**2. Epithelial Cells.**—A few cells from various parts of the urinary tract occur in every urine. A marked increase indicates some pathologic condition at the site of their origin. It is sometimes, but by no means always, possible to locate their source from their form. Most cells are much altered from their original shape. Any epithelial cell may be so granular from degenerative changes that the nucleus is obscured. They are usually divided into three groups:

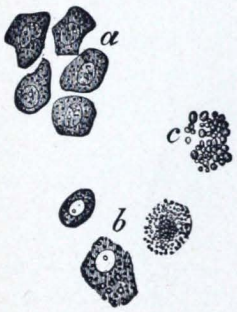



Fig. 52.—Renal epithelium from nephritic urine: *a*, Polyhedral epithelium in nephritis of scarlet fever; *b* and *c*, different grades of fatty degeneration in renal epithelium in chronic nephritis (×400) (after Bizzozero).

(1) **Small, round** or **polyhedral cells** are about the size of pus-corpuscles, or a little larger, with a single round nucleus. Such cells may come from the deeper layers of any part of the urinary tract. They are uncommon in normal urine. When they are dark in color, very granular, and contain a comparatively large nucleus, they probably come from the renal tubules, but their origin in the kidney is not proved unless they are found embedded in casts. Renal cells are abundant in parenchymatous nephritis, especially the acute form. They are nearly always fatty—most markedly so in chronic paren chymatous nephritis, where their substance is sometimes wholly replaced by fat-droplets ("compound granule cells") (see Figs. 48, 52, and 63).

(2) **Irregular cells** are considerably larger than the preceding. They are round, pear shaped, or spindle shaped, or may have tail-like processes, and are hence

named large round, pyriform, spindle, or caudate cells respectively. Each contains a round or oval distinct nucleus. Their usual source is the deeper layers of the urinary tract, especially of the bladder. Caudate forms come most commonly from the pelvis of the kidney (see Figs. 53, *b*, 54, 65, and 66).

(3) **Squamous** or **pavement cells** are large flat cells, each with a small, distinct, round or oval nucleus (Fig. 53, *a*). They are derived from the superficial layers of

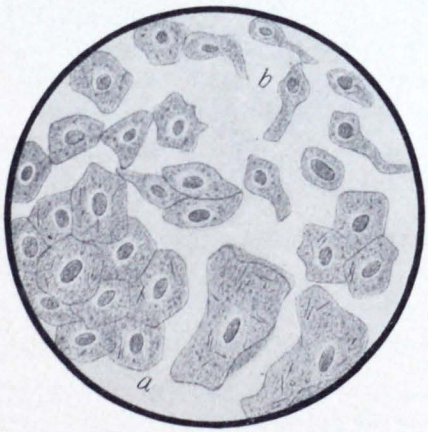



Fig. 53.—Epithelial cells from urethra and bladder: *a*, Squamous cells from superficial layers; *b*, irregular cells from deeper layers (Jakob).

the ureters, bladder, urethra, or vagina, and when desquamation is active, appear in stratified masses. Squamous cells from the bladder are generally rounded, while those from the vagina are larger, thinner, and more angular. Great numbers of these vaginal cells, together with pus-corpuscles, may be present when leukorrhea exists.

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

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

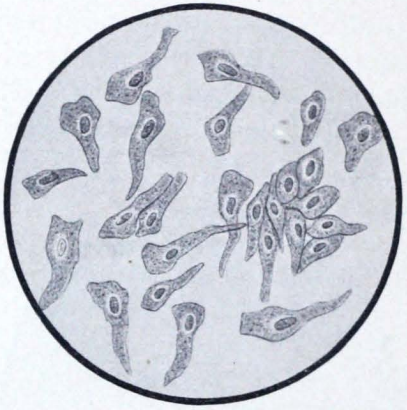


Fig. 54.—Caudate epithelial cells from pelvis of kidney (Jakob).

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

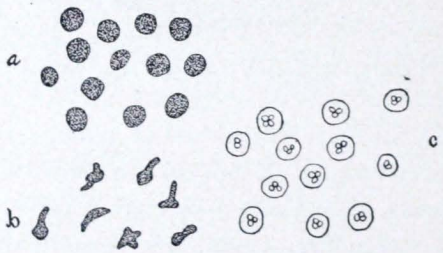



Fig. 55.—Pus-corpuscles: *a*, As ordinarily seen; *b*, ameboid corpuscles; *c*, showing the action of acetic acid (Ogden).

der the microscope the corpuscles appear as very granular cells, about twice the diameter of a red blood-corpuscle (Figs. 55 and 66). In freshly voided urine many

exhibit ameboid motion, assuming irregular outlines. Each contains one irregular nucleus or several small, rounded nuclei. The nuclei are obscured or entirely hidden by the granules, but may be brought clearly into view by running a little acetic acid under the cover-glass. This enables one to easily distinguish pus-corpuscles from small round epithelial cells, which resemble them in size, but have a single, rather large, round nucleus. In decomposing urine pus is converted into a gelatinous mass which gives the urine a ropy consistence.

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

A fairly accurate idea of the quantity of pus from day to day may be had by shaking the urine thoroughly and counting the number of corpuscles per cubic millimeter upon the Thoma-Zeiss blood-counting slide.

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

Red blood-corpuscles are not usually difficult to recognize with the microscope. When very fresh, they have a

normal appearance, being yellowish discs of uniform size (normal blood). When they have been in the urine any considerable time, their hemoglobin may be dissolved out, and they then appear as faint colorless circles or " shadow cells " (abnormal blood), and are more difficult to see (Fig. 56; see also Figs. 49 and 63). They are apt to be swollen in dilute and crenated in concentrated urines. The microscopic findings may be corroborated by chemic tests for hemoglobin, although the microscope may show a few red corpuscles when the chemic tests are negative.

When not due to contamination from menstrual discharge, blood in the urine, or *hematuria*, is always patho-

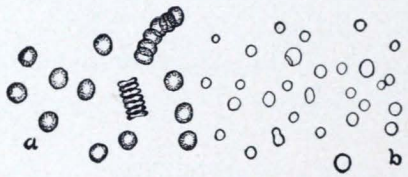



Fig. 56.—Blood-corpuscles: *a*, Normal; *b*, abnormal (Ogden).

logic. Blood comes from the *kidney tubules* in severe hyperemia, in acute nephritis and acute exacerbations of chronic nephritis, and in renal tuberculosis and malignant disease. An " idiopathic hematuria," probably of nervous origin, has been observed. The finding of blood-casts is the only certain means of diagnosing the kidney as its source. Blood comes from the *pelvis of the kidney* in renal calculus (Fig. 65), and is then usually intermittent, small in amount, and accompanied by a little pus and perhaps crystals of the substance forming the stone. Considerable hemorrhages from the *bladder* may occur in vesical calculus, tuberculosis, and new growths. Small amounts of blood generally accompany acute

cystitis. In Africa the presence of *Schistosomum hematobium* in the veins of the bladder is a common cause of hemorrhage (Egyptian hematuria).

**5. Spermatozoa** are generally present in the urine of men after nocturnal emissions, after epileptic convulsions, and in spermatorrhea. They may be found in the urine of both sexes following coitus. They are easily recognized from their characteristic structure (Fig. 57).

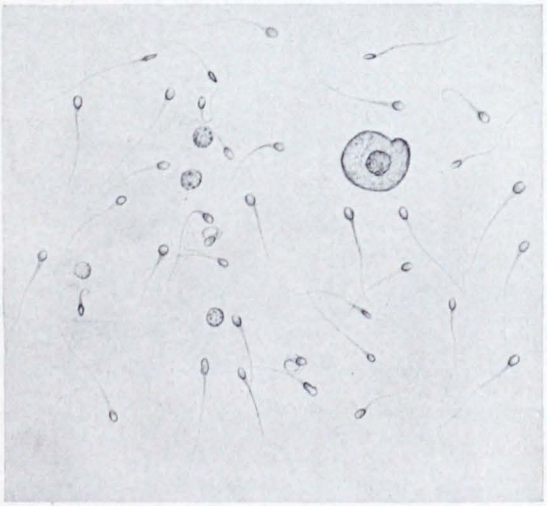


Fig. 57.—Spermatozoa in urine (Ogden).

The 4 mm. objective should be used, with subdued light and careful focusing.

**6. Bacteria.**—Normal urine is free from bacteria in the bladder, but becomes contaminated in passing through the urethra. Various non-pathogenic bacteria, notably *Micrococcus ureæ* (Fig. 58), are always present in decomposing urine. In suppurations of the urinary tract pus-producing organisms may be found. In many

infectious diseases the specific bacteria may be eliminated in the urine without producing any local lesion. Typhoid bacilli have been known to persist for months and even years after the attack.

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

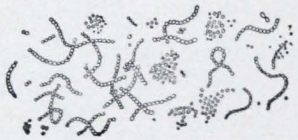


Fig. 58.—Micrococcus ureæ (after von Jaksch).

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

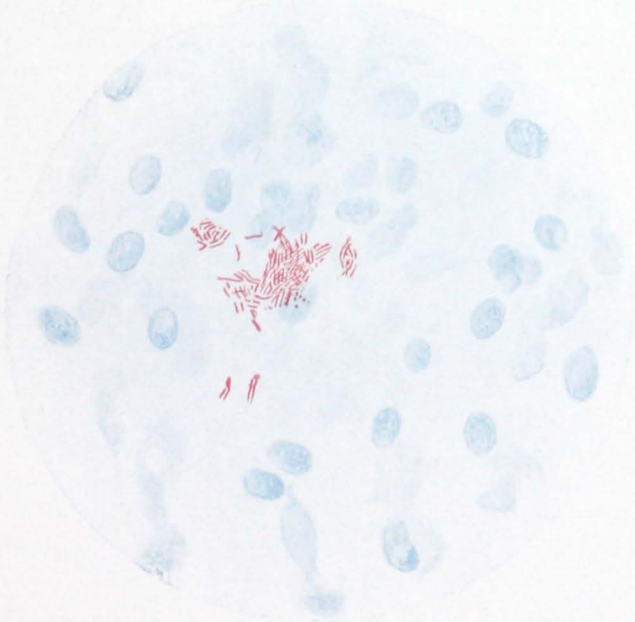
**Detection of Tubercle Bacilli in Urine.**—The urine should be obtained by catheter after careful cleansing of the parts.

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

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

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

# PLATE V



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

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

(5) Wash in water.

(6) Soak in alcohol fifteen minutes or longer. This decolorizes the smegma bacillus (p. 53), which is often present in the urine, and might easily be mistaken for the tubercle bacillus. It is unlikely, however, to be present in catheterized specimens. It is always safest to soak the smear in alcohol for several hours or over night, since some strains of the smegma bacillus are very resistant.

(7) Wash in water.

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

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

When the bacilli are scarce, the following method may be tried. It is applicable also to other fluids. If the fluid is not albuminous, add a little egg-albumen. Coagulate the albumen by gentle heat and centrifugalize. The bacilli will be carried down with the albumen. The sediment is then treated by the antiformin method (p. 52).

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

In gonorrhea **gonococci** are sometimes found in the sediment, but more commonly in the "gonorrheal threads," or "floaters." In themselves, these threads are by no means diagnostic of gonorrhea. Detection of the gonococcus is described later (p. 369).

**7. Animal parasites** are rare in the urine. Hooklets and scolices of *Tænia echinococcus* (Fig. 59) and em-

bryos of filariæ have been met. In Africa the ova, and even adults, of *Schistosomum hæmatobium* are common,

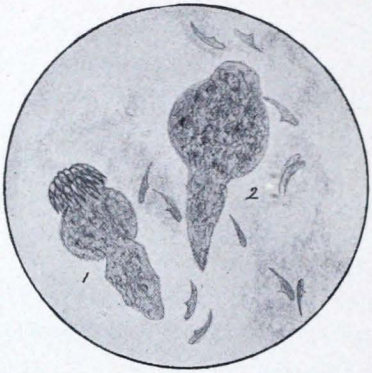



Fig. 59.—1, Scolex of tænia echinococcus, showing crown of hooklets; 2, scolex and detached hooklets (obj. one-sixth) (Boston).

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

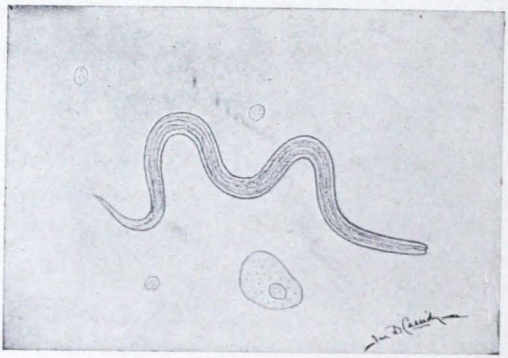


Fig. 60.—Embryo of "vinegar eel" in urine, from contamination; length, 340 μ; width, 15 μ. An epithelial cell from bladder and three leukocytes are also shown (studied with Dr. J. A. Wilder).

other protozoa may be mistaken for spermatozoa by the inexperienced.

A worm which is especially interesting is *Anguillula aceti*, the "vinegar eel." This is generally present in the sediment of table vinegar, and may reach the urine through use of vinegar in vaginal douches, or through contamination of the bottle in which the urine is contained. It has been mistaken for *Strongyloides intestinalis* and for the filaria embryo. It closley resembles the former in both adult and embryo stages. The young embryos have about the same length as filaria embryos, but are nearly twice as broad and the intestinal canal is easily seen (compare Figs. 60 and 134). For fuller descriptions of these parasites the reader is referred to Chapter VI.

### C. Extraneous Structures

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

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

in diabetic urine, and may reach the bladder and multiply there.

*Mold fungi* (Fig. 62) are characterized by refractive, jointed, or branched rods (hyphæ), often arranged in a network, and by highly refractive, spheric or ovoid spores.

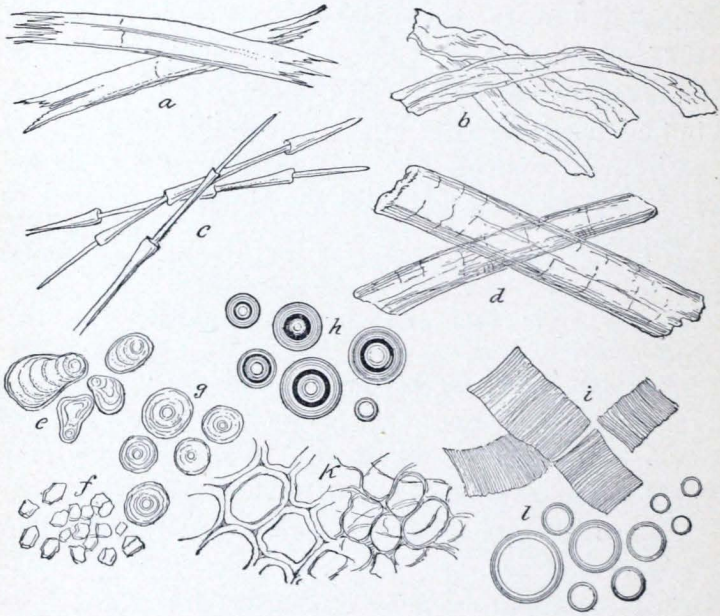



Fig. 61.—Extraneous matters found in urine: *a*, Flax-fibers; *b*, cotton-fibers; *c*, feathers; *d*, hairs; *e*, potato-starch; *f*, rice-starch granules; *g*, wheat-starch; *h*, air-bubbles; *i*, muscular tissue; *k*, vegetable tissue; *l*, oil-globules.

They are common in urine which has stood exposed to the air.

*Fibers* of wool, cotton, linen, or silk, derived from towels, the clothing of the patient, or the dust in the air, are present in almost every urine. *Fat-droplets* are most frequently derived from unclean bottles or oiled cathe-

ters. *Starch-granules* may reach the urine from towels, the clothing, or dusting-powders. They are recognized by their concentric striations and their blue color with iodin solution. *Lycopodium granules* (Fig. 5) may also reach the urine from dusting-powders. They might be mistaken for the ova of parasites. *Bubbles of air* are often confusing to beginners, but are easily recognized

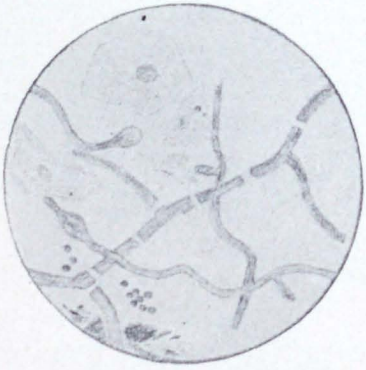


Fig. 62.—Aspergillus from urine (Boston).

after once being seen. *Scratches* and *flaws* in the glass of slide or cover are likewise a common source of confusion to beginners.

## IV. THE URINE IN DISEASE

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

1. **Renal Hyperemia.**—*Active hyperemia* is usually an early stage of acute nephritis, but may occur independently as a result of temporary irritation. The urine is generally decreased in quantity, highly colored, and strongly acid. Albumin is always present—usually in traces only, but sometimes in considerable amount for a

day or two. The sediment contains a few hyaline and finely granular casts and an occasional red blood-cell.

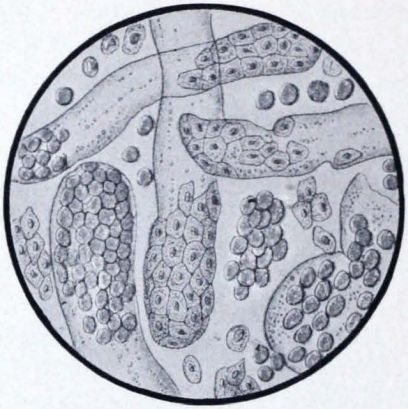


Fig. 63.—Sediment from acute hemorrhagic nephritis: Red blood-corpuscles; leukocytes; renal cells not fattily degenerated; epithelial and blood-casts (Jakob).

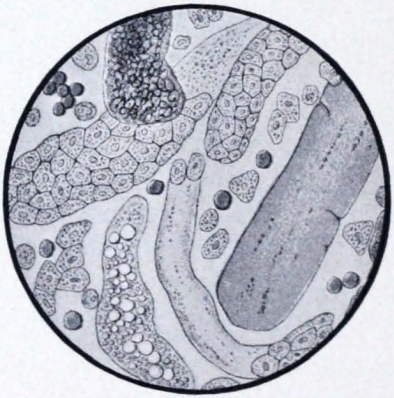


Fig. 64.—Sediment from chronic parenchymatous nephritis: Hyaline (with cells attached), waxy, brown granular, fatty, and epithelial casts; fattily degenerated renal cells, and a few white and red blood-corpuscles (Jakob).

In very severe hyperemia the urine approaches that of acute nephritis.

*Passive hyperemia* occurs most commonly in diseases of

the heart and liver and in pregnancy. The quantity of urine is somewhat low and the color high, except in pregnancy. Albumin is present in small amount only. The sediment contains a very few hyaline or finely granular casts. In pregnancy the amount of albumin should be carefully watched, as any considerable quantity, and especially a rapid increase, strongly suggests approaching eclampsia.

2. **Nephritis.**—The various degenerative and inflammatory conditions grouped under the name of nephritis have certain features in common. The urine in all cases contains albumin and tube-casts, and in all well-marked cases shows a decrease of normal solids, especially of urea and the chlorids. In chronic nephritis, especially of the interstitial type, there may be remissions during which the urine is practically normal. The characteristics of the different forms are well shown in the table on page 176, modified from Hill.

3. **Renal Tuberculosis.**—The urine is pale, usually cloudy. The quantity may not be affected, but is apt to be increased. In early cases the reaction is faintly acid and there are traces of albumin and a few renal cells. In advanced cases the urine is alkaline, has an offensive odor, and is irritating to the bladder. Albumin in varying amounts is always present. Pus is nearly always present, though frequently not abundant. It is generally intimately mixed with the urine, and does not settle so quickly as the pus of cystitis. Casts, though present, are rarely abundant, and are obscured by the pus. Small amounts of blood are common. Tubercle bacilli are nearly always present, although animal inoculation may be necessary to detect them.

## THE URINE IN NEPHRITIS

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

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

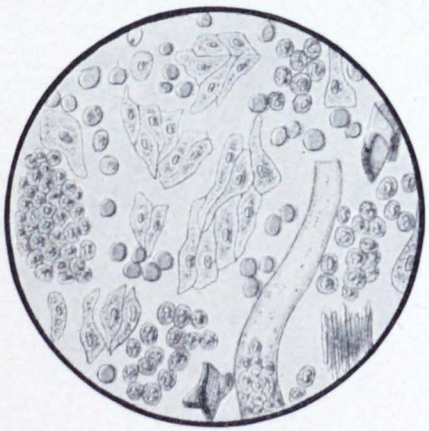


Fig. 65.—Sediment from calculous pyelitis: Numerous pus-corpuscles, red blood-corpuscles, and caudate and irregular epithelial cells; a combination of hyaline and pus-casts, and a few uric-acid crystals (Jakob).

pyelitis, and variable amounts of pus then appear, the urine remaining acid in reaction.

5. **Pyelitis.**—In pyelitis the urine is slightly acid, and contains a small or moderate amount of pus, together with many spindle and caudate epithelial cells. Pus-casts may appear if the process extends up into the kidney tubules (see Fig. 65). Albumin is always present, and its amount, in proportion to the amount of pus, is decidedly greater than is found in cystitis. This fact is

of much value in differential diagnosis. Even when pus is scanty, albumin is rarely under 0.15 per cent., which is the maximum amount found in cystitis with abundant pus.

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

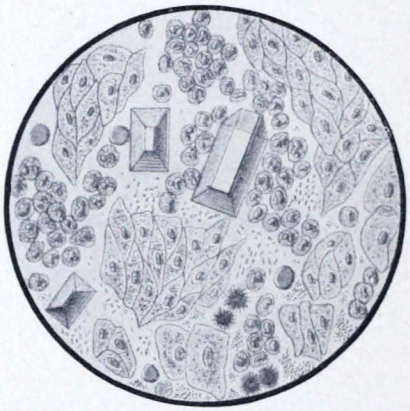


Fig. 66.—Sediment from cystitis (chronic): Numerous pus-corpuscles, epithelial cells from the bladder, and bacteria; a few red blood-corpuscles and triple phosphate and ammonium urate crystals (Jakob).

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

7. **Vesical Calculus, Tumors, and Tuberculosis.**—These conditions produce a chronic cystitis, with its

characteristic urine. Blood, however, is more frequently present and more abundant than in ordinary cystitis. With neoplasms, especially, considerable hemorrhages are apt to occur. Particles of the tumor are sometimes passed with the urine. No diagnosis can be made from the presence of isolated tumor cells. In tuberculosis tubercle bacilli can generally be detected.

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

9. **Diabetes Mellitus.**—The quantity of urine is very large. The color is generally pale, while the specific gravity is nearly always high—1.030 to 1.050, very rarely below 1.020. The presence of glucose is the essential feature of the disease. The amount of glucose is often very great, sometimes exceeding 8 per cent., while the total elimination may exceed 500 gm. in twenty-four hours. It may be absent temporarily. Acetone is generally present in advanced cases. Diacetic and oxybutyric acids may be present, and usually warrant an unfavorable prognosis. Accompanying the acidosis there is a corresponding increase in amount of ammonia.

## CHAPTER III

## THE BLOOD

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

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

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

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

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

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

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

The *reaction* is alkaline to litmus.

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

*Coagulation* consists essentially in the transformation of fibrinogen, one of the proteins of the blood, into fibrin by means of a ferment derived from disintegration of the leukocytes. The presence of calcium salts is necessary to the process. The resulting coagulum is made up of a meshwork of fibrin fibrils with entangled corpuscles and plaques. The clear, straw-colored fluid which is left after separation of the coagulum is called *blood-serum.* Normally, coagulation takes place in two to eight minutes after the blood leaves the vessels. It is frequently

desirable to determine the coagulation time. The simplest method is to place a drop of blood upon a perfectly clean slide, and to draw a needle through it at half-minute intervals. When the clot is dragged along by the needle, coagulation has taken place. This method is probably sufficient for ordinary clinical work. For very accurate results the method of Russell and Brodie, as modified by Boggs, is recommended. The instrument is shown in Fig. 67. A drop of blood is placed upon the cone, which is then quickly inverted in the moist chamber.

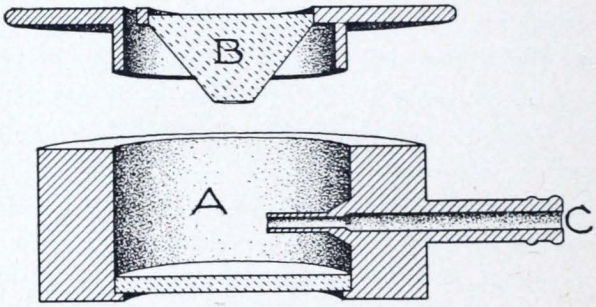



Fig. 67.—Boggs' coagulation instrument: A, moist chamber; B, glass cone; C, tube through which air is blown.

By means of a rubber bulb puffs of air are blown against the blood at intervals, while the motion of the corpuscles is watched with a low-power objective. Coagulation is complete when the red cells move only en masse and spring back to their original position when the current ceases. Coagulation is notably delayed in hemophilia and icterus and after administration of citric acid. It is hastened by administration of calcium salts.

For certain purposes, especially in bacteriologic and opsonic work, it is desirable to prevent coagulation

of the blood that is withdrawn. This may be accomplished by receiving it directly into a solution of 1 per cent. sodium citrate (or ammonium oxalate) and 0.85 per cent. sodium chlorid. This precipitates the calcium salts which are necessary to coagulation.

For most clinical examinations only one drop of blood is required. This may be obtained from the lobe of the ear, the palmar surface of the tip of the finger, or, in the case of infants, the plantar surface of the great toe. In general, the finger will be found most convenient. With nervous children the lobe of the ear is preferable, as it prevents their seeing what is being done. An edematous or congested part should be avoided. The site

Fig. 68.—Daland's blood-lancet.

should be well rubbed with alcohol to remove dirt and epithelial débris and to increase the amount of blood in the part. After allowing sufficient time for the circulation to equalize, the skin is punctured with a blood lancet (of which there are several patterns upon the market) or some substitute, as a Hagedorn needle, aspirating needle, trocar, or a pen with one of its nibs broken off. Nothing is more unsatisfactory than an ordinary sewing-needle. The lancet should be cleaned with alcohol before and after using, but need not be sterilized. It must be very sharp. If the puncture be made with a *firm, quick, rebounding stroke*, it is practically painless. The first drop of blood which appears should

be wiped away, and the second used for examination. The blood should not be pressed out, since this dilutes it with serum from the tissues; but moderate pressure some distance above the puncture is allowable.

When a larger amount of blood is required, it may be obtained with a sterile hypodermic syringe from one of the veins at the elbow, as described on p. 245.

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

## I. HEMOGLOBIN

Hemoglobin is an iron-bearing protein. It is found only within the red corpuscles, and constitutes about 90 per cent. of their weight. The actual amount of hemoglobin is never estimated clinically: it is the relation which the amount present bears to the normal which is determined. Thus the expression, "50 per cent. hemoglobin," when used clinically, means that the blood contains 50 per cent. of the normal. Theoretically, the normal would be 100 per cent., but with the methods of estimation in general use the blood of healthy persons ranges from 85 to 105 per cent.; these figures may, therefore, be taken as normal.

*Increase of hemoglobin*, or *hyperchromemia*, is uncommon, and is probably more apparent than real. It accompanies an increase in number of erythrocytes, and may be noted in change of residence from a lower to a

higher altitude; in poorly compensated heart disease with cyanosis; in concentration of the blood from any cause, as the severe diarrhea of cholera, and in "idiopathic polycythemia."

*Decrease of hemoglobin*, or *oligochromemia*, is very common and important. It is the most striking feature of the secondary anemias (p. 277). Here the hemoglobin loss may be slight or very great. In mild cases a slight decrease of hemoglobin is the only blood change noted. In very severe cases, especially in repeated hemorrhages, malignant disease, and infection by the hookworm and *Dibothriocephalus latus*, hemoglobin may fall to 15 per cent. Hemoglobin is always diminished, and usually very greatly, in chlorosis, pernicious anemia, and leukemia.

*Estimation of hemoglobin* is less tedious and usually more helpful than a red corpuscle count. It offers the simplest and most certain means of detecting the existence and degree of anemia, and of judging the effect of treatment in anemic conditions. Pallor, observed clinically, does not always denote anemia.

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

(1) **Von Fleischl Method.**—The apparatus consists of a stand somewhat like the base and stage of a microscope (Fig. 69). Under the stage is a movable bar of colored glass, shading from pale pink at one end to deep red at the other. The frame in which this bar is held is marked with a scale of hemoglobin percentages corresponding to the different shades of red. By means of a rack and pinion, the color-bar can be moved from end to end beneath a round opening in the center

of the stage. A small metal cylinder, which has a glass bottom and which is divided vertically into two equal compartments, can be placed over the opening in the stage so that one of its compartments lies directly over the color-bar. Accompanying the instrument are a number of short capillary tubes in metal handles.

Having punctured the finger-tip or lobe of the ear, as already described, wipe off the first drop of blood, and from the

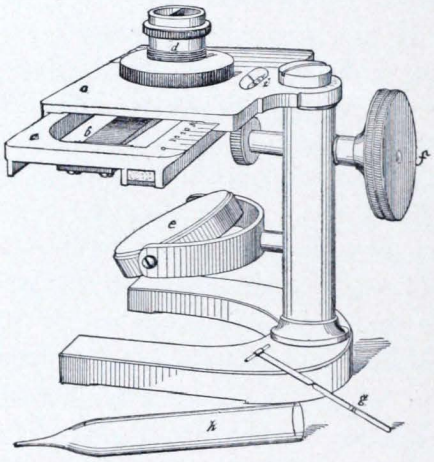


Fig. 69.—Von Fleischl's hemoglobinometer: *a*, Stand; *b*, narrow wedge-shaped piece of colored glass fitted into a frame (*c*), which passes under the chamber; *d*, hollow metal cylinder, divided into two compartments, which holds the blood and water; *e*, plaster-of-Paris plate from which the light is reflected through the chamber; *f*, screw by which the frame containing the graduated colored glass is moved; *g*, capillary tube to collect the blood; *h*, pipet for adding the water; *i*, opening through which may be seen the scale indicating percentage of hemoglobin.

second fill one of the capillary tubes. Hold the tube horizontally, and touch its tip to the drop of blood, which will readily flow into it if it be clean and dry. Avoid getting any blood upon its outer surface. With a medicine-dropper, rinse the blood from the tube into one of the compartments of the cylinder, using distilled water, and mix well. Fill both com-

partments level full with distilled water, and place the cylinder over the opening in the stage, so that the compartment which contains only water lies directly over the bar of colored glass. If there are any clots in the hemoglobin compartment, clean the instrument and begin again.

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

A recent modification of the von Fleischl apparatus by Miescher gives an error which need not exceed 1 per cent. It is, however, better adapted to laboratory use than to the needs of the practitioner.

(2) The **Sahli hemoglobinometer** (Fig. 70) is an improved form of the well-known Gowers instrument. It consists of a hermetically sealed comparison tube containing a 1 per cent. solution of acid hematin, a graduated test-tube of the same diameter, and a pipet of 20-c.mm. capacity. The two tubes are held in a black frame with a white ground-glass back.

Place a few drops of decinormal hydrochloric acid solution in the graduated tube. Obtain a drop of blood and draw it into the pipet to the 20 c.mm. mark. Wipe off the tip of the pipet, blow its contents into the hydrochloric acid solution in the tube, and rinse well. In a few minutes the hemoglobin is changed to acid hematin. Place the two tubes in the compartments of the frame, and dilute the fluid with water drop

by drop, mixing after each addition, until it has exactly the same color as the comparison tube. The graduation corresponding to the surface of the fluid then indicates the percentage of hemoglobin. Decinormal hydrochloric acid solution may be prepared with sufficient accuracy for this purpose

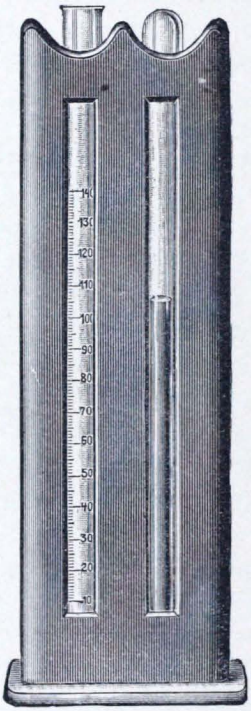


Fig. 70.—Sahli's hemoglobinometer.

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

This method is very satisfactory in practice, and is accurate to within 5 per cent. The comparison tube is said to keep its color indefinitely, but, unfortunately, not all the instruments upon the market are well standardized.

(3) **Dare's hemoglobinometer** (Fig. 71) differs from the others in using undiluted blood. The blood is allowed to flow by capillarity into the slit between two small plates of glass. It is then placed in the instrument and compared with different portions of a circular disc of colored glass. The reading must be made quickly, before clotting takes place. This instrument is easy to use, and is one of the most accurate.

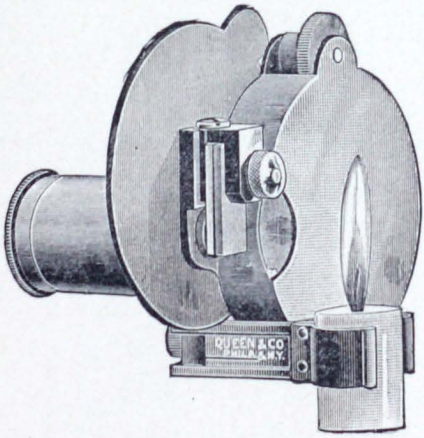


Fig. 71.—Dare's hemoglobinometer.

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

Mix chloroform and benzol in a urinometer tube, so that the specific gravity of the mixture is near the probable specific gravity of the blood. Add a drop of blood by means of a pipet of small caliber. A pipet like that shown in Fig. 161, A will be found satisfactory. If the drop floats near the surface, add a little benzol; if it sinks to the bottom, add a little chloroform. When it remains stationary near the middle, the mix-

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

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

For accurate results with this method, care and patience are demanded. The following precautions must be observed:

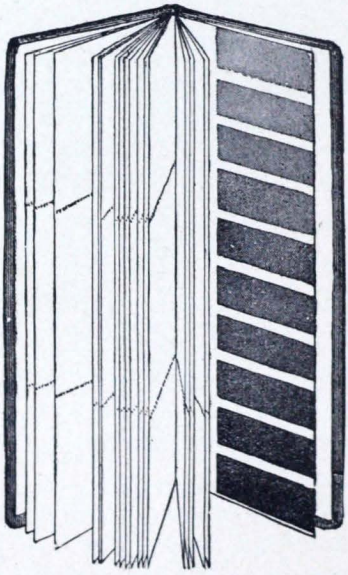


Fig. 72.—Tallquist's hemoglobin scale.

(*a*) The two fluids must be well mixed after each addition of chloroform or benzol. Close the tube with the thumb and invert several times. Should this cause the drop of blood to

break up into very small ones, adjust the specific gravity as accurately as possible with these, and test it with a fresh drop.

(*b*) The drop of blood must not be too large; it must not contain an air-bubble, it must not adhere to the side of the tube, and it must not remain long in the fluid.

(*c*) The urinometer must be standardized for the chloroform-benzol mixture. Most urinometers give a reading two or three degrees too high, owing to the low surface tension. Make a mixture such that a drop of distilled water will remain suspended in it (*i. e.*, with a specific gravity of 1.000) and correct the urinometer by this.

(5) **Tallquist Method.**—The popular Tallquist hemoglobinometer consists simply of a book of small sheets of absorbent paper and a carefully printed scale of colors (Fig. 72).

Take up a large drop of blood with the absorbent paper, and when the humid gloss is leaving, before the air has darkened the hemoglobin, compare the stain with the color scale. The color which it matches gives the percentage of hemoglobin. Except in practised hands, this method is accurate only to within 10 or 20 per cent.

Of the methods given, the physician should select the one which best meets his needs. With any method, practice is essential to accuracy. The von Fleischl has long been the standard instrument, but has lately fallen into some disfavor. For accurate work the best instruments are the von Fleischl-Miescher and the Dare. They are, however, expensive, and it is doubtful whether they are enough more accurate than the Sahli instrument to justify the difference in cost. The latter is probably the most satisfactory for the practitioner, provided a well-standardized color-tube is obtained. The specific gravity method is very useful when special instruments are not at hand. The Tallquist scale is so inexpensive and so

convenient that it should be used by every physician at the bedside and in hurried office work; but it should not supersede the more accurate methods.

## II. ENUMERATION OF ERYTHROCYTES

In health there are about 5,000,000 red corpuscles per cubic millimeter of blood. Normal variations are slight. The number is generally a little less—about 4,500,000—in women.

*Increase of red corpuscles*, or *polycythemia*, is unimportant. There is a decided increase following change of residence from a lower to a higher altitude, averaging about 50,000 corpuscles for each 1000 feet, but frequently much greater. The increase, however, is not permanent. In a few months the erythrocytes return to nearly their original number. Three views are offered in explanation: (*a*) Concentration of the blood, owing to increased evaporation from the skin; (*b*) stagnation of corpuscles in the peripheral vessels because of lowered blood-pressure; (*c*) new formation of corpuscles, this giving a compensatory increase of aëration surface.

Pathologically, polycythemia is uncommon. It may occur in: (*a*) Concentration of the blood from severe watery diarrhea; (*b*) chronic heart disease, especially the congenital variety, with poor compensation and cyanosis; and (*c*) idiopathic polycythemia, which is considered to be an independent disease, and is characterized by cyanosis, blood counts of 7,000,000 to 10,000,000, hemoglobin 120 to 150 per cent., and a normal number of leukocytes.

*Decrease of red corpuscles*, or *oligocythemia*. Red corpuscles and hemoglobin are commonly decreased together, although usually not to the same extent.

Oligocythemia occurs in all but the mildest symptomatic anemias. The blood-count varies from near the normal in moderate cases down to 1,500,000 in very severe cases. There is always a decrease of red cells in chlorosis, but it is often slight, and is relatively less than the decrease of hemoglobin. Leukemia gives a decided oligocythemia, the average count being about 3,000,000. The greatest loss of red cells occurs in pernicious anemia, where counts below 1,000,000 are not uncommon.

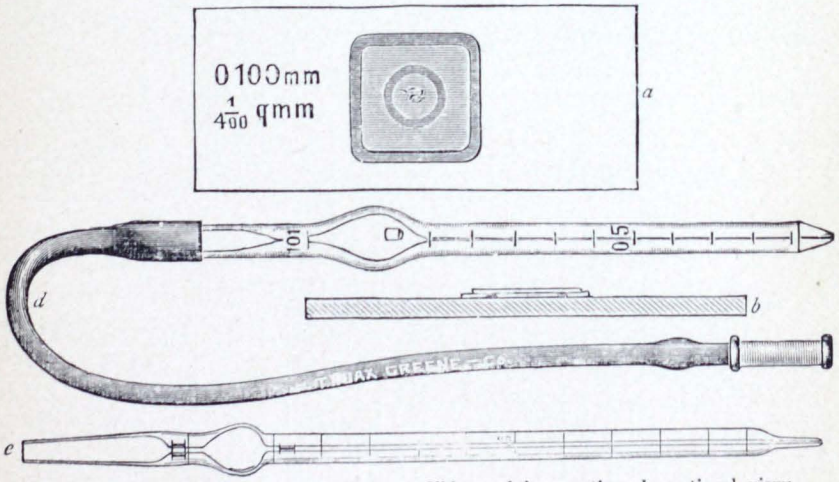



Fig. 73.—Thoma-Zeiss hemocytometer: *a*, Slide used in counting; *b*, sectional view; *d*, red pipet; *e*, white pipet.

The most widely used and most satisfactory instrument for counting the corpuscles is that of Thoma-Zeiss. The hematocrit is not to be recommended for accuracy, since in anemia, where blood-counts are most important, the red cells vary greatly in size and probably also in elasticity. The hematocrit is, however, useful in determining the relative volume of corpuscles and plasma (Volume Index, p. 200), and seems to be gaining in favor.

The **Thoma-Zeiss instrument** consists of two pipets for diluting the blood and a counting chamber (Fig. 73). The counting chamber is a glass slide with a square platform in the middle. In the center of the platform is a circular opening, in which is set a small circular disc in such a manner that it is surrounded by a "ditch," and that its surface is

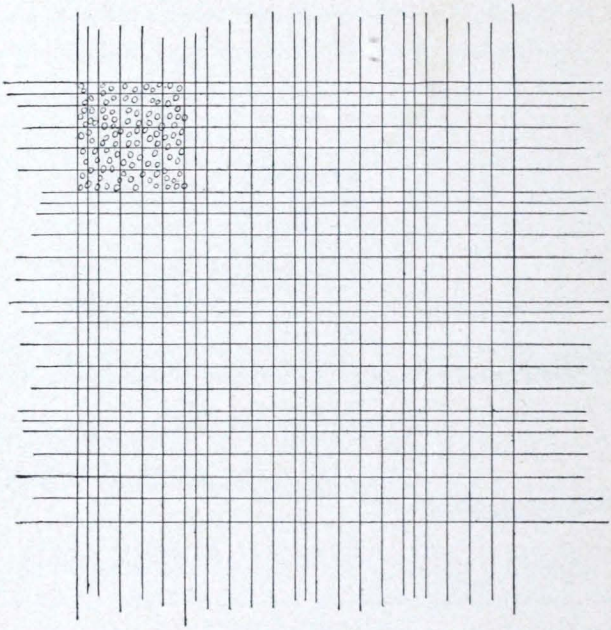


Fig. 74.—Ordinary ruling of counting chamber, showing red corpuscles in left upper corner.

exactly one-tenth of a millimeter below the surface of the square platform. Upon this disc is ruled a square millimeter, subdivided into 400 small squares. Each fifth row of small squares has double rulings for convenience in counting (Fig. 74). A thick cover-glass, ground perfectly plane, accompanies the counting chamber. Ordinary cover-glasses are of uneven surface, and should not be used with this instrument.

It is evident that, when the cover-glass is in place upon the platform, there is a space exactly one-tenth of a millimeter thick between it and the disc; and that, therefore, the square millimeter ruled upon the disc forms the base of a space holding exactly one-tenth of a cubic millimeter.

**Technic.**—To count the red corpuscles, use the pipet with 101 engraved above the bulb. It must be clean and dry. Obtain a drop of blood as already described. Suck blood

Fig. 75.—Method of drawing blood into the pipet (Boston),

into the pipet to the mark 0.5 or 1. Should the blood go beyond the mark, draw it back by touching the tip of the pipet to a moistened handkerchief. Quickly wipe off the blood adhering to the tip, plunge it into the diluting fluid, and suck the fluid up to the mark 101, slightly rotating the pipet meanwhile. This dilutes the blood 1 : 200 or 1 : 100, according to the amount of blood taken. Except in cases of severe anemia, a dilution of 1 : 200 is preferable. Close the ends

of the pipet with the fingers, and shake vigorously until the blood and diluting fluid are well mixed.

When it is not convenient to count the corpuscles at once, place a heavy rubber band around the pipet so as to close the ends, inserting a small piece of rubber-cloth or other tough, non-absorbent material, if necessary, to prevent the tip from punching through the rubber. It may be kept thus for twenty-four hours or longer.

When ready to make the count, clean the counting chamber and cover-glass, and place a sheet of paper over them to keep off dust. Mix the fluid thoroughly by shaking; blow two or three drops from the pipet, wipe off its tip, and then place a small drop (the proper size can be learned only by experience) upon the disc of the counting chamber. Adjust the cover immediately. Hold it by diagonal corners above the drop of fluid so that a third corner touches the slide and rests upon the edge of the platform. Place a finger upon this corner, and, by raising the finger, allow the cover to fall quickly into place. If the cover be properly adjusted, faint concentric lines of the prismatic colors—Newton's rings—can be seen between it and the platform when the slide is viewed obliquely. They indicate that the two surfaces are in close apposition. If they do not appear at once, slight pressure upon the cover may bring them out. Failure to obtain them is usually due to dirty slide or cover—both must be perfectly clean and *free from dust.* The drop placed upon the disc must be of such size that, when the cover is adjusted, it nearly or quite covers the disc, and that none of it runs over into the "ditch." There should be no bubbles upon the ruled area.

Allow the corpuscles to settle for a few minutes, and then examine with a low power to see that they are evenly distributed. If they are not *evenly distributed over the whole disc,* the counting chamber must be cleaned and a new drop placed in it.

Probably the most satisfactory objective for counting is the special 4 mm. with long working distance. To understand the principle of counting, it is necessary to remember that the large square (400 small squares) represents a capacity of one-tenth of a cubic millimeter. Find the number of corpuscles in the large square, multiply by 10 to find the

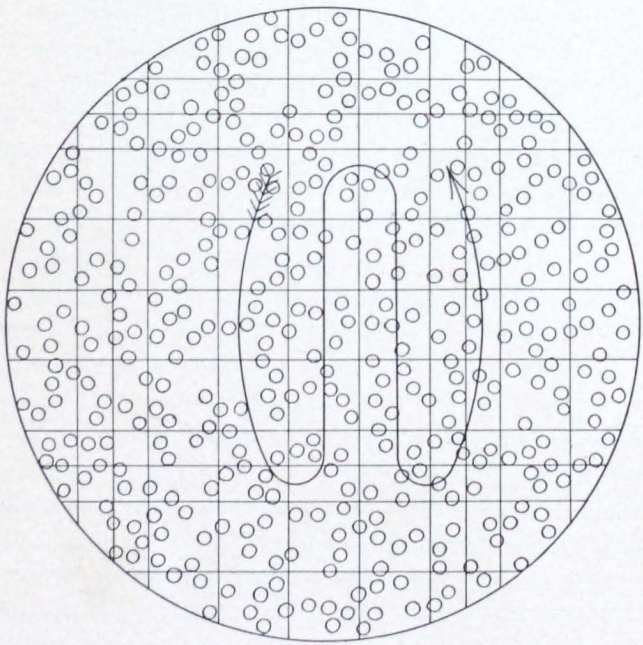


Fig. 76.—Appearance of microscopic field in counting red corpuscles. The arrow indicates the squares to be counted.

number in 1 c.mm. of the diluted blood, and finally, by the dilution, to find the number in 1 c.mm. of undiluted blood. Instead of actually counting all the corpuscles, it is customary to count those in only a limited number of small squares, and from this to calculate the number in the large square. Nearly every worker has his own method of doing this. The essential thing is to adopt a method and adhere to it.

In practice a convenient procedure is as follows: *With a dilution of* 1 : 200, *count the cells in* 80 *small squares, and to the sum add* 4 *ciphers; with dilution of* 1 : 100, *count* 40 *small squares and add* 4 *ciphers.* Thus, if with 1 : 200 dilution, 450 corpuscles were counted in 80 squares, the total count would be 4,500,000 per c.mm. This method is sufficiently accurate for all clinical purposes, provided the corpuscles are evenly distributed and three drops from the pipet be counted. It is convenient to count a block of 20 small squares, as indicated in Fig. 76, in each corner of the large square. Four columns of 5 squares each are counted. The double rulings show when the bottom of a column has been reached and also indicate the fourth column. In the writer's opinion it is easier to count in vertical than horizontal rows. If distribution be even, the difference between the number of cells in any two such blocks should not exceed twenty. In order to avoid confusion in counting cells which lie upon the border-lines, the following rule is generally adopted: *Corpuscles which touch the upper and left sides should be counted as if within the squares, those touching the lower and right sides, as outside; and vice versâ.*

*Diluting Fluids.*—The most widely used are Hayem's and Toisson's. Both of these have high specific gravities, so that, when well mixed, the corpuscles do not separate quickly. Toisson's fluid is probably the better for beginners, because it is colored and can easily be seen as it is drawn into the pipet. It stains the nuclei of leukocytes blue, but this is no real advantage. It must be filtered frequently.

| Hayem's Fluid. | |
|---|---|
| Mercuric chlorid | 0.5 |
| Sodium sulphate | 5.0 |
| Sodium chlorid | 1.0 |
| Distilled water | 200.0 |

| Toisson's Fluid. | |
|---|---|
| Methyl-violet, 5 B | 0.025 |
| Sodium chlorid | 1.000 |
| Sodium sulphate | 8.000 |
| Glycerin | 30.000 |
| Distilled water | 160.000 |

*Sources of Error.*—The most common sources of error in making a blood count are:

(*a*) Inaccurate dilution, either from faulty technic or inaccurately graduated pipets. The instruments made by Zeiss can be relied upon.

(*b*) Too slow manipulation, allowing a little of the blood to coagulate and remain in the capillary portion of the pipet.

(*c*) Inaccuracy in depth of counting chamber, which sometimes results from softening of the cement by alcohol or heat. The slide should not be cleaned with alcohol nor left to lie in the warm sunshine.

(*d*) Uneven distribution of the corpuscles. This results when the blood is not thoroughly mixed with the diluting fluid, or when the cover-glass is not applied soon enough after the drop is placed upon the disc.

*Cleaning the Instrument.*—The instrument should be cleaned immediately after using, and the counting chamber and cover must be cleaned again just before use.

Draw through the pipet, successively, water, alcohol, ether, and air. This can be done with the mouth, but it is much better to use a rubber bulb or suction filter pump. When the mouth is used, the moisture of the breath will condense upon the interior of the pipet unless the fluids be shaken and not blown out. If blood has coagulated in the pipet—which happens when the work is done too slowly—dislodge the clot with a horsehair, and clean with strong sulphuric acid, or let the pipet stand over night in a test-tube of the acid. Even if the pipet does not become clogged, it should be occasionally cleaned in this way. When the etched graduations on the pipets become dim, they can be renewed by rubbing with a grease pencil.

Wash the counting-chamber and the cover with water and dry them with clean soft linen. Alcohol may be used to clean the latter, but never the former.

### III. COLOR INDEX

This is an expression which indicates the amount of hemoglobin in each red corpuscle compared with the normal amount. For example, a color index of 1.0 indicates that each corpuscle contains the normal amount of hemoglobin; of 0.5, that each contains one-half the normal.

The color index is most significant in chlorosis and pernicious anemia. In the former it is usually much decreased; in the latter, generally much increased. In symptomatic anemia it is generally moderately diminished.

To obtain the color index, divide the percentage of hemoglobin by the percentage of corpuscles. The percentage of corpuscles is found by multiplying the first two figures of the red corpuscle count by two. This simple method holds good for all counts of 1,000,000 or more. Thus, a count of 2,500,000 is 50 per cent. of the normal. If, then, the hemoglobin has been estimated at 40 per cent., divide 40 (the percentage of hemoglobin) by 50 (the percentage of corpuscles). This gives $\frac{4}{5}$, or 0.8, as the color index.

### IV. VOLUME INDEX

The term " volume index " was introduced by Capps to express the average size of the red cells of an individual compared with their normal size. It is the quotient obtained by dividing the *volume* of red corpuscles (expressed in percentage of the normal) by the *number* of red corpuscles, also expressed in percentage of the normal.

The volume index more or less closely parallels the color index, and variations have much the same sig-

nificance. The following are averages of the examinations reported by Larrabee in the Journal of Medical Research:

| | Red corpuscles per cubic millimeter. | Hemoglobin per cent. by Sahli instrument. | Color index. | Volume index. |
|---|---|---|---|---|
| Normal males | 5,267,250 | 103.0 | 0.98 | 1.007 |
| Normal females | 4,968,667 | 106.0 | 1.06 | 1.001 |
| Primary pernicious anemia | 1,712,166 | 50.0 | 1.47 | 1.270 |
| Secondary anemia | 3,737,160 | 61.0 | 0.81 | 0.790 |
| Chlorosis | 3,205,000 | 34.5 | 0.55 | 0.695 |

**Method.**—The red cells are counted and the percentage of red cells calculated as for the color index.

The volume percentage is obtained with the hematocrit as follows: Fill the hematocrit tubes (Fig. 77) with blood, and before coagulation takes place insert them in the frame and centrifugalize for three minutes at about 8000 to 10,000 revolutions a minute. The red cells collect at the bottom and, normally, make up one-half of the total column of blood.

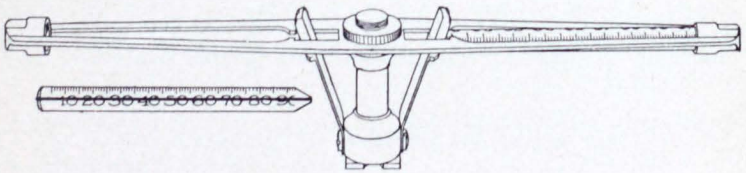


Fig. 77.—Daland hematocrit for use with the centrifuge.

Multiply the height of the layer of red cells (as indicated by the graduations upon the side of the tube) by 2 to obtain the volume percentage. When the examination cannot be made immediately after the blood is obtained, the method of Larrabee is available. This consists in mixing a trace of sodium oxalate with a few drops of blood to prevent coagulation, drawing this mixture into a tube of about 2-mm. caliber and waiting until sedimentation is complete—usually about three days. The height of the column is then measured with a

millimeter scale and the percentage relation to the normal calculated.

After the volume of the red cells and the red corpuscle count are thus expressed in percentages, divide the former by the latter to find the volume index. Example: Suppose the volume percentage is 80 (the reds reaching to mark 40 on hematocrit tube) and that the red count is 50 per cent. of the normal (2,500,000 per c.mm.), then $\frac{80}{50}$ or 1.6 is the volume index.

## V. ENUMERATION OF LEUKOCYTES

The normal number of leukocytes varies from 5000 to 10,000 per cubic millimeter of blood. The number is larger in robust individuals than in poorly nourished ones, and if disease be excluded, may be taken as a rough index of the individual's nutrition. Since it is well to have a definite standard, 7500 is generally adopted as the normal for the adult. With children the number is somewhat greater, counts of 12,000 and 15,000 being common in healthy children under twelve years of age.

### Decrease in Number of Leukocytes

Decrease in number of leukocytes, or *leukopenia*, is not important. It is common in persons who are poorly nourished, although not actually sick. The infectious diseases in which leukocytosis is absent (p. 206) often cause a slight decrease of leukocytes. Chlorosis may produce leukopenia, as also pernicious anemia, which usually gives it in contrast to the secondary anemias, which are frequently accompanied by leukocytosis. Leukocyte counts are, therefore, of some aid in the differential diagnosis of these conditions.

## Increase in Number of Leukocytes

Increase in number of leukocytes is common and of great importance. It may be considered under two heads:

A. Increase of leukocytes due to chemotaxis and stimulation of the blood-making organs, or *leukocytosis*. The increase affects one or more of the normal varieties.

B. Increase of leukocytes due to *leukemia*. Normal varieties are increased, but the characteristic feature is the appearance of great numbers of abnormal cells.

The former may be classed as a *transient*, the latter, as a *permanent*, increase.

### A. Leukocytosis

This term is variously used. By some it is applied to any increase in number of leukocytes; by others it is restricted to increase of the polymorphonuclear neutrophilic variety. As has been indicated, it is here taken to mean a transient increase in number of leukocytes, that is, one caused by chemotaxis and stimulation of the blood-producing structures, in contrast to the permanent increase caused by leukemia.

By *chemotaxis* is meant that property of certain agents by which they attract or repel living cells—positive chemotaxis and negative chemotaxis respectively. An excellent illustration is the accumulation of leukocytes at the site of inflammation, owing to the positively chemotactic influence of bacteria and their products. A great many agents possess the power of attracting leukocytes into the general circulation. Among these are many bacteria and certain organic and inorganic poisons.

Chemotaxis alone will not explain the continuance of

leukocytosis for more than a short time. It is probable that substances which are positively chemotactic also stimulate the blood-producing organs to increased formation of leukocytes; and in at least one form of leukocytosis such stimulation apparently plays the chief part.

As will be seen later, there are several varieties of leukocytes in normal blood, and most chemotactic agents attract only one variety, and either repel or do not influence the others. It practically never happens that all are increased in the same proportion. The most satisfactory classification of leukocytoses is, therefore, based upon the type of leukocyte chiefly affected.

Theoretically, there should be a subdivision for each variety of leukocyte, *e. g.*, polymorphonuclear leukocytosis, lymphocyte leukocytosis, eosinophilic leukocytosis, large mononuclear leukocytosis, etc. Practically, however, only two of these, polymorphonuclear leukocytosis and lymphocyte leukocytosis, need be considered under the head of Leukocytosis. Increase in number of the other leukocytes will be considered when the individual cells are described (pp. 230–243). They are present in the blood in such small numbers normally that even a marked increase scarcely affects the total leukocyte count; and, besides, substances which attract them into the circulation frequently repel the polymorphonuclears, so that the total number of leukocytes may actually be decreased.

The polymorphonuclear neutrophiles are capable of active ameboid motion, and are by far the most numerous of the leukocytes. Lymphocytes are about one-third as numerous and have little independent motion. As one would, therefore, expect, marked differences exist

between the two types of leukocytosis: polynuclear leukocytosis is more or less acute, coming on quickly and often reaching high degree; whereas lymphocyte leukocytosis is more chronic, comes on more slowly, and is never so marked.

**1. Polymorphonuclear Neutrophilic Leukocytosis.**—Polymorphonuclear leukocytosis may be either physiologic or pathologic. A count of 20,000 would be considered a marked leukocytosis; of 30,000, high; above 50,000, extremely high.

(1) **Physiologic Polymorphonuclear Leukocytosis.**—This is never very marked, the count rarely exceeding 15,000 per cubic millimeter. It occurs: (*a*) In the newborn; (*b*) in pregnancy; (*c*) during digestion, and (*d*) after cold baths. There is moderate leukocytosis in the moribund state: this is commonly classed as physiologic, but is probably due mainly to terminal infection.

The increase in these conditions is not limited to the polymorphonuclears. Lymphocytes are likewise increased in varying degrees, most markedly in the newborn.

In view of the leukocytosis of digestion, the hour at which a leukocyte count is made should always be recorded. Digestive leukocytosis is most marked three to five hours after a hearty meal rich in protein. It is absent in pregnancy and when leukocytosis from any other cause exists. It is usually absent in cancer of the stomach, a fact which may be of some help in the diagnosis of this condition, but repeated examinations are essential.

(2) **Pathologic Polymorphonuclear Leukocytosis.**—In general, the response of the leukocytes to chemotaxis

is a conservative process. It has been compared to the gathering of soldiers to destroy an invader. This is accomplished partly by means of phagocytosis—actual ingestion of the enemy—and partly by means of chemic substances which the leukocytes produce.

In those diseases in which leukocytosis is the rule the degree of leukocytosis depends upon two factors: the *severity of the infection* and the *resistance of the individual.* A well-marked leukocytosis usually indicates good resistance. A mild degree means that the body is not reacting well, or else that the infection is too slight to call forth much resistance. Leukocytosis may be absent altogether when the infection is extremely mild, or when it is so severe as to overwhelm the organism before it can react. When leukocytosis is marked, a sudden fall in the count may be the first warning of a fatal issue. These facts are especially true of pneumonia, diphtheria, and abdominal inflammations, in which conditions the degree of leukocytosis is of considerable prognostic value.

The classification here given follows Cabot:

(*a*) *Infectious and Inflammatory.*—The majority of infectious diseases produce leukocytosis. The most notable exceptions are influenza, malaria, measles, tuberculosis, except when invading the serous cavities or when complicated by mixed infection, and typhoid fever, in which leukocytosis indicates an inflammatory complication.

All inflammatory and suppurative diseases cause leukocytosis, except when slight or well walled off. Appendicitis has been studied with especial care in this connection, and the conclusions now generally accepted probably hold good for most acute intra-abdominal inflam-

mations. A marked leukocytosis (20,000 or more) nearly always indicates abscess, peritonitis, or gangrene, even though the clinical signs be slight. Absence of or mild leukocytosis indicates a mild process, or else an overwhelmingly severe one; and operation may safely be postponed unless the abdominal signs are very marked. On the other hand, no matter how low the count, an increasing leukocytosis—counts being made hourly—indicates a spreading process and demands operation, regardless of other symptoms.

*Leukocyte counts alone are often disappointing, but are of much more value when considered in connection with a differential count of polymorphonuclears.* (See p. 236.)

(*b*) *Malignant Disease.*—Leukocytosis occurs in about one-half of the cases of malignant disease. In many instances it is probably independent of any secondary infection, since it occurs in both ulcerative and non-ulcerative cases. It seems to be more common in sarcoma than in carcinoma. Very large counts are rarely noted.

(*c*) *Posthemorrhagic.*—Moderate leukocytosis follows hemorrhage and disappears in a few days.

(*d*) *Toxic.*—This is a rather obscure class, which includes gout, chronic nephritis, acute yellow atrophy of the liver, ptomain-poisoning, prolonged chloroform narcosis, and quinin-poisoning. Leukocytosis may or may not occur in these conditions, and is not important.

(*e*) *Drugs.*—This also is an unimportant class. Most tonics and stomachics and many other drugs produce a slight leukocytosis.

2. **Lymphocyte Leukocytosis.**—This is characterized by an increase in the total leukocyte count, accom-

panied by an increase in the percentage of lymphocytes. The word "lymphocytosis" is often used in the same sense. It is better, however, to use the latter as referring to any increase in the absolute number of lymphocytes, without regard to the total count, since an absolute increase in number of lymphocytes is frequently accompanied by a normal or subnormal leukocyte count, owing to loss of polymorphonuclears.

Non-phagocytic leukocytosis is probably due more to stimulation of blood-making organs than to chemotaxis. It is less common, and is rarely so marked as a polymorphonuclear leukocytosis. When marked, the blood cannot be distinguished from that of lymphatic leukemia.

A marked lymphocyte leukocytosis occurs in pertussis, and is of value in diagnosis. It appears early in the catarrhal stage, and persists until after convalescence. The average leukocyte count is about 17,000, lymphocytes predominating. There is moderate lymphocyte leukocytosis in other diseases of childhood, as rickets, scurvy, and especially hereditary syphilis, where the blood-picture may approach that of pertussis. It must be borne in mind in this connection that lymphocytes are normally more abundant in the blood of children than in that of adults.

Slight lymphocyte leukocytosis occurs in many other pathologic conditions, but is of little significance.

### B. Leukemia

This is an idiopathic disease of the blood-making organs, which is accompanied by an enormous increase in number of leukocytes. The leukocyte count sometimes reaches 1,000,000 per cubic millimeter, and leu-

kemia is always to be suspected when it exceeds 50,000. Lower counts do not, however, exclude it. The subject is more fully discussed later (p. 280).

### Method of Counting Leukocytes

The leukocytes are counted with the Thoma-Zeiss instrument, already described. Recently, several new rulings of the disc have been introduced, notably the Zappert and the Türck (Fig. 79), which give a ruled area of nine square millimeters. They were devised for counting the leukocytes in the same specimen with the red corpuscles. The red cells are counted in the usual manner, after which all the leukocytes in the whole area of nine square millimeters are counted; and the number in a cubic millimeter of undiluted blood is then easily calculated. Leukocytes are easily distinguished from red cells, especially when Toisson's diluting fluid is used. This method may be used with the ordinary ruling by adjusting the microscopic field to a definite size, and counting a sufficient number of fields, as described later. Although less convenient, it is more accurate to count the leukocytes separately, with less dilution of the blood, as follows:

**Technic.**—A larger drop of blood is required than for counting the erythrocytes, and more care in filling the pipet. Boggs has suggested a device (Fig. 78) which enables one to draw in the blood more slowly and hence more accurately. He cuts the rubber tube and inserts a Wright "throttle." This consists of a section of glass tubing in which a capillary tube drawn out to a fine thread is cemented with sealing wax. After sealing in place the tip is broken off with forceps, so that upon gentle suction it will just allow air to pass.

Use the pipet with 11 engraved above the bulb. Suck the blood to the mark 0.5 or 1.0, and the diluting fluid to the mark 11. This gives a dilution of 1:20 or 1:10, respectively. The dilution of 1 : 20 is easier to make. Mix well by shaking in all directions except in the long axis of the pipet; blow out two or three drops, place a drop in the counting chamber, and adjust the cover as already described (p. 196).

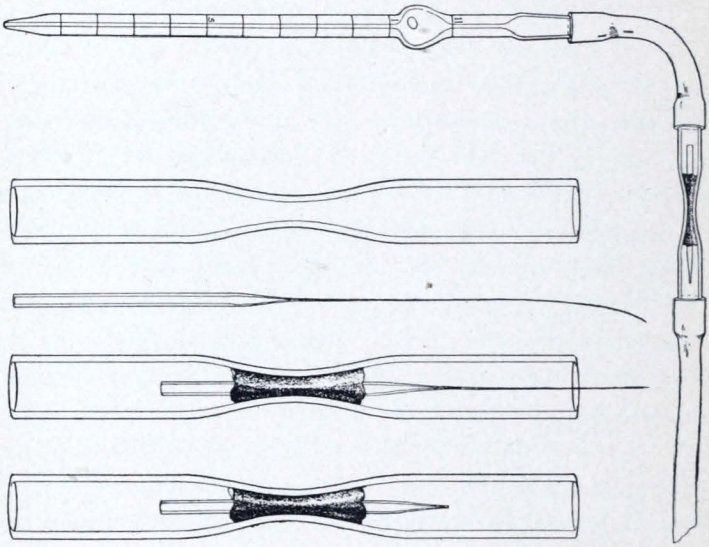


Fig. 78.—Boggs' "throttle control" for blood-counting pipet, and enlarged diagram showing construction of the throttle.

Examine with a low power to see that the cells are evenly distributed. Count with the 16 mm. objective and a high eye-piece, or with the long-focus 4 mm. and a low eye-piece. An 8 mm. objective will be found very satisfactory for this purpose. As one gains experience one will rely more upon the lower powers.

With the ordinary ruling of the disc, count all the leukocytes in the large square, multiply by 10 to find the number in

1 c.mm. of diluted blood, and by the dilution to find the number per c.mm. of undiluted blood. In every case at least 200 leukocytes must be counted as a basis for calculation, and it is much better to count 500. This will necessitate examination of several drops from the pipet. With the Zappert

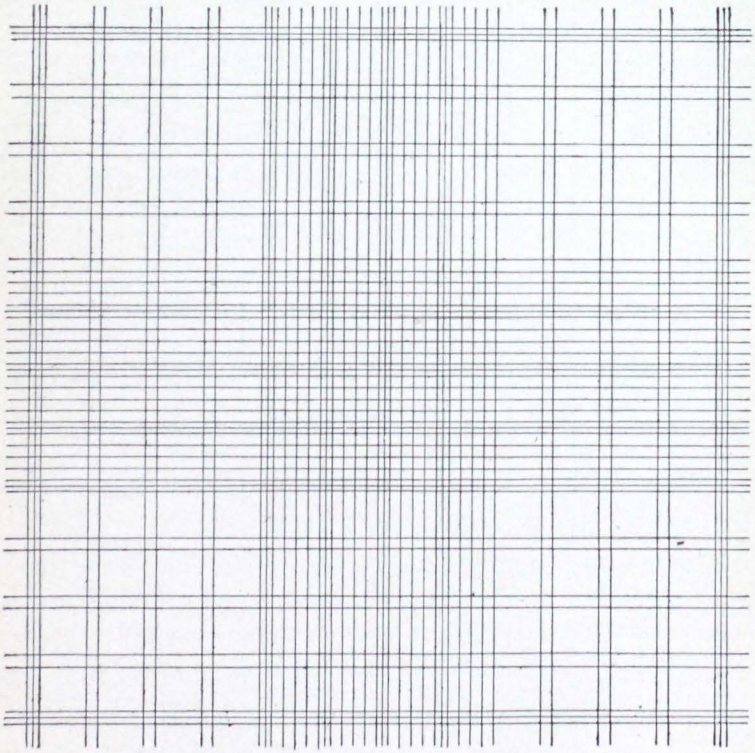


Fig. 79.—Türck ruling of counting chamber.

and Türck rulings a sufficient number can usually be counted in one drop, but the opportunity for error is very much greater when only one drop is examined.

In routine work the author's modification of the "circle" method is very satisfactory. It requires a 4 mm. objective,

and is, therefore, especially desirable for beginners, who are usually unable accurately to identify leukocytes with a lower power. The student is frequently confused by particles of dirt, remains of red cells, and yeast cells which sometimes grow in the diluting fluid. Draw out the sliding tube of the

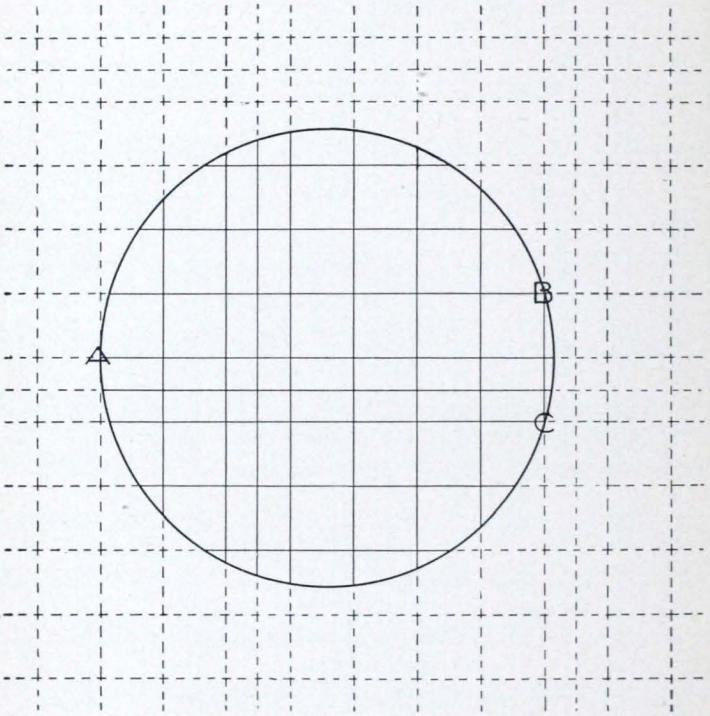



Fig. 80.—Size of field required in counting leukocytes as described in the text.

microscope until the field of vision is such as shown in Fig. 80. One side of the field is tangent to one of the ruled lines, A, while the opposite side just cuts the corners, B and C, of the seventh squares in the rows above and below the diameter line. When once adjusted, a scratch is made upon the draw-

tube, so that for future counts the tube has only to be drawn out to the mark. The area of this microscopic field is one-tenth of a square millimeter. With a dilution of 1 : 20, count the leukocytes in 20 such fields upon different parts of the disc without regard to the ruled lines, and to their sum add two ciphers. With dilution of 1 : 10, count 10 such fields, and add two ciphers. Thus, with 1 : 10 dilution, if 150 leukocytes were counted in 10 fields, the leukocyte count would be 15,000 per c.mm. To compensate for possible unevenness of distribution, it is best to count a row of fields horizontally and a row vertically across the disc. This method is applicable to any degree of dilution of the blood, and is simple to remember: *one always counts a number of fields equal to the number of times the blood has been diluted, and adds two ciphers.*

It is sometimes impossible to obtain the proper size of field with the objectives and eye-pieces at hand. In such case place a cardboard disc with a circular opening upon the diaphragm of the eye-piece, and adjust the size of the field by drawing out the tube. The circular opening can be cut with a cork-borer.

*Diluting Fluids.*—The diluting fluid should dissolve the red corpuscles so that they will not obscure the leukocytes. The simplest fluid is a 0.5 per cent. solution of acetic acid. More satisfactory is the following: glacial acetic acid, 1 c.c.; 1 per cent. aqueous solution of gentian-violet, 1 c.c.; distilled water, 100 c.c. These solutions must be filtered frequently.

## VI. ENUMERATION OF BLOOD-PLAQUES

The average normal number of plaques is variously given as 200,000 to 700,000 per cubic millimeter of blood. Many of the counts were obtained by workers who used unreliable methods. Using their new method, Wright and Kinnicutt find the normal average to range from 263,000 to 336,000. Physiologic variations are

marked; thus, the number increases as one ascends to a higher altitude, and is higher in winter than in summer. There are unexplained variations from day to day; hence a single abnormal count should not be taken to indicate a pathologic condition.

Pathologic variations are often very great. Owing to lack of knowledge as to the origin of the platelets and to the earlier imperfect methods of counting, the clinical significance of these variations is uncertain. The following facts seem, however, to be established:

(*a*) In acute infectious diseases the number is subnormal or normal. If the fever ends by crisis, the crisis is accompanied by a rapid and striking increase.

(*b*) In secondary anemia plaques are generally increased, although sometimes decreased In pernicious anemia they are always greatly diminished, and an increase should exclude the diagnosis of this disease.

(*c*) They are decreased in chronic lymphatic leukemia, and greatly increased in the myelogenous form.

(*d*) In purpura hæmorrhagica the number is enormously diminished.

Blood-plaques are difficult to count, owing to the rapidity with which they disintegrate and to their great tendency to adhere to any foreign body and to each other.

**Method of Kemp, Calhoun, and Harris.**—Wash the finger well and allow a few minutes to elapse for the circulation to become normal. Prick the finger lightly with a blood-lancet, regulating the depth of the puncture so that the blood will not flow without gentle pressure. Quickly dip a clean glass rod into a vessel containing diluting and fixing fluid, and place two or three good-sized drops upon the

finger over the puncture. Then exert gentle pressure above the puncture so that a small drop of blood will exude into the fluid. Mix the two by passing the rod lightly several times over the surface of the blended drop. (Some workers first place a drop of the fluid upon the finger and then make the puncture through it, this necessitating less care as to depth of the puncture.) Now transfer a drop of the diluted blood from the finger to a watch-glass which contains two or three drops of the fluid, and mix well. From this, transfer a drop to the counting slide of the Thoma-Zeiss hemocytometer, and cover. An ordinary thin cover will answer for this purpose, and is preferable because it allows the use of a higher power objective. Allow the slide to stand for at least five minutes, and then with a 4 mm. or higher objective count the plaques and the red corpuscles in a definite number of squares. At least 100 plaques must be counted. The number of red corpuscles per cubic millimeter of blood having been previously ascertained in the usual manner (p. 195), the number of plaques can easily be calculated by the following equation:

$$r : p :: R : P; \text{ and } P = \frac{p \times R}{r}.$$

$r$ represents the number of red corpuscles in any given number of squares; $p$, the number of plaques in the same squares; $R$, the total number of red corpuscles per c.mm. of blood; and $P$, the number of plaques per c.mm.

Beginners are apt to take too much blood and not to dilute it enough. Best results are attained when there are only one or two plaques in a small square. With insufficient dilution, the platelets are more or less obscured by the red cells.

The following diluting and fixing fluid is recommended:

Formalin ..... 10 c.c.
1 per cent. aqueous solution sodium chlorid ..... 150 c.c.
(Color with methyl-violet if desired.)

This fluid is cheap and easily prepared, and keeps indefinitely. It fixes the plaques quickly without clumping, and does not clump nor decolorize the reds. It has a low specific gravity, and hence allows the plaques to settle upon the ruled area along with the reds. Fluids of high specific gravity cause the plaques to float so that they do not appear in the same plane with the reds and the ruled lines.

**Method of Wright and Kinnicutt.**—This new method is simple, appears to be accurate, and certainly yields uniform results.

The plaques are counted with the Thoma-Zeiss hemocytometer already described, using a dilution of 1 : 100. The diluting fluid consists of two parts of an aqueous solution of "brilliant cresyl blue" (1 : 300) and three parts of an aqueous solution of potassium cyanid (1 : 1400). These two solutions must be kept in separate bottles and mixed and filtered immediately before using. After the blood is placed in the counting-chamber it is allowed to stand for ten minutes or longer in order that the plaques may settle. The plaques appear as rounded, lilac-colored bodies; the reds are decolorized, appearing only as shadows.

The leukocytes are stained and may be counted at the same time.

## VII. STUDY OF STAINED BLOOD

### A. Making and Staining Blood-films

**1. Spreading the Film.**—Thin, even films are essential to accurate and pleasant work. They more than compensate for the time spent in learning to make them. There are certain requisites for success with any method: (*a*) The slides and covers must be perfectly clean: thorough washing with soap and water and rubbing with alcohol will usually suffice; (*b*) the drop of blood

must not be too large; (*c*) the work must be done quickly, before coagulation begins.

The blood is obtained from the finger-tip or the lobe of the ear, as for a blood count; only a very small drop is required.

**Ehrlich's Two Cover-glass Method.**—This method is very widely used, but considerable practice is required to get good results. Touch a cover-glass to the top of a small drop of blood, and place it, blood side down, upon another cover-

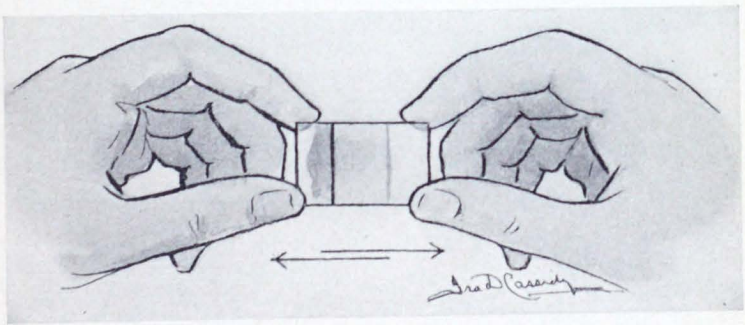


Fig. 81.—Spreading the film: two cover-glass method.

glass. If the drop be not too large, and the covers be perfectly clean, the blood will spread out in a very thin layer. Just as it stops spreading, before it begins to coagulate, pull the covers quickly but firmly apart on a line parallel to their plane (Fig. 81). It is best to handle the covers with forceps, since the moisture of the fingers may produce artifacts.

**Two-slide Method.**—Take a small drop of blood upon a clean slide about $\frac{1}{2}$ inch from the end. Place the end of a second slide against the surface of the first at an angle of 45°, and push it up against the drop of blood, which will immediately run across the end, filling the angle between the

two slides. Now draw the " spreader slide " back along the other. The blood will follow. The thickness of the smear can be regulated by changing the angle.

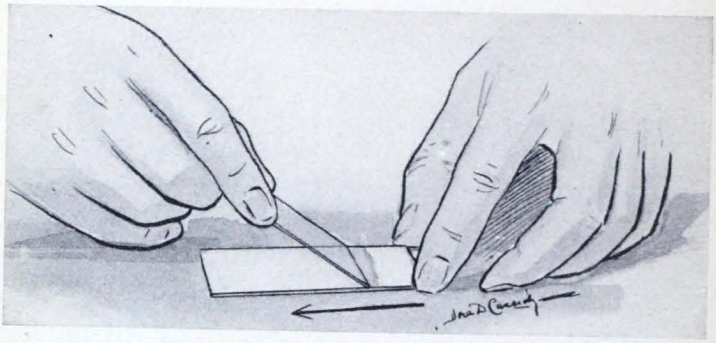


Fig. 82.—Spreading the film: two-slide method.

**Cigarette-paper Method.**—This gives better results in the hands of the inexperienced than any of the methods in general

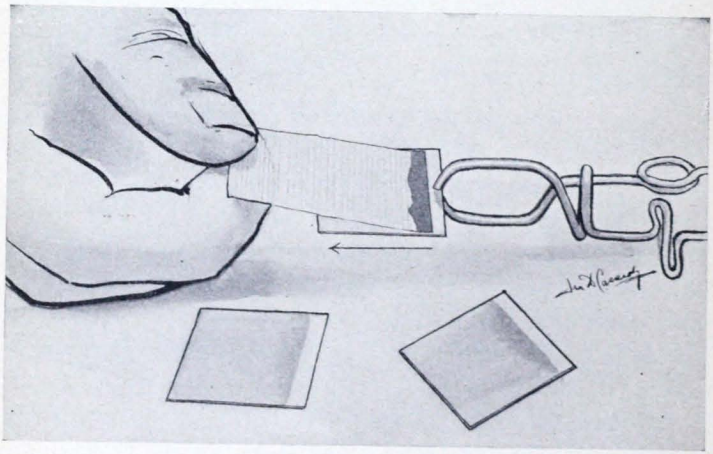


Fig. 83.—Spreading the film. Cigarette-paper method applied to cover-glasses.

use, and may be used with either slides or covers. A very thin paper, such as the " Zig-zag " brand, is best. Ordinary

cigarette paper and thin tissue-paper will answer, but do not give nearly so good results.

Cut the paper into strips about ¾ inch wide, *across the ribs*. Pick up one of the strips by the gummed edge, and touch its opposite end to the drop of blood. Quickly place the end which has the blood against a slide or a large cover-glass held in a forceps. The blood will spread along the edge of the paper. Now draw the paper evenly across the slide or cover. A thin film of blood will be left behind (Fig. 83).

The films may be allowed to dry in the air, or may be dried by gently heating high above a flame (where one can comfortably hold the hand). Such films will keep for years, but for some stains they must not be more than a few weeks old. They must be kept away from flies—a fly can work havoc with a film in a few minutes.

**2. Fixing the Film.**—In general, films must be "fixed" before they are stained. Fixation may be accomplished by chemicals or by heat. *Those stains which are dissolved in methyl-alcohol combine fixation with the staining process.*

**Chemic Fixation.**—Soak the film five to fifteen minutes in pure methyl-alcohol, or one-half hour or longer in equal parts of absolute alcohol and ether. One minute in 1 per cent. formalin in alcohol is preferred by some. Chemic fixation may precede eosin-methylene-blue and other simple stains.

**Heat Fixation.**—This may precede any of the methods which do not combine fixation with the staining process; it *must* be used with Ehrlich's triple stain. The best method is to place the film in an oven, raise the temperature to 150° C., and allow to cool slowly. Without an oven, the proper degree of fixation is difficult to attain. Kowarsky has devised a small plate of two layers of copper (Fig. 84), upon

which the films are placed together with a crystal of urea. The plate is heated over a flame until the urea melts, and is then set aside to cool. This is said to give the proper degree of fixation, but in the writer's experience the films have always been underheated. He obtains better results by use of tartaric acid crystals (melting-point, 168°–170° C.). The plate, upon which have been placed the cover-glasses, film side down, and a crystal of the acid, is heated over a low flame until the crystal has completely melted. It should be held sufficiently high above the flame that the heating will require five to seven minutes. The covers are then removed. Freshly made films of normal blood should be allowed to remain upon

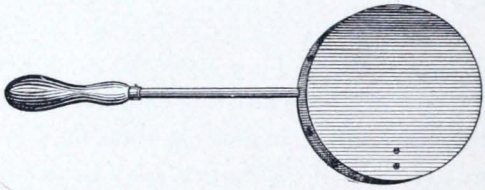


Fig. 84.—Kowarsky's plate for fixing blood (Klopstock and Kowarsky).

the plate for a minute or two after heating has ceased. Fresh films require more heat than old ones, and normal blood more than the blood of pernicious anemia and leukemia.

Fixation by passing the film quickly through a flame about twenty times, as is often done in routine work, is not recommended for beginners.

**3. Staining the Film.**—The anilin dyes, which are extensively used in blood work, are of two general classes: basic dyes, of which methylene-blue is the type; and acid dyes, of which eosin is the type. Nuclei and certain other structures in the blood are stained by the basic dyes, and are hence called *basophilic*. Certain struc-

tures take up only acid dyes, and are called *acidophilic*, *oxyphilic*, or *eosinophilic*. Certain other structures are stained only by combinations of the two, and are called *neutrophilic*. Recognition of these staining properties marked the beginning of modern hematology.

(1) **Eosin and Methylene-blue.**—In many instances this stain will give all the information desired. It is especially useful in studying the red corpuscles. Nuclei, basophilic granules, and all blood parasites are blue; erythrocytes are red or pink; eosinophilic granules, bright red. Neutrophilic granules and blood-plaques are not stained.

(1) Fix the film by heat or chemicals.

(2) Stain about five minutes with 0.5 per cent. alcoholic solution of eosin, diluted one-half with water.

(3) Rinse in water, and dry between filter-papers.

(4) Stain one-half to one minute with saturated aqueous solution of methylene-blue.

(5) Rinse well, dry, and mount. Films upon slides may be examined with an oil-immersion objective without a cover-glass.

(2) **Ehrlich's Triple Stain.**—This has been the standard blood-stain for many years, but is now little used. It is probably the best for neutrophilic granules. It is difficult to make, and should be purchased ready prepared from a reliable dealer. Nuclei are stained blue or greenish blue; erythrocytes, orange; neutrophilic granules, violet; and eosinophilic granules, copper red. Basophilic granules and blood-plaques are not stained.

Success in staining depends largely upon proper fixation. The film must be carefully fixed by heat: under-

heating causes the erythrocytes to stain red; overheating, pale yellow. The staining fluid is applied for five to fifteen minutes, and the preparation is rinsed quickly in water, dried, and mounted. Subsequent application of Löffler's methylene-blue for one-half to one second will bring out the basophilic granules and improve the nuclear staining, but there is considerable danger of overstaining.

(3) **Polychrome Methylene-blue Eosin Stains.**—These stains, outgrowths of the original Romanowsky method, have largely displaced other blood-stains for clinical purposes. They stain differentially every normal and abnormal structure in the blood. Most of them are dissolved in methyl alcohol and combine the fixing with the staining process. Numerous methods of preparing and applying these stains have been devised. Two only need be given here: Wright's stain and Harlow's stain:

**Wright's Stain.**—This is one of the best and is the most widely used in this country. The practitioner will find it best to purchase the stain ready prepared. Most microscopic supply-houses carry it in stock. Wright's most recent directions for its preparation and use are as follows:[1]

**Preparation.**—To a 0.5 per cent. aqueous solution of sodium bicarbonate add methylene-blue (B. X. or " medicinally pure ") in the proportion of 1 gm. of the dye to each 100 c.c. of the solution. Heat the mixture in a steam sterilizer at 100° C. for one full hour, counting the time after the sterilizer has become thoroughly heated. The mixture is to be contained in a flask, or flasks, of such size and shape that it

[1] Journal of the American Medical Association, Dec. 3, 1910.

forms a layer not more than 6 cm. deep. After heating, allow the mixture to cool, placing the flask in cold water, if desired, and then filter it to remove the precipitate which has formed in it. It should, when cold, have a deep purple-red color when viewed in a thin layer by transmitted yellowish artificial light. It does not show this color while it is warm.

To each 100 c.c. of the filtered mixture add 500 c.c. of a 0.1 per cent. aqueous solution of "yellowish water-soluble" eosin and mix thoroughly. Collect the abundant precipitate which immediately appears on a filter. When the precipitate is dry, dissolve it in methylic alcohol (Merck's "reagent") in the proportion of 0.1 gm. to 60 c.c. of the alcohol. In order to facilitate solution, the precipitate is to be rubbed up with the alcohol in a porcelain dish or mortar with a spatula or pestle. This alcoholic solution of the precipitate is the staining fluid.

**Application.**—1. Cover the film with a noted quantity of the staining fluid by means of a medicine-dropper.

2. After one minute add to the staining fluid on the film the same quantity of distilled water by means of a medicine-dropper and allow the mixture to remain for two or three minutes, according to the intensity of the staining desired. A longer period of staining may produce a precipitate. Eosinophilic granules are best brought out by a short period of staining.

The quantity of the diluted fluid on the preparation should not be so large that some of it runs off.

3. Wash the preparation in water for thirty seconds or until the thinner portions of the film become yellow or pink in color.

4. Dry and mount in balsam.

The stain is more conveniently applied upon cover-glasses than upon slides. Films much more than a month old do not stain well. In some localities ordinary tap-

water will answer both for diluting the stain and for washing the film; in others, distilled water must be used. Different lots of Wright's fluid vary, and a few preliminary stains should be made with each lot to learn its peculiarities.

When properly applied, Wright's stain gives the following picture (Plate VI): erythrocytes, yellow or pink; nuclei, various shades of bluish purple; neutrophilic granules, reddish lilac; eosinophilic granules, bright red; basophilic granules of leukocytes and degenerated red corpuscles, very dark bluish purple; blood-plaques, dark lilac; bacteria, blue. The cytoplasm of lymphocytes is generally robin's-egg blue; that of the large mononuclears may have a faint bluish tinge. Malarial parasites stain characteristically: the cytoplasm, sky-blue; the chromatin, reddish purple.

**Harlow's Stain.**—Probably the simplest modification of the Romanowsky stain, both in preparation and method of use, is that devised by W. P. Harlow of the University of Colorado. It differentiates granules particularly well, but is not so satisfactory for demonstrating *slight* grades of polychromatophilia, because it usually gives all the red cells a faint bluish tinge.

**Preparation.**—The stain consists of two solutions used separately:

No. 1. Eosin, yellowish, water soluble (Grübler) . 1 gram
Methyl alcohol (Merck's reagent) . . . . . 100 c.c.

No. 2. Methylene-blue ("B. X." or Ehrlich's rectified) (Grübler) . . . . . 1 gram
Methyl alcohol (Merck's reagent) . . . . . 100 c.c.

**Application.**—(1) Stain the film without previous fixation for one minute with the eosin solution.

(2) Shake off the excess, allowing a very little to remain, and at once cover with the methylene-blue solution for one or two minutes.

(3) Rinse quickly in distilled water, dry, and mount.

It is well known that pathologic bloods will sometimes not stain well with fluids which are satisfactory for normal bloods. Doctors Peebles and Harlow have shown that the various polychrome methylene-blue-eosin stains can be modified to suit any blood by adding a little alkali or acid. The alkali used is a weak solution of " potassium hydrate by alcohol " in methyl alcohol; the acid, glacial acetic in methyl alcohol. In the case of the Harlow stain it is added to the methylene-blue solution only. The alkali solution also serves to " correct " old fluids which, by reason of development of formic acid in the methyl alcohol, do not stain sufficiently with the blue. In general a stain is satisfactory when both nuclei and neutrophilic granules are clearly defined.

### B. Study of Stained Films

Much can be learned from stained blood-films. They furnish the best means of studying the morphology of the blood and blood parasites, and, to the experienced, they give a fair idea of the amount of hemoglobin and the number of red and white corpuscles. An oil-immersion objective is required.

**1. Erythrocytes.**—Normally, the red corpuscles are acidophilic. The colors which they take with different stains have been described. When not crowded together, they appear as circular, homogeneous discs, of nearly uniform size, averaging 7.5 $\mu$ in diameter (Fig. 104). The center of each is somewhat paler than the

periphery. The degree of pallor furnishes a rough index to the amount of hemoglobin in the corpuscle. As hemoglobin is diminished, the central pale area becomes larger and paler, producing the so-called "pessary forms" in which only the periphery of the cell is apparent. These forms indicate a low color index and are most abundant in chlorosis. Red cells are apt to be crenated when the film has dried too slowly.

Pathologically, red corpuscles vary in size and shape, staining properties, and structure.

(1) **Variations in Size and Shape** (See Plate IX and Fig. 104).—The cells may be abnormally small (called *microcytes*, 5 $\mu$ or less in diameter); abnormally large (*macrocytes*, 10 to 12 $\mu$); or extremely large (*megalocytes*, 12 to 20 $\mu$).

Variation in shape is often very marked. Oval, pyriform, caudate, saddle-shaped, and club-shaped corpuscles are common (Fig. 85). They are called *poikilocytes*, and their presence is spoken of as poikilocytosis.

Red corpuscles which vary from the normal in size and shape are present in most symptomatic anemias, and in the severer grades are often very numerous. Irregularities are particularly conspicuous in leukemia and pernicious anemia, where, in some instances, a normal erythrocyte is the exception. In pernicious anemia there is a decided tendency to large size and oval forms, and megalocytes are rarely found in any other condition.

(2) **Variations in Staining Properties** (See Plate IX).—These include polychromatophilia, basophilic degeneration, and malarial stippling. With exception of polychromatophilia they are probably degenerative changes.

(*a*) *Polychromatophilia.*—Some of the corpuscles partially lose their normal affinity for acid stains, and take the basic stain to greater or less degree. Wright's stain gives such cells a faint bluish tinge when the condition is mild, and a rather deep blue when severe. Sometimes only part of a cell is affected. A few polychromatophilic corpuscles can be found in marked symptomatic anemias.

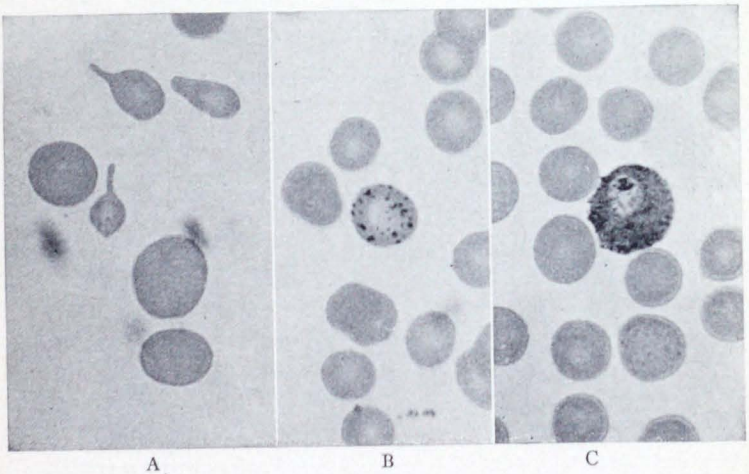



Fig. 85.—Abnormal red corpuscles: A, Poikilocytosis; B, basophilic granular degeneration; C, malarial stippling, the cell also containing a tertian parasite (× 1000) (courtesy of Dr. W. P. Harlow).

They occur most abundantly in malaria, leukemia, and pernicious anemia.

Polychromatophilia has been variously interpreted. It is thought by many to be evidence of youth in a cell, and hence to indicate an attempt at blood regeneration. There are probably several forms referable to different causes.

(*b*) *Basophilic Granular Degeneration* (*Degeneration of Grawitz*).—This is characterized by the presence, within

the corpuscle, of basophilic granules which vary in size from scarcely visible points to granules as large as those of basophilic leukocytes (Fig. 85). The number present in a red cell commonly varies in inverse ratio to their size. They stain deep blue with Wright's stain; not at all with Ehrlich's triple stain. The cell containing them may stain normally in other respects, or it may exhibit polychromatophilia.

Numerous cells showing this degeneration are commonly found in chronic lead-poisoning, of which they

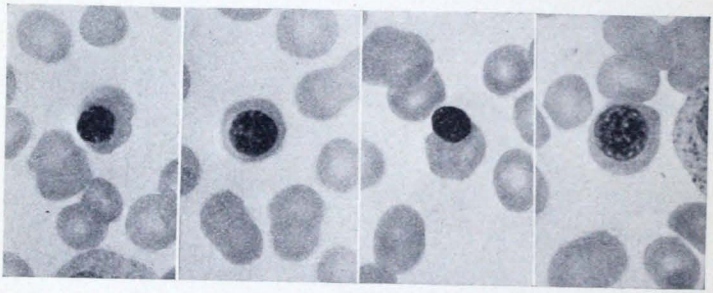


Fig. 86.—Normoblasts from cases of secondary anemia and leukemia (× 1000) (photographs by the author).

were at one time thought to be pathognomonic. Except in this disease, the degeneration indicates a serious blood condition. It occurs in well-marked cases of pernicious anemia and leukemia, and, much less commonly, in very severe symptomatic anemias.

(*c*) *Malarial Stippling.*—This term has been applied to the finely granular appearance often seen in red corpuscles, which harbor malarial parasites (Plates VI and VII and Fig. 85). It is commonly classed with the degeneration just described, but is probably distinct. Not all stains will show it. With Wright's stain it can be

brought out by staining longer and washing less than for the ordinary blood-stain. The minute granules stain reddish purple.

(3) **Variations in Structure.**—The most important is the presence of a nucleus (Plates VI and IX and Fig. 86). Nucleated red corpuscles, or *erythroblasts*, are classed according to their size: *microblasts*, 5 $\mu$ or less in diameter; *normoblasts*, 5 to 10 $\mu$; and *megaloblasts*, above 10 $\mu$. Microblasts and normoblasts contain one, rarely two, small round, sharply defined, deeply staining nuclei,

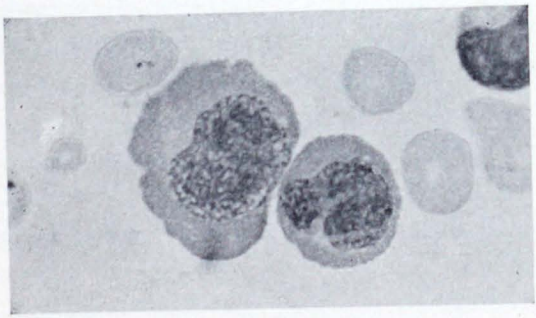


Fig. 87.—Megaloblasts from a case of pernicious anemia (× 1000) (courtesy of Dr. W. P. Harlow).

often located eccentrically. Occasionally the nucleus is irregular in shape, " clover-leaf " forms being not infrequent. The megaloblast (Fig. 87) is probably a distinct cell, not merely a larger size of the normoblast. Its nucleus is large, stains rather palely, has a delicate chromatin network, and often shows evidences of degeneration (karyorrhexis, etc.). In ordinary work, however, it is safer to base the distinction upon size than upon structure. Any nucleated red cell, but especially the megaloblast, may exhibit polychromatophilia.

Normally, erythroblasts are present only in the blood

of the fetus and of very young infants. In the adult, their presence in the circulating blood denotes an excessive demand upon the blood-forming organs to regenerate lost or destroyed red corpuscles. In response to this demand, immature and imperfectly formed cells are thrown into the circulation. Their number, therefore, is an indication of the extent to which the bone-marrow reacts rather than of the severity of the disease. Normoblasts occur in severe symptomatic anemia, leukemia, and pernicious anemia. They are most abundant in myelogenous leukemia. While always present in pernicious anemia, they are often difficult to find. Megaloblasts are found in pernicious anemia, and with extreme rarity in any other condition. They here almost invariably exceed the normoblasts in number, which is one of the distinctive features of the disease. Microblasts have much the same significance as normoblasts, but are less common.

**Cabot's ring bodies** are ring- or figure-of-8 shaped structures which have been observed in certain of the red cells in pernicious anemia, lead-poisoning, and lymphatic leukemia. They stain red with Wright's stain. Their nature is unknown.

**2. The Leukocytes.**—An estimation of the number or percentage of each variety of leukocyte in the blood is called a *differential count.* It probably yields more helpful information than any other single procedure in blood examinations.

The differential count is best made upon a film stained with Wright's, Harlow's, or Ehrlich's stain. Go carefully over the film with an oil-immersion lens, using a mechanical stage if available. Classify each leukocyte

seen, and calculate what percentage each variety is of the whole number classified. For accuracy, 500 to 1000 leukocytes must be classified; for approximate results, 300 are sufficient. Track of the count may be kept by placing a mark for each leukocyte in its appropriate column, ruled upon paper. Some workers divide a slide-box into compartments with slides, one for each variety of leukocyte, and drop a coffee-bean into the appropriate compartment when a cell is classified. When a convenient number of coffee-beans is used (any multiple of 100), the percentage calculation is extremely easy.

The actual number of each variety in a cubic millimeter of blood is easily calculated from these percentages, and the total leukocyte count. An increase in actual number is an *absolute increase;* an increase in percentage only, a *relative increase.* It is evident that an absolute increase of any variety may be accompanied by a relative decrease.

A record is generally kept of the number of nucleated red cells seen during a differential count of leukocytes.

The usual classification of leukocytes is based upon their size, their nuclei, and the staining properties of the granules which many of them contain. It is not altogether satisfactory, but is probably the best which our present knowledge permits.

The writer has found the table (Fig. 88, p. 232) very helpful in impressing this classification upon the student. It makes no attempt to indicate histogenetic relationships. The leukocytes of normal blood fall into two groups, each including three types. The cells in Group I contain single, round, oval or horseshoe-shaped nuclei, and have few or no granules in their cytoplasm.

The stippling of the cytoplasm shown in the diagram represents the finely granular appearance of protoplasm, not true granulation. The cells in Group II are polymorphonuclear and contain granules which are distinguished by their size and staining reactions. In its structure the chief abnormal leukocyte, the myelocyte, combines the

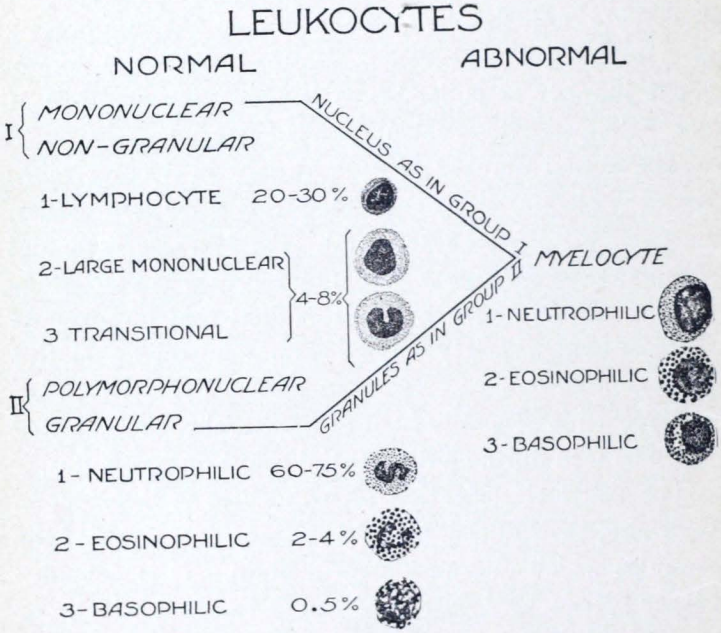



Fig. 88.—Outline of the classification of leukocytes.

two groups, being mononuclear like Group I, and granular like Group II.

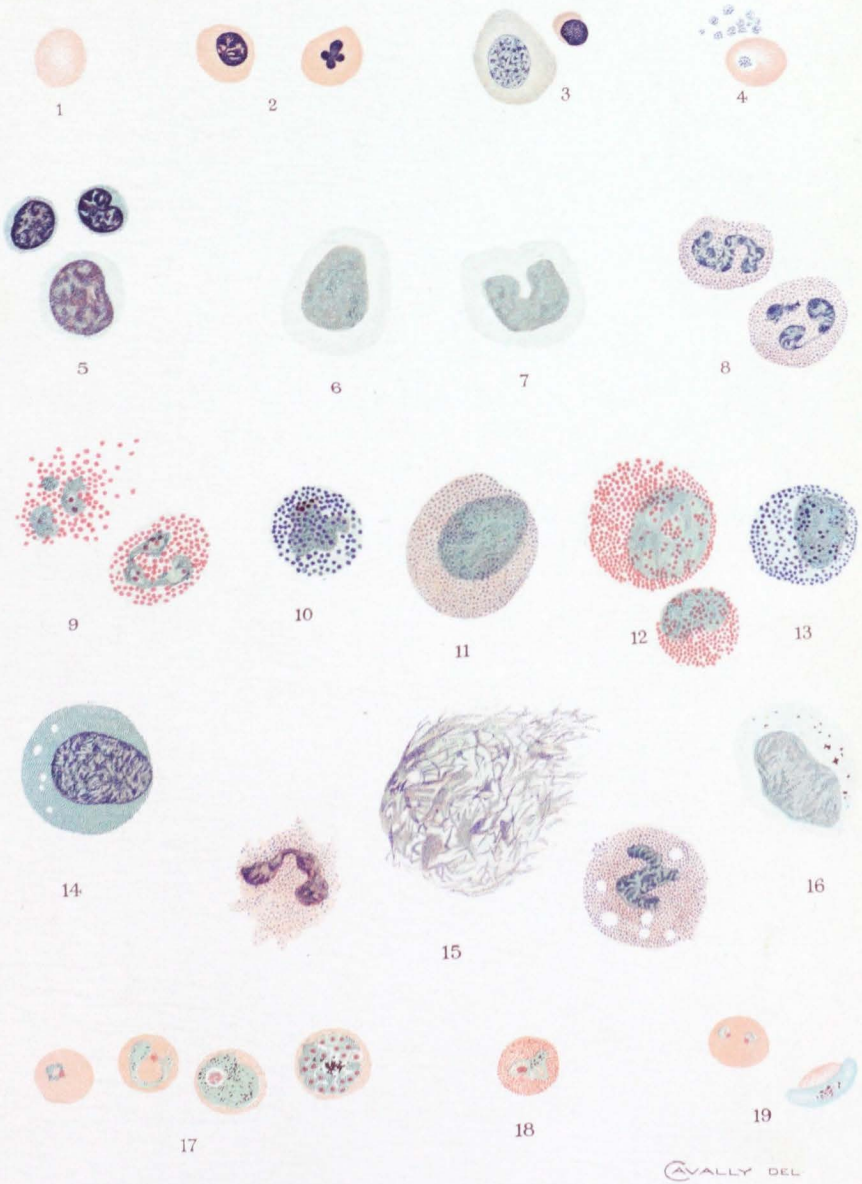
(1) **Normal Varieties.**—(*a*) **Lymphocytes.**—These are small mononuclear cells without granules (Plates VI and X). They are about the size of a red corpuscle or slightly larger (6–10 $\mu$), and consist of a single, sharply

## Explanation of Plate VI

Stained with Wright's stain. All drawn to same scale.

1, Normal red corpuscle for comparison; 2, normoblasts, one with lobulated nucleus; 3, megaloblast and microblast. The megaloblast shows a considerable degree of polychromatophilia; 4, blood-plaques, one lying upon a red corpuscle; 5, lymphocytes, large and small; 6, large mononuclear leukocyte; 7, transitional leukocyte; 8, polymorphonuclear neutrophilic leukocytes; 9, eosinophilic leukocytes, one ruptured; 10, basophilic leukocyte; 11, neutrophilic myelocyte. The granules are sometimes less numerous and less distinct than here shown; 12, eosinophilic myelocytes; 13, basophilic myelocyte; 14, "irritation" or "stimulation" form, with small vacuoles; 15, degenerated leukocytes: two polymorphonuclear neutrophiles, one ruptured, one swollen and vacuolated; and a "basket cell" composed of an irregular meshwork of nuclear material; 16, large mononuclear leukocyte containing pigment-granules; from a case of tertian malaria; 17, four stages in the asexual cycle of the tertian malarial parasite: the second and fourth were drawn from the same slide taken from a case of double tertian; 18, red corpuscle containing tertian parasite and showing malarial stippling; 19, estivo-autumnal malarial parasites: two small ring forms within the same red cell, and a crescent with remains of the red corpuscle in its concavity.

# PLATE VI



defined, deeply staining nucleus, surrounded by a narrow rim of protoplasm. The nucleus is generally round, but is sometimes indented at one side. Wright's stain gives the nucleus a deep purple color and the cytoplasm a pale robin's-egg blue in typical cells. Larger forms of lymphocytes are frequently found, especially in the blood of children, and are difficult to distinguish from the large mononuclear leukocytes. It is possible that the larger

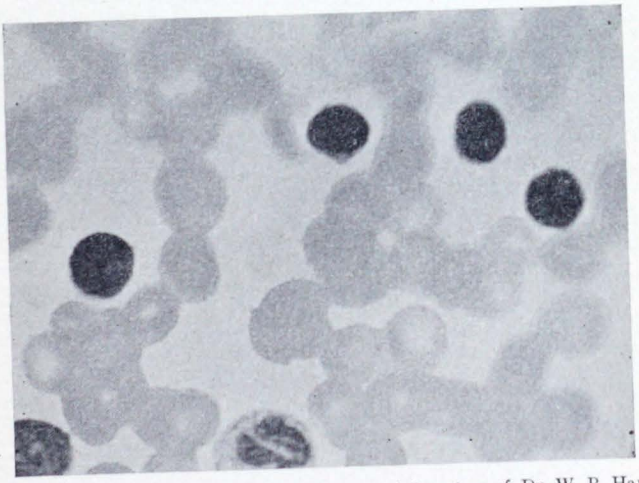


Fig. 89.—Lymphocytosis, case of pertussis (×1000) (courtesy of Dr. W. P. Harlow).

forms are young lymphocytes, which become smaller as they grow older.

Lymphocytes are formed in the lymphoid tissues, including that of the bone-marrow. They constitute, normally, 20 to 30 per cent. of all leukocytes, or about 1000 to 3000 per c.mm. of blood. They are more abundant in the blood of children.

The percentage of lymphocytes is usually moderately increased in those conditions which give leukopenia,

especially typhoid fever, chlorosis, pernicious anemia, and many debilitated conditions. A marked increase, accompanied by an increase in the total leukocyte count, is seen in pertussis (Fig. 89) and lymphatic leukemia. In the latter the lymphocytes sometimes exceed 98 per cent. Exophthalmic goiter commonly gives a marked relative lymphocytosis, while simple goiter does not affect the lymphocytes.

(*b*) **Large Mononuclear Leukocytes** (Plate VI).—These cells are two or three times the diameter of the normal red corpuscle. Each contains a single round or oval nucleus, often located eccentrically. The zone of protoplasm surrounding the nucleus is relatively wide. With Wright's stain the nucleus is less deeply colored than that of the lymphocyte, while the cytoplasm is very pale blue or colorless, and sometimes contains a few reddish granules. The size of the cell, the width of the zone of cytoplasm, and the depth of color of the nucleus are the points to be considered in distinguishing between large mononuclears and lymphocytes. When large forms of the lymphocyte are present, the distinction is often difficult or impossible. It is then advisable to count the two cells together as lymphocytes. Indeed, they are regarded by some hematologists as identical.

Large mononuclear leukocytes probably originate in the bone-marrow or spleen. Some hold that they are developed from the endothelial cells of the blood-vessels. They constitute 2 to 4 per cent. of the total number of leukocytes: 100 to 400 per c.mm. of blood. An increase is unusual except in malaria, where it is quite constantly observed, and where many of the cells contain engulfed pigment.

(*c*) **Transitional Leukocytes** (Plate VI).—These are essentially large mononuclears with deeply indented or horseshoe-shaped nuclei. A few fine neutrophilic granules are sometimes present in their cytoplasm.

They are probably formed from the large mononuclears, and occur in the blood in about the same numbers. The two cells are usually counted together, constituting 4 to 8 per cent. of the leukocytes.

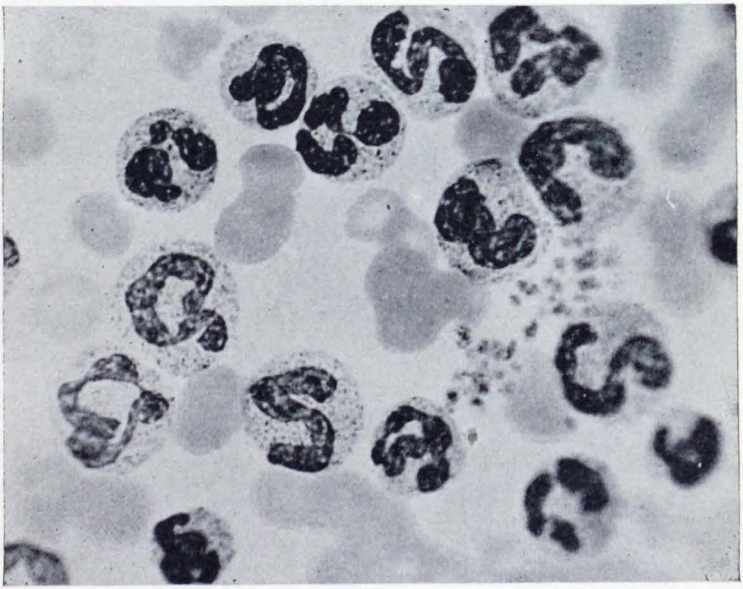


Fig. 90.—Marked polymorphonuclear neutrophilic leukocytosis (×1000) (courtesy of Dr. W. P. Harlow).

(*d*) **Polymorphonuclear Neutrophilic Leukocytes** (Plate VI).—There is usually no difficulty in recognizing these cells. Their average size is somewhat less than that of the large mononuclears. The nucleus stains rather deeply, and is extremely irregular, often assuming shapes comparable to letters of the alphabet, E, Z, S, etc.

(Fig. 90). Frequently there appear to be several separate nuclei, hence the widely used name, "polynuclear leukocyte." Upon careful inspection, however, delicate nuclear bands connecting the parts can usually be seen. The cytoplasm is relatively abundant, and contains great numbers of very fine neutrophilic granules (Fig. 93). With Wright's stain the nucleus is bluish purple, and the granules reddish lilac.

Polymorphonuclear leukocytes are formed in the bone-marrow from neutrophilic myelocytes. They constitute 60 to 75 per cent. of all the leukocytes: 3000 to 7500 per c.mm. of blood. Increase in their number practically always produces an increase in the total leukocyte count, and has already been discussed under Polymorphonuclear Leukocytosis. The leukocytes of pus, *pus-corpuscles*, belong almost wholly to this variety.

A comparison of the percentage of polymorphonuclear cells with the total leukocyte count yields more information than a consideration of either alone. In a general way the percentage represents the severity of the infection, or, more correctly, the *degree of toxic absorption;* while the total count indicates the patient's *power of resistance*. With moderate infection and good resisting powers the leukocyte count and the percentage of polymorphonuclears are increased proportionately. When the polymorphonuclear percentage is increased to a notably greater extent than is the total number of leukocytes, no matter how low the count, either very poor resistance or a very severe infection may be inferred.

Gibson has suggested the use of a chart to express this relationship graphically (Fig. 91). Its arrangement is purely arbitrary, but it may be found helpful in inter-

preting counts. An ascending line from left to right indicates an unfavorable prognosis in proportion as the

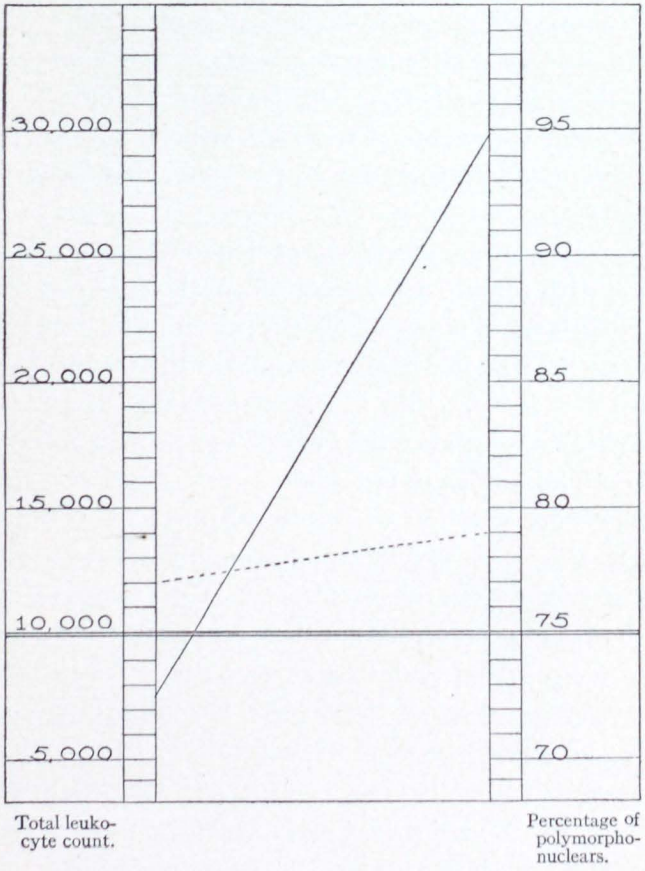



Fig. 91.—Gibson chart with blood-count in two cases of appendicitis: Dotted line representing a mild case with prompt recovery; the continuous line, a very virulent streptococcic case with poor resistance, peritonitis, and early death.

line approaches the vertical. All fatal cases show a rising line. A descending or horizontal line suggests a very favorable prognosis.

It is a matter of observation that in the absence of acute infectious disease or of inflammation directly in the blood-stream (*e. g.*, phlebitis, sigmoid sinusitis, septic endocarditis), a polymorphonuclear percentage of 85 or over points very strongly to gangrene or pus-formation somewhere in the body. On the other hand, excepting in children, where the percentage is normally low, pus is uncommon with less than 80 per cent. of polymorphonuclears.

Normally, the cytoplasm of leukocytes stains pale yellow with iodin. Under certain pathologic conditions the cytoplasm of many of the polymorphonuclears stains diffusely brown, or contains granules which stain reddish brown with iodin. This is called *iodophilia*. Extracellular iodin-staining granules, which are present normally, are more numerous in iodophilia.

This iodin reaction occurs in all purulent conditions except abscesses which are thoroughly walled off and purely tuberculous abscesses. It is of some value in diagnosis between serous effusions and purulent exudates, between catarrhal and suppurative processes in the appendix and Fallopian tube, etc. Its importance, however, as a diagnostic sign of suppuration has been much exaggerated, since it may occur in any general toxemia, such as pneumonia, influenza, malignant disease, and puerperal sepsis.

To demonstrate iodophilia, place the air-dried films in a stoppered bottle containing a few crystals of iodin until they become yellow. Mount in syrup of levulose and examine with an immersion objective.

Arneth classifies polymorphonuclear leukocytes into five groups, according to the number of lobes which the

nucleus shows. The percentage of cells in each group is fairly constant in health, but shows considerable variation in disease.

(*e*) **Eosinophilic Leukocytes, or "Eosinophiles"** (Plate VI).—The structure of these cells is similar to that of the polymorphonuclear neutrophiles, with the striking difference that, instead of fine neutrophilic granules, their cytoplasm contains coarse, round or oval granules having a strong affinity for acid stains. They are easily recognized by the size and color of the granules, which stain bright red with Wright's stain (Fig. 93). Their cytoplasm has generally a faint sky-blue tinge, and the nucleus stains somewhat less deeply than that of the polymorphonuclear neutrophile.

Eosinophiles are formed in the bone-marrow from eosinophilic myelocytes. Their normal number varies from 50 to 400 per c.mm. of blood, or 1 to 4 per cent. of the leukocytes. An increase is called *eosinophilia*, and is better determined by the actual number than by the percentage.

Slight eosinophilia is physiologic during menstruation. Marked eosinophilia is always pathologic. It occurs in a variety of conditions, the most important of which are: infection by animal parasites; bronchial asthma; myelogenous leukemia; scarlet fever, and many skin diseases.

(*a*) Eosinophilia may be a symptom of *infection by any of the worms*. It is fairly constant in trichinosis, uncinariasis, filariasis, and echinococcus disease. In this country an unexplained marked eosinophilia warrants examination of a portion of muscle for *Trichinella spiralis* (p. 363).

(*b*) *True bronchial asthma* commonly gives a marked eosinophilia during and following the paroxysms. This

is helpful in excluding asthma of other origin. Eosinophiles also appear in the sputum in large numbers.

(*c*) In *myelogenous leukemia* there is almost invariably an absolute increase of eosinophiles, although, owing to the great increase of other leukocytes, the percentage is usually diminished. Dwarf and giant forms are often numerous.

(*d*) *Scarlet fever* is frequently accompanied by eosinophilia, which may help to distinguish it from measles.

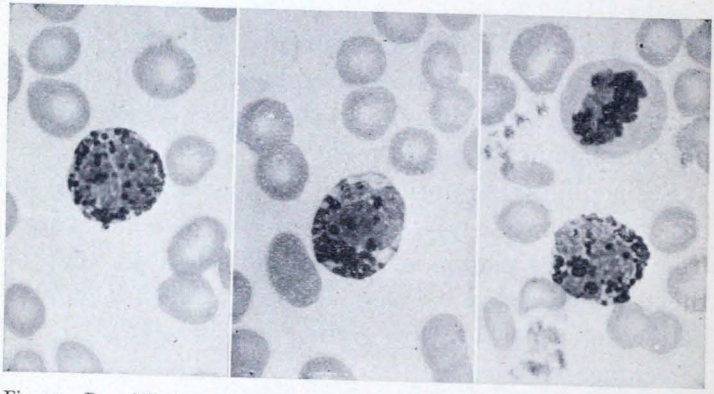


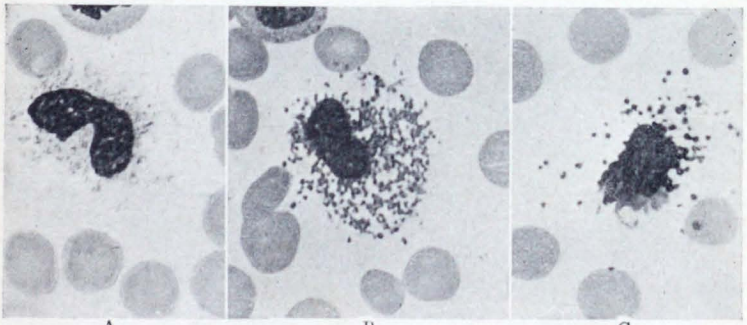
Fig. 92.—Basophilic leukocytes. At the right is also a normoblast undergoing mitosis (× 1000) (photographs by the author).

(*e*) Eosinophilia has been observed in a large number of *skin diseases*, notably pemphigus, prurigo, psoriasis, and urticaria. It probably depends less upon the variety of the disease than upon its extent.

(*f*) **Basophilic Leukocytes or "Mast-cells"** (Plate VI).—In general, these resemble polymorphonuclear neutrophiles except that the nucleus is less irregular and that the granules are larger and have a strong affinity for basic stains. They are easily recognized (Figs. 92 and 93). With Wright's stain the granules are deep purple, while

the nucleus is pale blue and is often nearly or quite hidden by the granules, so that its form is difficult to make out. These granules are not colored by Ehrlich's stain.

The nature of mast-cells is undetermined. They probably originate in the bone-marrow. They are least numerous of the leukocytes in normal blood, rarely exceeding 0.5 per cent., or 25 to 50 per c.mm. A notable increase is limited almost exclusively to myelogenous leukemia, where they are sometimes very numerous.



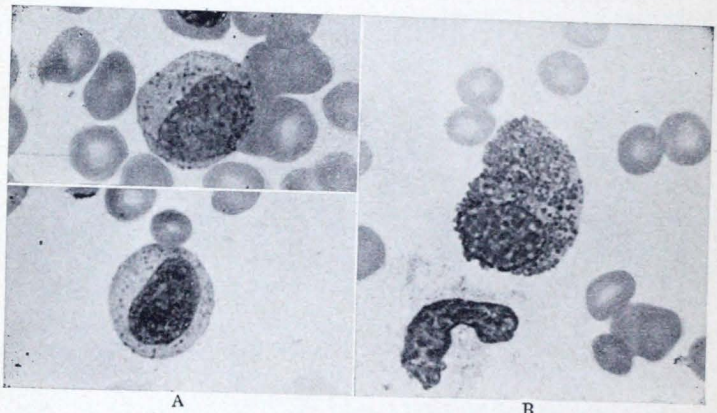
A B C

Fig. 93.—Ruptured leukocytes, showing relative size of granules: A, neutrophilic; B, eosinophilic; C, basophilic (×1000) (photographs by the author).

(2) **Abnormal Varieties.**—(*a*) **Myelocytes** (Plate VI and Fig. 94).—These are large mononuclear cells whose cytoplasm is filled with granules. Typically, the nucleus occupies about one-half of the cell, and is round or oval. It is sometimes indented, with its convex side in contact with the periphery of the cell. It stains rather feebly. The average diameter of this cell (about 15.75 $\mu$) is greater than that of any other leukocyte, but there is much variation in size among individual cells. Myelocytes are named according to the character of their granules—neutrophilic, eosinophilic, and basophilic myelocytes. These granules are identical with the corresponding

granules in the leukocytes just described. The occurrence of two kinds of granules in the same cell is rare.

Myelocytes are the bone-marrow cells from which the corresponding granular leukocytes are developed. Their presence in the blood in considerable numbers is diagnostic of myelogenous leukemia. The neutrophilic form is the least significant. A few of these may be present in very marked leukocytosis or any severe blood condition, as pernicious anemia. Eosinophilic myelocytes are found



A B

Fig. 94.—Myelocytes from blood of myelogenous leukemia: A, Neutrophilic; B, eosinophilic (× 1000) (photographs by the author).

only in myelogenous leukemia, where they are often very numerous. The basophilic variety is less common, and is confined to long-standing, severe myelogenous leukemia.

(*b*) **Atypic Forms.**—Leukocytes which do not fit in with the above classification are not infrequently met, especially in high-grade leukocytosis, pernicious anemia, and leukemia. The nature of most of them is not clear, and their number is usually so small that they may be

disregarded in making a differential count. Among them are:

(*a*) Border-line forms between polymorphonuclear neutrophiles and neutrophilic myelocytes.

(*b*) Small neutrophilic cells with a single round, deeply staining nucleus; they probably result from division of polymorphonuclear neutrophiles.

(*c*) "Irritation forms"—large non-granular mononuclear cells, whose cytoplasm stains fairly deep purple with Wright's stain, and intense brown with Ehrlich's:

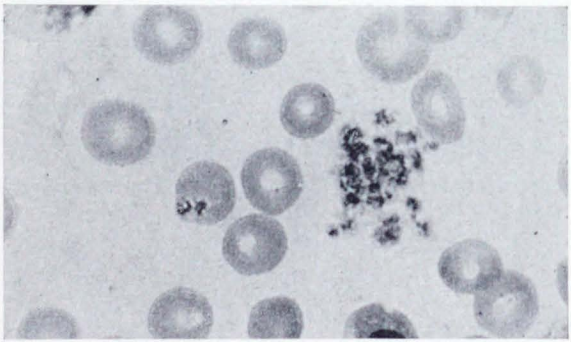


Fig. 95.—A cluster of blood-plaques and two plaques lying upon a red cell and simulating malarial parasites (× 1000) (photograph by the author).

they appear in the blood under the same conditions as myelocytes.

(*d*) Degenerated forms: vacuolated leukocytes, or merely palely or deeply staining homogeneous or reticulated masses of chromatin (the so-called "basket-cells," Plate VI).

**3. Blood-plaques.**—These are not colored by Ehrlich's stain nor by eosin and methylene-blue. With Wright's stain they appear as spheric or ovoid, reddish to violet, granular bodies, 2 to 4 $\mu$ in diameter. When well stained

a delicate hyaline peripheral zone can be distinguished. In ordinary blood-smears they are usually clumped in masses. A single platelet lying upon a red corpuscle may easily be mistaken for a malarial parasite (Plate VI and Fig. 95).

Blood-platelets are being much studied at present, but, aside from the facts mentioned under their enumeration (p. 213), little of clinical value has been learned. They have been variously regarded as very young red corpuscles (the "hematoblasts" of Hayem), as disintegration products of leukocytes, as remnants of extruded nuclei of erythrocytes, and as independent nucleated bodies. The most probable explanation of their origin seems to be that of J. H. Wright, who, from his recent studies, regards them as detached portions of the cytoplasm of certain giant-cells of the bone-marrow and spleen.

## VIII. BLOOD PARASITES

### A. Bacteria

Bacteriologic study of the blood is useful in many conditions, but in general, the elaborate technic involved takes it out of the reach of the clinician. As applied to the diagnosis of typhoid fever, however, the technic of blood-cultures has been so simplified that it can be carried through by any one who is competent to do the simplest cultural work.

**Typhoid bacilli** can be detected in the blood in practically every case of typhoid fever in the first week of the disease; in about 80 to 85 per cent. of cases in the second week; and in decreasing percentages in the later weeks. The blood-culture, therefore, offers the most certain means