge clears up. It is generally believed that the eosinophile cells in the blood are increased coincidently with their increase in the gonorrheal discharge. It has been shown that the eosinophiles are usually increased in all forms of ovarian disease with the exception of cancer. In syphilis a uniform increase of the eosinophiles has been observed, but some cases do not show such a regular finding.

Outside of the splenomyelogenous form of leukemia and the true bronchial asthma, infection with various parasites is accompanied by the most pronounced eosinophilia. Any parasite from the harmless pin-worm to the most malignant uncinaria may cause an eosinophilia (Emerson). This eosinophilia is not necessarily constant nor does its extent bear any relation to the severity of the infection. A differential diagnosis between typhoid fever and trichinosis is frequently possible on the basis of the marked eosinophilia in this latter condition, although eosinophilia does not always obtain here.

Mast-cell Leucocytosis.

In myelogenous leukemia we find these cells increased to a very large extent, often outnumbering the eosinophile cells. An increase of these cells is generally regarded as the sole isolated pathognomonic sign of this disease. Their increase may be as high as 20 per cent. of the leucocytes. These cells have been found in a few cases of cancer, tuberculosis, syphilis, and other lesions of the skin, while in bone disease complicated by septic infection a few reports of positive findings have been made.

Leucopenia.

This is a condition characterized by a reduction either in the total number of leucocytes or in one or more groups of leucocytes. The most usual condition showing a leucopenia is typhoid fever in which we find the polynuclear cells markedly diminished, thus lowering the leucocyte count, while the large mononuclear cells are relatively increased. Anything below 5,000 cells is regarded as a leucopenia. It must be considered in this connection that the typhoid leucopenia remains only when the disease is limited to the intestinal canal; when other organs become involved a leucocytosis supervenes. The count in typhoid fever may run as low as 2,000 cells, while in tuberculosis of the lymph-glands it may reach 500 cells. In cases of starvation or malnutrition the leucocyte count is always low and in chronic intoxication with the heavy metals, morphin, alcohol, and cocain, we observe a very low count, as a rule. A general rule to be observed in typhoid fever is that an increase in the number of white cells following a low leucocyte count is evidence of complications or of a mistaken diagnosis. It must be remembered that a relapse in typhoid may bring on a hyperleucocytosis, but this must occur during the afebrile

period, otherwise a leucopenia will remain. In measles also we observe a leucopenia following the eruption and a leucocytosis preceding the eruption. This leucopenia affects the polynuclear neutrophiles while the lymphocytes are relatively increased, with disappearance of the eosinophiles. In cases of uncomplicated influenza we usually find a diminution in the number of leucocytes, although a normal number may obtain. This enables one to differentiate influenza from pneumonia, in which latter condition a marked leucocytosis is practically always present. In pernicious anemia and in splenic anemia we find a marked leucopenia during the active periods of the disease, the count running, as a rule, between two and three thousand cells.

(*F*). Variations in Infancy and Childhood.

In the first few days after birth the leucocytes may reach as high as 20,000, while in the nursing period the cells average 13,000. In this connection the influence of a digestion leucocytosis must be remembered as the cells may reach as high as 25,000 of which the lymphocytes constitute about 60 per cent. As the age of the child increases, the number of leucocytes gradually diminishes until the age of puberty, when the average number is about 8,000. As the development of the child progresses, the number of lymphocytes is gradually diminished and that of the neutrophiles correspondingly increased. The relations between the lymphocytes and the neutrophiles must be constantly remembered in making a blood count, as the percentage of lymphocytes is high at first and then gradually diminishes until the age of puberty; the neutrophiles during the same period are at first low, reaching the normal 65 to 70 per cent. about the age of 15. There are no marked differences in the morphology of the granular and of the nongranular cells of the child as compared with those of the adult. The characteristic changes in the leucocytes of the blood of the growing child are more related to the changes consequent upon development and show no signs of degenerative changes. This statement has reference of course to the normal healthy child, the variations under the influence of the various infectious diseases being quite marked. These latter changes will be discussed under a separate heading.

(*G*). Functions of the Leucocytes.

The functions of the leucocytes are largely related to their powers of overcoming the effects of bacterial processes. These results are accomplished both through their ameboid powers and their characteristic property of phagocytosis. This latter property is largely influenced by chemotaxis as well as by the indefinite increase of so-called opsonins. Just what we are to regard as the basis of the opsonic index of the leucocyte is unsettled, but it is certain that the same leucocyte may show marked variations in its power of absorbing bacteria of different types. Whether this opsonic power is related in any way to the various assumptions embraced by Ehrlich's side-chain theory must be left for a later section. Besides the above important functions of the leucocytes, these cells show oxidizing, reducing, and fermentative powers, all

of which are of more or less importance in the study of immunity and of reaction to bacterial infection. The fermentative powers of the leucocytes are not resident in all types of these cells, being more marked in those of the polynuclear variety. It is highly probable that the ordinary leucocytosis is a conservative process, enabling the cell to overcome by these various actions the influences of the various conditions which have brought about the leucocytosis. In the leucocytosis of digestion we find the leucocytes playing a large rôle in the absorption of protein material so that it is highly probable that the protein is taken up entirely by these cells and transported to the various parts of the body. As is well known no increase in the amount of the protein of the portal vein is evident after a hearty meal, yet we must assume that protein is absorbed as such, although it has thus far escaped detection. This statement is borne out by the fact that we have a distinct alimentary albuminuria following ingestion of protein, such as egg albumin, which is foreign to the blood. Were such substances not absorbed as such there would be no possibility of their appearance in the urine, as the synthetic processes of the normal human cells are not capable of producing protein bodies foreign to themselves. It has been assumed, as a consequence of the teaching of Ehrlich, that the granules of the polynuclear leucocytes are specific secretory substances formed as neutralization products by the activity of various bacteria upon these cells. Whether this teaching is or is not true is undecided, but there can be little doubt that the leucocytes do have marked powers as antitoxins in neutralizing the poisonous metabolic products of bacterial activity. There is so much uncertainty in our knowledge regarding the development and the relation of the various types of leucocytes that speculation upon the function of these different types is useless. It must be admitted, however, that the leucocyte in the stage of a lymphocyte, in that of a transitional type, or in the form of a polynuclear granulated cell must have different functions, as the relations between these cells is so markedly changed in various disease processes. Whether anything specific will be forthcoming from later study is a matter of pure hypothesis, but it is interesting to speculate upon the reasons for a leucopenia with a relative lymphocytosis in typhoid fever, on the one hand, and a marked polynuclear leucocytosis in pneumonia, on the other. These two types of infectious diseases are, of course, not identical in their pathological findings, as in the former we have the lymphoid structures markedly affected, while in the latter the trouble is largely a direct result of reaction against infection. It is to be hoped for the sake of pathology that further study upon the function of the leucocytes will be made and less time devoted to research upon the more obscure problems of development which have so little apparent clinical value.

(7). Blood-plates.

(*a*). Appearance.

These bodies which have been called "third corpuscles" are probably not true cellular entities. Hayem has considered them as the direct forerun-

ners of the erythrocytes and has styled them, therefore, "hematoblasts." These bodies have been variously called plaques by Osler, blood-plates by Bizzozero, and by Arnold fragments of cells. These so-called corpuscles are small colorless bodies containing no hemoglobin. They are about 3 microns in diameter, round, oval, or rod-shaped without any biconcavity. They appear bluish, homogeneous or occasionally granular, and stained lightly by both basic and acid dyes. They do not contain any nucleus or membrane and become hyaline and glassy as soon as removed from the vessel, but on standing they become pale and unite to form granular masses. They are very sticky and adhere very extensively to one another, forming masses from which fibrinous threads radiate. This fact has lead to the belief that they have an important part in the formation of fibrin.

Specimens of the platelets are best obtained by puncturing the tip of the finger or the ear through a 10 per cent. solution of sodium metaphosphate. In this way the blood becomes at once mixed with the fixing fluid and the drop may then be placed on a slide and covered with a cover-glass. It has been customary to call anything a platelet which is smaller than a red-blood cell and which does not contain hemoglobin. The term platelet, however, should be reserved more particularly for those bodies which have a peculiar bluish refractility, no nucleus, show marked cohesive properties, and soon disintegrate.

(*b*). **Size.**

The normal size of the blood-platelet averages about 3 microns, although Preisich and Hein have reported them as high as 7 microns. As a rule, their size varies inversely as their number. Some of these bodies show clear areas either in the center or on one side, or on the whole periphery; others become crescents, triangles, spindles and threads (Emerson).

(*c*). **Number.**

The normal number of platelets per cmm. is approximately 250,000, this number varying in the same person at different times of day. The physiological factors influencing the number of these cells are not well understood and in pathological conditions we may find large or small numbers of these cells. It is generally accepted that they are increased in anemias from any cause and may be related to the red blood-cells as 1 to 10. They are increased in chlorosis, decreased in pernicious anemia, and in any severe secondary anemia. In splenomyelogenous leukemia we find a large increase of these bodies, while in the lymphatic leukemia these cells are diminished. They are increased in chronic diseases associated with cachexia and malnutrition, being more marked in cancer and nephritis than in anemia, due to other causes. The more acute, more severe, more threatening the disease, the fewer the number of platelets, so that we have a direct relationship between severity of disease and number of these bodies. The method of counting these cells has been given in a previous section to which the reader is referred.

Just exactly what significance is to be attached to their increase or decrease is uncertain, but it must be remembered that there is a certain relationship between their number and the severity of general conditions.

(*d*). **Staining Properties.**

These bodies stain very much like nuclear material with a basic stain, but also take the acid dyes under certain conditions. With the Wright stain these cells are seen as distinctly blue bodies grouped in numbers from 1 to 10 and seem to be composed of nucleus and protoplasm. The apparent nucleus consists of rows of blue or reddish dots occasionally arranged in spherical masses, while the indefinite poorly-defined protoplasm-like substance seems swollen to almost the size of a red corpuscle. As a rule, however, these bodies appear as grouped bluish masses of indefinite structure and outline.

(*e*). **Function.**

The function of these cells is very indefinite. It is possible that they have much to do with the formation of fibrin and may be the source of the so-called fibrin ferment, thrombogen. If we are to regard these bodies as derived from the leucocytes this function of fibrin formation is acceptable, but if they are to be considered as derivatives of red cells or as true independent bodies such an hypothesis is untenable. No facts of any clinical value have so far been forthcoming from the study of the blood plates, and it may be possible that they are really artefacts as Löwit has claimed.

(8). **Hemoconien.**

By this term we have reference to the presence in normal blood of very fine granules which are actively motile, not truly ameboid, but with motion more of the Brownian type. These granules, also called blood-dust by Müller, are small, round, colorless granules which vary in size from very fine dust-like particles to some as large as 1 micron in diameter. Their exact chemical nature is uncertain as they do not show, according to Müller, either the reactions of fat or of albumin. The general idea prevails that these bodies are the extruded granules of the leucocytes, as they resemble in size and in staining qualities those of various leucocytic types. The number of these granules is uncertain, but apparently the relation of these granules to the red cells, as shown by the ultra condenser, is about 50 to 1.

Although no clinical significance is known to attach to these granules, the writer has observed marked variations in their number in various pathological conditions, but cannot at present draw any conclusions from such observations.

(9). **Morphology of the Blood-forming Organs.**

As the bone-marrow is of such importance in the production of both red and white cells, it would seem advisable briefly to discuss the histology of this tissue. In it we find practically every cell which occurs in the blood

both in normal and abnormal conditions, and also many transitional forms between various groups of cells. The following brief outline of the histology of the bone-marrow is taken from a recent paper of Dickson.[1]

Varieties of Bone-marrow.

(1). **Primitive or Embryonic Marrow.**

A delicate interlacing network of mucoid cells, which later in the process of development go to form the connective-tissue framework or adenoid reticulum of the tissue.

(2). **Red "Lymphoid" or Formative Bone-marrow Proper.**

This is found in the adult in the short and flat bones, sternum, ribs and vertebræ, and to a varying extent in the ends of the long bones. This is the most important variety as in it are found the red and the majority of the white cells of the blood, and, according as one or the other of these series of cells predominates, the type of marrow may be classified as erythroblastic or leucoblastic, a varying admixture of these two types being practically always found in any given case.

(3). **Fatty or Yellow Marrow.**

This is found mostly, as age advances, in the central part of the long bones and is formed by a process of physiological transformation or degradation of the connective-tissue elements, together with the gradual disappearance of most of the blood-forming cells of the red marrow.

(4). **Fibroid Marrow.**

This is found in old persons, especially if debilitated by long-standing disease, and is characterized by the proliferation of the connective-tissue elements and by the progressive sclerosis of the marrow, followed by the gradual disappearance of the hemopoietic cells of the tissue.

(5). **Gelatinous Marrow.**

This is essentially a retrogressive change in the tissue and is in no way identical with that type already described as primitive or embryonic marrow. This change has been described by a previous writer as a chronic condition only, but has been frequently found by Dickson as an acute change in many of the acute infectious fevers and allied diseases.

Cytology of the Bone-marrow.

In this discussion little more will be taken up than a brief enumeration of the various varieties of cells, as the more important ones of these have been treated of in other sections.

[1] Jour. of Path., and Bact., 1907.

(*I*). **Blood-forming Cells.**

(*A*). **Leucocyte Series.**

(*a*). **Nongranular Cells with Basophile Protoplasm.**

(1). Large.

(2). Small.

(α). Cells similar to but smaller than the large variety.

(β). Cells identical in appearance and in staining reactions with the small lymphocyte of the blood. Of these there are also probably two varieties.

(*b*). **Granular Cells.**

(1). Neutrophile.

(α). Myelocytes with large rounded or oval nuclei. These have been definitely traced by Dickson to nongranular or hyaline cells in which the granules may be seen gradually developing. There are two types known as the larger and the smaller neutrophile myelocyte.

(β). Intermediate cells with indented or horse-shoe-shaped nuclei. These are developed from the myelocytes (α) and in turn go to form

(γ). Polymorphonuclear cells or adult leucocytes which pass out into the blood stream.

(2). Eosinophile.

The same types of these cells with eosinophile granulations are found in the marrow, as have been previously tabulated under the heading of neutrophile cells.

(3). Basophile.

(α). Mast cells.

The three types above discussed are also observed in the basophile mast cells of the bone-marrow.

(β). Cells resembling the eosinophile myelocytes but with granulations staining with the basic dye. These cells are probably altered eosinophiles.

(*B*). **Hemoglobin-holding Series.**

(1). Normoblasts.....Normocytes or ordinary red corpuscles.

(2). Megaloblasts { Normoblasts,.....normocytes. / Megalocytes (entirely pathological in the adult).

(*II*). **Giant Cells.**

(1). Mononucleated or megakaryocytes.

(2). Multinucleated or polykaryocytes.

(*III*). **Cells of Connective-tissue Type.**

(1). Fat cells.
(2). Cells of the reticulum.
(3). Various forms of phagocytic cells.
(4). Ordinary connective tissue cells.

(*IV*). **Endothelial Cells.**

(1). Found in their normal position in the vessel wall.
(2). Found proliferating and taking on phagocytic functions.

Reactions of the Bone-marrow in Disease.

Many of these reactions are connected with the production of the so-called inflammatory leucocytosis and take place with great rapidity. Other varieties of change are intimately concerned with the production of the hemoglobin-holding series of cells. These reactions may, according to the type of cell involved, be summarized as follows:

(*I*). **Leucoblastic.**

(1). Neutrophile
(2). Eosinophile.
(3). Basophile
(4). Hyaline or non-granular.

(*II*). **Erythroblastic.**

(1). Normoblastic.
Megaloblastic.

The above brief outline will show the reader that we have in the marrow all possible types both of red and of white cells and that any variation in the normal activity of the marrow will result in the overloading of the blood with cells of a particular type depending upon the kind and extent of the affection.

The histology of the other blood-forming organs, as the liver, spleen, and hemolymph nodes may be found in any text-book on histology.

IV. Pathology of the Blood.

(I). Special Pathology.

Under the head of the special pathology of the blood we have to consider the conditions which are manifested directly by changes in the composition and cellular structure of this tissue. In very few of the blood diseases proper is the blood picture so characteristic that a definite diagnosis is always possible, but in a few of them certain changes are more frequently found and more often lead to a presumptive diagnosis. The pathological conditions in the blood may be considered either primary or secondary, but it should be remembered that severe secondary changes may so closely simulate those found in primary conditions that differentiation is almost impossible. It is probably true that all pathological changes of the blood are really secondary, but in a certain number of these states the etiologic factors are so obscure that we can do no more than interpret the blood findings as primary conditions.

(*A*). Anemia.

This is a condition characterized by a deterioration, both qualitatively and quantitatively, in one or in all of the blood constituents. It is usually characterized by a diminution in the percentage of hemoglobin (oligochromemia) and by a decrease in the number of red cells (oligocythemia), but we should regard as essential factors in the anemic condition a reduction in the total volume of blood (oligemia) as well as a reduction in the amount of protein (hypalbuminosis). As much more attention has been paid to the first two factors than to the latter ones, anemia has come to mean a reduction in the amount of hemoglobin with a more or less extensive reduction in the number of red cells. Associated with these conditions we have, in the severer types of anemia, variations in form, size, and structure of the red cells as well as definite changes in the relationship of the different white cells. In the study of the anemic conditions we must differentiate the primary from the secondary form, by this we mean a differentiation between those forms which have no demonstrable cause from those types whose etiology is more or less secondary to other pathologic conditions.

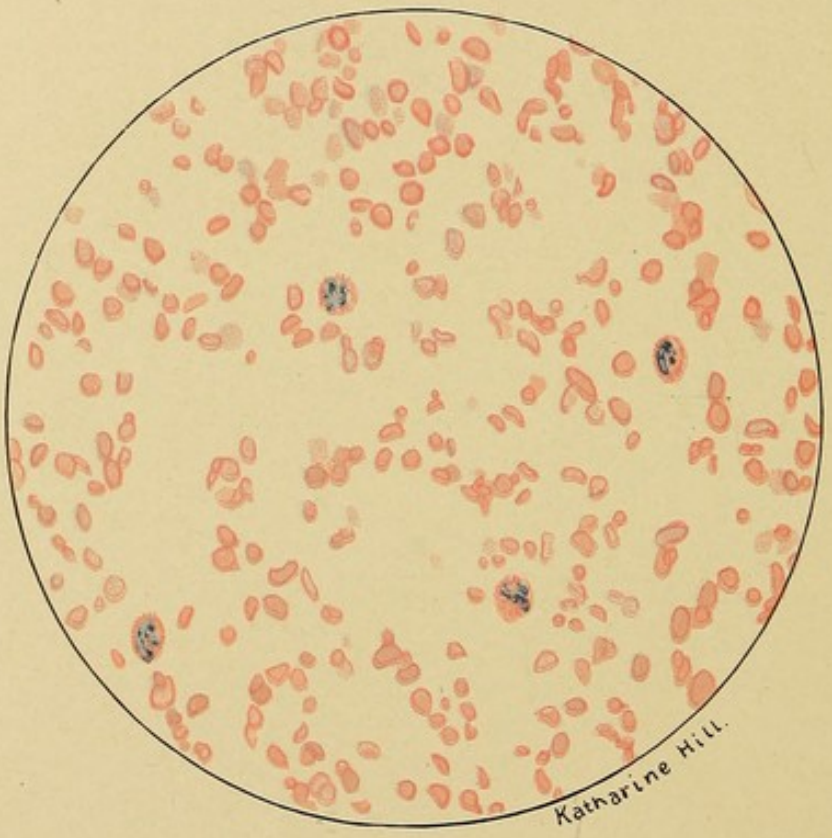
Primary Anemias.

(1). Simple Primary Anemia.

This form which has no demonstrable cause is difficultly separable from the secondary form as well as from certain other primary forms, such as primary pernicious anemia. It will probably be shown to be a true secondary anemia and must be sharply differentiated from the pernicious type from the point of prognosis. These cases are only recognizable when they are typical in form and are frequently not amenable to any form of treatment which we may institute. The rule, however, is that these forms of anemia yield more or less promptly to proper dietetic and therapeutic treatment. It is probable that the question of prognosis in these cases must depend largely upon the amount of degeneration of the red cells which occurs. In these cases we find that the diminution in the number of red cells is usually parallel to the reduction in the amount of hemoglobin, so that a high color index will usually obtain. More or less degeneration, as evidenced by the appearance of poikilocytes, normoblasts, Maragliano's polychromatophilia, etc., will be observed depending upon the severity of the case. The leucocytes are usually normal in number and in differential relations, while the blood plates are usually increased. The changes in the plasma in this primary anemia are not characteristic, although we do observe a diminution in the specific gravity which runs parallel to the oligochromemia.

(2). Chlorosis.

Chlorosis is a primary anemia occurring almost exclusively in girls about the age of puberty and characterized by a marked reduction in the amount of hemoglobin and a slight change in the number of red cells. Clinically, this state is evidenced by the appearance of a wax-like changing into a greenish



CHLOROTIC ANEMIA. (WRIGHT'S STAIN.)

tone of the skin and a sky-blue coloration of the cornea. Some cases may show a variety of colors of the skin. Certain changes are observed in the digestive and generative organs and certain general abnormalities are seen which are due to lessened production of blood-cells and to diminished oxidative and fermentative powers of the system. This form of primary anemia differs from all other forms in the absence of blood degeneration, as very rarely marked degenerative signs appear in the blood picture. The blood finding is not absolutely characteristic for this clinical entity, as it is simulated by many anemias of the secondary type. Clinically, this disease is so sharp that a diagnosis is often possible without a blood examination.

The chief characteristics of the blood in this condition are: (1) Reduction in the hemoglobin. This may run as low as 20 per cent., giving a color index of 0.5. Secondary anemias rarely reach such a low level. (2) Variations in the number and size of the red cell. The number of red cells is not reduced to a very great extent, the average being about 4,000,000, although counts as low as 1,000,000 have been reported. When these low counts do occur some complication should be suspected. Ordinarily the size of the cell is diminished, although we frequently find large "dropsical" cells which are due to absorption of fluid from the hypotonic plasma. These latter cells are usually few in number, the great majority of cells being smaller than the normal size. Poikilocytes and degenerated reds rarely occur except in the severer forms of this disease, while chromatophilia is usually regarded as a sign of active regeneration of the blood. When nucleated reds occur, which is a rare finding, they are practically always of the normoblastic type and rarely appear of the megaloblastic form.

The leucocytes in this condition are usually normal both in size and number and degenerative forms are rarely seen. It has been stated that the eosinophile cells are much increased in this condition, but the writer has found that their ratio is very rarely above the upper limit of the normal figure for these cells. The platelets are usually much increased and are usually large in size.

In chlorosis we find certain variations in the physical and chemical properties of the blood. The specific gravity is usually reduced in proportion to the reduction of the hemoglobin and may fall as low as 1028. The plasma is usually much diluted so that a condition of oligemia may be considered more or less characteristic of chlorosis. Whether or not a hydremia, as indirectly manifested by a diminution in the amount of albumin, obtains, is debatable.

Chlorosis belongs to the class of primary anemias and as such has no definite etiology. Various conditions such as hypoplasia of the arterial system, intestinal autointoxication, disturbances of the nervous system, such as a vasomotor neurosis, have been advanced to explain this condition, but none of them are tenable in all cases. A great importance must attach to hygienic conditions, poor food, and mental depression, especially about the age of puberty, as this disease is usually apparent under these conditions at this time

of life. A further factor which must necessarily bear upon the etiology of this condition is the defective power of absorption of iron compounds. Patients afflicted with chlorosis improve rapidly under the administration of iron, but not unless the digestive and absorptive powers improve at the same time. It does not concern us here as to the dynamics of the absorption and the effect of iron, but it should be accepted as an axiom that no therapeutic effects may be expected from iron unless the absorptive power is made better. Just exactly what the pathological conditions are which are accountable for the functional insufficiency of the bone-marrow is uncertain, but it must be recalled that gross as well as microscopic pathologic changes are not necessary to produce functional disturbance in any organ.

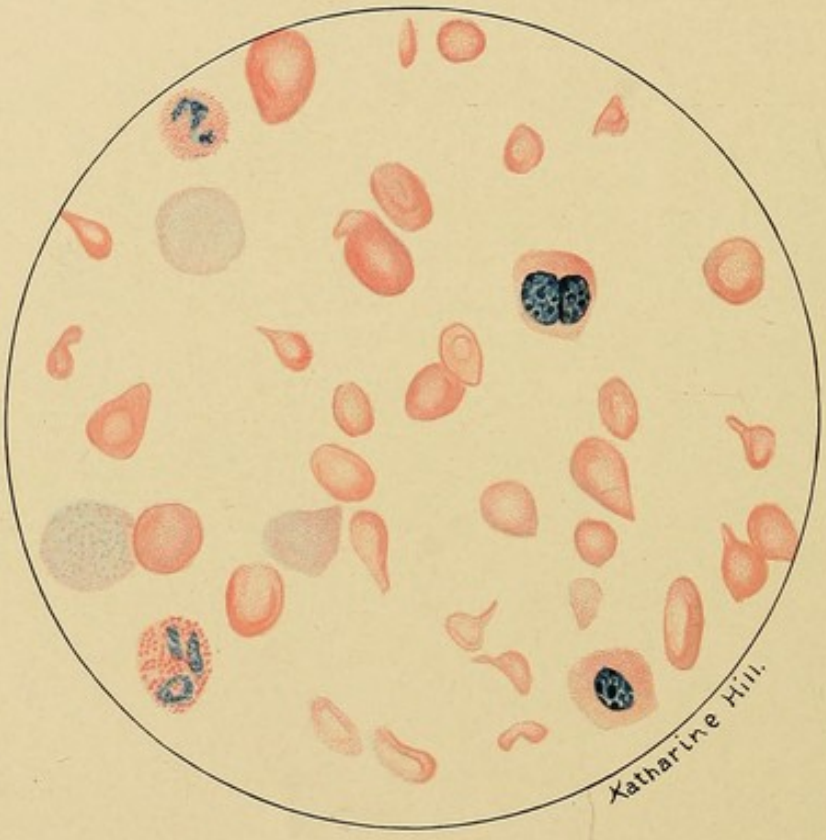
It should be remembered that we may have various types of chlorosis which show different prognostic characteristics: (1) Those in which the red cells are very slightly reduced (about 4,000,000), a marked diminution in the amount of hemoglobin, a low color index, and no change in the size and shape of the cell. Such cases usually recover promptly without showing any relapse. (2) Cases in which the red cells are below 4,000,000, which show a marked diminution in the amount of hemoglobin with a very low color index and which give very slight evidences of degenerative changes in the red cells. These cases are usually characterized by marked prostration, but usually recover more or less promptly although relapses frequently occur. (3) Cases in which the red cells are reduced as low as 2,500,000, a reduction in the hemoglobin with a very low color index, marked changes in the shape and size of the red cells. These cases respond slowly to treatment and have a bad prognosis (Ewing).

As these cases of chlorosis convalesce, we observe an increase in the number of red cells to a point somewhat above the normal and a later increase in the hemoglobin content of each individual cell. These changes are usually evident in from eight to ten days after institution of treatment, but as a rule a much longer time is necessary for any marked change to be observed in the number of the cells or in their hemoglobin content. The changes in the plasma are usually the first to appear and should be considered essential for the proper regeneration of the blood in chlorotic conditions.

This disease belongs in the group of curable conditions and usually has a good prognosis, but the susceptibility to intercurrent acute diseases during this period is much increased so that we should always bear in mind the probability of complications arising in the convalescent period.

(3). Progressive Pernicious Anemia (Biermer's Anemia).

This term is applied to a form of severe anemia which, in spite of all treatment, progresses steadily toward death. It results from defective hematogenesis and excessive hematolysis and is characterized by definite changes, both numerically and morphologically, in the red cells and by characteristic changes in the bone-marrow (Ewing). This condition has frequently been



BLOOD IN PERNICIOUS ANEMIA. (WRIGHT'S STAIN.)

described as the result of infection with certain intestinal parasites so that it cannot in all cases be considered a truly primary disease, although the larger majority of cases show no known etiology. According to Herter, certain anerobic bacteria, found in the large intestine, produce a substance of marked hemolytic power, which penetrates the intestinal wall and enters the portal circulation. In some cases of idiopathic purpura hemorrhagica the blood picture is that of pernicious anemia, but may be distinguished by the absence of megaloblastic change and by the prominence of hemorrhage. The blood picture in this disease is not absolutely characteristic, as certain forms of secondary anemia may show similar findings. The chief characteristics of the blood in primary pernicious anemia, as also in those severe types of secondary anemia which simulate this form, are (1) signs of rapid blood destruction, such as degenerated reds, endoglobular degenerations, polychromatophilia, increased iron compounds in the serum and corpuscles, an increase of iron in the liver and spleen; (2) poikilocytosis; (3) a high color index resulting from a marked diminution in the number of red cells and a correspondingly less degree of diminution in the amount of hemoglobin; (4) megaloblastic blood formation. This latter indicates a direct reversion to the embryonal type of blood formation, in which the presence of megaloblasts as direct precursors of megalocytes is observed. The red blood-cells in this condition are few in number being reduced to as low as 1,000,000 cells, counts of 500,000 having been observed without the patient suffering any marked inconvenience. Naegeli[1] reports a case showing 138,000 reds. This fact should be taken as evidence that the oligocythemia in pernicious anemia is not alone accountable for the symptomatology. This count may remain stationary, may show slight decrease, but usually progresses slowly until death ensues. The average diameter of the red cells is somewhat increased in pernicious anemia. While many of them may be normal in size and many very small, the cells average from 4 to 13 microns in diameter. A pernicious anemia is a distinct large cell anemia. A macrocytosis is much more characteristic of this disease than of any other, 70 per cent of the cells in these cases being of this type. Microcytosis is rare but may occur to such an extent that the average size of the red cell may be about normal. Poikilocytes are very common and often show extreme shapes and are frequently numerous in number. Polychromatophilic degeneration is very extensive in this form of anemia.

While both normoblasts and megaloblasts occur in large numbers in pernicious anemia, the megaloblasts usually outnumber the normoblasts. This megaloblastic increase may be considered pathognomonic of this anemia, as a preponderance of these large nucleated red cells does not occur, except in rare cases, in the other varieties of anemia. The hemoglobin may be markedly reduced showing values rarely above 50 per cent. and often as low as 10 per cent. The color index is always high; being more frequently above one than below. A low index is found, according to Ewing, in the chronic cases, while

[1] Leipzig, 1908

the acute forms are more frequently associated with a high index. If improvement occurs the index is always lowered, an increasing index denoting a bad prognosis.

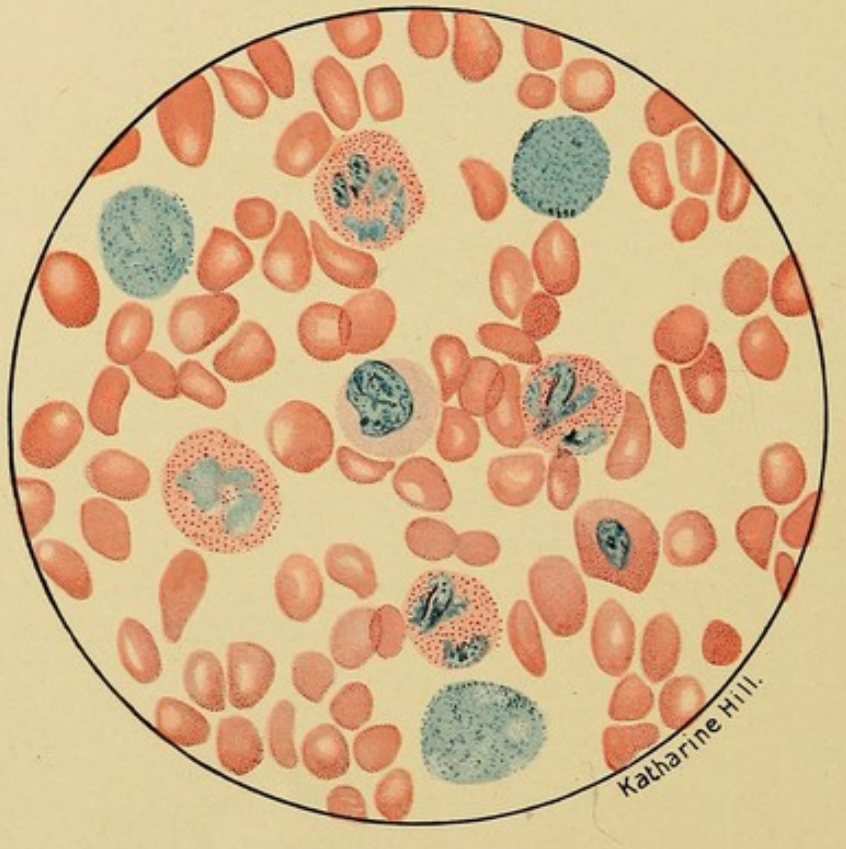
In this pernicious form of anemia we find the leucocytes practically always diminished in number, averaging about 4,000, a condition which almost never obtains in a secondary anemia. Their number usually runs parallel to that of the red cells, a leucocytosis pointing probably to a complication. As the case improves the neutrophile cells increase in number, the low leucocyte count being, as a rule, due to their diminution. The percentage of the nongranular mononuclear cells varies inversely to that of the granular form. As the disease progresses the percentage of the nongranular cells increases, while the granular cells are diminished. This disease shows, therefore, a high lymphocyte count which is relative and not real, being due to the diminution in number of the polymorphonuclear cells. The lymphocytes may constitute as high as 50 per cent. of the leucocytes, the eosinophiles may reach as high as 10 per cent., the myelocytes 2 per cent., while the mast cells may run as high as 3 per cent. Degenerations of all kinds are observed in the leucocytes, but nothing characteristic of pernicious anemia is found in the white cells.

The blood-platelets are largely decreased and may be as low as one-twenty-fifth of the normal number. Von Limbeck and Sahli claim that these cells are increased in number, but the usual finding is one of diminution.

(4). Splenic Anemia.

This is a form of chronic anemia characterized by idiopathic enlargement of the spleen without any involvement of the lymph nodes. A large number of conditions may be responsible for the anemia of the splenic type, so that a direct etiologic factor should be looked for in all cases. Among the conditions which may give rise to this type of anemia, we find gummata of the spleen, large round-cell sarcoma of the spleen, chronic splenitis of the malarial type, and splenomegaly associated with cirrhosis of the liver. This latter condition is known as Banti's disease and its etiology is uncertain.

While it is true that the splenic lesions do not differ essentially from certain stages of the lesions in ordinary pseudoleukemia, yet we do not find in this condition any involvement of the lymph-glands, although the spleen may be enormously enlarged. The blood picture in this condition is characterized by a relatively high red cell count, a marked reduction in the percentage of hemoglobin, and a consequent low color index. The white cells are rarely increased, a leucopenia being rather the rule. When we do find an increase in the number of white cells a relative lymphocytosis occurs usually associated with an increase in the number of basophiles. Poikilocytes and nucleated red cells are very uncommon while degeneration may occasionally be observed. Whether or not we have an increase or a decrease in the number of leucocytes, a relative lymphocytosis associated with enlarged spleen and no involvement of the lymph nodes must be considered characteristic of splenic anemia.



Blood in Leukanemia. (Wright's Stain.)

In Banti's disease we find an enormous increase in the size of the spleen and associated with this an extensive cirrhosis of the liver. Along with these factors we find a high-grade toxogenic protein decomposition, which is associated with very high values for the total nitrogen of the urine and of the output of purin bases. The number of erythrocytes diminishes corresponding to the degree of anemia, while the hemoglobin percentage is more markedly reduced. The leucocytes are either normal or more frequently diminished in number, a relative lymphocytosis existing as in the pure type of splenic anemia.

(5). **Anemia Infantum Pseudoleukemica.**

Von Jaksch[1] has described a rare form of anemia seen in children which is characterized by enlargement of the spleen, liver, and lymph nodes. The most striking points in this condition are the great diminution in the number of red cells, one case showing only 820,000; numerous nucleated red cells; diminution of hemoglobin; the leucocytes always increased in number, being from 20,000 to 50,000, as a rule, and displaying a remarkable variety of form and frequently attaining to unusual size. The morphological changes in the blood resemble both those seen in leukemia and in pernicious anemia, the disease passing either into one or the other of the previously mentioned conditions. The blood findings are not alone sufficient to warrant a diagnosis of infantile pseudoleukemia, but are significant when taken in conjunction with the clinical findings.

(6). **Leukanemia.**

This condition has been classed by some with the leukemias, as the blood findings are occasionally more prominent among the leucocytes, while by others it is classed with pernicious anemia owing to the frequent changes in the red cells. Von Leube considers this anemia a mixed form of pernicious anemia and of leukemia. Luce[2] regards this as a symptom of many conditions rather than an independent blood disease. In the majority of these cases the changes are more evident in the red cells, marked diminution in number (as low as 200,000 per cmm.) associated with extensive destruction of red cells with all irregular and unusual types of these cells being observed. The diminution in the amount of hemoglobin is great, but the color index is usually high just as we find it in pernicious anemia. The white cells show an extensive disturbance in the neutrophile and eosinophile blood picture with a large increase in the number of large lymphocytes. The number of white cells is usually increased, but not to as great an extent as in leukemia. The changes in the red cells and in the hemoglobin usually precede those in the white cells, so that the early stages may show us a typical picture of pernicious anemia, while later examinations may lead to the diagnosis of the mixed condition.

[1] Wien. klin. Woch., Bd. 2., 1889, S. 435 and 456.
[2] Deut. Archiv. f. klin. Med.. Bd. 77, 1900, S. 215.

(7). **Aplastic Anemia.**

Ehrlich[1] has reported a rapidly progressing anemia accompanied by hemorrhages into the mucous membranes, associated with hyperplasia of the bone-marrow, and not showing the ordinary changes in the blood which are supposed to accompany pernicious anemia. This type has been called aplastic anemia and has not been frequently reported.

The red cells are usually markedly reduced in number, being as low as 790,000 in a case reported by Wood. The hemoglobin may fall as low as 11 per cent. as reported by Muir, while the leucocytes are usually normal in number but showing much reduced percentages of the polynuclear neutrophiles. In this condition we find an enormous increase in the number of lymphocytes, the percentage being in Lipowski's case 93, the remaining 7 per cent. being neutrophiles. No nucleated reds have been found in the blood and only a very few in the marrow itself, which is fatty, almost white and contains few neutrophiles and no eosinophiles.

Secondary Anemia.

By secondary anemia we mean one in which etiological factors seem sufficient to explain the variations observed in the blood. The principal variation seems to be more directly observed in the reduction of the hemoglobin, although the number of red cells is coincidently reduced, but not to the same degree as is the hemoglobin. In the mild cases the color of the blood is but slightly paler than normal, but in secondary anemia of a severe type the color may resemble the watery drop observed in cases of typical pernicious anemia.

Cabot suggests a classification of the secondary anemias as follows: (1) Mild cases, showing a normal count of red cells, but having the hemoglobin diminished. (2) Moderate cases in which the count is normal, but the cells show signs of moderate degeneration, abnormal staining qualities, and a diminished tendency to rouleaux formation. (3) Severe cases, in which the count is not much reduced but in which the hemoglobin is very much lessened and the cells show marked qualitative and quantitative changes. (4) Very severe cases with a slightly lessened blood count, a marked diminution of the hemoglobin, and evidences of degeneration and destruction of the cells as well as evidence of regeneration. In some of the severer types of secondary anemia the blood picture may so closely resemble that of pernicious anemia that a diagnosis is possible only through the careful investigation of the etiology of the condition.

Blood Picture.

The general points observed in secondary anemia are as follows: A variable decrease in the amount of hemoglobin, a reduction in the number of red cells less in degree than the diminution of hemoglobin and a subnormal

[1] Charité-Annalen, Bd. 13, 1888, S. 300.

color index, which rarely reaches the low grade shown in chlorosis. The lowest color index is seen in those secondary anemias following cancer, severe hemorrhage, and gangrenous processes. The specific gravity of the blood is reduced corresponding to the degree of reduction of hemoglobin. The rapidity of coagulation is increased depending upon the grade of oligochromemia and of oligocythemia. The reduction in the number of red cells may be very marked as, for instance, in a case of von Limbeck the number was 306,000. The red cells show a lack of hemoglobin, frequently appearing as the pessary forms. Polychromatophilia is quite common, but bears no direct relation to the hemoglobin content of the cell. Only in the severer cases do we find poikilocytes, although anisocytes, especially microcytes, are frequently present, the larger "dropsical" cells being less common than in chlorosis. Nucleated erythrocytes are frequently seen in some cases, while in others even of severer grade of anemia they are absent. These nucleated reds are, as a rule, of the normoblastic variety, megaloblasts being exceedingly rare.

The leucocytes vary in number depending on the cause of the anemia, from a leucopenia, which is rare, to a leukemic condition. The increase in the number of leucocytes is more frequently in the polynuclear neutrophiles, the lymphocytes being rarely if ever increased, while the eosinophiles, although not increased, are usually at the upper limit of the normal value for these cells. In some cases of the severe chronic types of secondary anemia we may find a lymphocytosis, but this is rare. The blood-platelets are usually increased in number, in some cases being two and one-half times the normal values.

(1). Acute Hemorrhage.

The character of the anemia following an acute hemorrhage will depend upon the type of the hemorrhage, that is whether the loss of blood occurred suddenly and at one period, or whether slowly and at intervals. The loss of one-half of the total volume of blood at one time is usually fatal, as Panum has shown. If the loss be less following one large hemorrhage, regeneration takes place in from five to thirty days, depending upon the amount of blood lost. Regeneration is quickest in men between the ages of 20 and 40, slower in women, and slowest in children.

Immediately following a hemorrhage the blood picture will be normal qualitatively as there has been no time for the morphologic changes to take place. Shortly following the hemorrhage lymph pours into the blood to restore the volume and maintain the pressure, so that the blood count and the hemoglobin diminish usually to the same degree. As the formation of new cells goes on the color index decreases, as the new cells are, as a rule, deficient in hemoglobin. These cells are more or less easily degenerated, showing variation both in their shape and staining qualities. The number of cells reaches the normal much sooner than does the percentage of hemoglobin, so that we may find for weeks evidences of marked anemia.

The most frequent causes of acute hemorrhagic anemia are traumatism,

ectopic pregnancy, abortion, gastric and duodenal ulcers, uterine tumors, pulmonary tuberculosis, and hemorrhagic pancreatitis.

(2). **Chronic Hemorrhage.**

By a chronic hemorrhage we mean one in which repeated hemorrhages follow one another so closely that the blood has no time to regenerate before a second loss of blood occurs. This rules out of consideration those cases in which repeated hemorrhages occur but at sufficiently long intervals to permit of regeneration of the blood. This latter condition, although a chronic hemorrhage, gives the picture described under Acute Hemorrhage.

In the chronic hemorrhage we find the red cells markedly reduced, the hemoglobin very much diminished and usually a marked leucocytosis. The red cells are usually small and pale, show a low color index, and usually few nucleated forms, although the picture may rarely assume the pernicious type. Regeneration in this form of anemia is slow, as the blood-forming organs seem to lose their power of regenerating the blood after repeated hemorrhages.

The most frequent causes of chronic hemorrhage are scurvy, epistaxis, hemorrhoids, intestinal ulcers, gastric and other carcinomata, and intestinal parasites.

(3). **Inanition.**

In the discussion of the anemia of inanition, it must be remembered that other factors than starvation are necessary in its causation. That starvation alone will not cause anemia is shown from the examination of the blood of Cetti who fasted ten days. His blood showed about 6,000,000 red cells, a small diminution in the percentage of hemoglobin, and a leucocyte count of 4,200. Although the changes in the cells and the pigment of the blood are not marked following starvation, it is true that we have a loss of albumin of the plasma and a diminution in the total volume of blood. This is taken as evidence by Grawitz that a true anemia occurs. Such changes are not striking if the days of fasting are alternated with days of slight nourishment. It is not so much the quantity of food as the quality which is of importance in bringing about the anemic conditions. As is well known, the foods containing iron are the principal sources of the hemoglobin of the blood, and these are frequently, owing to disturbed gastric and intestinal functions, poorly digested and assimilated. Although the amount of iron contained in the ordinary food is sufficient under the best conditions to maintain the hemoglobin content of the blood, yet the methods of food preparation, as well as the abnormal methods of rapid eating, are important factors in the poor assimilation of the iron of the food.

The lack of sunlight as well as impure air are contributory factors in causing anemia through their influence upon the general body functions. No tissue, whether animal or plant, can flourish in air which does not have sufficient oxygen to support the combustion processes of the system. This statement needs no retraction in the case of anerobic bacteria, as it has been definitely shown that these organisms obtain the oxygen necessary for their develop-

ment from the culture media upon which they grow, although they are incapable of developing in an atmosphere of pure oxygen. Overwork, especially when associated with worry, has great influence upon all of the functions of the system. For this reason overwork has been credited with the power of producing anemia as well as many other serious systemic disturbances. However, it is rare to find an authentic case of anemia which can be traced directly to overwork without the mental influence of worry and the coincident nervous strain from this latter cause.

(4). Intestinal Parasites.

The anemia caused by intestinal parasites may be of such a severe grade as to resemble very closely the type of pernicious anemia. In many cases it is an impossibility to make a differential diagnosis between these types without the finding of an intestinal parasite which will clear up the diagnosis. In these cases the blood picture returns more or less quickly to normal after removal of the parasite in question, while in the pernicious anemia of unknown origin the progress of the disease is always toward a fatal termination. It is probable that the cause of the severe secondary anemia due to the presence of the intestinal parasite is a result of the toxic condition set up by the absorption of the hemolytic toxins elaborated by the parasite. A very severe anemia of the secondary type is frequently seen as a result of decomposition of the intestinal contents and in cases of chronic constipation in which the direct toxic agent is at present unknown. The most common intestinal parasites causing the severe types of secondary anemia are (1) uncinaria duodenale, (2) strongyloides intestinalis, and (3) bothriocephalus latus. The first of these causes an anemia which is very closely related to that shown by miners and tunnel diggers, and seems to be much more prevalent in the southern part of the United States, although it occurs in many different countries. The blood count may fall below one million red cells and the hemoglobin may be as low as 15 per cent., while all varieties of degenerative changes may be seen in the erythrocytes. In the anemia following infection with the bothriocephalus we find very marked similarity with the primary pernicious type. One-half to two-thirds of the nucleated reds in this variety may be of the megaloblastic type and yet may disappear within two to three weeks after the worm has been expelled. It is uncommon to find in secondary anemias caused by the parasites above mentioned any marked eosinophilia, which is so common in cases of infection with many of the other forms of intestinal parasites. The anemia of the severer type seems to prevent a chemotaxis toward eosinophile cells.

(5). Fever.

It is still very much of a question whether the blood in febrile cases shows the characteristics of a secondary anemia, as the result of the temperature increase. It has been shown that increased temperature, in itself, does not always produce anemia, although we do have marked destruction of the red

cells and a coincident loss in the amount of hemoglobin of the remaining cells. So great is the influence of the toxin of the febrile condition that it is highly probable that the anemia so frequently observed in febrile cases is due to a combination of causes, rather than to a specific effect of the increased temperature. The changes in the white cells in febrile cases are not always of the same character, the variations being dependent upon the specific causative factor of the fever. As the blood changes in the acute infectious fevers are of more or less importance, they will be discussed later under separate headings. The anemias which are secondary to both the acute and chronic infections are probably directly due to the influences of the toxins upon the blood and blood-forming organs. The condition of general nutrition as well as the state of digestion, especially in the chronic states, such as tuberculosis, leprosy, and syphilis, must be regarded as important factors in the causation of these secondary types.

(6). **Blood Poisons.**

There are a very large number of compounds which produce, when taken in toxic doses, very marked changes in the qualitative and quantitative composition of the blood. As is well known, iron compounds in therapeutic doses increase the amount of hemoglobin in the red cells and also increase the number of red cells up to a certain point. Many of the effects which are attributed to iron compounds may be due to the improved hygienic and dietetic conditions which usually prevail during the administration of these substances. Yet the therapeutic results following the administration of iron are such as to make it certain that a specific influence of this drug is present in anemic conditions, especially of the chlorotic type.

Many compounds produce a very marked secondary anemia, the most important of these being alcohol, opium, lead compounds, cocain, and acetanilid. While others, such as arsenic nitrobenzol, nitroglycerin, phenacetin, and poisonous mushrooms, cause dissolution of the red cells with marked hemoglobinemia. The anemia following the use of lead, either in toxic doses or after its slow absorption from constant contact with it in the arts, is of great practical importance. The causes of this lead anemia are rather complex. The lead compounds have a direct action on the red cells and on the blood-forming organs as well as upon the gastrointestinal tract and the eliminative organs. While the anemia shows no especial characteristics as regards the number of red cells and the amount of hemoglobin, yet the peculiar granular degeneration and the polychromatophilia are sufficient to differentiate this type from most of the other secondary anemias. The basophilic degenerations of the reds is more marked in lead anemia than in almost any other condition and usually runs parallel to the severity of the clinical symptoms of the case. In arsenical poisoning also we occasionally find a slight amount of granular degeneration of the red cells, but the hemoglobinemia in this latter condition will differentiate it from lead anemia.

(*B*). **Leukemia.**

Although acute forms occur, leukemia may be regarded as an essentially chronic condition which is characterized on the one hand by definite changes in the lymphatic and myeloid tissues of the body and, on the other hand, by certain peculiar changes in the number and relations of the various cellular constituents of the blood. These latter conditions must be regarded as purely symptomatic of the preceding states and not as the direct pathological condition in themselves. Leukemia has been classed as a primary anemia, although the changes in the blood are here more directly related to variations in the white cells than to characteristic changes in the red corpuscles; yet we do find a diminished red count as well as a diminution in the amount of hemoglobin in this condition. It is a disease marked by the constant presence in the blood of granular mononuclear or polynuclear cells, or an increase of the nongranular cells with round nuclei. While the leucocyte count is almost invariably increased to a marked extent, we find cases in which the number of cells is normal, but we find great deviations from the normal relations of these white cells.

While the tendency is becoming more and more general to regard this condition as a single entity, manifested by various blood pictures, yet we find the cells grouping themselves together in such definite ways that we are justified in dividing leukemia into three general types, with transitions from one to the other form. These types are (1) splenomyelogenous leukemia or "myelemia," (2) lymphatic leukemia or "lymphemia," (3) mixed leukemia. Each of these types shows a distinct blood picture which permits of the classification of the condition studied.

(1). **Splenomyelogenous Leukemia.**

This condition was formerly subdivided into two distinct types—the true splenic and the myeloid leukemia. However, practically none of the cases reported could be definitely classed under either one of these headings, as the blood picture was always referable to disturbance in both the spleen and marrow. This type of leukemia is characterized by a marked increase in all of the granular cells, especially of the neutrophile, eosinophile, and basophile types, while the nongranular cells are not so characteristically increased.

Gross Appearance.

The gross appearance of the blood is normal even though the leucocytes are increased to an enormous extent. In extreme cases it may appear pale and opaque and does not flow from a puncture as readily as normal blood. In making smears of such blood, the preparations appear granular and are not readily spread so that the future examination is rendered somewhat difficult.

Red Cells.

As a rule, these cells are diminished in number, but the oligocythemia is of a mild degree, the average count being about 3,000,000, although it may run

as low as 1,500,000. As a general rule, the red cells diminish in proportion to the increase in the number of white cells. Occasionally we find cases in which an oligocythemia persists with a normal or slightly increased leucocyte count. Such a condition might lead to the diagnosis of pernicious anemia, unless the differential count was carefully studied. The red cells are usually pale and of the chlorotic variety. Very little degeneration of the red cells is observed, microcytes and macrocytes are rare, but a few poikilocytes are seen in practically all cases. Polychromatophilia is more or less common and cells showing basophilic granulations appear with more or less frequency. Normoblasts are very common in this condition, yet their absence does not rule out the diagnosis of leukemia. Megaloblasts and gigantoblasts are frequently seen and are sometimes many in number, although they rarely if ever exceed the normoblasts in number.

Hemoglobin.

The hemoglobin is reduced to a somewhat greater extent than is the number of red cells, the color index being about 0.6 and the average percentage of hemoglobin about 40.

Leucocytes.

In this condition we find the leucocytes increased, as a rule, to a very marked degree, counts running as high as 750,000 having been seen by the writer. Osler gives as his average for the white count 298,700, while the average may vary to a slight extent at different periods of the day. Some of the cases show a uniformly high count, others a moderate count and a few others a low count of about 100,000 cells. It is this increase in the number of white cells which gives the blood its peculiar opacity in this condition and may make a diagnosis possible by mere inspection. In some cases of leukemia we find a normal count of white cells while in others the count may be similar to that of a simple leucocytosis. It is the differential count in combination with the large increase in cells which should be considered characteristic, rather than a simple increase in itself.

Differential Count.

Neutrophile Myelocytes.

These cells are large mononuclear cells with neutrophile granules. They are present in large numbers, averaging about 35 per cent. of all the leucocytes present. A diagnosis of leukemia is almost always possible when we have such an extreme neutrophile myelocytosis along with an extreme leucocytosis. These myelocytes appear in two forms: (1) the large myelocytes of Cornil, which may be as large as 30 microns in diameter and have a large, pale, eccentric nucleus which is poor in chromatin. These cells are seen only in splenomyelogenous leukemia and in some of the secondary leukemias of children and must be regarded as practically pathognomonic of this condition. (2) Small myelocytes about the size of the normal polynuclear leucocytes with



BLOOD IN SPLENO—MYELOGENOUS LEUKEMIA. (TRI-ACID STAIN.)

a centric, round nucleus staining deeply with the various aniline dyes. We find all gradations between these large and small myelocytes, sometimes observing a few which are about the size of the red cell. The granulations of these cells are sometimes numerous, but may be entirely lacking so that they may be indistinguishable from the large lymphocytes unless the pale quality of their nuclei is remembered. The degenerative changes in these myelocytes are few in number and are limited, as a rule, to the hydropic form usually seen in chlorosis.

Polynuclear Neutrophile Leucocytes.

These cells are relatively diminished, their average, according to Cabot, being about 46 per cent., although an absolute increase is present, amounting to as much as 60,000 to 75,000 cells. Marked variations in the size of these cells are common, some of them being very large, some very small and no definite relation existing between the numbers of the large and small cells. These variations in size of the polymorphonuclear cells are rarely if ever seen in ordinary leucocytosis. It is very common to find cells with irregularly-shaped nuclei and with more than one form of granule, which may vary in tint depending on the method of fixation. Marked degenerative changes in these cells are very common. Thus we find their stickiness is increased, their nuclei usually pale and frequently showing karyokinetic figures. All grades of variations in the granulations may be observed, the granular cytoplasm being occasionally replaced by a homogeneous highly refractive material.

Eosinophiles.

The eosinophile cells are usually much increased in the splenomyelogenous form of leukemia, but their percentage relations to the other leucocytic forms are practically normal. Their number may run from 3,000 to 100,000, the average absolute number being about 12,000, while their percentage is about five. The total number of these cells per cmm. greatly exceeds that found in any other condition so that we accept, with Ehrlich, such an increase as pathognomonic of splenomyelogenous leukemia. These cells appear in all modifications, some of them being very small, while some of them are very large. The eosinophile form of myelocyte occurs in large numbers, but never is as numerous as is the neutrophile myelocyte. We occasionally observe all forms of transition between the myelocyte and the eosinophile leucocyte, the eosinophile myelocytes occasionally forming the majority of the eosinophile cells. The granulations of these cells may be of uniform size and staining quality or there may be some basophile granulations among the eosinophiles while the granulations themselves may vary greatly in size. Ewing considers eosinophile myelocytes with granules of unequal size and density of stain as pathognomonic of myelocythemia.

Basophiles.

According to Ehrlich, we always find an increase in the number of mast cells in leukemia, their absolute increase being in some cases greater than

that of the eosinophiles and is always proportionately higher. This increase is so marked as to constitute a very reliable diagnostic feature of the blood. The number of basophiles may run as high as 140,000 (Taylor), while the percentage may vary from 5 to 47.

Lymphocytes.

The number and proportions of lymphocytes in the splenomyelogenous leukemia vary in different cases and at different times in the same case. As a rule, their percentage is reduced averaging about 10, while an absolute increase is usually present, this increase having no uniform relationship to the stage or character of the disease. These cells vary much in size, the large cells usually outnumbering the small ones. Large mononuclear cells with very faint cytoreticulum and vesicular nucleus occur in large numbers in this form of leukemia and seem to have no special significance, although they may be mistaken for the large lymphocyte or for the myelocyte. Degenerative changes are observed in both the small and large lymphocytes in leukemia; thus the nuclei of the small cells may become incurved and bilobed or even trilobed, while the cell body remains basophilic (Rieder).

Points in Diagnosis.

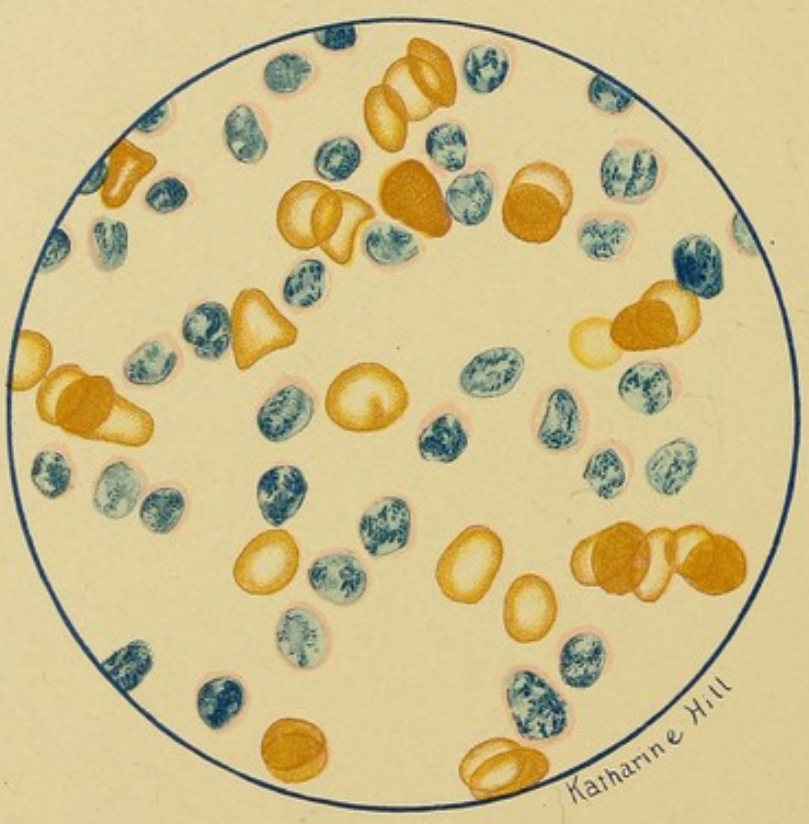
An excessive leucocytosis, with a large proportion of neutrophile myelocytes, the presence of a large number of eosinophile myelocytes and of basophile cells, the presence of atypical cells, both of the mononuclear and polynuclear variety, and large numbers of nucleated red cells are the chief characteristics. Any one of these points may fail for a time, but will usually be evident at some stage of the disease. The large size of the myelocyte is much more characteristic than the mere presence of these cells, so that we should confine our diagnosis to cases which show irregularity in size, staining qualities, and degenerative reactions of these cells rather than to those showing merely an increase. The presence of the large number of eosinophiles, especially those showing granules of irregular size and staining qualities, is a very important point to be remembered in the diagnosis of this condition.

(2). Lymphatic Leukemia (Lymphemia).

In this form of leukemia we observe a marked increase in the number of mononuclear nongranular cells in distinction from the previous form of leukemia in which the increase is rather in the number of the granular types. While a variety of the mononuclear nongranular cells are present, there is usually observed a predominance of one particular form and size, in some cases the small mononuclear cell with a narrow ragged rim of protoplasm, in others the cells of the large lymphocyte type, and in others large cells whose protoplasm is basophilic or in some cases distinctly acidophilic.

Red Blood-cells.

In this form of leukemia we find a much greater anemia than in the splenomyelogenous form, although we may observe a normal red count for



LYMPHATIC LEUKEMIA. (TRI-ACID STAIN.)

some time. The number of cells varies between 1,500,000 and 4,000,000, while the average percentage of hemoglobin is about 37 per cent. Nucleated red cells are rare in this condition, yet in the severer cases we may find them as numerous as in the splenomyelogenous type. All forms of degeneration noted under the previous type of leukemia are occasionally seen in this latter form.

Leucocytes.

The leucocytes are, as a rule, increased, the average being about 145,000, according to Osler. In this form we may find aleukemic periods which may last for a considerable period of time, the count usually rising just before death,

Differential Count.

According to Grawitz the cases of lymphatic leukemia may be divided into (1) those in which the increase of leucocytes is especially in the small mononuclear variety, (2) those showing an increase in the medium-sized cells with basophilic homogeneous protoplasm, and (3) those in which the cells which predominate are very large and usually degenerated. All these forms may occur together and may vary in the same case at different times. These mononuclear cells may constitute as high as 99 per cent. (Osler) of the total number of leucocytes. These leucocytes show in a very large number of cases much degeneration either of the protoplasm or of the nucleus, very few of the cells showing mitosis which is so common in the splenomyelogenous form. In this type of leukemia polymorphonuclear cells are rare, eosinophiles usually absent, and myelocytes and basophiles rarely if ever present. This type of leukemia is not easily amenable to diagnosis, especially in differentiating it from some cases of sarcoma in which the blood may show a similar picture.

In some cases we find a lymphatic leukemia with a considerable number of myelocytes both of the eosinophile and neutrophile type. This has led to the differentiation of a "mixed leukemia," which does not seem to be advisable as we may find myelocytes in the pure lymphatic type of this disease.

(3). **Acute Leukemia.**

This form of leukemia is characterized by its brief course (from six to eight weeks), by the severity of its symptoms, the frequency of the hemorrhagic diathesis, rapidly developing cachexia, and death. This condition occurs chiefly in young people and is usually of the lymphatic type, although a few cases of the myelogenous variety have been reported (Billings and Capps). In all cases the anemia is extreme, the red cells usually running below 1,000,000 and the hemoglobin as low as 10 per cent. There is no type of cell which is characteristic of this form, although the cells are much more uniform in size than in the chronic states of this disease. In some cases nearly all of the cells have a basophile protoplasm, while in others they show acidophilic properties Nucleated reds are usually rare, although they may be present in fairly large numbers. The drop in the count of red cells is usually sudden and denotes

rapid blood destruction. The leucocyte picture resembles closely that of acute infections.

(*C*). Pseudoleukemia.

Under the heading Pseudoleukemia have been grouped a great variety of diseases, which have in some cases the external appearances of the disease, such as the glandular swelling, splenic tumor and progressive cachexia without, however, showing the blood picture which is so characteristic of leukemia. On the other hand, we find conditions which have little in common with the clinical findings of leukemia and yet show a blood picture similar in some respects to that of leukemia. It is difficult to group all of these cases under one heading, as the blood-picture is not characteristic for any one of these conditions, but it seems wise to the writer to discuss certain of these states under the above heading of Pseudoleukemia.

(1). Hodgkin's Disease.

This condition, first described by Hodgkin in 1823, is characterized by chronically progressing cachexia with enlargement of the lymph-glands and spleen. It has been called, synonymously, lymphatic pseudoleukemia, lymphosarcoma, malignant lymphoma, and lymphatic anemia. It has nothing in common with the glandular tuberculosis, and gummatous lymphoma of syphilis, and may be sharply differentiated from these conditions. The blood characteristics in this disease are more particularly those of a true cachexia, the red cells showing a diminution in the number which may be as low as 2,200,000, but which is usually between 3,000,000 and 4,000,000. The more severe and pronounced the signs of anemia and cachexia the lower the number of cells, the number in these cases running as low as 1,500,000. Morphologically, the red cells show much less deviation from the normal than in other severe anemias, the average size of the cell being usually normal, degenerations of the red cells appearing only in the very severe conditions, microcytes and macrocytes, as well as nucleated erythrocytes, being very unusual except in the late stages. The hemoglobin content runs parallel to the number of red cells, being the lowest in those cases showing very low counts. The leucocytes are slightly increased, averaging about 12,000. This failure of a leucocythemia enables us to differentiate Hodgkin's disease from a true lymphemia. The differential count of the leucocytes may show a relative lymphocytosis, the relation of the lymphocytes to the polynuclear cells being as three to one instead of the normal one to three. Ehrlich and Pinkus consider this relative increase of lymphocytes characteristic of true pseudoleukemia in contradistinction to sarcomatous and other lymphomatous conditions. According to Grawitz, an increase in the leucocytes is associated with an unsatisfactory course of the disease, while a diminution in the number is observed as the disease progresses toward convalescence.

Cases have been reported which would seem to point to the transition

of Hodgkin's disease into a true leukemia, so that we may find irregular progress of a pseudoleukemia as an evidence of a transitional stage.

(2). **Tuberculosis of the Lymph-glands.**

Why this condition has been classed as a pseudoleukemia is questionable, as the blood picture shows nothing beyond a secondary anemia with cachexia or may show even a normal red and white count. A large increase in the number of leucocytes, which is so characteristic of leukemia, is rarely seen, although a true leukemia may arise in the course of a glandular tuberculosis. The differentiation of this condition should be based upon examination of an excised gland, which will show distinct tuberculous lesions and usually will contain the tubercle bacilli in demonstrable numbers. In other cases we may find a simple lymphoid hyperplasia without any distinct inflammatory changes and without demonstrable bacilli. Such nodes should be tested by inoculation experiments as advised by Ewing. Heredity plays a great rôle in the diagnosis of these tubercular conditions, while a scrofulous child should always be looked upon with suspicion. A splenic tumor appearing coincidently with the glandular swelling would speak rather against tuberculosis and in favor of a lymphatic pseudoleukemia. The diagnosis of this condition by examination of the blood alone is a practical impossibility. It is this type of case that is especially amenable to diagnosis by the use of the various tuberculin tests.

(3). **Lymphosarcoma.**

The lymphosarcomata usually run their course either as primary benign lymphomata or as the malignant sarcomata. The blood findings in these conditions show nothing beyond a slight anemia with nothing characteristic in the appearance of the white cells. The diagnosis must be based entirely on the examination of the excised gland or tumor.

(4). **Gummatous Lymphoma.**

An exact diagnosis of this syphilitic swelling of the lymphatic glands is at the present time a matter of more or less difficulty. The previous history, as well as other manifestations of syphilis, must be studied and a careful search made for the presence of the spirochæta pallida. It is to be said that these organisms have been frequently reported in lymphatic enlargements which seem to have no direct relationship to purely syphilitic conditions. The interpretation of one's findings is of the utmost import as artefacts, which commonly appear in preparations of broken-down glandular tissue, resemble very closely the spirochæte. The blood condition shows nothing characteristic and can have only incidental diagnostic importance. Application of the Wassermann serum test might throw much light on the diagnosis.

(II). **General Pathology.**

(*a*). **Blood Changes Following Surgical Intervention.**

Under this heading the writer will not attempt to take up all of the surgical

conditions, as the vast majority are not associated with any direct hematological characteristics.

As a rule, it may be said that pus formation anywhere in the system will cause a leucocytosis. The degree of this leucocytosis averages about twice the normal standard, but may greatly exceed this figure in individual cases. It must be said, however, that trivial as well as extensive pus formations may be accompanied by normal or even subnormal values for the leucocytes. This is due to the facts that small pus foci do not cause any systemic reaction and extensive pus formation may overcome the power of the system to react against the infection. If the pus cavity is well encapsulated, the absorption of the toxin from this focus is necessarily limited so that we may find no leucocytosis, even though a very large pus cavity is present. Thus we find in localized peritonitis following appendicitis that a leucocyte count may be normal, but may suddenly increase to a marked extent as an indication of the rupture of the cavity and an extension of the process. A general rule is that a distinct increase in the number of cells in excess of the figure originally obtained is indicative of the extension of pus formation and should put the surgeon on his guard as to operative interference.

If the absorption of toxic material from a focus of pus formation is great enough to produce a systemic effect upon a patient as manifested by a high leucocyte count, we find an anemia characterized by a marked diminution in the hemoglobin and the number of red cells, which is parallel in intensity to the severity of the poisoning.

Cases are frequently found in which a low leucocyte count prevails, although clinical evidence of severe sepsis is at hand. In these cases a differential count of the leucocytes should be made in all cases, as the low leucocyte count may throw one off his guard unless this precaution be taken. Should the polynuclear neutrophiles form 80 to 95 per cent. of the total leucocytes, a severe infection is indicated, even though the leucocyte count may be subnormal.

If a leucocyte count does not diminish or even return to normal within one to two days after operation, this should be taken as evidence that a reinfection has occurred or that the pus cavity has not been properly drained. Recourse must, therefore, be had to measures to overcome the secondary infection.

It has been found that administration of ether and chloroform causes a leucocytosis which usually lasts from 24 hours to 48 hours and which may interfere with the interpretation of a blood examination. This point must be borne in mind in the examination of blood of cases which have shown high leucocyte values prior to operative procedure. The differential count in such cases, however, will show only slight variations in the proportions of the different types of cells so that one may judge as to the cause of the secondary leucocytosis by determination of the polynuclear cells. The number of red cells as also the amount of hemoglobin are very markedly reduced in some cases following the administration of an anesthetic, so that a direct secondary

anemia may be the result. This fact has led to the refusal by many surgeons to resort to operative procedure in cases which show as low as 30 per cent. hemoglobin prior to operation.

As the blood changes in surgical conditions, which are not accompanied by pus formation, are not especially characteristic and are more especially associated with the diseases of the special organs, the writer will refer such discussions to other headings.

(*b*). Blood in Constitutional Diseases.

(1). Diabetes Mellitus.

In diabetes mellitus the changes in the cellular content of the blood are not very marked. The leucocytes may be subnormal, normal, or slightly increased, usually a very slight leucocytosis being observed. The amount of hemoglobin is usually reduced to a very slight extent, while the number of red cells may be slightly increased.

One of the most striking peculiarities of the blood in diabetes is the presence of an excess of fat (lipemia). Microscopic examination usually reveals the presence of the extracellular globules, but in some cases fat is present in sufficient amount to permit of demonstration by macroscopic methods. Glycogen has been found both in the plasma and in the leucocytes of diabetic blood and shows the peculiar characteristics discussed in the section on Iodophilia (p. 483). Certain peculiarities of the blood in diabetes have led to the introduction of tests supposed to be characteristic for such blood. These tests are occasionally of diagnostic value, although a diagnosis may, as a rule, be made even when these tests do or do not obtain.

Bremer's Test.—This test is based upon the fact that diabetic blood doses not stain to any appreciable extent when treated with certain aniline dyes. Thick smears of the blood are made upon slides and are fixed by dry heat. These smears are then covered with a 1 per cent. aqueous solution of Congo red and allowed to stain for a few minutes, after which they are rinsed in water and dried. Diabetic blood will be stained either a faint yellow or not at all, while normal blood will be colored a bright red. A 1 per cent. solution of Biebrich scarlet will stain the diabetic blood intensely while the normal blood is unstained. Bremer's original staining solution was made up as follows: Saturated watery solution of eosin and of methylene blue are mixed in equal proportion when a precipitate forms which is filtered, washed, dried and powdered. To 24 parts of this powder are added six of powdered methylene blue and one of eosin. One-twentieth of a gm. of this mixed powder is dissolved in 10 c.c. of 33 per cent. alcohol and forms the staining solution in which the specimens are stained for four minutes. The diabetic blood stained by this solution has a greenish tint, while normal blood is reddish violet.

Similar reactions have been found in normal blood, in leukemia, in exophthalmic goiter, in Hodgkin's disease, in multiple neuritis, and in some cachectic

conditions, but the reaction in all these cases is very inconstant. According to Bremer, cases of renal diabetes do not give this reaction, which is more characteristic of the pancreatic type of the disease.

Williamson's Test.—This test is performed as follows: Two drops of blood (20 cmm.) are dissolved in four drops (40 cmm.) of water and to the solution is added 1 c.c. of a 1 to 6,000 aqueous methylene blue solution. To this is added 4 drops (40 cmm.) of 6 per cent. solution of liquor potassæ and the test-tube placed in boiling water for four minutes. Diabetic blood will decolorize the solution, while normal blood leaves it a deep blue. The same effect is observed by using diabetic urine instead of the blood.

(2). **Gout.**

Little information is available as to the variations in the number of the red and white cells in the gouty condition. The recorded observations show that acute gout has little effect upon the number of red cells and upon the amount of hemoglobin, while chronic gout may be accompanied by an anemia which is more directly referable to causes other than the gouty condition itself. The leucocytes are usually increased in the acute attack, while in the chronic form the leucocytosis is of a more moderate grade. Neusser in working upon the blood of gouty patients found many polymorphonuclear leucocytes whose nuclei were surrounded by basophile granules—the so-called perinuclear basophile granules. These he considered diagnostic of the uric acid diathesis, but Futcher and Simon have found them in many other conditions, while Ehrlich regards them as artefacts.

While the chemistry of the blood in gout has been the subject of much investigation for a long period, nothing of diagnostic importance has been found in the chemical properties of the blood. The excess of uric acid has been shown not to be pathognomonic of gout, as it is present in many other conditions which are clinically far removed from the gouty state.

(3). **Addison's Disease.**

This disease is usually associated with a severe grade of anemia, the number of red cells being reported as low as 1,120,000 while the percentage of hemoglobin is coincidently reduced. The leucocytes are usually diminished, but may be slightly increased, while the relative proportions of the different cells are not markedly changed. As the disease progresses unfavorably, a relative lymphocytosis may be observed, but this is not always the case.

(4). **Rickets.**

The state of the blood in rickets varies with the extent and severity of the primary disease and is markedly affected by complications. Cases are reported in which the red cells are practically normal and in which the hemoglobin was only very slightly reduced. This disease is not associated with any special type of anemia, although the hyperemia of the bone-marrow might be expected to yield a large number of nucleated red cells. The usual condition of the

blood in rachitic children is of the type of simple chlorotic anemia. A grave secondary anemia is seen in many cases in which there are serious complications. The leucocytes in practically all cases of rickets are increased, but may not exceed the normal limits for the child. As is usual in the blood of a child, the lymphocytes are increased while the eosinophile cells are often relatively numerous. Just exactly what the cause of the leucocytosis in rickets is must be left to the realm of hypothesis, as neither the gastroenteritis nor the hyperplastic splenitis are sufficient to explain all cases.

(5). **Myxedema.**

In this disease we usually find an anemia of the secondary chlorotic type along with a moderate leucocytosis. The number of cells is usually somewhat diminished, although their size is usually increased. The proportion of the different leucocytes does not vary, although a few myelocytes are sometimes seen in the blood, which is numerically normal in other respects.

Although many studies of the chemistry of the blood in myxedema have been made, little knowledge has been forthcoming as to the exact cause of this toxemia. It is highly probable that the changed activity of the thyroid gland in this disease influences other organs to such an extent that slight anemia is the result and we should, therefore, assume that this anemia is more a secondary one than a primary result of thyroid insufficiency.

(*c*). **Blood in Acute Infectious Diseases.**

In a study of the blood in acute infectious diseases, we must remember that there are certain general rules which apply to all of such diseases with a very few exceptions. The interrelation of the fever with the resistance of the system in general is so close that it is hard to say in any given case whether certain changes in the blood are or are not due to the increased temperature in itself. There can be little doubt that a high temperature working over a considerable period of time will destroy large numbers of red cells and will bring on various changes in the blood which might be misinterpreted. A rather extensive concentration of the blood along with a progressive loss of albumin is observed in practically all conditions associated with fever. We should, therefore, expect to find the number of red cells increased at the outset of such condition, owing to the concentration of the fluid portion, while a distinct anemic condition may become evident only after the lapse of some time.

Fever in itself does not have a large influence upon the number of leucocytes, but it may be stated as a general rule that most infectious diseases (the exceptions being malaria, typhoid, tuberculosis, influenza, and measles) are associated with an increase in the number of white cells. This is not an invariable rule, as will be seen under the discussion of the various infectious types. The leucocytosis so commonly seen associated with infection is no doubt due to the action of the bacteria themselves and of their products upon the leucocytes. The positive chemotaxis which bacteria and thei

toxins exert upon the leucocytes is very marked. Moreover, as the blood becomes laden with these abnormal products, new leucocytes are thrown into the circulation to aid the old ones in their phagocytic action. Just what substances are accountable for the increased opsonic power of the serum in any specific infection must be left undecided for the present.

In the following discussion of the various infectious diseases, the writer will not attempt to give more than a brief discussion of the blood changes in these separate conditions, leaving associated questions to other writers.

(1). **Pneumonia.**

This disease is, hematologically, one of the most definitely characterized of all the infectious diseases. While the physical findings of this condition are largely local, the systemic effects are so marked that definite changes are seen in the blood both in the early and in the later stages of the infection. While showing so many characteristic findings in the blood, it is, at the same time, one of the most obscure in its relations to opsonins and to phagocytosis. Just why the virulent pneumococci should be so little capable of phagocytosis and why the addition of attenuated cultures of pneumococci or of extracts of virulent organisms should increase this phagocytic power of the leucocytes is at present very uncertain. We must, therefore, leave the discussion of this phase of pneumonia as well as of other infectious diseases to the section on Bacteriology of the Blood.

In pneumonia we find in the early stages that the blood is somewhat concentrated owing to the action of the increased temperature, while this concentration gradually increases as the exudate forms. Such a condition can lead only to an increased count of both the red and the white cells. As the disease progresses, the number of red cells shows a slight but a steady decline, which points not only to a destruction of the red cells, but to a diminished formation. This decrease in the number of red cells is occasionally seen only at the time of crisis, while in the cases in which the diminution is gradual the period of diminution does not usually exceed ten days. It will be seen, therefore, that the red cells in pneumonia may be about normal in number and at the same time an anemia may be present which becomes evident only after the disease has progressed for some time. The red cells are, as a rule, normal in appearance, but an occasional polychromatophilic cell may be seen, especially in the severe cases. Rarely normoblasts may be observed and very rarely megaloblasts.

The hemoglobin usually shows a greater reduction than does the number of red cells, which decrease may become evident only after the fever has subsided. A reduction in the hemoglobin below 60 per cent. is very unusual in pneumonia of the pure type.

Pneumonia is one condition in which the leucocyte count may prove of great value. A leucocytosis appears in most of the cases, being absent in very mild cases as well as in those very severe ones which show very feeble

resistance of the organism toward invasion by the pneumococci. Rieder's observations are very interesting on this subject. He has found that the leucocytosis of pneumonia is more a function of the intensity of the infection and the degree of resistance toward this infection than it is of the fever or of the extent of the exudate. A leucocytosis which may reach 12,000 to 20,000 appears very early in the course of this disease, and is usually evident at the time of the chill or immediately following. A steady increase is sometimes observed in the number of the white cells so that the maximum is usually reached just before the crisis. It is to be said that rapid extension of the disease as well as continuous high temperature may cause much irregularity in the count, cases being reported in which the leucocytes are high at first and steadily diminish as the patients grow worse. Others may show a sudden increase in the number of cells as the time of crisis is approached. According to Ewing, when the leucocytes increase slowly they usually diminish slowly and the disease defervesces by lysis. The degree of leucocytosis in pneumonia may reach any stage between the normal figure and that of 115,000 as reported by Laehr.

The increase in the number of white cells in pneumonia is largely referable to increase in the number of the polynuclear neutrophiles, these cells constituting as high as 97 per cent. of the total number of white cells. Associated with this polynuclear leucocytosis we have a marked diminution of lymphocytes, while the large mononuclear cells usually persist in considerable numbers. The eosinophile cells are always much reduced at the height of the leucocytosis, so that we may not be able to find a single one after very prolonged search. Cabot has reported a case in which the lymphocytes constitued 66 per cent. of a total of 94,600 white cells, but such a finding is not the usual one following infection with the pneumococcus. As defervescence goes on the polynuclear cells diminish very rapidly, while the lymphocytes increase and the large mononuclear leucocytes become very numerous, reaching as high as 16 per cent. in a case reported by Türk. The eosinophile cells usually appear about the time of crisis, but occasionally their appearance is postcritical. The degenerative changes seen in the leucocytes in pneumonia are in no way different from those observed in other infectious diseases.

It will be noted, from the above remarks, that the blood changes in pneumonia are those of a mild anemia associated with a high-grade polynuclear leucocytosis and a distinct lymphopenia. Too much reliance must not be placed on the blood finding in a case of pneumonia, owing to the fact that many abnormal cases are present and show results different from the above which can be interpreted only by a complete study of the complications in any special case. As a general rule, it is to be said that an absence of leucocytosis is strong negative evidence against pneumonia, while leucocytosis may serve to differentiate this condition from typhoid fever and malaria with which it might be confounded, especially where the systemic and cerebral symptoms are more pronounced than are the local pulmonary changes.

(2). **Typhoid Fever.**

This condition, like the preceding, is very often associated with such marked systemic disturbance that the local intestinal manifestations are obscured and the diagnosis rendered somewhat difficult. While typhoid fever is a purely infectious condition and subject to the ordinary laws governing such cases, yet we find for some reason that the invasion of the blood by the specific organism is not associated with a leucocytosis, although the febrile rise may be very marked. The study of the characteristic serum reaction for typhoid fever as well as of the bacteriology of the blood must be left for a later section, the discussion here being limited to the changes in the microscopic appearances of the blood.

In this condition we find the total volume of blood very much diminished in the early stages, both as a result of the high temperature and the diarrhea and repeated hemorrhages which may occur at any stage of the disease. This concentration of the blood leads to an initial polycythemia which may last for two or even three weeks. However the characteristic change in the red cells is one of a slight and gradual decline, the number of these cells not usually falling below 4,000,000. One must be on his guard in an examination of the blood in any infectious disease lest he conclude from a slightly increased count that no anemia is present. It is a very safe precaution, although rarely followed, to determine the specific gravity of the blood so that one may compute the degree of concentration. In this way he may be able to show that the number of cells normally present in such a concentrated blood is much higher than in the case of suspected typhoid fever which he is examining. At any rate, it is wise to make frequent determinations of both the number of cells and of the hemoglobin, as a reduction in both of these elements takes place gradually as in the cases reported by Thayer and Da Costa.

The reduction in the amount of hemoglobin is in some cases very marked, being as low as 50 per cent. in one case observed by the writer. The repeated hemorrhages which so often occur in typhoid fever may cause marked variation in this value. The morphological changes which occur in the red cells are not very marked, as a rule, but may be very severe in case much blood is lost by frequent hemorrhages. Polychromatophilia is more or less frequent and irregularity in the size of the red cells is occasionally seen, while nucleation of these cells along with formation of a few megaloblasts may occur in severe hemorrhagic cases.

The leucocytes are usually normal in number in the early stages of uncomplicated cases, but any complication may cause a polynuclear leucocytosis which may be confusing to the worker. The behavior of the leucocytes is very variable in the early stages, so that one should never rest his diagnosis of typhoid fever upon a negative leucocytosis. As the disease progresses, the leucocytes show a gradual reduction, especially in the number of the polynuclear cells, which reduction continues until the disease has reached its highest point, after which they slowly increase.

The more severe the action of the typhoid toxin the lower is the leucocyte count, the reduction not usually going below 2,500 cells, the majority of cases showing a count between 4,000 and 6,000. It is not an uncommon thing to observe a leucocytosis during the later course of typhoid fever and it is not always easy to explain such a condition. Marked hemorrhage, cold baths, severe diarrhea, and usually perforation may account for the increase in the number of cells, but we do not always find a leucocytosis after such conditions.

In typhoid fever we find quite characteristic changes in the relations of the various types of leucocytes. During the first week the neutrophile cells do not, as a rule, increase, while the lymphocytes, especially of the medium-size variety, show a progressive rise. The lymphocytes are rarely below 25 per cent. of the total number of cells and may reach as high as 65 to 70 per cent. The eosinophile cells are usually low in number during the febrile period, but reappear about the time of defervescense.

It will thus be seen that the characteristic changes in the blood of typhoid fever are a slight anemia, together with a leucopenia and a relative, and in some cases absolute, lymphocytosis. Such characteristics are the usual ones of typhoid fever, but it is to be remembered that suppurative processes do not always produce a leucocytosis, nor is a leucopenia always present in typhoid fever. For the relatively certain differentiation of typhoid fever from other diseases it is necessary to perform the Widal test, and even this may not always be present. A discussion of this test will be given in a later section.

(3). Scarlet Fever.

In this condition we find the usual effects of fever manifested in a slight concentration of the blood, leading in the early stages to a polycythemia. The usual change, however, in the red cells is one of gradual reduction in numbers to as low as 3,000,000 cells and occasionally much lower. The hemoglobin also suffers quite a diminution, so that the anemia may reach quite a severe grade.

The leucocytes in scarlet fever usually increase in number one or two days before the appearance of the rash and continue to increase until quite a marked leucocytosis, ranging from 10,000 to 50,000 cells, becomes evident at the time of the complete eruption. The degree of the leucocytosis corresponds as a rule with the severity of the disease and in some cases is diminished at the time of the eruption, but usually continues for several days and may even extend for weeks after the temperature has subsided.

The increase in the number of leucocytes is largely referable to the polynuclear cells, these constituting from 85 to 99 per cent. of the total number. The lymphocytes diminish in the early stages of the disease, but later rise to normal or slightly above normal figures. The eosinophile cells are usually normal or even subnormal at first, but steadily increase as the disease progresses and reach a degree of 10 to 20 per cent. in the second or third week, after which they slowly decline. These rules are not invariable in scarlet

fever, but a severe leucocytosis appearing prior to the period of eruption of an infectious fever is practically always suggestive of this disease. In some cases the polynuclear cells diminish about the end of the first week and the lymphocytes and eosinophiles rapidly increase, leading to a later secondary leucocytosis.

(4). **Measles.**

This condition shows in itself nothing particularly characteristic in the blood, but the absence of definite findings is of great importance in its differentiation from scarlet fever with which it might be confounded.

The red cells in this disease are not found to be greatly changed, although a slight reduction in their number is usual. A loss of hemoglobin is practically always noticed, so that we have a distinct anemia which will vary depending on the complications which may arise in the course of the disease.

The leucocytes are usually normal or slightly reduced in number at the outset of the disease, being the lowest at the height of the eruption when the figure may reach as low as 2,500 cells, returning to the normal within a few days after subsidence of fever. A complicating bronchitis may cause a moderate leucocytosis of 8,000 to 16,000 cells, but this should not lead one to a mistaken diagnosis, as the clinical symptoms of both scarlet fever and of measles should be well-established at the time of the complicating bronchitis. An eruptive fever in the second or third day of its course should be considered scarlet fever, or at least scarlatina, if a leucocytosis is present, while if the disease be measles the number of leucocytes will be normal or even subnormal in the absence of extensive bronchitis.

(5). **Variola.**

This condition is associated with more or less extensive destruction of the red cells. In the early stages of the disease the red count may be slightly above normal, owing to the concentrating effect of the fever, but later the red cells will show a sudden reduction. This reduction is especially noticeable in the cases associated with extensive pustulation, when the septic process has such a marked influence in destroying the red cells. The hemoglobin is usually reduced in degree parallel to the diminution in red cells, so that we may have an anemia ranging from the mild to severe type.

In most of the cases of small-pox we find a distinct leucocytosis which may run from 10,000 to 20,000 as a rule, but has reached as high as 41,000 in the severe cases. This leucocytosis begins with the appearance of the vesicle, increases as the exudate becomes purulent, and reaches its height when suppuration becomes extensive; that is, the degree of leucocytosis usually runs parallel to the severity of the septic process, the count returning gradually to normal as the suppuration subsides. The leucocytosis in small-pox is usually of the lymphocytic type, the number of these cells varying from 35 to 45 per cent. of the white cells. Associated with the increased lymphocyte value we find from 5 to 10 per cent. of the large mononuclear leucocytes and

usually an average of 3 per cent. of neutrophile myelocytes, in some cases these latter running as high as 16 per cent. Eosinophiles and basophiles are occasionally observed, especially in the hemorrhagic form of this disease.

It will thus be seen that small-pox causes a leucocytosis which may reach even the degrees given by scarlet fever, but the differential count as shown by the large percentage of lymphocytes in the former and the greatly increased number of polynuclear neutrophiles in the latter should make a mistake in diagnosis impossible. It is to be remembered that complications of true abscesses with the pustules of small-pox may increase the percentage of polynuclear cells in this disease, but never to such a degree as is shown in scarlet fever.

An examination of the blood of children, who have been vaccinated with small-pox virus, shows a distinct leucocytosis of the polynuclear type, reaching as high as 20,000 cells. This leucocytosis usually begins on the third or fourth day after inoculation and gradually subsides until the end of the period of vaccination. It is rather hard to understand why the leucocytosis in this condition should not take the same form as in the true small-pox, but no explanation is at present available.

The blood in cases of varicella seems to show the same characteristics as that of very mild cases of variola, or of vaccinia. The slight leucocytosis is usually of the polynuclear type, reaching a degree of 15,000. The large mononuclear cells which seem to play such an important rôle in the differential leucocyte picture of variola are for the most part absent in varicella, and myelocytes are practically never found in this condition.

(6). **Diphtheria.**

The number of red cells in diphtheria seems to be slightly increased, owing to the marked concentration of the blood in this condition. In practically all cases of diphtheria the number ranges from a high normal value to as high as 7,800,000 reported by Cuffer. As the disease progresses, especially after the temperature has fallen, the number of red cells is diminished and a coincident decrease in the percentage of hemoglobin is observed. This slight anemia is not evident in the early stages of the disease due, no doubt, to the abnormal concentration of the blood.

In this condition, like most infections, we find a leucocytosis ranging between 25,000 and 50,000, the higher the leucocytosis the more grave the prognosis. In one case reported by Felsenthal the leucocytes numbered 148,000, but this is very unusual, as the grade of leucocytosis is ordinarily proportional to the extent and depth of the membrane. The leucocytosis is usually of the polynuclear type, the lymphocytes being also slightly increased. In some cases a lymphocytosis of 60 per cent. (Ewing) has been observed, but this is not the usual finding. The eosinophile cells are reduced in number, but are relatively more numerous than in pneumonia. It is in diphtheria that we find quite marked degenerative changes in the leucocytes,

"the leucocyte shadows" and increased acidophile tendency of the neutrophile granules being especially worthy of mention.

(7). **Pertussis.**

The recent work of Barach[1] and others has so modified our ideas of the changes in the blood in pertussis that I can do no better than to give his summary upon these points:

"In the early stages of this disease there is a leucocytosis with increase of all the forms; then a small-cell lymphocytosis becomes conspicuous and continues to increase when the other forms have reached their limit. The large lymphocytes follow the course of the small ones, but they reach their greatest numbers after the small cells have reached theirs. During the stage of active lymphocytosis, bilobed, small lymphocytes are frequently seen as well as numerous degenerated large lymphocytes, especially the basket forms. Then comes the simultaneous falling of the leucocytosis and lymphocytosis, while the polynuclears begin to resume their normal proportion. A little later the mast cells are observed more frequently, and an occasional myelocyte may be seen. While the leucocytosis and lymphocytosis continue to fall by lysis, an eosinophilia is noted; this continues for a variable time, after which the blood formula resumes its normal proportions. During this entire cycle the transitionals seem unaffected.

"If we were to speak of the first and second half of the blood cycle in this disease, we would say that in the first half the lymphocytes are the prominent factors and in the second half the polynuclears and the eosinophiles.

"Clinically, leucocytosis is present at about the time the child first coughs; as the coughing goes on, the leucocytosis increases and the lymphocytosis becomes very marked. Churchill believes that a lymphocytosis exists in the prespasmodic stage and is of extreme importance in early diagnosis. The height of the leucocytosis is reached in the spasmodic stage, sometimes early, and sometimes in the latter part, the sickest children showing the highest grade of leucocytosis. About the time that a marked improvement is noted in the child the leucocytosis has decreased, the polynuclears have increased and the eosinophilia is present."

The degree of leucocytosis in this condition varies between 25,000 and 51,150.

(8). **Acute Rheumatism.**

In this disease we find that the red cells are quite markedly destroyed, causing, very frequently, a reduction of 2,500,000 cells. This reduction is not always evident in the early stages of the disease, as the blood becomes very much concentrated by the marked sweating which is such a prominent symptom of the disease. The hemoglobin suffers more than the red cells so that we may find the percentage of hemoglobin as low as 60 per cent. in this condition. This anemia is one of the characteristic signs of acute rheumatism

[1] Arch of Int. Med., vol. 1, 1908, p. 602.

and continues well on into convalescence, the hemoglobin not being as quickly restored as are the red cells. A largely increased formation of fibrin has been observed and may have some diagnostic importance.

The leucocytes are increased in proportion to the severity and acuteness of the disease, the grade being usually moderate and the type being polymorphonuclear. In the very mild cases we may find no leucocytosis and one reaching 20,000 or more is, according to Türk, always associated with complications. As the fever diminishes the leucocytes return to normal and are not as much affected by the subsequent attack as by the previous initial one. The eosinophile cells are absent only in the early stages, while they are present in moderate amounts later in the disease and show a distinct increase after defervescence (Loeffler).

It would be impossible in a work of this character to discuss in detail the blood changes in all diseases, whether infectious or noninfectious. The writer, therefore, has selected under the acute infectious diseases those which show the more characteristic changes in the blood and those in which an examination of the blood is more frequently called upon to aid in diagnosis. Many phases of these diseases have been left to the chapters on bacteriology of the blood, while those not taken up at all in this work must be looked for in the many books on general medicine.

(*d*). Blood in Chronic Infections.

(1). Tuberculosis.

The earlier studies of the blood of tubercular patients reveal the fact that the blood may show practically no changes which are comparable with the pallor of the skin and the degree of the emaciation of the subjects affected. An anemia is often seen of the very highest type, but usually one of moderate degree is present and even may not exist at all. The degree of anemia is independent of the localization of the disease, although pulmonary affections are more frequently associated with high-grade anemia than are other tubercular conditions. It is to be remembered that pulmonary tuberculosis is so frequently associated with extensive hemorrhage that one may not wonder at the severe anemia present, yet we find cases in which the regeneration is very rapid after severe hemoptysis. As a rule, a mild anemia of the chlorotic type prevails; that is, the count is practically normal with the hemoglobin somewhat reduced. Occasionally we find a slight lymphocytosis, especially of the smaller cells, and only when a secondary infection prevails does the leucocytosis take on the polynuclear type. The lymphocytosis in tuberculosis is so common that we usually find even in the sputum an excess of the small mononuclear cells in the pure tubercular affection of the lungs.

In tubercular infection of the meninges we practically always find a leucocytosis, but with this exception uncomplicated tuberculosis is not associated with an increase in the number of white cells. It is highly probable that the increased percentage of the mononuclear cells is more closely associated

with the poor nutrition which the tubercular patient shows than it is with any specific effect of the bacillus tuberculosis. Occasionally we find the eosinophile cells somewhat increased, especially in pulmonary conditions with cavity formation, but it must be remembered that a slight eosinophilia will obtain if tuberculin therapy is being used in such cases.

(2). **Syphilis.**

According to Becquerel and Rodier, a moderate grade of anemia is to be found in the majority of cases of syphilis, becoming more pronounced as the disease progresses. This anemia of syphilis is of the chlorotic type, but may increase until the pernicious type becomes established. As a rule, the reduction in the number of red cells is moderate, being rarely below 3,000,000 cells. The hemoglobin is usually relatively more decreased than are the cells, and the application of mercury in the treatment of this condition frequently lowers this percentage still further, establishing an anemia which is directly referable to the mercury. This reduction in the number of red cells and in hemoglobin becomes more marked as secondary symptoms appear, so that a diagnosis of an initial lesion becomes established by a later examination of the blood.

The increase in the number of leucocytes is largely limited to the secondary and tertiary stages of this disease, as the leucocytes usually remain normal up to the time of the eruptive stage. The increase in the secondary stage, which may reach as high as 20,000, is largely in the number of the small and large lymphocytes, but the eosinophile cells may be increased to as high as 5 per cent. in some cases. In very severe cases a progressive polynuclear leucocytosis is observed. As the tertiary stage comes on the leucocytosis usually persists, but the lymphocytosis becomes less distinct and constant through the increase in the number of polynuclear cells.

Justus' Test.

Justus has found, in studying the blood of patients suffering with florid syphilis, that injection or inunction of preparations of mercury cause a reduction in the percentage of hemoglobin of from 10 to 20 per cent. for a period of a few hours or days. After a certain time, varying with the general condition of the patient and the severity of the symptoms, the hemoglobin increases again. This test can hardly be considered diagnostic of syphilis, as the mercury salts all cause an anemia which may be directly traceable to their hemolytic action upon the red cells.

In the blood of patients suffering with congenital syphilis we always find a distinct anemia, associated with a slight leucocytosis, especially of the lymphocytic type. The red cells in this condition show many changes, such as polychromatophilia and nucleation, while the changes in the white cells often resemble the picture of a mild grade of leukemia. For a discussion of the causative factor, the spirochæta pallida, see the section on Parasitology (p. 552).

(3). **Leprosy.**

The blood in leprosy is quite different from that in either of the two previous conditions. The usual rule is a very slight reduction in the number of red cells, although cases have been reported with a red count of 1,900,000 and a blood picture of pernicious anemia. The hemoglobin does not seem to be reduced to any extent, the percentage usually being relatively higher than the number of red cells, so that a high color index almost invariably obtains. The leucocytes are rarely increased in number, being usually subnormal, with a relative increase in the number of lymphocytes, their percentage reaching as high as 47 per cent., according to Winiarski.

(4). **Carcinoma.**

Although discussed under the heading of Chronic Infections, carcinoma has at present no etiological relation to such conditions. This is one of the most important causes of anemia, owing to the frequent hemorrhages and the mechanical effects of the growth as well as to the unknown toxin, which may produce severe constitutional symptoms, even though the growth may have become latent. The anemia of malignant disease usually runs parallel to the progressive cachexia. The grade of anemia may vary, depending upon the location of the tumor, from a very mild chlorotic anemia to one with a perfect picture of pernicious anemia. It is natural to suppose that the more malignant the disease the greater will the blood changes be, so that we should expect to find the rapidly growing cancers which form numerous metastases usually associated with the more extreme blood picture. That this is not always the case is shown by the following statement of Emerson: "Our cases with rapidly developing metastases, with large nodules, are those with a slight chlorotic anemia; those which simulate pernicious anemia are more often those with few objective signs of cancer, an insignificant-looking little nodule." It is possible that this paradox may be explained by the fact that the development of the cancer is so rapid that the toxin has not had sufficient time to cause the blood changes which the more slowly developing growth may bring about. The changes in metabolism are much more marked in the slowly developing cancers than in the more rapidly growing ones, so that we might assume that the same rule applies to the blood changes as expressions of the general systemic disturbances.

When the anemia of cancer develops it is usually more severe than in any other chronic disease. The chief changes are at first in the size, shape, weight, and degeneration of the red blood-cells; later, as the cachexia develops the red cells are often as low as 2,500,000 or even as low as in pernicious anemia, 1,000,000. The hemoglobin is always reduced in amount, but is rarely as low as in chlorosis, the average, according to Cabot, being about 58 per cent. The hemoglobin value seems to be lower in cases of visceral cancer than in those of peripheral type. In a majority of the cases a moderate leucocytosis obtains which is never seen in the benign tumors unless these be complicated

by suppuration. This leucocytosis depends largely upon the amount of hemorrhage from the tumor and upon the position of the cancer. We find in carcinoma of the stomach and uterus, in which hemorrhages are very frequent, quite an extensive leucocytosis, while in cancer of the esophagus a leucopenia may obtain. The larger and faster the tumor grows the greater will be the degree of leucocytosis, a condition which is the reverse of that usually found in the case of the red cells. The leucocytosis of cancer is usually of the polynuclear type, but this may not be over 45 per cent., in which cases the lymphocytes are relatively increased. The eosinophiles are rarely as much diminished as in other conditions, but they are not always increased. Myelocytes are, perhaps, more frequently found in cancer than in the other types of anemia, excepting pernicious anemia and leukemia.

The degree of cachexia is often very extensive in cancer, but is not always so closely related as one would suppose to the changes in the blood. In those cases in which the cachexia is due to a combination of malnutrition with intoxication by the malignant toxin the blood changes are naturally very severe, but, as previously stated, the more fulminating types of cancer are not associated either with great cachexia or with severe changes in the blood. Cachexia, therefore, seems to be more a function of the chronicity of cancer than of its malignancy.

The changes in the blood in the numerous specific diseases of various organs show nothing characteristic in themselves. It is to be expected that in all chronic diseases of whatever organ, a slight anemia may be present owing to the effects of such disorders upon the general metabolism. These changes are, however, of the general type of simple anemia and are usually rapidly remedied by the application of the ordinary therapeutic agents. It is true that disease of the general organs causes changes in the composition of the fluid portions of the blood, which may bring about a secondary change in the cellular content. So closely correlated are the various organs that disorder of one necessarily brings about a disturbance in the normal functions of the others, so that we may find either the normal products of these organs lacking in the blood or abnormal products poured into it through the perverted metabolism induced by primary disease of the correlated organ. It would be useless to outline the blood changes in these general diseases, as nothing is found that could be used as a reliable diagnostic aid in case the blood were examined. It can only be by a careful study of the plasma that variations may prove of value. Our knowledge of such changes in the blood is, however, too meager to warrant any discussion.

(*e*). Effects of Splenectomy.

The effects of splenectomy are usually the combined results of severe hemorrhage, a preexisting anemia, of the loss of functions of this organ, and of intravenous infusion which has been performed following the operation (Ewing). In comparatively healthy subjects splenectomy has often been

performed without affecting the blood more than does any other abdominal operation. The most marked changes in the blood are seen in those cases in which the organ has been removed for rupture or idiopathic enlargement, the loss of blood and the shock of operation giving rise to a considerable degree of secondary anemia.

The red cells are frequently restored to normal in one to three months, but in some cases which progress less favorably the anemia may be more persistent. The restoration of hemoglobin does not take place, as a rule, as rapidly as does that of the red cells. Following the operation we usually observe a polynuclear leucocytosis which may run, as in one case observed by the writer, as high as 75,000 cells. This leucocytosis usually lasts from one to two months, but may persist for several months, in which case the polynuclear cells are replaced by lymphocytes. Eosinophilia usually develops early and has been observed in some cases two or three years after operation. In some cases, especially those suffering from extensive hemorrhage, a very profound anemia characterized by great diminution in the number of red cells, the presence of polychromatophilic and degenerated cells, nucleated red cells, and a high degree of leucocytosis is observed. The leucocytes in these cases may take on the picture of an acute leukemia, but this condition is transitory as the blood improves more or less rapidly. A leucocytosis or permanent lymphocytosis are probably the only specific effects of splenectomy.

V. Parasitology of the Blood.

(1). Malaria (Paludism; Hemamebiasis).

Malaria is a disease caused by the entrance of an animal parasite into the blood and its development within the red blood-corpuscle. This parasite was first studied by Laveran and belongs to the class of sporozoa. It was not until the recent work, especially of Grassi, Ross, and Nuttall, that we were enlightened as to the source of this invader. It is at present well established that the malarial parasite runs its sexual cycle (*sporogone*) within the body of the anopheles mosquito (Anopheles maculipennis). The old idea that malaria was an air-borne disease, the contagion arising from stagnant pools in swampy regions must now be replaced by the modern mosquito theory. It is true that the anopheles lays its eggs upon the surface of almost stagnant water and that the larvæ hatch in these places. The eggs are boat-like in shape (each separate, the groups being arranged in ribbons) and float upon the surface, while the larvæ lie just below the surface and are in a plane parallel with it. These facts have led to the adoption of the modern methods of prevention of malaria by covering the surface of such stagnant pools with oil, which prevents access of air to the larvæ and, in consequence, causes death.

In a discussion of malaria it must be remembered that there are several types of this disease, depending on (1) the kind of parasite causing the infection and (2) the period at which the various groups of the same parasite run their

asexual course in the host. We have, therefore, to discuss the three types of infecting organism, each of which is a protozoan form and is found in the red cells. The cycle of development of the tertian organism is approximately 48 hours, so that with a single infection paroxysms will occur on alternate days. With the quartan organism the cycle of development requires 72 hours, while with the estivo-autumnal form it is variable, running from 24 to 72 hours. It is, of course, possible to have infection with more than one form of parasite or with more than one series of the same parasite, so that we may have daily exacerbations through infection with any one of these three types of parasite.

In the study of the blood in malaria there are definite changes both in the red and white cells and in the hemoglobin. These, however, are of secondary importance to the study of the parasite causing the disease. We will, therefore, discuss the parasite before taking up the changes in the cellular composition of this tissue.

In an examination of the blood for the malarial parasite a study of the fresh specimen is always desirable when possible, as the peculiar ameboid movements of the parasite as well as the rapid oscillatory movements of its granules can, of course, not be seen in the fixed specimen. Moreover, the peculiar brassy tone of the red cell and the irregularity in shape and size of these cells may best be studied in the fresh specimen. The beginner, however, will find that a stained specimen will yield much more definite results, providing his staining technic is good than will a study of the fresh specimen, as the slight refractility both of the cell and of the parasite in the fresh specimen makes it difficult in every case to get the proper illumination of the specimen. In the hands of an expert the examination of fresh blood is practically all that is required for a diagnosis in the average case, and when the parasites are moderately numerous the beginner can scarcely make a mistake. It would seem, therefore, inadvisable to rest a diagnosis upon an examination of the fresh specimen in cases in which no organisms are found, but to control this examination by a careful study of a stained specimen in which one may frequently be surprised at the number of parasites to be seen, although a negative result has been observed in the unstained specimen. A word of caution, however, is necessary at this point. Frequently one observes in stained specimens many artefacts due to deposition of staining pigments upon the red cell, while in the fresh specimen areas of coagulation necrosis are not infrequently seen, so that the untrained observer may assume the presence of malarial organisms. For an absolute diagnosis of malaria it is necessary to find intracellular organisms, and not to be content with a single examination in doubtful cases.

Examination of Fresh Blood.

The technic of making a fresh specimen of suspected blood is the same as that previously outlined and consists in touching a perfectly clean cover-slip to a drop of blood and allowing the cover-slip to fall upon a clean glass slide. The quantity of blood should be rather small so that the red cells may

PLATE XXVIII



THE TERTIAN PARASITE.

1. *Normal erythrocyte.*
2, 3, 4, 5. *Intracellular hyaline forms.*
6, 7. *Young pigmented intracellular forms.* In 6 two distinct parasites inhabit the erythrocyte, the larger one being actively ameboid, as evidenced by the long tentacular process trailing from the main body of the organism. This ameboid tendency is still better illustrated in 7, by the ribbon-like design formed by the parasite. Note the delicacy of the pigment granules, and their tendency toward peripheral arrangement in 6, 7, and 8.
8. *Later developmental stage of 7.* In 7, 8, and 9 enlargement and pallor of the infected erythrocyte become conspicuous.
9. *Mature intracellular pigmented parasite.*
10, 11, 12. *Segmenting forms.* In 10 is shown the early stage of sporulation—the development of radial striations and peripheral indentations coincidentally with the swarming of the pigment toward the center of the parasite. The completion of this process is illustrated by 11 and 12.
13. *Large swollen extracellular form.* Note the coarse fused blocks of pigment. (Compare size with that of normal erythrocyte, 1.)
14. *Flagellate form.*
15. *Shrunken and fragmenting extracellular forms.*
16. *Vacuolation of an extracellular form.*

NOTE.—The original water-color drawings were made from fresh blood specimens, a Leitz $\frac{1}{12}$-inch oil-immersion objective and 4 ocular, with a Zeiss camera-lucida, being used.

(E. F. FABER, *fec.*)

(From *Da Costa's "Clinical Hematology."*)

be distinctly separated from one another. The examination is best made by the use of a 1/12 immersion lens.

(*a*). **The Tertian Organism (Hemameba vivax; Plasmodium vivax).** The youngest form of the tertian parasite as it appears in the red cell resembles very closely the spore of the parent rosette. It is a small, compact, colorless, nonpigmented disk (hyaline form) about 2 microns in diameter and shows an undulating outer rim of basophilic protoplasm which encloses a single large nuclear body which does not stain with methylene blue but shows a distinct chromatin stain with any of the modifications of the Romanowsky stain. This nuclear body is usually surrounded by a clear space which does not take the stain and which has been termed by Gautier "*the milky zone.*" The parasite has a very rapid ameboid movement and shows a great number of changes in shape and position. It sometimes assumes a typical ring-like form which is usually a little thicker at one point, from which the name "*signet ring*" has been given. Occasionally several of these rings may be seen within a single blood-cell. After about 12 hours the corpuscle increases slightly in size, becomes somewhat paler, but still has the sharp, smooth, round outline of the normal cell. At this stage the ameboid powers of the organism are very marked, so that many pseudopodia may be seen, connected to the larger part of the organism by very thread-like pale and rather indistinct bands of union. This gives the appearance of disconnected globules of protoplasm, which is very slightly refractile. At this period (12 hours) pigment (melanin) appears in the parasite in the form of very fine light brown granules which have a very rapid dancing motion and are clustered especially at the ends of the pseudopodia. The organism continues to increase in size and, at the same time, the host becomes somewhat larger, paler, but still round in outline. At the end of 24 hours the organism fills about one-third of the cell, is still ameboid and shows increased pigment, which is somewhat darker in color and is less actively motile, being distributed throughout the substance of the parasite. In this form one may occasionally see the nucleus as a globular body at the end of a pseudopod. In the last half of the cycle of development of the tertian organism the growth is much more rapid than in the first half, the parasite being fully developed within 40 hours. The cell at this time is about one and a half times its normal size and is so little refractile that its outline can scarcely be seen. The organism is from eight to ten microns in diameter, is round, and is even less refractile than is the corpuscle. The pigment is much more abundant at this time and is still evenly distributed throughout the organism. The next stage in the development of the organism is known as the *presegmenter stage.* The cell becomes practically invisible, the pigment collects in one or more irregular clumps throughout the organism, the granules moving in irregular lines. At this time the periphery of the organism shows slight crenation and refractive dots appear irregularly in the periphery of the organism. The line of demarcation between the presegmenter and the segmenter is

very slight. The corpuscle is now practically eliminated and the organism becomes more dense and highly refractile. The refractive dots which were visible in the presegmenter stage are now seen to be in the center of lines of separation which pass from the irregular crenated border down toward the center of the organism, thus marking off future segments, which are as a rule from 15 to 20 in number. As development proceeds the segments become more sharply defined until the clumps form into discrete circular masses with a distinctly refractile spot in the center. The pigment in these segment forms seems to be left in masses between the segments without any definite arrangement. Each segment now splits off from the mother segmenter cell and becomes free in the blood in the form of the original hyalin type which becomes attached to a red cell and soon enters it to pass through the various stages discussed. It is to be remembered that the hyalin forms do not modify their host, either in shape, color, or size, such changes being observed only at the time when the pigment first becomes evident.

The preceding is a concise description of the cycle of development within the cell (*asexual generation or schizogone*), but we occasionally find tertian forms other than hyaline which are extracellular. These seem to be of two types, the degeneration forms and the *gametocytes*, a cell capable of sexual development. The degeneration forms or, as they are sometimes called, extruded intracellulars, are sometimes the only ones seen in the specimen. These are parasites which have passed from the cell and have died, the organism sometimes appearing as if it had passed out through a very fine hole. If it be entirely extruded, the hemoglobin leaves the cell with it and only a shadow of the red corpuscle remains behind. However, this does not always occur, so that we meet with typical dumb-bell-shaped organisms in the plasma. If the blood be observed while this process of extrusion is going on, the pigment will still be extremely active but it gradually becomes quiet as the organism dies. The organism may break up into fragments forming several pigmented spherical masses or it may become deformed and vacuolated, constituting the so-called "sporulating" forms. The gametocytes are found at all times in the blood after the infection has been established for a few days. These are not in reality extracellular forms as one sees them in the stained specimens surrounded by the shell of the corpuscle. These gametocyctes are of two forms, the *macrogamete* or female cell and the *microgamete*, the male cell, which is one flagellum of the *microgametocyte* (parent male cell). The macrogametes are large organisms, pale, indistinct, and three or four times as large as the red cell. Some of them show no trace of the corpuscle, their pigment is abundant and exhibits very active movements. Their nucleus is about three and one-half microns in diameter and is sometimes seen in the fresh specimen; either its outline is distinct or its size and shape may be recognized, as it is the only portion of the parasite which is not invaded by the pigment granules. The function of these macrogametes seems to be to continue the life of the organism within the mosquito after the organism has become fertilized by the male element or microgamete. The

PLATE XXIX



THE QUARTAN PARASITE.

1. *Normal erythrocyte.*
2. *Intracellular hyaline form.*
3. *Young pigmented intracellular form.* Note the coarseness, dark color, and scantiness of the pigment granules.
4, 5, 6, 7. *Later developmental stages of 3.* Note the peripheral distribution of the pigment in all the parasites from 3 to 8. (Compare size and color of the erythrocytes in 5, 6, and 7 with 7, 8, and 9, Plate VI.)
8. *Mature intracellular form.* Note that the stroma of the erythrocyte is no longer demonstrable.
9, 10, 11. *Segmenting forms.* In 9 are shown the characteristic radiating lines of pigment. (Compare with 10, 11, and 12, Plate VI, and with 10, 11, and 12, Plate VIII.)
12. *Large swollen extracellular form.* (Compare with 13, Plate VI.)
13. *Flagellate form.* (Compare with 14, Plate VI.)
14. *Vacuolation of an extracellular form.*

(E. F. FABER, *fec.*)

(From *Da Costa's "Clinical Hematology."*)

microgametocyte is smaller than the macrogamete, being eight to ten microns in diameter. Its pigment is very active, but soon forms a circle around the center and becomes stationary. Occasionally this pigment may become even more active than before, the margin of the cell may undulate and several flagella protrude. These flagella are the microgametes and are two to three times the length of a red blood-cell and often contain pigment granules which enable them to be followed when they break loose from the parent cell. After these flagella separate, the parent cell is seen as a small cell with central motionless pigment. This process of flagellation is not seen in the fresh specimen, but occurs 15 or 20 minutes after the blood has been drawn, which would point to the fact that such a process does not occur within the body. Normally, this change takes place only in the stomach of the mosquito.

(*b*). **The Quartan Organism (Hemameba malariæ).**

This organism is much more rare than is the tertian. Its cycle of development requires 72 hours so that the normal paroxysm occurs every fourth day. If two groups are causing the infection there will be two days with paroxysms, one free day, and then two days of paroxysm following. If more than two groups are introduced we may have daily chills and fever, but only when the groups are large enough in number to cause a paroxysm. According to Ross, 250,000,000 organisms are necessary before a chill follows. The small hyaline forms of the quartan parasite are not distinguishable in the early stages from those of the tertian type, but are easily recognized at the time pigment appears, as the granules of the former are coarser, darker in color, and not so actively motile. As the parasite grows in size the corpuscle becomes smaller and stunted with an irregular crenated margin. The protoplasm of the organism is more refractile than that of the tertian organism and hence the outlines of the pseudopodia are more easily seen, although the parasite is less actively motile. In 24 hours the red cell is quite small, crenated, and distinctly brassy in color. The organism is round or oval, quite distinct, slightly ameboid, and its pigment blackish-brown in color and gathered at the periphery, especially on one side, thus differing from the tertian organism in which the pigment is scattered throughout the organism. The pigment granules have practically no motion at this stage of the development. As development proceeds the parasite fills from one-third to one-half of the cell, becomes rounder, and loses its ameboid power. The protoplasm is very distinct and highly refractile. During the third day only a rim of the cell is left and this usually takes on a dark, brassy tone. The organism is at this time full grown and is about seven microns in diameter. The pigment now passes from the periphery of the organism toward the center in definite radial lines, giving a wheel-like formation, with the pigment granules forming the spokes. Later the pigment collects in the center and we have the formation of the presegmenter form. Following this the organism becomes opaque, refractive dots appear in a single regular circle about the periphery, and crenations of the border appear with these dots as a

center. Lines of division start from these crenations and run to the center, forming from six to twelve segments, like the petals of a flower, giving rise to the name "daisy," "marguerite," or "rosette" form. These quartan segments are much more perfect than are the tertian forms, and later separate to form the hyaline types which take up the development as outlined above. This whole cycle of the development of the quartan organism takes place in the peripheral blood as it does in the tertian organism, but the number of segmenter forms is much more numerous in the quartan type than it is in the tertian form. This is probably due to the fact that a large number of the tertian forms accumulate in the internal organs.

The gamete forms are not as frequently seen as are those of the tertian organism. They are similar in appearance, but somewhat smaller than those of the tertian organism and give rise to flagellation in the same manner. The extracellular forms are occasionally found, but not so frequently as those of the tertian form.

The distinguishing marks between these two types or organisms may be summarized as follows: The cycle of development of the tertian organism is 48 hours, while that of the quartan is 72. The quartan organism is smaller, more refractile, less ameboid, and its pigment is coarser, darker, less motile, and more peripheral in position. The corpuscle infected by the quartan organism is smaller, shrunken, crenated, and more brassy. The presegmenter and segmenter stage are much more distinctive in the quartan than in the tertian type and more of the segment forms of the former are found in the peripheral blood, although the number of segments of the quartan type are less than those of the tertian parasite (Emerson).

(*c*). **The Estivo-autumnal Parasite (Plasmodium precox; Hematozoon falciparium.)**

This is the most dangerous type of malarial infection. The duration of the cycle of development varies from 24 to 72 hours. In infection with this organism the members of the same group do not always develop in the same unity, so that we may find at times an intermittent fever, but one which becomes more and more continuous. The hyaline forms are similar to those of the tertian and the quartan types, but are slightly smaller and assume the "signet-ring" form much more commonly and maintain it longer. This early form of the estivo-autumnal parasite is distinguishable from the tertian by the shrinkage of the red blood-cell and from the quartan parasite by the smaller dimensions. In some cases the rings do not show the thickening of one segment, but remain of a uniform fine caliber throughout. These rings which do not show the distinct "signet-ring" type nearly always present two nuclear bodies lying at opposite poles or close together. Occasionally these rings appear as if unfolded and stretched across the cell like a thread, the nuclei appearing at irregular intervals. These rings may at times lose their refractility and become ameboid. As the parasite develops, a slight amount

PLATE XXX



THE ESTIVO-AUTUMNAL PARASITE.

1. *Normal erythrocyte.*
2, 3. *Young hyaline ring-forms.*
4, 5, 6. *Intracellular hyaline forms.* In 4 the parasite appears as an irregularly shaped disc with a thinned-out central area. In 5 and 6 its ameboid properties are obvious.
7. *Young pigmented intracellular form.* Note the extreme delicacy and small number of the pigment granules. (Compare with 6, Plate VI, and with 3, Plate VII.)
8, 9. *Later developmental stages of 7.*
10, 11, 12. *Segmenting forms.*
13, 14. *Crescentic forms at early stages of their development.*
15, 16, 17, 18, 19. *Crescentic forms.* In 15 and 19 a distinct "bib" of the erythrocyte is visible. Vacuolation of a crescent is shown in 18, and polar arrangement of the pigment in 17.
20. *Oval form.*
21, 22. *Spherical forms.*
23. *Flagellate form.*
24. *Vacuolation and deformity of a spherical form.*
25. *Vacuolated leucocyte apparently enclosing a dwarfed and shrunken crescent.*
26. *Remains of a shrunken spherical form.*

(E. F. FABER, *fec.*)

(From *Da Costa's "Clinical Hematology."*)

of pigment appears, usually seen as one or two granules which are motionless, as a rule, and are located at the periphery of the parasite or at the inner edge of the biconcavity. The cell is very commonly much shrunken, crenated, and brassy, even in the early stages. Some cells, which do not contain parasites, show the same injurious effects of the organism. The parasite at this time occupies about one-fifth of the cell. The infected cells now usually disappear from the circulation and continue their development in the lymph-glands, especially in the spleen. In some cases, however, the parasite does continue its development in the peripheral blood, but this is rare. In such blood or in that obtained from the spleen, the pigment appears much increased and seems to be rather coarse and dark in color, thus resembling very closely the quartan organism at this stage. It seems to be a general rule that the more malignant the type of estivo-autumnal malaria, the fewer older forms are seen in the peripheral blood, although numerous young parasites are present. In some cases the hemoglobin becomes concentrated around the parasite, leaving an almost colorless ring at the periphery of the cell. The cycle of development in the internal organs seems to take place within the macrophages, which are best studied in the fresh specimen. The parasite develops to about five microns in size, which is about half the size of the cell, and when full-grown has its pigment all in the center, never diffusely scattered as in the tertian organism or peripherally located as in the quartan type. This form is rarely seen in the peripheral circulation. The segmenters vary in size from 2 1/2 to 5 microns in diameter, the process of segmentation being similar to that of the other organisms giving rise to the formation of 15 or 16 very small segments.

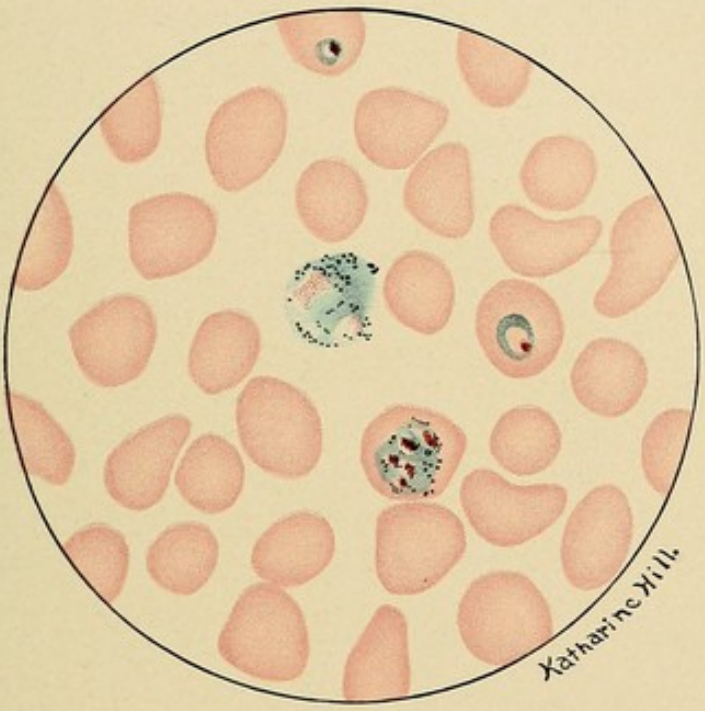
Certain characteristic forms of this type of malaria appear in the peripheral blood from about the seventh day of infection and in the internal organs as early as the fifth day. These forms are known as the *crescents and the ovoids*. The crescents are slightly longer than the red blood-cells and show a distinctly crescentic shape with rounded ends, although irregular forms are at times observed. They are very refractile and usually show a fringe of the degenerated red blood-cell, which is more abundant in the concavity of the crescent and forms the so-called "bib." The pigment is large in amount and is massed at the center of the crescent, occasionally in the form of a sheaf or ring. The granules are usually coarse and rod-shaped. These crescents very frequently change their shapes, becoming oval, dumbbell-shaped, or circular and then may resume their original crescentic form. In the circular types no trace of the corpuscles is seen and the protoplasm is not as distinctly refractive as is that of the crescent. In this form of malaria we also find pigmented leucocytes, both the polynuclear neutrophiles and the large mononuclears assuming this function. In these phagocytic cells one may see masses of pigment or even parasites, especially the segmenting and flagellating forms. These pigmented cells are also seen in the other form of malaria, but only just after the chill, while in the estivo-autumnal form they may occur at any time during the infection.

Examination of Stained Specimens.

The technic of making preparations of malarial blood for staining is practically the same as that outlined previously. Precaution must be taken to make thin smears so that the parasite may be brought out more clearly. The stains to be used will depend largely on the experience of the worker, but the writer would recommend the thionin and Nocht stains above the others, although the Wright and Giemsa stains will frequently give beautiful pictures. Stains which have been kept for some time are not always reliable, so that it is well to have fresh specimens of the stain on hand for use. If the blood has been kept for some time before staining a diffuse plasma staining with methylene blue will be observed.

The Tertian Parasite.

The young hyaline form consists of a mass of blue protoplasm usually grouped in ring-form with a mass of reddish-violet stained chromatin, usually situated at the thinner portion of the ring and extending for a large part within the clear achromatic or vesicular part of the parasite. These hyalines are two to three microns in diameter. There is some discussion as to what portion of the parasite the name nucleus should be applied. Some give this term only to the chromatin staining part, while others include both the chromatin and achromatic portion. However this may be, it is necessary for the recognition of the tertian organism that the blue protoplasm and the red chromatin be both observed. There are frequently artefacts in the blood which resemble very closely these hyaline forms so that the worker must be constantly on his guard. Such artefacts are the Maragliano degenerations so commonly seen in red blood-cells and also the cases in which blood-platelets lie upon a red blood-cell. Any structure which lies upon a red blood-corpuscle appears surrounded by a colorless zone, while the true malarial ring is in direct contact with the hemoglobin of the cell. Moreover, such artefacts will not show the chromatin staining portion which is so characteristic of the hyaline ring. In the specimens examined at the end of 24 hours, one will observe that the achromatic area has become somewhat larger, while the chromatin portion seems to be grouped in more irregular masses, some cells appearing to have several nuclei. In the full-grown parasite the chromatin breaks up into a cluster of fine granules which is scattered diffusely through the cell in the form of strands and masses. These chromatin clumps separate into from 15 to 20 dense round masses, around which the protoplasm collects with them as a center. The protoplasm at this stage is distinctly achromatic and is always so in the segmenting cell. The distinct, achromatic, milky zone surrounds each segmentary chromatin clump, while the general protoplasm shows a diffuse faintly basic staining. The pigment which must not be confused with the chromatin is pushed toward the periphery and, after segmentation is complete, collects in masses near the center. It is to be recalled that at the time the pigment collects in the center in the fresh specimen there is no distinct evidence of



TERTIAN MALARIAL PARASITE. (WRIGHT'S STAIN.)

segmentation, although this segmentation shows quite distinctly in the stained specimen.

The sexual development of the parasite is easily followed in some cases in the stained specimen. According to Stephens and Christophers the young gamete is characterized by the position of the chromatin as it lies in the center of the vacuole instead of at the edge. During the development the gamete is occasionally filled with basophile particles which are known as Plehn's karyochromatophilic granules or Schügner's granules. The full-grown macrogamete contains an abundance of protoplasm which stains a deep blue and a small amount of chromatin in a compact mass, which is peripherally placed and surrounded by a thin vacuole-like area. The pigment of these female cells is uniformly distributed throughout the cell and the inclosing red blood-cell can be seen only with difficulty. The chromatin is much more voluminous in the microgametocytes, but is looser and centrally placed in a large achromatic zone arranged in the form of a band which stretches clear across the cell. The protoplasm is in the form of a ring around the nucleus and stains more of a grayish-green color than does the bluish protoplasm of the macrogamete.

The Quartan Parasite.

The structure of the quartan parasite resembles very closely that of the tertian form, but in the hyaline type the chromatin mass is less distinct and is in the form of an irregular clump of granules in the older forms, while in the younger a cluster of fine granules without any distinct achromatic zone is seen. As development proceeds the parasite generally takes a form extending across the cell and usually occupies the larger portion of the red cell which has become shrunken and irregular in shape. The segment forms are much more distinct in the quartan type than in the tertian and show much more regular and geometric lines of cleavage with the chromatin exactly in the center of the crenated surface. The pigment granules are coarser and much more distinct than in the tertian form and are more peripherally located.

The Estivo-autumnal Parasite.

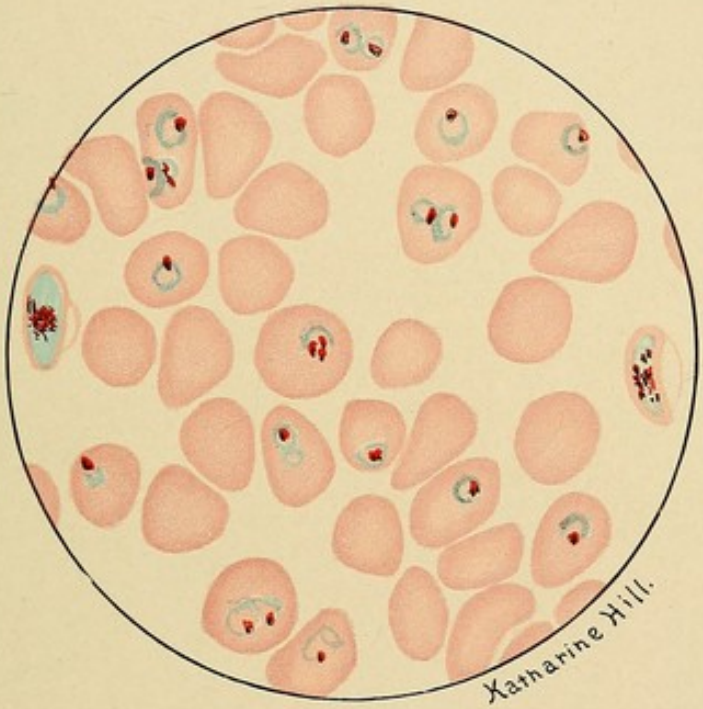
The hyaline forms in this type show the chromatin in two or more masses or filaments. The protoplasm is scantier than in the other forms and remains so throughout the cycle of development of this parasite. A very characteristic appearance of the hyaline rings of this type seems to be the thickening of the protoplasmic layer opposite the chromatin mass. The gamete forms are distinctly spherical, being of the same thickness all the way round. Their nucleus forms a portion of the ring, but does not project as in the schizonts (the asexual parasites). The red blood-cells in these sexual types usually show no coarse granular stippling. The crescent forms, which are characteristic of this type of malaria, show the chromatin in a loose network which occupies the larger portion of the cell, has little blue staining protoplasm, and has its pigment scattered throughout its body. This is the male form and is somewhat kidney-shaped and is shorter and broader than the female type. The female

crescent is longer and narrower, its chromatin more or less compact and centrally located, its pigment in a ring around the nucleus or in a clump at the center, while its protoplasm is more or less extensive and takes a distinct bluish tinge. We also find two types of the circular form. The microgametocyte is smaller than the red cell, distinctly spherical in shape, with its chromatin in the center in a large irregular mass, or in several dense masses near the periphery. These masses containing chromatin material are later extruded and form the flagella or microgametes. The macrogamete is two or three times the size of the microgametocyte, is often of a triangular shape, and has abundant blue-staining protoplasm. The chromatin is in a single mass at the periphery and is surrounded by a circle of pigment.

The examination of the stained specimens does not give as great an opportunity for study of the developmental cycle of these parasites as does the examination of a fresh specimen. The conditions found in the fresh blood resemble more nearly those found in the stomach of the mosquito than in the circulating blood, so that many pictures seen in the fresh specimen are practically never found in the stained slide. It is further to be said that we do not always find malarial organisms either in the fresh or stained specimen, although the patient may be at the time suffering from malaria. It may be stated, as a rule, that in all well-marked initial attacks of malarial fever the parasite may be found in the blood if it be examined within 18 hours of the chill. The energetic use of quinin has so much influence upon the ameboid types of the parasites, that the blood may fail to show any of these organisms, although the patient may die from the effect of the infection. It is safe, however, to state that there is practically no case of malaria in which parasites may not be found in the blood, if frequent and repeated examinations are made. It is, therefore, absolutely necessary to make many examinations of the blood, both in the fresh and stained state, before a negative diagnosis can be sustained.

Development of the Organism Within the Mosquito (Sporogony).

The cycle of development of the malarial organism has been more closely followed in the mosquito in the case of the estivo-autumnal parasite. For any development to occur within the body of the mosquito it is necessary that the macrogamete become fertilized, so that the course in the mosquito is one of sexual development. The microgametocytes throw out their flagella (microgametes) and the macrogamete ripens in the stomach of the mosquito by casting off karysomes (polar bodies consisting of chromatin), and in so doing causes the formation of a slight mound at one portion of the organism through which the free flagellum enters. This process occurs in from one to one and a half hours after the mosquito has bitten a patient infected with malaria. The nuclear material of the macrogamete and microgamete then unite. The cell then forms a distinct motile spindle shape called the *vermiculus or oökinet.* The size of this fertilized macrogamete is from 20 microns up and may be found in



ESTIVO—AUTUMNAL PARASITE. (WRIGHT'S STAIN)

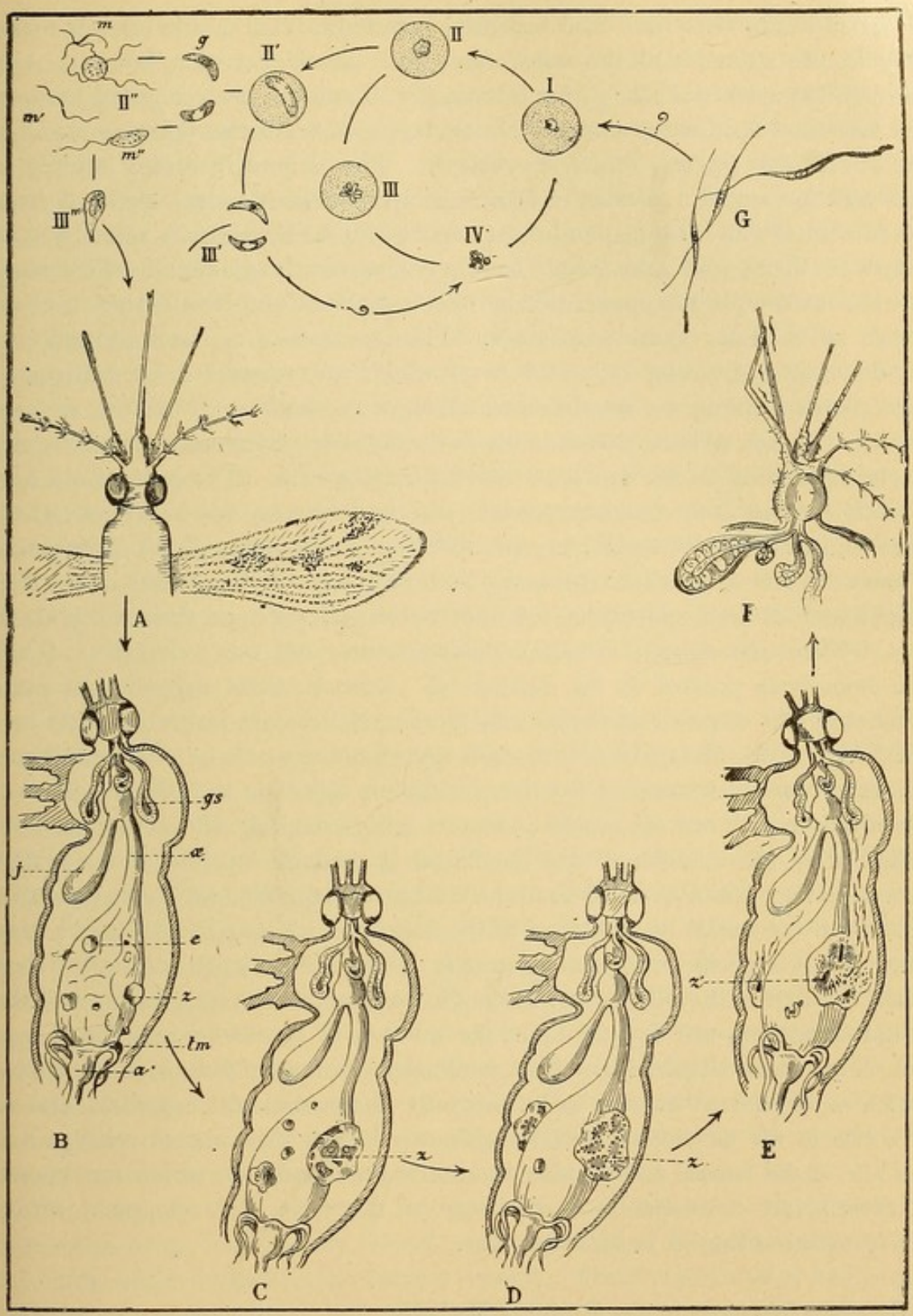


FIG. 144.—Cycles of the Malarial Parasite. (*Deguy et Guillaumin.*)

I, Beginning of the development of the parasite as the ameboid intra-cellular body. *I*, *II*, *III*, and *IV* represent the endoglobular cycle or schizogony. *I*, *II*, *II'*, *II"*, *III"*, and *III'* represent the extra-cellular cycle or sporogony. In *II'*, there are two free gametes (*g*), one microgametocyte (*m*), one microgamete (*m'*), and the union of a macrogamete and a micro amete (*m"*). *III'*, free gametes; *III"*, fertilized macrogamete, taken up by the mosquito, *A*. In *B*, *C*, *D* and *E* it becomes encysted in the gastric musculature forming the zygotes (*z* and *z'*). In *E*, the sporozoits are formed and in *F* they are thrown out by the saliva of the mosquito. In *G*, these are free to enter the cell forming the ameboid bodies.

about 48 hours after the blood has been ingested. This motile form is found only in the stomach of the mosquito. The vermiculus then bores its way through the epithelial cells of the intestinal wall and becomes encysted between the intestinal epithelium and the elastic layer, which forms the membrane of the spore cyst (*zygote, oöcyst, sporoblast*). This zygote increases rapidly in size and the nucleus divides rapidly. In its growth it bulges outward from the intestinal wall forming pendulous tumors into the body cavity which growth may vary from four and a half to ninety microns in diameter. This stage is associated with the appearance of much pigment and is called the *medium zygote* or medium sporoblast stage. The protoplasm gathers around the divided nuclei, forming daughter cysts which are connected by bridges of protoplasm forming the stage known as *large zygote, large sporoblast, or large oöcyst.* In each of these divisions the nucleus divides many times, the daughter nuclei remaining on the surface of each daughter cyst. The protoplasm now collects around each daughter nucleus, the first forming spherical cells which then elongate into threads lying parallel over the remains of the sporoblasts. These threads are called *sporozoits* and have an elongated nucleus. The final length of these sporozoits is about fourteen microns and their width about one. Their protoplasm is thick, homogeneous, and very refractive. They are sometimes present to the number of 10,000 in some zygotes, but more frequently are not so numerous. As the oöcyst becomes larger it bursts into the body cavity, the sporozoits of each cyst ripening at about the same time. These sporozoits wander at first free, but soon collect in the salivary gland of the mosquito. They are motile and move with a bending and gliding motion. When they are inoculated into the blood of man by the bite of the female mosquito they attach themselves to the red blood-corpuscle and finally penetrate it to form the initial hyaline type of the organism. The period of incubation after the bite of the mosquito is usually between the eighth and twelfth day, when the first chill will appear, although the exact time of appearance of the initial symptoms will depend upon the number of sporozoits introduced into the circulation (Emerson). The anopheles is the only type of mosquito which is at present known to be the host of the malarial organism and to give rise to the development of the *gameto-schizonts* (the sexual cells), while the bite of the female animal introduces into the blood cells which are known in their future development as schizogones; the cycle of development within the mosquito is known as the sporogone.

General Changes in the Blood in Malaria.

There are few conditions which lead so rapidly to such an extreme reduction in the red cells as does acute malaria. An acute attack may reduce the red cells to as low as 500,000 cells as reported by Kelsch. Frequently a reduction of 1,000,000 is observed during the first day, with a progressive reduction as the time goes on. In the afebrile period of the disease a continuous fall is observed, but this is much less rapid. The regeneration of the cells is

very active so that an increase in the number of cells has been observed directly after an attack in some cases. In cases of chronic malaria the red cells are commonly reduced to as low as 583,000 (Kelsch), while when attacks occur only at intervals and are promptly stopped by quinin no reduction in the red cells may follow (Marchiafava). In cases of moderate severity the usual changes of secondary anemia are present in the red cells. Polychromatophilia and granular degeneration of the reds progresses steadily, while the hemoglobin content of the cells may be markedly reduced. Frequently cases are seen in which the anemia takes on the absolute pernicious type, so that the parasites seem to have been massed in the bone-marrow. As Ewing states, "there can be no doubt that the tendency of the estivo-autumnal parasite to be massed in the bone-marrow, in both ameboid and crescentic phases, and the excessive demand on red-cell production arising in the disease render pernicious malaria an extremely favorable condition for this disturbance of the structure of the marrow and the development of specific megaloblastic changes." Besides the changes which can be directly referred to anemia or toxemia, changes in the size of the cell are quite constant, the tertian parasite causing from the start swelling of the cell and progressive loss of hemoglobin, while the quartan and estivo-autumnal forms cause the red cell to shrink and take on a peculiar brassy tone.

The leucocytes do not show very characteristic changes. In the acute malarial attacks of average severity the absence of leucocytosis is of considerable corroborative value, although a slight leucocytosis amounting to about 10,000 with an increase in the percentage of polynuclear cells has been observed by Billings and others. Except during the three or four hours immediately following a chill malarial blood usually shows a diminished number of leucocytes with a distinct relative lymphocytosis, which finding is that seen in typhoid fever. In the more severe estivo-autumnal attacks a definite leucocytosis has been distinctly observed, especially in the hemoglobinuric or black-water type of malarial infection. The extent of the leucocytosis varies between 10,000 and 35,000 although many attacks fail to cause any distinct increase. During the afebrile periods the eosinophile cells are usually increased and may be observed throughout the course of the attack. Neutrophile myelocytes are occasionally present and rarely eosinophile myelocytes. Pigmented leucocytes are seen in the majority of cases, especially in the severe and fatal cases, the pigmented leucocytes being more closely related to the severity of the paroxysms than to the extent of the deposits in the various viscera. These pigmented or phagocytic cells include mononuclear and polynuclear leucocytes and a few endothelial cells. The large and small mononuclears usually contain pigment or rosettes, while many of the polynuclear leucocytes also contain the parasites. These phagocytes may contain, besides parasites and malarial pigment, hematoidin, hemosiderin, red blood-cells, leucocytes, and occasionally an unknown crystalline pigment.

(2). **Relapsing Fever (Famine Fever).**

The cause of this peculiar fever is the *spirillum of Obermeier* and is not a member of the class of bacteria but, more properly speaking, belongs to the class of spirochete. This organism is between 16 and 40 microns in length and about one micron in width, but is subject to considerable variation in size. It is thin, sharply curved, and appears to be structureless. It takes a deep chromatin stain and also stains with methylene blue in from two to five minutes. It is seen in the blood only during the febrile period of the disease and at that time is actively motile with a rapid wavy motion, much resembling the movements of a coiled spring in its stretching and collapsing. It moves rather slowly among the corpuscles, but does not disturb them to any extent. Cases have been reported in which these spirochete are present in the blood 24 hours

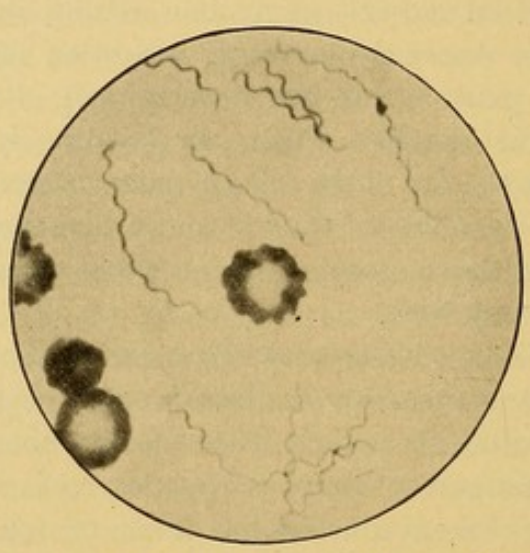


FIG. 145.—Spirillum of Obermeier. (*Pitfield.*)

before the chill, but they are usually to be found in larger numbers at the time of the rise in temperature, increasing rapidly from day to day. The fever, as a rule, continues about six days, at the end of which time these parasites leave the blood. Strangely enough these organisms have been found in varying numbers in different parts of the circulation, while there does not seem to be any strict parallelism between their number and the height of the fever. Loewenthal has applied the agglutination test to the blood of suspected cases and finds the reaction positive in 85 per cent. of the cases in the periods in which the parasites are absent.

The changes in the blood are not characteristic in this condition. The red blood-cells seem to be diminished to a slight extent for several days after the attack, but increased during the afebrile period. The hemoglobin may be reduced to as low as 50 per cent. so that a very distinct anemia may be present. The leucocytes seem to be distinctly increased in this disease, the most marked leucocytosis occurring just after the crisis.

Cases of relapsing fever are practically never found in the United States, unless imported through the medium of emigrants from Russia and especially

India. The cases reported by Wellman show that relapsing fever, as found in West Africa, may arise from the bite of a tick transmitting the spirillum of Obermeier.

(3). Sleeping Sickness.

This very interesting condition which is so prevalent in Central and West Africa seems to be due to an actively motile fusiform flagellate known as the *trypanosoma Gambiense,* which can be found in the blood free in the plasma (never intracorpuscularly), moving with a screw-like motion among the red cells which it does not seem to disturb. This parasite doubtless has a sexual development, its host being the common fly, Glossina palpalis, while closely related trypanosomata are transmitted by the bites of various flies,

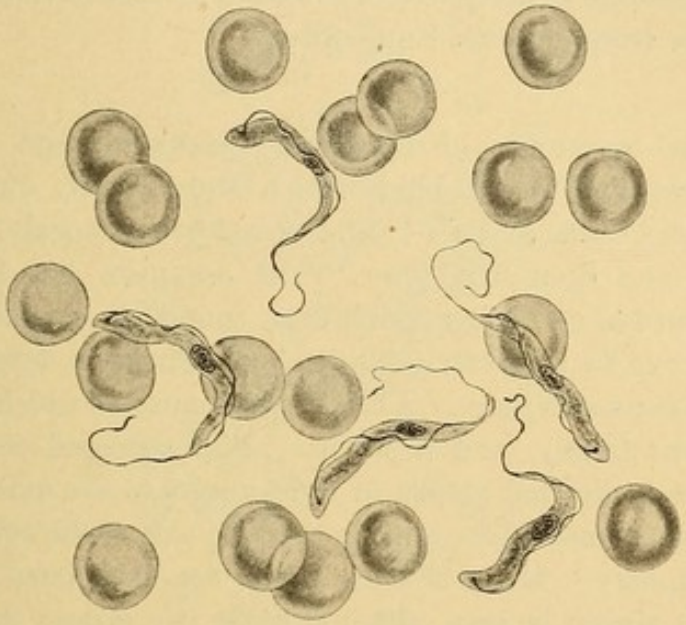


FIG. 146.—Trypanosoma gambiense. (*Da Costa.*)

especially one of the seven varieties of the tsetse fly. This organism is from two to three times as long as a red blood-corpuscle (18 to 25 microns) and 2 to 2 1/2 microns wide, having a flagellum anteriorly and an undulating membrane extending its entire length. In the fresh blood specimen these parasites should be looked for with only a medium magnification. These parasites vary much in number sometimes being absent for a long period and then suddenly reappearing in large numbers. Symptoms of the disease seem to bear little relation to the number of parasites in the peripheral blood, so that in some cases it may be necessary to examine the fluid in the edematous areas or even to puncture the cervical lymph-glands. When these parasites are stained with a polychrome dye they show a rather large red nucleus about the middle, a centrosome staining intensely in a vacuole-like area near the blunt posterior end, and a line of chromatin taking a dense red stain running down the edge of the undulating membrane and terminating in the flagellum which is also stained red. The protoplasm of the body takes a distinct blue stain.

The parasite contains no pigment and, therefore, obtains its nourishment from the plasma and not from the red cell.

This disease may take an acute course, but as a rule is exceedingly chronic, running for years, but becoming fatal as soon as the parasite reaches the cerebrospinal fluid. The true sleeping sickness appears only when the cerebrospinal fluid is invaded and seems to be, according to the recent work of Koch, directly amenable to treatment with atoxyl. In examining the cerebrospinal fluid for these parasites it is best to gently centrifuge the fluid for five minutes, after which the sediment may be examined under a vaselined cover-glass.

There are many other types of trypanosomata, but the Gambiense form is the more important. This is pathogenic toward man, but cannot be distinguished from the trypanosoma of the tsetse fly which is so fatal to the horse and mule (trypanosoma Brucei), that of the surra disease (trypanosoma Evansi), or that of dourine (trypanosoma Equiperdum).

(4). **Kala-azar.**

Through the researches of Donovan, Leishman, and Ross, parasites have been demonstrated in the blood which are probably directly associated with the condition known as kala-azar, tropical splenomegaly, spiroplasmosis, cachexial fever, and dum-dum fever. The organism has been called the *Leishman-Donovan body*, and is a small oval, round, or oat-shaped body from 2 1/2 to 3 microns in diameter. These bodies have a definite cell outline and contain two chromatin masses, a larger one, a nucleus which is almost round or oval and stains faintly, and a smaller bacillus-shaped centrosome which stains deeply and is directed almost at right angles to the axis of the nucleus. These two chromatin masses are both in the long axis of the cell, the larger one being at the periphery. Many of these forms are vacuolated and the outline of the cell cannot always be seen, although these two masses thus arranged are distinctive. They are easily stained with the various polychrome dyes and are best studied with the highest lenses. These bodies probably represent a stage in the development of a trypanosome as shown by the work of Leishmann and Statham. They are not found in the circulating blood as a rule, but they have occasionally been reported in the form of intracellular bodies in fatal cases. They are easily seen in the blood obtained by splenic puncture and also in the granulation tissue taken from the ulcers. Many are found in the mesenteric lymph-glands, bone-marrow, and liver. Some of these bodies lie free, but most of them are intracellular, either in the leucocytes, endothelial, or splenic cells, and frequently in large masses in the macrophages.

The changes in the blood are those of a moderate anemia, associated with a leucopenia with a relative and absolute increase in the number of the large mononuclears. The average leucocyte count is about 2,000.

(5). **Filariasis.**

This is a condition associated with the presence of filariæ in the blood (*filaria sanguinis hominis*). While many of these filariæ are known, the

most common one is the *filaria Bancrofti* (filaria nocturna). These are from 270 to 340 microns (0.2 to 0.3 mm.) long and from 7 to 11 microns broad. They are enclosed in a sheath which is considerably longer than is the parasite and shows fine cross striations. The anterior end of the parasite is abruptly rounded and has a six-tipped prepuce and a sharp fang, while the posterior end tapers for about two-fifths of the length of the parasite. The median axis of the parasite is granular. The movement of these parasites is distinctly progressive at first as seen under the microscope, but they soon become motionless, appearing to attach themselves to the glass slide at their anterior end. Strangely enough these embryos appear in the circulation only toward evening, their numbers gradually rising to a maximum about midnight and diminishing

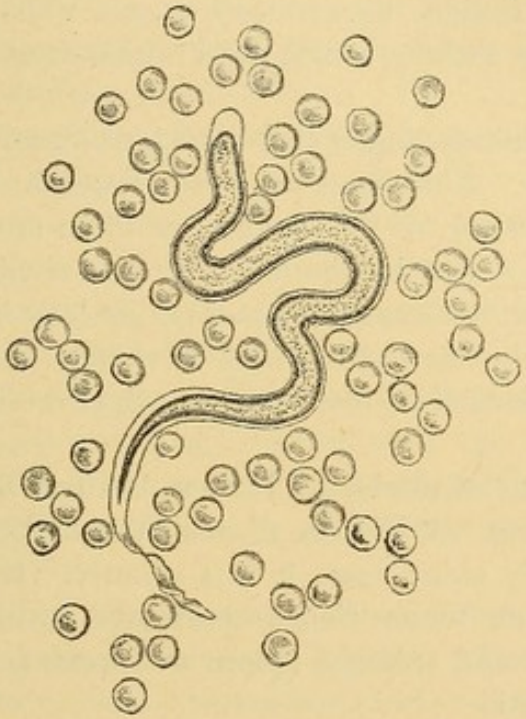


FIG. 147.—Filaria bancrofti. (*Da Costa.*)

toward dawn. During the day they are found in the internal organs, especially the lungs. The forms appearing in the blood are practically all embryos, as the adult types lie in the lymphatics where they obstruct the lymph flow.

The obstruction in the lymph-glands may also be brought about by the eggs, which are 25 to 38 microns long by 15 broad. The embryos reach the general circulation only through the thoracic duct. The female filaria is 85 to 150 mm. long, with a distinct neck, a head with a simple, minute, terminal mouth, and a plain cylindrical body covered by a striated cuticle and tapering toward the neck and tail. The tail ends bluntly and has a small depression surrounded by two lips. The male is about 80 mm. long, without a neck and having a tendril-like tail rolled into one or more spirals.

Like the malarial organisms, the filaria has an intermediate host in the mosquito, both of the culex and anopheles variety. The embryos, which

are taken up by the bite of the mosquito, cast off their sheath in about one hour in the stomach of a mosquito. Some of these embryos die at this stage, but others bore actively through the intestinal wall to the muscle, where they remain. In the next two or three days the embryo becomes larger and its alimentary tract develops. On the seventh day the worm is about 1 1/2 mm. long and is perfectly developed. It now travels toward the head and takes its position in the labium, whence it enters the blood of its new host during the biting by the insect. A large number of these adult forms is necessary to cause very severe cases and many years may pass before any symptoms are manifest.

In examining the blood for the filaria, it is best to take a specimen late at night and to make a very thick, fresh specimen which should be examined with a low power. Besides the ordinary anemia which may develop in such cases, we find a very striking eosinophilia which may run from 4 to 17 per cent. in such cases.

A very characteristic finding in such cases is the condition of hematochyluria followed by chyluria. This hematochyluria seems to be due to rupture of the varicose lymph-vessels of the bladder, as these form a large part of the collateral circulation when the thoracic duct is occluded (Emerson). Such attacks may occur for years and be separated by long intervals. Their onset is spontaneous or following exertion and is usually associated with pain and fever. The urine shows the presence of blood, chyle (as high as 3.8 per cent. fat), and embryos.

Many other forms of filariæ are known, but this Bancroft type seems to be the more important. While this disease occurs endemically in the tropics there are undoubtedly many cases in this country. It is, therefore, wise in a case showing lymph tumor, elephantiasis, and hematochyluria, especially when pain and fever and enlarged spleen are present, to examine the blood for the filaria Bancrofti.

(6). **Syphilis.**

The search for the causative factor of syphilis has extended over a period of many years and various agents have been advanced from time to time, but none of them has remained fixed as the distinct etiologic factor. Through the work of Schaudinn and Hoffmann in 1905, a parasite has been found which seems to be so constantly associated with syphilitic lesions, whether primary, secondary, or tertiary, that it is highly probable that the causative unit has been found. It is true that in many conditions which at present bear no known relation to syphilis, such as pseudoleukemia, similar parasites are found in the enlarged glands, but whether or not these are the true spirochæte pallidae remains to be seen.

Zeit has very exactly summed up the points which must be met before the spirochæta pallida is definitely accepted as the etiologic factor of syphilis, as follows: "If we are to consider the spirochæta pallida as the unquestionable etiologic factor of syphilis it will be necessary, however, to explain, by further

exhaustive investigations, the discrepancies which the cytorrhyctes defenders have been most active to point out and still uphold, of which I will mention the following:

The difficulty of finding spirochætes in cover-glass smears from tissues which are teeming with silver-stained spirochætes.

The morphological similarity of the Pallida type and certain mouth spirochætes which I have named pseudopallida.

The practically total absence of spirochætes in the highly infectious organs of slaughtered syphilitic monkeys, although the organs of the macerated syphilitic fetus are teeming with them.

The Jancke experiment of successful infection with syphilitic virus which was filtered through a Pasteur filter, whereas the sprochætes do not pass the filter.

The fact that pallidæ can be kept alive for days and weeks, whereas syphilitic virus loses its virulence in 6 to 8 hours.

The destruction of pallidæ when kept in glycerin, although the virulence of syphilitic virus can be preserved for days by glycerin.

The presence of typical pallidæ in the skin of the macerated pig fetus.

The only cultures (not pure cultures) which ever were produced of the pallida by Levaditi, grown in collodium sacs in the peritoneal cavity of monkeys and rabbits, did not produce syphilis in monkeys.

By fulfilling Koch's classic postulates in the way of culture, the whole question could be definitely and finally decided with one stroke. Everything speaks for it that the pallida is the etiologic factor of syphilis, and many of the discrepancies can easily be explained by considering spirochæte pallidæ as protozoa and not bacteria."

The spirochæta pallida (*treponema pallidum*) derives its name from its low refractive power and the difficulty with which it takes up anilin dyes. It has a very delicate structure, usually presenting 10 to 40 deep spiral incurvations in the larger specimens or only a few in the smaller ones. Its length varies between 4 and 10 microns and its width does not exceed 1/2 micron. The organism has been demonstrated in the circulating blood, in the scrapings obtained from the chancre, in the incised papules, in smears from the mucous patches, and in the fluid aspirated from the inguinal glands. It seems to be easily demonstrable in the blood from a splenic puncture, while in the congenital forms it is found in the internal organs and in the peripheral blood. A characteristic difference between this spirochæta and some other types (spirochæta buccalis), with which it might be confused, is that its ends lie above and below a longitudinal line drawn through the center of its curvatures, while in the other forms the ends lie on the projection of such a line. The organism moves in an oscillatory manner about its longitudinal axis, its movements being winding, bending and whipping, while in the spirilla the longitudinal axis remains rigid. Schaudinn demonstrated the existence of a flagellum at each end, while the other spirochæte have an undulating membrane.

These organisms are seen only with great difficulty in the specimens of fresh blood, but thanks to the introduction of the ultra-condenser (the dark-field illuminator) we are in a position to see these organisms, both in the splenic and peripheral blood, although considerable practice is necessary to properly adjust the light. These organisms do not take anilin dyes readily, so that special methods have been advanced for their demonstration in smears. A very good stain for them is the Goldhorn stain. The smears are fixed with pure

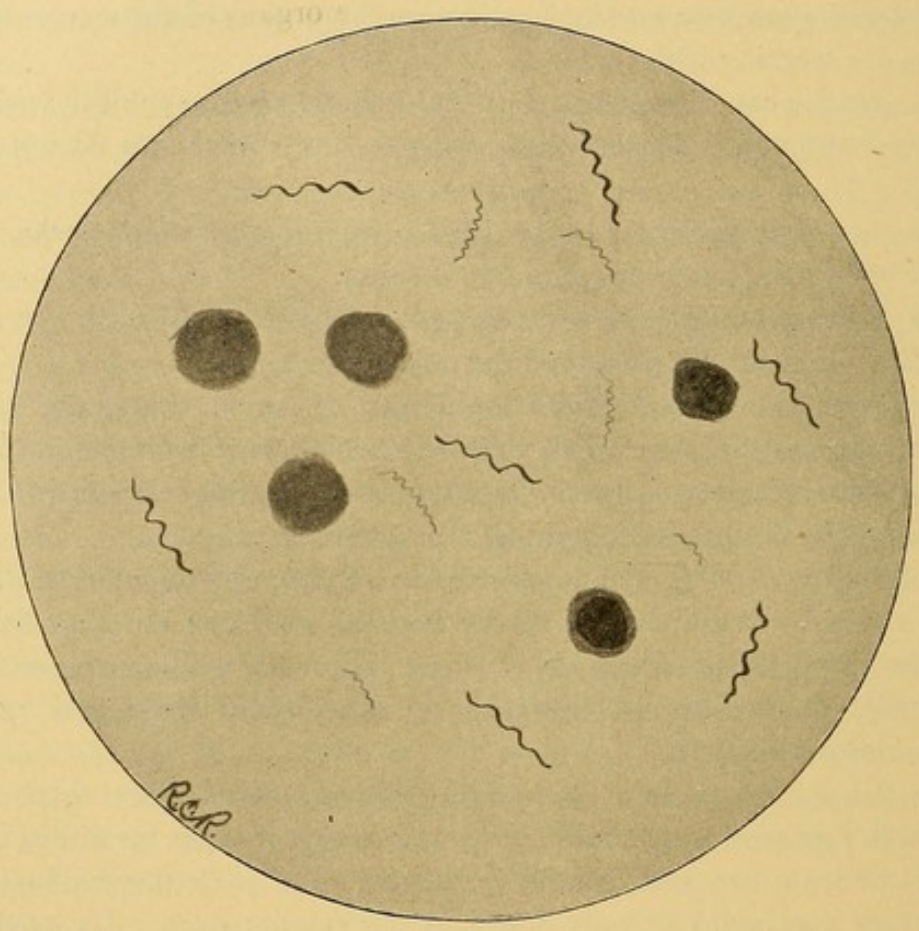


FIG. 148.—Spirochæte pallidæ and refringens. (*Pitfield.*)
The darker ones are the refringens.

methyl alcohol for 15 minutes and are then covered with the stain (polychrome methylene blue) for three to five seconds, when the excess is drained off. The specimens are then slowly introduced into clean water with the film sides down. Keep the slide in this position for four to five seconds and then shake in the water to remove the excess of the dye. The spirochæte appear of a violet color. This violet tint may be changed to a bluish-black by covering the specimen with Gram's iodin solution for 15 to 20 seconds, after which it is washed and dried as usual and the examination made with the immersion lens. The writer has also found the use of the Giemsa stain very reliable, especially when the staining is continued for 18 hours (see Exudates). Other stains, such as that of Levaditi, have been advocated, but they do not seem to

give any better results and are more complicated. For staining the spirochæte in tissues the Levaditi stain is admirable.

The examination of the blood is very often disappointing, owing to the fact that few spirochæte may be present in the specimen. Better results are obtained by examination of specimens from a curettage which has been carried sufficiently far to allow serum to appear. This serous fluid is then spread upon slides and treated in the usual manner (see Exudates).

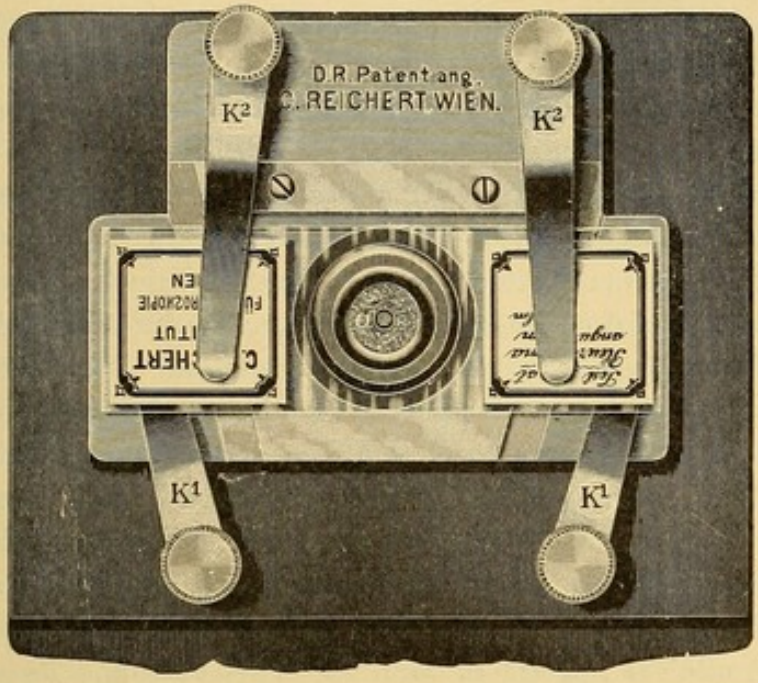


FIG. 149.—Ultra-condenser of Reichert.

Wassermann's Serum Reaction.

Much interest has been aroused by the application of certain principles of serum pathology to the diagnosis of syphilis. The writer cannot go into great detail in this discussion and must refer the reader to other works for full accounts. The terms used will be found in the section on Ehrlich's Side-chain Theory.

The discovery by Klebs, Metschnikoff and Roux that syphilis could be transmitted to monkeys, coupled with the work of Wassermann, Bruck, and Neisser showing that the serum of these syphilitic apes contained a material, an antibody, which was not found in normal serum, led the way for the application of an earlier line of investigation; that is, Bordet and Gengou had shown that emulsions of bacteria, when mixed with inactivated serum with the addition of complement, did not cause hemolysis in the presence of the hemolytic amboceptor because the complement bound itself with the bacteria and thus prevented the union of complement with the red cell through the medium of the amboceptor. Wassermann and his colleagues then showed that extracts from infected organs could be used, as well as solutions of bacterial extracts or bacterial suspensions, in preventing the characteristic action of the antigen.

They then applied this principle to syphilis by treating the blood serum of monkeys, rendered inactive or immune by previous infections of syphilitic material of either man or monkey, with the serum of syphilitic men or with the extract of organs (preferably the liver) of congenitally affected children or of the placenta of secondarily affected mothers, under the conditions outlined by Bordet and Gengou. As no hemolysis occurred, it was clear that the immune serum of the monkey contained antibodies against the antigens of the syphilitic material. A number of later observers have shown, however, that this reaction is not specific for luetic antigens, but that lipoid substances act quite as well in this respect.

In the application of these principles to the diagnosis of syphilis, we use a known substance as antigen (either extract of syphilitic organs, lecithin or sodium oleate solution as advised by Sachs[1]) and add to it (1) the complement (fresh normal guinea-pig serum), (2) the suspected serum heated to 56° C., (3) amboceptor (the heat-inactivated serum of a rabbit injected with calf's blood), and (4) the washed red corpuscles of the sheep as the cells to be hemolyzed. The failure of hemolysis is called a positive reaction. Such a result is due to the fact that the complement has been bound, the antigen and serum tested being homologous. Recently Noguchi[2] has advocated the use of an anti-human hemolytic system instead of the original anti-sheep hemolytic system of Wassermann. He has further simplified the technic by the introduction of test-papers saturated with amboceptor, complement, and antigen. This brings the test directly home to the general practitioner.

The chief clinical interest of this test centers around its specificity. It does not always appear in syphilis, although 80 per cent. of syphilitic individuals give a positive reaction. A negative reaction is, however, not a proof that syphilis is not present. Experiments covering a large number of cases have shown that nonsyphilitic individuals never give this reaction, so that a positive result is a certain indication of syphilis. It is probable that this test will be as frequently used as is the Widal test in typhoid fever, as it seems to be even more certain in its positive phase and no more uncertain in its negative phase.

(7). Yellow Fever.

This infectious noncontagious febrile disease is caused by a specific organism not yet discovered. This etiologic factor is, however, blood-borne, as shown by the fact that injection of blood from a yellow-fever patient into a healthy subject causes the disease. Whatever the organism be it must be very minute, as the blood serum retains its infecting power after passage through a Berkefeld filter. The various organisms which have been held responsible for this disease, among them the Bacillus X of Sternberg and the Bacillus icteroides of Sanarelli, have all been abandoned and even the myxococcidium stegomyiæ of the Yellow Fever Commission is at present considered as foreign to yellow fever.

[1] Berl. klin Woch., Bd. 45, 1908, S. 494.
[2] Jour. of Exp. Med., vol. 11, 1909, p. 392.

In 1881 Finlay advanced the hypothesis that yellow fever was transmitted to man only through the bite of a mosquito of the Culex group, the stegomyia fasciata. The United States Commission, consisting of Reed, Carroll, Agramonte, and Lazear, furnished the experimental proof that this hypothesis was valid and showed that the unknown organism of the disease required a period of 12 days' development in the body of the mosquito before it could be transmitted from the stegomyia as an infecting agent. A second U. S. Commission, consisting of Parker, Pothier, and Beyer with the help of Smith stated in 1903 that yellow fever was due to a parasite of the sporozoan type, the myxococcidium stegomyiæ, which developed in the stegomyia. This organism has never been found in the human body, hence its schizogony (asexual development) is unknown. The French Commission, Marchoux, Salimbeni, and Simond, as well as Schaudinn and Carroll, do not believe that this organism has anything to do with yellow fever, but associate this myxococcidium with a yeast fungus which is usually found in the mosquito.

As this disease is beyond question blood-borne, its hematological changes are of some interest. Jones shows that anemia is infrequent, that fibrin formation is deficient, that the globucidal action of the serum is greatly increased, and that both cholemia and hemoglobinemia occur. The red cells show little variation in number, Pothier never finding them below 4,280,000. The hemoglobin suffers considerable loss, being usually between 50 and 75 per cent. This loss is restored very slowly in convalescense. Albertoni draws attention to the lack of parallelism between the percentage of hemoglobin and the specific gravity of the blood, the latter falling much more than the former. Morphological changes in the reds are rare. An occasional normoblast may be seen.

The leucocytes range between 4,660 and 20,000, the higher the count the more favorable the prognosis. In this leucocytosis the polynuclear neutrophiles are in higher proportion, only rarely being normal. Eosinophiles are few and myelocytes only occasional.

(8). Rocky Mountain Spotted Fever (Tick Fever).

This disease is not to be confused with typhus fever or epidemic cerebrospinal meningitis, to both of which the term "spotted fever" has been occasionally applied.

Wilson and Chowning have reported that the blood of man affected with Rocky Mountain spotted fever shows the presence of an erythrocytic parasite which they call Piroplasma hominis. These parasites are ovoid in form, have ameboid motility, and are unpigmented. Three forms of these intracellular ovoids were found, (1) a small, nonmotile form, 1 to 2 microns in length by 1 micron in width; (2) a larger actively ameboid form, 3 to 5 microns in length by 1 to 1.5 microns in width, and showing a dark granular spot at one end; (3) a twin form, consisting of two pear-shaped bodies, lying with their tapered ends approaching and bearing a granular spot at each end. These bodies stain best with the polychrome dyes.

Through the work of Ricketts and King it has been definitely established that the parasite of spotted fever finds its host in the wood tick (dermacentor venestus (Banks) or, as Stiles states, the dermacentor andersoni). There is apparently no cycle of development in the tick as an intermediate host. Ricketts advances much evidence against the piroplasma as the causative factor, but is unable to say that such might not be the etiologic unit. He[1] has recently succeeded in finding an extra- and intra-cellular pleomorphic polar-staining bacillus, which is extremely small and is constantly present in the blood of infected animals as well as in the infecting tick. Whether this is the true etiologic factor is still unsettled, but Ricketts believes it is.

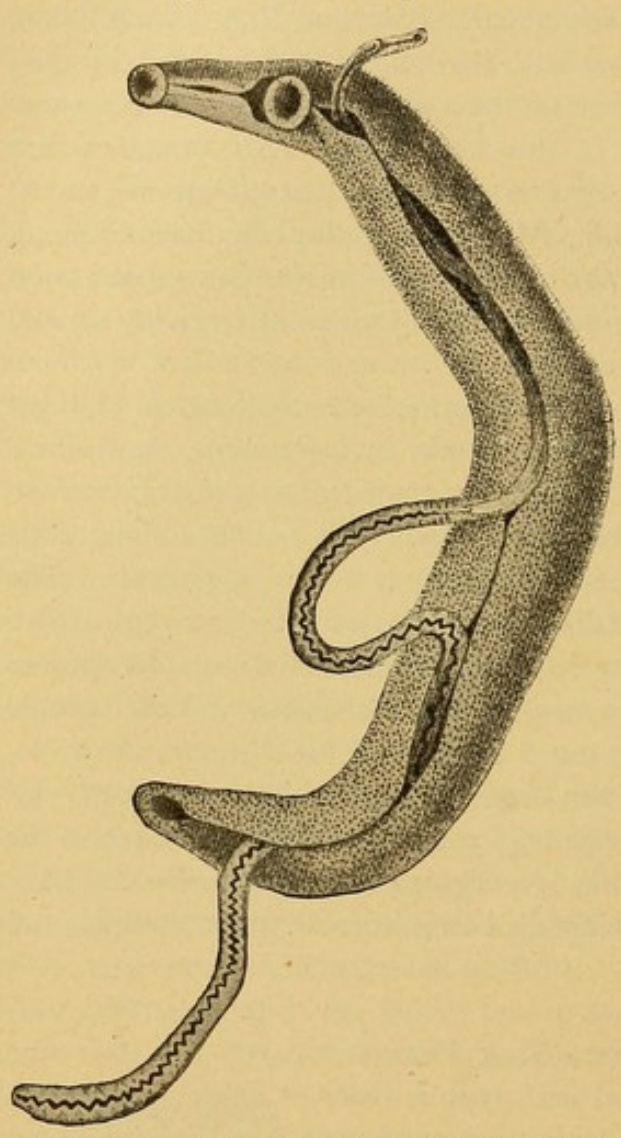


FIG. 150.—Schistosomum hematobium; male with female in gynecophoric groove. (*Tyson after Loos.*)

The red cells in this disease are reduced to about 4,000,000, while the hemoglobin content may be as low as 50, thus giving a low color index. Degenerations and atypical staining qualities are rare. The leucocytes are increased to 12,000 or more and show nothing differentially abnormal, except a slight increase of the large lymphocytes.

(9). **Distomiasis (Bilharziasis).**

This is a chronic parasitic disease due to the deposition in the tissues of the eggs of the worm, *Schistosomum hematobium*, also called Bilharzia hematobia, gynecophorus, distomum hematobium, distoma capense, and Thecosoma. It is a very common condition in Africa, but has been found but six times in America, according to O'Neil. Infection appears to be more commonly induced by drinking unfiltered infected water, but occasionally it may come through the skin.

The adult parasites inhabit the blood of the portal vein and the vessels of the pelvis, rectum, and bladder. The male is smaller and thicker than the female, is 12 to 15 mm. long and 1 mm. broad, is flat and so folded as to form a gynecophoric canal which holds the female. The female is about 20 mm. long and 0.25 mm. thick and is the active agent in the infection.

[1] Jour. A. M. A., vol. 52, 1909, p. 379.

The eggs of the parasite are oval or spindle-shaped, measure about 0.16 mm. in length and 0.05 mm. in breadth, and have a distinct spine-like projection from the posterior end or from one side. These ova are particularly frequent in the urine of such cases. Occasionally they may be found in the circulating blood.

VI. Bacteriology of the Blood.

In a clinical analysis, it is frequently necessary to make examinations of the blood for the presence of various bacteria. Such an examination requires a rather minute knowledge of the methods of bacteriology, but the writer cannot take space to outline such procedures. It is occasionally desired to make cultures from the blood in suspected cases, so that a few words seem advisable.

Blood Cultures.

For the ordinary blood culture the amount of blood required is from 5 to 20 c.c., so that one must obtain it by puncture of a blood-vessel. The one usually selected is the median basilic vein at the bend of the elbow. The site of puncture of the vein should be as carefully cleansed as is done in any preparation for surgical interference. In some cases it is absolutely essential, in order to avoid any chance of infection, that an incision be made over the site of puncture so that the needle may be passed directly into the vein without first penetrating the skin. As a rule, such precautions as the latter are not taken, as the slight infection which might thus arise would be recognized as a contamination more than as a direct growth from the blood. The instrument best adapted for such purposes is, in the writer's opinion, the Lüer syringe, which is made entirely of glass and has a tightly-fitting platinum needle. Sterilization is best done by the use of dry heat to 150° for one hour or boiling in 1 per cent. sodium carbonate solution for 15 minutes. These instruments should never be sterilized with carbolic acid or bichlorid solution owing to the danger of inhibiting the growth of organisms if present.

When the above preparations have been carried out, an elastic band or a towel is tied about the arm so as to produce constriction of the vessels and distention at the point of puncture. The needle is plunged into the vessel against the direction of the blood current. Very slight aspiration is necessary, as the blood tends to flow into the syringe through its own force. The usual amount of blood drawn is 10 c.c., but occasionally 20 c.c. are preferable, especially if the patient is full-blooded and can stand the loss easily. The blood is now placed in a series of tubes, containing melted agar and also in a vessel containing nutrient bouillon. In the case of the blood and agar mixture, this is poured as soon as possible into Petri dishes and placed in the incubator. The ordinary rules for studying bacterial cultures will then apply. If only a few colonies develop in the agar, especially if they are superficial, one should think strongly of contamination, while if the colonies be deep and are observed

in several plates, contamination may be ruled out. The methods of identification of the various bacteria must be learned elsewhere.

The routine use of blood cultures in the study of cases of infection very often furnishes information which is of great diagnostic and prognostic importance, especially if the specific organism is isolated. Such a result, however, can follow only when relatively large amounts of blood are used. It is also necessary to make subcultures and to apply a great many bacteriological principles before definite knowledge of the invading organism can be gained. For these reasons such investigations are usually left to hospitals and the larger clinical laboratories.

Organisms Found in the Blood.

As a rule it may be said that in practically every infectious disease of known origin the specific organism may be obtained from the blood, at some stage of the infection, in practically pure culture. Thus the streptococcus, staphylococcus, pneumococcus, colon bacillus, typhoid bacillus, micrococcus melitensis, and many others have been isolated. Libman has recently called attention to the significance of negative results in such examinations and concludes that many factors may combine to prevent positive results at the especial time of the examination.

Rosenberger[1] has reported a series of 300 cases, in all of which the tubercle bacillus was found in the blood by the following technic. About 5 c.c. of blood are withdrawn from a vein of the arm and immediately placed in an equal quantity of a 2 per cent. solution of sodium citrate in normal salt solution. The mixture is well shaken and placed in the refrigerator for twenty-four hours. The sediment is pipeted off and thick smears are made upon clean glass slides. These smears are dried with moderate heat and are then placed in distilled water until the blood-cells are completely laked. The delicate film which remains is fixed by heat and stained as previously described for the tubercle bacillus. While it is possible in advanced cases to demonstrate tubercle bacilli in the blood by this method, the writer's experience is not such as to lead him to agree with Rosenberger in ascribing great importance to this test in incipient tuberculosis. If it is proven that a primary bacteriemia exists in tuberculosis, our ideas of the lymphatic extension of this disease must be revised.

VII. Serum Reaction.

Since the early work of Pfeiffer in 1894, the study of certain peculiar reactions in the serum has proceeded through many modifications until Widal[2] elaborated his diagnostic test in connection with typhoid fever. It is to be said that this so-called Widal reaction is simply the application to typhoid fever of a general principle which is well established in serum pathology. The principle is as follows: The addition of a serum of a patient, suspected of

[1] Amer. Jour. of Med. Sci., vol. 137, 1909, p. 267; N. Y. Med. Jour., vol. 89, 1909, p. 1250.
[2] Bull. Méd., Tm. 10, 1896, pp. 618 and 766.

having an infectious disease, to a pure bouillon culture of the specific organism of this suspected disease has the effect of bringing about agglutination of the organism in the culture. Naturally associated with this we find loss of motility of the motile organisms.

Widal Reaction.

Technic.

Although the specific Widal reaction has been obtained from many of the secretions and excretions of the body, either the blood or its serum seems to be the most available material for such examination. Many methods have been advanced for obtaining the blood for examination so that the writer will necessarily limit himself to those with which he is most familiar. If the whole blood can be obtained by the one who is to make the examination, the best method would seem to be to dilute the blood in a definitely known proportion with distilled water. This can be very readily done by the use of the leucocytometer in which dilutions from 1 to 10 to 1 to 50 may be easily obtained. The greatest objection to this method is the fact that the blood does not always dissolve completely in water, although hemolysis occurs to a great extent.

More frequent, however, is the employment of dried blood for such examinations. In the use of dried blood one or two drops of blood are allowed to fall from a puncture upon a glass slide and are then dried in the air. Such specimens may be easily transported and furnish very often a practical means of sending material to the laboratories for examination. In some cases strips of mica are used instead of the glass slide, as these will not break and can be mailed with perfect safety. The great disadvantage of using blood dried in this way is the impossibility of securing an exact dilution of the blood when it is redissolved in distilled water. The experienced worker, however, learns to control the dilution by the color of his drop. The detritus of the red cells may be confusing to the beginner, but should not be mistaken for masses of bacilli when the examination is made. In the writer's laboratory the practice has been to use pieces of filter-paper upon which is ruled a square of known dimensions (1/2 inch). The physician is directed to fill this square with blood and allow it to dry. It has been found that the solution of this blood in five drops of distilled water yields a dilution as near 1 to 50 as is possible with any method which does not make use of absolute dilution.

Many workers prefer the use of serum rather than of the whole blood, so that simple methods for obtaining serum have come into use. The simplest clinical method is the use of a capillary tube with a central bulbar enlargement into which the blood is drawn by capillarity from the puncture of the ear. The ends are sealed and coagulation of the blood allowed to proceed. In a short time the serum will be separated from the coagulum and may be drawn off and diluted with accuracy. Another method of obtaining serum is by the use of the cantharides blister which will furnish a large amount of serum in

from 6 to 18 hours. This serum may be drawn into capillary tubes and used as previously mentioned.

The culture of typhoid bacilli must be fresh and must show many actively motile organisms in the hanging-drop specimen. Great difference exists in different strains of bacteria so that the worker should always know just what

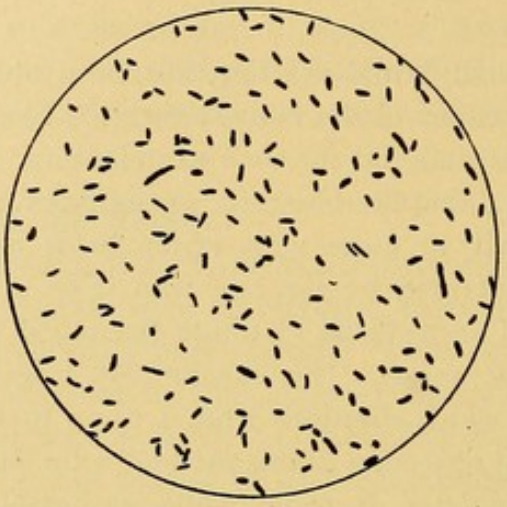


FIG. 151.—Bacillus typhosus at beginning of Widal test. (*Da Costa.*)

sort of culture he has with which to deal. It seems to be fairly well established that a freshly isolated culture is not suitable for diagnostic use, but must be transplanted many times into artificial media. The stock culture of typhoid bacillus is best kept in sealed tubes of nutrient agar in the ice-box, from which fresh agar cultures should be made every few weeks. In laboratories in which

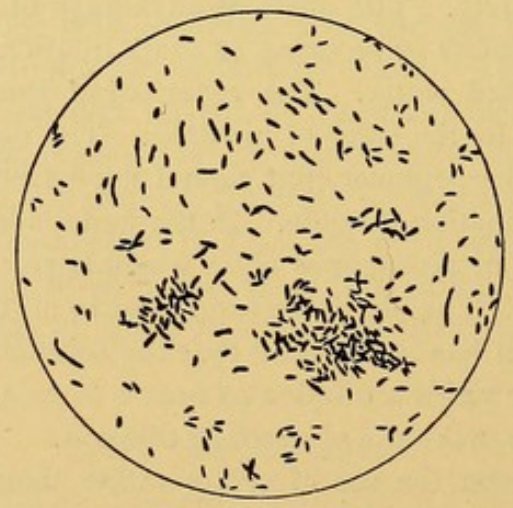


FIG. 152.—A Pseudo-Widal reaction. (*Da Costa.*)

the Widal test is a matter of daily routine, fresh bouillon cultures should be made daily from the stock cultures, so that one may be certain of having actively motile organisms at all times.

As the general practitioner does not always have facilities at hand for the propagation of live cultures of bacteria, advantage has been taken of the fact,

first shown by Widal, that cultures of the bacillus typhosus which had been killed by heating to 60 ° C. for 45 minutes did not lose their capacity to agglutinate when brought into contact with suspected serum. For this reason, dead cultures of typhoid bacilli, which have been killed by heat or by various chemical agents, have been introduced for the use of the general worker. The use of such dead cultures does not seem in any way advisable to the writer, as such a method entirely eliminates the factor of diminished or lost motility, which is such an important one in deciding as to the character of the reaction. These tests are always macroscopic in character; that is, the suspension of typhoid bacilli gradually settles out in tubes on the addition of the suspected serum, so that the reaction may be observed by the naked eye.

It has been shown that the healthy serum of many patients or that of subjects suffering with diseases other than typhoid fever frequently shows a

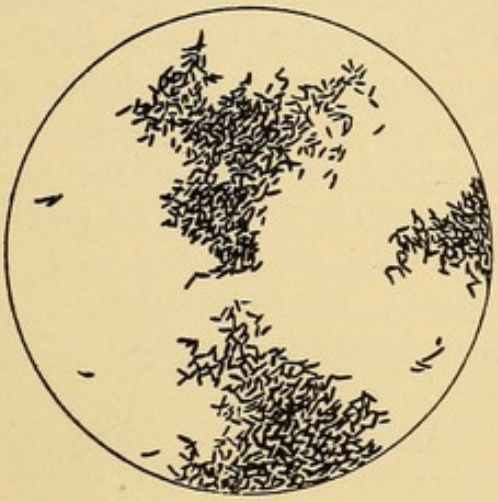


FIG. 153.—A positive Widal reaction. (*Da Costa.*)

reaction similar to that given by typhoid fever. This statement is true only when the question of dilution of the serum is not taken into account. The characteristic Widal reaction is shown when the blood is diluted 1 to 50 with water or, in many cases, at very much higher dilutions, while in other diseases and in healthy subjects the reaction practically disappears at a dilution of 1 to 20. The element of time at which this reaction is evident is also of great importance in judging the result. While the reaction in cases of typhoid fever may occur instantly in a dilution of 1 to 10 or 1 to 20, and in dilutions of 1 to 50 it may take one-half to three-quarters of an hour, yet a period ranging from one-half to one hour is necessary with other sera at a dilution of 1 to 10 or 1 to 20.

In order, therefore, to demonstrate the specific typhoid reaction it seems necessary to increase the dilution and to limit the time. It is the practice in the writer's laboratory to use in every case a dilution of 1 to 50 and to wait 45 minutes before judging of a reaction. A reaction is considered positive only when marked clumping is evident and when complete loss of motility is present. An incomplete reaction, pointing to the possible presence of typhoid

fever is shown by a moderate clumping and by a diminished rate of motility, although some motion may still be present. It should be remembered that when dried blood is used instead of the whole blood or serum, that a slight tendency to produce agglutination is observed, due to the presence of the fibrinous masses, so that marked clumping without loss of motility should not be considered even as significant of typhoid fever.

In performing this test the serum is diluted, preferably 1 to 50, with distilled water, and a drop of this diluted serum is placed upon a cover-slip. A loopful of a bouillon culture of actively motile typhoid bacilli is then mixed with this drop of diluted serum and the ordinary bacteriological hanging-drop preparation made. The specimen is best studied with the high-power dry lens, as the oil-immersion is much more apt to break the cover-slip. The organisms will be seen, in specimens which are properly lighted, as actively motile bacilli moving with their twisting motion throughout the field. In some cases this motility will be observed to diminish almost at once, while in the ordinary case 20 to 45 minutes are necessary before complete loss of motility ensues. In some cases loss of motility without any tendency to agglutination is observed. This may be referable to the fact that the specimen has become dry, so that it should, therefore, be discarded for a fresh one.

The time at which the Widal reaction may appear in the course of typhoid fever is of some importance. The earliest date of appearance of a positive reaction is rather hard to determine, but definite reports of positive results as early as the second day have been given by Fraenkel and others. It is frequently found before the appearance of rose spots, splenic tumor, or the diazo reaction in the urine, but it is practically never found when the blood shows a normal or an increased number of leucocytes. The writer has found this association of leucopenia with the Widal reaction so constant that he is frequently inclined to omit a Widal reaction when a leucocytosis exists. In practically all cases of typhoid fever a definite Widal reaction may be obtained at some stage of the disease, but there have been cases reported in which no Widal reaction was evident throughout the course of the condition. It would seem that such negative cases might be explained by faulty technic or by the fact that they were of a very mild type so that a bacteriemia did not obtain. The agglutinating power of the blood seems to reach a maximum at the period of defervescence and often falls rapidly during convalescence. There are, however, some cases in which the Widal reaction has been evident one year after the acute attack without any signs of intermediate relapses.

Other organisms beside the typhoid bacillus give such agglutination tests. The technic is the same and the results are comparable. Differentiation of the etiologic factor in many obscure conditions is often possible through the application of this principle of Pfeiffer. This test is clinically almost completely identified with the bacillus typhosus, although the paratyphoid and colon bacilli are occasionally studied in this way and may give rise to much confusion and misinterpretation, if the typhosus culture be not pure.

VIII. Special Characteristics of the Blood.

This section of hematology is very closely associated with pathology and biochemistry and can, therefore, be taken up only in brief outline. Our knowledge of the great importance of the fluid portion of the blood is so rapidly increasing that soon the changes in its cellular composition will be of secondary importance. As a matter of fact, changes in the number of the cells as well as in the percentage of hemoglobin must be dependent, to a certain extent, upon the more obscure changes which are taking place in the plasma in various diseases. Our ability to fathom the secrets of the many physical and chemical changes of the plasma, has been so slight that we have hitherto neglected to take into consideration anything but the changes in the cells, which can be so easily studied by the various methods previously outlined. By the elaboration of the side-chain theory of Ehrlich and of the opsonic theory of Wright, we have come somewhat nearer to a proper realization of the importance of the plasma in all infections as well as in many diseases in which great metabolic disturbance is evident. For that matter one can hardly realize a condition in which the blood plasma may not show some characteristic change, inasmuch as the nutrition of the entire body can come only through the blood. When one considers the close correlation of the various organs he may see at once that pathological changes in any of the viscera will result in an abnormal blood, which may show no changes at present capable of detection. For this reason one hails with delight any advance in serum pathology and could but wish that his knowledge would more rapidly increase.

Phagocytosis.

According to the theory of Metschnikoff, the leucocytes are capable of incorporating into their substance materials which are foreign to the blood in which they circulate. This process is known as phagocytosis and is one of the greatest protective measures which the system has for its fight against bacterial invasion. When the blood becomes laden with bacteria, as in the various infectious diseases, we find a leucocytosis in practically all cases, the exceptions having been previously noted. This is the natural sequence if the system is to rid itself of these invaders. The leucocytes are drawn by chemical attraction (chemotaxis) toward the bacteria and attempt to swallow them by throwing pseudopodia about them and drawing them into the protoplasm. This is successful in many cases, while in others it is not, so that the question of ascendency of the leucocyte or of the bacterium will depend upon the degree of phagocytosis. Strangely enough much variation is shown in the susceptibility of different organisms to phagocytosis. Thus the pneumococcus at times is very difficultly amenable to phagocytosis, while at others it is easily acted upon. The recent work of Rosenow[1] has thrown much light upon the mechanism of this action.

[1] Jour. of Inf. Dis., vol. 3, 1906, p. 683.

Opsonins.

Realizing that there was a more definite basis for phagocytosis than was embraced in the older conceptions, Wright[1] introduced the idea of opsonins to designate the presence in the blood serum of substances which render the various bacteria subject to phagocytosis. The normal blood serum contains such opsonic material for the various bacteria with which it may be infected, but this varies greatly toward the different organisms. Thus we may find individuals showing much more opsonin (a higher opsonic index) toward one organism than toward another. This explains, in a way, the well-known fact that different people are variably susceptible to the same disease, while the same individual may be strongly resistant toward infection with one organism, but easily a victim of another infection.

Regarding their clinical nature very little is known. There seems to be a certain amount of evidence which points to the fact that these opsonins belong to the class of globulins, while Simon and Lamar[2] have shown that they are apparently associated with the euglobulin fraction. Quite as little is known of the structure of the opsonins, so that it is at present doubtful in what position to place them in the side-chain theory. According to Hektoen,[3] they may contain a haptophoric group which unites with the bacterial or other receptors and also an opsonipherous group which brings about changes in the cell, making it capable of phagocytosis. According to Savtchenko and Dean, the opsonins should be regarded as amboceptors, while Greig-Smith looks upon the process of opsonification as the first stage of agglutination. All of these theories must wait for future confirmation. The opsonins are thermolabile and are usually destroyed by heating for ten minutes to 60° C. They occur in all classes of vertebrates and show here a peculiar characteristic, namely, that the serum of different animals is capable of activating various organisms for phagocytosis by leucocytes of animals of different species. This would bring the opsonins into the same field as the agglutinins, precipitins, and hemolysins.

From a clinical standpoint, opsonins are frequently found to be diminished in certain bacterial infections. It is, therefore, conceivable that the resistance of the patient or, in other words, his phagocytic power might be increased by the addition of substances which could act as opsonic material. Such substances are the bacterial vaccines of Wright, suspensions in physiological salt solution of dead cultures of the organism to which the patient shows a diminished power of phagocytosis. The relation of the phagocytic power of the patient, as evidenced by the number of organisms which a definite number of leucocytes takes up under the opsonifying influence of this serum, as compared with the same condition in the case of the serum of a normal individual, is known as the *opsonic index* toward the organism investigated. The

[1] Proc. Royal Soc., vol. 62, 1903, p. 357.

[2] Johns Hopkins Bull., vol. 17, 1906, p. 27.

[3] Jour. A. M. A., vol. 46, 1906, p. 1407; ibid. vol. 48, 1907, p. 1739; Ill. Med. Jour., vol. 13, 1908, p. 9.

number of bacteria taken up by the leucocytes of the normal individual is taken as one.

According to Wright, the injection of a dose of vaccine is followed by a decrease in the opsonic index (the negative phase), which is of variable degree and duration, depending upon the dose. This negative phase is followed by an increase in the opsonic power of the leucocytes (the positive phase), which is associated with improvement in the condition of the patient. The various doses of the vaccine should be so administered that it is never given during a negative phase. While a low opsonic index is the rule in chronic cases, high indices may be observed with active systemic manifestations of acute cases. As a rule, it is more generally beneficial to use a vaccine prepared from the discharges of the patient than it is to use an already prepared vaccine of the same organism. The reason for this is that so much difference exists in the various strains of the same organism that little or no result may follow the administration of a stock vaccine. These autovaccines are very easily prepared by one having bacteriological facilities and can be administered by the attending physician. It is not always easy to gauge the dosage of a vaccine in any particular case, as the idiosyncracy of the patient plays such an important rôle. It is better practice, therefore, to start with a small dose and obtain no reaction than it is to give an overdose which will prove very harmful. Failure to observe this precaution is, in the writer's opinion, the reason that tuberculin so soon fell into disrepute and is now being restored to such great favor.

It has been found by various workers that the administration of a proper dose of a vaccine is followed by certain local symptoms, such as swelling, redness, and tenderness at the point of injection, while certain systemic symptoms, such as rise in temperature, general malaise, and pains in the joints, are practically always evident. It has, therefore, become the practice of many of our workers, to gauge the results of vaccine treatment more by the reaction than by the determination of the opsonic indices of their patients. The routine of such treatment is to give an injection of bacterial suspensions about once a week, oftener only in cases which have shown no reaction following a previous injection. One great advantage of an autovaccine over a stock vaccine is that the former contains all of the organisms bringing about the infection. Affections which are amenable to treatment by vaccine therapy are practically always of the mixed variety so that a stock vaccine will increase the opsonic index and thus clear up only the infection with that specific organism. Recent work, especially in cases of gonorrhea, has shown that autovaccines do not always give better results than stock vaccines, for the reason that the patient has become so accustomed to his own autogenous material that a difference in strain is sometimes very beneficial. As a rule, the beginning dose of a staphylococcic vaccine is 500,000,000 organisms, while that of gonorrhea is approximately 40,000,000. There does not seem to be at present a well-established dosage for the streptococcic vaccine, so that each case must be a law unto itself. The most amenable cases for treatment by bacterial vaccines seem to be cases of

long-standing suppurative conditions induced by the various pus-forming organisms. Some obscure infections, which the writer has seen treated with the colon vaccine, have cleared up in a surprising manner.

The technic of the opsonic determinations as well as of the preparation of the vaccines is at present so little used by the general worker that the writer must refer to the original work of Wright and of Douglass for such detailed information.

The general principles of such work are as follows. For the opsonic determinations four factors are essential: (1) the patient's serum, (2) the normal control serum, (3) the washed leucocytes, and (4) the bacterial emulsions. One volume of washed corpuscles is drawn into a capillary tube and is followed by one volume of the patient's serum and then by one of the bacterial emulsion. The contents of the capillary are then thoroughly mixed by blowing the material into a dish and redrawing it into the capillary. A similar mixture is then made using the control serum. Both capillary tubes are now incubated for 15 minutes at 37° C. A drop of the mixture is blown onto a glass slide, a smear is made, fixed, and stained with any of the polychrome dyes or with special bacterial stains.

The bacillary index is then determined by counting a series of 50 to 100 polynuclear leucocytes and observing the number or organisms in each. The phagocytic index represents the average number per leucocyte. The opsonic index is then calculated by dividing the patient's value by that of the normal control.

Ehrlich's Side-chain Theory.[1]

The early work of Ehrlich, published in 1885, advanced a theory to account for various phases of immunity, especially of the action of the blood in producing antitoxins against various poisons elaborated by infectious agents. It is to be said that no such formation of antitoxin against the ordinary medicinal poisons has been found.

According to this theory, the protoplasm of the cell consists of a central group of molecules (Leistungskern), in which the inherent vital characteristics of the cell are located and whose integrity is necessary for normal cell life. At different portions of the cell certain other molecular groups are attached exactly as side-chain groups are attached to the benzene nucleus of organic chemistry. These groups or, as Ehrlich styles them, side-chains are capable of uniting with various material which is brought into intimate relationship with the cell structure. Such materials are foods, toxins, and other injurious agents. In order that food may be taken up by the cell it must possess certain groups which will enable it to combine with the groups in the side-chain of the cell. It must be, in other words, homologous or, as Ehrlich states, must bear the same relationship to the side-chain which Fischer has applied in his assumption of the "key in the lock" hypothesis regarding ferment action upon the

[1] See Adami's Pathology, 1908; Sachs, Wiesbaden, 1902.

various types of hexoses. In the nomenclature of Ehrlich the side-chains are styled *receptors* and the group of the food or of the toxin which combines with these receptors is known as the *haptophore* group. These receptors, as well as haptophores, possess specific affinity, uniting with one another only when homologous.

It has been found that a toxin molecule has certain injurious effects upon the cell; it is, therefore, necessary to ascribe this action to other than the two groups above mentioned, as the union of a haptophore with a receptor would form an inert substance. Ehrlich, therefore, assumes the presence in the toxin molecule of a second group which he styles the *toxophore* group, which exerts the untoward effect upon the cell. The toxophore group in itself can unite with the cell only through the medium of its haptophore group. As the

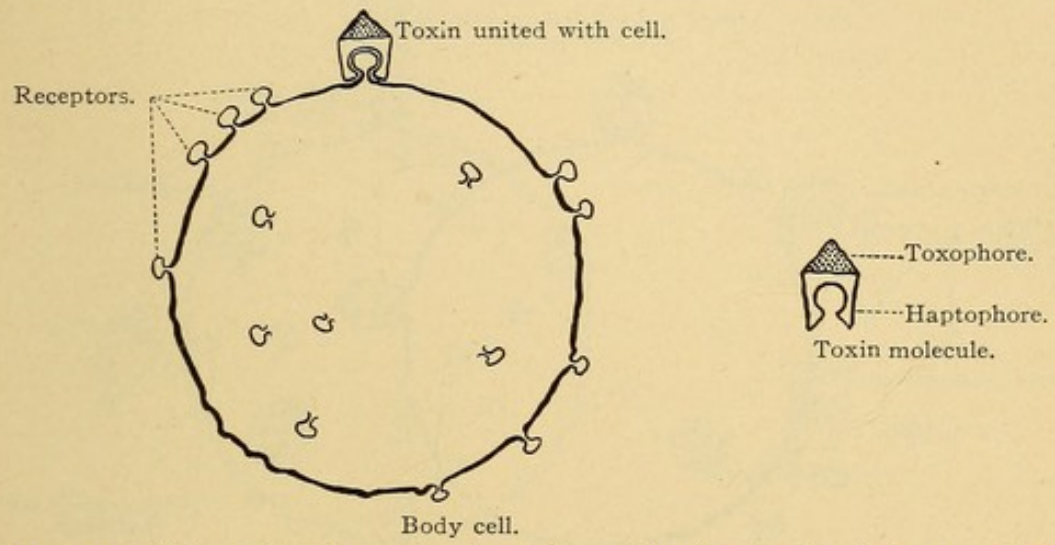


FIG. 154.—Illustrating the mechanism of the toxin-cell union by the intermediation of receptors. (*Da Costa.*)

cell becomes irritated by the presence of the toxophore group, it endeavors to overcome this by a new formation of receptors. According to the strength of the irritation, many more receptors will be found than can combine with the toxin material present, so that many of the extra receptors pass out into the circulation in the form of free receptors. This is a graphic explanation of the fact that the cell when irritated by toxic material elaborates substances from its own protoplasm, which have a neutralizing effect upon the toxic substance. These free receptors (haptines) form the antitoxins. They combine only with homologous toxin material and are, therefore, specific. Welch believes that these antitoxins have a second function beside that of neutralization of toxin, namely, an irritating one upon the bacterial invaders so that these organisms are forced to elaborate similar substances for their own protection. Toxins which have been deprived of their toxophore group are known as *toxoids* and can combine with the receptors of the cell, exerting no untoward effect upon the cell. These toxoids may also unite with antitoxin through the medium of their haptophore group. Occasionally toxins are incompletely combined with the anti-

toxins; that is, the antitoxic material is not sufficient in amount to completely neutralize the toxin, so that such toxins may still combine with the cells and exert a modified poisonous effect. Such attenuated toxins are known as *toxones*.

It has been found that the injection into animals of bacteria, various body cells, and certain secretions of some animals, as for instance, snake venom, gives rise to the development of specific antibodies in the blood serum of the animal so treated, the substances injected being named antigens. Such blood or serum will be found to have a lytic (destructive) action, upon cells similar to those injected. Such sera are specific; that is, they act only upon the kind of cell used in the injection. The term hemolysis has been introduced to express the destructive action upon the erythrocytes shown by the dissolving out of the hemoglobin from the red cell. The stroma or discoplasm of the

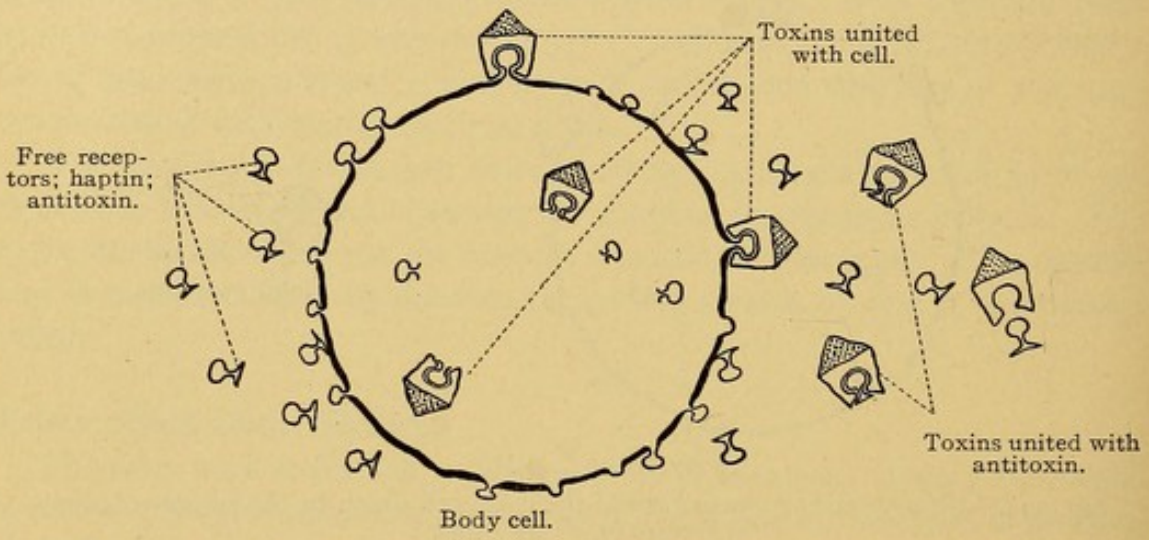


FIG. 155.—Illustrating the elaboration and action of antitoxin. (*Da Costa.*)

red cell is a membrane which shows peculiar relations to diffusion of various materials into the cell and to the passage of hemoglobin and other cellular material from the cell. Its chief function seems to be to prevent, as far as possible, any loss in hemoglobin. If this membrane becomes permeable, then we must assume the action of some toxic material. The term *hemolysis* has reference merely to the abnormal loss of hemoglobin and not to any disturbance beyond increased permeability of the stroma. The stroma of these cells remains behind and may be seen in the centrifuged specimen as the so-called shadows. Hemolysis must, therefore, be considered as a sign of protoplasmic death. Substances (hemolysins) bringing about such change belong, necessarily, in the class of blood poisons. Such hemolysins are increased or lowered temperature, various inorganic compounds, such as distilled water, ammonium salts, and organic compounds, such as urea, bile acids, ether, alcohol, chloroform, solanin, saponin, and digitalin. The saponins are among our strongest hemolytic substances, acting in dilutions of 1 to 100,000. Besides these we

have various secretions, such as those of the cobra, spider, and the bees which are active hemolytic agents.

It has been observed by various workers that hemolysis is prevented when the serum is heated to 60°. This points to the fact that some substance is destroyed which is of great importance in this process. In addition it has further been found that the renewing of the activity of the old heated serum by adding a supply of fresh isologous serum will restore the hemolytic activity. It is evident, therefore, that there are two factors which must be taken into consideration, one is a thermostable (heat-resisting) substance, while the other is thermolabile (destroyed by heat). To the first of these Ehrlich has given the name *amboceptor* and to the second the name *complement*. The amboceptor

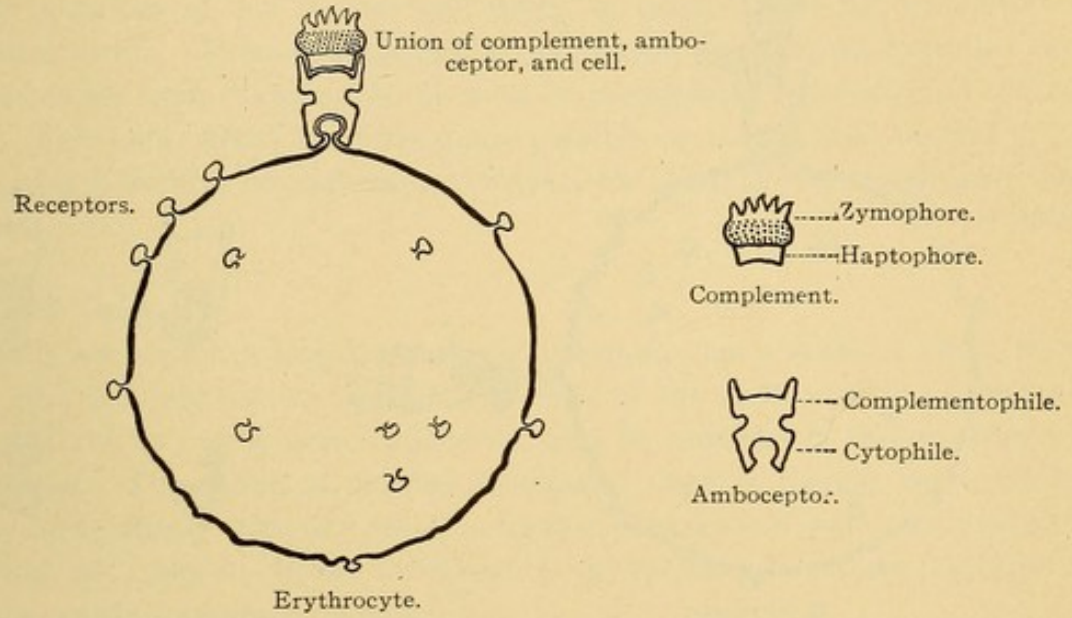


FIG. 156.—Illustrating the mechanism of hemolysis. (*Da Costa.*)

has been shown to have two haptophore groups, with one of which it unites to the receptor of the cell and with the other to the complement. The haptophore group which unites with the cell is known as the cytophile, while the one uniting with the complement is termed the complementophile. For hemolysis, therefore, we must have the cell receptor, the amboceptor, and the complement. The complement has also been shown to have two groups analogous to those of the toxin molecule. The first is the haptophore group, while the second is the zymophore group, through which the destructive action upon the cell is manifest.

The amboceptor is formed within the body as the result of cellular hyperactivity aroused by the irritant action of the toxic material. The complement is probably derived, for the most part, from the leucocytes, and acts very much as an enzyme. It can exert its toxic action only when united with the cell by means of the amboceptor, so that free complement has no injurious effect.

It has been frequently observed that the red cells are more resistant than normally, while in many cases they appear less resistant to hemolysis. This is explained by the side-chain theory very much as it explains the formation of antitoxin. These antihemolysins are formed within the blood plasma after inoculation with hemolytic material. The hyperactivity of the cell causes it to throw off two types of such bodies, namely, *anticomplement* and *anti-amboceptors*. The former combines with the haptophore group of the complement and the latter with the cytophilic group of the amboceptor, each of these combinations making it impossible for the necessary union of cell, amboceptor, and complement to occur.

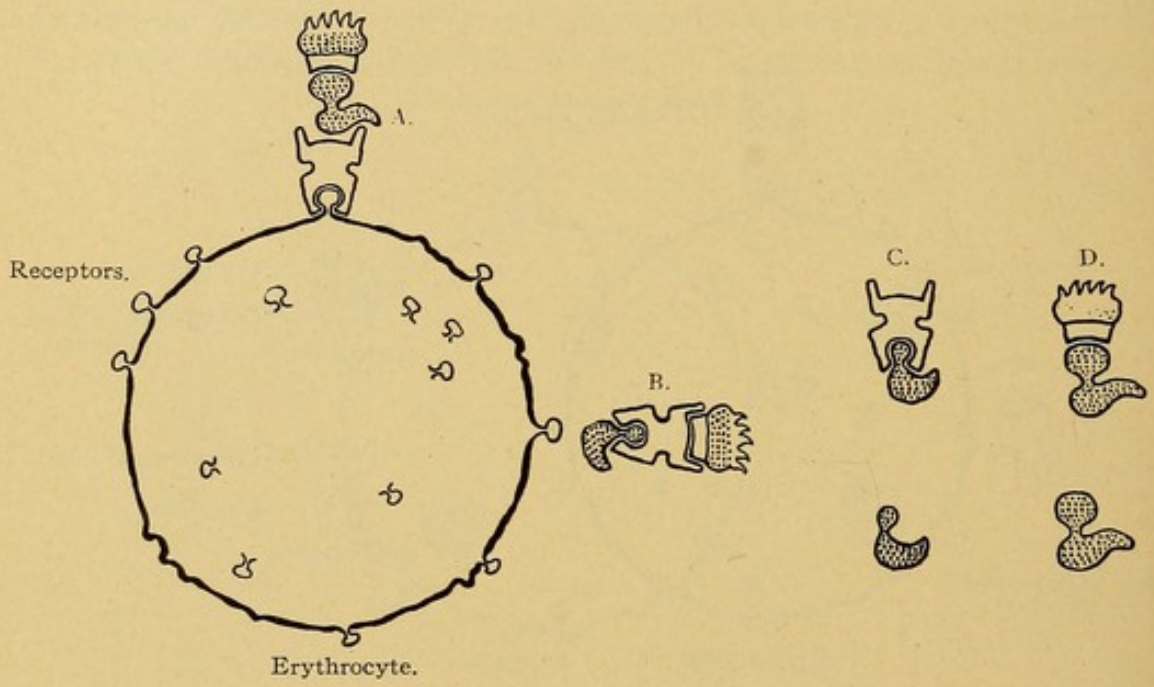


FIG. 157.—Illustrating the Mechanism of Antihemolysis. (*Da Costa.*)

A, Interference of anticomplement with complement-amboceptor union. B, interference of antiamboceptor with amboceptor-cell union. C, antiamboceptor-amboceptor union. D, anti-complement-complement union.

It has been found that frequently the serum of an animal, which has been injected with certain bacteria or with certain body cells, shows the peculiar property of agglutinating or clumping such bacteria or cells when these latter are added to it. This condition is known as agglutination and the agents bringing it about are styled *agglutinins*. These substances are developed in the blood of the animal during the process of adaptation toward the presence of such foreign material. Agglutinins from the standpoint of the side-chain theory are free receptors, having a haptophore group which unites with the receptor of the cell or of the bacterium, and cause agglutination through the presence of a second group known as the zymophore or agglutiniphore group. Agglutination, therefore, does not require the presence of a complement.

In some cases, intraperitoneal injection of body fluids or of solutions of certain proteins into animals brings about a condition which enables the blood

serum of such animals to cause a precipitation of the protein to which the animal has been adapted. This fact has been taken advantage of in formulating a medicolegal method for the detection of blood of different animals and will be taken up in a later section. The *precipitins* consist of free receptors combining, by means of their haptophore group, with the receptor of the cell and exerting their precipitating effect through the medium of their zymophore or precipitinophore group.

IX. Medicolegal Aspects.

In medicolegal examinations one is frequently called upon to determine the identity of various suspected stains. Such examinations may tax the entire resources of the worker and have, in many cases, led to absolutely negative results. It is not sufficient at the present day that the examiner state that a certain stain is blood, but he must be prepared to say what kind of blood it is. This latter point has been made possible by recent work so that one is fairly sure of limiting his statement at least to the blood of very closely associated animals.

(1). Red Cells.

It is not always a simple matter to determine that a stain on cloth, wood, iron, etc., is really due to blood. The color of the stain may be of any hue from dull red to a dirty gray, depending upon its exposure to various elemental conditions. The action of various substances, such as mortar, brick, or lime in any form, strong acids and alkalies, leather, chemicals in wall-paper, starched clothing, etc., may so change the blood or its reactivity to certain tests that no definite conclusions can be obtained.

It is self-evident that the demonstration under the microscope of blood-cells is the surest proof of the presence of blood, the size and shape of the corpuscles frequently giving a clew as to the source of the blood. It becomes, therefore, necessary to make a suspension of the stained material preferably in isotonic (0.9 per cent.) sodium chlorid solution. The degree of solubility of the stain will depend upon the age of the stain, the heat to which it has been subjected, the amount of sunlight or of moisture to which it has been exposed, and the material upon which the stain is formed. Various fluids have been advanced, from time to time, as dissolving agents for the stains, many of these are active laking or hemolytic agents, especially for dried red cells, so that one sees in such preparations only the shadow of the red cell. Such laking agents are distilled water, 0.85 per cent. ammonium chlorid solution, 50 per cent. glycerin solution and many others. Frequently a strong 30 per cent. potassium hydrate solution will enable the worker to obtain a very good idea of the presence or absence of red cells. Marx's fluid (made as follows, hydrochlorate of quinin 10 c.c. of a 1 to 1,000 solution added to 10 c.c. of 33 per cent. potassium hydrate solution, and tinted with eosin which will

stain the erythrocytes a characteristic reddish hue) is a very excellent examining fluid.

As the presence of the red cells is so hard to demonstrate, search for them is frequently omitted and reliance placed more upon the demonstration of the hemoglobin in such stains. If the stain is upon a hard surface it may be scraped off with a clean piece of glass. If it be upon cloth, a portion of the stained part and also of the unstained part is removed and cut into small pieces with scissors which are absolutely clean. Such small pieces not broader than 1 mm. are very easily handled and can be readily teased out if desired.

(2). **Guaiac Test.**

This test originally devised by van Deen is one of our oldest for the detection of blood coloring matter, but seems to be much more reliable in its negative phase than when positive. The principle of the test is as follows: To a watery solution of the suspected stain is added an equal portion of fresh tincture of guaiac. This tincture is best made by dissolving the guaiac resin in alcohol when needed. The addition of the guaiac tincture to the suspected solution causes a milky turbidity. On now adding ozonized oil of turpentine, peroxid of hydrogen, or oil of eucalyptus, so that these latter substances float upon the guaiac and blood solution, a distinct blue color will be manifested at the point of contact, and, on shaking the tube, the coloration will spread throughout the mixture. This test seems to be very delicate, demonstrating the presence of blood in a dilution of several thousand. The blue coloration observed in this test is due to the oxidation of the guaiac to guaiaconic acid, which is then further oxidized into guaiac blue by the catalytic action of the oil of turpentine. It has been shown by Taylor that this reaction is also given by many substances among which we find manganate and permanganate of potassium, peroxid of manganese, peroxid of lead, chlorin, bromin, iodin, nitric and chromic acids, ferric chlorid, salts of copper, ferro- and ferricyanid of potassium, gum acacia, gluten, unboiled milk, raw potato pulp, pus, and any living cell or its intracellular enzymes. Various enzymes such as the oxidases will give this reaction. Buckmaster advises the use of the pure guaiaconic acid along with peroxid of hydrogen in the place of the guaiac resin and believes that the test increases in delicacy and accuracy under these conditions. He states that fluids containing hemoglobin or most of its derivatives give this test when it is impossible to detect pigments by any other methods and rightly adds that boiling the fluid suspected of containing blood does not interfere with the reaction but, on the contrary, throws out of consideration the action of milk, pus, fibrin, or of any enzyme. For the proper performance of this test the fluid to be tested, should not be alkaline and only very slightly, preferably not at all, acid.

(3). **Schaer's Test.**

This test is similar in many ways to the van Deen test, but employs, instead of guaiac, a 1 to 4 per cent. solution of aloin in alcohol. On adding this tincture to the suspected solution a red color soon becoming distinctly cherry-red appears

upon the addition of ozonized oil of turpentine. Many other substances give a pink color, but only after the lapse of one or two hours, while the color with blood appears in a very short time. The tincture of aloin should always be freshly prepared, as of itself it undergoes this color change after standing.

(4). **Teichmann's Test.**

This is one of the most important tests for the presence of blood and when positive is conclusive proof of the kind of stain with which one is working. A drop of blood or a portion of the suspected stain is spread upon a glass slide and covered with a drop of a very dilute solution (0.85 per cent.) of common salt. The salt solution is then evaporated at a low temperature. A few drops of glacial acetic acid are then placed upon the salted stain and the preparation



FIG. 158.—Hæmin Crystals from Human Blood. (*Hawk.*)
Reproduced from a micro-photograph furnished by Prof. E. T. Reichert, of the University of Pennsylvania.

is covered with a cover-glass. The acid is now slowly evaporated by holding the slide over a flame in such a way that the fluid steams but does not boil. As the acid evaporates, more is allowed to run under the cover-glass, this addition and evaporation being done thrice. The specimen is cooled and mounted in glycerin or distilled water, after which it may be examined with the dry lens. A successful specimen, which is not always obtained, will show the presence of numerous brownish rhomboid crystals, which are separate or grouped in sheafs or rosettes. These are the *hemin crystals* or hydrochlorate of hematin, called, after their discoverer, *Teichmann's crystals*.

It very frequently happens that the specimens are not successful, so that several slides should be made before a negative report is given. Reasons for failure may be found in the excessive heat applied to evaporate the acid, in the fact that the salt solution may have been too concentrated, or that the

stain may have been very old. The addition of salt seems to be unnecessary except in the case of old blood stains or when the blood is poor in salts. It should even then be added in only very small amounts. As pointed out by Rose, when blood is mixed with iron rust the hemin test is usually negative.

(5). **Spectroscopic Examination of Blood.**

Certain characteristic appearances of the blood are noted on spectroscopic examination. The spectrum of the various blood pigments has been given in previous sections so that only a few additional remarks are necessary in this place. In spectroscopic examinations, the use of the ordinary hand spectroscope is all that is necessary, the more complicated ones adding little to the differentiation of the various spectra. If the blood be fresh and the hemoglobin unaltered the spectrum is, of course, that of oxyhemoglobin; but as the usual stains submitted for examination are frequently very old ones, such a spectrum is practically never seen. The end-product of the alteration of hemoglobin as found in old blood stains is hematoporphyrin, which is iron-free and can be identified easiest by the spectroscope. The suspected stain is dissolved in concentrated sulphuric acid yielding a reddish-violet fluid, which is then examined spectroscopically. The spectrum of hematoporphyrin has been given previously. Many blood stains can be recognized only by this test, so that it is always advisable to make a spectroscopic examination, especially if other tests for hemoglobin have failed.

(6). **Precipitin Test.**

The work of Wassermann and of Uhlenhuth[1] has shown that blood stains may show their origin on the application of the precipitin test to such work. The principle of this test is as follows: A rabbit is injected intraperitoneally with 5 to 10 c.c. of blood serum. At intervals of three to five days other injections are given until six or eight in all have been made. As stated previously, the serum of the rabbit so injected shows the power, when added to a solution containing serum or hemoglobin of the same species of animal against which the rabbit is immunized, of precipitating the albumin in the form of a light flocculent precipitate. In testing a suspected stain the material is dissolved in physiological salt solution or in 1/10 per cent. sodium hydrate. Ziemke recommends, if the stain is old or fails to dissolve readily, the use of concentrated potassium cyanid solution which is later neutralized with tartaric acid. The solution if turbid should be cleared by filtration or centrifugation. To this clear filtrate is then added the active serum in a dilution of 1 to 50 or even 1 to 100. The very small-sized test-tubes, about 2 inches in length and 1/4 inch in diameter, are very satisfactory for such dilutions and tests. It is necessary at the same time to run controls of this reaction. We therefore prepare a solution of known human blood, one of the solutions used in dissolving the blood stain, one of blood from another animal, one of human blood to which normal rabbit serum is added, and one of the dissolved stains which remains

[1] Jena, 1905.

untreated. These specimens are then placed in the thermostat at 37° C. A positive reaction is indicated by a distinct turbidity appearing instantly or within a few minutes and by the formation of a flocculent precipitate within three hours. This reaction seems to become less distinct the older the specimen, but in no case does it absolutely fail. This test is specific in the sense that only animals of closely related species will react to the same immune sera. Although reactions may occur with sera from different animals, most reactions with heterologous blood may be avoided by the use of dilutions of 1 to 50 or more. According to Ewing, the following sources of error may creep into this reaction. (1) turbidity or precipitation may occur in any blood from too slight dilution of serum. (2) Potent sera considerably diluted (1 to 20) cause turbidity in solutions of blood from closely related animals. (3) The chemicals used may be impure and cause precipitates. (4) Chloroform when added to prevent bacterial growth does not always inhibit all bacterial development and precipitates occurring after three hours may be referable to this cause. (5) The reaction of the solution may be too acid or too alkaline and the test fails or occurs in heterologous blood. (6) Human exudates or excretions may be present in the solution and react as blood albumins.

The active serum precipitates albumins from exudates, from sputum, from pus, urine, and feces of the same animal species, so that the suspected stain must be previously identified as blood by the tests outlined above. In no other phase of blood work is a faultless technic so necessary as in the application of the precipitin test. It is, therefore, wise to give a positive report on the origin of a stain only when a distinct flocculent precipitate has occurred at a dilution of 1 to 50 in a period of one to three hours. Any other result should be reported as suspicious or as probably of human origin.

X. Value and Limitations of Blood Examinations.

It has been truly said that the value of a blood examination "is measured by the practical use which may be made of it, and not by any interesting yet useless information it may throw on the case." In clinical work we are prone to follow well-established lines of procedure and to forget, when engaged in counting the cells in an obscure case, that "the meaning and aim of the clinical study of the blood covers a much wider field than is embraced in the mere investigation of the histological elements." Yet our knowledge regarding the variations in the plasma is so meager that the busy practitioner may be pardoned for such neglect.

There can be little question that the so-called "blood diseases" have the most characteristic variation from the normal, yet even here it is not so easy, as one might assume, to make an indisputable diagnosis in all cases. Thus, aside from the pure parasitic diseases, leukemia and advanced pernicious anemia seem to be the only ones capable of a certain diagnosis from the examination of the blood. True the various anemias may be detected, but the etiology

is not always clear nor the differentiation of a primary from a secondary form invariably possible.

It is, of course, evident that bacteriologic examination of the blood and the application of the agglutination reaction may clear up many an obscure case, but such work requires much more time and much more attention to detail than is at the disposal of the busy practitioner. It is, however, in just this class of infectious fevers that much benefit, in a confirmatory way, is forthcoming from a careful study of the blood. Thus, a leucocytosis or a leucopenia in any suspected case might lead to a differentiation of central pneumonia from typhoid fever or a scarlet fever from a case of measles. Moreover, a leucocytosis following a leucopenia in typhoid would strongly indicate perforation and consequent surgical interference. Suppurative processes anywhere in the system are usually associated, unless very limited in extent, with a polynuclear neutrophile leucocytosis; so that the leucocyte count may be of great importance in judging of the extension of a pus infection. Immediate operation is occasionally decided upon after a leucocyte count, but this should not be made the only basis for such intervention unless frequent counts have given the surgeon the definite knowledge that a leucocytosis has suddenly occurred.

It should be stated as an axiom that blood findings may never be interpreted except in the light of the clinical findings. To the clinician it should be said, never trust the laboratory report implicitly unless it agrees with the clinical manifestations; to the laboratory worker we would say, never report a blood finding as diagnostic without knowing something of the clinical history of the case.

A single blood examination will rarely be of any greater value than will a single temperature determination. It is the series that enables one to decide either as to diagnosis or as to operative or therapeutic procedures. What we need most is not so much blood work, but much better work when done. Too much reliance is often placed on blood reports, so that a word of caution does not seem ill advised. Never rely solely on the blood findings, but use them merely as one of many links in your chain of clinical evidence. In this way mistaken diagnoses will occur less frequently.

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CHAPTER IX.

TRANSUDATES AND EXUDATES.

I. General Considerations.

The serous membranes are normally kept moistened by liquids whose quantity is only sufficient in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under pathological conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues or under the epidermis. If such conditions be the result of circulatory disturbance, the kidneys are usually unable to eliminate the normal amount of fluid from the system and, as a result, the retained fluid collects both in the serous cavities and in the areolar tissue. Such accumulations of fluid, known as *transudates*, are similar to the lymph, being, as a rule, poor in cellular elements and yielding little or no fibrin. These transudates must be sharply differentiated from the accumulations of fluid, which are the result of direct inflammatory processes in the membranes lining the serous cavities and are known as *exudates*. These latter fluids are generally rich in cellular elements and yield relatively more fibrin. As a rule, the richer a transudation is in leucocytes, the closer it stands to pus, while the poorer it is in these elements the closer it resembles true lymph.

The formation of true transudates is largely a question of filtration under the influence of the rate of blood flow, the blood pressure, the irritation of the capillary endothelium, and the variable permeability of the endothelial cells. We should expect, therefore, that the passage of dissolved substances from the blood would be regulated by the same laws that control the secretion of physiologic fluids, namely the laws of passage of fluids through semipermeable membranes. The crystalloids would be, therefore, in approximately the same concentration as in the blood plasma, while the colloids must be in far less concentration, the actual values being influenced, of course, by the special membrane through which the fluid passes. The condition of the blood would hence affect the chemical composition of such transudates, hydremic plasma yielding a fluid poorer in solids, while anhydremic blood is associated with a transudate of higher specific gravity.

From a clinical standpoint a differentiation between transudates and exudates is not infrequently impossible, so that it is advisable to resort to aspiration of the fluid and to chemical and microscopical examination of the material withdrawn. The chief phases of such examinations are: (1) the chemical and physical properties of the fluid; (2) the bacteriological aspect of the fluids, and (3) the morphological characteristics of the cellular elements.

Obtaining the Specimen.

Whenever fluid is to be withdrawn, either for diagnostic or therapeutic purposes, it is necessary to resort to puncture of the cavity containing the fluid. In all cases the site of puncture must be as carefully prepared as in any surgical procedure. Puncture may be performed with the ordinary trocar or, preferably, with a large needle which has a rather large lumen. The instruments must be carefully sterilized before use. If the skin is especially tough, it is advisable to make a small incision through the skin and insert the needle through the incision. Very little pain is felt by the patient during this procedure, but the writer is accustomed to invariably resort to the use of ethyl chlorid to anesthetize the part.

In the withdrawal of pleuritic effusions the spot selected should be neither too high nor too low. It may be in the seventh intercostal space in the axillary line or in the eighth intercostal space at the outer angle of the scapula. The arm of the patient should be brought forward with the hand resting on the opposite shoulder, in order to widen the intercostal spaces. In inserting the needle it is wise to make the thrust close to the upper margin of the rib so as to avoid the intercostal artery. In all cases the fluid should be withdrawn slowly and the excess above that required for examination allowed to drain until the desired amount is obtained. If the puncture is for diagnostic purposes, 10 to 20 c.c. are sufficient, while the therapeutic withdrawal of fluid will vary with the amount present and with the clinical symptoms of the case. Should the patient show signs of shock or of depression during the operation, the procedure should be interrupted as quickly as possible. Aspiration is rarely necessary or advisable.

In the withdrawal of fluids from the abdominal cavity, the needle or trocar is thrust through the lower abdominal wall and the fluid collected. In this procedure more or less danger of puncturing the bowel exists if the effusion be small, so that the needle should not be carelessly inserted lest this complication arise. Naturally, in well-marked ascites no such danger is present.

II. Physical and Chemical Properties.

Transudates are, as a rule, serous in character, usually transparent, colorless, or light yellow in color, but at times showing a milky, reddish, or a greenish tinge, the latter practically always being observed after the fluid has stood exposed to the air. Such solutions are, as a rule, dichroic, yellow by transmitted light and green by reflected light. They are alkaline in reaction and show a specific gravity, which varies, according to the origin of the fluid, from 1006 to 1018, while serous exudates from the same cavities show a much higher specific gravity. The variations in specific gravity depend largely upon the amount of albumin present in the transudate, this practically never being over 3 per cent. and usually much lower. The chief proteins present are albumin and globulin,

these being related to one another in the transudates as one and one-half to one, while in the exudates the globulin is relatively much increased. The determination of the total protein may be made by methods of fractional precipitation as previously discussed. The transudates from the pleura contain the largest percentage of albumin, while edematous fluids rarely show over 1 per cent. Transudates do not coagulate spontaneously. Glucose is present both in transudates and exudates in amounts varying between 0.04 and 0.1 per cent. The mineral constituents of transudates are somewhat higher than in the exudates, the former averaging 0.96, the latter 0.89 per cent. Under pathological influences fat, blood, uric acid, and biliary pigment may find their way into both types of fluid. In diabetes an excess of sugar and the presence of acetone bodies may be detected.

The exudates are usually straw- or lemon-yellow in color depending on the degree of inflammation, or they may assume colorations ranging from a deep red (hemorrhagic) to a milky (purulent) shade. Biliary pigments may cause a bright green, while various medicaments, such as methylene blue, may produce a greenish-blue coloration. The specific gravity is almost invariably above 1018, the reaction is alkaline, the albumin content is usually above 3 per cent., reaching as high as 7 per cent., while the globulin is relatively much increased in comparison with the albumin. This globulin increase is largely due to paraeuglobulin. Nucleoprotein is especially abundant in purulent exudates in which the autolytic processes are more or less marked. The total nitrogen of the various fluids varies from 0.22 to 1.38 per cent. The nitrogen partition may be seen from the following table of Gerhartz.[1] Exudates coagulate spontaneously on standing.

Figures in terms of % (grams per 100 cc.)	Total N	Precipitable N	Protein N	Ammonia N	Purin N	Urea N	Amino-acid N
Transudate	0.22–0.58	0.18–0.53	0.17–0.52	0.007–0.01	0.002–0.007	0.01–0.05	0.002–0.005
Serous exudate....	0.43–1.09	0.39–1.07	0.37–1.03	0.01 –0.03	0.01	0.01–0.06	0.007
Purulent exudate..	1.11–1.33	0.94–1.22	1.14	0.01	0.007	0.02–0.1	0.004–0.28

The exudates, which, accurately speaking, are always of inflammatory origin may be serous, serofibrinous, seropurulent, hemorrhagic, purulent, putrid, chylous, and chyloid.

Serous Exudates.

These are clear, of a light straw color, and show a specific gravity above 1018. There is a large amount of fibrin, as shown by the dense network microscopically, containing a few red cells which may be derived from the bleeding at the point of puncture, a few leucocytes which may vary in type according to the kind of bacteria causing the infection, and large endothelial cells from the serous membrane lining the cavity. If the blood-cells be present in sufficient numbers to give a distinct red color to the fluid it is termed a hemor-

[1] Chemie der Transudate und Exsudate, Jena, 1908.

rhagic exudate, while if a few pus-cells are found it may be called a seropurulent type. The gradations between the true serous and serofibrinous types of exudate are exceedingly varied, so that it is difficult to tell which type is really present. Even from a purely serous exudate a certain amount of coagulation, with formation of a distinct fibrin network, may be obtained, so that the only criterion would be one of degree.

The type of leucocyte present is usually of the polymorphonuclear variety, although other forms may be present. It is, therefore, an important part of the examination of an exudate to determine the percentage relations of these various cellular elements. This will be discussed in the section on Cytology.

Chylous Exudates.

On account of the close relationship between the abdominal, thoracic, and pericardial cavities, on the one hand, and the large lymphatic trunks, on the other, it is possible for lymph to pass directly into these cavities in case rupture of these vessels occurs. Such exudates show all the properties of chyle. The fluid is white and milky, contains between 1.5 and 2.5 per cent. of protein, and a considerable amount of fat, which may be demonstrated by staining with osmic acid or Sudan-III or by alkalinizing with sodium hydrate and shaking out with ether.

Chyloid Exudates.

It has been found that carcinoma, tuberculosis, extreme cardiovascular changes, hepatic disturbances, puerperal sepsis, and infection with the filaria may give rise to a chyloid type of ascites. The fluid in these cases is less milky than that of the true chylous type, contains less fat, and is not so completely cleared by shaking with ether. It contains rather large amounts of pseudo-globulin and lecithin. Sugar is usually absent, while present in the true chylous type. Considerable difference is observed between these two types of exudate as to the rate of accumulation of fluid after tapping. In the chylous variety the accumulation is rapid, while in the chyloid form it is usually much slower.

Hemorrhagic Exudates.

This type is, in reality, a serofibrinous form containing large numbers of blood-cells. It is observed in patients with hemorrhagic diathesis, in connection with active tuberculosis, with neoplasms of the serous cavities, and following injuries to the chest or abdomen. In this form of exudate, the exciting bacterial agent, usually the tubercle bacillus, may occasionally be found, but not always. The type of leucocyte (mononuclear) would be very strong presumptive evidence in favor of tuberculosis, even though no bacilli were found. If the exudate be due to a malignant growth, it is sometimes possible to obtain shreds of the tumor tissue and thus make a probable diagnosis. In judging of the malignancy of the cells, it is sometimes difficult to differentiate the abnormal from the normal, especially in the affections of the pleura. These

malignant cells are usually extremely large and are characterized by their vacuolation and fatty degeneration (see cut). It is not infrequent to find in hemorrhagic exudates, which have remained in the body cavity for some time, a large number of cholesterin crystals and occasionally small masses of hemosiderin.

Purulent Exudates.

These are composed either of true pus or of seropus. They are more or less yellow in color, thick and occasionally tenacious, and separate on standing or centrifuging into a cellular deposit and a pus serum. The cells forming the pus are not infrequently in a condition of advanced fatty degeneration and may contain numerous bacteria. The addition of acetic acid will usually clear up the cells so that the nuclei become recognizable. Fat is present in amounts as high as 7 per cent., while a relatively large amount of various proteins and extractives is shown on chemical examination. Fatty acid crystals and cholesterin may be abundant in the sediment, especially in old abscesses. Fresh pus is usually alkaline, but may become distinctly acid, owing to the development of lactic acid in the process of autolysis.

Purulent exudates are investigated with special regard to the type of cell present and to the organism associated with the pus formation. In ordinary pus, the cell is of the polymorphonuclear type, although occasionally the mononuclear form may be predominant. The bacteria are very numerous and include many of the most important types. While the pyogenic bacteria are more frequently the various types of staphylococci and streptococci, it is to be remembered that other organisms may produce pus under certain conditions. Thus the typhoid bacilli, colon bacilli, pneumococci, Friedländer's bacilli, gonococci, diphtheria bacilli, Morax-Axenfeld and Koch-Weeks bacilli, and influenza bacilli, among others, may be the causative factor in the formation of a purulent exudate. It will be seen, therefore, that the bacterial examination of a purulent exudate may require a very extensive research. As it is frequently impossible to decide, from a stained specimen, as to the special organism causing the infection, cultures should be made in every doubtful case. The peculiarities of such cultures may be found in any work on bacteriology.

Putrid Exudates.

These may be observed in various cavities of the body or in the substance of various organs, especially the liver and lungs. They arise from the entrance of pus into these cavities from perforation of a gangrenous area, gastric or intestinal ulcer, malignant growths, etc. The material obtained by puncture is usually brownish or greenish in color, has a very offensive odor, and is usually alkaline, but may be acid. Microscopically degenerated cells, numerous bacteria, cholesterin, fatty acid, and hematoidin crystals are observed. In some cases various portions of an echinococcus cyst may be found in the exudates. Bilirubin crystals and various amino acids may be found in rupture of an hepatic abscess.

III. Bacteriology.

It is always advisable to make cultures of the material obtained by puncture in order to discover the organism which is acting as the exciting cause of the condition under investigation. Usually a few c.c. of the fluid are allowed to drop into a flask containing 50 c.c. of sterile nutrient bouillon and the mixture incubated for 24 to 48 hours. From the growth, obtained in this preliminary work, subcultures are made on various media and microscopical examination employed to identify the organism. It is always advisable to make at least two microscopical specimens, staining one with the ordinary methylene blue stain and the second with Gram's stain.

Tubercle Bacilli.

If these organisms are suspected, the technic of staining is the same as outlined under Sputum. It frequently happens, in the examination of suspected tubercular exudates, that the presence of a large amount of fibrin, either with or without spontaneous coagulation of the specimen, makes it exceedingly difficult to find the organism, even though it be present. In such cases, advantage is taken of a procedure, recommended by Jousset,[1] known as *inoscopy*. If the fluid has not coagulated, it is allowed to do so in order that the coagulation may enclose the bacilli within the fibrinous network. The coagulum is separated as completely as possible from the supernatant fluid, is washed with distilled water, and is treated with 30 to 40 c.c. of the following mixture, which will digest the fibrin.

Pepsin,	2 grams.
Sodium fluorid,	3 grams.
Glycerin,	10 c.c.
Hydrochloric acid (conc.),	10 c.c.
Distilled water, q.s., ad.,	1000 c.c.

This mixture is placed in the incubator for 24 hours, when the fluid becomes homogeneous. The digested fluid is centrifuged and smears are made from the sediment as previously described. It is always advisable to treat such smears with a small quantity of albumin fixative, so that the organisms may not be washed from the slide. The smear is fixed in the flame and stained in the usual way with carbol fuchsin. The tubercle bacilli may not appear as deeply stained after such treatment, as both their morphological and staining characteristics are slightly altered by the digestion.

Gonococcus.

The gonococcus appears in stained specimens as small biscuit-shaped or coffee-berry-shaped cocci, which are arranged in pairs separated by a narrow unstained portion. Occasionally two of these pairs of hemispheres are joined together, forming tetrads. They may be stained by any of the anilin dyes, but are recognized especially by their reaction toward Gram's method. In

[1] Sem. Méd., Tm. 23, 1903, p. 22.

this connection it must be said that other organisms, among which we find the diplococcus intracellularis meningiditis of Weichselbaum and the micrococcus catarrhalis, resemble the gonococcus both in morphological and staining characteristics, so that a differentiation by this method is not always possible. Fortunately, however, the cultural peculiarities of these organisms absolutely differentiate them.

Gram's Stain.

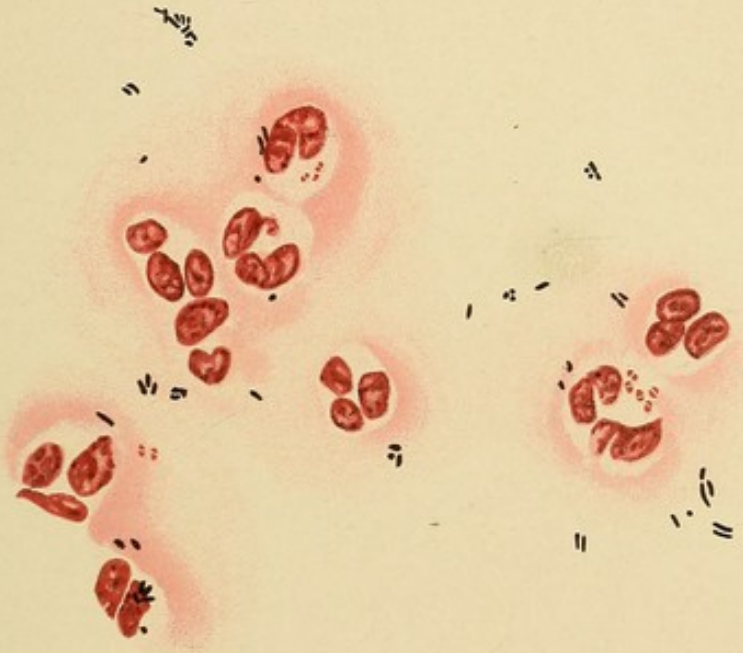
The principle of the staining of various organisms by Gram's method is that certain organisms retain the primary color after treatment with decolorizing agents, while others lose this primary stain and must be treated with a contrast stain for their recognition. If the organism retains the primary blue color it is called Gram-positive, while if it lose the primary color and take on the contrast stain it is called Gram-negative. Among the organisms which are Gram-negative we find the gonococcus, meningococcus, micrococcus catarrhalis, influenza bacillus, typhoid bacillus, colon bacillus, Koch-Weeks bacillus, and the Morax-Axenfeld bacillus; while the tubercle bacillus, smegma bacillus, diphtheria bacillus, pneumococcus, streptococcus, staphylococcus, and various saprophytic cocci found in the smears both of the male and female urethra are Gram-positive.

The solutions required in Gram's method are: (1) an anilin oil—gentian violet mixture, consisting of 84 c.c. of anilin water (water saturated with anilin and filtered), and 16 c.c. of a saturated alcoholic solution of gentian violet; (2) a solution of iodin consisting of one gram of iodin, 2 grams of potassium iodid, dissolved in 300 c.c. of water; (3) a dilute solution of carbol fuchsin, a dilute aqueous solution of safranin, or a 1 per cent. aqueous solution of Bismarck brown as a contrast stain.

Technic.

Smears of the exudate are made, in the manner previously described for making blood smears, by receiving a drop of the purulent material upon one end of a glass slide and spreading it in a thin even layer by means of a second slide. These smears are then fixed by passing several times through the flame. When the slides have cooled, they are covered with the gentian-violet solution which is allowed to act for one to three minutes. The solution is then poured off and the excess removed by washing in water. Without drying, the iodin solution is placed on the slide and allowed to act for one-half to one minute. It is then washed in water and the preparation treated with 95 per cent. alcohol until no more color is removed by it. The alcohol is then removed by washing with water and the smear is covered with one of the contrast stains above mentioned, in the writer's laboratory the safranin solution, which is allowed to act for only a few seconds. The stain is then washed off with water and the slide dried between folds of filter-paper.

The specimen under the microscope shows the Gram-positive organisms stained deep blue, while the Gram-negative bacteria and the bodies of the pus-



GONOCOCCI IN URETHRAL DISCHARGE (GRAM'S STAIN.)

cells take the red safranin stain. In such specimens the gonococcus, if present, will be observed both intracellularly and extracellularly, the former being the more characteristic. The ordinary pus cocci may likewise be intracellular, but these are distinctly Gram-positive, while the gonococcus is Gram-negative.

In the purulent exudate from the urethra, large masses of mucoid material may be present, which are known as gonorrheal threads. These may be found in the urinary sediment and are usually easily recognized. They contain masses of pus cells, within which may be found numerous gonococci. These shreds may persist for years in anyone with a history of a previous gonorrhea, but they may then contain no organisms. The cytology of gonorrheal pus presents nothing characteristic beyond the presence of numerous eosinophiles with large numbers of polynuclear neutrophiles and an occasional lymphocyte.

Smegma Bacilli.

In the exudate of the preputial follicles, known as *smegma preputii*, are found fat globules, ammonium soaps, cholesterin crystals, and a few epithelial cells. In this smegma are found many bacilli, smegma bacilli, which show the same morphological and practically the same staining characteristics as the tubercle bacillus. This is differentiated, as described under Sputum. If the urine is to be examined for tubercle bacilli, it is much better technic to use a catheterized specimen than it is to resort to methods of questionable differentiation. If this is done any acid and alcohol-fast bacilli may be regarded as the tubercle bacilli, but in all cases the smegma bacilli must be excluded.

Ducrey's Bacillus.

This bacillus is the organism causing soft chancre and is known as the bacillus ulceris cancrosi. It is found in the purulent discharge from the chancre, but rarely, if ever, in pure culture. The sections of tissue may, however, show none but this organism.

In making preparations for study of this organism, the ulcerated surface is scraped with a platinum loop, the pus spread upon a slide, dried in the air, and fixed with alcohol-ether or over the flame. It stains readily with all bacterial stains, but decolorizes with Gram's stain.

In stained specimens the organism appears as a short, thick, oval bacillus with rounded ends and two lateral indentations, which occasionally give it the appearance of a figure 8. Ordinarily the extremities are more deeply stained than the central portion, this appearing almost clear. The organism has a tendency to form chains or groups, which are rarely found in the pus-cells but are frequently seen within the epithelial cells.

Spirochæta Pallida.

This organism has been practically established by Schaudinn and Hoffmann [1] as the causative factor of syphilis. It may be obtained from the primary chancre, the incised papules, condylomata, mucous patches, inguinal glands, and from tertiary lesions in the internal organs, as well as in congenital syphilis.

[1] Arbeiten aus der kais. Gesundheitsamte, Bd. 22, 1905, S. 527.

The organism is known as the spirochæta pallida or treponema pallida, and is so-called on account of its morphology and low refractility as well as from the difficulty with which it takes the anilin dyes. Its size, form, and type of motility have been discussed in the section on Blood.

Technic.

To obtain this organism from the primary lesion, the chancre is thoroughly cleansed either with normal salt solution or with soap and water, rinsed with salt solution and dried. If the sore is healed, the epithelial covering is removed. The chancre is lightly curetted or the edges scraped, the blood, which should be avoided, is wiped away, and the serum which exudes used for the later work. A thin smear is made from the serum, transferring a drop by means of a platinum loop to a slide and using a rapid circular motion in spreading. The specimen is dried in the air and fixed for 5 to 10 minutes in absolute alcohol or by passing through the flame. It is then stained in the following freshly prepared solution for 3 to 20 hours, using one drop of solution to 1 c.c. of water as the staining agent.

Giemsa's Azur-eosin Solution.

12 c.c. of eosin solution (2.5 c.c. of 1 per cent. eosin solution in 500 c.c. of water).
3 c.c. of a 1 to 1000 aqueous solution of azur I.
3 c.c. of a 0.8 to 1000 aqueous solution of azur II.

This solution should always be made up fresh to insure the best results. In some cases the Giemsa solution obtained from Grübler works nicely, but not always, so that it is always advisable to know that the special stain with which one is working really stains the organisms before pronouncing a result as negative. At the end of the time selected for staining, preferably at least 18 hours, the specimen is washed with water, dried, and mounted in cedar oil. It is then examined with a Welsbach light, as the organisms do not appear well in diffuse daylight. The spirochæte pallidae are stained a violet-red color by this method, while the spirochæte refringens, with which they might be confused, stain blue.

In many cases it is possible to find these organisms by the use of the ultra-condenser. The characteristic corkscrew-shaped organism with its twisting, rotating, and bending motion is then sharply differentiated from the other types of spirochæte, with which it might be confused in the stained specimens. This latter method is, of course, not suited to the work of the general practitioner as it requires much more apparatus than is at his command.

IV. Cytology.

The cytology[1] of transudates and exudates has reference to the study of the various types of cells found in such fluids. As a rule, such investigations,

[1] See Labbé, Cytodiagnostic, Paris, 1903; Ravaut, Cytodiagnostic, Paris, 1901; Brion, Centralbl. f. allg. Path., Bd. 14, 1903, S. 609.



Katharine Hill

SPIROCHETAE PALLIDAE IN TISSUE. (LEVADITI'S STAIN.)

known as cytodiagnosis, are carried out more frequently on the nonpurulent types of fluid, as the examinations of the purulent fluids are more especially concerned with the bacteria present.

Technic.

The technic of obtaining the cellular components of the puncture fluid will vary according as the effusion does or does not contain fibrin. If this be present in fairly large amount, the fluid must first be defibrinated before the next steps are possible. Here, again, the procedure may be complicated by the presence or absence of coagula. If the fluid be not coagulated, it is placed in a large sterile flask which contains a few sterile glass beads, the mixture is actively shaken until a firm clot is obtained, in the meshes of which a few of the cells will necessarily be enclosed, but the majority will remain suspended in the liquid. The fluid is then separated from the clot and placed in centrifugal tubes which are drawn out to a rather fine point. If the fluid be coagulated when collected, it should be shaken with glass beads to break up the clot and liberate the cellular elements. The fluid is then separated and treated as follows.

The remaining portion of the technic is the same for fluids which contain or do not contain fibrinous material. The centrifuge is rotated rather rapidly for about ten minutes in order to collect the cells as a sediment. In most of the exudates the number of these cells is very small so that a concentration is absolutely essential. As Widal and Ravaut have shown, the polynuclear cells seem to be somewhat more affected by the defibrinization than the other types, so that these cells may show a relative diminution. After the cells have collected, the fluid is removed by rapidly inverting the tube in such a way that the sediment does not follow the liquid. Some workers advise the withdrawal of the sediment by a pipet with a long fine tip, but the writer has not found this method any more advantageous than the one spoken of above. The sediment in the tube is shaken so as to mix it thoroughly and a drop placed upon the glass slide and spread as described under Blood. Where very few cells are present it is wise to allow the drop to dry on the slide without spreading, in order to concentrate the cells in a small area. The specimen is then treated with Wright's stain and examined under the immersion lens. If stains are to be used which contain no fixative, such as methyl alcohol, it is necessary that the specimen be fixed by methods previously discussed in the section on Blood. The stain to be used will depend largely upon the points to be studied, the eosin-hematoxylin stain being especially serviceable in differentiating the nuclear structures of the cell. As the cells in the various pathologic fluids frequently show more or less degeneration, the nuclear portion is more suitable for study than is the cell protoplasm. For this reason the writer prefers the use of the eosin-hematoxylin method along with the Wright stain or the triacid stain for the granules of the cells.

These specimens may also be used for the study of the bacteria present.

When this object is to be subserved, it is advisable to make two specimens, staining one with the ordinary methylene blue stain and a second by Gram's stain. If the material be very limited in amount, it is possible to combine the above staining methods by treating first with the eosin-hematoxylin method, washing in water and then following the ordinary procedure of the Gram method of staining. Such specimens are extremely panoptic and are especially to be recommended.

Cytology of Normal Fluids.

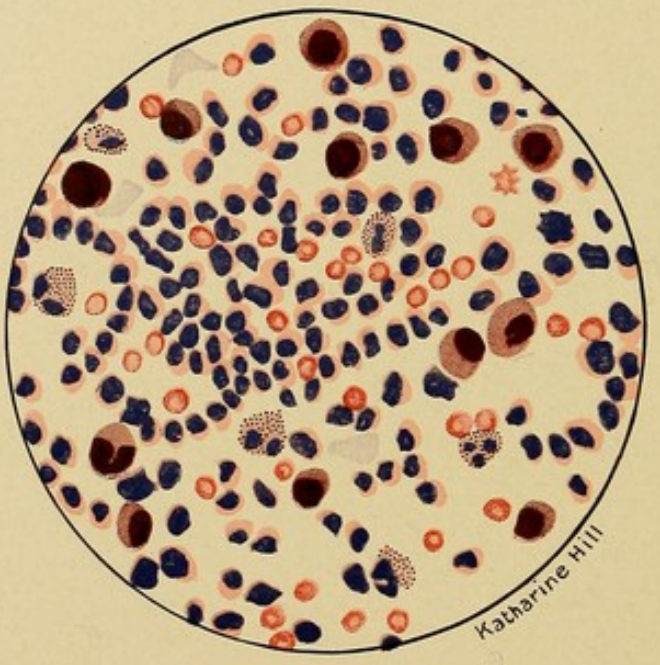
The number of cellular elements in fluids from the various serous cavities of the body may vary from a very few to a large number. The cells observed are the red and white corpuscles of the blood, the latter of which are usually relatively more numerous than in the circulating blood and are usually largely of the polynuclear type, although mononuclear forms are frequently present. Neutrophiles and eosinophiles are present under normal conditions, the latter being relatively more abundant than in the blood. If a large number of red cells are found, an injury of the small vessels during puncture usually explains their presence. Besides these types of cell, which are exactly similar to those of the blood, a few endothelial cells are practically always seen. These cells are observed of different shapes, may be single or grouped in sheets, and may be very much degenerated. They are larger than the other cellular elements, their contour is usually circular, but may be irregular; they are mononuclear, and frequently contain round vacuoles.

In examining normal as well as pathologic fluids for their cellular content, 100 cells should be counted if possible and the percentage of each type thus determined. This constitutes the cytologic formula of the exudate.

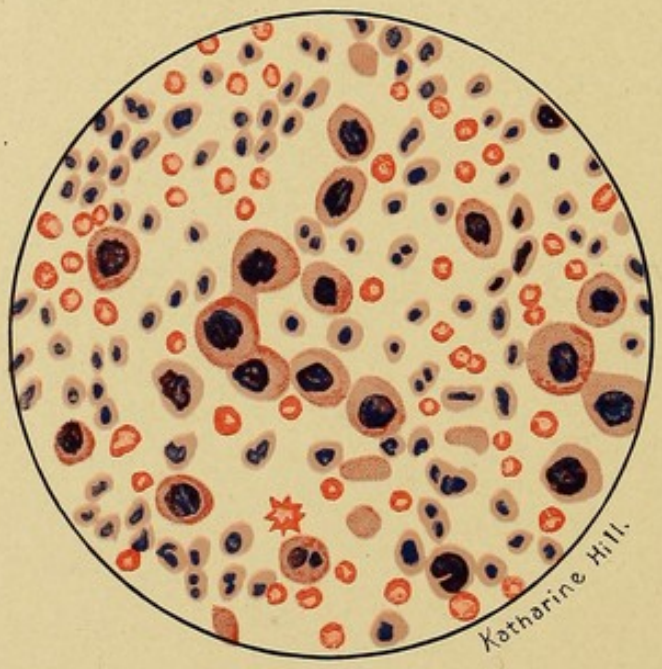
Cytology of Pathologic Fluids.

According to the theory of Metschnikoff, the presence of a bacterial infection is associated with attraction of the leucocytes to the infected area. These cells then enter into combat with the bacteria and either destroy the organism or are destroyed by them. As has been shown, certain organisms, especially the tubercle bacillus and probably the typhoid organism, attract the lymphocytes, while most of the other organisms attract the polymorphonuclear neutrophiles. Theoretically, therefore, it should be possible to decide as to a tubercular or nontubercular condition by the presence or absence of an increased number of the mononuclear types of leucocytes. This is the basis of the attempt at differential diagnosis by means of cytodiagnosis.

Not infrequently one finds in malignant conditions the so-called specific cells which are either sarcomatous or carcinomatous. These, although specific, are not easy to absolutely identify. These cells are very large, frequently showing fatty degeneration, extensively vacuolated, and showing a mitotic mulberry-like nucleus. Although differing from the endothelial cell, confusion is very apt to arise, so that it is difficult to make a diagnosis in all cases from the appearance of such cells.



EXUDATE FROM TUBERCULAR PLEURISY. (EOSIN-HEMATOXYLIN STAIN.)



EXUDATE IN PNEUMONIC PLEURISY (EOSIN-HEMATOXYLIN STAIN.)

Pleural Exudates.

Primary Tubercular Pleurisy.

This is characterized especially by an increase, both relative and absolute, in the number of lymphocytes. A pleural lymphocytosis exists when there is an excess of mononuclear cells, with abundant protoplasm, a large nucleus, and smaller than the endothelial cells. In the very early days of the infection a neutrophilia may exist, but this is rarely seen, as attention may not be drawn to the condition sufficiently early. Associated with these polynuclear cells in the early stage we may find an increase in the number of endothelial cells. Eosinophile cells are frequently observed, but do not have any definite relation to tuberculosis as an infection. The red cells may be occasionally numerous, but are usually small in number.

Secondary Tubercular Pleurisy.

As a rule, tubercular pleurisy secondary to a pulmonary tuberculosis yields a liquid which is poor in cells, practically all of which are very much altered and in some cases very difficultly recognizable. The polynuclear types may predominate to such an extent that a distinct polynucleosis exists as evidence of a septic rather than a true tubercular pleurisy. The polynuclear cells are usually old, much deformed, and their nature recognizable only by staining their neutrophile granules. Where the infection is directly tubercular and not mixed (the latter, however, usually being the case), an approximately equal division of the polynuclear and mononuclear forms may obtain. In this type of pleurisy the eosinophile cells may be very numerous, in one case of Widal and Ravaut constituting 54 per cent. of the cellular elements. The endothelial cells may be, as in the primary tubercular pleurisy, sufficiently numerous to constitute a distinct endotheliosis. They are, however, single and very rarely grouped in masses.

Pneumococcus Pleurisy.

This is a truly septic type of pleurisy and is characterized by a distinct polynucleosis. In the early stages of this type of pleurisy, the endothelial cells may be very numerous, while in the later stages they may be much diminished. In this, as in all types of pus accumulations, marked autolysis is present so that the cells may show extreme degeneration. As this condition progresses toward recovery, some of the polynuclears may be replaced by the mononuclear lymphocytes; while if suppuration becomes extensive the polynuclears increase and autolysis becomes extremely marked. In such exudates the pneumococcus may be demonstrated by staining methods.

Streptococcus Pleurisy.

This type is especially associated with a polynucleosis. These cells are frequently observed undergoing karyolysis, the cell body usually being markedly degenerated. In its early stage it may be accompanied by an endotheliosis, but when well developed is usually associated with the presence of only a few isolated endothelial cells. Stained smears show streptococci in large numbers.

Typhoid Pleurisy.

In this type a lymphocytosis is usually observed along with an endotheliosis, these latter cells being in large masses instead of in single isolated forms as observed in the secondary tubercular pleurisy. This point may be valuable in differentiating these two conditions, which are associated with an increase in the number of lymphocytes. The eosinophiles may be increased and red cells may be present in large numbers. The identification of the specific organism will serve as a positive differentiation from tubercular conditions.

Malignant Pleurisy.

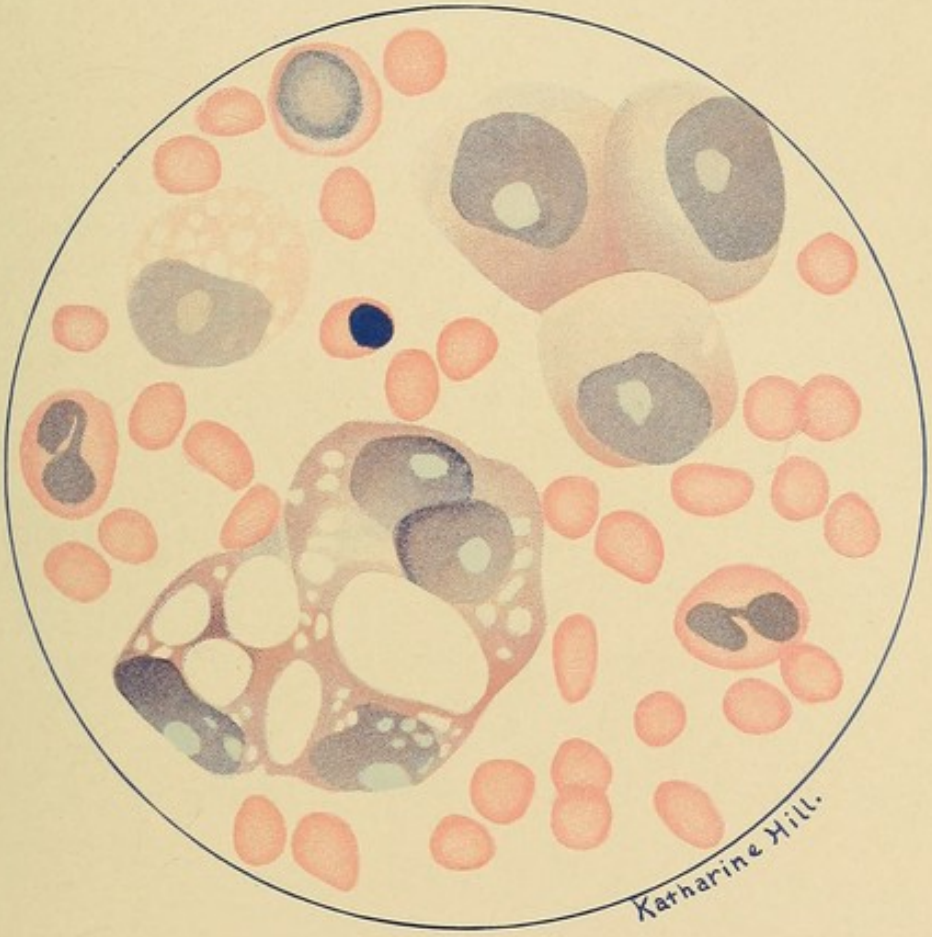
This is a type of the aseptic pleurisies and may accompany malignant growths of the lung or pleura. Nothing characteristic is found in the cytologic formula of such exudates, but occasionally portions of the tumor mass may be obtained or certain cells may be found which are more or less distinctive, although not absolutely pathognomonic. These cells are frequently confused with the larger endothelial cells of the pleura, but are characterized, according to Deguy and Guillaumin, as follows: Malignant pleural cells contain glycogen which is recognized by the brown coloration shown on treatment with dilute iodin solutions, they contain large amounts of fatty material, they are extensively vacuolated, these vacuoles may be large or so numerous and small that the cell resembles a sponge, the cells are extremely large, and the nucleus usually shows mitotic figures.

Nephritic and Cardiac Pleurisy.

The secondary exudate observed in cardiac and renal conditions is characterized especially by the marked endotheliosis. These endothelial cells are grouped in masses of 5 to 10 cells, which show more or less degeneration, their contours and their large nuclei being distinct. This endotheliosis is not complicated by the presence of many other cells in the pure nephritic pleurisy, but in the cardiac type we usually find a polynucleosis at the same time. This polynucleosis is much more marked where infarcts or emboli have complicated the condition than when it is due to a pure congestion. Numerous red cells are especially observed in association with a congestive pleurisy.

Peritoneal Exudates.

The cytological examination of peritoneal exudates has, as yet, yielded fewer diagnostic points than has that of pleural exudates. It is, however, sometimes possible to differentiate a tubercular peritonitis from an ascites or an ovarian cyst by means of such examinations. Tubercular peritonitis usually shows a lymphocytosis and also a relative polynucleosis. A few endothelial cells may occasionally be found, but these do not yield much information. In ascites of hepatic origin few cellular elements are observed beyond the peritoneal endothelial cells. In ovarian cysts there are fewer cellular elements as a rule, but those present are usually large, round, or oval, and filled with vacuoles. Moreover, cylindrical ciliated epithelial cells as well as goblet cells or red cells are frequently relatively numerous.



EXUDATE IN MALIGNANT PLEURISY. (EOSIN-HEMATOXYLIN STAIN. AFTER DEGUY AND GUILLAUMIN.)

V. Cyst Fluids.

(1). Ovarian Cysts.

(*a*). Serous Cysts.

Such cysts are true retention cysts formed by dilatation of the Graafian follicles and retention of the ovarian secretion and are known as *Hydrops folliculorum Graafii*. They contain a clear, watery, serous liquid, which has an amber color, a specific gravity ranging between 1005 and 1022, and a chemical composition practically identical with that of other serous fluids.

(*b*). Myxoid or Colloid Cysts.

These are proliferating cysts developed from the epithelial tubules. "We sometimes find in small cysts a semisolid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called colloid because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and, as this mass in the different cysts is more or less diluted with serous liquids, their contents may have a variable consistency. In other cases the small cysts may also contain a thin watery fluid. The color of the contents is also variable. In certain cases it is bluish-white, opalescent, and in others yellow, yellowish-brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or reddish-brown, due to the decomposed blood pigment. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1015 to 1030, but may be, in a few cases, 1005 to 1010 or 1050 to 1055. Though the contents of the proliferating cyst may have a variable composition, still it may be characterized, in typical cases, by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity. Such a liquid does not ordinarily show a spontaneous coagulation" (Hammarsten).

Microscopical examination of the sediment shows red and white blood-cells, large epithelial cells, which may be filled with vacuoles, cylindrical or goblet cells, granular cells showing more or less fatty degeneration, fatty granules, cholesterin crystals, and colloid corpuscles in the form of large, circular, highly refractile bodies.

Chemically, these cysts are characterized by the presence of colloid, which is not a distinct chemical entity. It is a gelatinous substance, insoluble in water and acetic acid, soluble in alkalies, and yields a reducing body on boiling with acids. Not infrequently pseudomucin (metalbumin) is found, especially in the extremely viscid fluids. For its detection the serum albumin must be previously removed by the addition of acetic acid, boiling, and filtering. The filtrate is treated with alcohol when a thready precipitate forms. If this precipitate be boiled with HCl, a substance is formed which reduces copper solutions quite markedly. Pseudomucin is distinguished from true

mucin by the fact that it is not precipitated by acetic acid. Mitjukoff has isolated a further colloid body from certain ovarian cysts, to which he gives the name of paramucin. It is precipitated by acetic acid and is soluble in an excess. If treated with alkali it first swells up and then dissolves in an excess of the reagent. It differs from mucin and pseudomucin in the fact that it reduces copper solution without previous boiling with acids. McConnell[1] has recently reported the finding of a multi-locular cyst of the ovary, which contained true mucin.

(*c*). **Papillary Cysts.**

These are intraligamentary types and contain a yellow, yellowish-green, or brownish-green fluid which contains only traces of pseudomucin. The specific gravity ranges between 1032 and 1036.

(*d*). **Dermoid Cysts.**

These may be seen in the shape of small cysts not larger than a pea, but usually they are much larger, in some cases reaching the size of a man's head. The cyst usually contains a fatty unctuous material, which is derived from the epidermal lining of the cyst, and associated with it fat, desquamated epithelial scales, hair, teeth, bone, cartilage, etc.

(2). **Parovarian Cysts.**

These cysts of the organ of Rosenmüller contain a clear, pale yellow, or colorless, limpid fluid or occasionally one showing slight opalescence. The specific gravity ranges from 1002 to 1010 and differs from that of the ovarian cyst by its usual limitation to these lower figures. Albumin may be present in small amounts or be entirely absent, while pseudomucin is rarely, if ever, present.

(3). **Hydrocele.**

The contents of such a cyst are usually clear, show a color which may range from yellow to green, have a specific gravity of 1015 to 1030, and usually coagulate spontaneously. They contain a relatively large percentage of albumin, of which about 50 per cent. is globulin. In ordinary hydrocele many large oval cells may be seen, which have an eccentric nucleus and may be grouped in masses, although more frequently they appear as isolated cells; in some cases many eosinophiles, but this is rare in uncomplicated hydroceles. If the hydrocele be of tubercular origin a marked lymphocytosis usually exists.

(4). **Spermatocele.**

Fluids from such cysts are usually thin, colorless, and cloudy like thin milk. They may have an acid reaction, but are ordinarily alkaline. The specific gravity ranges between 1006 and 1010. Such fluids do not coagulate either spontaneously or after the addition of blood. Microscopically, one observes cell detritus, fat granules, leucocytes, and spermatozoa.

[1] Jour. of Med. Res., vol. 20, 1909, p. 105.

(5). **Hydronephrosis.**

This is a true retention cyst of the kidney, due primarily to obstruction of the ureter, which may be either congenital or acquired. Material aspirated from a renal cyst is usually clear, but may be yellowish or reddish and distinctly turbid. Its specific gravity varies from 1010 to 1015, while the chemical composition is usually suggestive of urine. For some time the presence of urea and uric acid in hydronephrotic cysts was supposed to be pathognomonic, but it has been shown that these substances may be present both in ovarian and pancreatic cysts and may even be lacking in old renal cysts. Occasionally epithelial cells, derived from the uriniferous tubules may be found, but such cells are not always present nor are they sufficiently characteristic to be of great importance from the diagnostic standpoint.

(6). **Hydatid Cysts.**

The fluid obtained by puncture of an echinococcus cyst is usually clear and of an alkaline reaction, has a specific gravity varying between 1005 and 1015, is practically free from albumin, and contains a large amount of inorganic salts, especially of sodium chlorid. The characteristic findings of such a cyst are the hooklets, scolices, and shreds of faintly laminated membrane. In some cases no trace of any morphological elements can be found, but usually the diagnosis is rendered certain, especially if careful search be made, by the presence of some portion of the parasite or the cyst membrane.

(7). **Pancreatic Cysts.**

The puncture fluid from a pancreatic cyst varies in its physical properties, depending upon the nature of the cyst as well as the length of time the fluid has remained in the cyst. It is usually bloody in character, has a specific gravity ranging from 1010 to 1030 and may contain methemoglobin, hematin, and cholesterin. As characteristic constituents of such a cyst one finds ferments, which will digest all types of food material. Such tests may be performed as outlined in the sections on Gastric Contents and Feces.

VI. Cerebrospinal Fluid.

Since the introduction of lumbar puncture by Quincke, the cerebrospinal fluid has gained more or less importance from the diagnostic point of view. So much may be learned, either from the standpoint of direct or differential diagnosis, that every practitioner should be able to perform a lumbar puncture and to examine the fluid obtained. There is little danger in the procedure as the spinal cord does not reach to the point of puncture and the fibers of the cauda equina are sufficiently movable to escape the needle. While few bad effects are observed in the ordinary run of cases, a few have been reported in which symptoms of collapse were evident. It should be a rule, therefore, to stop proceedings if such symptoms arise and also to keep the patient quiet in bed for at least 24 hours following the puncture, so that the pressure in the cerebrospinal cavity may become equalized.

Lumbar Puncture.

The patient is placed upon his left side near the edge of the bed, the knees should be flexed upon the abdomen, and the site of puncture prepared as for any surgical procedure. The needle used for puncture should be from 5 to 10 cm. long and have a lumen from 1 to 2 mm. in diameter. It is always wise to provide the larger needles with a stylet, so that tissue fragments or blood may not gain entrance accidentally to the tube and thus lead to possible diagnostic errors. This stylet may be removed the moment the needle penetrates the dura mater.

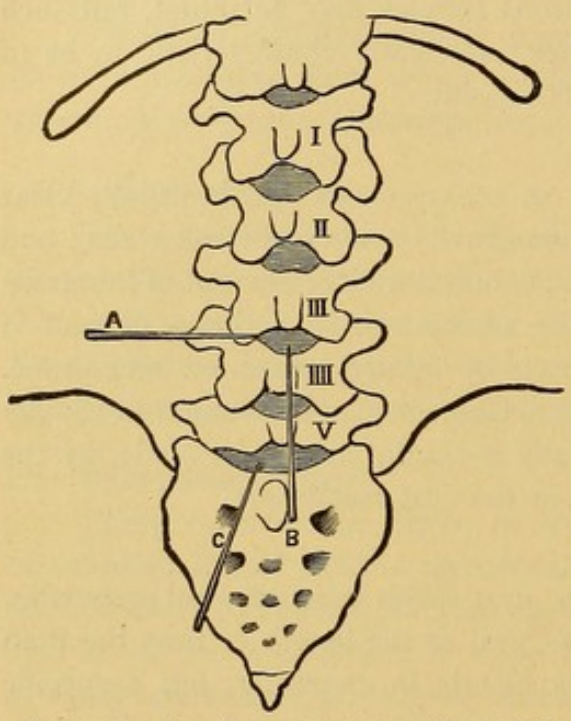


FIG. 159.—Lumbar puncture: A, Quincke's site; B, Maran's site; C, Chipault's site. (*Tyson.*)

The site of puncture should be on a level with the junction of the third and fourth lumbar vertebræ at a point about 1 cm. to the side (preferably the upper) of the median line. The needle is directed slightly upward and inward, the depth to which the puncture should be made varying with the age of the patient, the younger the child the less the depth. This puncture should be made carefully and yet with sufficient force to penetrate easily the musculature. If any marked resistance arises, it is probable that the needle has struck the vertebra, in which case the pressure must be reduced or the needle may break. This is not an infrequent occurrence with those not used to the technic, so that it may be advisable for the student to practice the procedure upon the cadaver. As soon as the dural sac is reached, the cerebrospinal fluid will flow from the canula, the rate of flow indicating in a general way the pressure of the fluid. No aspiration should be used at any time, as this procedure is extremely dangerous.

It is frequently advisable to know exactly what this pressure is, so that one may resort to the following method as used by Sahli. As soon as the needle penetrates the dura, a connection is made with a mercury manometer by means of a rubber tube filled with a 1 per cent. solution of carbolic acid. The portion of the manometer above the level of the mercury, forming the connection between it and the carbolic acid tube, must also be filled with the fluid. The manometer is filled with mercury to the zero point and held in such a manner that this point is on a level with the point of the aspirating needle, which is possible with ordinary manometers only when the connecting tube is of considerable length. Under normal conditions the dural pressure, in the dorsal position, ranges between 5 and 7.5 mm. of mercury, or 60 to

100 mm. of water if a water manometer be used. In pathologic conditions, such as meningitis or brain tumor, it ranges between 15 and 60 mm. of mercury or 200 to 800 mm. of water.

Normal cerebrospinal fluid is colorless, limpid, and free from morphological elements. Its specific gravity ranges between 1002 and 1010. It is alkaline in reaction, the degree of alkalinity varying between 15 and 20. It contains a trace of protein and about 0.1 per cent. of glucose. The salt content of this fluid is, according to Zdarek, 0.836 gram, of which 0.429 gram is referable to sodium oxid and 0.017 to potassium oxid. This would rather militate against the older statements that a large amount of potassium salts as compared with sodium salts was present. The relation of KCl to NaCl is, according to Nawratzki, 1 to 18, while Zdarek gives this ratio as 1 to 40. The former figure agrees closely with that obtained by various workers with pathological cerebrospinal fluids. The amount of fluid obtained by lumbar puncture is extremely variable. In normal individuals this amount is unknown as puncture is rarely performed upon normal cases. Pathologically, the amount obtained varies between 10 and 100 c.c. Naturally, if the communication between the subarachnoid spaces of the brain and of the spinal cord is blocked by a tumor or inflammatory adhesions, or if the aqueduct of Sylvius or the foramen of Magendie be obliterated, little fluid may be obtained by puncture, although large amounts may be present above the obstruction. The largest amounts are seen in cases of serous or tubercular meningitis, so that such conditions may usually be ruled out if a small amount of fluid is obtainable.

Pathologically, we may observe a very cloudy fluid, due to the presence of leucocytes, erythrocytes, and endothelial cells. This cellular admixture may be so extensive that the fluid resembles pure pus. In cases of cerebral hemorrhage from the ventricles, hemorrhagic pachymeningitis, or traumatic lesions of the spinal cord, so much blood may be present as to give the appearance of practically pure blood, the color varying from a bright red to a brownish or greenish-red, depending upon the length of time it has remained in contact with the remaining portion of the fluid. This admixture with blood may lead to the spontaneous coagulation of the fluid. This may serve as a differentiating point between inflammatory and noninflammatory lesions. Thus, in tubercular meningitis very slight coagulation may be observed, while in the epidemic cerebrospinal meningitis the coagulum may be very firm.

The chemical examination of the cerebrospinal fluid has as yet shown practically no diagnostic or clinical value. While the albumin content normally is much less than 0.1 per cent., it may vary under pathologic conditions to as high as 0.8 per cent. Glucose is usually present, but may entirely disappear under pathologic influences due to the autolysis controlled by the leucocytic ferments, the glucose being converted into lactic acid. Cholin is present normally in traces, while pathologically it may vary, according to Donath, between 0.021 per cent. and 0.046 per cent. This factor may be determined, both qualitatively and quantitatively, as follows: Ten c.c. of spinal fluid are

faintly acidified with dilute HCl and evaporated to dryness on the water bath. The yellowish residue is extracted with absolute alcohol and filtered. The filtrate is treated with an absolute alcohol solution of platinum chlorid and the mixture set aside for 24 to 48 hours. The chlorplatinate of cholin separates out on standing. This is filtered off, dried in a drying oven at 100° C., and weighed. The crystals may be colorless or slightly yellow, are usually serrated and lanceolate and arranged in the form of rosettes, or they may appear as needles arranged in sheaths or as hexagonal or rhombic plates.

Variations in the phosphorus content of the cerebrospinal fluid are apparently associated with marked degeneration of nerve tissue. Normally this appears to be something like 0.003 per cent., while it may run, in pathological conditions, as high as 0.05 per cent. Koch has recently introduced a method which permits of a more or less complete chemical examination of this fluid, using only 5 c.c. for the entire examination. This method promises to furnish us more reliable and extensive figures of the chemical composition of the cerebrospinal fluid than are at present available.

Microscopic Examination.

By far the most important part of the clinical examination of cerebrospinal fluid is a study of the bacteriology and cytology of the fluid. Normally the fluid contains practically no morphologic elements, while under pathologic conditions large numbers of various types of cells may be present. The material for examination is obtained, as previously described, in the section on cytology. In true tubercular meningitis a lymphocytosis is almost invariably observed, while in the epidemic type, due to the meningococcus of Weichselbaum, the cells are of the polynuclear type. In the chronic cases of epidemic meningitis as well as during convalescence from this disease, the lymphocytes may be present to such an extent that a slight degree of lymphocytosis is present, but never to the same extent as observed in the tubercular type. A lymphocytosis is also observed in syphilitic lesions of the central nervous system. This is important from the standpoint of differential diagnosis. In the meningitis due to the pneumococcus, a polynucleosis is the rule, although occasional cases are seen in which a lymphocytosis obtains. Preble has recently advanced the view that the meningococcus of Weichselbaum and the pneumococcus of Fraenkel are identical organisms, at least from the clinical standpoint. This view would seem to have considerable clinical support as well as true bacteriologic foundation.

Tubercular Meningitis.

The fluid in such cases is usually clear, but may be slightly opalescent. The cellular elements are largely mononuclear while a few red cells may be present. Sections stained for the tubercle bacillus usually give positive results in at least 75 per cent. of the cases. If tubercle bacilli cannot be found, the presence of a lymphocytosis will be at least suggestive, while animal inoculation or the tuberculin test will absolutely clear up the diagnosis.

Epidemic Cerebrospinal Meningitis.

In these cases the fluid may be transparent, but is usually somewhat opalescent and may be thick and purulent. The cellular elements are usually polynuclear in type and red cells may be more or less numerous.

Smears made from the sediment show the presence of numerous diplococci, which closely resemble the gonococcus in morphological and staining characteristics. This organism, the diplococcus meningitidis intracellularis of Weichselbaum, appears as a diplococcus, each element forming a hemisphere with its parallel side contiguous to that of its mate. It is sometimes seen in the form of tetrads or as isolated cocci, which appear as true spheres of variable size and showing a clear space in their interior. It is stained with the ordinary dyes and is negative to Gram's stain. For its cultural peculiarities the reader should consult works on bacteriology. Not infrequently one observes specimens of the meningococcus which show a Gram positive reaction, so that it is difficult to distinguish them, especially when they are in the form of isolated cocci or in groups of two or four from the pneumococcus. This type has been described as the meningococcus of Bonome, while Jaeger and Heubner describe a diplococcus which is Gram-positive and may be confused with the unusual types of Weichselbaum's meningococcus.



FIG. 160.—Diplococcus intracellularis meningitidis. (*Councilman.*)

Recently Flexner has succeeded in preparing an anti meningococcic serum which appears to have remarkable results in controlling this hitherto unmanageable disease. In using this serum, the injection must be made directly into the spinal cavity. More or less frequent injections of the serum and examinations of the lumbar fluid are made and the influence of the serum estimated by bacteriological and cytological examinations. In the cases of mixed cerebral infection, in which the meningococcus is associated with the pneumococcus, streptococcus, typhoid bacillus, staphylococcus, and other organisms, this serum does not seem to have as much influence as in the pure meningococcus infections.

It is to be remembered that a purulent meningitis may be secondary to infection with practically all of the pus-forming organisms found within the system. It is, therefore, essential that any infection showing meningeal symptoms should be investigated by an examination of the cerebrospinal fluid.

In sleeping sickness, a study of the cerebrospinal fluid frequently reveals the presence of the trypanosoma Gambiense. These parasites are not found in all cases, but when present usually furnish a grave prognosis.

Recent investigation of the cerebrospinal fluid, applying Wassermann's serum reaction for syphilis, has shown that in the large majority of nervous

cases of syphilitic origin a positive reaction is obtainable. Such investigations are advisable, therefore, in any case of doubtful etiology, as a prompt recognition of the true cause will undoubtedly lead to amelioration of the symptoms, even though a cure be not effected.

In the microscopic examination of the cerebrospinal fluid it is sometimes of interest to know the number of cells per cmm. of the fluid. This may be done by the use of the counting chamber and leucocytometer mentioned under Blood, using the undiluted spinal fluid. As a rule, from 5 to 10 cells may normally be found per cmm. In many pathologic conditions the number may vary from 10 to several hundred.

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CHAPTER X

SECRETION OF THE MAMMARY GLANDS.

I. General Considerations.

The normal secretion of milk takes place in the mammary glands of the female after delivery. It is true that a small quantity of milk may be secreted by the new-born of both sexes for a few days after birth. Moreover, cases have been reported in which the adult male secreted sufficient milk to act as a wet-nurse, but these must be regarded as cases of extreme rarity.

During the course of a normal pregnancy a small amount of a thin, yellowish fluid may be expressed from the mammary glands, but as a rule the first real secretion is observed following delivery of the child. This secretion is thin and watery, more or less translucent, and shows a distinct yellowish color.

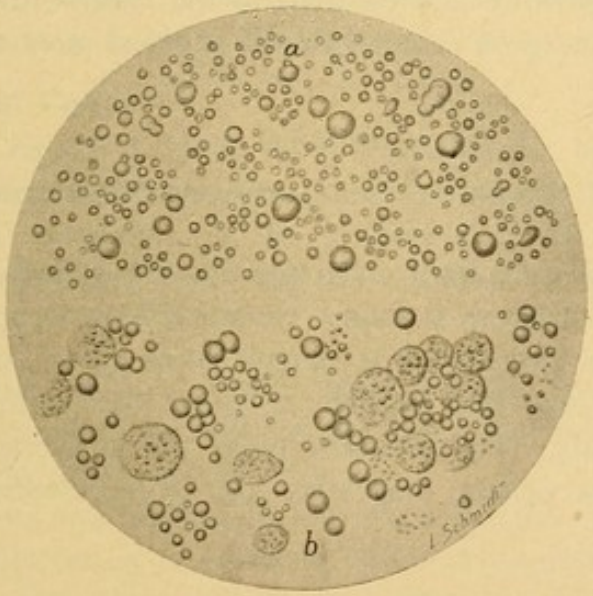

Fig. 161.—Normal Milk and Colostrum. (*Hawk.*)
a, Normal milk; *b*, colostrum.

Microscopic examination shows the presence of rather large cells in which are many fat granules and occasionally a distinct nucleus. This secretion, which is called *colostrum*, continues for three to four days and is distinguishable from the later secretion by the fact that it contains relatively more salts than normal milk and, according to the usual statements, more sugar. In a series of determinations of breast milk collected during the fourth day after delivery, the writer could not show any marked increase in the sugar in all cases, but in three out of eight cases examined a percentage higher than eight of lactose was obtained.

It is probable that the function of the colostrum is gradually to accustom the child to the taking of food by diminishing to a slight extent the elements, protein and fat, which are more apt to cause digestive disturbances than is the lactose.

The secretion of true milk begins about the fourth day and continues for a variable length of time. Marked variations are observed both in the quantity and the quality of this secretion in various women so that no hard and fast rule can be given as to the composition of normal mother's milk. While it is probably true that the normal woman should nurse her child during a large part of the first year, it is rare to find, especially in private practice, many such cases. Either the milk becomes scanty and loses in nutritive power or becomes excessive and consequently diluted. In either case the child is not receiving the most suitable nourishment, so that breast-feeding is abandoned under these circumstances. It seems to be a general rule, which is difficultly explicable, that the more highly socially developed the woman the less apt she is to nurse her child with any success. It is possible, and this seems to the writer the most probable explanation, that the child usually receives more or less constant attention from the physician, and variations in the breast milk are more frequently noticed than in the case of the poorer child who rarely has the advantage of medical attention unless more or less serious illness occurs.

II. Physical and Chemical Properties.

Various figures have been given for the composition of human milk so that it is difficult to strike an average. So much depends upon the nourishment of the mother, upon the amount of exercise taken by her, and upon the general condition of the system that marked variations exist in the proportion of the chemical constituents. The following table, taking averages of examinations reported by various writers, may serve as one representing more nearly the normal condition.

Water,	87.24
Salts,	0.26
Protein,	1.50
Fat,	4.00
Lactose,	7.00

These figures are not as high as regards protein as those usually given, but are higher as far as the lactose is concerned. Although many writers have given the percentage of protein much higher than 1.5 per cent., the writer has never been able, in several hundred examinations of milk in his laboratory, to find one showing a protein percentage of two or more.

As it is frequently necessary to modify cow's milk so that it may more

nearly approach mother's milk in composition, the writer inserts the following table for comparison.

Water,	87.25
Salts,	0.75
Protein,	3.50
Fat,	4.00
Lactose,	4.50

It will be seen that cow's milk shows a higher per cent. of protein and a lower per cent. of carbohydrate. It is necessary, therefore, that this be modified by diluting the milk so as to diminish the protein and by adding lactose to make up for the deficiency of carbohydrate. In the dilution the fat content will necessarily be lowered so that this may be remedied, as suggested by Backhaus, by the addition of cream. The writer must refer to works on pediatrics for the various methods of modifying cow's milk. It is to be remembered that no modification is equal to mother's milk. The casein of human milk forms a much finer clot with the gastric juice than does that of cow's milk so that the latter may not be well tolerated by the child. Moreover, some unknown principle present in human milk is accountable for a distinct biologic difference in these two types of nutritive material.

(1). **Appearance and Color.**

Normal human milk is a white fluid which usually has a slight bluish tinge except immediately after birth when the color may be distinctly yellowish from the presence of colostrum. If the percentage of fat be relatively high the color will be more nearly a pure white with little of the blue tone.

(2). **Specific Gravity.**

The specific gravity of human milk varies between 1028 and 1034. An increase in the percentage of fat will usually lower the specific gravity while the protein and carbohydrates will increase it.

Cow's milk shows approximately the same specific gravity. If a low specific gravity is obtained it is evidence either of an increased percentage of fat or of dilution with water. If the percentage of fat be low the milk is unquestionably a watered one. If the milk tested shows a high specific gravity the chances are that most of the fat has been removed either by skimming or by centrifugation.

The determination of the specific gravity may be made by a special instrument known as Quevenne's lactodensimeter or by the use of the ordinary hydrometer used in urine work. As it is never of any clinical importance or even of any marked practical importance that the specific gravity should be absolutely accurately determined, the writer is accustomed to use the urinometer for such determinations. Corrections for variations in temperature are necessary only when great differences exist between the temperature of the room and the temperature at which the instrument is calibrated.

(3). **Reaction.**

Normal human milk as well as cow's milk shows an amphoteric reaction to litmus-paper and an acid reaction to phenolphthalein, cow's milk being somewhat more acid than mother's milk toward the latter indicator.

(4). **Coagulation.**

If milk be allowed to stand, the reaction gradually becomes more and more acid owing to the development of bacteria, especially of the bacillus acidi lactici. When the degree of acidity reaches a certain point, casein separates first in the form of flocculi and later the entire fluid may coagulate to a jelly-like mass. This mass soon contracts and settles out leaving a slightly turbid fluid known as milk plasma or acid whey. In order to inhibit the development of bacteria and prevent this coagulation, known as souring, certain preservatives are frequently added to market milk and should be capable of detection by the practitioner. These will be discussed in a later section.

Besides this type of coagulation of cow's milk a second form is observed which takes place under the influence of chymosin without any change in the reaction of the fluid. In this case the whey is sweet and contains practically all of the lactose originally present in the milk.

(5). **Total Solids.**

Five to ten c.c. of the well-mixed milk are placed in a weighed platinum dish, evaporated to dryness on a water-bath, and dried to constant weight in the oven at 105°. The difference in weight between the platinum dish and its contents, on the one hand, and the platinum dish, on the other, gives the amount of total solids in the milk taken. A simple calculation will yield the percentage of total solids.

Under normal conditions the total solids of both human and cow's milk should average between 12 and 13 per cent. Variations in this figure are, of course, due to fluctuations in the various constituent elements.

(6). **Ash.**

The platinum dish containing the dried residue of the milk is heated over a direct flame until the residue is completely incinerated. This is then placed in the desiccator and dried to constant weight. The difference in weight between the dish and contents and the dish alone represents the amount of salts present in the milk originally taken.

(7). **Protein.**

The methods for the determination of protein are divided into those for estimation of the total protein material present and into those in which separate determinations are made of the casein, on the one hand, and albumin and globulin, on the other.

(*a*). **Total Protein.**

Method of Sebelien.

Ten c.c. of milk are diluted with 90 c.c. of water, 5 c.c. of a saturated sodium chlorid solution, and 15 c.c. of Almén's tannic acid solution are added. The mixture is thoroughly stirred and the dense precipitate which forms is allowed to settle. The composition of Almén's tannic acid solution is as follows:

Tannic acid,	4 grams.
Acetic acid (25 per cent.),	8 c.c.
Alcohol (50 per cent.), qs., ad.,	200 c.c.

The precipitate, which consists of the total protein of the milk and a large portion of the fat carried down by the precipitate, is then filtered off through a fine filter and is washed with cold water. The filter-paper and its contents are then placed in a Kjeldahl flask and a nitrogen determination made as described under Urine. It is advisable to use 20 c.c. of sulphuric acid instead of the ten employed in the case of urine, as the mixture oxidizes much more rapidly under these conditions. If the nitrogen obtained in this determination be multiplied by 6.37, the result will be the protein in 10 c.c. of milk.

Method of Boggs.

For routine work this method is perhaps more advisable for the general practitioner than is the preceding, but it does not always give confirmatory results. It is based upon the fact that the total protein of milk is precipitated by phosphotungstic acid in hydrochloric acid solution, the amount of precipitate being measured in an Esbach tube. The reagent used has the following composition:

Phosphotungstic acid,	25 grams.
Concentrated hydrochloric acid,	25 c.c.
Distilled water, q. s., ad.,	250 c.c.

It has been found that the milk should be diluted with water before adding the reagent if the results are to be accurate. As a rule, a dilution of 1 to 10 for human milk and 1 to 20 for cow's milk suffices.

The diluted milk is poured into the Esbach tube to the mark *U* and the reagent added to the mark *R*. The tube is then closed with a stopper and inverted several times to thoroughly mix the contents. It is then set aside for 24 hours when the percentage of protein in the milk is read off directly from the calibrations on the tube in case the dilution was 1 to 10, while with a dilution of 1 to 20 the figures are multiplied by 2.

This method, while convenient, is open to the objection that many factors may influence the depth to which a precipitate settles. Moreover, the Esbach tubes reading as high as 12 parts do not give as satisfactory results as those with readings from one to seven. Such being the case the method must be used more for clinical purposes than for scientific estimations.

(*b*). **Casein.**

Twenty c.c. of well-mixed milk are measured into a beaker and approximately 380 c.c. of water are added. The mixture is thoroughly stirred and very dilute acetic acid added *drop by drop* with constant stirring until a flocculent precipitate is observed. When this point is reached a stream of carbon dioxid is passed through the mixture for one-half hour, after which the vessel is allowed to stand until the next day. The above part of the technic is directly applicable to cow's milk. If human milk is being examined it is necessary to heat the vessel to 40° C. during the passage of the carbon dioxid.

After the mixture has stood overnight, it is filtered through a nitrogen-free filter and washed with water. The residue on the filter contains casein which is mixed with a portion of the fat present. The filter-paper and contents are then placed in a Kjeldahl flask and a nitrogen determination is made as previously described. Multiplication of the nitrogen value by 6.37 yields the amount of casein in the 20 c.c. of milk originally taken.

(*c*). **Albumin and Globulin.**

The filtrate from the above precipitation of casein contains the remainder of the protein material and the carbohydrate of the milk. This filtrate is placed in a porcelain dish and heated for a few minutes to the boiling-point. The protein material is coagulated and may be filtered through a nitrogen-free filter and washed several times with cold water. A nitrogen determination is then made and the value multiplied by 6.37 to obtain the amount of albumin and globulin present in the 20 c.c. of milk.

The filtrate from this latter precipitation contains the lactose, which may then be determined by titration with Fehling's solution, as described under Urine. It is to be remembered that 10 c.c. of Fehling's solution are reduced by 0.0678 gram of lactose, and not by 0.05 as in the case of glucose.

(8). **Fat.**

It is important in the determination of the fat content of milk that a thoroughly mixed specimen be examined. As the fat tends to rise to the surface of the milk, the fluid should be poured from one vessel into another several times to insure thorough mixing and an immediate measurement made of the portion to be tested.

For clinical purposes as well as for examination of market milk the method of Babcock is to be recommended. For accurate results, however, this method is not to be advised.

Babcock's Method.

This method consists in the destruction of the organic matter, except the fat, by means of sulphuric acid. The fat is then separated by centrifuging and determined by reading off the percentage from the calibrations in the neck of the bottle used.

In the case of cow's milk or where sufficient human milk may be obtained,

17.6 c.c. of milk are measured into the bottle and 17.5 c.c. of sulphuric acid added. These fluids are then mixed by shaking and rotating the bottle in such a way that no curds pass into the neck of the bottle. As soon as the mixture becomes homogeneous and dark brown or even black in color, the bottles are placed in a special centrifugal machine and whirled for five minutes. If the room be very cold it is advisable to fill the holder of the centrifuges with boiling water in order to keep the fat melted while it is being centrifuged. At the end of five minutes, centrifugation is discontinued and the neck of the bottle filled with boiling water. The melted fat will rise in the neck of the flask and may be read off from the calibration. In order to facilitate this the bottles are again centrifuged for one minute.

If a small amount of milk only is available the smaller tubes shown in cut may be used. Milk is added to the mark five and sulphuric acid poured in so as to fill the body of the tube. It is usually necessary to add the milk and acid by means of thin narrow pipets, as the neck of the tube is too small to permit of easy entrance of the fluids otherwise. The milk and sulphuric acid are mixed by rotation of the tube until a homogeneous fluid results. The mixture is centrifuged for a few minutes, the neck of the tube being filled with a mixture consisting of equal parts of concentrated hydrochloric acid and amyl alcohol. The percentage of fat is then read off from the calibrations on the tube.

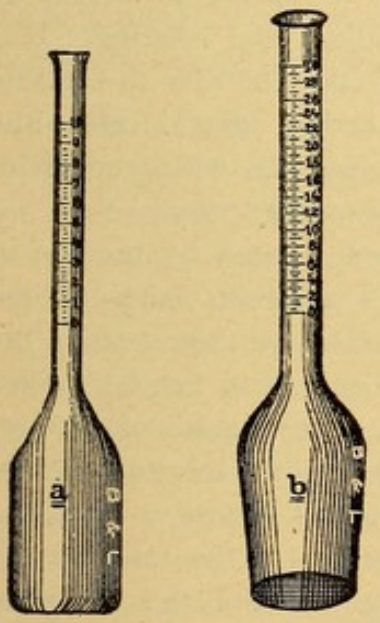

FIG. 162.—Babcock Bottles. *a*, Milk bottle; *b*, cream bottle.

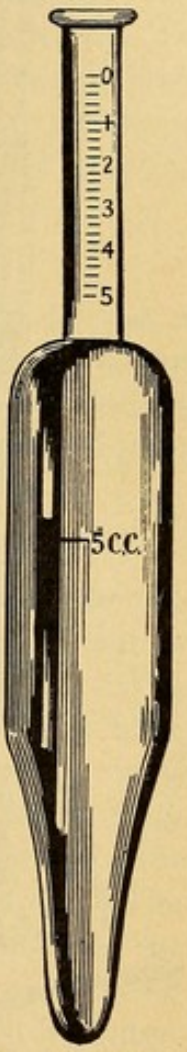

FIG. 163.—Bottle for human milk.

Extraction Method.

A few grams of dried washed sand are placed in the extraction shell of a Soxhlet apparatus and 10 c.c. of well-mixed milk are allowed to fall upon it drop by drop. This is dried at a temperature of 100° C. for one to two hours and is placed in the tube of the extraction apparatus. The fat is extracted in the usual way by the use of gasoline or anhydrous ether, complete extraction usually requiring from two to three hours. The apparatus is disconnected, the ether evaporated from the distilling flask, the residue in the flask dried at 100° C., and the flask and contents dried to constant weight in the desiccator. The difference between the original weight of the flask and its weight including the extracted residue, yields the amount of fat in the 10 c.c. of milk. This method

is the most accurate one, but is not as convenient as the preceding for the general practitioner.

(9). **Lactose.**

In general routine analyses of milk the lactose may be determined by difference. By this is meant that subtraction of the values for the sum of water, ash, protein, and fat from 100 will yield the percentage of lactose. For clinical purposes this is usually sufficient, but for the more accurate work it does not give exact figures, as slight amounts of other undetermined substances are present.

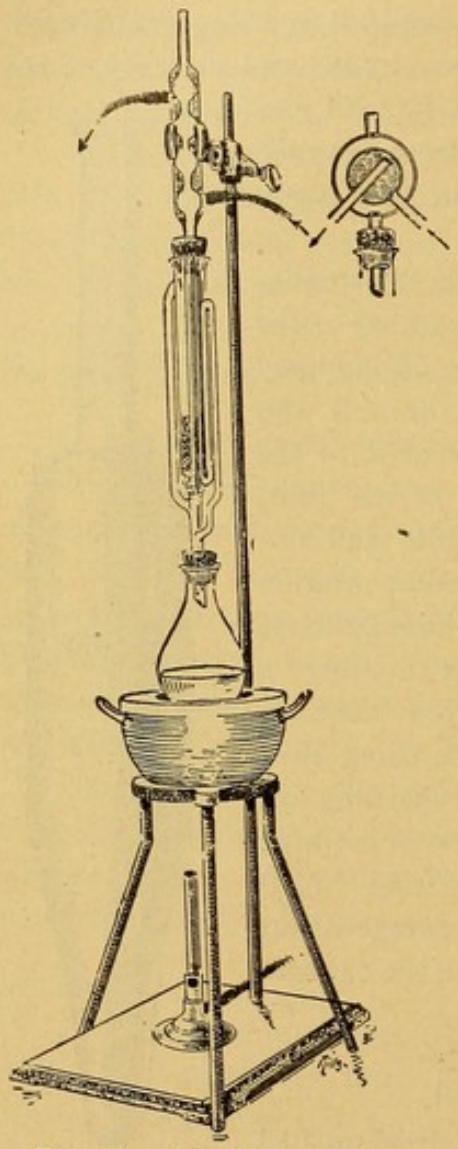

FIG. 164.—Soxhlet apparatus. (*Hawk.*)

For any direct determination of lactose it is necessary that the larger portion of the protein material be removed previously. This may be done by the method outlined under Determination of Casein, Albumin, and Globulin; or for clinical purposes sufficiently accurate results may be reached by acidifying with acetic acid, boiling, and filtering. It is advisable to always take the time to saturate the mixture with carbon dioxid after the casein has been precipitated with acetic acid, as the results are more satisfactory.

The fluid is then titrated by the use of Fehling's or Purdy's solution, using all the precautions mentioned under these tests in the section on Urine. Ten c.c. of Fehling's solution are reduced by 0.0678 gram of lactose, while the 35 c.c. of Purdy's solution are reduced by 0.02712 gram of lactose.

If the polarimeter is to be used for the estimation of lactose in milk the following procedure may be used. Fifty c.c. of the well-mixed milk are placed in a flask, 25 c.c. of a solution of neutral lead acetate are added, the flask is closed with a stopper through which passes a glass tube approximately 30 cm. in length. The mixture is then heated over a small flame to boiling. After the mixture has cooled it is filtered through a dry filter into a dry vessel and polarized.

(10). **Preservatives in Cow's Milk.**

(*a*). **Sodium Carbonate.**

To hide the acid reaction of a spoiled sample of milk sodium carbonate is often added, and may be detected as follows: Ten c.c. of milk are mixed with ten c.c. of 96 per cent. alcohol and a drop of rosolic acid solution. Pure

unadulterated milk produced a brownish-yellow color, but, in the presence of sodium carbonate or bicarbonate, a rose color is obtained. For greater precision in doubtful cases the questionable sample should be compared with known unadultered milk. Phenol-phthalein solution may be used as an indicator in place of rosolic acid. By this method 0.05 per cent. of carbonates may easily be detected.

(*b*). **Salicylic Acid.**

Twenty c.c. of milk are treated with two or three drops of sulphuric acid and shaken with an equal amount of ether. The greatest possible part of the ethereal solution is drawn off and evaporated, the residue extracted with 40 per cent. alcohol, filtered, and 5 c.c. of the filtrate treated with a few drops of ferric chlorid solution. A violet color shows the presence of salicylic acid or some other hydroxy derivative of benzol.

(*c*). **Formaldehyd.**

Two c.c. of concentrated sulphuric acid are placed in a test-tube and a drop of ferric chlorid solution added. A few c.c. of milk are allowed to run from a pipet upon the surface of the mixture in such a way that a distinct line of contact forms. A violet color at the point of contact of the two liquids is characteristic of formaldehyd in the presence of casein.

(*d*). **Boric Acid and Borax.**

Fifty c.c. of milk are alkalinized with milk of lime, evaporated to dryness and incinerated. The resulting white crystalline residue is treated with a few drops of tincture of turmeric and very dilute hydrochloric acid and is then dried on the water bath. The presence of the slightest trace of boric acid gives to the dry residue a beautiful vermilion or cherry red color. It is possible by this method to detect 0.001 per cent. of boric acid in milk. Only very dilute hydrochloric acid must be used in testing for boric acid, since the concentrated acid itself gives with tincture of turmeric a red color. The coloration produced by boric acid is distinguished from that produced by hydrochloric acid by the fact that it does not disappear by treatment with water in the cold, but only after long boiling, while the color caused by hydrochloric acid disappears as soon as it is diluted with water.

If the crystalline residue obtained as above described be treated with alcohol and the alcohol ignited, a flame tinged with a beautiful emerald-green color is obtained in the presence of boric acid.

III. Bacteriological Examination of Milk.

The bacteriological examination of human milk is frequently desirable from a clinical standpoint as milk may become contaminated as it passes along the lacteal ducts. In pathologic conditions many types of organisms, such as typhoid bacilli, pneumococci, and tubercle bacilli may be obtained, although the latter are extremely rare.

With cow's milk the chief question at issue is whether the milk contains sufficient bacteria to be harmful to the child. Aside from the presence of the tubercle bacillus in the milk of infected animals, large numbers of saprophytic organisms must find their way into this fluid and will, if in large numbers, influence the intestinal activity of the child.

The methods of examining milk for bacteriological differentiation are the same as for any other fluid and will be found in works on bacteriology. It is, however, a matter of some interest to the practitioner to determine the number of bacteria present, as a control of the sanitary conditions or as a check on the efficacy of pasteurization, without regard to the various types present. It is to be remembered that bacteria develop rapidly so that a specimen should be examined as fresh as possible in order to determine with more certainty the degree of original contamination. The method of performing this enumeration may be found in general works on bacteriology.

The number of bacteria in milk may vary from a few thousand to many millions. It is an impossibility to obtain a specimen which is sterile so that the presence of as high as 100,000 organisms should not be regarded as dangerous unless pathogenic types are present. If the freshly examined specimen contains between 10,000 and 50,000 bacteria per c.c., it is probable that all possible precautions have been taken to prevent contamination, although the writer has found some specimens showing a count of only 2500 per c. c.

In the examination of milk it is customary also to make a cytological examination. Normally milk contains only a few scattered leucocytes and epithelial cells. If examination reveals numerous leucocytes, absolute evidence is present of infection of the animal and consequent unfitness of the milk for use. The methods used in this cytological examination are the same as outlined in previous sections.

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