# CLINICAL DIAGNOSIS

EWING

# NOTES

ON

# CLINICAL DIAGNOSIS

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# TRANSUDATES AND EXUDATES.

## TRANSUDATES.

*Transudates* are fluids which pass from the vessels into serous cavities or tissue spaces as a result of mechanical disturbance in the circulation, altered conditions of the blood, and changes in the walls of vessels, but in the absence of any true inflammatory process.

*Gross Appearance.*—Transudates are usually serous in character, and of a pale straw color; they may be mixed with blood, especially when rapidly formed, in which case they may clot; rarely they are chylous, chyloid, or opalescent.

On standing, they may yield a very scanty deposit of lining endothelial cells, leucocytes, red blood cells, and altered chemical elements.

*Microscopical Examination* reveals, in the sediment, endothelial cells, leucocytes, red blood cells, fat globules, cholesterin crystals in cases of long standing, and granular or globular detritus. They are usually free from bacteria.

*Chemical Examination.*—The *specific gravity* of pure ascitic transudates usually varies between 1.005 and 1.015, but in many old cases a slight inflammatory element may raise the gravity beyond this point. The *reaction* is faintly alkaline. Most transudates contain considerable *albumen,* on the proportion of which the gravity principally depends. As a rule transudates contain much less albumen (1-3 per cent.) than do exudates (4-6 per cent.). Pleuritic transudates contain more albumen than do the peritoneal fluids. The most abundant form of albumen in these fluids is *serum-albumen,* which readily transudes through slightly altered vascular walls.

*Serum-globulin* is present in small proportions as it passes with difficulty through unbroken vascular walls, and its proportion to serum-albumen in transuded fluids is far below that in the blood.

In exudates, on the other hand, the ratio of serum-globulin to serum-albumen may approach that of the blood (1-1.5), and any considerable percentage of globulin in a supposed transudate points toward an inflammatory origin or to some complication. Traces of *nucleo-albumen* are commonly present in transudates, and in some cases of chronic ascites nucleo-albumen may be present in considerable quantities and constitute the only form of albumen demonstrable in the fluid. *Fat* in the form of globules, crystals of fatty acids, or the derivative cholesterin in crystals, is occasionally present, less frequently than in exudates.

Fat globules and cholesterin crystals usually indicate that the transudate is of old formation. In true *chylous ascites*, which is usually referable to filariasis, the fat globules are extremely minute and may escape detection.

In chronic ascites the presence of nucleo-albumen may give an opacity and opalescence to the fluid, causing it to resemble the true chylous fluid. Such fluids are termed "chyloid."

Traces of *sugar* and *uric acid* are usually present. *Sodium chloride* (.6 per cent.) is the chief inorganic principle.

### *Methods of Examination.*

The examination of transudates should include the study of their

Gross appearance,
Reaction,
Specific gravity,
Total content of albumen (Esbach's method),
Ratio of albumen and globulin,
Microscopical characters of sediment.

In most details the fluid is to be treated as one of urine, under which subject the tests will be found described.

## EXUDATES.

*Exudates* are fluids which pass from the vessels or are extruded from tissues as the result of inflammatory processes.

The main varieties of exuded fluids are *serous* and *purulent*.

## SEROUS EXUDATES.

*Gross Appearance.*—Serous exudates may be simply *serous* or may contain varying quantities of blood (hemorrhagic), or fibrin (sero-fibrinous), or pus (sero-purulent), or chyle (chylous). They may clot spontaneously, especially when mixed with blood, either in the serous cavity or after aspiration. On standing, they usually yield a distinct sediment. Granules, or large masses of cells, fibrin, or mucus, may be visible to the naked eye. Fluids from joint cavities are often viscid from the presence of mucus.

*Microscopical Characters.*—The sediment from serous exudates usually contains, in moderate number, red blood cells, leucocytes, and exfoliated endothelial cells, and these cells are more abundant than in transudates. *Fat* is present in the form of a minute emulsion in chylous exudates, and as globules, or fatty acid or cholesterin crystals, *in cases of long standing*. The decomposition of blood often yields hematoidin crystals. Isolated cells or larger particles of a tumor may be found in the sediment of exudates complicating carcinoma or endothelioma of serous surfaces.

*Chemical Analysis.*—The specific gravity of serous exudates is usually higher than 1.010, depending upon the quantity of albumen present. The reaction is alkaline.

*Serum-albumen* is present in considerable quantity (4-6 per cent.). *Serum-globulin* occurs in larger proportions than in transudates, and in active inflammatory lesions may approach or equal the ratio of the blood. *Peptone* makes its appearance only when the fluid contains a considerable admixture of pus. *Sugar, uric acid* and *sodium chloride* are present in about the same proportions as in transudates.

*Bacteria* are frequently present in serous exudates, but in such small numbers that their demonstration usually requires the test by culture or inoculation.

### *Special Varieties of Exudates.*

*Tuberculous exudates* are usually serous or hemorrhagic, the latter form indicating the more severe inflammation. A hemorrhagic pleural exudate is usually tuberculous, and tuberculous pleural exudates are more frequently bloody than those of the peri-

toneum. Bacilli are usually present in tuberculous exudates in very small numbers, but when there are necrotic foci in the serous membrane they become more abundant. Rarely they may be found by staining the sediment; usually their demonstration requires the inoculation of 10 c.c. (or more) of the fluid into the peritoneal cavity of a guinea-pig.

*Carcinoma or endothelioma* of serous membranes is frequently accompanied by an exudate which is usually serous, but often bloody. It is sometimes possible to demonstrate in the sediment of such cases isolated cells or small masses of the tumor. For positive identification such masses must show very typical characters of a neoplasm, among which the presence of *mitotic nuclei* is most convincing.

*Joint-fluids* usually contain mucus and are slightly viscid. In *traumatic* and *rheumatic synovitis* the exudates are usually clear and sterile.

In *tuberculous synovitis* the fluid is serous, bloody, or sero-purulent, and tubercle bacilli may be demonstrated either by staining the sediment or by inoculation. In *gonorrhœal* synovitis the fluid is serous or sero-purulent, and the gonococcus may often be demonstrated by staining methods or by culture on chest serum.

### *Methods of Examination of Exudates.*

The gross and chemical examination of exudates is similar to that of transudates; but the microscopical tests include the demonstration of specific germs by staining the sediment secured after standing or by means of the centrifuge. The staining methods will be described later. For the demonstration of the tubercle-bacillus in serous exudates by inoculation at least 10 c.c. of the fluid, containing, if possible, the sediment, should be injected into the thighs or peritoneal cavity of a guinea-pig, observing strict antisepsis throughout the entire procedure. After three weeks the animal may be killed and examined for tuberculous lesions.

## PURULENT EXUDATES.

*Gross Appearance.*—The gross appearance of pus is determined by the relative quantities of serous fluid, leucocytes, and red

blood cells composing it, and upon changes occurring after its formation. When a serous exudate contains a large quantity of leucocytes the straw-colored fluid becomes turbid; when the exudate is largely composed of leucocytes the pus is thick and creamy. The thin, *sanious pus* of gangrenous inflammation contains few leucocytes, decomposed blood cells, and many bacteria. When a mucous membrane is involved in the purulent process, mucus is added to the exudate, giving the characteristic appearance of *muco-pus.*

Various putrefactive processes may quickly alter the gross character of pus, as indicated by changes in color and odor. In pus obtained from old abscess cavities the *effects of age* may be seen in the separation of solid and fluid constituents. Such pus contains small granules or large flakes of coherent and degenerated cells, and granular detritus, floating in turbid or clear serous fluid (cold abscess). When desiccation occurs the pus may become thick and caseous. Fat globules and crystals of fatty acids or cholesterin may give peculiar characters, such as a greasy consistence or glittering lustre. In *actinomycosis,* the colonies of the ray-fungus are seen as *small, light-colored granules,* much firmer in consistence than the somewhat similar granules composed of adherent cells seen in old pus.

Various other micro-organisms such as saccharomyces, leptothrix, aspergillus, bacillus pyogenes filiformis, and actively growing pyogenic or putrefactive germs may form zoogloea, become entangled with particles of necrotic tissue, and produce *semi-solid opaque masses* visible to the naked eye.

*Chemical Analysis.*—The *reaction* of fresh pus is alkaline, but decomposition may early cause the formation of fatty and lactic acids and a change to a neutral or acid reaction.

The *specific gravity* varies between that of serous exudates, 1.010, and that of the blood, 1.060, being usually about 1.030-5.

The fluid portions are rich in serum-albumen and serum-globulin, while *peptone* is added from the destruction of leucocytes. Glycogen, glucose, uric acid, urea, acetone, and xanthin bases are demonstrable in small amounts. Soon after formation, fats make their appearance in the form of globules, soon changing to fatty acid crystals, and later to leucin, tyrosin, and cholesterin. Other

crystalline bodies sometimes seen are hematoidin and triple phosphates.

*Microscopical Characters.*—The vast majority of pus cells are polynuclear leucocytes with neutrophile granules. In fresh, unstained specimens these cells are small spheroidal bodies containing a central highly refractive nucleus, surrounded by many *fine* and *perfectly opaque granules,* which serve at once to distinguish them from any other cell in the body. *Eosinophile cells* are rarely seen in pus; but some specimens, for reasons not clear, contain them in marked proportions. *Mononuclear leucocytes* are usually present in small numbers. *Tissue cells* are demonstrable in considerable numbers or masses only under special conditions. Leucocytes may very early undergo fatty or hydropic degeneration, with distortion or destruction of the cell body and loss of granules. Tissue cells are very apt to swell and lose their original shape, becoming circular in outline. They may still be distinguished from leucocytes by their larger size, absence of opaque granules, vesicular nucleus, and condensed border. *Red blood cells* are usually present, and in intense inflammations may be abundant.

## MICRO-ORGANISMS OF PUS.

The full bacteriological examination of pus requires the services of a bacteriologist and the equipment of a laboratory. When such an examination is desired, a specimen of the pus should be taken, under aseptic precautions, with a sterilized swab and test tube, and sent to the laboratory for examination.

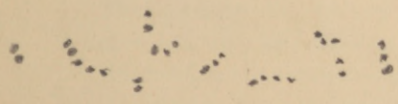
Most specimens of fresh pus contain a specific micro-organism in such numbers that its demonstration is possible by staining a specimen dried on a glass slide. In this way we may secure indications pointing to the presence of the *staphylococcus pyogenes, streptococcus pyogenes, diplococcus pneumoniæ, bacillus anthracis,* the *gonococcus, bacillus diphtheriæ, amœba coli,* and may positively identify the *tubercle bacillus* and the *ray fungus* (actinomyces).

The peculiarities in occurrence and staining qualities of these germs require special consideration.

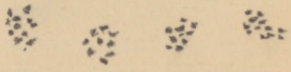
The *common pyogenic germs, staphylococcus* and *streptococcus*

The Streptococci ~~Staphylococcus~~ are positively chemotactic for polynuclear neutrophiles.

Streptococci



Staphylococci



Pneumococci (Welch)

*pyogenes,* are found in most cases of wound infection and acute suppuration, and may be recognized, as far as is possible from morphological examination alone, by staining a specimen smeared and fixed on a glass slide by aqueous methylene blue. The pus of old abscesses is, however, frequently sterile.

The *pneumococcus* is usually found, in the procedures resorted to for clinical diagnosis, in the pus of empyema and meningitis.

This germ presents a characteristic appearance of lanceolate diplococci surrounded by a capsule, and growing usually in short chains. The cocci and capsule are readily demonstrated by Gram's stain. (See gonococcus.)

*Welch's method* is specially adapted to the demonstration of the pneumococcus and capsule.

1. The exudate is smeared, dried, and fixed on a glass slide, passing the slide through the flame until it is as hot as the hand can bear.

2. Flood the specimen with glacial acetic acid, which is immediately drained off.

3. Flood the specimen two or three times with anilin-water-gentian-violet.

4. Wash in 2 per cent. aqueous solution of sodium chloride, in which it may be mounted.

*Washing in water must be avoided.*

To prepare anilin-water-gentian-violet, which must only be used when fresh, shake well nine parts of distilled water with one of anilin oil, and filter. To the filtrate add one-tenth as much of saturated alcoholic solution of gentian-violet.

By this method the pneumococci appear as deep blue diplococci, and the capsule is lightly, but *distinctly,* stained bluish. Care must be taken not to mistake the *clear areas* surrounding many bacteria, and resulting from shrinkage of the surrounding medium, for capsules.

The *gonococcus* occurs principally in genito-urinary discharges, but may be found in gonorrhœal infections of the rectum, mouth, eyes, serous cavities, and blood. Its natural habitat is the genito-urinary tract, where it thrives best on surfaces lined by *columnar epithelium,* growing less actively on adult stratified squamous epithelium.

There are some important peculiarities in the occurrence of this germ in gonorrhœa.

1. *In gonorrhœa in the male* the gonococcus is found in all acute and chronic urethral discharges, and may persist several years after infection. In gonorrhœal cystitis it is most abundant in the "threads" found in the urine.

2. *In the female,* the gonococcus is most abundant in and is often limited to the urethal pus. Next in abundance it is found in the discharge from the cervix uteri. In the vaginal discharge it is often overgrown by other diplococci, and may thus escape detection. It is therefore important to examine the pus from the *urethra* or *cervix,* and not to trust to the examination of the vaginal discharge, which is more readily obtained.

3. *In female children,* it is found abundantly even in the vaginal discharge, but is not usually found in the urethra, and children rarely develop gonorrhœal cystitis.

In most cases of *chronic gonorrhœa* the morphological examination of the pus by Gram's method and counterstain will successfully demonstrate the germ; but when this test is negative, resort must be made to cultural methods. In acute cases the results of morphological examination are practically certain.

The identification of the gonococcus in stained specimens requires that the germ should show the following characters:

(1.) In morphology it must be a *biscuit-shaped diplococcus.*

(2.) It must be found *within the bodies of the pus cells.*

(3.) It must *decolorize by Gram's method.*

### *Method of Staining the Gonococcus.*

(1.) The pus is smeared and fixed on a glass slide.

(2.) Flood the specimen for 30 seconds with fresh anilin-water-gentian-violet, ~~and wash in water~~.

(3.) Flood the specimen with Gram's iodine sol. (I. 1.0—KI, 2.—$H^2O$, 300) for 1 to 5 minutes, and ~~wash in water~~.

(4.) Decolorize in alcohol 97 per cent. for 2 to 4 minutes. Then wash in water.

(5.) Counterstain in sat. aq. sol. of Bismarck-brown for 1 to 3 minutes. Carbol-fuchsin


gonococci

### *Rationale of Above Method.*

Gram's stain decolorizes the gonococcus, but leaves other urethral diplococci and pyogenic cocci deeply stained by gentian-violet. The gonococci are then counterstained by Bismarck-brown. The effect of iodine is not fully understood. In decolorization by alcohol it has been found that under 2 minutes alcohol will not decolorize all gonococci, while over 4 minutes it may decolorize some pyogenic cocci. The progress of decolorization should be followed under the low power lens, and should be at once suspended when the nuclei of the leucocytes have lost their blue stain.

*Bacillus anthracis* is commonly present in large numbers in the exudate of malignant pustule, and its peculiar morphology admits of its demonstration in specimens of the pus stained with methylene blue. This germ is 1-1.2 $\mu$ in thickness, 6-8 $\mu$ in length. Longer threads are often seen, especially in cultures. The ends are sharply cut across or *concave*. Spores are common. The protoplasm is finely granular, and the bacillus may appear to be surrounded by a thin, unstained capsule.

When such bacteria are found in suspected pustules a biological test should at once be made to confirm the diagnosis.

## PHARYNGEAL EXUDATES.

The examination of pharyngeal exudates is of great importance in the diagnosis of diphtheria and other diseases of this region.

The complete investigation of such cases may involve three procedures:

(1.) *The morphological examination of the exudate.*

(2.) *The biological examination.*

(3.) *The test by inoculation.*

(1.) *Morphological Examination.*—The results of the morphological examination of a croupous or other pharyngeal exudate may conveniently be grouped in three classes:

(*a*) The stained specimens may show an abundance of *Klebs-Lœffler bacilli* and few or no other germs.

The morphology of the *bacillus diphtheriæ* being usually quite

characteristic, it may be identified with fair certainty on this feature alone. Moreover, in many cases of diphtheria the germ is present in such large numbers, and nearly pure growth, that the stained exudate leaves no doubt of the nature of the disease, and one is justified in making a positive diagnosis of the presence of the *bacillus diphtheriæ*.

(*b*) The stained specimens may show many *cocci* as well as typical Klebs-Lœffler bacilli.

Cases of this type are very commonly encountered, and the evidence thus obtained is hardly less positive than before that we are dealing with a case of "mixed infection" with Klebs-Lœffler bacilli and pyogenic cocci.

(*c*) The stained specimens may show the presence of many *cocci,* but *few or no Klebs-Lœffler bacilli.*

Such cases may or may not be true diphtheria, but the scant number or absence of Klebs-Lœffler bacilli leave the question in doubt, and resort must be had to the *biological test.*

(2.) *Biological Examination.*—In all cases remaining doubtful or negative from the morphological examination, as well as for confirmation of positive results, a smear from the throat must be streaked over coagulated blood serum and kept in the thermostat for 12 to 24 hours. On blood serum in the thermostat, the Klebs-Lœffler bacillus outstrips in growth most pyogenic or other germs occurring in the mouth and throat, and when the culture tubes are examined after 24 hours the colonies of Klebs-Lœffler bacilli may be recognized as a sharply outlined, well-raised, granular, creamy yellow or reddish growth.

*Streptococcus pyogenes* produces discreet, pin-point, rounded, pale, bluish, translucent colonies.

The *staphylococcus* produces more abundant opaque, shiny, rounded colonies of white, orange, or lemon-yellow color.

The identification of all these germs is to be completed by transferring the growth to a glass slide and staining with methylene blue.

### *Morphology of the Klebs-Lœffler Bacillus.*

The *bacillus diphtheriæ* commonly grows in the form of *slender* rods, 3-5 $\mu$. in length, a little thicker than the tubercle bacillus,

Klebs-Loeffler Bacilli.

and straight or slightly curved. A marked characteristic is their variation in shape and size. They may stain deeply and uniformly with methylene blue or a *beaded or cross-striated* appearance is common. The ends may be distinctly *clubbed,* especially in cultures, and these swollen ends may appear to contain discreet spore-like bodies staining violet with methylene blue. The tendency toward "clubbing" probably causes some short individuals to appear as *cocci.* They do not form chains, but rather long threads may occur.

### *Staining.*

Smears from the throat or from cultures of the Klebs-Lœffler bacillus are usually stained by Lœffler's alkaline methylene blue solution—

Sat. alcoholic sol. Methylene blue..... 30
Aq. sol. KOH (1-10000).....100

Or, stain by a 1 per cent. aqueous sol. of methylene blue.

Fraenkel has devised a method which seems to differentiate the true from the false diphtheria bacillus.

Cultures on blood serum 10 to 20 hours old are stained 1 to 3 seconds in the following solution: Meth. blue, 1; alcohol, 96 per cent. 20; Ac. glac., 50; Aq., 950. Wash in water and counter-stain in a watery solution of Bismarck-brown. The "polar granules" in the true *bacillus diphtheriæ* are stained blue, those of the pseudo-diphtheria bacillus appear brown.

(3.) *Inoculation.*—Having identified the *bacillus diphtheriæ* by the above methods, it is found that some patients whose throats harbor such germs do not suffer from a disease which the clinician is satisfied to regard as diphtheria. Park has shown that many such cases sooner or later develop diphtheritic inflammation. Others, however, do not. Some authorities believe that in the cases remaining immune the infecting germ is not the Klebs-Lœffler bacillus, but a *pseudo-diphtheria bacillus.* Others claim that the pseudo-diphtheria bacillus is only a non-virulent form of the true Klebs-Lœffler germ. It is impossible at present to decide which of these views is correct, but practically all germs of typical morphology may be regarded as of the Klebs-Lœffler variety, while

in doubtful cases it becomes necessary to determine the virulence of each infection.

*Without the test by inoculation it is therefore impossible to state positively whether or not a case of mild pharyngitis is true diphtheria.*

### *Method of Inoculation.*

As a routine procedure it is safe to rely upon the following test, as used by the New York Health Department: One-half cubic centimetre of a 48 hours broth culture of the bacillus to be tested is injected subcutaneously into a guinea-pig.

Cultures of ordinary virulence will cause the death of the animal in 36 hours.

Cultures of slight virulence may require 3 to 4 days to cause death, or may fail to kill.

Non-virulent cultures produce no distinct effect upon the animal.

### EXAMINATION OF THE EXUDATE IN DISEASES OF THE BUCCAL MUCOSA, ETC.

*Leptothrix.*—Various forms of a micro-organism related to the fungi or algæ, growing in the form of long filamentous threads not distinctly jointed, are found in and about decaying teeth, in the tonsillar or other pharyngeal crypts, or the pharyngeal wall, in the lachrymal ducts, in the nostrils, stomach, and vagina, and in necrotic pulmonary foci. These forms are all grouped under the term *leptothrix*. They may produce pharyngitis with persistent and recurring ulceration.

They may be recognized in specimens of the exudate stained by Gram's iodine solution, in which they appear as pale bluish threads arranged in parallel columns.

*Mycoderma (Oidium) Albicans.*—This fungus grows in the superficial ulcerations of the buccal mucosa in "*thrush.*" It is best examined in the fresh exudate, and presents long threads, *hyphæ*, with double refractive contours, rounded ends, and containing or surrounded by numerous large spores.

It is probable that several varieties of fungi are related to the

stomatitis of children, and their identification requires full biological analysis.

*Mucor, Aspergillus, and Eurotium.*—Varieties of this fungus group are occasionally seen in many situations, but its demonstration is specially important in some forms of refractory and recurrent inflammation of the external auditory canal. It is readily recognized in the fresh condition from the large spheroidal mass of conidia spores mounted on rather coarse hyphæ.

*Trichopyton Tonsurans.*—This fungus causes a variety of cutaneous diseases, including *Herpes or tinea tonsurans* (Ringworm), (Parasitic sycosis), *Eszema marginatum,* and *Impetigo contagiosa.* The hyphæ and spores may be demonstrated in the fresh condition, in the scales from the affected skin, and in the sheaths and shafts of the hair. The hyphæ are long and narrow and contain few spores.

They may be stained by Gram's method.

*Achorion Schonleinii.*—This fungus grows in the scabs and hairs of regions affected by *favus.* It produces hyphæ and spores very similar in appearance to those of trichophyton, and is best demonstrated in the fresh condition.

*Microsporon furfur* is the fungus of pityriasis versicolor or chromophytosis. Its hyphæ and spores are somewhat smaller than those of the other fungi infecting the skin.

It is at present impossible to accurately identify all the fungi of the skin. It is probable that their variety is much greater than present classifications would indicate. Practically, the important object is to demonstrate by microscopical examination simply the fungoid nature of these lesions.

# SPUTUM.

The sputum is an exudate composed of mucus and pus, to which are often added serum, fibrin, red blood cells, necrotic tissue, and micro-organisms, derived from the respiratory tract.

## *Gross Characters.*

*Mucoid sputum* is the simplest form of this exudate, of which good examples are sometimes seen in chronic bronchitis. Purely mucoid sputum with very little admixture of pus is unusually light in color, often slightly translucent, very viscid, tenacious and elastic, and very slightly aerated. A pure specimen is not often obtained.

*Muco-purulent sputum* is the ordinary expectoration of acute bronchitis. The addition of pus gives a yellowish color and greater opacity to the mass, greatly reduces its tenacious elastic quality, and usually leads to more complete aeration.

*Purulent Sputum.*—The quantity of pus in the sputum often greatly exceeds that of the mucus, and when the viscid quality of the mucus is no longer noticeable to the naked eye it is usual to describe the expectoration as purulent. Such specimens are seen in severe bronchitis and in tuberculosis of the lungs. The expectoration of *pure pus* of the usual character occurs in abscess of the lung, and after rupture into the lung of abscesses in contiguous viscera and cavities.

*Blood-stained Sputum.*—A few red blood cells not appreciable to the naked eye may be found microscopically in many specimens of sputum. Blood in visible quantities is often streaked over the sputum in its passage through inflamed bronchi. When exuded with the mucus it is more evenly mixed, but bright red if fresh. Both varieties of blood-stained sputum are most often seen in acute bronchitis of severe onset.

*Bloody Sputum.*—When the blood in the expectoration exceeds the mucus, the viscidity is greatly diminished, although the sputa remain coherent. Such specimens are commonly spoken of as bloody sputum, and are seen in acute bronchitis, pneumonia, in pulmonary tuberculosis either during the few days following a hemorrhage or without distinct hemorrhage, in tumors of the lung, and in hemophilia.

*Pure blood* is expectorated in pneumonia, in phthisis in small quantities frequently repeated from eroded bronchi and in large, fatal losses from ruptured arteries, in aneurism, in asphyxia and various septic conditions of infants, in hemophilia and other blood diseases of adults, as well as from trauma.

*Rusty sputum* is the peculiar pathognomonic expectoration of acute lobar pneumonia. It consists of gelatinous pellets of mucus slightly mixed with pus and uniformly tinged a rusty color. The color is due to the presence of undissolved red cells, which are exuded with the mucus in the terminal bronchioles and in the air passages and vesicles, and are very evenly mixed with the mucus. It is seen almost exclusively in pneumonia, but has been noted after an attack of acute pulmonary œdema.

*Serous Sputum.*—When serum is added to the expectoration it becomes semi-fluid in consistence, and on standing the serum separates from the mucus, giving two distinct layers of the sputum. *Saliva* is usually more frothy than serum, and too minute in amount to yield a separate layer on standing. The serum is often slightly blood-stained. Such mixtures of mucus and serum are expectorated in the terminal stages of bronchitis, in Bright's disease, pulmonary tuberculosis, pneumonia, endocarditis. Many patients with œdema of the lungs fail to expectorate the serum (e. g., morphine poisoning).

*Pure serum* may be expectorated in considerable quantity in acute œdema of the lungs, occurring in the initial stages of pneumonia, or in nephritis with arterio-sclerosis.

*Fibrinous coagula* are often mixed with the sputum in chronic bronchitis and pneumonia, and there is a type of the former disease in which very large dendritic fibrinous casts of the bronchi are occasionally expectorated.

*Gangrenous sputum* is characteristic of acute necrosis with putrefaction of lung-tissue. The bloody sputum of pneu-

monia may have the dark brown color of prune-juice, indicating decomposition of the blood. At post-mortem such cases usually show some small area of dead lung-tissue. In *gangrene of the lung* a very fetid discharge occurs of dark brown or greenish color, which usually separates on standing into three layers. The uppermost layer consists of frothy mucus and pus, the middle layer of serum, while the heavier solid portions of tissue sink to the bottom.

In some severe cases of the infectious diseases, in pneumonia, and in jaundice, the sputum may be colored yellowish or greenish by dissolved *bile pigments.*

*The Minute Features of the Sputum Distinguishable by the eye.*—On close inspection it is possible and important to detect some elements in the sputum which are commonly overlooked in a hasty examination.

On careful scrutiny *minute air-bubbles* may be seen throughout the mucus, although the sputa are compact and the specimen has appeared entirely unaerated.

*Small fibrinous coagula* are discernible as solid, rounded, whitish, opaque bodies of the size of a pin-head or larger. Most of the small white, opaque particles in sputum are composed of adherent leucocytes or necrotic granular matter.

The *colonies of actinomyces* appear as small white granules, partly calcific, and resisting considerable pressure.

*Curschmann's spirals* can usually be detected by the naked eye, as whitish, opaque spiral threads, 1-10mm. in length.

Small *masses of lung-tissue* are usually grayish in color, and of very irregular outline.

Masses of *hematoidin* are dark brown particles resisting much pressure.

### *Microscopical Examination.*

*Mucus* appears in the specimen, smeared on a glass slide, fixed by heat and stained by methylene blue, in the form of a fine network of deep blue threads. Very tenacious mucus may yield thicker threads. *Fibrin* occurs in coarse, dense threads, masses, and rounded coagula, all staining very deeply with methylene blue. (Weigert's fibrin stain may be applied for its positive identifica-

tion, as follows: Stain half an hour in anilin-water-gentian-violet. Wash in three-fourths per cent. salt sol. Dry with blotting-paper, and flood for two minutes with Gram's iodine solution. Dry with blotting-paper, and decolorize with anilin oil. Mount in balsam. Fibrin then appears very dark blue or black.)

*Red blood cells* usually retain their ordinary appearance.

*Epithelial cells* in sputum may be derived from the mouth, where they are of the large, flat, *squamous* variety; or from the bronchi (or nares), when they appear with or without *cilia* and *condensed border,* but of distinctly *columnar* type; or from the pulmonary parenchyma, when they are *rounded,* of large size, and usually contain *black pigment.* In pulmonary congestion from endocarditis the alveolar epithelium contains large *brownish grains* of blood *pigment.* Leucocytes may be recognized by their multiple nuclei and clear cell bodies (methylene blue).

In resolving pneumonia, all pulmonary cells show *vacuolation* and *fatty degeneration.*

Large and small *fat globules* are seen in many forms of sputum. Unrecognizable granular and globular detritus forms a considerable portion of most specimens of sputum. Much of this globular material is probably *altered mucin.*

*Elastic fibres* occur in the sputum either singly or in masses in which they show a distinct alveolar arrangement. The single fibres may usually be recognized as long, wavy threads, highly refractive, but lacking the double contour of vegetable fibres. They can be positively identified when found in the basket-shaped network of the pulmonary alveoli, and only under such conditions is it justifiable to conclude from their presence that destruction of lung-tissue has taken place. They are most abundantly seen in pulmonary tuberculosis, but have been found in bronchiectasis, abscess, and rarely in pneumonia. They are not seen in the sputum of grangrene of the lung.

The *crystalline bodies* found in sputum include the elongated, diamond-shaped *Charcot-Leyden crystals,* which are often very abundant in chronic bronchitis; *hematoidin* masses, which are recognized from their dark brown color, opacity, and irregular shape; long, acicular *crystals of the fatty acids;* and, occasionally, *cholesterin-plates* and crystals of calcium carbonate and triple phosphate.

*Curschmann's spirals* consist of a central convoluted fibrinous mass, formed probably in the terminal bronchioles, and surrounded by many spirally wound threads of mucus added during the passage through the bronchioles. These spirals frequently hold a large number of Charcot-Leyden crystals. They occur in chronic bronchitis.

### *Bacteriology.*

*Moulds, Aspergillus, Leptothrix* and *Yeast-fungi* are occasionally seen in the sputum of abscess of the lung and phthisical cavities, where they are commonly regarded as innocuous. It is probable that they facilitate the putrefactive processes in these conditions. In some refractory cases of chronic bronchitis, with spasmodic asthma, aspergillus and other moulds have been found in abundance in the sputum, and are regarded by some as an etiological factor. From what is known of their effects in respiratory diseases of lower animals, their presence in sputum from the human subject must always be regarded with suspicion.

The white granular colonies of the *"ray fungus"* are discharged in considerable numbers in pulmonary actinomycosis. The colonies consist of a central mass of interwoven threads. These threads have many of the characteristics of bacilli, differing mainly in that they are branched. The outlying ends of the threads are *swollen and clubbed,* probably as a result of a degenerative process, and the radiate arrangement of the clubbed ends gives the characteristic appearance to the colony from which it is called the "ray" fungus. Masses of threads may occur without the clubbed ends. Actinomyces may be well demonstrated by staining with hematoxylon and eosin or by Gram's method and eosin, either in smeared specimens or in sections of the sputum hardened in formalin and cut in celloidin.

*Bacteria.—Non-pathogenic* cocci and bacilli are present in nearly all specimens of sputum and multiply rapidly after expectoration, decomposing the mucus and destroying the viscidity of the specimen.

*Pathogenic Species.*—The *streptococci* of the sputum are frequently derived from the mouth, and may have no pathological significance. In some cases of *severe acute bronchitis* strepto-

Welch's method - Glacial acetic acid followed by Anilin Water - Gentian Violet. 2% to 4% Salt can be used to wash off the color. Generally the preparations cannot be kept. Do not dry the slide by heat cover & examine in the salt.

cocci have been found in large numbers in the bronchial exudate, and there is little doubt that they may at times be an important, if not prime, factor in this disease. *Streptococci* and *staphylococci* are abundant in the sputum from cavities, and are chiefly responsible for the active inflammation of the lung in pulmonary tuberculosis.

*Diplococcus lanceolatus (pneumococcus)* is found in the mouth scrapings of many healthy individuals. It may be found in most specimens of sputum, and is specially abundant in the expectoration of lobar pneumonia, of which disease it is the bacterial cause. It may be stained as in pus. The *pneumobacillus of Friedlander* is found in a few cases of pneumonia. It is capsulated and resembles the *diplococcus lanceolatus,* but is rod-shaped, broader, and has rounded ends. It may be stained by Welch's method, but decolorizes by Gram, being thus differentiated from the pneumococcus.

The *bacillus of influenza* is found in enormous numbers in the sputum of this disease, and may be identified with considerable accuracy by its morphological characters.

This bacillus grows in the form of minute rods, measuring 1.5x.3 $\mu$ straight, with rounded ends, often staining more deeply at the ends. They occur singly or in clumps of 100 or more. In the later stages of the disease they are often found within the leucocytes. They persist for some weeks after the acuteness of the disease has subsided. They stain faintly with methylene blue and are best demonstrated by staining for five minutes with a weak solution of carbol-fuchsin.

The *tubercle bacillus* may be found in the sputum in most cases of pulmonary tuberculosis.

The tubercle bacillus grows in the form of slender, straight, or slightly curved rods. 2.5-3.5 $\mu$ in length, .3 $\mu$ in thickness. The younger germs stain uniformly with carbol-fuchsin; the older ones frequently exhibit unstained points resembling spores or vacuoles, and this feature may become so marked as to cause the bacillus to appear as a row of small cocci. They occur singly, or two or three individuals lie side by side or end to end. They resist the effects of the ordinary decomposition of sputum for months, but probably do not multiply after expectoration.

### *Method of Staining the Tubercle Bacillus.*

(1.) Fix the thinly spread sputum upon a glass slide by means of heat.

(2.) Flood the specimen with carbol-fuchsin for two minutes, gently heating—(carbolic acid (5 per cent. sol.), 100 parts; sat. alcoholic sol. of fuchsin, 10 parts).

(3.) Wash thoroughly in water, and decolorize by 10 per cent. $H_2SO_4$, until, after washing in water, all traces of red color are removed from the thin portions of the specimen. Two or more additions of the acid may be required.

(4.) Treat the specimen with 95 per cent. alcohol for one minute.

(5.) Wash in water and counterstain by dilute aqueous solution of methylene blue.

(6.) Wash in water, dry in air or by blotting-paper; mount in balsam.

The tubercle bacillus then appears bright red, while the sputum and all other bacteria are stained blue.

### *Rationale and Details of the Method.*

The tubercle bacillus takes up the anilin dyes very slowly, but when once stained it retains the dye with much greater tenacity than most other germs. Carbolic acid (anilin oil, etc.) acts as a mordant, carrying in the dye more rapidly than when it is in simple aqueous solution. Heating greatly facilitates this process. The application of carbol-fuchsin stains all the bacteria in the specimen, but on the addition of sulphuric acid all the germs lose their stain except the tubercle bacillus, which firmly retains the dye and is unaffected by the acid, at least for several minutes. Alcohol, likewise, has no decolorizing effect upon the stained tubercle bacillus; in fact it *heightens the red color* of the stain, while completing the decolorization of the other germs. Methylene blue is then used to counterstain all the uncolored germs and elements of the sputum. Several important technical details here deserve mention.

Tubercle bacilli

The choice of the portion of sputum to be stained should be made with care. The tubercle bacillus is very unevenly distributed in most specimens of sputum, being often entirely absent in the bronchitic exudate while abundant in the pulmonary detritus. The specimen should be examined in a glass dish on a black background and a search made for the *opaque whitish granules* which usually come from the pulmonary parenchyma, and will yield the bacilli if any are present in the specimen. In the absence of such masses, the most opaque and purulent portions should be chosen.

A somewhat less reliable and more laborious method is to dissolve the mucus by heating in a strong solution of KOH, and staining the sediment thrown down by the centrifuge.

In heating the specimen after flooding with the dye it is important not to raise it to the boiling point, and to evaporate as little as possible along the edges. These dried edges, as well as the thicker portions of the specimen, cannot safely be decolorized, but there is little danger of decolorizing the bacilli in well-stained and thinly-spread specimens if the treatment with acid is not continued longer than *five minutes. Alcohol will not decolorize the tubercle bacillus,* and may be added repeatedly to complete the effect of the acid. It develops a brilliant red color in the stained bacilli, and decolorizes other elements not affected by the acid.

Strong solutions of methylene blue darken the tubercle bacillus and render it more difficult of recognition. It *is better to use a weak solution for a longer time.*

Most specimens of tuberculous sputum contain elements other than the tubercle bacillus which retain the red dye, but which can readily be passed over on account of their atypical shape. At times one meets with an abundance of fine, needle-shaped bodies, shorter and thinner than the tubercle bacillus, with pointed ends, and deep red stain, which are probably crystals of fatty acids.

*Inoculation.*—When the microscopical examination for tubercle bacilli proves negative, the tuberculous nature of the disease may sometimes be demonstrated by the inoculation of a portion of the sputum into the groin of a guinea-pig. If any tubercle bacilli are present tuberculous lesions will be found in the adjoining lymph nodes after three weeks.

*Differentiation of the Tubercle Bacillus from the Bacillus of Leprosy and the Smegma Bacillus.*

The bacillus of leprosy may be stained by the same procedure as the tubercle bacillus, but the former decolorizes more rapidly than the latter. In staining the *bacillus lepræ* it is necessary therefore to use a *five per cent. solution of* $H_2SO_4$ instead of the ten per cent. solution, and to *omit the treatment with alcohol.*

To fully exclude the presence of the bacillus lepræ, the decolorization by 10 per cent. $H_2SO_4$ should be thorough, and the specimens should be treated with strong alcohol for fifteen minutes.

The *smegma bacillus* stains almost as tenaciously as does the tubercle bacillus, and the ordinary treatment with acid and alcohol fails entirely to decolorize it. It may be decolorized, however, by treatment with ninety-five per cent. alcohol for *eight to twelve hours. In staining urinary sediment for tubercle bacilli it is extremely hazardous to risk a diagnosis unless the specimens have been treated with alcohol for the above period.*

*The Sputum in Diseases of the Lungs.*

*Acute Bronchitis.*—A variable period of congestion precedes the catarrhal stage in most cases of bronchitis. During this congested stage the expectoration is scanty, tenacious, and largely *mucoid.* In severe cases it is frequently *blood-stained.* Usually within twenty-four to thirty-six hours the exudation of mucus and pus has become freer, the sputum is more abundant, looser, and yellowish or greenish-yellow in color. The staining with blood may continue. In some cases, at the height of the disease, the pus may greatly exceed the mucus of the sputum, which is then light yellow and of slight viscidity. *Fibrinous plugs* and *casts* are seen in some forms of acute bronchitis. An important prognostic feature of the sputum of acute bronchitis is the degree of aeration. Mucus from the larger bronchi is usually *well aerated,* that from the smaller bronchioles is *heavy and unaerated,* as in the so-called *capillary bronchitis* which is attended with much dyspnœa.

Eaten by cattle
The Timothy bacilli, found in hay, have the
same power of retaining Carbol-fuchsin.
They are found in milk & butter. These bacilli
lose in virulence by progressive inoculations

The smegma bacillus can be differentiated
from Tbc. bacillus by the Pappenheim stain:
Stain with carbol-fuchsin in the ordinary way,
& decolorize with

Sol. Rosolic acid 1.
Alcohol, 97% 100.
Methylene Blue to saturation
then add Glycerine 20

Rosolic acid decolorizes the smegma bacillus
faster than the Tbc. bacillus. This Pappenheim
can be used as a decolorizer in ordinary
Sputum work.

*Microscopically* one may identify mucus, fibrin, red cells, leucocytes, epithelium, and various bacteria.

*Chronic Bronchitis.*—In the exacerbations of chronic catarrhal bronchitis the sputum usually resembles that of acute bronchitis and may be chiefly mucoid, or muco-purulent, or purulent. Considerable serous fluid is often added in cases of emphysema and nephritis.

In some cases of chronic bronchitis with spasmodic dyspnœa *Curschmann's spirals* and *Charcot-Leyden crystals* are found in the sputum. Many of the leucocytes in such sputum are of the eosinophile variety.

In the acute exacerbations of a peculiar form of chronic bronchitis, fibrin is expectorated either in small *plugs,* or rarely in the form of *large dendritic casts of the bronchi.*

In chronic bronchitis with *bronchiectasis* the sputum is often excessive in amount and is discharged from the dilated bronchi at very irregular intervals. Besides the ordinary variations in character, it may be brown or green in color, and of moderately fetid odor. Microscopically the evidences of the growth and effects of putrefactive micro-organisms are abundant.

Either with or without bronchiectasis, the *mycelia (hyphæ)* and *spores* of various *fungi* may be found in the sputum of chronic bronchitis. These cases are usually marked by severe spasmodic asthma, are very refractory to treatment, and many believe that these peculiarities are owing to the presence and irritative effects of the fungi.

*Lobar Pneumonia.*—The sputum throughout pneumonia may be derived almost exclusively from the bronchi and present only the characters of the sputum of acute bronchitis. The severity of the bronchitis complicating pneumonia may be estimated by the quantity of this bronchitic exudate.

*Rusty sputum,* composed of mucus from the bronchioles, and blood, leucocytes, and epithelia from the pulmonary parenchyma, is pathognomonic of acute lobar pneumonia, but has been observed in acute œdema of the lungs.

The color of rusty sputum is usually caused by the red blood cells mixed with the mucus, but in some instances the blood cells appear to have been dissolved and their hemoglobin distributed throughout the mass.

*Bloody sputum,* or pure blood, is expectorated at the onset of some cases of pneumonia, an occurrence which properly raises the suspicion of a tuberculous infection, but may indicate only a severe inflammation.

*"Prune-juice"* expectoration results from the decomposition of blood extravasated in the lung. It is an unfavorable sign, and usually originates from small foci of necrosis. This and other forms of pneumonic sputum may have a peculiar fetid, acid odor.

*Serous fluid* is a significant addition to pneumonic sputum, indicating more or less œdema of the lungs.

Severe cases of pneumonic infection may be accompanied by *jaundice,* and the sputum may then be colored by *bilirubin or biliverdin.* It is said that biliverdin may also originate by transformation of the dissolved hemoglobin of rusty sputum.

*During resolution* the expectoration becomes more fluid, frothy, and less tenacious, owing to fatty degeneration of the exudate. Fibrinous plugs may then be seen in considerable numbers.

*Microscopically* one finds, in addition to the elements of bronchitic sputum, an abundance of alveolar epithelium, red blood cells and detritus, hematoidin masses, fibrinous coagula and spirals. During resolution the evidences of fatty degeneration are very marked.

The *diplococcus lanceolatus (pneumococcus)* may be demonstrated by Gram's or Welch's method in most cases of lobar pneumonia. Occasionally one finds instead the *pneumobacillus of Friedlander. Streptococci,* and the germs of various infectious diseases, are found in the sputum of secondary and catarrhal pneumonia.

In the *pneumonia of heart disease* the sputum usually contains abundant evidences of extravasated blood. The alveolar epithelium in such cases is often loaded with large reddish-yellow grains of blood pigment. Small hemoptyses are common, and the formation of an infarct is usually indicated by a considerable hemoptysis.

In *abscess of the lung,* the sputum usually resembles that of pneumonia, until the discharge of the abscess is indicated by the appearance of pure pus mixed with elastic fibres or larger shreds of lung tissue.

In *gangrene of the lung* the sputum may be pneumonic for a few days preceding the necrosis, when it becomes *dark, serous,* and *extremely fetid.* It separates in three layers, as previously described. Microscopically, it shows enormous numbers of putrefactive bacteria, as well as leptothrix and other fungi. The necrotic process usually destroys the lung-tissue so completely that elastic fibres are not to be found in the sputum. Fat globules, fatty acid crystals, leucin, tyrosin, cholesterin, and triple phosphate crystals may often be found.

*Tuberculous Sputum.*—The sputum in pulmonary tuberculosis may be derived from the inflamed bronchi, from the pneumonic process in the pulmonary parenchyma, from the necrosis of lung-tissue, from the walls of cavities, and from the pleural cavity.

*Bronchitic tuberculous sputum* is expectorated in small amounts from chronic apical lesions in the absence of cavities. When small bronchiectatic cavities form, the sputum is considerably increased in amount.

In *acute miliary tuberculosis* of the lungs the bronchi contribute nearly all the expectoration, which is often very scanty. In nearly all cases of active tuberculous inflammation of the lungs the bronchi are inflamed and largely increase the quantity of the expectoration. *Blood-streaked and stained* sputum is very frequently derived from the inflamed and ulcerated bronchi, and many of the small hemoptyses are from their walls.

*Tuberculous pneumonia* is frequently ushered in or marked during its course by hemoptysis. The sputum may often be blood-stained, but seldom or never rusty. It differs but slightly from the sputum of lobar pneumonia, except for the presence of tubercle bacilli and the changes following the formation of cavities.

When a *cavity* forms in a consolidated tuberculous lung the contents are often discharged into the bronchi, causing irritation and increased bronchial secretion. Consequently the formation of a cavity is frequently indicated by a marked increase in the quantity of expectoration containing much pus, broken-down lung-tissue, and elastic fibres, and often accompanied by hemoptysis. Old cavities with suppurating walls produce a large quantity of sputum, which is largely purulent, often necrotic, contains elastic fibres and lung-tissue, and is frequently expectorated in the form of discreet purulent coin-shaped masses, called *"nummular sputa."*

When serous fluid or pus in the pleural cavity ruptures into the lung the discharge appears in large and characteristic amounts in the expectoration.

### *Occurrence of Bacilli in Tuberculous Sputum.*

Tubercle bacilli are usually *scarce,* and may be *absent* from the sputum in pure *miliary tuberculosis,* either acute or chronic.

Similarly in *chronic lesions* of considerable extent, when the inflammation has largely subsided, the bacilli frequently become *very scarce,* and may *disappear* from the sputum entirely. The number of bacilli in the sputum is, therefore, a valuable indication of the progress of the inflammation in the lung.

In the early stages of *tuberculous pneumonia* bacilli may be absent in the sputum, but appear in great numbers even before cavities form.

It is probable that some cases of pneumonia that are followed by tuberculosis are tuberculous from the first, but the tuberculous element is overshadowed by the complicating infection and the bacilli, if present in the sputum, are overlooked in the examination. Nevertheless, in the vast majority of such cases of pneumonia in which tuberculosis is suspected the result of the examination of the sputum is reliable.

Throughout the course of most tuberculous lesions bacilli are *abundantly present* in the sputum, and, with some care in selecting the portions to be examined, are readily detected by the microscope.

Very large numbers of bacilli are found in the sputum from actively suppurating cavities and rapidly necrosing lung.

*Actinomycosis.*—The sputum in pulmonary actinomycosis closely resembles that of subacute phthisis. The colonies of actinomyces may be recognized by the naked eye as *small, solid, whitish bodies* of the size of a millet-seed or pin-head.

# STOMACH-CONTENTS.

*Vomitus* is largely composed of food-products partly digested and of fluids derived from the alimentary tract. Its examination may give very definite information regarding the nature of diseases of the gastro-intestinal tract.

## *Gross Appearances.*

In the early stages of digestion most *food-products* may be identified by the naked eye, including milk-curds, particles of meat, vegetable detritus, and fluidified fat.

*Mucus* is nearly always secreted by the stomach during nausea and appears in the vomitus in the form of thin, translucent, stringy masses. Mucus resulting from acute or chronic gastritis is more opaque and more uniformly mixed with the food.

*Blood* is sometimes streaked through the mucus in acute gastritis and is discharged little altered and red in color. Fresh blood is otherwise rather rarely seen in the vomitus, for even when rapidly poured into the stomach from a ruptured vessel it usually remains in the organ for some minutes, is soon altered in color by the gastric fluids, and when discharged is commonly of a dark red or brownish color. Frequently large hemorrhages are retained in the stomach for longer periods, and when vomited the blood is dark brown or black in color. The usual vomitus of ulcerating carcinoma of the stomach resembles *"coffee-grounds"* in form and color.

In cases of *pyloric stenosis with dilatation* of the stomach, large quantities of *blackish fluid* may be vomited, of which the color is usually, but not always, due to old and extensively altered blood.

Vomitus is frequently discolored by *bile* regurgitated from the duodenum. Usually the bile pigment present is the brownish-yellow bilirubin, less frequently it has become oxidized to biliverdin and the vomitus is greenish. A variety of conditions ap-

pears to lead to oxidation of bilirubin, such as the administration of calomel, but its causes are not fully understood.

*Pus* sometimes appears in considerable quantities in the vomitus, as after rupture of an abscess in the neighborhood.

*Fæcal matter* sometimes appears in the vomitus in cases of intestinal obstruction or peritonitis, usually in small quantities, in fluid form, and recognizable principally by its odor.

*Round-worms* (Ascaris lumbricoides) are rather frequently seen in the vomitus,—*Oxyuris vermicularis* and *Anchylostoma duodenale,* very rarely.

### *Microscopical Examination.*

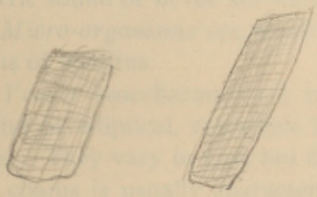
*Muscle fibres* in nearly all stages of digestion may be identified by their yellowish color and cross-striation. In the last stages of digestion they appear as oval or elliptical, colorless, slightly refractive bodies, devoid of striation. Elastic fibres are colorless, slightly curling, refractive threads of single contour.

A great variety of *vegetable cells* are commonly revealed by the microscope, and most of them are readily identified by their large size, geometrical shape, thick double border, and often coarsely granular contents. *Starch grains* sometimes exhibit a concentric striation; usually this ordinary character is absent in the stomach contents and they appear as colorless, highly refractive, sometimes scaly masses, often showing radiating lines of fracture. On the addition of nitric acid, followed by solution of iodine, they assume a blue color.

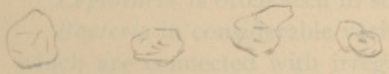
*Fat* is usually present in the form of globules of the ordinary appearance, or it may have been split up and its derivatives, the *fatty acids,* may appear in the crystalline form of long, colorless needles.

*Mucus,* which is usually identified by its viscidity, appears as thin, slightly granular threads, entangling much granular detritus. It sometimes appears in the form of jelly-like grains which, under the microscope, exhibit a twisted, spiral structure.

In fresh *blood* the cells retain their usual morphology; more often the dissolved blood-pigment alone remains, in the form of brownish-red grains and masses of hematoidin.

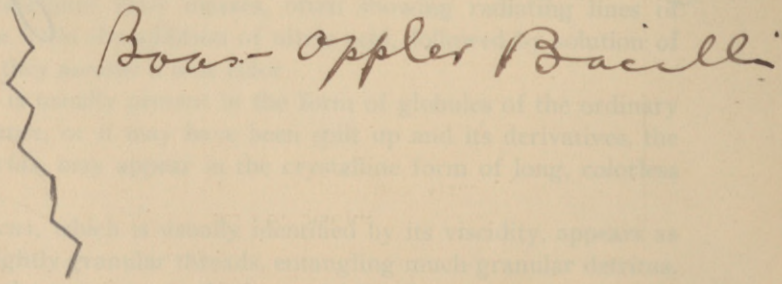


Muscle-fibres



Starch grains

Blood is determined by Teichmann's test & Korzyuski's & Jaworski's tests spectroscopic test.


Boas-Oppler Bacilli

*Leucocytes* may be recognized by their fine, opaque granules and small, refractive nuclei.

*Columnar epithelium* from the stomach and *squamous epithelium* from the œsophagus and mouth may usually be found in the masses of mucus. In acute gastritis the exfoliation of columnar cells may be abundant.

In some cases of chronic gastritis *particles of mucous membrane* may be found whose microscopical structure may indicate the nature of the lesion.

In ulcerating carcinoma of the stomach *shreds of the new growth* may often be identified by careful search in the vomitus.

Such masses of tissue are most readily found adhering to the gastric sound or in the shreds of vomited mucus.

*Micro-organisms* are usually present in abundance in all specimens of vomitus.

*Yeasts* (saccharomyces) occur in groups of three or more round or elliptical, refractive bodies, about the size of red blood cells. They vary in size, but their arrangement in short bifurcating chains is usually characteristic. They stain brownish-yellow by Gram's fluid.

*Sarcinæ* are cocci growing in cuboidal packets of *eight* individuals or its multiple. They stain deep mahogany-brown with Gram's fluid.

*Leptothrix* is often seen in stagnating stomach contents.

*Bacteria* in considerable variety occur in the vomitus, some of which are connected with irregular forms of fermentation (*bac. acidi. lactici.*), but their full identification is not usually attempted. *Streptococcus pyogenes* has been isolated in some infectious cases of acute gastritis.

The *Boas-Oppler bacillus* is a non-motile germ growing in jointed, irregular chains of long bacilli, occasionally in great abundance, and nearly constantly present in stagnating stomach contents if free from hydrochloric and rich in lactic acid.

The presence of free hydrochloric acid favors the growth of sacinæ while inhibiting that of the Boas-Oppler bacillus. On the other hand, the presence of lactic acid favors the growth of the Boas-Oppler bacillus and appears to inhibit the growth of sarcinæ (Boas).

### *Chemical Analysis of Stomach-contents.*

While the chemical analysis of vomitus often yields valuable information, the more accurate determination of the digestive powers of the organ requires that the stomach-contents should be secured at a known period during the digestive process. For this purpose test-meals are employed.

### *Test-meals.*

*Ewald's test breakfast* is generally used. It consists of 30 to 70 grams of white bread and 300 c.c. of water.

During the early stages of the digestion of this breakfast considerable *traces of lactic acid* may be produced, so that when special importance attaches to the presence of this acid it is customary to substitute for the test breakfast the *test-meal of Boas,* which does not contain lactic acid, and consists of: Water, 1 quart; rolled oats, 1 ounce, boiled down to 1 pint.

Test-meals must be given in the morning on an empty stomach. When there is retention of food the stomach must be washed out on the previous evening.

The stomach-contents should be expressed through the stomach-tube one hour after the ingestion of the meal. The quantity of fluid obtained in this way varies from 15 to 50 c.c. It is sometimes impossible to obtain a sufficient quantity for the tests, and the operation must be repeated. The following chemical tests are applied principally to the *filtered stomach-contents* obtained by the above methods:

### *Albumens.*

*Syntonin,* or acid albumen, is one of the products of incomplete petonization of albumens, and its demonstration may at times indicate the stage to which digestion has progressed.

Syntonin may be demonstrated in the filtrated stomach-contents by neutralizing the filtrate with solution of potassium hydrate, when the presence of syntonin is indicated by more or less turbidity or precipitate.

Riegel's test meal consists of pork, beef-steak, bread + water. This is used for observation of digestive power of stomach.

*Propeptone* in solution gives a marked turbidity on the addition of acetic acid and sat. sol. NaCl., the turbidity being cleared up by heating.

*Peptone* in traces is present in nearly all specimens of stomach-contents, but its presence in considerable amounts indicates the complete digestion of albumens. It gives a rose-red color when the filtered fluid is alkalized by KOH and treated with 1 to 5 gtt. of a 1 per cent. solution of cupric sulphate (Biuret reaction).

*Starch,* during digestion, is converted into maltose, which responds to Fehling's test. The intermediate products are erythrodextrin and achroodextrin. Starch grains turn blue on the addition of nitric acid followed by Gram's fluid—erythrodextrin violet—and achroodextrin remains unchanged. These tests may be made on a glass slide by the aid of the microscope.

In strongly acid stomach-contents erythodextrin is usually found at the completion of gastric digestion, while in the absence of free HCl achroodextrin is almost exclusively present.

### *Hydrochloric Acid—Qualitative Test for Free HCl.*

The presence of free HCl is best determined by means of *Gunzberg's reagent,* which consists of:

| | |
|---|---|
| Phloroglucin | 2. |
| Vanillin | 1. |
| Absolute alcohol | 30. |

Over a clean porcelain dish a drop of the reagent is thinly spread and evaporated. A few drops of the stomach-contents are then added and the dish is gently heated over the flame. In the presence of hydrochloric acid a *carmine-red color* appears along the edge of the evaporating stomach-fluid.

The test is very delicate, is not obscured by the ordinary ingredients of the vomit, and is not produced by the organic acids. Other inorganic acids give the same reaction.

### *Quantitative Estimation of HCl.*

The *total acidity* of the stomach-contents is due to (1) HCl, free, and combined in various ways, to (2) organic acids, lactic,

butyric, acetic, etc., and to (3) acid salts. A considerable proportion of the hydrochloric acid secreted in the gastric-juice may at once combine with food products and fail to react to Gunzberg's reagent. Thus several centimetres of strong HCl may be added to a pint of milk, which will yet fail to show the presence of any *free* HCl by any reagent. The acid has loosely united with the alkalies and albumens of the milk, and is therefore termed *combined hydrochloric acid.*

In the analysis of stomach-contents it is obviously important to estimate the *total production* of HCl. For this purpose Toepfer's method is to be recommended.

### *Toepfer's Method.*

This method of estimating the total production of HCl in the gastric-juice is based upon the sensitiveness of certain coloring agents to various acid principles as found in stomach-contents.

*a. Di-methyl-amido-azo-benzol* (or *amido-benzol*) reacts only to *free inorganic acids,* such as:

Free HCl.

*b. Alizarin* reacts to:

Organic acids (lactic, butyric, etc.).

Acid salts.

Free HCl, but not to *loosely combined HCl.*

*c. Phenol-Phthalein* reacts to:

Organic acids.

Acid salts.

Free HCl.

Combined HCl.

Phenol-Phthalein may therefore be used to estimate *the total acidity* of the stomach-contents.

Now, if a drop or two of a solution of amido-benzol is added to 5 c.c. of normal filtered stomach-contents the fluid assumes a brilliant red color from the effects of the free HCl upon the dye. If, then, the free HCl is neutralized by the addition of an alkali, the red color is no longer maintained by the other acid principles and the fluid quickly changes to a lemon tint.

Similarly in the presence of alizarin, when by the addition of

Add about 10 c.c. of water to the 5 c.c. of stomach contents.

In the Alizarin test, add the alkali until there is a purplish color

In the phenol-phthalein test, add the a few drops of the alkali more than required to bring a light pink color

an alkali, the free HCl, the organic acids, and the acid salts have become neutralized, the dye promptly changes color.

Finally, in the presence of phenol-phthalein, the further addition of an alkali will neutralize all acid principles, including the loosely combined HCl, and not until this has been accomplished will the phenol-phthalein change color.

If, in each of these procedures, we note the quantity of alkali required to bring the end-reaction we may estimate, in units of HCl, the quantity of the various acid principles concerned. On this account the *deci-normal solution of NaOH* is used in the titrations.

One cubic centimetre of deci-normal soda solution neutralizes .00365 grams of HCl. If, then, it requires 5 c.c. of deci-normal soda solution to bring the end-reaction with amido-benzol, the fluid contains 5x.00365 grams of *free HCl.*

The *combined HCl* may be found by subtracting the quantity required in the second titration (with alizarin) from that required in the third (with phenol-phthalein). For, by consulting the lists of the principles to which alizarin and phenol-phthalein react, it will be seen that

Group c — group b = the combined HCl.

Finally, adding the free and the combined HCl, we get the *total production* of HCl in the gastric fluid.

While the total acidity of the stomach-contents is not due to HCl alone, it is customary and convenient to deal with this acidity in terms of HCl.

### *Fluids Required.*

(1.) Amido-benzol, .5 per cent. alcoholic solution.

(2.) Alizarin, 1 per cent. aqueous solution.

(3.) Phenol-phthalein, 1 per cent. alcoholic solution.

(4.) Three beakers each containing 5 c.c. of filtered stomach-contents, and each diluted with an equal quantity of distilled water.

(5.) Deci-normal solution of NaOH.

To prepare a normal solution of NaOH the following procedure may be used: Dissolve 63 grams of oxalic acid C. P., ac-

curately weighed, in distilled water, bringing the solution up to 1 litre at a temperature of 60° F., 15° C. Dissolve about 40 grams NaOH C. P. in distilled water and bring the solution up to 1 litre.

Place 10 c.c. oxalic acid solution in a beaker, add 1 drop of phenol-phthalein, and titrate from a burette with the soda solution.

If it takes 9.5 c.c. of the soda solution to neutralize 10 c.c. of oxalic acid, .5 c.c. of water must still be added to each 9.5 c.c. of the soda solution to render it of standard normal strength, and to 950 c.c. of soda solution 50 c.c. of water must be added. Water may readily be added in whatever proportion is found necessary. Nine parts of water added, then, to one part of the normal gives the deci-normal solution.

### *Method of Procedure.*

Into each of three beakers are measured 5 c.c. of filtered stomach-contents and diluted with an equal quantity of distilled water. To one of these, 1 to 2 drops of amido-benzol is added, which in the presence of free HCl immediately turns a bright red color. From a graduated burette deci-normal soda solution is cautiously added till on agitating the beaker the fluid begins to turn to an orange-yellow. The fluid is then added drop by drop until the *last traces of red* have disappeared and the fluid is of *bright lemon-yellow* color, indicating the end of the reaction. The quantity of soda solution used is then noted, from which may be computed the amount of free hydrochloric acid present.

To the second beaker 1 to 2 drops of alizarin are added, and the solution titrated as before. The end-reaction is indicated when a *deep violet color* is reached. From the quantity of soda solution used may be computed the acidity due to all acid principles excepting loosely combined HCl.

To the third beaker 1 to 2 drops of phenol-phthalein are added and titrated as usual. The end-reaction is reached when the *rose color* first appearing *no longer darkens* on further addition of the alkali. From the quantity of soda solution used in this titration the *total acidity* of the specimen may be computed.

As an illustrative example of the computation now required it may be supposed that 2.5 c.c. of soda sol. are required in the first

titration, 5.5 c.c. in second, and 7.5 in the third. The quantity of free HCl per cubic centimetre is then $2.5 \div 5 \times .00365 = .001095$ grm., or .109%.

The quantity of combined HCl = 7.5—5.5 = 2 c.c.

2 c.c. ÷ 5 = .4.

$.4 \times .00365 = .00146$ grams, or .146%.

The total HCl is then

.109 + .146 = .255%.

### *Sources of Error in Toepfer's Method.*

(1.) It requires some experience in order to determine accurately the full end-reaction in the different titrations.

(2.) Amido-benzol reacts to organic acids, lactic, acetic, and butyric, as well as to free HCl, and in the presence of these acids there may be a considerable error in the estimate. On the other hand, the organic acids are not usually present when the stomach contains free HCl, and even when present it is claimed that their disturbing effects are practically nullified by the dissolved albumens.

### *Significance of Changes in the Content of HCl.*

(1.) The presence of a *normal amount* (.1 to .2 per cent.) of free HCl in the vomitus or stomach-contents, expresssed one hour after a test-meal, is strong evidence against any organic disease of the stomach. When the symptoms point, nevertheless, toward a disease of the stomach, the normal percentage of HCl indicates either nervous dyspepsia or atony of the muscular wall.

2. *Continuous hyperacidity* (over .2 per cent.) occurs most frequently in neurotic dyspepsia, is very often present in simple ulcer, and may characterize the early stages of chronic gastritis. It speaks strongly against carcinoma, except when a simple ulcer is undergoing carcinomatous metaplasia.

(3.) *Continuous subacidity* (under .1 per cent.) is seen in

chronic gastritis, especially with dilatation and atony; in some cases of simple ulcer with chronic gastritis; and in incipient carcinoma.

(4.) *Anacidity* is a frequent and persistent symptom of the later stages of chronic gastritis, when pepsin is also lacking; it may be found in neuroses, when pepsin is usually present; and, when other signs are confirmatory, it speaks strongly in favor of carcinoma.

(5.) Markedly *variable acidity* indicates, with strong probability, a neurosis.

### *Lactic Acid.*

*Uffelmann's reaction* is generally employed as the qualitative test for lactic acid. The procedure is as follows: To 10 c.c. of a 5 per cent. sol. of carbolic acid add 20 c.c. of distilled water, and 1 drop of a 5 per cent. sol. of ferric chloride. The resulting solution is of deep amethyst-blue color, which may soon be altered, so that the solution must be used when fresh. A few drops of the stomach-contents are then added, when, in the presence of .1 per cent. of lactic acid, a *lemon-yellow color* is produced.

### *Sources of Error in Uffelmann's Test.*

(1.) Phosphates, glucose, and alcohol give a very similar reaction, but their presence, except in vomitus, can usually be guarded against.

(2.) Mineral acids decolorize the solution, but do not give the characteristic lemon-yellow color. In the presence of much HCl (.2 to .3 per cent.), which, however, is rarely associated with lactic acid, the solution is decolorized. One may then shake up a few cubic centimetres of the specimen with ether and apply the test to the ethereal extract, with or without the addition of water and evaporation of the ether.

(3.) Butyric and acetic acids give a yellowish color, and usually a slight precipitate.

Strauss' test – Extract the lactic acid out by shaking up with ether. Then test with dil. sol of $Fe_2Cl_6$, when a yellow-green color is formed.

Kelling's test – Shake up the gastric fluid with ether and add the $Fe_2Cl_6$ directly to the ether.

Strauss' reaction easily takes place with 1 part of Lactic acid to 1,000. It is a very good test.

There is increased quantity of HCl in a carcinoma that is developed on the bed of an ulcer. The presence of HCl is against Carcinoma but its absence does not prove Carcinoma.

*Significance of Lactic Acid.*

(1.) Lactic acid is often ingested with the food, or rapidly forms from it during the early stages of normal digestion, especially when milk or bread (Ewald's test breakfast) have been taken.

(2.) Lactic acid is seldom present after a diet of carbohydrates (Boas' test-meal), and is usually found in traces only during the course of non-malignant disease of the stomach.

(3.) It may be present in larger proportions in dilatation of the stomach with stagnation of its contents.

(4.) When associated with retention of stomach-contents and absence of HCl, its presence speaks very strongly in favor of carcinoma.

*Pepsin.*—When associated *with free HCl* in the stomach-contents pepsin may be demonstrated by the digestion test, as follows: To 10 c.c. of filtered stomach-contents a small piece of coagulated egg albumen, or better, a stick of dried commercial fibrin, is added in a test-tube, which is kept in the thermostat (37° C.). The presence of pepsin is indicated by the more or less complete digestion of the albumen. The solution may be tested for peptone, which, with specimens of normal stomach-contents, will be readily demonstrable *within one hour.*

*In the absence of HCl* pepsin may yet be present, but will require, in order to digest albumen, the addition to the test-tube of 1 to 2 drops of HCl.

Marked diminution in quantity or absence of pepsin indicates a corresponding disturbance of the glandular activity of the stomach, which, however, may exist with a considerable variety of lesions.

*Chymosin* may be demonstrated by adding 3 to 5 drops of stomach-filtrate to 5 c.c. of warm milk. If chymosin is present the milk should coagulate in 10 to 15 minutes. To demonstrate minute quantities of chymosin the filtrate must be neutralized by deci-normal soda solution.

Normal stomach-filtrate may be diluted 1 to 30 or 1 to 40 and yet coagulate milk. Rough quantitative analyses may be made by noting the effects of various dilutions.

In the absence of HCl the persistence of chymosin in considerable quantity speaks strongly against an organic lesion of the stomach. In advanced chronic gastritis the chymosin is usually reduced below 50 per cent. of the normal, and a dilution of 1 to 10 or 1 to 15 prevents its digestive action.

*Bile.*—The presence of bile in the stomach-contents may be demonstrated by the tests employed for its detection in the urine.

### *Character of the Vomitus, etc., in Disease.*

*Acute Gastritis.*—In acute gastritis the stomach is early emptied of food products, which therefore appear in the first specimens of vomitus.

In most cases, but not in all, there is considerable secretion of mucus, which is sometimes blood-streaked, and usually entangles leucocytes and exfoliated epithelium. The latter may be found in masses of moderate size. Regurgitated bile is often present, especially when vomiting is excessive.

*Chemical examination* usually discloses a high grade of acidity in the vomitus, resulting from the presence of organic acids (lactic, butyric, acetic, and fatty). Free HCl is usually absent, and its total production greatly diminished. In the presence of much mucus the reaction of the vomitus may be neutral or alkaline.

The addition of free HCl usually develops some digestive power in the vomitus, indicating the presence of traces of pepsin and chymosin. The examination of the vomitus in cases of acute poisoning is an important branch of legal medicine. Such vomitus usually contains much blood and the poisonous agent, mineral or alkaloidal, may usually be demonstrated by special tests.

*Chronic Gastritis.*—Ewald classifies the results of the examination of stomach-contents in chronic gastritis in the following groups:

(1.) *Simple Gastritis.*—Free HCl, pepsin, and chymosin are diminished, but on the addition of HCl some digestive power is developed. The total acidity is not high, and depends upon the presence of organic acids, principally lactic and fatty acids.

(2.) *Acid Gastritis.*—The stomach-contents are similar to

those of simple gastritis, except that the secretion of HCl is excessive.

(3.) *Mucus Gastritis.*—Considerable quantities of mucus appear in the vomitus and washings. Free HCl is absent, but on its addition reduced digestive power is developed.

(4.) *Atrophic Gastritis.*—The stomach-contents are lacking in HCl, pepsin, and chymosin. Mucus is also scanty or absent. Shreds of mucous membrane, indicating the character of the lesion, may occasionally be discovered in the vomitus or washings.

*Ulcer of Stomach.*—The ordinary vomiting in ulcer of the stomach occurs at *the height of the paroxysms of pain,* which are relieved thereby, and the vomitus is usually highly acid from HCl; the food is well digested, and moderately mixed with mucus.

*Hematemesis* usually occurs apart from paroxysms of pain, during the night or early morning, or during exertion. It is frequently associated with more active secretion of HCl which changes the hemoglobin to hematin, and the blood, when discharged, usually some minutes or hours later, is of dark brown color. When the hemorrhages are frequently repeated the blood may be a brighter red. It may be passed into the intestine and not vomited at all.

In ordinary cases the analysis of stomach-contents (aspiration is usually contra-indicated) shows .2 to .5 per cent. of free HCl, and normal or slightly diminished ferments.

*Carcinoma of Stomach.*—Vomiting is irregular in occurrence, and when the tumor involves the curvatures only, may be absent. The vomitus is usually acid in reaction, often distinctly sour or fetid, and in ulcerating cases frequently exhibits the peculiar appearance of "coffee-grounds" from the altered blood derived from small repeated hemorrhages.

Careful search will rather frequently be rewarded by the discovery in the vomitus, washings, or on the tube, of particles of the ulcerating new growth. The presence of *pus,* which is often discharged in considerable quantities from an ulcerating growth, and the presence of the *Boas-Oppler bacillus* are always worth consideration.

*Chemical examination* of the expressed contents or vomitus shows, in a large proportion of cases, an *absence of free HCl* and the *presence of lactic acid* in considerable quantity. Yet in a few

cases of gastric carcinoma free HCl is present and lactic wanting, and in some cases of chronic gastritis free HCl may be absent and lactic acid present. Nevertheless, even in the absence of a palpable tumor, the loss of HCl and production of lactic acid are a valuable and often an early diagnostic sign of carcinoma.

*Dilatation of Stomach.*—Gastro-ectasis may follow carcinoma of the pylorous, simple ulcer in the pyloric region, or chronic gastritis, and the stomach-contents may show many of the characters of, or be largely affected by, the underlying disease.

The vomitus and stomach-washings in this condition are in many respects characteristic.

The *quantity* of the vomitus and washings greatly exceeds that seen in other conditions. The increased capacity of the stomach may be shown by the large quantity of fluid which may be ingested through the tube, and when completely filled, the outlines of the organ on percussion are widened.

When allowed to stand in a vessel the vomitus usually *separates into three layers,* the heavier portions falling to the bottom, other masses, inflated by the abundant production of gases, rising to the top.

On gross examination the remains of articles ingested days, or even weeks, previously may be detected.

The *odor* has frequently a penetrating, sour quality. Occasionally there is the odor of $H_2S$.

Inflammable carbureted hydrogen gases have been demonstrated.

*Microscopical examination* shows marked evidences of abnormal fermentation and putrefaction.

Bacteria are usually very abundant, including *sarcinæ, yeasts, bac. subtilis, leptothrix, and the Boas-Oppler bacillus.* Sarcinæ are more abundant when HCl is present; the Boas-Oppler bacillus when HCl is absent.

*Chemical analysis* shows in all cases markedly *increased total acidity,* which varies with the character of the ingesta, the degree of retention, and the state of the gastric mucosa.

The acidity is largely due to the production of *organic acids*—fatty, butyric, and acetic. In non-malignant cases lactic acid may long be absent. With carcinoma of the pylorus lactic acid is usually abundant.

*Absorption and Motility.*

The *absorptive activity* of the stomach may be roughly indicated by *Penzoldt's test.*

When 5 grams of KI are taken in a gelatine capsule, iodine appears in the urine and saliva of the normal subject within six to fifteen minutes, while with deficient absorbtive capacity its appearance is much later. The iodine may be detected by applying a few drops of saliva or urine, and one drop of strong $HNO_3$ to starch paper, which, in the presence of iodine, turns blue or violet.

The *motility of the stomach* is best determined by giving a test-meal at night on an empty stomach, and examining the washings on the next morning. Normally no traces of the ingested food should remain.

# FÆCES.

## *Gross Characters.*

*Quantity.*—The quantity of fæcal matter in physiological conditions varies greatly with the character of the food, being increased by a vegetable diet, in which there is much indigestible residue. The normal daily limits are probably between 60 to 250 grams, of which 75 per cent., in formed stools, is water.

During fasting, fæcal matter is still formed, principally of mucus, which is the basis of fæces, but in small quantity. Following the relief of constipation, and in some forms of children's diarrhœa, enormous quantities are passed.

*Consistence.*—The consistence varies from that of the serous discharge of cholera to that of the hard scybalous masses of chronic constipation, and depends upon the quantity of fluid ingested, the amount of exudate added from the alimentary tract, and the character and duration of intestinal absorptive processes.

*Odor.*—The natural odor of fæces is that of their decomposed albuminous principles, indol, skatol, phenol, ammonia; and of fatty acids, hydrogen sulphide, etc. Most of these principles are produced apart from the digestive function and in the large intestine.

The odor is characteristic in the stools of fatty diarrhœa in infants, and often in other, acholic, stools; in cholera; and in amœbic dysentery.

*Color.*—The color of the normal fæces depends largely on the character of the food, partly on the admixture of a derived biliary pigment, *stercobilin*, which is probably identical with urobilin.

In infants' stools the large proportion of fat and undigested milk gives a whitish color, more or less tinged with bile pigments, bilirubin or biliverdin.

In adults the usual color is brownish-yellow. The *green* stools, after the use of calomel, contain biliverdin. Meat diet,

huckleberries, and red wines often produce dark or black stools. Iron, manganese, and bismuth *blacken* the fæces, forming sulphides. Many stools containing old blood are dark brown or black. Santonin, senna, and rhubarb produce bright *yellow stools.* In obstructive jaundice the absence of bile and presence of undigested fat yield *whitish, acholic* stools. In cholera and often in typhoid fever the absence of bile and presence of leucocytes, epithelial cells, and bacteria give "rice-water" or very light-colored stools.

*Alimentary Detritus.*—A great variety of alimentary detritus may be recognized in the stools. These are principally vegetable products imperfectly masticated or accidentally swallowed and are easily identified. Orange cells and strings of spaghetti are sometimes mistaken for parasites. Coagulated albuminous masses are often mistaken for mucous plugs, but give the xantho-proteic reactions.

Glittering white deposits or streaks are often seen in diarrhœal stools and are composed of long crystals derived from fat.

Foreign bodies of almost every description have been found in the fæces, after having been added to the stools after passage.

*Gall stones* should be searched for by passing the semi-fluid fæces through a sieve. These concretions may be only crumbly, partly calcific masses, or solid stones with characteristic facets.

*Cholesterin* is found in nearly all biliary calculi, some of which are composed exclusively of this principle. Pure cholesterin calculi are translucent, somewhat greasy, and their surface is coarsely jagged from the projecting angles of the masses of plates in which cholesterin crystallizes. They are largely dissolved in alcohol and ether.

The addition of *calcium salts* and *bile pigments* gives more solid, brittle, and opaque calculi of various colors.

Calculi, composed principally of biliary pigments, are dark brown, hard, and heavy. Calculi containing principally calcium salts have rough granular surfaces, are opaque, usually light-colored, and often brittle.

*Pus* may be found in the stools in considerable quantities after the rupture of abscesses into the intestinal tract, the most common origins being pelvic abscess in the female, appendicitis, and abscess of the liver. Its presence in impure form and smaller quantity

markedly affects the appearance of the stools in many diarrhœal diseases, to which it tends to add a light yellowish color, especially noticeable about flakes of mucus. Pus may be seen in distinct form in chronic ulcerative colitis and in carcinoma of rectum or colon.

*Mucus* in small flakes tinged with bile and mixed with leucocytes, epithelial cells, and food-detritus constitutes the bulk of many diarrhœal stools.

When abundantly formed in the *small intestine* and *upper colon* it is usually well mixed with the fæces but more readily identified. In many cases of *colitis* the masses of mucus are well separated from the semi-fluid or fluid fæcal detritus. In *proctitis* the mucus appears mostly in discreet masses which have adhered to the surface of the stool.

Discharges of considerable masses of nearly pure mucus are sometimes seen in colitis. Mucous cylinders and casts of large size are characteristic of *membranous colitis* in the adult. The masses are several inches to one foot in length, ribbon-shaped or spirally twisted, often of the consistence of jelly, sometimes dense, opaque, and irregularly constricted, so as to resemble a tape-worm. Coagulated albuminous matter is sometimes mistaken for mucus.

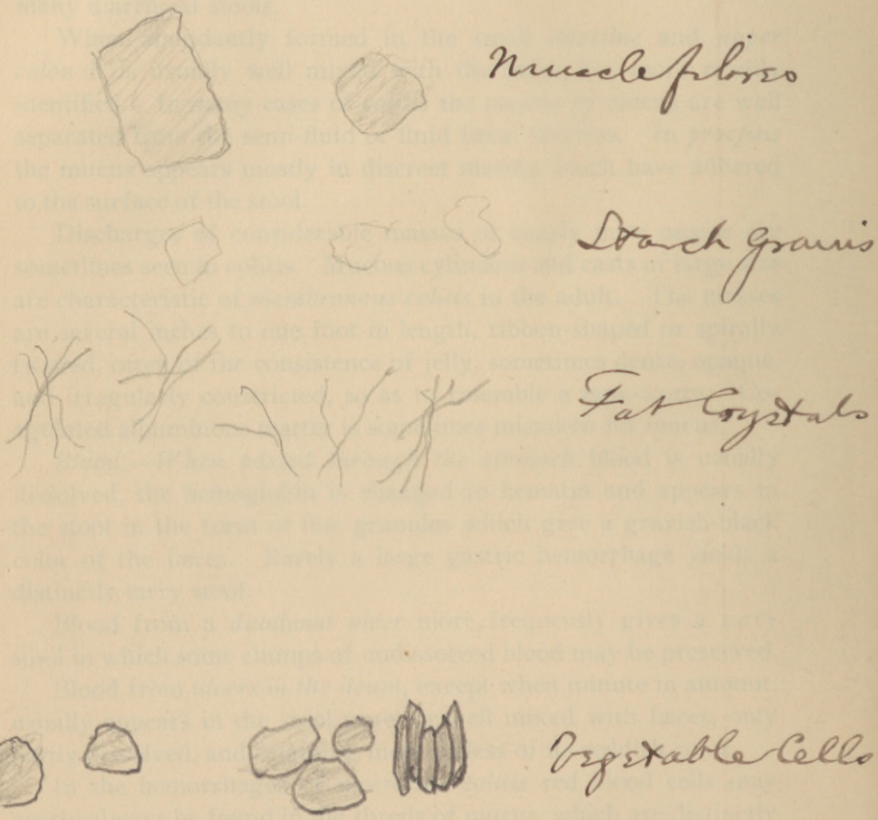
*Blood.—When passed through the stomach* blood is usually dissolved, the hemoglobin is changed to hematin and appears in the stool in the form of fine granules which give a grayish-black color of the fæces. Rarely a large gastric hemorrhage yields a distinctly tarry stool.

Blood from a *duodenal ulcer* more frequently gives a tarry stool in which some clumps of undissolved blood may be preserved.

Blood from *ulcers in the ileum,* except when minute in amount, usually appears in the stool pure, or well mixed with fæces, only partly dissolved, and retaining more or less of its reddish color.

In the hemorrhages of *ulcerative colitis* red blood cells may nearly always be found in the shreds of mucus, which are distinctly blood-stained. Large hemorrhages in the colon are discharged with little alteration, while blood from the *rectum* merely covers the formed or semi-fluid stool and is bright red in color. The bright red blood from piles usually follows the defecation.

*Korzynski's and Jaworski's test* seems to demonstrate small amounts of dissolved blood. To a small portion of fæcal matter is


Muscle fibres
Starch grains
Fat Crystals
Vegetable Cells

added a few grains of $KClO_3$ and a drop or two of conc. HCl. Heat gently until the mixture is decolorized and the chlorine is driven off. The addition of one or two drops of a dilute solution of potassium ferrocyanide ($K_2FeCy_6$) will then, in the presence of blood-pigment, develop a distinctly blue color from the formation of Prussian blue.

### *Microscopical Examination.*

*Alimentary detritus.—Muscle fibres* often preserve their striation throughout the passage of the intestinal tract, but are sometimes reduced to colorless, homogeneous, translucent, oval, or elliptical bodies, 2 to 5 times larger than leucoctyes.

*Starch grains* have usually lost their striation in the stools and appear as coarse, highly refractive bodies with scaly surface or radial fracture. On treatment with dil. $HNO_3$ and Gram's fluid they turn blue or deep brown.

*Fat* may appear in the form of *globules*, especially in acholic stools, or in the various crystalline forms of its derivatives, such as the long acicular crystals of the fatty acids, cholesterin plates, or as irregular, polygonal, yellowish, refractive masses. All of these may be transformed into fat-droplets by heating with conc. $H_2SO_4$.

Fat is nearly always to be found in the stools in health. In large quantities it frequently indicates a diminished production of bile. Its presence is characteristic of most acute and chronic diseases of the small intestine. In the fatty diarrhœa (acute enteritis) of children, fat crystals are often present in enormous abundance.

*Coagulated proteids* constitute a considerable proportion of the coarsely granular detritus found in the fæces.

*Vegetable cells* are found in the fæces in quite as great variety as in the vomitus. They are often much swollen and their contents partly dissolved.

*Diatoms* are frequently encountered in the stools.

*Mucus* from the small intestine forms much of the unrecognizable detritus of the stools.

Small particles composed of hyaline globules of mucus are

often discharged, intimately mixed with the fæces, in diseases of the small or large intestine. When derived from the upper portion of the jejunum they are often bile-stained. The larger masses of mucus in catarrhal conditions almost invariably entangle epithelium and leucocytes.

*Pus cells* are scanty in most simple catarrhal conditions of the intestine, but a few are commonly found mixed with the mucus. In ulcerative processes, however, they are abundant, being associated with blood-detritus and intimately mixed with the fæcal matter, and in the absence of fæcal matter may with epithelial cells and bacteria constitute the bulk of the stool.

*Blood cells* may sometimes be found when the gross inspection of the stool fails to show the presence of blood. The degree of solution of blood and alteration of its pigment may best be determined by the microscope.

*Epithelial cells.*—The examination of the stools for epithelial detritus is often of great importance.

A moderate number of columnar epithelial cells, usually isolated, may be found in *normal fæces.* In most *catarrhal conditions,* especially in children, exfoliated epithelium is very abundant in the stools.

In *choleraic discharges* the stools are largely composed of serum, epithelial cells, leucocytes, and bacteria.

With *typhoid ulcers,* epithelial cells and shreds of the necrosing ulcers may often be demonstrated in the stools. The *diagnosis between catarrhal and ulcerative processes* may be determined by finding clumps of epithelial cells, with adherent leucocytes and blood cells, in the masses of mucus discharged from ulcers. The diagnosis between *meningitis and enteritis* in children with marked meningeal symptoms may require the examination of the stools.

Shreds of an *ulcerating malignant new growth* of the colon may be found in the mucus particles of the stools.

*Crystals.*—Of the crystalline bodies found in the fæces, the long acicular *crystals of fatty acids* have been mentioned as of nearly constant occurrence. *Cholesterin plates* are occasionally seen, but their presence is without definite significance.

*Charcot-Leyden crystals* are sometimes found in great abundance, especially in cases suffering from intestinal parasites.

Crystals of *calcium oxalate, sulphate, and phosphate* are found


Bismuth Sulphide

especially after vegetable diet. *Triple phosphates* are much more frequently seen, especially in alkaline diarrhœal stools.

Irregular brownish-red crystals or polygonal masses of *hematoidin* indicate the presence of altered blood.

When *bismuth* has been administered by mouth black crystals of the sulphide are seen in abundance and may be recognized by their oblong form, black color, and serrated edges.

## *Micro-organisms.*

The intestinal tract harbors a great variety of micro-organisms, the presence of most of which is without important significance.

(1.) Many of these are carried in by the food and remain for a short period only, while others are natural denizens of the mouth and stomach, but may, under some conditions, flourish in the intestine.

(2.) A considerable group finds favorable biological conditions in the intestine, is uniformly present there, exercises an important influence on digestive and putrefactive processes, and sometimes becomes pathogenic.

(3.) Others are distinctly pathogenic, and indicate by their presence a diseased condition.

In the *first group* may be mentioned *oidium albicans*, rarely seen in the stools of children if suffering from thrush; yeasts, frequently found in the acid stools of milk-fed children; and *leptothrix*, usually derived from the mouth or stomach.

In the *second group* belong principally *bac. coli communis*, *bac. lactis aerogenes*, and *proteus vulgaris*.

*Bacillus coli communis* is nearly constantly present in fæces, and is found in all parts of the intestine, most abundantly in the colon. It is a short bacillus, 3 to .5 $\mu$ long by .4 $\mu$ broad, rather actively motile, and decolorizes by Gram's method. In typhoid fever it is enormously increased in numbers, and in some intestinal diseases it may be the only germ that can be isolated from the fæces.

Besides its usual capacity to ferment glucose with the production of gas, to ferment milk with the production of acid, and to

produce indol, it may develop distinct pathogenic qualities, the exact importance of which have not yet been determined. Thus it has been found exclusively in peritonitis, angiocholitis, pyelitis, cystitis, and largely in many terminal infections of near or distant organs.

*Bacillus lactis aerogenes* is present in most specimens of fæces, and is very abundant in the stools of milk-fed infants, being most numerous in the upper part of the small intestine. It is a short bacillus, 1 to 3 $\mu$ long by 1 to .5 $\mu$ broad, non-motile, decolorizing by Gram's method. It rapidly coagulates milk and actively produces acid and gas. Distinct pathogenic qualities in the human subject have not been demonstrated, but it is nearly constantly present and is often the predominating germ in the stools of *cholera infantum* and *catarrhal enteritis.*

*Proteus vulgaris* is one of the most widely distributed of putrefactive organisms and is frequently present in abundance in the stools in diarrhoeal diseases, less abundantly in the normal fæces. It is a short bacillus, 1.25 to 3.75 $\mu$ long by .6 $\mu$ broad, exhibits very active but intermittent motility, decolorizes by Gram's method. It is probably an important etiological factor, if not the specific cause, of many cases of *cholera infantum,* and has been found in the pus of cystitis, pyelitis, and peritonitis.

In the *third group* are the germs of several specific intestinal diseases.

The *bacillus typhosus* is the specific germ of typhoid fever and is present in the stools in great abundance during the febrile period, diminishing in number during convalescence. It can usually be isolated by the ordinary plate methods during the first 10 days of the illness, but after ulceration has occurred it appears to diminish in number in the stools and is usually overgrown by other species.

The *bac. typhosus* averages 2 to 4 $\mu$ in length, .5 $\mu$ in breadth, but may grow in threads. Its ends are oval; it is actively motile. It decolorizes by Gram's method. The isolation of this germ from the colon bacillus requires very careful biological analysis

There are three procedures for the isolation of *bac. typhosus* which require notice.

(1.) Elsner's method.

On a mixture of potato-gelatin and iodide of potash the growth

of most intestinal saprophytes is inhibited, while the colon and typhoid bacilli develop actively. At the end of 24 hours the colon bacillus has produced typical opaque brownish colonies while the typhoid growth is invisible. After 48 hours the typhoid growth appears as very fine granular colonies resembling drops of water, which can readily be distinguished from the larger opaque colonies of the *bac. coli com.* (Details of the preparation of this medium may be found in Abbott's Bacteriology.)

(2.) *His's method.*

This method is based upon the fact that the typhoid bacillus is more actively motile than the colon bacillus, and this difference in motility results in a difference in growth upon semi-fluid media.

A *tube medium* and a *plating medium* are required.

The *tube medium* consists of 5 grams of agar, 80 grams of gelatin, 5 grams of Liebig's beef extract, 5 grams of sodium chloride, and 10 grams of glucose to 1 litre of water. The gelatine is added after the agar has melted and the glucose after clearing. The medium must be titrated with normal HCl or NaOH until it shows 1.5 per cent. of normal acid.

The *plating medium* consists of 10 grams of agar, 25 grams of gelatine, 5 grams of Liebig's beef extract, 5 grams of sodium chloride, and 10 grams of glucose. It must contain at least 2 per cent. of normal acid.

The suspected stool is plated on the second medium, and after 18 hours' growth in the thermostat the typhoid colonies appear as fine grayish points among the larger yellowish colon colonies. Thread formation is abundant in the typhoid colonies, rare in the others.

The growth of most intestinal saprophytes is inhibited.

From a suspected typhoid colony a tube of the first medium is inoculated. After 18 hours in the thermostat the more actively motile typhoid bacillus has clouded nearly the entire tube, while the growth of the colon bacillus is much more restricted.

(Details of this method may be found in the Journal of Experimental Medicine, Vol. II., 1897, p. 677.)

(3.) *Widal's test.*

A hanging drop preparation from a suspected colony of the typhoid bacillus obtained by any of the biological methods is treated with blood serum from a case of typhoid fever. A distinct

reaction indicates with great certainty the presence of the typhoid bacillus. (See Widal's Test on Blood.)

The *comma bacillus* is constantly present in the intestine in cases of Asiatic cholera. They are slightly curved rods with rounded ends, .8 to 2 $\mu$ in length, .3 to .4 $\mu$ in breadth. Two or more individuals may form a semi-circular, or S-shaped, or spiral figure. They stain slowly with aniline dyes and are decolorized by Gram's method. They are actively motile, exhibiting usually a single flagellum, and do not form spores.

In distribution they are usually limited to the intestine, are most abundant in the ileum, where they penetrate the mucosa, rarely reaching the sub-mucosa. They are discharged in enormous numbers in the stools, and in the rice-water exudate may be found in nearly pure culture. In irregular cases the bacilli may be scanty in the stools. They have been found in the vomitus.

The identification of the cholera bacillus and diagnosis of cholera is based upon (1) the microscopical characters of the stools; (2) the biological characters of the suspected germ, including especially the growths in gelatin, agar, peptone solution, and the cholera-red reaction; (3) the effects of intraperitoneal inoculation of guinea-pigs with pure cultures. (See Text Books on Bacteriology.)

*Streptococcus pyogenes* is probably the chief etiological factor in some forms of entero-colitis in children and of colitis in adults. Its abundant presence in the shreds of mucus and epithelium may be regarded as indicating that this germ is probably of etiological importance in the disease.

The identification as well as the isolation of any of these germs require the usual bacteriological procedures.

*Tubercle bacilli* may be found in the stools in cases of pulmonary tuberculosis without intestinal lesions, having been swallowed with the sputum. When symptoms of enteritis or colitis are present the discovery of tubercle bacilli in the mucus of the stools, especially if they occur repeatedly and in considerable numbers, establishes the diagnosis of intestinal tuberculosis.

*Protozoa.—The amœba dysenteriæ* is probably the specific cause of one form of colitis. These organisms are protozoa, 25 to 35 $\mu$ in diameter. When at rest they are of spheroidal form; their protoplasm is *finely granular* and *slightly refractile*. There is a

single vesicular nucleus, and often a fine nucleolus, both apt to be centrally placed. Coarser granules, bacteria, red blood cells, and *vacuoles* are often contained in the protoplasm.

In the fresh state they exhibit for a few hours distinct and often active amœboid movements, on which character only can positive identification be based. Smaller amœbæ, 10 to 15 $\mu$ in diameter, with well-marked capsule, have been described. These are supposed to be encysted forms of the *amœba dysenteriæ*.

Several recent investigations lead to the conclusion that there is a third form of amœba often to be found in the normal intestine, or in other diseases than dysentery. This organism measures 10 to 15 $\mu$ in diameter, is very finely granular, and is not wont to englobe red blood cells and other coarse detritus. The term *amœba coli* (*mitis, vulgaris*) should be restricted to this form.

The *amœba dysenteriæ* occurs *in considerable abundance in* the *masses of mucus* discharged in the stools of tropical dysentery, and in the pus of secondary abscesses. When such an abscess ruptures into the lung they may be found in the sputum.

The *demonstration* of the *amœba dysenteriæ* requires that the particles of mucus should be secured from the stool very soon, at least twenty-four hours after its passage, and be examined microscopically, and, if possible, on a warm stage. The amœba may then be identified by its morphology and motility.

Various other protozoa have been discovered in the stools, but their occurrence is rare, and their pathological importance is as yet largely undetermined.

*Vermes*—(1) Cestodes (Tape-worms).

*Tænia solium*, or pork tape-worm, is a rather rare parasite in America. The adult worm reaches a length of two or three metres. Its head is spherical in shape and about the size of a pin-head. It presents four prominent suckers. The pointed tip or rostellum is often pigmented and supports a row of twenty-six coarse hooklets. The head is followed by the unsegmented neck, which is about one inch in length. The first segments are very small, but gradually increase in size, the adult forms measuring 9 x 6 mm. The corners of these adult segments are rounded, the

sexual orifice is situated on the lateral edge behind the middle point, and the uterus presents 7 to 10 lateral branches.

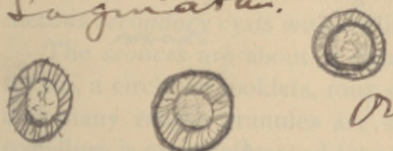
The *eggs in the ovary* are thin-skinned, pale yellow cells; *in the uterus* they are spherical, brownish bodies, .03 mm in diameter, surrounded by a dense chitinous, radially-striated shell, and containing a young formed embryo in which hooklets are usually visible. The ova are usually surrounded by a second envelope composed of an albuminous envelope and a limiting vitelline membrane. *In the stools* the eggs regularly contain embryos with hooklets, and lack the yolk-membrane.

*In the further development of the embryos,* the shell is dissolved in the stomach of the pig, the liberated embryo makes its way into the tissues of the host, and there becomes *encysted,* developing into a cyst filled with serum (*cysticercus cellulosæ*), from whose wall a new tape-worm head buds off (*scolex*). The uncooked flesh of the animal being eaten by man, the worm then develops in the intestinal tract, the head being fastened in the duodenum or upper jejunum. In some countries, rarely in America, the cysticercus develops in the human subject.

*Tænia saginata* (or beef tape-worm) is a common parasite in Europe and America. The adult worm measures 4 to 8 metres in length; its head is flat, and presents four suckers, which are usually fringed with pigment. It lacks rostellum and hooklets. The segments are longer and broader than those of tænia solium. The uterus presents many lateral branches closely packed together, and divide only dichotomously instead of branching like a tree. The ova are practically indistinguishable from those of tænia solium.

*Bothriocephalus latus,* or fish tape-worm, is a common intestinal parasite in many parts of Northern Europe, and is not rare in America, although usually found among the foreign-born population. Infection results from eating uncooked fish. It measures five to nine metres in length, being composed of three to four thousand short, broad segments, the largest of which measure 3.5 x 12 mm. The head is of elongated club shape, presenting on either side a slit-like depression. The sexual orifices are near the anterior margin of the ventral surface, and the uterus is a simple coiled canal. The segments are distinctly pigmented at their central portions. The eggs are oval and measure about

Measly beef is beef infected by embryos of T. Saginata.



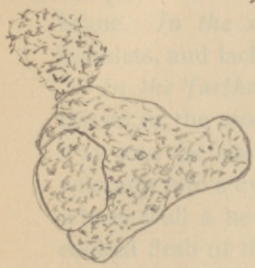
Ova of Taenia Saginata

Ova are abundant in the stools.

B. Latus causes severe pernicious anaemia. The anaemia is probably due to poisonous excretions from the worms, mostly

Ovum B. Latus.

T. Echinococcus present in the intestinal tract of almost all dogs.



Scolex

The hooks are diagnostic.

.07 x .045 mm. They are surrounded by a thin, doubly refractive shell, on the anterior pole of which a sharply limited cap-like cover may sometimes be seen. When secured from the adult segments, the egg contains a number of globular protoplasmic masses. The free embryo is surrounded by a ciliated envelope.

*Tænia echinococcus* lives in the intestinal canal of the dog, but its eggs may reach the intestine of man, whence it may wander into any of the viscera, producing cysts (echinococcus cysts). The adult worm measures 4 mm. in length, and consists of a head and three segments, the last of which comprises the bulk of the parasite. The head is provided with a rosette of 30 to 40 hooklets.

When echinococcus cysts develop in a viscus their walls are composed of an *outer layer* of connective tissue derived from the infected organ, and of an *inner layer* developed by the parasite. The wall developed by the parasite consists of an *outer* laminated cuticular layer, and an *inner* parenchymatous layer composed of cells, muscle bundles, and circulatory system. From this parenchymatous layer are developed small heads of new parasites (scolices), which may become very numerous.

The cysts *may remain single,* or from the cuticular layer numerous *secondary cysts* with scolices may develop.

The *scolices* are about .3 mm. long, are provided with a rostellum, a circle of hooklets, four suckers, a water-vascular system, and many coarse granules are seen in their parenchyma. The rostellum is often telescoped into the posterior part of the body, in which case the scolex is spheroidal. The *cysts* contain clear non-albuminous fluid, many cholesterin plates, and usually free scolices and hooklets are floating in the aspirated fluid. The fluid may in some cases be turbid, and in old, degenerated cysts may be caseous. The cyst wall may suppurate or become calcific, and in some old cases nothing may remain except a mass of fatty, caseous material in which are the swollen remnants of the parasitic wall, but no traces of lamination, and no remnants of scolices.

## *Nematodes.*

*Ascaris lumbricoides* (round-worm) is a common intestinal parasite in children. It is a light brownish-red, cylindrical worm,

the female measuring 25 to 40 cm. in length, the male being one-third shorter. The posterior end of the male is bent and provided with two chitinous spicules. The mouth is provided with very fine teeth. The eggs are 50 to 60 $\mu$ in diameter, are surrounded by a double contoured shell, an irregular albuminous envelope, and contain coarsely granular material. They are matured in the intestine of the host, and infection occurs directly from one human subject to another. The eggs and worms are often discharged in the fæces, and the worms travel into many ducts leading from the intestinal tract, being frequently vomited.

*Oxyuris vermicularis* (thread or pin worm) is a small, round worm, the female measuring 10 mm., the male 4 mm. in length. The male has a blunt spiculated posterior extremity. The eggs are 24x50 $\mu$ in dimensions, and consist of a thin, colorless shell, an albuminous envelope, and coarse globular contents. The males and young individuals infest the lower ileum: the female is found in the colon and infests the rectum and anus, being specially active at night, when excessive pruritus results from their movements. After being discharged in the fæces the ova reach the stomach of man or beast and repeat their development.

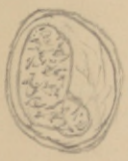
*Anchylostoma duodenale* is a small cylindrical worm residing in the upper part of the small intestine. The female is 5 to 18 mm. long; the male, 6 to 10 mm. On the ventral surface of the dorsally-curved head is the mouth, which, on the dorsal surface, is deeply cleft and provided with two teeth, while on the ventral edge are four curved teeth. The posterior extremity of the male is flattened, presenting three flaps, or bursæ, and two long, projecting spicules. In the female the posterior extremity is pointed and presents an awl-like prong. The eggs are oval in shape, about as large as those of *bothriocephalus latus;* they are surrounded by a colorless, double-contoured shell, and contain coarsely globular protoplasm. They begin their development in the intestine, continue to grow in muddy water, whence they may, on reaching the human intestine, at once develop new individuals. They burrow into the intestinal mucosa, producing a small hemorrhagic spot, in which they are partly concealed, and they may be discharged with their ova in the stools.

This parasite is very common in the tropics.

*Trichocephalus dispar* (whip-worm) is a rather common but

These may cause intestinal obstruction at the ileo-caecal valve or jaundice by obstructing bile duct.

very severe
This worm also causes anaemia.



Egg of A. Duodenale.

Ovum of T. Dispar

harmless parasite inhabiting the neighborhood of the cæcum. They measure 4 to 5 cm. in length, the anterior two-thirds being thread-like, the posterior portion being much thicker, and in the female containing the uterus.

The *ova* are very characteristic, being distinctly *reddish in color,* elongated oval in shape, and exhibiting at either end a projecting, colorless, highly refractive knob.

The early development occurs in water or damp earth.

### *Chemical Examination of Fæces.*

The chemistry of the fæces is an extensive and important subject, but in the present state of knowledge it is of little assistance in clinical diagnosis.

*Reaction.*—The normal reaction of the fæces in the adult is usually alkaline, owing to ammoniacal fermentation, sometimes acid from lactic and butyric acid fermentation. Infants' stools are normally acid. In catarrhal enteritis, especially in children, the reaction is strongly acid.

Acholic stools are usually alkaline, and when there is much intestinal exudation, as in typhoid fever and cholera, the reaction is strongly alkaline.

*Albuminoid Principles.*—Mucin is always present, sometimes in increased quantity. It may be demonstrated by dissolving the fæces in lime-water, filtering and testing the filtrate with acetic acid, which precipitates mucin.

*Serum albumen* occurs in considerable quantity in diarrhœal and typhoid stools. For its demonstration an acidified watery extract may be tested, as in urine.

*Peptone* is usually present in the stools in all forms of intestinal ulceration, and often in acholic stools.

The watery extract should be boiled, filtered, and peptone demonstrated as in urine.

*Biliary acids, fatty acids,* and fat derivatives, *indol, skatol,* and *phenol,* are present in all normal and most pathological specimens, but their demonstration is of little practical value.

Blood-pigment may be demonstrated by Teichmann's test (see Blood), and bile pigment by Gmelin's test (see Urine).

*Character of the Fæces in Intestinal Diseases.*

*Catarrhal Enteritis.*—A catarrhal affection limited to the small intestines usually leads to constipation, but when there is irritation or inflammation of the colon diarrhœa supervenes. In children enteritis is nearly always associated with colitis, and diarrhœa is commonly present.

There is a great variety in the character of the stools in the enteritis, or gastro-entero-colitis, of children. Usually the stools are small, very frequently voided, of sour odor, often frothy, yellowish or greenish from admixture of bile, containing much undigested food-residue, strongly acid in reaction, and microscopically show an abundance of crystals of fatty acids and exfoliated epithelial cells.

In some more rapid, choleraic cases the stools contain much serous fluid, little fæcal matter; epithelial cells and leucocytes are very abundant, and fat crystals are less numerous. In other less severe cases the stools are composed largely of mucus.

*Cholera.*—The thin "rice-water" discharges of cholera are alkaline in reaction, lacking in fæcal matter, and are composed principally of serum, leucocytes, epithelium, and the comma bacillus, usually in large numbers.

Ordinary diarrhœal stools may precede the "rice-water" discharges, and convalescence is sometimes marked by dysenteric stools.

*Dysentery.*—In colitis the number and character of the stools vary with the seat and character of the lesion. An affection of the *rectum* and *lower sigmoid* flexure is marked by the frequent discharge of small stools of blood and mucus. A lesion of the *higher* portions of the *colon* leads to the discharge of larger stools at less frequent intervals. These larger stools are diarrhœal; they contain mucus in small particles intimately mixed with the fæces, or larger and more abundant masses or membranous casts; epithelial cells may be abundant, but leucocytes are usually scanty. When ulceration occurs blood is added to the mucous particles, leucocytes become abundant, and shreds of mucous membrane or clumps of epithelial cells may frequently be detected by the microscope. In the more severe stages fæcal matter may be entirely absent.

In one form of productive colitis with ulceration the stools are composed largely of serum mixed with blood, and deposit, on settling, leucocytes and epithelial cells.

In *amœbic colitis* the evidences of necrosis of mucous membrane are usually very marked, bloody mucus, and necrotic shreds of mucous membrane are discharged in considerable quantity, the strong sour odor is often characteristic, and the *amœba dysenteriæ* may be demonstrated in the masses of mucus.

*In advanced cases of ulcerative colitis, as well as at other times, the contents of the ileum may pass through the colon unchanged.*

*Typhoid Fever.*—The ordinary diarrhœal stool of typhoid fever is ochre yellow in color, of the consistence of pea-soup, and of powerful odor. It contains a moderate admixture of fæcal matter, and milk curds may be seen when the ingestion of milk is excessive. In severe cases the stools may be of light color, or even white, but are not necessarily acholic. When colitis is present the stools are more frequent and contain considerable mucus, which otherwise is usually absent.

*Hemorrhages* in typhoid fever occur under three distinct conditions:

(1.) There may be a preliminary bleeding from the congested mucous membrane during the first week of the disease. This blood is usually moderate in amount, well mixed with the fæces, and much altered.

(2.) Large hemorrhages from the eroded vessels of Peyer's patches begin usually after the tenth to twelfth day. This blood is well mixed with the fæces. Small quantities may be retained until much altered and of a tarry-black color. Large amounts may be passed while still of a dark reddish color, or fatal hemorrhages may be found in the ileum after death.

(3.) *Hemorrhages from the colon* are usually moderate in quantity, quickly and frequently voided while the blood is red, and from ulcers in the lower part of the colon there may be a continuous bleeding from the anus.

*Microscopically,* the remnants of food-products are recognizable in moderate quantity, diminishing as the disease progresses. Isolated epithelial cells are always greatly increased, and after ulceration has occurred shreds of epithelium, an abundance of leucocytes, and disintegrated blood are present.

The *lighter-colored stools* are composed mostly of blood-serum, epithelial cells, leucocytes, and an enormous number of bacteria. Fatty crystals and triple phosphate crystals are often encountered in typhoid stools.

*Chemical analysis* shows a reaction usually alkaline, and the presence of albumen and peptone. In the early stages of the disease altered bile pigments are usually increased in amount; in the later periods of severe cases the secretion of bile is greatly diminished, and the stools may be nearly acholic, but the transformation of bile pigment into the colorless leukourobilin of Nencki may be partly responsible for the whitish color of these stools.

# URINE.

*Quantity.*—The healthy adult passes from 1 to 2 litres of urine daily, but the normal and pathological variations in quantity of this secretion are very marked.

Most of the physiological variations are slight and clinically unimportant, but attention must always be paid to the diuretic effects of excessive fluids in the diet, and to the diminished quantity of urine passed when there has been profuse and prolonged perspiration. In pathological conditions the most striking cases of *polyuria* are those referable first to *diabetes,* in which the polyuria may be extreme and persistent; to *chronic diffuse nephritis* with waxy degeneration of glomeruli and cardiac hypertrophy, conditions accompanied by less marked and much less constant polyuria; to the *rapid absorption of serous effusions,* especially in cases of ascites, when a sudden relief of renal congestion leaves the kidneys free to act; to an *epicritical polyuria* of moderate grade, sometimes observed in many acute diseases, attended with oliguria; and to many *nervous diseases* associated with paroxysmal or continuous polyuria.

*Oliguria* is observed in nearly all *febrile conditions;* in *congestion of the kidneys* from the venous stasis of heart disease or the local congestion of nephritis; while *anuria* may result from extreme grades of congestion or from obstruction to the urinary passages.

*Color.*—The color of urine is due principally to the presence of pigments, the origin and nature of which are not fully known. The most important is *normal urobilin. Uroerythrin* colors crystalline urates and uric acid. Considerable recent study of urine has been devoted to the presence and significance of a *pathological urobilin* (in anæmia) and to *hemato-porphyrin.*

The color of normal urine varying, often with the density, from light straw to brownish-red, is usually much deepened in *fever. Blood* may add the reddish tinge of oxyhemoglobin or the dark

brown (rarely black) of methemoglobin. *Bile* gives the yellowish or greenish colors of bilirubin or biliverdin.

In *poisoning* by carbolic acid or related drugs the urine is often "smoky" or black. Green urine has been observed after taking *salol*. *Rhubarb* and *senna* may give a brown or deep red color.

*Pale or colorless urines* may be observed in *chronic nephritis*, especially with waxy glomeruli, and in most varieties of *polyuria*.

*Solid particles in suspension* often alter the color of urine, as in the persistent turbidities due to pus, bacteria, epithelial cells, casts, and chyluria.

*Odor.*—The normal odor of urine is due to volatile acids. Characteristic odors are developed in ammoniacal fermentation, diabetic coma, carbolic acid poisoning, and by the ingestion of many vegetable products.

*Reaction.*—Normally the reaction of the twenty-four-hour urine is nearly always acid, owing to the presence, not of free acids, but of acid salts and phosphates. The reaction varies promptly with the diet, being alkaline with a vegetable diet, and acid with a mixed diet. Shortly after a full meal the urine is often alkaline.

The *acidity* is usually *increased* in rheumatism, gout, fevers, leukemia, and diabetes; by the administration of alcohol, benzoic, cinnamic or mineral acids, and on standing by the production of acids, as in diabetic urines, from fermentation of carbohydrates. *Alkalinity* of the urine may result from a vegetable diet or full meal, from the administration of drugs, the carbonates, or organic acids which are converted into carbonates; and pathologically from ammoniacal fermentation. This process results from the action of a ferment elaborated by *micrococcus ureæ* or *bacterium ureæ*, etc., which decomposes urea into carbonate of ammonia or ammonia, water, and carbon dioxide.

The reaction of the urine is tested by red and blue litmus-paper.

*Specific Gravity.*—The gravity of normal urine usually varies between 1.015 and 1.025. Urine being practically a solution of urea and salt, variations in gravity indicate corresponding variations in the amount of these excretory principles. But in drawing from the specific gravity conclusions as to the daily excretion

of solids, it is imperative to know the entire quantity of urine passed in the twenty-four hours.

It is important to remember also that organic principles (urea) are of lower gravity than the inorganic (NaCl), so that a urine rich in chlorides and of high specific gravity may yet be deficient in urea, or a urine poor in chlorides and of low gravity may contain a normal percentage of urea.

The *specific gravity is low,* but the solids excreted are not diminished, after liberal ingestion of fluids. Low gravity and diminished excretion of solids are combined in *chronic nephritis with the growth of new connective tissue,* and often in diabetes mellitus. Very low specific gravity and excretion of urea is seen in nephritis with waxy changes in the blood-vessels.

The specific gravity of the urine is high and excretion of solids increased in lesser degree in *fevers,* and during remissions in the course of chronic nephritis. Specially high gravity and markedly increased excretion of solids are seen in *diabetes mellitus.* The specific gravity of urine is taken by means of the *urinometer,* in the use of which ordinary care must be observed. All instruments should be tested in distilled water, in which they should read 1.000. The sinker must be clean; it must not touch the sides of the bottle, and air-bubbles and foam must be removed.

## CHEMICAL ANALYSIS.

### *Chlorides.*

Chlorides of Na, K, $NH_4$, Ca, and Mg. occur in the urine, but NaCl is most abundant, and as the others are found in traces only, it is usual to estimate the entire group in terms of the sodium salt.

From 10 to 15 grams of chlorides are normally eliminated during twenty-four hours, but the excretion is subject to marked *physiological variations,* the most important of which results from the quantity and quality of the food. A *large elimination of chlorides* occurs with a full diet, especially if rich in potassium salts. Under ordinary conditions an increased excretion of urine carries with it an increased elimination of chlorides.

*Pathologically*, the chlorides are *diminished* in most febrile diseases, from (1) diminished ingestion of chlorides in the food, (2) diminished excretion of urine, and (3) from their elimination by other channels, in vomiting, diarrhœa, and exudations.

Their total excretion is lessened in chronic nephritis with the diminution of urine; in gastric hyperacidity with vomiting of HCl; and in many chronic conditions attended with malnutrition. The chlorides are *increased* after the relief of all conditions which have caused a previous decrease, and in most cases of polyuria.

Considerable importance attaches to the daily estimation of the chlorides in the diagnosis and prognosis of all forms of pneumonia, in which the combination of a diminished ingestion of food and an exudation rich in chlorides commonly reduces the excretion to a very low grade.

In normal urine the addition of one drop of 5 per cent. sol. of $Ag.NO_3$ precipitates the chlorides in thick curds, which settle to the bottom of the test-tube, leaving the supernatant fluid clear; but in pneumonia the resulting precipitate may be hardly visible. Intermediate grades of turbidity occur with various proportions of chlorides, and these may be designated as are precipitates of albumen, as a *faint trace*, *a trace*, or *a small amount*.

Using this nomenclature, several rules may be stated regarding the behavior of the chlorides in pneumonia.

During all pneumonic exudations there is a marked diminution of chlorides in the urine.

The diminution of the chlorides in lobar pneumonia is proportionate to the general severity of the case, and especially to the extent of the exudate. In very severe and usually fatal cases of pneumonia the chlorides are reduced to a *faint trace*.

At the crisis, and often a few hours before the appearance of other symptoms of improvement, the chlorides are sharply increased—e.g., from a *faint trace* to a *well-marked trace*.

The chlorides are unaffected during pseudocrises.

In addition to pneumonia the chlorides are markedly reduced during most purulent exudations, such as empyema, meningitis, and peritonitis.

In general, the estimation of the urinary chlorides is of most value in the diagnosis between pneumonia and tuberculosis, typhoid fever, and malaria,—in which diseases the chlorides are only

There is an enormous increase in phosphates in some Neurasthenias.

Practically, oxalates are of no importance in urine.

moderately reduced—and between purulent and tuberculous meningitis.

In drawing conclusions from such analyses of the urine it is important to know the exact stage of the exudate and to remember the effects of low diet, vomiting, and diarrhœa. Thus, the urinary chlorides may be normal with the chest full of pus, the exudative process having ceased, or they may be reduced to a trace in typhoid fever with severe vomiting and diarrhœa.

### *Clinical Method of Estimating the Urinary Chlorides.*

(1.) Remove albumen by boiling the urine acidified with acetic acid. Filter.

(2.) Add one drop of $HNO_3$ conc. to 2 in. of clear urine in a test tube, to prevent precipitation of phosphates.

(3.) Precipitate the chlorides by one drop of 5 per cent. sol. $Ag.NO_3$.

This method is sufficiently accurate when dealing with the small quantities of chlorides present in pneumonia. In other cases their estimation requires more elaborate quantitative analysis.

### *Phosphates and Sulphates.*

Clinical deductions of value concerning the excretions of urinary phosphates and mineral sulphates cannot at present be formulated.

*Urea.*—$CO{<}^{NH_2.}_{NH_2.}$

Urea, representing the bulk of the nitrogenous excretion of the urine, is elaborated principally in the liver, and is derived from two main sources—(1) the nitrogenous principles of the food, (2) the nitrogenous elements of the tissues.

The *absorbed nitrogenous principles of the food,* over and above those required in the metabolic processes of the body, are soon excreted in the form of urea, and the quantity of urea derived from this source is subject to marked physiological varia-

tions. The quantity of *urea derived from the tissues* varies but little in health, being but slightly different after exercise and during starvation.

These considerations may serve to explain the wide limits of the physiological excretion of urea. On a mixed diet 25 to 40 grams are usually excreted daily. On a diet poor in proteids it may sink to 15 grams, and on a richly proteid diet may reach 100 grams. The ordinary proportion of urea in urine is 2 per cent.

*Pathologically*, the excretion of urea is *increased* in febrile diseases from more active tissue destruction, and the urine is at the same time concentrated. In diabetes mellitus the urea is usually much increased in amount.

Urea excretion is *diminished* by disturbances in the functions of the *liver*, either with or without organic lesions, and in acute yellow atrophy urea is replaced in the urine by leucin and tyrosin. In diseases of the *kidney*, in which the functions of the renal epithelium are impaired, the excretion of urea is markedly diminished.

The estimation of urea is of great importance in following the course of nephritis.

The total daily excretion of urea must invariably be determined by analyzing a specimen of the mixed urine of the twenty-four hours, and computing the total excretion from the total quantity of urine.

To fully establish the presence of a diminished excretion of urea the observations must extend over a period of several days, for accidental daily variations are not infrequent.

In cases of chronic nephritis a continuous low excretion of urea is one of the most reliable signs of an established and serious renal lesion. An improvement in the excretion of urine and urea is not unfrequently observed before the onset of uræmic symptoms.

*Qualitative Tests for Urea.*—Urea crystallizes *rapidly* in fine white needles, or *slowly* in rhombic prisms. It melts at 130 ° C., and is transformed by further heating into ammonia, which evaporates, and cyanuric acid, a residue which yields a reddish-violet color with one drop of dilute solution of cupric sulphate (biuret reaction). *When heated with nitric acid* urea crystallizes in rhombic or hexagonal plates of *urea nitrate*, which are apt to be arranged like tiles on a roof. The form and arrangement of these crystals furnishes a ready and reliable qualitative test for urea.

*Quantitative Estimation of Urea.*—The best clinical method is that based upon the decomposition of urea by sodium hypobromite into carbon dioxide and nitrogen, as follows:

$$CON_2H_4+3NaOBr.=NaBr.+CO_2+2H_2O+2N.$$

The $CO_2$ is absorbed by an excess of NaOH in the bromite solution, and from the volume of the nitrogen gas alone is estimated the quantity of urea in the tested specimen.

*Preparation of Bromite Solution.*—The bromite solution is of the following composition:

30% sol. NaOH. 70 c.c.
Aq. 150 c.c.
Bromine 5 c.c.

The 30 per cent. sol. of NaOH should be kept in stock, and the water and bromine should be added just before using. In the solution thus formed there is NaOBr. and an excess of NaOH.

*Preparation of Urine.*—A few cubic centimetres of the mixed urines of twenty-four hours should be obtained.

Albumen must be removed by boiling after acidification with acetic acid. The urine should be diluted, if necessary, until its specific gravity is about 1.010.

*Method of Procedure.*—The ureometer (Doremus instrument is convenient) is filled with the solution of bromite and freed from air-bubbles, and by means of a graduated curved pipette 1 c.c. of urine is introduced and slowly discharged into the long arm of the ureometer, care being taken not to expel any air with the urine. After the resulting gas has fully collected in the long arm, the quantity of urea may be read in grams per cubic centimetre.

Strictly accurate readings would require corrections for temperature and barometric pressure, and while the latter correction may be ignored, the effect of changes in temperature is large enough to render it desirable, though not essential, that the reading should be corrected according to the tables computed for that purpose and found in most text-books.

A further correction must, of course, be made if the urine has been diluted.

## *Uric Acid.*

Uric acid is a product of nitrogenous metabolism, related to, but less highly oxidized, than urea. It appears to be formed in the tissues and organs, especially in the liver and spleen. It is excreted by the kidney normally in amounts varying from .2 to 1 gram daily, averaging about .7 grams on a mixed diet. The ratio of excretion of uric acid to urea is 1 to 50 or 70 in adults, 1 to 7 or 17 in children, while in birds uric acid largely replaces urea in the urine. It is increased by meat diet (.2 grams), and diminished by vegetable diet. There are many indications that it originates from the nuclear principles of the tissues.

*Pathologically,* uric acid is distinctly increased in several conditions.

In the *uric acid diathesis* and its acute manifestations there is a very markedly increased excretion of uric acid. Frequently these cases suffer from a mild form of *chronic nephritis,* the urine containing traces of albumen, and large amounts of urea, uric acid or oxalic acid, and the specific gravity being high. In the attacks of *acute gout* the excretion of uric acid is diminished before, and increased during and after the paroxysm. During attacks of *acute rheumatism* the elimination of uric acid is increased.

In most febrile diseases the total excretion of uric acid is high.

In leukemia, and in most cases of chronic hypertrophy of the spleen, the elimination of uric acid is increased, and may reach a very high figure (5 grams).

*Qualitative Tests for Uric Acid.*—The *crystalline* form and the reddish or brownish color of the precipitated uric acid and urates of the urine are characteristic.

*Muxeride Test.*—A few crystals are dissolved by heating with conc. $HNO_3$ in a porcelain dish until the nitric acid is evaporated and a reddish-yellow residue is obtained, which turns purple on the addition of a drop of ammonia, from the formation of ammonium purpurate (muxexide). The further addition of NaOH sol. changes the color to reddish-blue, while heat causes it to rapidly disappear.

At present, results of determination of uric acid are of no clinical value.

There is as yet no simple method applicable for clinical purposes of estimating the excretion uric acid. Rough indications, not always reliable, may be obtained by observing the width of the colorless ring above the albumen in the nitric acid test for albumen (see Simon, p. 397); or by finding an abundant spontaneous deposit of uric acid or urates soon after the urine is passed.

In gout and rheumatism the increase of urates is plainly observable in most specimens in the abundant brick-dust deposit settling after a few hours in the cold.

On the other hand, the urates may be precipitated, although deficient in quantity, and a marked excess may yet remain in complete solution, so that quantitative analysis gives the only reliable information concerning the state of their excretion.

*Hippuric acid, kreatin, and the Xanthin bodies* are other products of nitrogenous metabolism, the significance of which in the urine is imperfectly understood.

*Oxalic acid* occurs in minute traces in normal urine, and its excretion is much increased by many vegetables which contain this acid, by defective digestion of carbohydrates, and in the *oxaluric diathesis.* In this last condition there is transient albuminuria and an increased excretion of uric acid, which appears to be replaced at intervals by oxalic acid.

Crystals of calcium oxalate are characteristic, but the quantitative estimation requires somewhat elaborate chemical analysis.

## *Indican.*

*Indol* is a product of bacterial fermentation of albumens in the intestinal tract. It is oxidized and united with $H_2SO_4$ in the blood, and excreted in the urine as a sodium salt, which is called *indican,* or indoxyl-sodium-sulphate. It is normally excreted in the proportion of 6 milligrams per litre of urine. Pathologically, it is often increased in amount, when it indicates abnormal *bacterial putrefaction of albumens.* This abnormal process may occur:

(1.) *In the Intestinal Tract.*—Intestinal putrefaction and the absorption of indol is largely dependent upon the activity of the gastric-juice. Indicanuria is usually marked in carcinoma of the stomach and in gastritis.

In digestive and muscular torpor of the small intestine from obstipation, peritonitis, intestinal diseases, etc., indican is much increased in the urine. In simple constipation and in diseases of the colon alone increased indicanuria is never seen.

(2.) *In the Tissues.*—Indican is greatly increased in cases of empyema, especially if gangrenous, in putrid bronchitis, gangrene of lung, and gangrenous cellulitis.

### *Test for Indican (Obermayer's).*

To 5 c.c. of urine add 5 c.c. of strong HCl containing two drops of a 5 per cent. sol. of ferric chloride. Shake well and allow to stand 5 minutes. Then add 2 c.c. of chloroform, shake again, and after 10 to 20 minutes note the intensity of the bluish color of the sediment.

In normal urine the color of the sediment is light blue. With an increased quantity of indican the sediment is intensely blue.

This test is based upon the fact that ferric chloride in the presence of acid oxidizes indican to indigo. The chloroform is added to dissolve out and concentrate the indigo. The blue color begins to fade after twenty-four hours. In very highly pigmented urine it is well to precipitate the pigment by adding 20 per cent. solution of lead acetate and proceed with the clear filtrate. In the presence of much pigment, chloroform fails to dissolve all the indigo.

### *Acetone.*

*Acetone* is a metabolic product of the proteids of the blood and tissues. It occurs in traces in normal urine, and is usually increased by a nitrogenous diet and diminished by ingestion of carbohydrates.

*Pathologically,* increased amounts are found in the urine in

Empyema + Intest. Obstruction give the greatest quantity of Indican in the urine.

Obermayer's reagent = 1000 c.c. HCl
2-4 gms. $Fe_2Cl_6$.

$H_2O_2$ may be used instead of $Fe_2Cl_6$.

diseases in which proteids are extensively destroyed, as in fever, diabetes, cachexia, and starvation.

*In diabetes* the excretion of acetone varies with the excretion of nitrogen, but not with that of glucose. It may become so abundant that the urine has a distinct odor of acetone. Acetonuria increases usually with the intensity of the disease, often heralding the onset of diabetic coma, and diminishing with the ingestion of carbohydrates.

*In prolonged fevers* acetonuria may be marked, being here referable to the exclusive nitrogenous diet as well as to the destruction of tissues.

In short acute fevers it is not increased.

A *digestive acetonuria* is seen in cases of carcinoma of stomach, when it may be produced in part in the stomach-contents, and in a considerable variety of conditions in which feeding is improper, and gastric or intestinal digestion is impaired. It is usually increased in pregnancy.

*Tests for Acetone.*—In marked acetonuria the aromatic odor of the urine is characteristic.

*Lieben's Test.*—A few cubic centimetres of urine should be *distilled* and treated with a few drops of Gram's fluid and sodium hydrate, when the presence of minute traces of acetone can be detected by the resulting *odor of iodoform*, while larger amounts give a precipitate of iodoform scales. When acetone is present in traces only, the odor of iodoform does not develop till after several hours.

### *Glucose.*

Glucose, lactose, maltose, and levulose, are occasional pathological principles in the urine, but the presence of glucose only is of clinical importance.

*Physiological glycosuria* may often result from the ingestion of 100 to 250 grams of pure glucose, the result varying with the idiosyncrasies of the subject and upon minor peculiarities in the absorption and elaboration of food.

A *digestive glycosuria* may be observed after the ingestion of considerable amounts of sugar or saccharine foods, especially when there is disturbance of function or organic lesion of the liver

or pancreas. In most conditions associated with a tendency to glycosuria, sugar appears in the urine when much glucose or saccharine food is ingested.

The occasional glycosuria observed in some febrile diseases and with lesions of the central nervous system are probably often of digestive origin.

A *neurotic glycosuria,* apart from disorders of digestion, and usually continuous, has been noted in a small proportion of cases of organic nervous diseases, such as tumor of the brain, apoplexy, diffuse cerebral syphilis, etc. As neurotic or toxic glycosuria may be classed those cases which occur in sunstroke and other extreme pyrexias, or in the later months of pregnancy.

*Toxic glycosuria* is often observed after poisoning by many drugs, but in some of these cases the reducing substance is now believed to be glycuronic acid, and not glucose.

In general it may be said that transitory glycosuria of the several varieties is rather frequent, while a continuous glycosuria apart from diabetes is rather rare.

*Diabetic glycosuria* is the characteristic symptom of *diabetes mellitus.*

### *General Characters of Diabetic Urine.*

*Glucose.*—As a rule, the greater the excretion of sugar the worse is the disease, but some rapidly fatal cases excrete only a few grams daily, while others do well while discharging very large amounts of sugar. In established cases the excretion of glucose usually reaches 4 per cent. (60 grams) daily, while 24 per cent. (360 grams) has been observed, and either from careful treatment or spontaneously, the glycosuria may temporarily disappear, so that the diagnosis requires repeated examination of the urine.

The safest *prognostic signs* are obtained by noting the effect of nitrogenous diet on the glycosuria. In the *most favorable cases,* on careful diet, the glycosuria disappears. In *less favorable cases,* the glycosuria is much reduced by proper diet, but never disappears. In *unfavorable cases,* nitrogenous diet has little effect on the glycosuria.

Nylander's test – If urine contains sugar, Bi will be reduced to metallic black ppt. This is a very good test.

Associated with glucose, diabetic urine often contains much acetone, fatty acids, and occasionally free fat.

*Polyuria* is a very early and constant symptom, the amount of urine in cases of moderate gravity varying between 2 to 5 l. Rarely the quantity is normal, and with terminal nephritis and fever the amount may be subnormal.

The *specific gravity* commonly reaches 1.030-40, is occasionally much higher, in the earlier stages may be normal, and rarely is subnormal.

*Urea* is nearly always excreted in excessive amount by diabetics, ranging from 2 to 5 times the normal quantity. It is derived largely from the increased ingestion of food and partly from the destruction of nitrogenous tissues.

*Albumen* is frequently present in diabetic urine and is referable to the character of the food, to minor disturbances in the renal epithelium, and to chronic nephritis.

### *Tests for Glucose.*

Glucose reduces certain metallic oxides in alkaline solution, and on this character several qualitative tests are based.

In all tests for glucose the urine should be freed from albumen.

*Trommer's Test.*—Strongly alkalize 5 c.c. of urine with NaOH; add 5 per cent. $CuSo_4$, drop by drop, till it no longer dissolves; heat carefully, but do not boil. In the presence of glucose the blue $CuOH_2O$ changes through a yellow stage to the red $Cu_2O$.

Possible errors in this test arise from the presence of uric acid, kreatin, mucin, lactose, and bile pigment, or from the presence of many drugs, such as benzoic and salicylic acids, glycerine, chloral, sulphonal, all of which reduce the oxide only *if boiled.*

Traces of sugar may escape detection by Trommer's test.

*Fehling's Test.*—This is subject to the same limitations as is Trommer's test. Fehling's solution must be used *fresh,* being composed of equal parts of the following *stock* solutions:

(1.) 34.64 grams cryst. $CuSO_4$ dissolved in water and the solution brought to 500 c.c.

(2.) 173 grams Rochelle salt and 125 grams KOH dissolved in water and brought to 500 c.c.

These solutions, used also for the quantitative test, are of such strength that 10 c.c. of the mixed fluids (5 c.c. of each) are completely reduced by .05 gram of glucose.

The qualitative test is made by heating 5 c.c. of Fehling's solution, without boiling, and adding a drop or two of the suspected urine. A distinct yellow or reddish precipitate indicates the presence of glucose, but *flaky precipitates* and *simple discolorations must be ignored.*

*Phenyl-hydrazin Test.*—To 5 to 10 c.c. of urine two knife-points of *phenyl-hydrazin hydrochlorate* and three of sodium acetate are added, dissolved, and boiled for 5 to 20 minutes. On cooling a yellow crystalline deposit of phenyl-glucosazon results in the presence of minute traces of glucose. The characteristic appearance of these crystals and their formation in minute quantities should be determined by microscopical examination of the sediment.

The phenyl-hydrazin test is the most delicate and reliable of tests for glucose, the peculiar crystals not resulting from any urinary ingredients other than glucose, lactose, and maltose. The two last substances are rare and the form and melting-points of their crystalline products are different from those of glucose.

### *Quantitative Test for Glucose.*

*Fehling's Test.—Preparation of Fluid*—5 c.c. of each of the stock solutions previously described are placed in a 4 to 6 ounce beaker and diluted with about 40 c.c. of water. The beaker is then heated close to the boiling point, *but not boiled.* (boil gently)

*Preparation of Urine.*—The urine must be freed from albumen by boiling after slight acidification with acetic acid and filtering.

The ordinary diabetic urine (s. g. 1030-5) requires to be diluted with water in the proportion of 1 in 5.

A graduated burette is then filled with the diluted urine.

*Procedure.*—The urine is slowly added to the hot Fehling solution until all traces of blue color have disappeared. This point can be most accurately judged by filtering a small portion of the solution and examining the filtrate by transmitted light. When the last traces of blue color have disappeared the quantity of urine required is read from the burette.

Einhorn's fermentation tubes are best for quantitative test. Fill the tube with the urine and yeast and set aside for 24 hrs. This is best test for practitioner

If you add 30 cc. Aqua Ammonia to Fehling's, instead of water, there will be no danger of forming a yellow ppt., because the $NH_3$ keeps the Cu in sol.

*Computation.*—Fehling's solution is of such composition that 10 c.c. require for complete reduction .05 gram of glucose. It is customary to estimate the excretion of glucose in percentage, which may be computed by the following formula:

$$y : .05 :: 100 : x$$

in which y = number of c.c. of diluted urine employed;
x = grams of glucose per 100 c.c. of urine, i. e. the percentage. ∴

$$x = \frac{5}{y}$$

A final correction is to be made for the dilution of the urine.

*Hematuria.*—Blood *from the urethra* is usually passed in the intervals between micturition. In *hemorrhage from the bladder* the blood is often well mixed with the urine, but clots of considerable size, too large to have passed the ureters, are sometimes forced with strangury through the urethra. Profuse hemorrhage *from the kidney* may give rise to clots only on reaching the bladder. Usually renal hemorrhages are smaller in amount, the blood is well mixed with the urine, and the presence of blood-casts indicates a renal origin.

In the diagnosis of the origin of genito-urinary hemorrhage the principal evidence obtained from the urine concerns abnormal characters other than the hematuria.

The *microscopical examination* of the sediment is the most delicate test for the presence of blood in the urine, and in most specimens of fresh urine the blood cells are perfectly preserved.

*Hemoglobinuria* (or methemoglobinuria) may result from the solution of the blood of genito-urinary hemorrhages or from the rapid destruction of the circulating blood and imperfect elaboration of its pigment by the liver.

Hemoglobinuria resulting from the destruction of circulating blood is usually of *toxic* origin, being seen in *severe infectious diseases,* smallpox, scarlatina, yellow fever, pernicious malaria, *icterus neonatorum,* etc., and in *poisoning* by potassium chlorate, hydrochloric acid, carbolic acid, iodine, and carbon monoxide. There is a peculiar form of *paroxysmal* hemoglobinuria of unknown origin.

*Tests for Hemoglobinuria.*

*Heller's Test.*—5 c.c. of urine are boiled with KOH, precipitating the phosphates which, in the presence of blood, are colored red. With highly pigmented urines the precipitate may be filtered and dissolved in acetic acid when any reddish tinge is readily detected.

(2.) *Spectroscopic Test.—Browning's hand spectroscope* is a very convenient instrument for detecting hemoglobin in solution. About 10 c.c. of urine acidified with acetic acid and diluted, if very deeply colored, are examined in a test-tube with the spectroscope by means of a strong light.

Oxyhemoglobin gives two absorption bands between D and E. Reduced hemoglobin gives one band between D and E. Methemoglobin gives one band between E and F, one between C and D —i. e., in the orange near the red—and also two *faint* bands between D and E.

Not only the presence but the exact nature of dissolved blood-pigment may thus be determined. *Methemoglobin* is very much the commoner form in urine.

*Bilinuria.*—In many cases of obstruction to the flow of bile bilirubin appears in the urine before any other symptoms of jaundice are noted. Usually its presence may be suspected from the peculiar yellowish appearance of the urine or its foam, but the urobilin of highly colored urines may give a similar appearance, while traces of bilirubin may escape detection by the unaided eye.

*Smith's Test for Bilinuria.*—Over 10 c.c. of urine in a test-tube are carefully floated 2 to 3 c.c. of Gram's iodine solution diluted 1 to 10 with alcohol. In the presence of bilirubin a distinct *green ring* appears between the two layers of fluid owing to a change of bilirubin to biliverdin. This test is very delicate.

*Gmelin's Test.*—Smith's test is inapplicable with solutions of biliverdin which must be submitted to Gmelin's test as follows: On a piece of filter-paper soaked in the urine, or better, through which the urine has been filtered, is placed a drop of fuming nitric acid, when a play of colors is observed in the changing bile-pigment.

*Ehrlich's Diazo-reaction.*—This reaction depends upon the presence and peculiar properties of a chromogen occurring in the

Rapid & fatal phthisis cases give an Diazo reaction.

Pink foam at top of test-tube is characteristic

urine, most abundantly in typhoid fever and pulmonary tuberculosis.

Ehrlich finds that it never occurs in normal subjects and rarely in afebrile conditions. In *febrile diseases* it is either *absent* in the majority of cases, as in rheumatism and meningitis, or *occasionally present* in moderate intensity as in pneumonia, scarlet fever, diphtheria, and erysipelas, or almost *constantly present* as in typhoid fever, measles, and phthisis.

In *typhoid fever* the reaction may usually be obtained on the fifth or sixth day, continuing through the third week, when it usually disappears. It is absent in mild cases and rarely also in cases of average severity.

In *pulmonary tuberculosis* it is usually not obtainable till after the third week, when, in severe cases of unfavorable prognosis, it becomes persistent.

In the second or third week of an obscure fever, therefore, a distinct reaction may prove of considerable value in the diagnosis between typhoid fever and the diseases which simulate it.

The failure of the reaction in a severe continuous fever is even stronger evidence against its typhoid nature. In relapses of typhoid fever the reaction reappears, while in recrudescences of fever from pneumonia and suppurative complications it is usually absent.

*Method of Procedure* (Simon).

Two solutions are required.

(1.) HCl 50 c.c.
Aq. 950
Sulfanilic acid to saturation.
(2.) Sodium nitrite .5 per cent. solution.

To 40 c.c. of sulfanilic acid solution add 1 c.c. of sodium nitrite solution. The resulting nitrous acid acts upon sulfanilic acid, producing diazo-benzene-sulfonic acid, and is itself neutralized.

A few c.c. of urine in a test-tube are treated witth an equal quantity of the above *mixed* solution of sulfanilic acid and sodium nitrite, and thoroughly shaken. Two c.c. of ammonia are then floated over the mixture. At the junction of the layers a *deep carmine color* appears when the reaction is distinct.

Some experience is necessary before one can detect the characteristic color of this reaction.

### *Albuminuria.*

Serum-albumen, serum-globulin, albumose (or peptone), nucleo-albumen, and mucin are varieties of albumen found in the urine, the presence of each of which is of definite pathological significance.

### *Chemical Properties.*

*Xantho-proteic Reaction.*—Serum-albumen, globulin, albumose, and vegetable albumens are proteids which respond to the xantho-proteic test. To the solution of albumen add a few drops of nitric acid and boil, when the solution turns yellow. Add ammonia and the solution turns orange.

Nucleo-albumen and mucin are albuminoids and do not give the xantho-proteic reaction.

*Serum-albumen is soluble* in water, in dilute saline solutions, and in saturated solutions of sodium chloride and *magnesium sulphate.*

It is *precipitated* by boiling, by nitric acid, and by potassium ferrocyanide.

*Serum-globulin is insoluble* in water and in saturated solutions of sodium chloride and *magnesium sulphate,* but is soluble in dilute saline solutions.

It is *precipitated* by heat, by nitric acid, and by potassium ferrocyanide.

*Albumoses* (peptone) are the products of the hydration of proteids. They are soluble in water. They are *not precipitated* by heat, or nitric acid, or potassium ferrocyanide, but may be precipitated by phosphotungstic acid, and they give the *biuret reaction.*

*Mucin* is an albuminoid substance which contains no phosphorus and which when heated with hydrochloric acid yields a substance which reduces copper oxides.

It is soluble in dilute alkalis and is precipitated by acetic acid.

*Nucleo-albumen* is an albuminoid substance resembling mucin,

It is thought that in all cases of functional albuminuria, there must be some antecedent lesion of kidney.

in physical properties, but contains phosphorus, and when heated with hydrochloric acid does not yield a substance reducing copper oxides. It is soluble in dilute alkalis and is precipitated by acetic acid.

### *Significance of Albuminuria.*

*Serum-albumen* is always derived directly or indirectly from the blood, passing readily through animal membranes and vessel walls, and its presence in the urine is always pathological.

*Functional albuminuria,* in the absence of any demonstrated renal lesion, occurs in a great variety of cases. It may be seen after (1) severe muscular *exercise* or mental emotion; (2) a *dietetic albuminuria* has been observed after the injection of excessive amounts of albumen, such as raw eggs; (3) a *cyclical albuminuria* is sometimes seen in patients giving no other symptoms of nephritis, or (4) a *simple* persistent or intermittent *functional albuminuria* may occur in subjects without distinct evidence of nephritis and without known etiology.

It is characteristic of functional albuminuria that serum-albumen is largely or exclusively excreted and only in small amounts.

### *Albuminuria Referable to Diseased Conditions of the Kidneys.*

Most forms of albuminuria are referable to a renal lesion which, however, is not always demonstrable after death.

In all forms of *acute* and *chronic nephritis* albumen may be excreted in considerable quantity, depending upon the severity of an exudative process, the venous congestion, the state of the blood, and the condition of the capillary walls and renal epithelium.

In the more *acute lesions* the percentage of serum-globulin is high and variable, while in the *chronic cases* the percentage of globulin is lower and uniform.

The *quantity of albumen* excreted in acute nephritis is generally proportional to the severity of the disease, varying usually from 5 to 8 grams daily, but being at times much greater.

In chronic productive nephritis the albumen is usually low (2 to 5 grams) and *may long be absent* (contracted kidney), but

when exudation occurs in severe exacerbations very large amounts (30 grams) may be observed.

In most *febrile diseases* transient or continuous albuminuria may be observed, and is here referable to an acute congestion and degeneration of the renal epithelium or to an exudative nephritis.

In typhoid fever, pneumonia, meningitis, ulcerative endocarditis, scarlatina, diphtheria, and smallpox a trace of albumen is usually found on boiling and peptone may frequently be demonstrated.

In *yellow fever* albumen usually appears in the urine within twenty-four hours after the onset of the disease.

In *pernicious malaria* it may occur, but usually appears much later.

In cases of *irritant poisoning* albuminuria is nearly constant.

### *Albuminuria from Lesions of the Genito-Urinary Passages.*

Albumen may be added to the urine in the absence of renal disease from lesions in the urinary passages.

In cases of *hematuria,* hemoglobin may be detected and the globulin ratio is high.

*Pyelitis* is usually associated with exudative nephritis, but in the early stages most of the albumen in the urine of this disease may be derived from the pus and blood exuded from the pelvic mucosa.

In *cystitis* the albuminuria is usually proportional to the quantity of pus and blood in the sediment.

### *Globulinuria.*

Globulin is associated *in the blood* with serum-albumen in the proportion of about 1 of globulin to 1.5 of albumen, but varies greatly in health and disease. An important property of globulin is its relatively slow diffusibility through animal membranes. Serum-albumen, on the other hand, diffuses readily through such membranes. The passage of globulin into the urine, therefore,

requires an active exudative process with destruction or serious alteration of epithelial and endothelial cells.

In *acute nephritis* the globulin ratio is high, varies much, and diminishes with the general improvement.

In *chronic nephritis* a steady and rather low ratio of globulin indicates an established but slowly progressive lesion (1 :10, 1 :20).

High globulin ratios are often seen with *waxy changes* in the kidneys (1 : .8+).

In *transitory albuminuria* without nephritis globulin is usually absent or occurs in traces only.

### *Albumosuria (Peptonuria).*

True peptones, representing the end product in the hydration (digestion) of proteids, are not precipitated by saturation with ammonium sulphate and do not occur in the urine. Various intermediate forms of hydrated proteids, precipitated by saturation with ammonium sulphate and called *albumoses,* are found in the urine in many pathological conditions.

Albumosuria is most marked and constant when there is an *accumulation, with more or less absorption, of pus* in the body (*pyogenic albumosuria*), as in empyema, cellulitis, suppurative meningitis, resolving pneumonia, suppurating cavities in phthisis.

*Enteric albumosuria* is usually marked and constant in ulcerative lesions of the intestine, as in typhoid fever, tuberculosis, dysentery, and carcinoma.

Albumosuria of supposed *hematogenic origin* occurs in scurvy, pernicious anæmia, leukemia, diphtheria, the exanthemata, acute yellow atrophy, pregnancy, and various nervous diseases.

The detection of albumose in the urine may thus prove of great value in the diagnosis between:

(1.) Suppurative and non-suppurative, especially simple tuberculous, lesions.

(2.) Typhoid and other ulcerative intestinal lesions, from catarrhal conditions.

(3.) Exanthemata, diphtheria, etc., and simple fevers.

*Nucleo-albuminuria and mucinuria* occur principally in dis-

cases in which there is destruction of the epithelium of the renal tubules and bladder.

In acute nephritis they are frequently demonstrable, but rarely in chronic nephritis. In cystitis they are very commonly seen and in jaundice and leukemia almost constantly. Simon has found nucleo-albumen in many cases of functional albuminuria and occasionally when other forms of albumen were absent.

## *Tests for Albumens.*

(1.) *Boiling with Acetic Acid.*—Two inches of urine in a narrow test-tube are acidified with a few drops of dilute (2 per cent.) acetic acid, and the upper portion is boiled. The presence of albumen is indicated by a faint cloud or abundant precipitate.

This test is very delicate and is the best method for routine clinical work.

It is subject to several sources of error.

(a) It is not reliable in strongly alkaline urine in which the *alkali-albumen* is not always precipitated by heat.

(b) By an excess of acid, soluble *acid albumen* may be produced and fail to coagulate by heat.

(c) Mucin and nucleo-albumen are precipitated by acetic acid, but after shaking, this turbidity is general and not limited to the boiled layer.

(d) Phosphates may precipitate on boiling. They promptly dissolve on the addition of one drop of $HNO_3$, which increases an albuminous turbidity.

(2) *Nitric Acid Test.*—In a conical glass place 1 c.c. of strong $HNO_3$ and float above it 10 c.c. of urine. The presence of albumen is indicated by a white ring between the acid and the urine. This test is fairly delicate and very reliable.

Heller

Turpentine, tolu, copaiba, and cubebs give a turbidity simulating albumen.

A play of colors, yellow, green, red, etc., indicates the presence of bile (Gmelin's test). A single light red or brick red band results from the presence of *normal urobilin*, while a mahogany red is produced from *pathological urobilin*. The presence of *indi-*

When patient has been taking Copaiba, his urine will give this reaction.

Roberts' test consists of strong 5% nitric acid with sat. sol. magnesium sulphate.

Spiegler's Reaction

8 Hg Cl$_2$
20 glycerin
4 Tartaric acid
200 aqua

Acidulate the urine in this test & see that the reagent contains an excess of tartaric acid - A mug is formed in presence of albumen. This is the most delicate test.

Sulpho-Salicylic test is used also

*can* is shown by a light or deep blue or violet ring above the ordinary color-ring.

The presence of *uric acid* is indicated by the appearance of a distinct whitish ring in the clear supernatant urine. If uric acid is present in normal *proportion* the ring appears in 10 minutes and is of slight breadth (1 to 2 mm.). An excessive *proportion* gives a ring in 5 minutes which soon becomes much broader, while with scanty uric acid the ring may fail.

(3.) *Potassium Ferrocyanide Test.*—Strongly acidify with acetic acid and add a few drops of a 10 per cent. solution of $K_2FeCy_6$.

A white ring, cloud, or precipitate, indicates the presence of albumen. This test is very delicate and reliable.

(4.) *Trichloracetic Acid Test.*—One to two cubic centimetres of an aqueous solution 33 per cent. of trichloracetic acid are placed in a test-tube and above it are floated 10 c.c. of clear urine. The presence of albumen is indicated by a white ring between the fluids.

This test is the most delicate and reliable yet devised for the demonstration of albumen, and should be used in all doubtful cases.

The last three of the above tests precipitate serum-albumen, globulin, and albumose, but the albumose is readily redissolved by heating.

### *Quantitative Estimation of Total Albumen.*

*Esbach's method* is most applicable for clinical purposes.

Esbach's reagent consists of

| | |
|---|---|
| Picric acid | 10 grams |
| Citric acid | 20 grams |
| Aq. dest. | to 1,000 |

Special test-tubes are employed in this method, which are to be filled with urine up to the mark U, then with the reagent to the mark R, thoroughly agitated, and after 24 hours the grams of solid albumen per litre of urine are read off from the depth of the sediment at the bottom of the tube which is graduated for this purpose.

The precipitate consists of all forms of albumen, uric acid, and kreatinin. The urine must be rendered acid by acetic acid.

Highly albuminous urines should be diluted to a specific gravity of 1.005 to 1.010, and the reading is most accurate at a temperature of 60° F.

### *Separation of Serum-Albumen and Serum-Globulin.*

The urine is rendered alkaline by NaOH and after standing a few minutes any precipitate of phosphates is filtered off.

The filtrate is then saturated with magnesium sulphate, adding an excess of the crystalline salt with thorough shaking.

At the end of 10 to 20 minutes the serum-globulin is fully precipitated. The filtrate may then be tested for serum-albumen by $HNO_3$ or Esbach's reagent, and the tests may be conveniently performed in the Esbach tube.

### *Test for Albumose* (Salkowski-Hofmeister).

Albumen and globulin must be removed by boiling with acetic acid and filtering. Fifty c.c. of the filtered urine in a beaker are acidified with 5 c.c. HCl and the albumoses, etc., precipitated with 2 to 3 c.c. of 10 per cent. phosphotungstic acid. The beaker should be *very gently heated* so that the precipitate may fall in a *coherent resinous mass.*

When the precipitate is complete and the mass brownish gray the supernatant fluid is carefully decanted and the mass washed twice with distilled water.

The mass is now dissolved by heating and the addition, drop by drop, of NaOH (30 per cent. sol.). When fully dissolved the fluid, if of a bluish color, should be boiled with the addition of more alkali until it becomes colorless.

The clear fluid now contains albumose in solution and readily yields the *biuret reaction* as follows:

Pour the fluid into a narrow test-tube and after cooling add one drop of a very dilute (1 per cent.) solution of cupric sulphate. An amethyst-red color of variable depth indicates the presence and quantity of albumose.

Peptones practically never appear in urine
Albumose appears in urine in ulceration of intestine.

With a little practice this test occupies 3 to 5 minutes and readily demonstrates .015 gram of albumoses per 100 c.c.

When urine is very rich in urobilin 10 c.c. only should be used, as such urines in larger quantity may give the biuret test in the absence of albumose.

### *Test for Nucleo-Albumen and Mucin.*

The urine, freed from albumen by boiling, is treated with concentrated acetic acid, added drop by drop. When uric acid is abundant it may precipitate in the above procedure, but may be dissolved by gentle heat, or the urine may previously be diluted 1 to 3 with water.

### *Urinary Sediments.*

*Gross Examination.*—(1.) *In acid urines* a reddish precipitate forming early is almost always composed of *uric acid or urates.* A precipitate of urates may be whitish, but usually has the tinge of brickdust.

These sediments are rapidly cleared up by heating and by alkalis.

(2.) *In alkaline urines* a whitish precipitate, present on passing and settling immediately, is often seen in cystitis and is composed of pus, epithelial cells, bacteria, and phosphates.

After a few hours, or sooner, a precipitate of granular *phosphates* and carbonates usually occurs, dissolving in $HNO_3$. When a urine originally acid and rich in uric acid ferments, *alkaline urates* are deposited in abundance, but dissolve in $HNO_3$.

In *stale* urines a considerable variety of precipitates form which resist ordinary solvents and are difficult of identification.

(3.) *Persistent turbidities* are often seen in pyelitis and cystitis and are composed of *pus, epithelial cells, and bacteria.*

Fermenting urine usually shows a persistent turbidity of moderate grade, due to *bacteria.* It may be cleared by alum.

In acute nephritis *casts* may be so abundant as to give a persistent turbidity, partly clearing as the casts slowly settle.

In *chylous urine* the persistent turbidity results from the *emulsion of fat.* It may be largely removed by extraction with ether.

## MICROSCOPICAL EXAMINATION OF URINE.

### *Crystals.*

(1.) *Acid Urine.—Uric acid* usually crystallizes in the well-known whetstone form, the crystals occurring either singly or in radiating groups. They are often found in rosettes composed of longer narrow slabs with rounded ends. When thrown down by the addition of acid large flat, ragged plates may be produced.

The dumb-bell crystals of uric acid are larger than those of calcium oxalate.

Crystals of uric acid are nearly always stained dark red by uroerythrin, but small rhombic plates with rounded edges, devoid of color, are sometimes seen.

Uric acid crystals dissolve readily on heating and by the addition of alkalis. The muxexide test is characteristic. (See uric acid.)

*Urates* are often abundantly precipitated in acid urines, usually in *granular form* and almost always of the color of brickdust. They rapidly dissolve on the addition of acids or by heat.

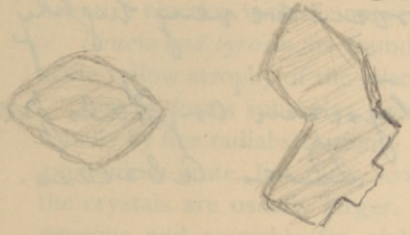
*Calcium oxalate* crystallizes in characteristic diamond-shaped *octahedra.*

One or two opposite *axes* may be *elongated. Dumb-bell* crystals may sometimes be found usually associated with or adherent to casts. These crystals differ from the dumb-bell crystals of uric acid in being radially striated, shorter and thicker, and lacking the red stain of uric acid crystals. Simon speaks of highly refractive, more or less angular bodies occurring in urines rich in oxalates, which he is inclined to regard as an *amorphous* variety.

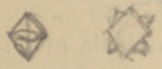
*Phosphates.*—Ammonia-magnesium or triple phosphates rather frequently crystallize in feebly acid urines. Their usual form is that of a large rhombic prism or "coffin-lid." Very small crystals of this type may resemble the octahedra of calcium oxalate, but dissolve with acetic acid. Other types are rare in acid urine.

*Hippuric acid* crystals are occasionally seen in the acid urine of fevers, diabetes, after ingestion of cranberries, or the administration of benzoic and salicylic acids. It forms transparent colorless four-sided prisms or finer needles. These are often massed

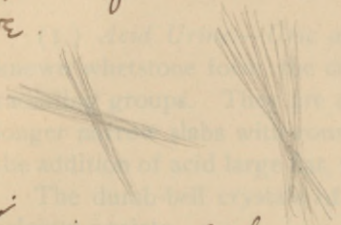
Uric Acid Crystals



Calcium Oxalate Crystals

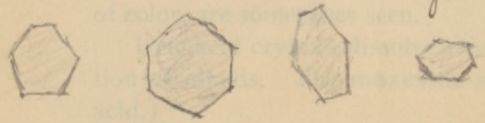


Crystals of Leucin & Tyrosin are very highly refractive.



Tyrosin crystals from Liver Abscess.

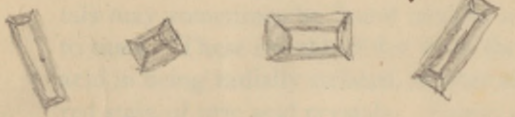
Cystin is a sulphur compound. We do not know what it indicates. Cystin calculi are seen.



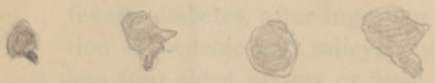
Cystin Crystals.

Oxalates are very hard to find in alkaline urine.

Triple Phosphate Crystals



Ammonium Urate Crystals.



in radiating bundles. Some of the thicker prisms closely resemble triple phosphate crystals, but are insoluble in HCl, and other forms may be distinguished from uric acid by their lack of color and negative reaction with the muxexide test.

*Leucin and tyrosin* are found almost exclusively in the urine of acute yellow atrophy of the liver. ~~+~~ phosphorus poisoning.

Leucin forms spherules, marked sometimes by concentric and usually by fine radial striations. These have the brownish color of ammonium-urate, but their striation is much more delicate and the crystals are usually larger. Some leucin crystals are homogeneous and resemble fat globules, but are insoluble in ether. Tyrosin forms fine, long needles, often grouped in sheaves like ammonium urate, but the needles are finer and longer.

*Cystin,* rarely seen in urinary sediments, crystallizes in colorless hexagonal plates, which differ from uric acid in dissolving in HCl.

*Bilirubin and Hematoidin* form coarse, irregular, reddish-brown rhombic prisms, or, rarely, needles, which are soluble in strong NaOH, and exhibit a bluish or greenish rim when treated with $HNO_3$.

(2.) *Alkaline Urine.*—The bulk of most mineral precipitates in alkaline urine is composed of triple and granular phosphates and ammonium urates. They readily dissolve with acetic acid.

*Triple phosphates,* in addition to the usual "coffin-lid" prisms, when abundantly precipitated in alkaline urines, form a variety of star-shaped, "snow-flake" crystals, with four, five, or more arms, and feathery borders.

*Calcium phosphates,* often numerous in alkaline, and rarer in feebly acid urine, form a variety of colorless acicular crystals, usually grouped in radiating masses.

*Basic magnesium* phosphate forms large colorless, refractive plates with scalloped edges.

The *granular phosphates* are basic salts of calcium and magnesium.

*Ammonium urate* in granular or crystalline form is precipitated in urines undergoing ammoniacal fermentation. Two principal varieties of these crystals are seen: (1) Spheres with granular surface, often beset with one or more prominent spicules; (2) sheaves of rather short, coarse needles.

In any form their prominent character is their *opacity* and dark *yellowish color.*

They readily dissolve in acetic acid.

*Calcium carbonate* appears in the sediment of fermenting urine in the form of coarse granules, sometimes approaching a minute dumb-bell in shape. They dissolve in acetic acid with the evolution of gas.

## *Organized Sediment.*

### *Casts.*

*Casts* are cylindrical masses of coagulated albuminoid matter or cellular detritus formed in the renal tubules, and often discharged in the urine.

They usually consist of a *matrix* composed of an albuminous substance, not fibrin, but closely related to it, and of a variety of *adherent cellular detritus.* They are *formed* in nearly all parts of the uriniferous tubules, but probably only those reach the urine which lie below the constricted portion of Henle's loop.

*Hyaline casts* are composed of an albuminoid matrix, which usually entangles a few fine granules. They are very slightly refractive, as viewed in urine, their edges are faint, their ends are rounded, and their surface mostly homogeneous. Occasionally there is a curled thread attached to one end. They are soluble in weak mineral acids.

*Significance.*—Hyaline casts probably always require for their formation an exudative or catarrhal process in the renal tubules, but this may be very slight and transitory, and if, as is probable, their formation is accompanied by a functional albuminuria, the discharge of albumen is often too scanty or transient for demonstration. They are even less significant of renal disease than is transient albuminuria, especially since their discharge may occur long after formation.

They are seen in a large percentage of all urines when carefully centrifuged. In the urine of old subjects, and in nearly all chronic diseases, their presence, *in small numbers,* is nearly constant.

In *chronic nephritis* they nearly always are found, but here they occur in larger numbers.

Stephen Crane.

Waxy casts may appear without waxy degeneration of the kidneys. They indicate a severe nephritis.

Waxy Casts.

Hyaline casts with a deposit of urates at the ends must not be mistaken for granular casts.

Granular Casts

Sometimes hyaline casts are drawn out into long cylindrical slightly twisted masses, often ending in more delicate threads, which are called *cylindroids*. These have the same significance as hyaline casts. They are to be carefully distinguished from mucous threads, which may have very much the same appearance, but are usually very irregular in thickness and are insoluble in acids.

*Waxy casts* resemble hyaline casts in being homogeneous, but they are usually broader, their ends are sharply cut off or fractured, they are usually more opaque and refractive than hyaline casts, and they turn mahogany brown on addition of tincture of iodine. They are found only in chronic nephritis with waxy degeneration.

*Granular casts* of two distinct varieties are to be differentiated in the urine:

(1.) Finely granular casts composed of a hyaline matrix beset with few or many fine granules are very commonly seen, and their significance is little more than that of hyaline casts. They are most abundant in the quiescent stages of chronic nephritis, but may be found wherever purely hyaline casts occur.

(2.) Coarsely granular casts composed of coarse, yellowish, opaque granules, and little or no matrix, are most abundant in the urine of acute renal lesions. In some cases of severe pneumonia with nephritis, and in some exacerbations of chronic nephritis, these casts occur in great abundance. They indicate an active degenerative process in the renal epithelium. It cannot be denied that imperfect examples are occasionally seen where their significance can be little more than that of the finely granular variety.

*Epithelial casts* are cylindrical masses composed of renal epithelial cells, whose borders, nuclei, and granules are partially or completely preserved. They are usually of larger size than other casts and are often much contorted.

They may have a hyaline matrix, and occasionally a hyaline cast may be seen with one or two epithelial cells attached.

There are all intermediate stages between a coarsely granular and an epithelial cast, but when masses of distinct epithelial cells are discharged it indicates an *active desquamative* process in the renal tubules.

Epithelial casts are pathognomonic of acute nephritis or of an acute exacerbation of chronic nephritis.

*Pus Casts.*—An occasional leucocyte is sometimes seen attached to a cast of another variety, but when leucocytes with or without hyaline or granular matrix compose the bulk of the cylinder it forms a true pus cast. The leucocytes are smaller than epithelial cells, rounded, and fully distinguishable in most instances by their fine opaque neutrophile granules. Pus casts indicate an acute lesion with the exudation of leucocytes, and are principally seen in severe acute and suppurative nephritis.

*Blood Casts.*—A few red blood cells are often found attached to casts, and occasionally casts composed largely of red blood cells, with or without other matrix, are encountered. They are seen in acute exudative nephritis, after the hemorrhages of acute or chronic nephritis, and rarely in renal hemorrhages without nephritis.

*Cylindrical masses of urates* resembling granular casts are sometimes seen in urine rich in urates. These granules are perfectly opaque and black, and dissolve on heating.

*Cylindrical masses of bacteria*, evidently compressed in growth by the renal tubules, are sometimes discharged in the urine of suppurative nephritis, usually from ascending infection.

### *General Significance of Casts in Nephritis.*

The presence of *a few hyaline or finely granular casts* is of little import in the diagnosis of nephritis. When, however, these varieties of casts become abundant and constant they strongly indicate the existence of chronic nephritis.

The presence of a single epithelial or pus cast, or of a typical coarsely granular cast, assures the diagnosis of an organic renal lesion.

Casts, as well as albumen, may be temporarily absent from the urine in established cases of nephritis. This absence is especially noted in advanced cases of chronic diffuse nephritis without exudation.

In all cases it is essential in judging of the significance of casts to consider the other characters of the urine, especially the specific gravity and excretion of urea.

### *The Examination of Urine for Casts.*

The urine should be as fresh as possible, as casts are dissolved by acids and fermentative processes. The urine should be allowed to settle a few hours in a conical glass, and, if possible, a scanty sediment should be further concentrated in the centrifuge.

A few drops of the sediment should be examined on a glass slide, without covering. The cover glass frequently crushes casts beyond recognition.

Leitz eyepiece No. 2, and objectives 3 and 5, are the only magnifications suitable for this purpose. All casts may be seen with this lower power, and may be more minutely examined by the higher power. A *flood of light obliterates hyaline casts*, and students are especially recommended to secure a *moderate illumination* for the present purpose.

*Casts may be preserved*, best in concentrated sediment, by the addition of 5 per cent. formalin, or 1 per cent. of chloroform.

### *Special Genito-Urinary Diseases.*

*Urethritis.*—A common expedient for distinguishing urethritis from cystitis is to have the patient pass urine in two portions. *The first portion* flushes out the urethra and contains pus and gonorrhœal threads. *The second portion*, in the absence of cystitis, is clear. The threads may then be examined for gonococci.

In the absence of complications the urine in urethritis exhibits no other abnormalities.

*Cystitis.*—In acute cystitis the urine usually undergoes ammoniacal fermentation in the bladder, and on passage it is alkaline in reaction, shows a persistent turbidity, due to bacteria, pus, and epithelium. Mucus, often in the form of threads, may be abundant. Flat epithelial cells and leucocytes are usually much more abundant than with pyelitis. The pus usually settles rapidly, but incompletely. Many red blood cells or clots of moderate size may be found.

*In chronic cystitis* the reaction varies, being usually alkaline with exacerbations, while at other times it may be acid. Pus and

mucus are present as in acute cystitis. When ulceration occurs clumps of mucus, pus, and blood may indicate its effects.

In acute or chronic cystitis the masses or threads of mucus are very apt to contain specific bacteria, such as the gonococcus or tubercle bacillus, and should be selected in the examination for this purpose. The centrifuge may greatly assist in the search for these germs, especially in the absence of distinct masses of mucus.

When the morphological examination of the sediment for the gonococcus is negative, in a large proportion of chronic cases of gonorrhœal origin a successful result may be obtained by planting the sediment or threads in chest-serum-agar.

Particular attention may here be called to the great danger of mistaking, especially in females, the smegma bacillus for the tubercle bacillus. (See Sputum.)

*Pyelitis.*—The characters of the urine in pyelitis vary considerably.

Usually the reaction is acid, unless there is a complicating cystitis. The pus is well mixed with the urine, is usually less abundant than in cystitis, settles more rapidly and completely, often leaving the supernatant urine entirely clear, and is often found in the form of little clumps of 10 to 12 leucocytes. Masses and threads of mucus are less abundant and of smaller size than in cystitis.

The stratified epithelial layers of the pelvis contain *long cylindrical epithelium,* which are not found in the epithelial lining of the bladder. If such cells are found in the urinary sediment the diagnosis of pyelitis may be justified; but in the writer's experience they are of infrequent occurrence in the urine of pyelitis. These cells are 4 to 5 times the diameter of a leucocyte, while the longest cylindrical epithelium from the bladder rarely exceeds 2 to 3 times the diameter of a leucocyte.

Pyelitis is usually associated with more or less nephritis, and the urine then contains albumen and casts. The albuminuria of cystitis and simple pyelitis is slight and proportionate to the pyuria.

Specific bacteria may usually be demonstrated in the masses of mucus and pus, or sediment, from the urine.

### *Epithelial Cells.*

*Flat epithelium* from the bladder or vagina may be found in the sediment of most normal urines, but occurs in great abundance in cystitis and vaginitis.

*Cuboidal and short columnar epithelium* is found abundantly with acute pyelitis and cystitis, especially with the latter disease.

*Very long columnar epithelium* appears to be derived exclusively from the renal pelvis.

The epithelium from the adult *seminal vesicles* is almost always pigmented and columnar.

*Renal epithelium* is of comparatively small size, cuboidal, granular, and with rather faint, delicate borders.

*Spermatorrhœa, Etc.*—Spermatozoa may be found in normal urine in certain physiological conditions, and in chronic constipation, after convulsive seizures, severe traumatism, and in many specimens of post-mortem urine.

A persistent spermatorrhœa occurs in some cases of cystitis, in chronic prostatitis, in vesiculitis, and in sexual neuroses. In chronic spermatorrhœa, especially with prostatitis, the urine commonly shows a deposit of phosphates, in which are somtimes seen crystals of *spermin*, which resemble long and irregular crystals of triple phosphate.

### *Bacteriuria.*

The normal bladder and its contents are free from bacteria, but during micturition, especially in the female, a few micro-organisms may be added to the urine from the urethra or vagina. The presence of any considerable number of bacteria in fresh urine is always pathological. Urine submitted to bacteriological examination for the purpose of ascertaining its condition in the bladder should be the last portions passed through a sterile catheter which has been introduced under aseptic precautions.

### *Non-pathogenic Micro-organisms.*

*Moulds* and *yeast* grow abundantly in diabetic urine, and infrequently in other specimens.

*Micrococcus ureæ* and a *group of bacilli* have been isolated

from urine undergoing ammoniacal fermentation as a result of a ferment elaborated by these germs. They may be found in ammoniacal urine on its passage, and regularly develop in most urines on standing. They are usually carried into the bladder on dirty catheters.

The *smegma bacillus* is often encountered, sometimes in large numbers, during the examination of urinary sediment for the tubercle bacillus. The importance of using a differential staining method to distinguish this germ from the tubercle bacillus, especially in the female, has been mentioned.

### *Pathogenic Micro-organisms.*

In *cystitis* the gonococcus, tubercle bacillus, pyogenic cocci, and the colon bacillus may be isolated.

In *pyelonephritis* the colon bacillus, *proteus* (Hauseri), *staphylococcus pyogenes,* and tubercle bacillus may be found.

In *suppurative nephritis, staphylococcus pyogenes, streptococcus pyogenes,* the colon, and the tubercle bacillus, have been isolated as the specific germ of the disease.

In *many infectious diseases* the specific germ of the disease has been found in the urine, having been excreted by the kidneys. Such diseases are, especially, typhoid fever, diphtheria, pyæmia, septicæmia, erysipelas, and tuberculosis.

In *pulmonary tuberculosis,* tubercle bacilli may pass into the urine in small numbers in the absence of tuberculous lesions of the genito-urinary organs.

### *Filaria Sanguinis. Chyluria.*

The embryos of *filaria sanguinis* are found in the urine in most cases of chyluria from filariasis. They are identical in appearance with the embryos in the blood, but are usually dead when found, and few in number.

*Chylous urine,* which is usually an intermittent or continuous symptom of filariasis, is of pale milky appearance, owing to the presence of very fine emulsion of fat. These subdivided globules are very minute and are readily overlooked on microscopical examination. The fat may be demonstrated by extraction with ether. Hematuria occasionally accompanies chyluria.

F. Nocturna only come out in blood at night.
Mode of infection is through mosquito drinking water.
Filaria are found in lymph.
Chyluria is due to blocking of lymphatics in bladder wall.

# BLOOD.

## CHEMISTRY.

In a very limited field the knowledge of the chemistry of the blood is available in clinical diagnosis.

REACTION.—The blood is alkaline in reaction owing to the presence of disodium phosphate and sodium bicarbonate. The alkalinity is reduced in all forms of anæmia excepting chlorosis. The alkalinity of the blood and its bactericidal power is progressively diminished during the course of infectious diseases. The alkaline reaction of the blood may be demonstrated by soaking a piece of litmus-paper in fresh blood and washing it in salt solution. The present methods of measuring the alkalinity of the blood are too complicated for routine application.

SPECIFIC GRAVITY.—Normal blood has an average gravity in the male of 1.055-60, being slightly lower in the female (1.054), and in children 1.050. In new-born infants the gravity is high. The normal limits may be placed between 1.046 to 1.067.

There is a moderate *physiological* diminution of the gravity from fasting, ingestion of solid and fluid food, muscular exercise, pregnancy, and in arterial as compared with venous blood.

*In disease* the specific gravity is usually diminished in proportion to the anæmia, but in febrile conditions the gravity may be increased, as also in general venous congestion with cyanosis, or in jaundice.

Pathological variations in specific gravity depend principally upon changes in the hemoglobin of the red cells and to a lesser degree upon changes in the albumens of the plasma.

It has been found, therefore, that the specific gravity of the blood *in the absence of hydræmic conditions* is an accurate measure of the content in hemoglobin, and some prefer to estimate the percentage of HB from the specific gravity rather than by the more

direct colorimetric methods. For this purpose reference may be made to tables showing the usual relation between specific gravity and percentage of Hb. There are, however, both theoretical and practical objections to this custom.

*Hammerschlag's method of estimating the specific gravity of the blood* is to be recommended for clinical purposes:

A small urinometer of suitable dimensions is partly filled with a mixture of chloroform (s. g. 1.526) and benzine (s. g. .889) of a specific gravity (usually 1.060) slightly higher than that of the specimen of blood, and in which blood is insoluble.

By means of a pipette a drop of blood, expressed from the finger-tip with the usual precautions, is transferred to the fluid. The red cell mixer of Thoma may be used for this purpose. In expelling the blood, the tip of the pipette should be submerged in the fluid and no air should be allowed to pass out with the blood. The drop should not be very minute in size, and should best float on the fluid. If it is allowed to sink to the bottom it will probably be lost by spreading out on the bottom of the vessel. By adding chloroform or benzine, drop by drop as required, and carefully stirring, a mixture is secured in which the drop of blood neither rises nor sinks, but which is of exactly the same density as the blood. The specific gravity of the mixture and of the blood may then be taken as with urine.

The aerometer should be minutely graduated and tested in distilled water at 60° F. All parts of the apparatus should be clean and dry. The chloroform mixture may be filtered and used repeatedly.

Color.—The *color* of the blood is that of its iron-holding albumen, hemoglobin, which is of *dark red* tinge in the veins (reduced Hb) and *bright red* in the arteries (oxyhemoglobin). The loss of Hb in anæmia is often plainly indicated in the *pale red* color of the drop. A faint *milky appearance* may be noted in advanced cases of leukemia. A bright *cherry-red* color is characteristic of the blood in cases of poisoning by CO (carbonic-oxide Hb). A *brownish chocolate color* results from the action of certain blood-poisons, potassium chlorate, hydrocyanic acid, anilin derivatives, snake poisons, etc. (methemoglobin). In pernicious malaria the blood may, in advanced cases, be of a *brownish* color from the presence of much *pigment*.

## *Hemoglobin* (Hb).

Hemoglobin is an albuminous body rich in an iron-pigment (albumen 96 per cent., hemochromogen 4 per cent.), which constitutes the bulk of the red cell, being held in semifluid form in the stroma of the cell. Its important physiological property is a capacity to absorb oxygen (1.16 c.m. of $O_2$ per 1 gram), and under other conditions to yield it up again. Simple Hb (reduced Hb) is dark red in color, but when saturated with oxygen (oxyhemoglobin) is bright red. The spectra of the two substances are different (as noted later).

Hb constitutes from 10 to 14 per cent. of the weight of normal blood, and this amount, when judged by colorimetric methods, is called 100 per cent.—i. e. a normal quantity of Hb. The Hb *index*—i. e. the relation of the Hb-content of the blood to the number of the red cells—is said to be normal (1) when 100 per cent. of Hb is found with 5,000,000 red cells.

The percentage of Hb is slightly higher in the male than in the female or child. High percentages are seen in the blood of young infants.

*Physiological variations* are considerable, many subjects, especially females, never registering above 85 per cent., while others, usually males, may reach 110 per cent. The effects of diet and exercise are slight and transient.

*In disease* a reduction in Hb is the essential lesion of anæmia, and is noted in nearly all acute and chronic diseases.

In chlorosis the loss of Hb is much greater than the reduction of red cells, and the Hb *index* is less than 1. In secondary anæmias the loss of Hb may be more nearly proportionate to the reduction in red cells, and the Hb *index* may be normal or slightly reduced. In some forms of pernicious anæmia the Hb *index* may be higher than normal owing to the increased quantity of Hb in the large red cells.

The percentage of Hb follows the bulk rather than the number of red cells.

### *Qualitative Tests for* Hb.

(1.) *Teichman's Test.*—This test may be applied to fresh blood or to old blood stains. It is based upon the production of

characteristic crystals of *hæmin*, a derivative of Hb, and it furnishes evidence of medico-legal value. The procedure is as follows:

Evaporate on a glass slide a drop of solution of NaCl containing the fresh blood or particles of the clot, and cover with a cover-glass.

Fill the space beneath the cover-glass with glacial acetic acid and heat until bubbles appear.

Add acetic acid and heat till a brown color appears.

Evaporate slowly to dryness, and examine microscopically after the addition of glycerine.

Traces of Hb have now been converted into characteristic rhombic prisms of *hæmin*.

(2.) *Spectroscopic Test.*—The fresh blood or clot is dissolved in distilled water, thereby securing a dilute solution of the blood pigment (best .5 per cent.). This is placed in a small flask with flat sides and examined with *Browning's spectroscope.*

*Reduced* Hb gives one broad absorption band between D and E, while one-half of the blue and indigo and part of the red are absorbed.

*Oxyhemoglobin* gives two bands between D and E, while the blue and indigo are completely absorbed.

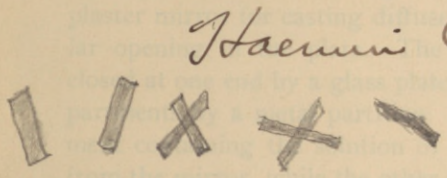
The essential part of this test for *medico-legal purposes* consists in the ready transformation of the spectrum of oxyhemoglobin into that of reduced Hb by the addition of ammonium sulphide.

When blood-pigment has been changed in methemoglobin, CO hemoglobin, or hematin, the blood must be dissolved in weak acids or alkalies in order to obtain the spectra of these bodies. (See text-books for special spectra.)

### *Quantitative Estimation of* Hb.

(1.) *Fleischl's Hemoglobinometer.*—This method is based upon the comparison of the color of a solution of blood with that of a stained-glass wedge.

The instrument consists first of the glass *wedge*, to which is



attached a graduated scale varying from zero at the thin edge and 120 at the thick end of the wedge. This wedge and scale travel in the *stand* of the instrument by means of a rack and pinion. The stand is furnished with a plate for holding the *cell,* and with a plaster mirror for casting diffused artificial light through a circular opening in the plate. The *cell* is a cylindrical metal tube closed at one end by a glass plate and divided into two equal compartments by a metal partition. When in position one compartment containing the solution of blood receives the light directly from the mirror, while the other compartment, filled with distilled water, lies over the colored-glass wedge. A comparison of the colors in the two compartments is thus readily obtained. Capillary tubes in metal handles accompany the instrument and are used to convey a measured quantity of blood to the cell, where it is dissolved in distilled water.

These tubes do not always measure the same quantity of blood, but on each handle is stamped a number showing the capacity of the tube, which must correspond to a number stamped on the *stand.*

A slowly flowing medicine dropper is used to add the distilled water.

*Procedure.*—The finger-tip is cleansed with soap and water and dried in alcohol.

A free puncture of the skin is made with an acne-lancet or needle, and a drop of blood expressed, using very slight pressure only. Having previously seen that the capillary tube is in working order, one end of it is applied to the surface of the drop, when it becomes instantly filled with blood.

The blood is then dissolved in one chamber of the cell half filled with distilled water. The dissolved blood is thoroughly mixed by means of the tube, which is then washed into the chamber by a few drops of water. Both sides of the cell are then filled *level full* with distilled water, taking care that the fluid of one side does not run into the other.

The specimen is now examined in a *dark room* by *yellow candle or lamp-light* and the colored-glass wedge is moved till its color exactly matches that of the dissolved blood. The percentage of Hb is then read from the graduated scale.

*Precautions in the Use of Fleischl's Apparatus.*

The capillary tube must be clean, and should be blown out several times with distilled water to insure its rapid filling. It must be dry.

When filled the tube must show neither positive nor negative meniscus, but be *level-full.*

Any adherent blood may be very carefully wiped off.

To prevent oxidization a glass cover may be applied to the filled cell, dropping it down first on the side of the water-compartment, but using great care not to inclose air-bubbles or to expel any solution of Hb.

The eye may be assisted in the comparison by looking through an improvised yellow paper tube. Do not strain the eye, but alternate one with the other. A candle gives the best light, which should be dim.

The Fleischl apparatus is inaccurate with low percentages of Hb. In very anæmic cases it may be well to use a double quantity of blood and divide the result accordingly.

The capillary tube may be dispensed with and a known quantity of blood (usually $8\frac{1}{2}$ divisions on the capillary tube), be measured in the red cell mixer of Thoma-Zeiss.

After some experience in matching colors and constant care in the details of the test, very accurate and constant results may be obtained by Fleischl's apparatus.

(2.) *Gower's Hemoglobinometer.*—This instrument consists of two glass tubes mounted side by side perpendicularly on a stand. One is closed and is filled with carmine-colored gelatine representing the color of a 1 per cent. solution of normal blood. The other tube, graduated from 10 to 120 divisions, receives the blood dissolved in distilled water. There is also a capillary tube for measuring 20 cubic millimeters of blood.

In the test, the capillary tube is filled with blood to the 20 cmm. mark, which is dissolved in the graduated cylinder with a little distilled water. Distilled water is then added drop by drop until the blood solution shows the same color as the gelatine. The middle of the meniscus then indicates on the scale the percentage of Hb.

Gower's instrument is less accurate than Fleischl's, but simpler and cheaper.

## HISTOLOGY.

ERYTHROCYTES.—*Structure.*—Fresh blood examined microscopically is seen to contain a large number of yellowish biconcave disc-shaped cells floating in the plasma, either singly, or very soon gathering in rows (*rouleaux*). In the fresh condition these cells stain with great difficulty (achromatic) and appear homogeneous, but their behavior when stained in the dry condition indicate that they are composed of a delicate *investing membrane* continuous with a *reticulated stroma*. In the meshes of the stroma lies the semifluid HB, which is limited to the periphery of the cell, while, according to some authorities, the centre of the cell is occupied by a homogeneous colorless and nearly *achromatic substance.*

In dried specimens of blood the HB shows a strong tendency to unite with acid dyes (eosin, fuchsin), and when stained by a mixture of eosin and methylene blue the erythrocyte appears bright red with a *central area* which is clear or faintly stained. *When thinly spread and rapidly dried* this central clear area is usually obliterated and *the cell stains evenly throughout.*

In most specimens of dried blood stained by eosin and methylene blue some cells are found to be ruptured and their achromatic substance extruded in the form of coarse granules faintly stained by methylene blue, and identical in appearance with the "blood plates." Sometimes such bluish granules may appear in unruptured cells.

In many diseases attended with anæmia the red cell stains with eosin, diffusely or irregularly, of a violet or brownish color (*polychromatophilia*).

The normal red cell in the human adult is non-nucleated. *Infants'* blood usually shows an occasional nucleated red cell of normal size (*normoblast*). Normoblasts are seen in many forms of anæmia.

In *severe anæmia* very small nucleated red cells (*microblasts*) and, more frequently, very large nucleated red cells (*megaloblasts*) are encountered.

*Size.*—In the *adult,* normal red cells average 7 to 8 $\mu$ in diameter, but there are physiological variations between 6 and 9 $\mu$.

In *infants' blood* the larger cells are usually more numerous and variations in diameter are more frequent.

*In disease,* variations in size become very marked, usually in proportion to the severity of the anæmia. In pernicious anæmia very small cells (2 to 3 $\mu$) may be seen (*microcytes*) lying beside very large cells (12 to 16 $\mu$), (*megalocytes*).

*Form.—Physiological* variations of the normal form of red cells are very slight. In fresh blood exposed to the air the red cells rapidly shrink, become *crenated,* and exhibit in fresh or dry condition numerous projecting points and marked irregularity of outline. In *anæmia* microcytes and megalocytes fail to show a biconcave appearance, they do not form rouleaux, and many irregular pear-shaped cells are seen, called *poikilocytes.*

*Number.*—In the normal *adult male* subject the red cells usually number about 5,000,000 per cubic millimeter, and in the *female* about 4,500,000. Variations between 4,000,000 and 5,500,000 in the male and between 4,000,000 and 5,000,000 in the female are not infrequently seen. In new-born *infants* 6 to 7 millions are usually found, but the number rapidly declines during the first two weeks of life to four or four and a half millions.

In *high altitudes* six to seven millions of red cells are commonly found in healthy individuals, but the origin and significance of this polycythæmia is imperfectly understood.

In *disease* the numbers of red cells vary greatly. They may be temporarily increased to six or six and a half millions by severe *diarrhœa,* by which the fluids of the blood are reduced.

Nearly all *acute and chronic diseases* are associated with anæmia, and a reduction in the number of red cells.

The anæmia of the acute infectious diseases, without complications, is usually not severe or persistent. In acute malaria, however, there is an exception to this rule, as the red cells are here rapidly reduced in number.

In *chronic diseases and cachexias* the loss of red cells and albumens of the blood may reach an extreme grade, depending upon the actual loss of blood, the chronic toxæmia, and the imperfect nutrition of the patient.

In *diseases of the blood,* the loss of red cells is usually propor-

tionate to the general severity of the anæmia, but in chlorosis the loss of Hb exceeds that of the red cells, while in pernicious anæmia the reduction of red cells is greater than that of Hb.

### *Leucocytes.*

In the fresh condition the white blood cells are colorless spheroidal bodies of *homogeneous,* or *finely* or *coarsely granular* appearance, and present a spherical or irregular, highly refractive *nucleus.* Shortly after the escape of the blood some of these cells begin to exhibit *amœboid movements.*

### *Classification of Leucocytes.*

The accepted classification of leucocytes is based upon (1) the character of their nuclei, (2) the staining qualities of their protoplasm, and (3) their places of origin.

(1.) According to the *form of the nucleus* leucocytes are described as:

(a) *Small* and *Large Mononuclear Leucocytes.*—The small nuclei are usually compact, while the larger nuclei are vesicular. Sometimes the large nuclei are of horseshoe shape, and these cells are sometimes denominated *"transitional leucocytes."*

(b) *Polynuclear leucocytes* are the most numerous variety, in some of which the irregular nucleus is composed of two or more lobes united by delicate or coarse nuclear threads (*polynuclear neutrophile leucocytes*), while in others the several lobes are completely separated, as in the *eosinophile leucocytes.*

(2.) The classification based upon the *staining reaction* of leucocytes was the result of Ehrlich's studies.

The aniline dyes fall into two distinct classes, according to their chemical relations. Some of them act as bases uniting more or less selectively with the acid principles of the cells and failing to stain the basic portions. These stains are called *basic dyes,* among which are hematoxylon, methylene blue, thionin, etc., the selective quality diminishing in the order named.

Other stains act as acids, uniting only with the basic principles of the cells. These are called *acid dyes,* of which those most used are eosin and fuchsin.

Finally, when certain basic dyes are mixed with acid dyes a compound is formed with modified staining tendencies and uniting with certain elements in the leucocytes, which are difficult to stain by any simple dye. Such a mixture or compound is called a *neutrophile stain,* of which Ehrlich's triacid mixture is an example.

When specimens of dried blood are stained by basic dyes, e. g. methylene blue, the bodies of the *large and small mononuclear* leucocytes are stained intensely blue, and these cells are therefore called *basophile leucocytes.* The bodies of the small mononuclear leucocytes usually contain small basophile *granules,* while in the large mononuclear leucocytes the bodies show no granules, but a fine basophile *reticulum.*

When blood is stained by Ehrlich's triacid mixture most of the polynuclear leucocytes are seen to contain many fine brownish *neutrophile* granules, which cannot be readily stained by either basic or acid dyes. These cells are therefore called *polynuclear neutrophile leucocytes.* (The neutrophile granules may be stained by the prolonged action of strong solutions of eosin, and have therefore a slight acidophile tendency.)

Again, when an acid dye, eosin, is used in weak solution, some polynuclear leucocytes are found to contain large *eosinophile* granules, stained bright red by the eosin, and these cells are called *eosinophile leucocytes.*

(3.) *According to their places of origin,* the small mononuclear leucocytes, being probably derived exclusively from the lymphatic structures of the body, are called *lymphocytes.* Of these a *small* and a *large* variety are usually distinguished.

Some large mononuclear leucocytes with reticulated cell body are by some believed to be derived from the splenic pulp and are called *splenocytes.*

Authorities are not agreed as to the mode of origin of polynuclear neutrophile leucocytes, but there is strong reason for believing that they are derived almost exclusively from the mononuclear cells with neutrophile granules found in the bone-marrow (*myelocytes*).

Eosinophile leucocytes are normally derived from the eosinophile cells of the marrow, but in pathological conditions they appear to develop in many other tissues.

### *Resume on Classification.*

The leucocytes of *normal blood* may now be described as follows:

(1.) *The small mononuclear leucocyte,* or *small lymphocyte,* with a single compact nucleus and basophile granules.

(2.) *The large mononuclear leucocyte,* or *large lymphocyte,* (or splenocyte), with a single vesicular nucleus and a basophile reticulum.

(3.) *The polynuclear neutrophile leucocyte,* with nucleus of two or more lobes united by delicate threads, and with many fine neutrophile granules.

(4.) *The eosinophile leucocyte,* with a nucleus composed of two or more separated lobes, and with large eosinophile granules.

In pathological blood two other forms of leucocytes occur which are not seen in normal conditions.

(1.) *Myelocytes* are mononuclear cells with *neutrophile* or *eosinophile granules.* As the name indicates, they are probably derived exclusively from the bone-marrow, and they are found principally in leukemic blood.

(2.) *Mast-cells.*—These are mono- or polynuclear cells with *large basophile granules.* The granules retain basic dyes with great tenacity, and must be demonstrated with Ehrlich's dahlia stain. Mast-cells are found almost exclusively in myelogenous leukemia.

### *Proportions of Leucocytes in Normal Blood.*

Leucocytes are found in normal blood in about the following proportions:

| | |
|---|---|
| Small mononuclear leucocytes..... | 6 per cent. |
| Large mononuclear leucocytes..... | 29 per cent. |
| Polynuclear neutrophile leucocytes.... | 64 per cent. |
| Eosinophile leucocytes..... | 1 per cent. |

In children the mononuclear often outnumber the polynuclear leucocytes.

The number of leucocytes per cubic millimeter of normal blood usually varies between 7,000 and 10,000.

Both the numbers and proportions of leucocytes in the blood are subject to many variations, to be considered later.

## METHODS OF EXAMINING RED AND WHITE BLOOD CELLS.

(1.) *Thoma's Hematocytometer.*—This instrument consists of a *pipette* or *mixer,* by which a measured quantity of blood is diluted in known proportion with a diluting and staining fluid. The capillary tube of the mixer is graduated so that when the tube is filled with blood up to the mark *1,* and then with diluting fluid up to the mark *101,* the blood has been diluted in the proportion of *1 to 100.* When the capillary tube has been filled to the mark *0.5* the resulting dilution is in the proportion of *1 to 200.*

The bulb of the pipette contains a glass ball to aid in mixing the blood.

The *diluting fluids* to be recommended are: (1) A 3 per cent. solution of common salt deeply tinged with gentian-violet. (2) *Toisson's fluid:*

| | |
|---|---|
| Sodium sulphate | 40 grm. |
| Sodium chloride | 5 |
| Glycerine | 150 |
| Distilled water | 800 |
| Methyl violet | .125 |

The latter solution is more permanent, the former more easily prepared, and better adapted for the enumeration of leucocytes.

The *counting chamber* is a large glass slide, in the centre of which a large circular area has been excavated. In this area is cemented a circular shelf of such thickness that its surface is *exactly 1-10th mm. lower than the surface of the slide.* On the shelf an area of 1 sq. millimeter is subdivided by fine intersecting lines, making in all 400 small squares, *each of which is 1-400th square millimeter in area.* Every fifth square is subdivided by an additional line. When a drop of diluted blood is placed on the shelf and covered with a flat cover glass the depth of the fluid is everywhere 1-10th mm. and the cubic contents lying over one small

square is 1-10 x 1-400=1-4000th cubic mm. When the cells have settled out of the diluted blood on the shelf, the number lying in each square can readily be counted.

The cover glasses accompanying the instrument are made perfectly flat, so that the depth of fluid is uniform.

*Method of Procedure.*—The finger-tip, cleaned as before, is punctured, and, with light pressure only, a drop of blood is secured. The capillary tube is filled to the mark (1) or (0.5) and its tip cleaned of adherent blood. The mixer is then filled with diluting fluid to the mark 101 and thoroughly shaken.

The counting-chamber and cover glass having been thoroughly cleaned, two drops of the diluted fluid are expelled from the mixer, and a small drop is then placed on the centre of the shelf. As quickly as possible the cover glass is adjusted and firmly pressed down. If Newton's color-rings appear at the corners of the cover glass the preparation is successful. Dust particles may prevent intimate contact of slide and cover glass and the formation of these rings, and must in every case be avoided. *The rapid and successful adjustment of the cover glass is the most important detail in the process of counting red cells.*

The size of the drop to be deposited on the shelf can only be learned by experience. When the cover glass is in place, the blood must at least cover all, or nearly all, the shelf. It may project into the moat, but if it runs out underneath the cover glass the specimen must be discarded.

The preparation appearing thus far successful, it is examined in the light *to see that the red cells are evenly distributed.* If the cover glass has not been rapidly adjusted it will be found that the central portions of the blood contain more cells than the peripheral, in which case the specimen must be rejected.

After the cells have settled for a moment, counting may proceed, using a mechanical stage and Leitz No. 7 lens. One hundred small squares at least must be counted over. To avoid counting the same cells twice it is customary to include in any square those cells lying on the lines below and to the right of the square, omitting such as lie on the lines above and to the left of the square.

*Computation.*—Having counted all the cells in 100 small squares, the number per cubic millimeter may be estimated as follows:

100 small squares compose an area of $\frac{1}{400}$ sq. mm., or a cubic contents of $\frac{1}{400} \times \frac{1}{10}$ (depth) $=$1-40th cubic mm. The dilution of the blood being 1-100 the number of cells counted is therefore to be multiplied by $40 \times 100 = 4000$. If 400 small squares are counted for complete accuracy, the multiplier is 1000.

*Cleaning the Instrument.*—After every use of the capillary tube it is absolutely essential that both chamber and tube be thoroughly and properly cleaned and dried.

The *counting chamber* is to be wiped with a cloth moistened with water. *Alcohol and ether dissolve the cement under the shelf.*

The *mixer* is first emptied of blood, the last particles of which are to be dissolved by refilling with water.

The bulb is then filled with alcohol or ether, or both, and after this fluid has been expelled, *the tube must be thoroughly dried before using again.* Minute particles of water remaining in the tube cause the partial or complete solution of red cells in the subsequent specimen and the appearance of faint "shadow corpuscles."

## *Enumeration of Leucocytes.*

Leucocytes are best counted in the same specimen and chamber with the red cells. A special chamber has been constructed for this purpose in which 9 square millimeters are outlined in large squares. The limits of these square millimeters can readily be recognized by examining the chamber with the low power of the microscope.

In order to enumerate a sufficient number of leucocytes for accurate results, it is necessary to count all the white cells appearing in 3, 6, or 9 square millimeters, according to their abundance.

Only 8 to 10 leucocytes are found in one cubic millimeter of diluted normal blood, but at least 50 must be counted to secure any reliable indication of their numbers, while at least 100 or more must be counted when minor variations in number are being followed.

For the purpose of counting over this larger area, a mechanical stage is almost essential.

The leucocytes are readily identified in salt solution, after a

To get a 1/10 dilution use 1/2 % Acetic acid.
In this the leucocytes stand out very distinctly
This is used in Leukaemia, Etc.

little practice, by their refractive appearance, and usually also from their bluish stain from gentian-violet. The small lymphocytes are the only ones that can be overlooked with ordinary care.

*Computation.*—If 54 leucocytes are found in 6 sq. mm., the average is 9 per sq. mm., or (9×1000) 9000 per cubic mm.

### PREPARATION OF DRY SPECIMENS OF BLOOD.

Blood for this purpose must be secured under the usual precautions from the finger-tip or lobe of the ear. It is important that the drop expressed should be compact and of medium size. *Absolute cleanliness* of the punctured skin and of all apparatus is the first and great essential for satisfactory specimens of this sort.

*Glass slides* should be used on which to spread the blood. They should be scoured with *soap and water,* and immediately before using they should be *polished* with a clean piece of old cloth free from alkalies, oils, and dust. The best instrument for spreading the blood is a glass slide with a smoothly ground edge.

The drop of blood is scraped from the finger tip with the edge of such a glass slide, taking care to touch the skin as lightly as possible. The slide with its adherent blood is then applied obliquely to one end of a second slide, and *after the blood has spread to the edges of the smearer* it is slowly and carefully drawn across the slide.

By varying the pressure the thickness of the layer of blood may be controlled. It is important that the blood should be pushed before the edge of the smearer, and not trailed after.

The specimen thus spread is allowed to dry in the air, but the access of moisture shrinks the cells or dissolves them before they dry. The thoroughly dried specimen may also be entirely dissolved by subsequent access of moisture, and it is best to fix the specimens at once.

Specimens simply dried in the air may be safely transported, or kept for days, if wrapped in tissue-paper and kept dry.

### *Methods of Fixation.*

(1.) *Dry Heat.*—The specimens are heated in a small oven for

10 minutes, at a temperature of 120° C. This is the best method for general use.

(2.) *Alcohol and Ether.*—The specimens may be immersed in equal parts of alcohol (95 per cent.) and ether for fifteen minutes. This method is reliable for most purposes, is easily carried out, and is specially recommended in staining the malarial plasmodium.

(3.) *Vapor of Formalin.*—The specimen may be exposed for five minutes over a wide-mouthed bottle containing 25 per cent. solution of formalin. This method is satisfactory, except that formalin alters the staining reaction of some of the elements of the blood.

(4.) *Vapor of Osmic Acid.*—The specimen may be exposed for two minutes over a bottle containing 2 per cent. solution of osmic acid.

This is a reliable method, but osmic acid causes the red cells to stain yellowish, and the solution must frequently be replenished.

## *Methods of Staining.*

Two methods of staining are required in the full examination of blood.

(1.) *Eosin and Methylene Blue.*—Flood the slide with a saturated alcoholic solution of alcoholic eosin and wash immediately in water. Flood the slide with a saturated watery solution of methylene blue for one minute. Wash in water, and dry in the air.

This method is to be recommended for all ordinary examinations of blood. ~~*It fails to demonstrate neutrophile granules and "mast-cells."*~~ In staining for the malarial plasmodium *very light staining with eosin is essential,* and the saturated alcoholic solution should be diluted one-half. The effect of methylene blue is much sharper if this dye is added after the specimen has thoroughly dried.

(2.) *Ehrlich's Triacid Solution.*—The slide is flooded with the above solution for one minute, and washed in water.

Staining by this method is required for the demonstration of neutrophile granules and myelocytes, and is specially applicable in cases of suspected leukemia. It is also a rapid and reliable method for general use, but fails to demonstrate the malarial plasmodium.

2. Alcohol
3. Alcohol + Ether
4. Alcohol + Formalin.
5. Methyl Alcohol ( Jenner Stain)

Dry slide with blotting paper before adding methylene blue

This stain shows Bacteria, malarial, Filaria, nuclei, Eosinophile ~~granules~~ cells, mast-cells-basophile + occasionally neutrophile granulations when Eosin is very ~~strong~~.

~~Jenner~~ Jenner Stain

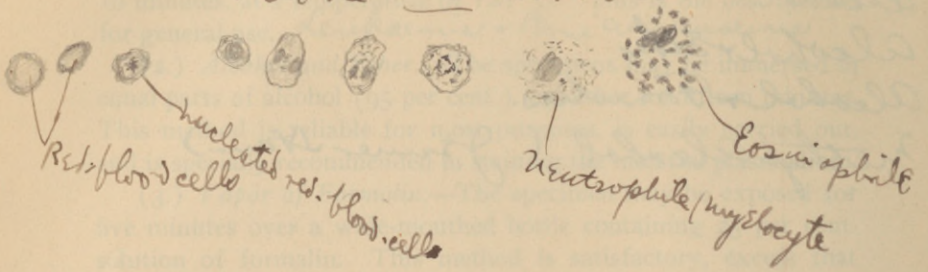
Eosin + methylene Blue dissolves in methyl alcohol. Leave the slide in this mixture for 1½ min + then take it out + ~~dry~~ wash off

Before staining by Ehrlich's stain, fix the stain by dry heat.

In Ehrlich's stain lymphocyte cell-body is faint pink; in Jenner's stain, they are deep blue.

In Bone-marrow, you find normal red-cells, nucleated red-cells, myelocytes, polynuclear neutrophiles, Eosinophile granulations, lymphocytes and large mononuclear leucocytes & rarely mast-cells

Bone-marrow.



*Ehrlich's Dahlia Solution.*

For the demonstration of mast-cell granules this solution is adapted, being of the following composition:

| | |
|---|---|
| Alcohol (absolute) | 50. c.c. |
| Glacial acetic acid | 12.5 c.c. |
| Distilled water | 100. c.c. |

Add Dahlia to saturation.

Stain several hours, wash in water, decolorize in alcohol, or, more rapidly, in 20 per cent. acetic acid, till the nuclei fade; wash in water and dry.

*General Directions in the Examination of Stained Specimens.*

When red cells are rapidly dried, as usually occurs on the edges of the streak or in very thinly spread areas, they stain evenly with eosin. Where thickly spread the red cells dry less rapidly, the Hb runs to the periphery of the cell and a central clear area is formed in each cell. The size of this central clear area is a rather accurate indication of the cell's content in Hb, *but in judging of this character one must inspect only those cells found in rather thickly spread portions of the smear.* A cell very deficient in Hb may, if rapidly dried, be uniformly and intensely stained by excessive action of eosin.

On the other hand, variations in the size of the cells are best judged in thinly spread sections.

*Changes in the number and variety of leucocytes* may be estimated in stained specimens with moderate accuracy. For this purpose the slide should be examined with a moderately low power and all parts of the specimen should be included in the estimate. At least 500 leucocytes should be counted in order to estimate the percentage of the different varieties present in many specimens. On the other hand, a distinct lymphocytosis or polynuclear leucocytosis may be apparent at the first glance.

DISEASES OF THE BLOOD.

*Chlorosis.*—The chief lesion in the blood of chlorosis is a diminution in the Hb-content of the red cells and a consequent reduction of the total albumen of the blood and of its specific gravity.

The *hemoglobin* ranges from 60 per cent. in mild cases to 20 per cent. or less in severe cases.

The *hemoglobin-index*, that is, the ratio between the percentage of Hb and the number of red cells, is low.

The *number* of red cells may be nearly normal in mild cases, while in severe cases they are rarely less than two millions.

In *morphology* the red cells exhibit in stained specimens a large central clear area, indicating a loss of Hb.

In mild cases the *shape and size* of the red cells remain normal. In severe cases there are moderate variations in shape and size.

*Nucleated red cells* of normal size, *normoblasts*, are often seen in severe cases of chlorosis.

The *leucocytes* in chlorosis are usually but little affected. There may, however, be an increase in all varieties of leucocytes—i. e. a *mixed leucocytosis*.

A periodical increase of leucocytes associated with the appearance of nucleated red cells is sometimes observed in chlorosis, constituting the so-called "*blood crisis*."

### *Prognostic Indications.*

(1.) In a first attack of chlorosis in which there is only a loss of Hb, the prognosis is good for early recovery.

(2.) When to the loss of Hb there is added a considerable diminution in the number of red cells, recovery follows more slowly.

(3.) When to a marked loss of Hb, and considerable diminution in number of red cells, there are added changes in the shape and size of the red cells, recovery is usually slow, relapses are common, and the disease has been known to run into pernicious anæmia.

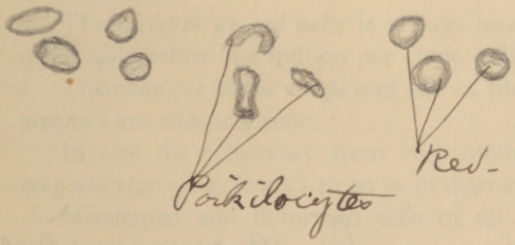
*Secondary Chlorotic Anæmia.*—In all diseases attended with malnutrition, toxæmia, or hemorrhage, the blood is impoverished and the changes may resemble those of chlorosis.

It is not always possible to distinguish secondary chlorotic anæmia from chlorosis. For this purpose it is important to recognize the presence of a primary disease, such as carcinoma, ulcer of

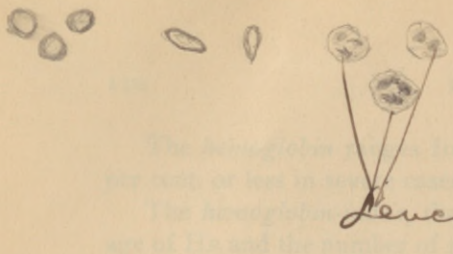
Poikilocytes are seen

Megaloblasts are never seen

Chlorosis – I



Secondary Anaemia - Cancer - II



Leucocytes

Polychromatophilia appears in Lead Poisoning and all the Anaemias & Malaria.

Microblasts = small nucleated red cells

Normoblasts are normal sized red cells with nuclei.

Megaloblasts are diagnostic of Pernicious anaemia. The megaloblasts have a nucleus which is larger in proportion to the cell than that of the normoblasts; their nuclei are also composed of a net-work, which is not found in normoblasts

stomach, nephritis, rachitis, malaria, syphilis, etc., while in the blood examination attention should be given to the following features which usually distinguish secondary chlorotic anæmia from chlorosis:

(1.) The percentage of HB and the number of red cells may be very low (15 per cent. HB; 1,000,000 red cells).

(2.) The HB *index* is not as low as in chlorosis—i. e. the reduction in HB and in the number of red cells is apt to progress uniformly.

(3.) Most diseases that lead to secondary anæmia cause moderate *polynuclear leucocytosis,* the presence of which often distinctly characterizes the blood of secondary chlorotic anæmia.

### *Pernicious Anæmia.*

*Primary pernicious anæmia* is a disease of the blood-producing organs characterized by an extreme reduction in the number of red cells and HB of the blood, and tending to a fatal issue.

The HB is usually below 25 per cent. The HB-*index* is usually *increased,* owing to the presence of an excess of HB in the red cells which remain, and which are often of large size. In rarer cases the HB *index* may be normal, or in some very rapid cases it may be diminished.

The *number of red cells* is always less than two million, and often falls below one million per cubic millimeter.

The *changes in the shape and size* of the red cells of pernicious anæmia are characteristic.

In size the cells vary from very minute *microcytes* to large *megalocytes*—i. e. from 1 to 20 $\mu$ in diameter.

Misshapen and deformed cells of all sizes are encountered, which are called *poikilocytes.*

In many of the cells of pernicious anæmia the HB suffers an alteration which causes it to stain brownish with eosin, and this change in staining quality is called *polychromatophilia.*

*Nucleated red cells* of large size are nearly constantly present in this disease, and are called *megaloblasts.* Their presence in considerable numbers is pathognomonic of the disease. *Microblasts* are also sometimes seen. The HB of these cells usually stains brownish with eosin, and may contain basophile granules.

The *absence of rouleaux* in stained specimens is a characteristic feature of the blood of pernicious anæmia. In dry specimens the *plasma* may be distinctly *stained* by eosin, indicating a solution of Hb in the plasma (hemoglobinæmia).

The *leucocytes* in primary pernicious anæmia are usually diminished in number, and those remaining are principally of the mononuclear variety.

Leucocytosis arising in the course of the disease usually indicates an exudative complication. A few myelocytes are not infrequently seen in these cases.

### *Types of Primary Pernicious Anæmia.*

In the great majority of cases all of the changes above described are well marked in the blood, but in some acute cases, which must at present be classed with this disease, a fatal issue is reached before the appearance of the usual morphological changes. In such instances the red cells are extremely deficient in number and Hb-content, and are diminished rather than increased in size. This type of the disease has been called the *microcytic form* of primary pernicious anæmia. It is most frequently seen after parturition.

*Splenic anæmia* is a slowly progressive anæmia, usually of microcytic type, reaching often a pernicious grade, and associated with enlargement of the spleen. It is regarded by some as the splenic form of Hodgkin's disease.

In *Hodgkin's disease* the changes in the blood are those of the microcytic type of pernicious anæmia.

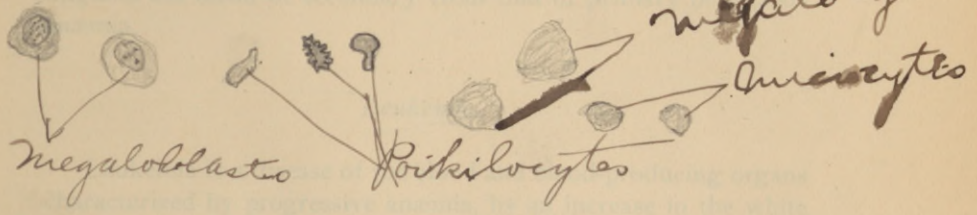
### .... *Secondary Pernicious Anæmia.*

Severe grades of secondary anæmia most frequently result from malaria, syphilis, carcinoma, and nephritis.

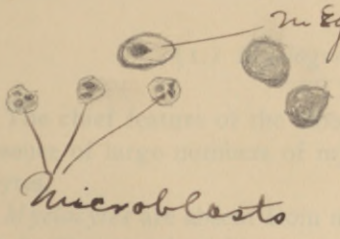
The red cells may be increased in size and show an excess of Hb (megalocytic type), or, as is usual in the very acute cases, they may be normal or reduced in size and show a deficiency in Hb

Pernicious Anaemia – I


Pernicious Anaemia – II

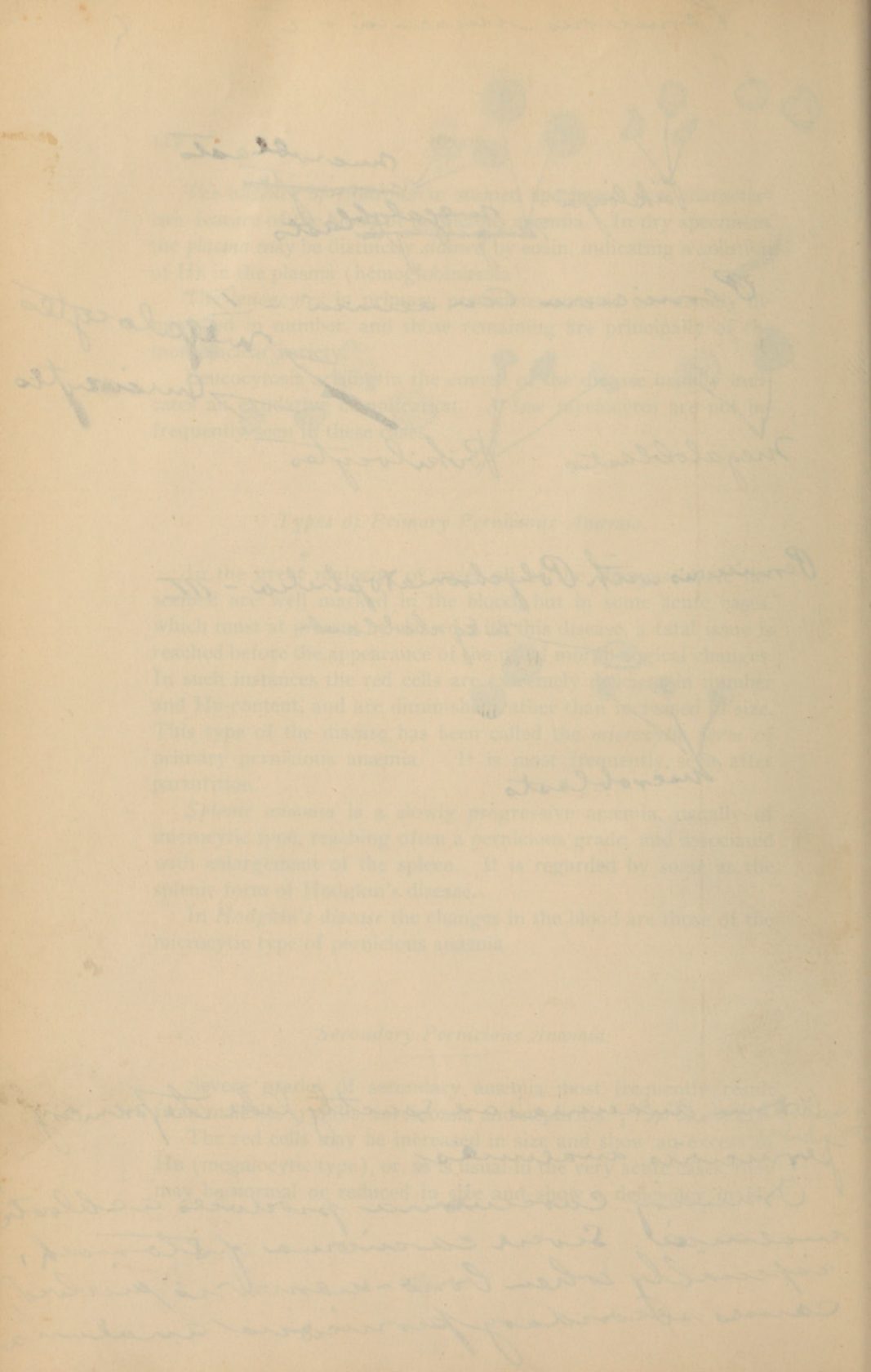



Pernicious with Polychromatophilia – III




Worm anaemias are exactly like the primary pernicious anaemias.

Ordinary carcinoma produces a chlorotic anaemia. Severe carcinoma of stomach, especially when bone-marrow is involved, causes secondary pernicious anaemia



(microcytic type). The increase of Hb in the large red cells is almost always less marked and uniform than in primary pernicious anæmia. Megaloblasts are usually absent in secondary pernicious anæmia.

A persistent polynuclear leucocytosis usually serves to distinguish the blood of secondary from that of primary pernicious anæmia.

## *Leukemia.*

Leukemia is a disease of the blood and blood-producing organs characterized by progressive anæmia, by an increase in the white cells of the blood, and by certain changes in the viscera. In many respects it resembles a tumor-formation in a fluid tissue.

Based on the changes in the blood, there are two distinct types of leukemia.

### (1.) *Myelogenous Leukemia.*

The chief feature of the blood in this form of leukemia is the presence of large numbers of myelocytes and of polynuclear leucocytes.

*Myelocytes* are absent from normal blood, but are occasionally seen in scant numbers in severe secondary anæmias. In leukemia they are present in very large numbers, and constitute the essential diagnostic sign of this disease.

It is important to distinguish three varieties of myelocytes.

(1.) *Ehrlich's myelocyte* is of the same size as the polynuclear leucocyte, but possesses a single pale central nucleus and neutrophile granules. This is usually the form of myelocyte seen in secondary anæmias, but is abundant in myelogenous leukemia.

(2.) *Cornil's myelocyte* is a very large cell with a single pale eccentric nucleus and neutrophile granules. This cell is seen almost exclusively in myelogenous leukemia.

(3.) *The eosinophile myelocyte* is a mononuclear leucocyte with unusually large and darkly staining eosinophile granules (triacid stain). This cell has been observed only in myelogenous leukemia.

*Polynuclear leucocytes* are usually very abundant in myelogenous leukemia, and may outnumber the myelocytes. They exhibit a variety of forms of degeneration, including especially a subdivision of nuclei, hydropic swelling of nuclei, and loss of neutrophile granules.

*Mast-cells* are seen in considerable numbers only in chronic myelogenous leukemia.

The *red cells* in leukemia usually show the changes of severe chlorotic anæmia, being reduced in number and very deficient in Hb. In prolonged cases the red cells may show the changes of pernicious anæmia. Nucleated red cells are abundant in myelogenous leukemia, but rather scarce in the lymphatic variety.

*Course of the Disease.*—Myelogenous leukemia usually runs a *chronic* course, and all the above changes in the blood become distinct. The leucocytes then usually exceed 100,000 per cubic millimeter, and may even outnumber the red cells. An acute infectious disease, such as pneumonia, arising in the course of leukemia, may replace the mixed with a pure polynuclear leucocytosis, the myelocytes temporarily disappearing. With this rare exception the examination of the blood furnishes at all times a positive diagnosis.

In *acute* myelogenous leukemia, on the other hand, the increase of leucocytes may not exceed the limits of a polynuclear leucocytosis, and the diagnosis must at times rest upon the discovery *that 5 to 10 per cent. of the leucocytes are myelocytes.*

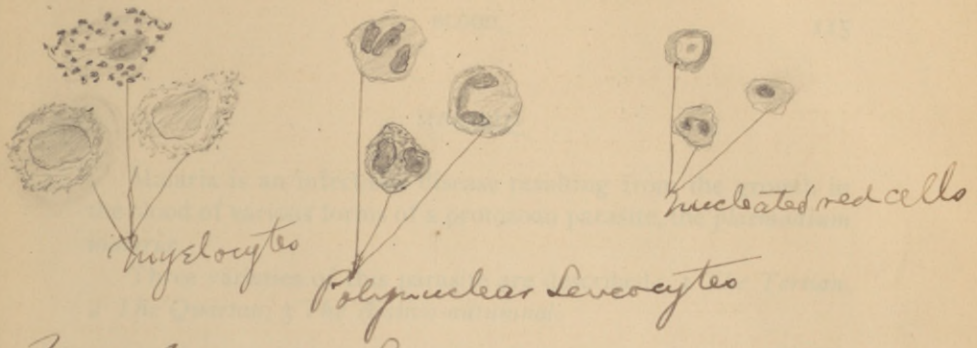
### (2.) *Lymphatic Leukemia.*

In lymphatic leukemia the increase is of the small and medium sized lymphocytes, while polynuclear leucocytes are scanty, and myelocytes and mast-cells are absent. The number of white cells present is often quite as large as in myelogenous leukemia.

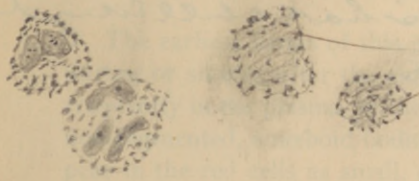
When closely examined after staining by eosin and methylene blue, some of these lymphocytes are found to exhibit small basophile granules, while others possess a reticulated cytoplasm.

In the diagnosis of early lymphatic leukemia it is important to remember that the blood of children sometimes shows an extreme but temporary lymphocytosis.

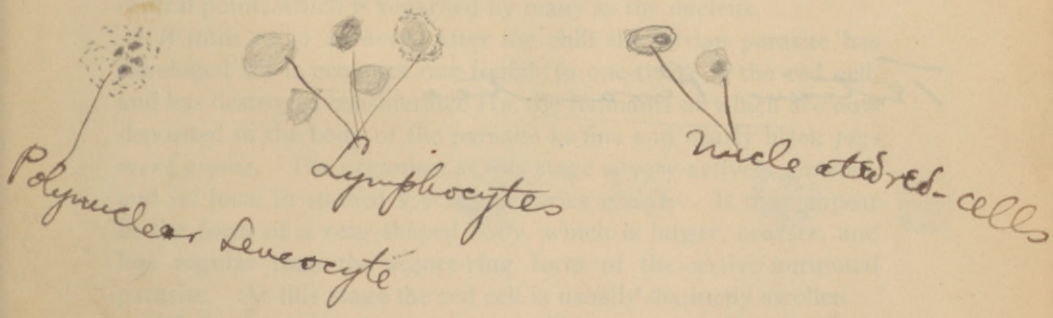
Myelogenous Leukaemia – I



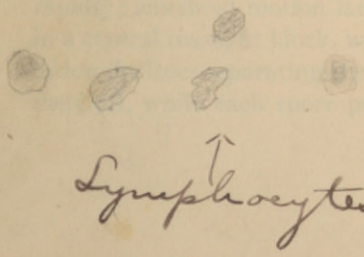

Myelogenous Leukaemia – II




Mixed Leukaemia – III




Lymphatic Leukaemia – IV



Reports that patients have chill every day
so due to double infection.

Tertian Parasites.

## MALARIA.

Malaria is an infectious disease resulting from the growth in the blood of various forms of a protozoan parasite, the *plasmodium malariæ*.

Three varieties of this parasite are described: 1 *The Tertian*, 2 *The Quartan*, 3 *The Æstivo-autumnal*.

### (1.) *The Tertian Parasite.*

The earliest forms of this parasite are found in the circulation during or shortly after the chill, usually within the red cells, less frequently in the plasma, in the form of small, spheroidal, hyaline, non-pigmented, amœboid bodies. In fresh blood these bodies appear in the red cells as small, colorless, highly refractive spheres, exhibiting slight but acive changes in shape and position. In dry specimens they stain deeply with methylene blue, and they are solid or vesicular in form, and sometimes exhibit a densely stained central point, which is regarded by many as the nucleus.

*Within 12 to 18 hours* after the chill the tertian parasite has developed till it occupies one-fourth to one-third of the red cell, and has destroyed considerable Hb, the remnants of which are now deposited in the body of the parasite as fine and nearly black *pigment grains*. The organism at this stage is very actively amœbid, and its form in stained specimens varies greatly. It may appear in the form of a ring-shaped body, which is larger, coarser, and less regular than the signet-ring form of the æstivo-autumnal parasite. At this stage the red cell is usually distinctly swollen.

The parasite continues to grow till it occupies almost the entire and greatly swollen cell. Pigment grains become abundant, the body of the parasite begins to show a more definite reticulum, and its amæboid movement gradually diminishes.

*Toward the end of 48 hours* segmentation begins and proceeds rapidly; amœboid motion largely ceases, and the pigment gathers in a central mass, or block, with radiating lines. In the fresh condition the lines separating the new spores are very distinct and geometrical, while each spore presents a fine central point, supposed

to be the nucleus. In stained preparations, during the segmenting process, the reticulum becomes denser and sharper, and then breaks up into 15 to 20 small spheroidal segments or spores.

The cycle is completed by the discharge of the new brood of spores into the plasma, which is simultaneous with the chill.

The pigment is discharged into the plasma, often englobed by leucocytes, and lodged principally in the liver, spleen, and lymphatic organs.

### (2). *The Quartan Parasite.*

The quartan parasite differs from the tertian in the following main particulars:

The early hyaline bodies are more refractive than the tertian. Their amœboid movement is slower throughout the cycle. The quartan pigment is coarser than the tertian.

The segmenting rosette is much smaller than the tertian and exhibits 6 to 12 spores only. Quartan rosettes are usually more abundant in the peripheral circulation.

The infected red cell instead of swelling is distinctly shrunken. This difference results in part from the smaller size of the fully developed quartan parasite.

The cycle of development of the quartan parasite requires 72 hours.

### (3). *The Æstivo Autumnal Parasite.*

The spores of this parasite closely resemble those of the tertian, but when seen in the plasma or soon after entering the red cell they are readily distinguished. *In the plasma* they move rapidly about with a peculiar rolling and darting motion, and exhibit three or four slightly projecting knobs, so that their shape is often that of a minute star with blunt points. *In the cell* they are slightly refractive, spheroidal or ring-shaped bodies, showing rather active changes in form.

In stained specimens, the earliest intracellular bodies are usually of the typical *signet-ring* form, but may be quite minute in size.

*After 12 to 18 hours* the ring-shaped organism has developed

Mucous Polypus Quinsy is a rare disease.

Crescent

into a characteristic signet-ring form of larger size, occupying now from one-fourth to one-third the diameter of the cell. The signet is very prominent, but the bow of the ring may be very thin.

*After 24 hours* the æstivo-autumnal parasite tends to disappear from the circulation and gather in the spleen, marrow, or other viscera, to complete its development. In these situations the ring-shaped body develops into a pigmented spheroidal body, and on the third or fourth day into an elliptical or young crescentic body, which is very apt to reach the general circulation by the end of the *third or fourth* day. By the *fifth to seventh* day the fully developed crescentic body usually appears in the peripheral blood.

The appearance of the crescent is very characteristic. It measures from 9 to 12 μ in length, exhibits a central group of large brownish-yellow pigment grains, and a remnant of the red cell membrane usually stretches across its concavity.

The *segmenting body* of the æstivo-autumnal parasite appears to develop principally in the marrow and spleen from the younger spheroidal bodies. The crescents are believed to be sterile forms. In the marrow the segmenting bodies are slightly smaller than the tertian forms and almost always show 18 spores. They are rarely seen in the circulation.

The time required for the fertile cycle of the æstivo-autumnal parasite is not definitely known. Clinically, the paroxysms, if undisturbed by quinine, usually follow one another every seven days.

### *Irregular Forms of Malarial Parasites.*

*Flagellate bodies* may develop from the adult forms of the parasite. They are composed of a central spheroidal pigmented mass to which are attached one or more long flagella. The flagella may become free, but are still actively motile. Their biological significance is unknown. They are best obtained by allowing the fresh blood to stand on the finger-tip or under the cover glass for several minutes.

*Pigmented extra-cellular bodies* are probably sterile adult forms which have completely destroyed the red cell and maintain a temporary existence in the plasma. Some of these bodies are said to reach a considerable size, but it sometimes is difficult to distinguish

such forms from pigmented and cystic leucocytes. The diagnosis of malarial parasites should not rest on their presence alone.

*Pigmented Leucocytes.*—Typical pigmented leucocytes are very characteristic signs in malarial blood, and on their presence alone the diagnosis must often rest: (1) in severe acute cases after the administration of much quinine; (2) in remittent malarial fevers; and (3) in chronic malarial fever and cachexia. They persist in the blood long after all traces of parasites have disappeared.

The pigmented leucocytes of malaria are usually the mononuclear forms, being derived from the spleen, marrow, and lymph nodes. Malarial pigment may be identified in them in the form of single grains or clumps of dark brownish yellow lustre.

The identification of *free malarial pigment* is usually hazardous, and the diagnosis of malaria should never be based on its presence alone.

### *The Significance of the Examination of the Blood in Malaria.*

In all severe acute seizures, if the blood is examined within twenty-four hours after the beginning of the paroxysm, and before much quinine has been administered, the plasmodium can readily be found, usually in considerable numbers. Under such conditions the result of the examination is positive evidence for or against the existence of malaria.

In some very mild initial paroxysms the plasmodia may be scanty and difficult to find.

Large doses of quinine, especially if administered subcutaneously, may clear the blood of parasites within twelve hours.

In æstivo-autumnal malaria, while quinine is being administered, there is a period between the second and fourth day when no organisms may be found in the blood, but on the fourth or fifth day crescents almost always make their appearance, notwithstanding the use of quinine.

The crescentic bodies are very resistant to quinine, and may persist in actively treated cases for at least two weeks, and in other cases much longer.

In the remittent malarial fevers the blood may (1) show the

presence of a few tertian organisms or crescentic bodies, or (2) the diagnosis must rest on the presence of considerable anæmia and of pigmented leucocytes; (3) a severe remittent fever may also result from infection with several generations of one or more varieties of the parasite.

In chronic malarial fever and cachexia the plasmodia are usually absent from the circulation, but pigmented leucocytes and severe anæmia are nearly constant signs.

### *General Characters of the Blood in Malaria.*

Malarial infection very often rapidly impoverishes the blood, and the evidences of secondary chlorotic anæmia are usually marked after the first or second severe paroxysm.

In the severe remittent fevers the anæmia is always pronounced, and beginning changes in shape and size of the red cells are usually noted. Nucleated red cells may be abundant.

In the chronic cases with cachexia, the changes of pernicious anæmia may be fully established in six to eight weeks.

The *leucocytes* are usually reduced in number during and after acute malarial paroxysms. In the remittent fevers there may be a mixed or polynuclear leucocytosis, or lymphocytosis.

In chronic malaria there may be a moderate polynuclear leucocytosis. In all, except the very early stages, pigmented leucocytes may be found in moderate numbers.

### *The Method of Examining Malarial Blood.*

After acute paroxysms, when the plasmodia are abundant, a drop of blood may be covered on a glass slide and examined in the fresh condition. This method has the advantage of permitting observation of the amœboid motion of the parasite, and of the presence of flagellate bodies. A negative result by this method should always be controlled by a thorough search, repeated, if necessary, on following days, through stained dry specimens.

In staining the blood for this purpose a *very light stain with eosin is necessary*, otherwise the early ring-shaped form of the

æstivo-autumnal parasite may fail to stain with methylene blue and be overlooked.

The best demonstration of these delicate bodies requires also a *thinly spread layer* and *instant drying*.

## LEUCOCYTOSIS.

During the course of many infectious diseases the number of leucocytes in the circulation is subject to marked variations, the significance of which is not fully understood.

The influence of *chemotaxis* is probably of considerable importance in these variations.

If a capillary tube containing a fluid culture of *bacillus pyocyaneus* be inserted beneath the skin of a rabbit, the wound closed, and after half an hour the tissues examined, it is found that the leucocytes in the neighboring vessels are scanty and that a little serous exudation has occurred. The leucocytes appear to be repelled by the bacterial culture, and the repelling influence is termed *negative chemotaxis*. After 2 to 3 hours, however, the appearance of the tissues changes, leucocytes are abundantly present, they gather about the ends of the tube, and even plug its opening, and the attractive force is called *positive chemotaxis*. How much these phenomena depend upon the variable calibre of small vessels and upon the increased cohesiveness of leucocytes and tissue cells is not known.

Similarly when a culture of *bacillus pyocyaneus* is thrown into the ear-vein of a rabbit, the leucocytes in the circulation are for a time reduced in number (*hypoleucocytosis*), being lodged in the capillary vessels, but after 1 to 2 hours a reaction sets in and many new leucocytes are poured into the circulation, so that their numbers are greatly increased (*hyperleucocytosis*).

In the course of many infectious diseases the initial stage of hypoleucocytosis has been observed, followed by hyperleucocytosis, which usually continues throughout the course of the disease.

From the data derived from the clinical and experimental study of this subject it appears that *the leucocytosis of infectious diseases represents the effort of the blood and blood-producing organs to rid the system, by means of leucocytes and their derivatives, of bacteria and toxines.*

## *Occurrence of Leucocytosis.*

The term *"leucocytosis"* usually refers to an increase of the polynuclear neutrophile leucocytes, which is the variety usually increased during infectious diseases. *"Mixed leucocytosis"* refers to an increase of all varieties of leucocytes. *Lymphocytosis* signifies an increase of the mononuclear cells only.

The forms of leucocytosis observed may be classified as follows:

*Physiological Leucocytosis:*
- Digestion leucocytosis.
- Leucocytosis of pregnancy.
- Leucocytosis of the new-born.

*Pathological Leucocytosis:*
- Inflammatory leucocytosis.
- Cachectic leucocytosis.
- Post-hemorrhagic leucocytosis.
- Ante-mortem leucocytosis.

*Digestion Leucocytosis.*—In subjects with active powers of digestion, a full meal, especially if including meats, raises the number of leucocytes from 1,000 to 10,000. The increase begins 1 to 3 hours after the meal and continues during active gastric digestion. It is more pronounced in infants than in adults. It is often absent in cases of advanced chronic gastritis and carcinoma, a fact which has been applied in the diagnosis between gastric ulcer and cancer.

*Leucocytosis of Pregnancy.*—During the latter months of first pregnancies a rather inconstant leucocytosis is usually present, the numbers reaching 12,000 to 15,000. It disappears soon after childbirth.

*Leucocytosis of the New-born.*—During the first few days after birth, infants' blood usually shows from 20,000 to 25,000 leucocytes, the numbers gradually becoming normal in two to three weeks. This leucocytosis is probably in part referable to continuous digestion and to the venosity and concentration of infants' blood.

Lymphocytosis is very frequently observed in infants and children.

*Inflammatory Leucocytosis.*—In infectious diseases leucocytosis occurs, in general, with those diseases associated with an exudative lesion, and is absent in most others.

Pronounced leucocytosis has been observed in pneumonia, diphtheria, scarlatina, erysipelas, rheumatism, and all acute purulent processes. In the absence of complications, it is usually absent in typhoid fever, miliary tuberculosis, malaria, and measles.

*Leucocytosis of Pneumonia.*—An initial hypoleucocytosis has been observed during the first hours of the disease. When the leucocytes remain low during the course of severe pneumonia, the disease is almost invariably fatal.

At the height of the disease 25,000 to 85,000 leucocytes may be found in the blood, the increase showing considerable relation to the extent of the lesion, but measuring more accurately the systemic reaction against the infectious process.

At, or just before, the crisis, eosinophile leucocytes begin to reappear in the blood.

In diagnosis, the absence of leucocytosis is strong negative evidence against pneumonia. As against other diseases unaccompanied by leucocytosis, such as typhoid fever and tuberculosis, a marked increase of leucocytes favors the diagnosis of pneumonia.

*Leucocytosis of Diphtheria.*—The leucocytosis of diphtheria follows the same general rules as in pneumonia. The increase of leucocytes is rather greater, especially in children; but may be slight or absent in mild cases. Persistent hypoleucocytosis has been observed in malignant cases. The extent of the membrane has considerable influence upon the grade of leucocytosis. Lymphocytosis may be very marked with or without extensive lymphadenitis.

### THE BLOOD IN TYPHOID FEVER.

During the course of uncomplicated typhoid fever the leucocytes are usually diminished in number. In the first week of the disease the polynuclear leucocytes are principally decreased, leaving a relatively large number of lymphocytes.

With the increasing hyperplasia of the lymphatic structures of

relative or absolute

Eosinophilia, takes place in Tape-worms &c -
pecially Anchylostoma, Trichinosis and in
Spasmodic Asthma. Eosinophiles are abundant
in Sputum of Asthma, and pus of acute
Gonorrhoea.

— Eosinophile

When there is reaction with dilution of 1 to 40, Typhoid is almost certain.

In Typhoid agglutination, the bacilli are quiet. In Acute Miliary Tbc., agglutinating bacilli are motile to some extent.

Colon bacillus will agglutinate 1 to 100 or 200

Widal reaction persists for two or three years in the blood.

the ileum, mesentery, and spleen, the lymphocytes are distinctly increased in number, so that a true lymphocytosis characterizes the blood in the second, third and fourth weeks. In the later stages of the disease the suppuration of ulcers and frequent inflammatory complications may cause polynuclear leucocytosis.

WIDAL'S TEST.—This test is based upon the action on cultures of *bacillus typhosus* of bactericidal substances developed in the blood of subjects suffering from typhoid fever.

When to a tube of a twenty-four hours' broth culture of *bacillus typhosus* is added 1-10th as much blood serum from a patient suffering from typhoid fever and showing the typical reaction, the bacteria are rendered motionless and precipitated from the fluid in clumps. The reaction may be performed with a hanging drop preparation of the culture to which, when a drop of dissolved blood is added from a platinum loop, the typical reaction is indicated by the rapid *loss of motility* of the bacilli and their *clumping* in characteristic masses.

When Widal's test is properly performed, by adding 1 part of serum to 10 of the culture a prompt reaction renders the diagnosis of typhoid fever *extremely probable.*

When 1 part of serum is added to 20 of the culture a prompt reaction is almost certainly indicative of typhoid fever.

On the other hand, a negative reaction is of little value as against typhoid fever.

The partial reactions often secured with less diluted blood are unreliable.

*Occurrence of Widal's Reaction.*—From the compilation of a large number of cases the New York Health Board concludes that Widal's reaction is present in typhoid fever—

From the fourth to seventh day in 70 per cent. of the cases.

From the eighth to fourteenth day in 80 per cent. of the cases.

During the third and fourth weeks in 90 per cent. of the cases.

It is absent throughout in 5 to 10 per cent. of the cases.

Widal's reaction persists in the blood for months, or even years, but after three to four months is usually feeble.

*Method of Procedure.*—It is usually required to make the test from specimens of dried blood which do not permit of exact dilution.

Sufficiently accurate results may be obtained by dissolving the

clot in an equal quantity of distilled water, and mixing one part of the solution with nine parts of the culture by means of a capillary tube. The mixing pipette of the hemocytometer is graduated in ten divisions, and may be used for this purpose, the mixed fluids being carefully expelled on a cover glass for the hanging drop preparation.

With a *sharp reaction* the loss of motility and clumping is complete by the time the preparation is ready for examination. A *distinct reaction* may not be complete till after 5 to 15 minutes have elapsed. In *feeble reactions* the clumping is not complete till after 30 minutes, and some bacilli are apt to remain permanently motile. Such reactions are unreliable evidences of typhoid fever.

A rough demonstration of Widal's reaction may be made by dissolving the clot in distilled water and preparing the hanging drop by means of a platinum loop.

The culture to be used should be a 24-hour broth culture of medium virulence, actively motile, and grown in the thermostat.

### THE BLOOD IN TUBERCULOSIS.

Acute or chronic miliary tuberculous lesions are nearly always unaccompanied by leucocytosis.

A slight relative or absolute increase of mononuclear leucocytes is usually seen in pulmonary and lymphatic tuberculosis.

The moderate chlorotic anæmia and increase of lymphocytes is so frequently noted that the term *"lymphatic anæmia"* has been applied to these cases of chronic tuberculosis. When leucocytosis arises in the course of pulmonary tuberculosis it usually signifies a purulent complication, or hemorrhage, or suppuration of cavities, or cachexia.

### SUPPURATIVE VS. NON-SUPPURATIVE LESIONS.

The examination of the blood for leucocytosis is often of decisive value in the diagnosis between:

Tuberculous and concealed suppurative processes;

Tuberculous and suppurative meningitis;

Typhoid fever and appendicitis.

# Milk

Holt's apparatus for testing human milk is best. In cow's milk, test the Sp. Gr. The fat in Cow's milk can be determined by Feser's Lactoscope or by Babcock method. Sugar can be tested by ppt. the proteids then using Fehlings Sol. Proteids Can be determined by a modification of Esbach's method; remove fat first and then put the serum in the Esbach tube.