the cover slip. This is the third step, and is shown in Fig. 594. The cover slips thus separated are now held from eighteen inches to two feet above the spirit lamp until the blood is quite dry. This is the fourth and last step, and is shown in Fig. 595.

It is recommended by some hæmatologists to use the thumb and forefinger in drawing the upper slip away, rather than to use the open forceps. When this plan is

FIG. 595.—Method of Taking Dried Specimen of Blood. Fourth step.

followed the corners of the cover slips should not exactly correspond, but one cover slip should be allowed to project beyond the other, so as to offer something to hold on to. As it is better not to touch the cover slip except with the forceps, and as the moisture of the skin readily adheres to the glass and ruins the blood specimen, the forceps method should certainly be preferred.

Thayer mentions that flies attack the dried specimens, eating holes in them. While this is undoubtedly true in some cases, yet the holes which one sees in dried specimens are more commonly caused by allowing the blood to dry in the air instead of using a spirit lamp. The writer has seen these holes in specimens taken in winter.

The prepared specimens may now be placed in a labelled pill box and kept indefinitely. The method of preparation here given permits of the preserving of specimens for a number of years, to be used for class purposes; or of sending them, packed in a little cotton, by mail to an expert. By learning this simple technique, therefore, a physician engaged in practice in a section of the country removed from laboratory opportunities, or one occupied in military duties, may take specimens and send them to a laboratory for examination.

The painful mistakes made in establishing the differential diagnosis between typhoid and malaria among the American soldiers in the late Spanish war might have been avoided, and many lives saved, had the microscope been given more place and some of the simple methods above described been carried out. The indiscriminate administration of quinine, while perhaps benefiting many malarial cases, must have produced injurious effects upon the ulcerated intestine.

Before proceeding to the work of staining it is necessary to "fix" the contents of the corpuscles so that they shall not become dissolved; and this is called fixation. A number of methods have been employed, which may be divided into fixation (1) by heat; (2) by fluids; (3) by vapors.

(1) *Fixation by Heat.*—(*a*) Ehrlich's copper-bar method. (1878). A spear point made of copper and about eighteen inches in length is placed upon a support, as shown in cut (Fig. 596). A Bunsen burner is so located that the flame will strike the extreme point of the bar. The flame of an ordinary Bunsen burner, if applied to the extreme tip of a bar of this size, will be able to heat only a certain amount of the bar to a certain degree of heat; beyond which the temperature will not rise, no matter how long the bar is subjected to the flame. In other words, the flame has a limited heating power. This would at once be appreciated if we should attempt to heat with the ordinary Bunsen burner a copper bar five or ten feet long. We should not be surprised to find the far end of the bar cold, no matter how long the copper may have been submitted to the flame. We should realize that the little flame, with its limited heating power, could not heat so large a bar. The heating power of an ordinary Bunsen burner does not extend, on a metal bar, beyond the distance of eighteen inches. What we wish to obtain by this heating procedure is the fixed boiling line on the bar. This we accomplish by placing drops of water upon the surface of the bar, about twenty minutes after it has been subjected to the flame; and directly the point farthest from the apex of the bar at which a drop of water boils away is found, we draw a chalk line across the bar at that point. This marks the temporary boiling line. After the lapse of a few minutes we put another drop of water just beyond the line; if the boiling point has extended the drop will boil away, and we then draw a second line at the new point and still farther from the apex. This procedure is repeated until a point is reached at which the water does

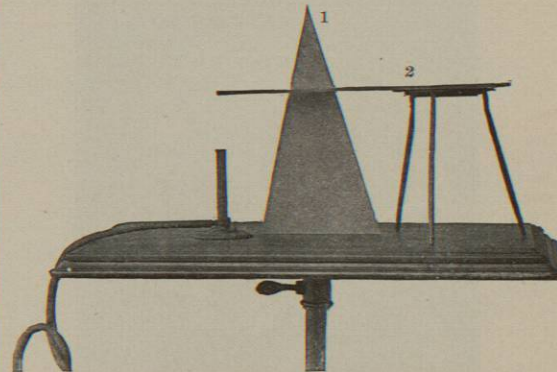

FIG. 596.—Ehrlich's Copper-Bar Method of Fixation of Blood Specimens by Heat. 1, Bar placed in upright position to show shape; 2, bar in place with Bunsen burner at tip.

not boil away, when we know that the limit of the heating power of the flame has been located. Once the permanent boiling line is thus located, no matter how long the flame is allowed to attack the apex of the bar, the line will never extend any farther from the apex. The confirmation of the fact that this line has been reached is

afforded when a drop of water boils away almost at once on the apex side of the line, but remains until slowly dried off on that side of the line away from the apex. This, then, is the line we seek, and it is called the fixed boiling point or line. The blood specimens, as above prepared, are now laid *blood side up* either upon this line or directly to the boiling side of it. If the boiling line has been accurately located, it is, in the writer's experience, rather severe treatment to place the specimens blood side down on this line, as recommended by Cabot ("Clinical Examination of the Blood," third edition, p. 44). Specimens so placed are allowed to remain from forty minutes to an hour, except in cases of pernicious anæmia or of grave secondary anæmia, when a much shorter time—perhaps only twenty minutes—is sufficient. This is not satisfactory for malaria specimens. One should be careful not to allow draughts to influence the direction of the flame, as in this way the bar is chilled and the boiling point thereby altered for a period of several minutes. Ehrlich's copper-bar method is a good one, in the thoroughly equipped laboratory, for specimens prepared for class purposes; but it is tedious and calls for much watching, and has been replaced by quicker and simpler methods which seem, so far as one can at present judge, equally good.

(*b*) A quick heat method, and one applicable to office work, is that by means of the spirit lamp. The specimens, taken as above described, are simply held about two feet above the flame until the blood is thoroughly dried; or the cover slips may be heated to 60° or 70° C. (a temperature which can be borne by the skin) before taking specimens. This serves for immediate staining and examination; but for specimens which are to be kept for a long time, or in which accurate differential examination is desired, it will be found unsatisfactory.

(*c*) Another fairly quick heat method is that by means of the thermostat, brought gradually to a temperature of 150° C. and kept at this degree of heat for from forty minutes to an hour. This is as satisfactory as the copper-bar method, but not as readily carried out.

(*d*) Another simple means is to make use of the top of a steam register, upon which the specimens are allowed to remain for from forty minutes to an hour. This is a very good and convenient method. Care must be taken that no moisture shall be on the metal.

(2) *Fixation by Fluids.*—(*a*) Of these, Nikiforov's method is the best. It calls for equal parts of absolute alcohol and ether. The specimens are immersed in this liquid for from two to four hours. This, next to the formalin method given below, is the best for malarial specimens. For quick work fairly good results may be obtained from an immersion lasting twenty minutes.

(*b*) Dr. G. A. Tuttle has lately devised a method which is described by Dr. Fred P. Solley, in the reports of the Pathological Department of the Presbyterian Hospital, New York City. It was kindly brought to my attention by Dr. W. G. Thompson. The following is taken from those reports:

"Experiments were made by Dr. G. A. Tuttle with chromic acid, and after trials of different strengths for varying periods of time he found that a two-per cent. solution poured on the film and allowed to remain for exactly thirty seconds gave uniformly good results, both red and white cells taking up their special stains clearly and surely. The nuclei and granules of the leucocytes stain quite as sharply as in the best specimens fixed by heat—while the protoplasm of the mononuclear lymphocytes is usually better stained than by the latter method.

"By this method, then, the whole process of preparing the specimens for examination is reduced to five or six minutes, and when the films are evenly and thinly spread the preparations are certain to be available for accurate differential computation. The red cells occasionally show defects as a result of the action of the acid; but this usually means that the solution has been left on too long, or that the films were unevenly spread. Indeed, the spreading of the films is of the utmost importance in securing satisfactory results by any method. . . . The two-per-cent. chromic acid solution is spread from a dropper over the film, the cover glass being held in a staining forceps. After thirty seconds the acid is thoroughly washed off and the excess of water removed by tapping a corner of the square on filter paper. The stain is then poured over the film in the same manner, and washed off quickly after three minutes. The specimen may then be dried between layers of filter paper and is ready to be mounted in balsam.

"By employing this method a complete blood examination may be carried out in a little over an hour at the bedside of the patient, often affording valuable information in cases in which the desirability of surgical intervention is in question."

This method has proved most satisfactory in my hands.

(*c*) Bichloride of mercury and Müller's fluid (formula, see p. 48, footnote) may be used, but the results are uncertain.

(*d*) Formalin method. Five drops of ten-per-cent. formalin solution are mixed with 10 c.c. of 95 per cent. alcohol. The cover slips are immersed in this for thirty seconds.

Futcher and Lazear report this as a most satisfactory method for malarial blood. The writer has found that when using the Ehrlich eosin hæmatoxylon stain this is a thoroughly reliable fixing method. Futcher and Lazear's statement as to its value for malarial specimens is, in the writer's opinion, to be indorsed.

(3) *Fixation by Vapors.*—In this method cotton soaked in two-per-cent. formalin is kept at the bottom of a wide-mouthed bottle. The specimens are subjected to the vapor from this, with the cork in the bottle, for from fifteen to thirty seconds. This is a good office-table and classroom method. During one winter my classes at the Cornell University Medical College prepared all their blood specimens by this method. While quickly obtained, the stains were not permanent, so that in a few months they had almost wholly faded out.

Blood Stains.

The following is not an exhaustive article upon blood staining; it treats of this subject only in so far as it is part of the apparatus used in the clinical examination of the blood. So much has lately been accomplished in the differential staining of the malarial parasite that this part of the subject of blood staining will be treated of under other heads (*Histological Technique*, *Malaria*, etc.).

In the process of distillation of coal gas from bituminous coal, for ordinary lighting purposes, there condenses upon the pipes a "thick, black, opaque, viscid liquid" which is known as coal tar. From this coal tar have been separated great numbers of useful products, such as paraffin, naphtha, benzol, creosote, carbolic acid, pitch, etc. When benzol is acted on by nitric acid, nitrobenzol is formed, and this, when treated with nascent hydrogen, produces aniline, which is an oily liquid, aniline oil occurring as a by-product of the process. This aniline is a chemical base and unites with acids to form salts. These salts are of a great variety of most beautiful colors—violet, green, purple, etc.—and are the origin of our valuable stains in microscopic work. Benzol being the source commonly employed in the preparation of the aniline dyes for commerce, this would make these dyes apparently by-products of coal tar. Aniline, however, is a direct product, but in too small quantities to make it profitable to separate it. The word anil comes from the name of a plant from which indigo is made; the word signifies purple, and is applied to this product of coal tar as purple is one of the most striking colors produced by the combination of aniline with an acid.

The stains used in microscopic work are manufactured by Grübler in Germany and sold in powder form. From these, various strengths of stain may be made. The system by which these powders are classified is at times confusing. It is to be regretted that the names are selected so as purposely to convey no information beyond that of enabling the compounder to refer to his records and

there learn the secret process which produced the stain. So no further explanation can be given of this classification. Many of these names, as tropaeolin ōō and orange "G," were given by the commercial compounders and have been adopted by Grübler, who confines himself to the preparation of stains for scientific purposes. Eosin is from the Greek word meaning the dawn. Methyl blue and methylene blue differ the one from the other in that the former, mixed with sodium hydrate, changes color to a reddish brown, the latter to violet. Methyl blue is the stain usually employed in blood work.

The stains are grouped into two general classes—acid and basic. These terms do not indicate a litmus reaction, and are therefore not to be taken in the strict chemical sense. The stains are for the most part neutral salts, that is, made up of a base and an acid, but neither acid nor basic in reaction. Now if the acid part of a salt is the staining element in the salt, the stain is called an acid stain. Example: ammonium picrate, which is a salt made up of ammonia, *the base*, and picric acid, *the acid*. Picric acid is the element in the salt which stains; hence the name *acid stain*. Another stain is rosanilin acetate—rosanilin is *the base* and acetic acid is *the acid*. The base in this salt is the staining element, and hence the name *basic stain*. The term *neutral stain* refers rather to a peculiarity of the tissue than to the stain.

According to this grouping of the stains the tissues are also classified. Those tissues which stain with an acid stain only are called eosinophilic; those which stain with a basic stain only are called basophilic, and those requiring both an acid and a basic stain are called neutrophilic. Tissues which consistently stain with an acid stain were termed by Ehrlich eosinophilic, because eosin was the most striking stain of the acid group. Now that we have all our stains clearly classified, however, it is better to adopt a term referring to the entire group rather than to a single stain in the group. Oxyphilic (ὀξύς, acid; φιλέω, I take to) is therefore a better word.

THE STAINS.

(This list contains only a few of the more common stains.)

Acid.	Basic.
Eosin,	Rosanilin acetate,
Fuchsin (acid),	Fuchsin (basic),
Orange "G,"	Saffranin,
Picric acid,	Methyl blue,
Aurantia,	Methyl violet,
Indulin,	Methyl green, etc.
Nigrosin,	
Tropaeolin ōō, etc.	

The *Tissues*, according to the explanation just given, are grouped by Ehrlich as follows:

1. Oxyphilic (ὀξύς, acid; φιλέω, I take to), synonymous with eosinophilic (acidophilic is a hybrid, and should therefore be discarded), refers to tissues taking only acid stains. The Greek letter "α" is used to designate oxyphilic granules. Example: eosinophiles of human blood.

2. Amphiphilic (ἀμφί, both)—refers to those tissues staining equally well with either an acid or a basic stain. Example: the bone marrow of rabbits. There are no amphiphilic granules in human blood. The Greek letter "β" is used to designate amphiphilic granules.

3. Basophilic refers to tissues taking only basic stains. Example: Mastzellen. The Greek letter "γ" is used to designate basophilic granules.

4. Neutrophilic refers to tissues requiring both an acid and a basic stain. Example: polynuclear leucocytes. The Greek letter "ε" is used to designate neutrophilic granules. (The word neutrophilic is a hybrid—neutron, neither, and φιλέω, to take to. Oudeterophilic (οὐδέτερος, neither), would be more correct but not as simple, perhaps, as the present word.)

In order to demonstrate these staining reactions of the various blood corpuscles and their granules, the stains should be each in turn applied to specimens of blood; then the specimens should be examined, and those parts which take the stain should be noted.

For example, subject a specimen of blood to an acid stain—eosin. Result: Red cells stained; eosinophile granules stained; granules of polynuclear leucocytes very faintly tinged; *all else unstained.*

Subject a specimen of blood to a basic stain—methyl blue. Result: Nuclei of all leucocytes stained; granules of polynuclear leucocytes very faintly tinged; *all else unstained.*

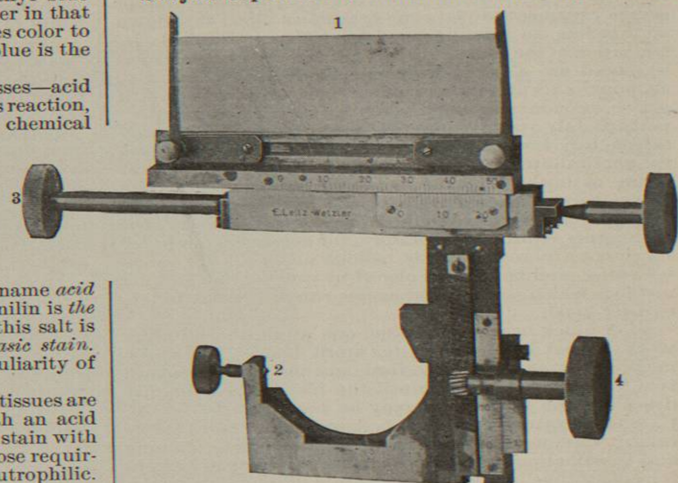

FIG. 597.—Mechanical Stage. 1, Slide in place; 2, space and screw for reception of and fastening to upright of microscope; 3, screw for lateral motion of slide; 4, screw for forward and back motion of slide.

Subject a specimen of blood to a neutral stain—eosin and methyl blue. Result: Red cells stained red; eosinophile granules stained red; nuclei of leucocytes stained blue; granules of leucocytes stained reddish blue.

The blood thus prepared is now examined and the percentage of normal cells, peculiarities of staining, and cells not normally in peripheral circulation are noted. In order to examine these fields accurately and not to recount the cells we employ the mechanical stage (Fig. 597). This is so constructed as to enable one to move the specimen a microscopic distance at a time from side to side, up and down, and across diagonally. It is moreover supplied with a register by means of which the observer may take note of his whereabouts in the field, so that having moved away from the field, he may return to it again by causing the figures upon the register to stand according to the position previously noted. In this way corpuscles may be restudied and at the same time the whole specimen thoroughly examined.

When the blood has been fixed as already described it is then subjected to the stains for varying lengths of time. The following are a few of the stains and formulæ employed in blood work:

1. Eosin: 0.5-per-cent. alcohol solution; one-half to one minute. Methyl blue—saturated aqueous solution; one-half to one minute.

2. Ehrlich's triple stain:

	Ingredient	Amount	Brackets
1	Acid fuchsin	3.5	1 } 3 } 4
1	Aquæ	2.0	1 } 3 } 4
2	Orange "G"	6.0	2 } 3 } 4
2	Methyl green	6.5	2 } 3 } 4
	Aquæ	10.0	3 } 4
	Absolute alcohol	5.0	4

The brackets show the order in which ingredients should be mixed in preparing the stain.

NOTE.—The sero-diagnosis test for typhoid fever is not included in this article, being considered under the head of *Typhoid Fever*.

TABLE SHOWING THE SOURCE, APPEARANCE, STAINING REACTION, GREEK LETTER EXPRESSING STAIN, AND THE NUMERICAL VALUE OF EVERY CELL FOUND IN THE PERIPHERAL CIRCULATION UNDER NORMAL OR PATHOLOGICAL CIRCUMSTANCES.

Name according to source.	Name according to appearance.	Name according to stain. Protoplasm / Granules.	Name according to stain. Nucleus.	Name according to Greek letter expressing stain.	Numerical value of each cell.
Non-granular. 1.	Erythrocytes. Red cell	Oxyphilic		(α)	5,000,000 to cubic millimetre.
Non-granular. 2.	Erythrocytes. Normoblast (Nucleated red cells)	"	Basophilic	(α)	Found normally in bone marrow only. In peripheral circulation in pernicious and grave secondary anæmia.
Non-granular. 3.	Erythrocytes. Gigantoblast or megaloblast (Nucleated red cells)	"	"	(α)	
Non-granular. 4.	Plates	Basophilic		(γ)	300,000 to cubic millimetre.
5. Lymphocyte	Leucocytes. Small mononuclear	Neutrophilic (Sometimes basophilic granules at periphery.)	Basophilic	(ε) (δ)	6,000 to 10,000 in c.mm.: 20–30 per cent.
6. "	Leucocytes. Large "	Neutrophilic	"	(ε)	2–3 "
7. "	Leucocytes. Transitional "	"	"	(ε)	2–3 "
8.	Leucocytes. Polynuclear	" (Basophilic in leukæmia)	"	(ε)	60–70 "
9.	Leucocytes. Coarse granular (Eosinophile)	Oxyphilic (Eosinophilic)	"	(α)	2–3 "
10. Myelocyte		Neutrophilic	"	(ε)	Found in bone marrow only. In peripheral circulation in spleno-medullary leukæmia.
11. Mastcell		Basophilic (Requires dahlia to stain.)	"	(γ)	¼ of 1 per cent. (Virchow). Increased in leukæmia.
12. Eosinophilic myelocyte		Oxyphilic	"	(α)	Found in bone marrow only. In peripheral circulation in spleno-medullary leukæmia.

NOTE.—The mastcell of Cornhill was described by him prior to Ehrlich's observations upon the staining peculiarities of these cells, and may have been a myelocyte or a large mononuclear, and had best, therefore, be dismissed from the list of blood cells.

3. Carbol thionin: Saturated solution of thionin in 50-per-cent. alcohol, 20; 2-per-cent. carbolic acid, 100. (Should stand several weeks before being used.) Stain for from ten to fifteen seconds. Especially good for malarial parasite.

4. Eosin hæmatoxylon of Ehrlich: Eosin (cryst.), 0.5; hæmatoxylon, 2; absolute alcohol, 100; distilled water, 100; glycerin, 100; acetic acid, 100; glacial acetic acid, 10; alum (in excess), 50. (Should be made several weeks before using.) Alum aids in dissolving hæmatoxylon.

5. Müller's fluid, see p. 48, footnote. 6. Formalin solution for fixing blood, see p. 65. 7. Hayem's solution, see p. 40. 8. Pacini's solution, see p. 40. 9. Polychrome methylene blue (methylene blue, 1; potassium carbonate, 1; water, 100. Keep for several months before using.) 10. Toison solution, p. 43.

CHART ARRANGED BY THE WRITER FOR CLINICAL REPORT ON BLOOD; USED IN THE CORNELL DIVISION OF BELLEVUE HOSPITAL, NEW YORK CITY.

Name............ Record No............
Address.............. ....

Red Blood Corpuscles:

Size...... { Normally (1 $\mu = \frac{1}{25000}$ of an inch), 7 μ.
Microcytes, 2–5 μ.
Macrocytes, 8–10 μ. }

Shape .... { Normally, biconcave discs.
Poikilocytes, irregular outline.
Crenated, spiculated. }

Color ..... { Normally, pale yellow.
Colorless, shadow corpuscles
Vacuolated, degeneration areas—deficiency in hæmoglobin. }

Number per cubic millimetre. { Normal, 5,000,000.
By Gowers' instrument...............
By Thoma and Zeiss instrument........
By Daland's hæmotocrit..............
By Oliver's hæmocytometer........... }

Nucleated red cells. { Normally, not present.
Normoblasts.
Megaloblasts or gigantoblasts. }

Staining .. { Normally uniform.
Polychromatophia = different intensity of stain in cells. }

Malarial parasite.
- Hyaline. { Amœboid.......... Non-amœboid.......... Pigmented.......... }
- Pigmented.
  - Intra-cellular. { One-fourth grown... One-half grown.... Full grown........ Pigment motile.... }
  - Extra-cellular. { Non-flagellating ... Vacuolated........ Flagellating........ Non-vacuolated.... Pigment motile..... }
- Segmenting..........
- Crescents .... { Pigment motile.......... Flagellating.......... }
- Round bodies. { Pigment motile.......... Flagellating.......... }

Hæmoglobin:
Normal, 85 to 95 per cent.
By Gowers' hæmoglobinometer..................
By von Fleischl's hæmoglobinometer..............
By Oliver's tintometer.........................
By Roy's specific-gravity method...............
Color index (normal, 1).........................

White Blood Corpuscles:

Number per cubic millimetre. { Normal 6,000 to 10,000.
By Gowers' hæmocytometer..........
By Thoma-Zeiss hæmocytometer.......
By Daland's hæmatocrit..............
By Friedländer's instrument.......... }

Number per each 100 leucocytes.

	Normal.
Small mononuclear lymphocytes	20–30%
Large mononuclear lymphocytes	3
Transitional lymphocytes.......	3
Polynuclear leucocytes.........	60–70
Eosinophiles..................	2.4
Mastcells.....................	0.25
Myelocytes...........	not present.
Eosinophilic myelitis	"

Plates .... { Present (normal, 250,000 per cubic millimetre).
Absent. }

Plaques... { Present.
Absent. }

Fibrin..... { Present.
Absent. }

Blood Dust. { Present. Absent.
Coagulation time (normal, two to three minutes).
Gross test.
Wright's coagulometer tubes.

Staining . . { Fixation { Heat { Flame. Copper bar. Sterilizer. } Alcohol and ether āā, equal parts. Formalin (10 per cent. in 95-per-cent. alcohol). } Stain. }

Remarks.

Ehrlich's Eyepiece (Fig. 598) supplies an important need. This instrument is so devised that accurate fields may be measured off on the dried specimen of blood and the relative numerical values of white and red corpuscles thereby ascertained. The instrument is constructed like an eye-piece, and may be fastened to the microscope in place of the eyepiece. A handle projects from one side of the instrument, and when this is moved the square aperture noted on looking through the eye-piece is seen to increase and decrease equally on all sides according to the direction in which the handle is turned. On the uppermost side of the square (see Fig. 598) three notches will be noted. When the handle is placed so that the square is at its greatest size the dimensions of this square will be just sixteen times that of the square formed when the first notch is covered. The second notch indicates a square four times as large as when the first notch is covered; the third notch indicates a square nine times as large; and the largest square, as stated, is sixteen times as large as the smallest.

Thus by counting all the red cells in the first or smallest square of a number of fields, and all the white cells in the fourth or largest square (which is sixteen times as large as the smallest) of a number of fields, and multiplying the number of red cells by sixteen, we get the ratio of red to white cells in a dried specimen.

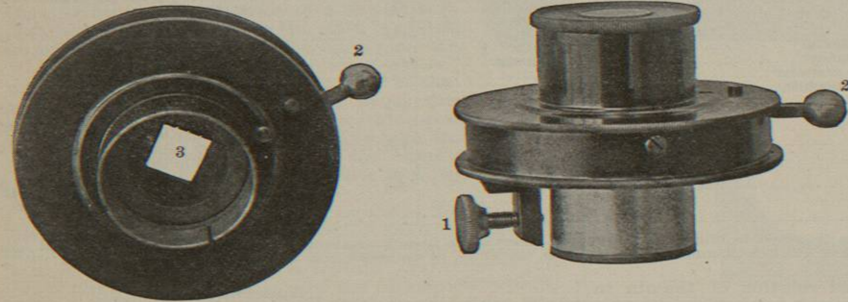

FIG. 598.—Ehrlich's Eyepiece for Determining the Relative Values of White and Red Cells in a Dried Specimen of Blood. 1, Screw for fastening eyepiece to microscope; 2, handle by means of which the square aperture (3) may be altered in size; 3, aperture reducible to definite relative sizes by observing notches seen on upper margin of square.

ALKALINITY OF THE BLOOD.—Engel's (*Berliner klin. Woch.*, 1897) modification of the Löwy-Zuntz method for estimating the degree of alkalinity of the blood places the apparatus among those applicable for this purpose in bedside examinations. It consists of the following (Fig. 599):

1. A capillary pipette similar in construction to that used in the Thoma-Zeiss corpuscle-counting apparatus, but of somewhat larger size. The capillary portion is marked off into ten equal divisions. The fifth mark indicates 0.025 c.cm. and the tenth indicates 0.05 c.cm. Above the bulb portion is a mark that indicates 5 c.cm.
2. A receiver and a stirrer made of glass.
3. A burette measuring 5 c.cm., and marked 1, 2, 3, 4, 5 in equal divisions.
4. Litmus paper.

To Make the Test.—Blood from the ear (taken in the usual way) is drawn up to the 0.05 mark and distilled water to the 5.0 mark. These are mixed by carefully shaking the pipette, and then the mixer is blown out into the glass receiver. In the burette is placed a $\frac{1}{25}$ normal tartaric acid solution.

As stated elsewhere in this article, a normal solution in chemistry is the sum of the atomic weights of the chemical employed (in grams) in 1,000 c.c. of distilled water. The formula for tartaric acid is $C_4H_6O_6$, and the sum of its atomic weights would be $\frac{C_4H_6O_6}{2}$—*i.e.*, $\frac{48+6+96}{2} = 75$. Therefore 75 gm. of tartaric acid in 1,000 c.c. of water would represent a normal tartaric acid solution. A $\frac{1}{25}$ normal solution, however, is all that is required, which would be: 1 gm. tartaric acid to 1 litre of distilled water.

This solution is then allowed to drop from the burette into the receiver containing the diluted blood, which is stirred from time to time with the glass stirrer. Blue litmus is immersed from time to time, and directly this turns red, showing an acid reaction, the titration is at an end, and the amount of acid solution required to accomplish this acid reaction (that is, the amount required to overcome the alkalies of the blood) is ascertained

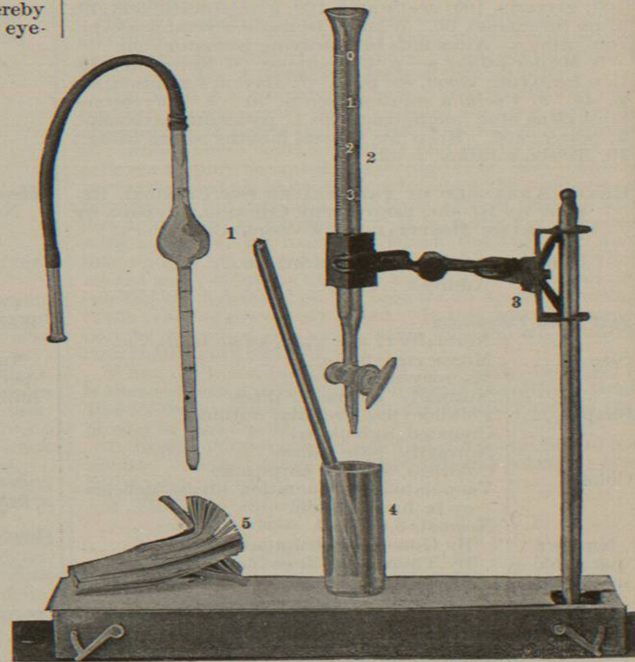

FIG. 599.—Engel's Apparatus for Determining at the Bedside the Degree of Alkalinity of the Blood. 1, Capillary pipette for taking and mixing definite quantities of blood; 2, burette for acid solution; 3, litmus paper; 4, receiver and stirrer for diluted blood while titrating.

TABLE OF DISEASES, THE DIAGNOSIS OF WHICH MAY BE MADE THROUGH THE BLOOD. THE CHARACTERISTICS OF EACH DISEASE ARE SHOWN IN THIS TABLE. THE DISTINCTIVE FEATURES ARE MARKED THUS (☞)

	Red blood cells.	Hæmoglobin.	Color index.	White blood cells.	Proportion of red to white blood cells.	Poikilocytes.	Microcytes and macrocytes.	Degeneration areas and polychromatophilia.	Normoblasts and gigantoblasts.	Small mononuclear lymphocytes.	Large and transitional lymphocytes.	Polynuclear leucocytes.	Eosinophilic leucocytes.	Myelocytes.	Mastcells.	Plates.	Fibrin.	Coagulation time.	Blood dust.
1. Normal blood	5,000,000 to c.mm.	90 to 100%	1	6,000 to 10,000 to c.mm.	700 to 1	Absent	Absent	Absent	Absent	20 to 30 in every 100 leucocytes; 20 to 30%	6 to 8 in every 100 leucocytes; 6 to 8%	60 to 75 in every 100 leucocytes; 60 to 75%	2 to 3 in every 100 leucocytes; 2 to 3%	Absent	1 in every 400 leucocytes; ¼ of 1%	300,000 to c.mm.	Absent	2 to 3 minutes	Present
2. Primary anæmias. *A*, Chlorosis	4,000,000 to c.mm. normal	☞ Below 80% reduced	Low	Normal	Normal	Present	Present	Present	Absent	Normal	Normal	Normal	Normal	Absent	Normal	Normal	Absent	Normal	?
B, Pernicious	1,000,000 to 3,000,000 to c.mm.; markedly reduced	Reduced, but above the reduction in red cells	High	Normal	Altered from normal 300 to 1	Present	Present	Present	☞ Present	☞ Increased 30 to 40%	Normal	Decreased 50 to 60%	Normal	Absent	Normal	?	?	?	?
3. Secondary anæmia	☞ 2,000,000 to 4,000,000 to c.mm.; reduced	Reduced in proportion to decrease in number of red cells	Fairly high	12,000 to 15,000 to c.mm.; moderate increase	Altered from normal 400 to 1	Present	Present	Present	Absent	Normal or decreased in proportion to polynuclear increase	Normal	Increased 75 to 80%	Normal	Absent	Normal	?	?	?	?
4. Addison's disease and 5. Hæmochromatosis	☞ Normal, may be slight anæmia	Normal	According to grade of anæmia	Normal	Normal	Absent	Absent	Absent	Absent	Normal	Normal	Normal	Normal	Absent	Normal	Normal	Absent	Normal	Present
6. Anæmia, pseudo-leukæmia infantum	☞ Reduced	Reduced	do.	☞ Greatly increased 100,000 to c.mm.	Altered. 18 to 1	Present	Present	Present	☞ Present	☞ Increased	Normal	Decreased	Normal	☞ Present	Normal	?	?	?	?
7. Leucocytosis. *A*, Leukæmia. (1) Lymphatic	Normal or slightly decreased	Normal or slightly diminished	do.	☞ Marked increase, 70,000 to c.mm.	Altered, 30 to 1	May be present	May be present	May be present	Not present unless anæmia exists	☞ Increased 70 to 80%	Normal or slightly increased	Decreased	Normal	Absent	Normal	?	?	?	?
(2) Spleno-medullary or lienomyelogenous myelocytic	Normal or slightly decreased	Normal or slightly decreased	do.	☞ Very greatly increased 150,000 to c.mm.	May be as low as 1 to 1	May be present	May be present	May be present	Not present unless anæmia exists	Decreased	Normal	Decreased	Normal	☞ Present	☞ Increased	?	?	?	?
B, Inflammatory. Polynuclear	Normal	Normal	do.	Increased 12,000 to 40,000 to c.mm	Slightly altered	Absent	Absent	Absent	Absent	Decreased	Normal	Increased 75 to 80%	Normal	Absent	Normal	?	?	?	?
C, Trichinotic, etc. Eosinophilic	Normal	Normal	do.	Increased	Normal	Absent	Absent	Absent	Absent	Normal	Normal	Decreased	Increased as high as 89%	Absent	Normal	?	?	Normal	?
8. Hodgkin's disease	Normal. May be reduced	Normal. May be reduced	do.	☞ Normal	Normal	Absent. May be present	Absent. May be present	Absent. May be present	Absent	Normal	Normal	Normal	Normal	Absent	Normal	?	?	?	?

by noting the level at which the liquid stands in the burette.

There is no one alkali in the blood which we can hold responsible for its alkaline reaction. It is therefore necessary to measure this alkalinity by taking a known alkali, say NaHO, as the unit. By ascertaining the amount of a $\frac{1}{15}$ normal tartaric acid solution required to neutralize a known quantity of blood, and then ascertaining the amount of sodium hydrate neutralized by this amount of a $\frac{1}{15}$ tartaric acid solution, we learn in terms of NaHO the amount of tartaric acid required to neutralize a known quantity of blood. Things which are equal to the same are equal to one another.

Thus by experiment we find that 0.5 c.cm. of $\frac{1}{15}$ normal tartaric acid solution neutralizes 0.05 c.cm. of blood; *then* 1,000 c.cm. of $\frac{1}{15}$ normal tartaric acid solution neutralizes 100 c.cm. of blood.

Again, by experiment, we find that 75 gm. of tartaric acid neutralizes 40 gm. NaHO ($23 + 1 + 16 = 40$). 1 gm. of tartaric acid neutralizes ($\frac{1}{75}$ of 40) 533 mgm. of NaHO.

Since 1,000 c.cm. of $\frac{1}{15}$ tartaric acid solution neutralizes 100 c.cm. of blood, 100 c.cm. of blood has an alkalinity of 533 mgm. of NaHO.

0.5 c.cm. of $\frac{1}{15}$ normal tartaric acid solution neutralizes 0.05 c.cm. of blood. 1,000 c.cm. of $\frac{1}{15}$ normal tartaric acid solution neutralizes 100 c.cm. of blood. 75 gm. of tartaric acid solution neutralizes 40 gm. of NaHO.

$C_4H_6O_6 = \frac{48 + 6 + 96}{2} = 75$ neutralizes NaHO $= 23 + 1 + 16 = 40$.

Then 1 gm. of tartaric acid solution neutralizes ($\frac{1}{75}$ of 40) 0.533 NaHO.

This is the quantity of tartaric acid in the $\frac{1}{15}$ normal solution (1 gm. in 1,000 c.c. of water). This, therefore, is the value in alkalinity—so to speak, in terms of NaHO—of the tartaric acid solution employed.

Now on titration it is found that from 9 to 10 drops of such a solution are required to neutralize 0.05 c.cm. of blood.

10 drops equal 0.5 c.cm., which equal 0.5 gm.

0.5 gm. equals 0.266 NaHO.

Therefore 10 drops equal an alkalinity of 0.266 NaHO.

1 drop = 0.0266 NaHO.

It is perhaps better to bear in mind that 9 or 10 drops represent the normal, and to report examinations accordingly. For example, "6 drops required to neutralize," "12 drops required to neutralize," etc. It would also simplify the proceeding to use a solution of litmus in the blood solution instead of the papers.

The Eyepiece Micrometer (Fig. 600) is of so simple construction and mechanism that its employment is to be recommended for more accurate clinical reports on the size of the blood corpuscles.

The following explanation is taken from the Leitz advertisement of the instrument:

Micrometric Measurements.—The scale of the eyepiece micrometer is divided into $\frac{1}{10}$ mm. Each of these divisions represents, according to the objective used, a certain absolute linear measure of the object, as shown in the following table:

Number of objective.	Absolute length of object represented by one division of the eyepiece-micrometer scale. Millimetres.	Number of objective.	Absolute length of object represented by one division of the eyepiece-micrometer scale. Millimetres.
1	0.054	7	0.0026
2	.028	8	.0020
3	.015	9	.0017
4	.012	Immersion $\frac{1}{10}$	.0022
5	.0048	" $\frac{1}{12}$	.0018
6	.0034	" $\frac{1}{18}$	.0014

When making micrometer measurements it is absolutely necessary accurately to maintain the tube length at 170 mm. If this is neglected the measurements become unreliable or even worthless.

The above micrometer values are measured with eyepiece II.; in the other eyepieces they differ in an inappreciable degree.

Example: Let a scale of *Hipparchia Janira*, as seen with objective 6, cover 54 divisions of the scale longitudinally and 20 divisions transversely. Its actual length will then be $54 \times 0.0034 = 0.184$ mm., and its breadth $20 \times 0.0034 = 0.068$ mm.

Suppose a valve of *Pleurosigma angulatum*, measured with objectives 4, 6, and 7, to cover 21, 74, and 98 divisions respectively; then the measurements of its length represent the following absolute dimensions:

Objective 4: $21 \times 0.012 = 0.252$ mm.
" 6: $74 \times 0.3004 = 0.252$ "
" 7: $98 \times 0.0026 = 0.255$ "

Tables of Magnifications.—The image seen in the microscope produces upon the eye the same effect as an object seen at the normal distance of distinct vision, *i.e.*, 10 inches.

If, therefore, a rule be placed at the foot of the microscope at a distance of 10 inches from the eye, it can be compared with the microscopical image of a scale divided into $\frac{1}{100}$ mm. The quotient is the magnification of the objective and the eyepiece combined.

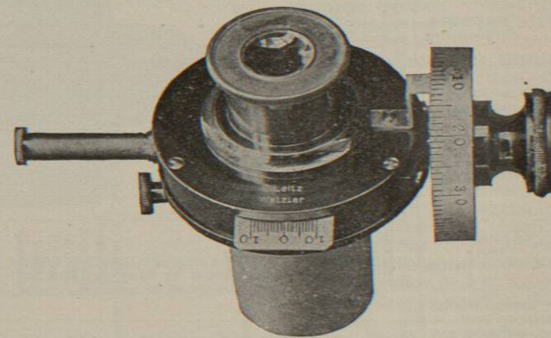

Fig. 600.—The Eyepiece Micrometer.

If, *e.g.*, 92 mm. of the rule are found to cover $\frac{7}{10}$ of the image of the micrometer scale, it follows that the magnification is $\frac{92}{0.7} = 130$. The tables of magnification have been compiled in this manner and are sufficiently accurate for practical purposes.

Thus, supposing the same specimen of *Pleurosigma angulatum* to be measured—

(1) with objective 7 and eyepiece 0,
(2) with objective 4 and eyepiece II.,

and supposing the length of its image to be 67 mm. in the first and 22 mm. in the second case, then, since the actual size of the object is found by dividing the length of the image, as seen at a distance of 10 inches from the eye, by the magnification of the objective and eyepiece combined, the length of our specimen of *Pleurosigma angulatum* is:

(1) $\frac{67}{270} = 0.248$ mm.
(2) $\frac{22}{90} = 0.244$ mm.

In all these measurements a tube length of 170 mm. must be strictly adhered to.

(Note.—As this article goes to press I learn that Jolles's ferrometer has undergone such simplification as to place it among those instruments useful for clinical work.

The eyepiece spectroscope is also a valuable addition to our clinical apparatus.

Both of these instruments are as yet untested, but their simple construction and application commend them to the clinician.)

Bibliography.

As space does not permit of a long list being introduced, the reader is referred to the very full bibliographical note in von Jaksch's "Clinical Diagnosis" and to the writings of the observers who are quoted throughout the article. *Charles N. B. Camac.*

BLOOD-LETTING.—This title includes all methods of abstracting blood for therapeutic purposes, whether they are for a general or for a local effect. The terms *venesection* and *phlebotomy* are restricted to bleeding from the larger veins, for the purpose of influencing the system generally; whilst *leeching*, *wet-cupping*, and *scarification* are means of abstracting blood from the capillaries, the effect of which is almost entirely local.

General blood-letting, or venesection, is of very ancient origin. References to it have been found in history prior to the time of Hippocrates, and in the writings of this early authority it occupies an acknowledged position as a valuable therapeutic agent. During the many years that it has been employed it has been viewed with a varying degree of favor, and at times its advocates have employed it in all forms of disease and in a most extravagant manner. The seventeenth century and the early part of the present century mark the periods of its greatest use, during which excessive and repeated bleedings were constantly employed. The amounts of blood removed seem astounding at the present day. For a pleurisy 5,520 gm. were abstracted during a period of several days; in a case of pericarditis it was found necessary to abstract, on different occasions, 721, 720, 960, 1,200, and then 1,440 gm. before the patient was relieved; and, in a case of inflammatory rheumatism, twenty pounds of blood were taken during the progress of the attack. In the early part of the century, medical opinion went to the other extreme; the practice fell into disrepute and was almost entirely abandoned. During the last twenty-five years its use has been revived. Many of the older practitioners who had never forsaken its use have been more outspoken in advocating its therapeutic powers. At medical gatherings, many papers have been read and numerous discussions have followed, in which venesection has been very generally supported. Its employment has now assumed a more rational character. The advance in our knowledge of physiology and a closer clinical observation have made clearer what its effects are upon the system, and we now employ it with a definite object in view and restrict its use to a much more narrow sphere.

Venesection exercises what may be termed a mechanical effect upon the circulation, as well as a general effect upon the system. When a certain proportion of the blood is removed the tension of the blood-vessels is at once lessened, the degree depending upon the amount withdrawn. The effect is but temporary, as that which is lost is rapidly renewed; but, if any disturbance of the circulation exists, it is sufficient to allow the equilibrium to be regained, the heart beating more easily and the blood flowing more freely through the vessels.

The general effect upon the system is of the utmost importance. Accompanying the lowering of blood pressure and loss of blood cells, there is a diminished activity of the various functions. The heart's action is quieter, respiration goes on more slowly, tissue changes are less active, and there is a lowering of body heat. This depression is but temporary. In a few hours there begins a renewal of the blood, tissue changes are accelerated, the nervous system is improved by a stimulation of the nerve centres, and general bodily improvement is the result. At the International Medical Congress for 1900, in the discussion upon this subject, M. A. Robin stated, as the result of many years' observation, he was satisfied that after moderate bleeding of 150 to 250 gm., polyuria is regularly observed, and the excretion of solids is increased. A greater amount of air is taken into the lungs, as much as sixty-one per cent., and the proportion of oxygen consumed by the tissues is correspondingly increased. When the bleedings are renewed the reaction is slower, and when they are frequently repeated, a state of anæmia ensues, with a tendency to degenerative changes.

Indications.—The indications for bleeding may be summed up under three heads: (*a*) when there is excessive vascular tension; (*b*) when it is desired to obtain the benefit of its physiological action upon the various tissues and organs; (*c*) when it is believed that good may result from removing a definite amount of blood, and with it a certain proportion of toxic material, from the system.

There is no difference of opinion as to the value of venesection in all conditions in which there is venous engorgement. It may be thought desirable to try the nitrites and allied drugs for the purpose of "bleeding into the arteries," or to employ hydragogue cathartics or diuretics to unload the congested vessels; but if these measures fail, all are in favor of bleeding. The cause of the obstructed circulation may lie in the heart or in the pulmonary tissue. The effect of either of these causes is an overfilled and possibly a dilated right heart, distended veins, and more or less congestion of the various organs. In this condition the removal of blood from the venous system affords prompt relief. The laboring heart beats more freely, the arteries become filled, and the congested veins and organs return to the normal. The dyspnœa disappears, the dusky hue of the skin fades, and the general condition of the patient is at once improved. Mitral disease, when compensation is failing, and a feeble heart that is suddenly overtaxed, are the two conditions that most frequently give rise to these distressing symptoms. In such cases, when the dilatation is extreme and the force of the heart very low, venesection must be prompt to be of service. Among these cases may be included many instances of cardiac failure that occur during the administration of a general anæsthetic, when the dilated heart becomes suddenly overfilled and unable to empty its cavities. A sudden blow over the heart may suffice to cause a powerful contraction, or the withdrawal of blood will relieve the pressure and allow regular contractions to be re-established. Of the pulmonary causes of venous congestion, emphysema is the most common, and in this condition marked benefit will follow the withdrawal of blood. Cases of bronchitis and those in which there is a tendency to pulmonary œdema afford favorable conditions for this treatment.

Venesection is also of service when the arteries show a condition of increased tension, when the pulse is full and bounding, and when a condition of general plethora prevails. In these cases the relief afforded by the withdrawal of blood is also very marked, the reduced blood pressure relieving the congested organs and often preventing cerebral hemorrhage. In the convulsions of uræmia, and especially in puerperal eclampsia, when there is the same arterial tension, the value of venesection is unquestionable. The relief is immediate, and the severity of subsequent attacks is likely to be lessened. In puerperal states there should be no hesitation in resorting to it, if the arterial tension is abnormally high; and the fact that a free loss of blood has occurred during labor does not warrant the belief that venesection will be any less effective in relieving the tension. In these cases much of the benefit is due to the reduction of blood pressure, and much also may be explained by the favorable influence exerted on the tissue changes and by the increased oxidization. In addition, it is also suggested that the increased nutritive changes neutralize the poisons circulating in the blood and convert them into harmless products.

It is difficult to determine to what extent the benefit should be ascribed to the actual removal of toxic material from the body (with the blood that is drawn off), yet we find that there are many eminent authorities who lay great stress upon this explanation. To increase the usefulness of the procedure in these toxic cases, it is recommended that the bleedings should be very free and that the blood lost should be replaced by normal saline solution, either administered subcutaneously or injected directly into the veins.

The effect of venesection in lowering temperature and allaying the symptoms of inflammation would suggest