a circular shape with fine spiculated appearance at periphery. 3. Is thinner in the centre than at margin of specimen. (See Plate XIII., Fig. 1.)

*Microscopic:* 1. Corpuscles each lying separate with some little space between—not in rouleaux. 2. Majority of corpuscles stationary. 3. Each corpuscle circular—*not crenated.* (See Plate XIII., Fig. 6.)

Poikilocytosis is of course pathologic and cannot be avoided; crenation is not, however, and is usually due to faulty technique. (See Plate XIII., Figs. 3 and 5.)

*Characteristics of a Poor Fresh-Blood Specimen, and Such as Condemn It for Examination.—Gross:* 1. Blood thick and oozing out from under cover slip indicates that too much blood has been taken. 2. Irregular shape indicates either that too much blood has been taken, or that dust has been on the slip or slide, or that the blood has been chilled. 3. Thick at one point and partially spread at another indicates dust or moisture. 4. Lump of blood—no spreading—indicates general uncleanliness.

*Microscopic:* 1. Structureless masses. 2. Abundance of rouleaux. 3. Much crenation. 4. Corpuscles closely placed, giving tiled-floor appearance. 5. Corpuscles rapidly floating about specimen. 6. Corpuscles ragged and mutilated. 7. White corpuscles broken into granular collections. (See Plate XIII., Figs. 3 and 5).

*What can be Learned from Fresh Specimens of Blood.*—When the precautions laid down in the foregoing have been observed, the following objects may be distinguished:

1. The Red Blood Corpuscles—non-nucleated in the normal.

Size—$7\mu$. (Note: $\mu$ = micron = $\frac{1}{25400}$ of an inch. It is well to become familiar with the size of the red cell, and to use it as a unit of measurement when speaking of other bodies, as twice, thrice, etc., the size of the red cell.)

Shape—biconcave discs.
Color—pale yellow.
Character—elastic.
Tend to form rouleaux.
Become crenated.
May contain bright round spots termed "artifacts."
} Conditions due to faulty technique.

The adjective artifactitious, or simply factitious, might be applied to the last three conditions, as they are the result of manipulation.

In anæmia:
2. Microcytes or Macrocytes.
3. Poikilocytes.
4. Nucleated Red Cells. { Normoblasts. Gigantoblasts.
5. Lake-like Areas with irregular shape and margin in the red cells, due to deficiency of hæmoglobin, termed by Maragliano "degeneration areas," called also vacuoles.

6. Shadow Corpuscles, described first by Norris, are seen in the blood of anæmia from toxic agents and burns. These are red cells deprived of hæmoglobin. They can be artificially produced by adding water to the blood specimen.

The White Blood Corpuscles—Nucleated Cells.
7. Small Mononuclear or Small Lymphocytes.
8. Large Mononuclear or Large Lymphocytes.
9. Transitional Mononuclear or Transitional Lymphocytes.
10. Polynuclear Leucocytes.
11. Eosinophiles.
12. Myelocytes in leukæmia. (The Mastcells cannot be detected in the fresh specimen.)

An approximate idea of an increase in the white cells as a whole, and one or other variety of these individually, can be had from the fresh specimen. More than four or five white cells in a *well-spread* field with a one-twelfth immersion lens may be taken to indicate a leucocytosis. In lymphatic leukæmia the increase in the small mononuclear leucocytes is readily appreciated; so also the presence of the cells normally found in the bone marrow, as the myelocytes, can be detected in the fresh specimen in cases of spleno-medullary leukæmia. In inflammatory leucocytosis the increase in the polynuclear leucocytes can be noted. The increase in the eosinophiles, so important a diagnostic sign in trichinosis, etc., can also be detected.

13. The Plate * and the Plaque—non-nucleated.
Size—One-seventh the size of the red cell ($1\mu$).
Shape—irregular.
Color—colorless.
Not visible normally.

By placing a drop of Hayem's or Pacini's solution † over the point of puncture (the finger must be used for this purpose) and allowing the blood to mingle with this preservative fluid the plates may be seen. The cover slip is applied to the drop in the same way as when taking a fresh specimen.

We have no systematic work upon the significance of the presence of these bodies.

The writer has noted them in great numbers (without the use of a preservative fluid) in the blood of pneumonia cases, in a case of grave anæmia secondary to malaria, and in a case of Hodgkin's disease.

The work of Osler, Bizzozero, Schimmelbusch, Welch, and Eberth has been more to prove that these bodies are separate corpuscular elements than to explain their association with disease. An exception to this statement should be made in favor of Welch's work, which goes to show that these bodies are the active agents in the formation of the white thrombus; but even this has not, as yet, been of any clinical value.

14. Fibrin does not, normally, appear for some time in a well-taken fresh specimen. Its presence shortly after taking the specimen is therefore of pathological significance. We have not been able as yet, however, to attach any diagnostic value to this condition.

15. Blood Dust (hæmokoniosis, literally, blood full of dust, of Müller) can be seen as floating particles about one-twentieth or one-thirtieth the size of the red cell. No satisfactory explanation of the significance of these particles has as yet been given.

16. The Tertian and Quartan Malarial Parasite in all its stages of development, and many of the stages of the Æstivo-autumnal Malarial Parasite, may be seen with the one-twelfth oil-immersion lens.

17. The Filaria Sanguinis Hominis may be seen with the 7 or 9 Leitz lens.

18. Spirochaëte of Relapsing Fever may be seen with the 7 or 9 Leitz lens.

All this information is to be had by the simple procedure of taking, in the way described, a specimen of blood. All these points will be found grouped in the table at the end of this article.

Having ascertained, then, from the fresh specimen that there is a decrease in the number of red cells, or an increase in the number of white cells, it becomes necessary to determine this decrease and increase definitely, for which purpose additional instruments are required.

## II. Blood-Corpuscle Counting.

*History.*—It is only of late years that the methods of enumerating the blood corpuscles have been simplified so as to be clinically applicable, the complicated apparatus heretofore used having had place in works on physiology only. It is therefore of value to trace the development of our present simple apparatus from its complicated beginnings. When we look over all the methods employed to accomplish this last, we find that investigators have endeavored to construct apparatus along three separate lines, as follows: 1. Actual enumeration. 2. Centrifugalizing and estimating cells according to amount of sediment. 3. Color changes and opacity due to decrease in number of cells.

This will therefore be, not a history of blood counting in chronological order, but rather the record of the development of apparatus along these three lines.

* The term plate is here used to refer to the corpuscles; plaque to the aggregation of these plates. Platelet, a term synonymous with plate, is best discarded.

| † *Hayem's Solution.* | |
|---|---|
| Bichloride of mercury | 0.5 |
| Sodium sulphate | 5. |
| Sodium chloride | 1. |
| Distilled water | 200. |

| *Pacini's Solution.* | |
|---|---|
| Bichloride of mercury | 2. |
| Sodium chloride | 4. |
| Glycerin | 26. |
| Distilled water | 226. |



EXPLANATION OF PLATE XIII.

## EXPLANATION OF PLATE XIII.

(Drawn by Dr. E. Dunning.)

| Figure | Reference |
| --- | --- |
| Fig. 1.—Gross appearance of well-taken fresh-blood specimen.<br>Fig. 6.—Microscopic appearance of well-taken fresh-blood specimen. | See p. 39: *Characteristics of a Good Fresh-Blood Specimen.* |
| Fig. 3.—Microscopic appearance of poorly taken fresh-blood specimen.<br>Fig. 5.—Microscopic appearance of poorly taken fresh-blood specimen. | See p. 40: *Characteristics of a Poor Fresh-Blood Specimen.* |
| Fig. 2.—Microscopic appearance of the Thoma-Zeiss counting stage with normal blood diluted with Toison solution. Red cells unstained; white cells stained blue. This is with the low-power objective (3 Leitz) which takes in all sixteen squares (see Fig. 555, p. 44), but does not magnify the cell as much as the higher power (see Plate XIII., Fig. 4), and which therefore greatly increases the task of counting. | See p. 45. |
| Fig. 4.—Same as Fig. 2, Plate XIII., except that the blood is that of leukæmia (120,000 leucocytes to the cubic millimetre), and the objective is of high power (7 or 9 Leitz). This takes in only one-sixteenth of the entire field, but it renders the corpuscles much more readily distinguishable. | See p. 45. |

Fig. 1.

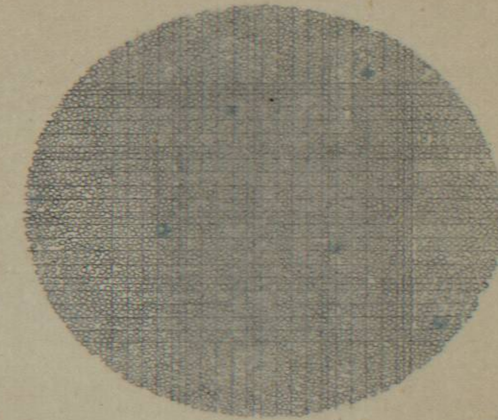

Fig. 2.

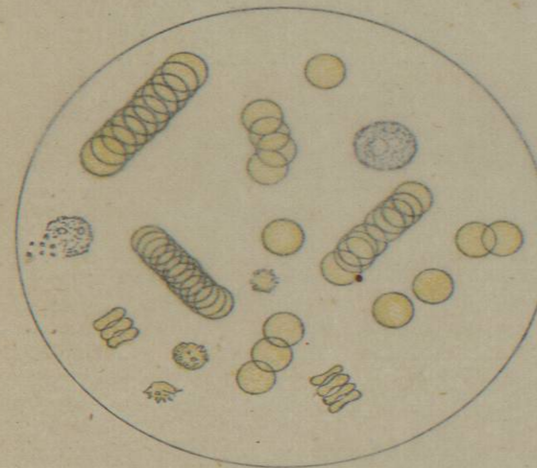

Fig. 3.

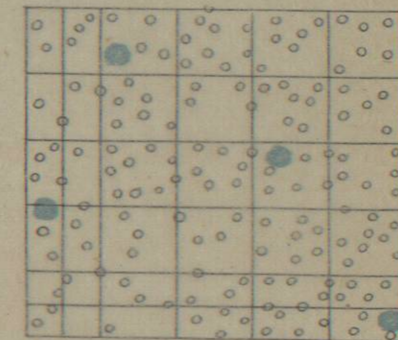

Fig. 4.

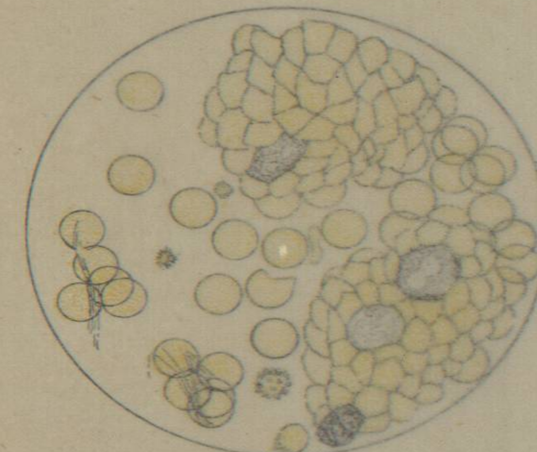

Fig. 5.

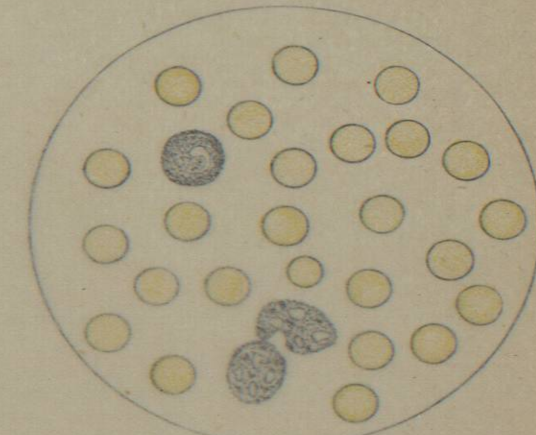

Fig. 6.

HUMAN BLOOD

## EXPLANATION OF PLATE XIII.

(Drawn by Dr. E. Dunning.)

Fig. 1.—Gross appearance of well-taken fresh-blood specimen.

Fig. 6.—Microscopic appearance of well-taken fresh-blood specimen.

See p. 39: *Characteristics of a Good Fresh-Blood Specimen.*

Fig. 3.—Microscopic appearance of poorly taken fresh-blood specimen.

Fig. 5.—Microscopic appearance of poorly taken fresh-blood specimen.

See p. 40. *Characteristics of a Poor Fresh-Blood Specimen.*

Fig. 2.—Microscopic appearance of the Thoma-Zeiss counting stage with normal blood diluted with Toison solution. Red cells unstained; white cells stained blue. This is with the low-power objective (3 Leitz) which takes in all sixteen squares (see Fig. 555, p. 44), but does not magnify the cell as much as the higher power (see Plate XIII., Fig. 4), and which therefore greatly increases the task of counting.

See p. 45.

Fig. 4.—Same as Fig. 2, Plate XIII., except that the blood is that of leukæmia (120,000 leucocytes to the cubic millimetre), and the objective is of high power (7 or 9 Leitz). This takes in only one-sixteenth of the entire field, but it renders the corpuscles much more readily distinguishable.

See p. 45.



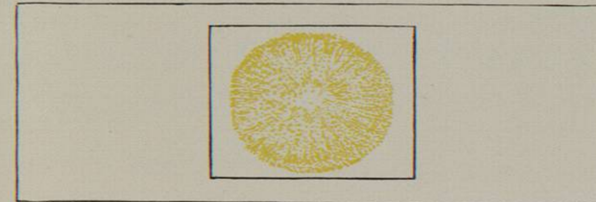

Fig. 1.

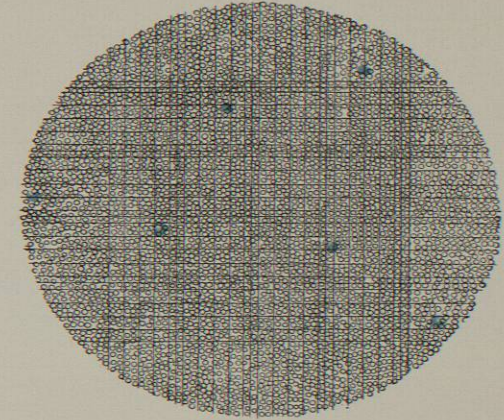

Fig. 2.

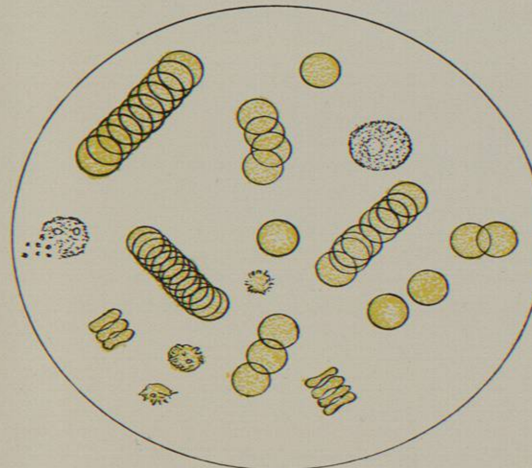

Fig. 3.

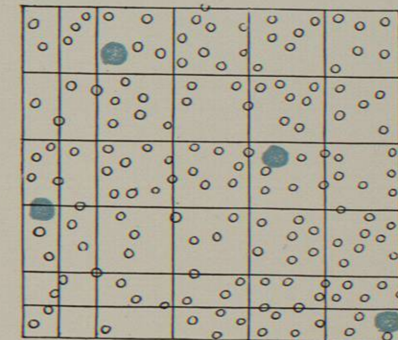

Fig. 4.

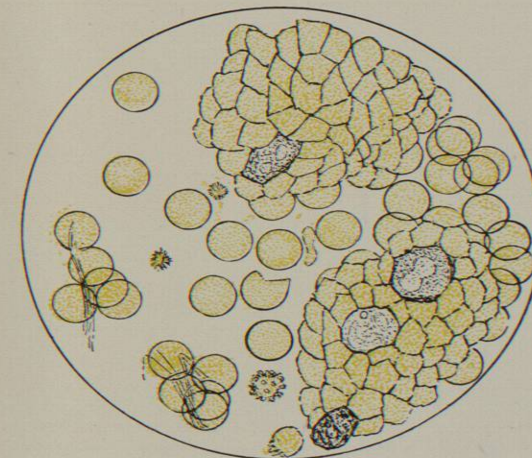

Fig. 5.

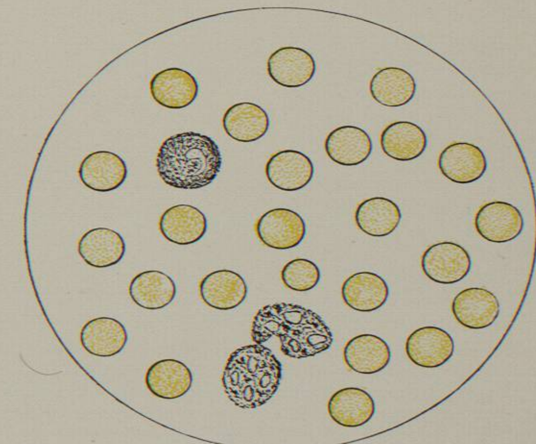

Fig. 6.

HUMAN BLOOD

Blood.

1. Actual Enumeration of Blood Corpuscles.—Vierordt was among the first actually to enumerate the red blood cells; and though his apparatus was most

Fig. 544.—Potain's Mixer.

crude and his method most complicated, yet his results have been abundantly confirmed by subsequent observers working with varied styles of apparatus. Vierordt's figures were, 5,174,000 red blood cells per cubic millimetre.

Vierordt diluted with a fixed quantity of sugar solution a cubic millimetre of blood and then spread this upon a slide. By means of a micrometer he counted every corpuscle. For one enumeration it took an entire week. The result of this work was to establish the following:

(1) A known quantity of blood must be taken, that quantity being the amount contained in a cubic millimetre.

(2) A known dilution.

(3) A cubic millimetre of normal blood contains 5,174,000 red cells.

At this time, Kölliker, after declaring that "owing to the difficulty of the subject" the most careful estimates can "only be described as approximate," adds: "One method only can be successful, consisting in the direct enumeration of the globules in accurately determined quantities of blood." Welker adds the next improvements (1) in using a stage micrometer, and (2) in counting the corpuscles in a fraction of a large dilution and multiplying the result of this count by the figures required to make the whole cubic millimetre.

Potain's mélangeur or mixer afforded the first means of accurately diluting the blood. This was nothing else in principle, but of cruder make, than the mixer now employed in the Thoma and Zeiss blood-counting apparatus. Fig. 544 shows this mixer.

Potain's mixer was divided precisely as is the Thoma-Zeiss mixer, into a dilated and a capillary portion, the capillary portion being exactly $\frac{1}{100}$ part of the whole. The dilated portion of the Potain mixer contained the lit-

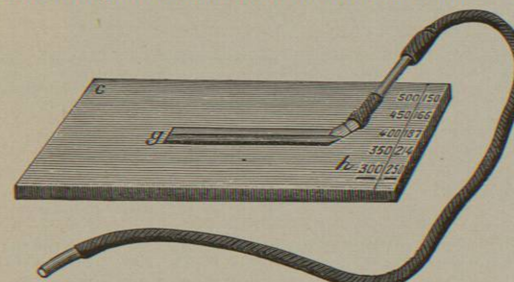


Fig. 545.—Malassez' Artificial Capillary.

tle glass ball, as in the Thoma-Zeiss apparatus. Malassez combined this suction capillary pipette with an artificial capillary in the following manner: Blood is drawn up by suction into the Potain mixer to the 1 mark, and a diluting fluid, called the "artificial serum," consisting of —gum arabic specific gravity 1.020, one volume; sodium sulphate and sodium chloride, equal parts and each of specific gravity 1.020, two volumes—to the 100 mark, making dilution of 1 in 100. The pointed end of the mixer is then fastened to a rubber tubing connected with the artificial capillary, as seen in Fig. 545.

The artificial capillary is set in a plate of glass which is divided into fractions of a cubic millimetre, as seen in the illustration. The microscope is adjusted so as to magnify the corpuscles as they lie in the artificial capillary (see Fig. 546), and their number ascertained for a fractional part of this capillary as marked on the glass plate. According to the fraction of a cubic millimetre which that portion selected to count represents, the number counted is multiplied.

Suppose one counts the corpuscles in $\frac{1}{400}$ of a cubic millimetre; then 400 times that number multiplied by the amount of dilution represents the number of corpuscles in a cubic millimetre.

This method offered many difficulties· 1. Complicated artificial serum for dilution. 2. Introduction of blood without air into artificial tube difficult. 3. Difficult to clean apparatus.

Hayem and Nachet devised an instrument (Hématimètre) by which the blood and serum are obtained in

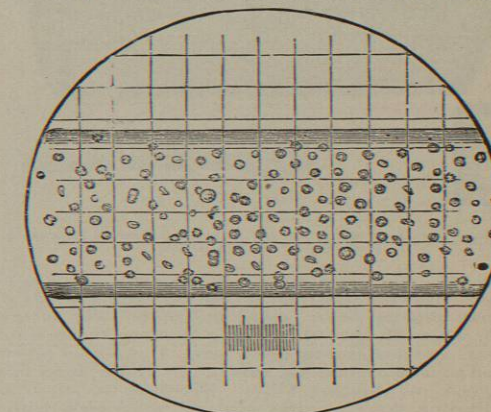

Fig. 546.—Artificial Capillary as It Appears Under the Microscope. × 180 diameters. (From Ranvier.)

two separate pipettes and then mixed in a glass receiver. A drop of this mixture is then placed upon a glass slide arranged as follows: A circular well, similar to that employed in the Thoma and Zeiss apparatus, is constructed with the accurate measurement of 1 cm. in diameter and 0.2 mm. in depth. An eyepiece micrometer ruled in a large square and divided into sixteen little squares is then attached. One side of the large square measures exactly $\frac{1}{5}$ mm.* (see Fig. 547).

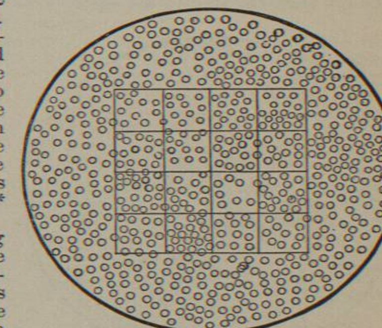

Fig. 547.—Appearance of Corpuscles with Hayem and Nachet's Instrument.

By counting the cells in the square and multiplying this count by the fraction of a cubic millimetre which this square represents, and the number of volumes of the diluting fluid, the number of cells in a cubic millimetre is ascertained. This

* One-fifth millimetre in depth by one-fifth millimetre on two sides of square makes the block of blood one-fifth (0.2) cubic millimetre—$5 \times 5 \times 5 = 125$.

is complicated, but, as will be seen later, it has contributed certain points toward the simple apparatus used to-day.

In *The Lancet* for December 1, 1877, Gowers describes an instrument which he calls the hæmacytometer, and which more nearly approaches perfection than any already described; it therefore marks what may be considered the border line between the ancient and modern

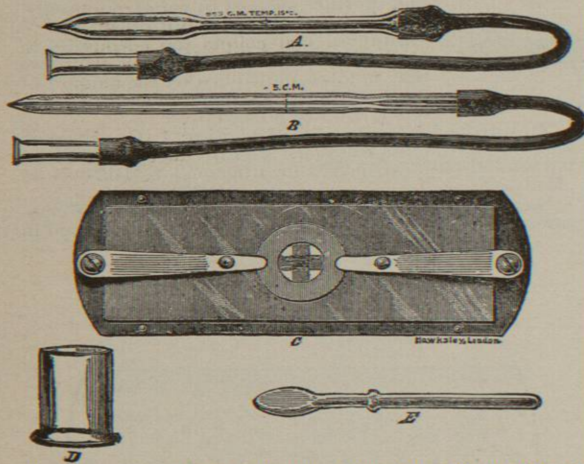


FIG. 548.—Gowers' Hæmacytometer. (From Kirkes' "Handbook of Physiology," twelfth edition.)

history of blood-counting by the actual enumeration method. Gowers' instrument (see Fig. 548) consists of: A, Small pipette (with rubber mouthpiece for suction) which, when filled (with diluting fluid) to the mark on its stem, contains exactly 995 c.mm. B, Another pipette marked to hold 5 c.mm. (of blood). D, Glass jar for mixing (blood and diluting fluid). E, Stirrer (to stir blood and diluting fluid in glass jar). C, Brass plate with a cell $\frac{1}{5}$ (0.2) mm. in depth and with the floor divided into $\frac{1}{10}$ (0.1) mm. squares and a cover glass held in place by springs.

A standard saline solution of sodium sulphate of specific gravity 1.025 is employed; 995 c.mm. of solution is mixed with 5 c.mm. of blood with the pipettes. These are mixed in the glass jar. A drop is placed in the cell in the brass plate and the cover slip held down by the springs. The cells are then counted in ten squares, and the calculation for 1 c.mm. of which this is a fraction made.

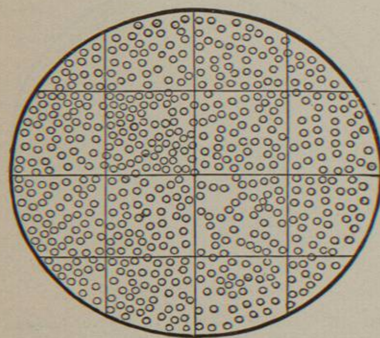

FIG. 549.—Appearance of Corpuscles Under Microscope with Gowers' Instrument.

As we review these descriptions of apparatus we see that the effort of the inventors has been to overcome certain obstacles, *i.e.:*

(1) Reduction of the corpuscles to a countable number. Vierordt accomplished this by dilution and by fixing the area to be counted to the cubic millimetre.

(2) Obtaining the blood so as to prevent clot, and to dilute accurately. Welker showed the value of accurate dilution. Potain's mélangeur best accomplished this.

(3) Spreading the blood over an area thin enough to see each corpuscle. Malassez accomplished this with a long narrow tube; Hayem and Nachet with a cell whose depth was a fraction of a cubic millimetre.

(4) Mapping out the area so that the eye could navigate upon it. Hayem and Nachet accomplished this with an eye-piece micrometer. Gowers, without an eye-piece micrometer, but with a marked-out slide.

All these methods depend (as Kölliker has pointed out) upon the following priniciples: (1) A known quantity of blood for estimation (1 c.mm.); (2) a known dilution of a known quantity of blood; (3) a known fraction of a cubic millimetre to be counted.

Upon these principles and with the information obtained from the foregoing inventors, Thoma and Zeiss have constructed their apparatus. This combines the mélangeur of Potain and the mapped-out cell of Gowers.

*Principle of the Thoma-Zeiss Mixer.*—The mixer consists of a capillary portion and a dilated or bulb-like portion. The capillary portion enables one to procure a fixed quantity of blood that is free from clotting, if promptly taken; and the bulb portion enables one to procure a fixed dilution. The principle of this mixer is best understood by reference to the diagram shown herewith (Fig. 550).

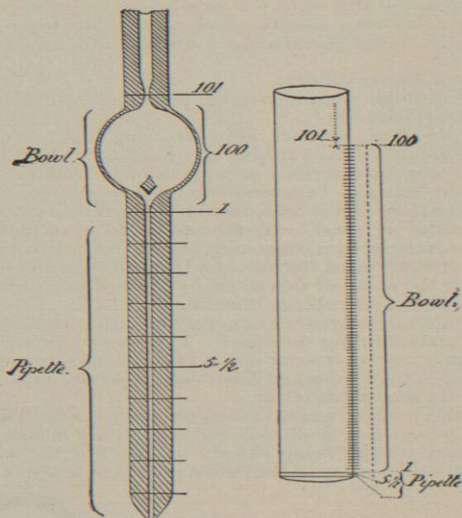


FIG. 550.—The Mixer of Thoma and Zeiss Corpuscle-Counting Apparatus. Shows principle of dilution by comparison of pipette (a necessary instrument in dealing with a coagulable fluid like blood) with an ordinary receiver (in which a non-coagulable fluid could be diluted).

Suppose the fluid to be diluted were wine or some non-coagulable fluid. Then we should pour the wine into the receiver, as shown in the diagram, up to the mark 1, and the diluting fluid up to the mark 100. This would be 1 to 100. Were we to pour the wine up to the .5 mark only (or $\frac{1}{2}$ of 1), and the diluting fluid up to 100, we should have half as much wine as before to the same amount of diluting fluid, or in the proportion of 1 to 200. Half the fluid to be diluted with the same amount of diluting fluid has the same result as doubling the amount of the latter.

Now blood cannot be poured in this way. An instrument must therefore be devised by which the blood can be taken free from clotting. Our receiver, therefore, is converted into a capillary tube and our pouring is replaced by suction. That portion of the receiver marked off as 1 is converted into the pipette, and that portion of the receiver marked off as 100 is converted into the bowl. We now have an instrument by which a definite quantity of blood can be drawn up together with a definite quantity of diluting fluid. This instrument, when full, represents 101 of blood and diluting fluid, or 1 of blood to 100 of diluting fluid. If the blood be drawn to the .5 or $\frac{1}{2}$ mark and the diluting fluid to the 100 mark we have 1 to 200.





The Thoma and Zeiss apparatus is usually provided with two mixers similar in all respects except that one is larger than the other: one for counting the white cells, the larger one; and one for counting the red cells, the smaller one. That for counting the red cells is all that is necessary, and, as will be seen later, is preferable to two separate proceedings.

The diluting fluids, at first so complicated, have been much simplified. The object has been to obtain a medium as nearly as possible like that in which the corpuscles normally float.

*Diluting Fluids:* (1) Normal, better named physiological, salt solution.* (2) Acetic acid 1 in 300 destroys the red cells and accentuates the nuclei of the white, and is therefore valuable in counting the white cells alone. (3) By using a basic stain together with the preservative salts the results obtained separately by the other fluids are combined. The most satisfactory of these is the Toison solution.

*Formula.* †

| | |
|---|---|
| Sodium chloride | 1.000 gm. |
| Sodium sulphate | 8.000 gm. |
| Glycerin | 30.000 c.c. |
| Water | 160.000 c.c. |
| Methyl violet | 0.025 gm. |

In the preparation of this solution it is advisable first to mix the sodium chloride and sodium sulphate thoroughly, and then to add these to the glycerin and water—the methyl violet being added last. The brackets in the above formula show these steps in the preparation.

*Principle of the Counting Well.*—Having thus an apparatus for taking a fixed quantity of blood with a fixed quantity of diluting fluid, we must next have a means of counting the corpuscles. For this a glass slide is constructed, with a well in the centre of the following known dimensions. It must be remembered that each of the various parts of this apparatus described has been constructed to overcome an obstacle. The mixer overcame the clotting and diluted the drop, thus reducing the number of corpuscles to a countable figure. The well which we are about to describe, (1) reduces the cubic millimetre to a depth through which it is possible to see each corpuscle separately (a cubic millimetre of blood and fluid to the dilution of two hundred times would be so thick and contain so many corpuscles that the task would be beyond human skill); (2) maps out that area so that the eye can find, as it were, the longitude and latitude of any point in the sea of corpuscles.

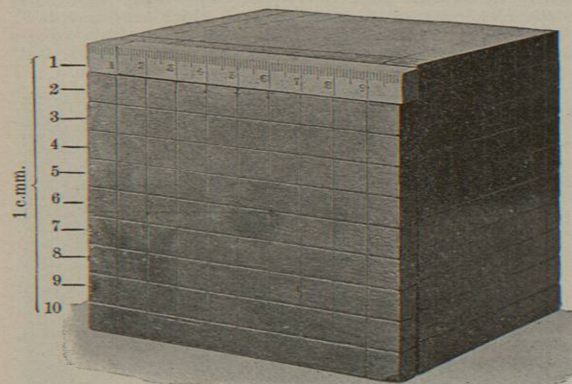


FIG. 551.—A Cube Representative of the Amount of Blood from Which Corpuscle Enumeration is Made. The block represents 1 c.mm.

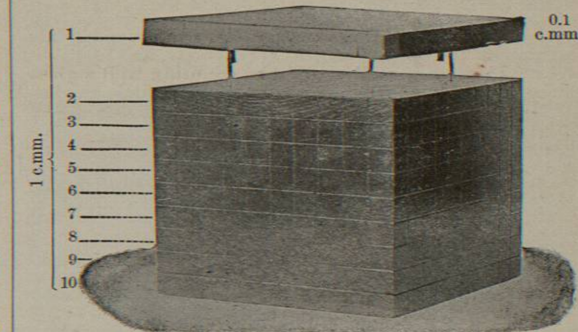


FIG. 552.

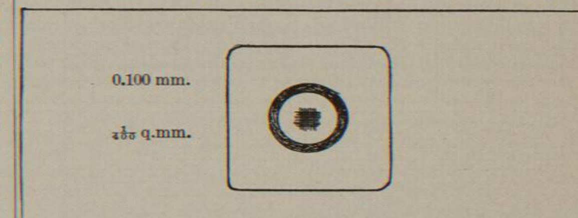


FIG. 553.—Full-Face View of Counting Stage. (Drawn by Dr. E. Dunning.) [0.100 mm. = depth – $\frac{1}{10}$ of a millimetre; $\frac{1}{400}$ q.mm. (German quadrat-square) = smallest square which measures $\frac{1}{400}$ of a square millimetre (25 × 16 = 400).]

What fraction of a cubic millimetre offers the depth and contains that number of corpuscles appreciable by the human eye? This fraction is found to be one-tenth of a cubic millimetre in 1 to 100 or 1 to 200 dilution of blood.

The illustration (Fig. 551) represents a millimetre cube. If this consisted of blood, the depth would be too great for the human eye to penetrate and would contain so many corpuscles as to be beyond human skill to enumerate. We therefore retain two dimensions of the cube, but take only one-tenth of the depth. This layer of blood is therefore one-tenth of a cubic millimetre in a 1 to 100 or 1 to 200 dilution (see Fig. 552).

The counting stage, to fulfil these requirements, must be constructed as follows: The bottom of the well upon which the corpuscles rest must be laid off with a square whose dimensions are 1 mm. each way, and the well must be one-tenth of a millimetre in depth. The illustration (Fig. 553) shows the full face view of the counting stage. In the centre is seen the bottom of the well with a square laid off measuring 1 mm. each way.

The second illustration (Fig. 554) shows the profile of this stage with the cover slip in place. The measurement from the under surface of the cover slip to the bottom of the well is exactly 0.1 mm.

When, therefore, a drop of the diluted blood is placed

* Normal in chemistry indicates a solution containing the sum of the atomic weight of the salt employed in grams to 1,000 c.c. of distilled water. As this is not the case with so-called normal salt solution, the term is misapplied. Physiological salt solution is, roughly, NaCl ʒ j. to the O j. distilled water.

† This solution must be kept in the dark and must be filtered from time to time as it loses its color and develops a fungus growth which seriously plugs the pipette. This solution preserves the red cells and by means of the methyl violet stains the white cells a light purple, making the latter readily distinguishable and enabling one to make the count of both red and white cells in one process.

in this well and the cover slip is in place, those corpuscles which we see contained within the square, the dimensions of which are 1 mm. each way and 0.1 mm. in depth, are the contents of a cube of diluted blood measuring $\frac{1}{10}$ of a cubic millimetre.

(NOTE.—These microscopic dimensions, but for the present simple method of construction, would render this instrument in the making a very costly and delicate affair. In the manufacture of this counting well a glass slide similar in length and breadth, but a little thicker

FIG. 554.—Profile View of Counting Stage. (Drawn by Dr. E. Dunning.)

than that employed for general microscopic work, is used. Upon a round block of glass the millimetre square is cut or pressed. This block of glass is cemented to the centre of the glass slide and forms the bottom of the counting well. Another piece of glass, like a thick cover glass but with *a round hole* cut in the centre, is cemented upon the slide, so that the block of glass already in place occupies, but does not exactly fit, the hole. The difference in measurement, from above down, between the upper surface of the round block of glass and the upper surface of the glass with the hole through it is just 0.1 mm.

FIG. 555.—Subdivisions of Counting Field of Thoma and Zeiss Apparatus. [1 sq.mm.—$\frac{1}{16}$ sq.mm.—$\frac{1}{400}$ (25 × 16) sq.mm.]

Thus the well with a millimetre square marked at the bottom and 0.1 mm. in depth is formed. The fact that the block of glass does not completely fill the hole in the outer piece of glass explains why there is left about it a groove into which the blood flows, if more than is required exactly to cover the block of glass is blown into the well. This seems an objection, as currents are thus produced allowing many corpuscles to flow out of the mapped area. There seems no good reason for the presence of this groove except possibly that it obviates the additional expense of making the block of glass exactly fit the hole.)

*Principle of the Ruling of the Counting Well.*—We now turn to the marking out of a millimetre square which is to render it navigable. Here, as before, an obstacle is to be overcome, viz., the inability of the human eye to keep its bearings when viewing several thousands of corpuscles.

The first subdivision of this area (as first done by Nachet and Hayem on the eye-piece and by Gowers on the stage) is into 16 squares. But even this does not overcome the difficulty, and it is necessary still further to subdivide each one of these into 25 squares (Fig. 555). It now becomes necessary to establish some boundary mark for

FIG. 556.—Shows Method of Drawing Blood into Mixer. Diluting fluid near *at hand*.

these 16 large squares, each consisting of 25 small squares. This is done by bounding each of the 16 squares on two sides with double lines. Now we can readily count the corpuscles in one of the large squares which is bounded on two sides by the double lines, and realize that this represents $\frac{1}{16}$ of the field; or in one of the small squares, and realize that this represents $\frac{1}{25}$ of $\frac{1}{16}$, or (16×25 = 400), $\frac{1}{400}$ of the whole millimetre square. On the glass slide (Fig. 553) will be noticed the figures 0.100 mm., referring to the depth, which is $\frac{1}{10}$ of a mm.; also $\frac{1}{400}$ q.mm., referring to the smallest squares, each one of which measures $\frac{1}{400}$ sq.mm. ($\frac{1}{25}$ of $\frac{1}{16} = \frac{1}{400}$). (Fig. 555.)

Once these mathematical facts are clearly understood, one may count in many different ways. One small square may be counted, and the result multiplied by 400; or 25 small squares, and the result multiplied by 16. The best method,

FIG. 557.—Thoma and Zeiss Corpuscle-Counting Apparatus. Drawing diluting fluid into mixer. The blood has already been drawn in.



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Blood.
Blood.

however, will be given in the following description of the use of the apparatus.

Now that we understand the principle of the apparatus, let us proceed to its use:

In order to obtain the blood the ear is punctured as for a fresh specimen, and the tip of the capillary tube *inserted into* the drop and retained there while *gentle* suction is exerted, and the blood drawn either to the 1 mark, or the .5 or $\frac{1}{2}$ mark, as shown in Fig. 556. The latter is preferable, for the following reasons: (1) Less blood. (2) Difficult to stop sharp at the 1 mark without the blood passing on into the bowl and necessitating cleaning the instrument and beginning again. In using the .5 mark, if the blood pass the mark it may be soaked off the tip of the pipette with linen or blotting paper until the column stands opposite the .5 mark—cleaning, etc., not being necessary.

As promptly as possible, to avoid clotting, the pipette is *submerged* into the diluting fluid; and then, but not till then, suction is again made until the fluid stands opposite the 101 mark (Fig. 557). Suction must be gentle throughout, and especially so as the fluid is approaching the 101 mark, as it readily pops into the rubber tubing. The tip of the pipette must *always be immersed* in the fluid being sucked up, whether it be blood or diluting fluid, otherwise air enters the pipette and destroys the specimen.

Directly the diluting fluid has reached the 100 mark the finger is placed on the tip of the pipette and the whole shaken in order that the corpuscles may, by the aid of the small glass ball in the bulb portion of the mixer, be thoroughly mixed with the diluting fluid (Fig. 558). As the pipette contains the diluting fluid and no corpuscles, this must be emptied; and to be certain that this is done the bulb of the mixer is emptied one-third by blowing the fluid out. The diluting fluid should be allowed to act upon the blood in the mixer for five or ten minutes before the drop for counting is taken from the mixer.

The counting stage is now employed. This stage and the cover slip must be free from dust particles. The latter (the cover slip), grasped with the clamp forceps, is placed conveniently at hand. A thick cover slip with accurately level surfaces, to prevent bending, is provided with the apparatus; but as this interferes with the use of the high-power lens, it is an objection. An ordinary cover slip may be used, if care is taken not to press down upon it with the objective. From the mixer a drop is blown on to the central raised table of the counting well

FIG. 558.—Position of Hands in Shaking Mixer.

(Fig. 559). The drop should not (1) flow into the well, (2) or contain air bubbles, (3) or particles of dirt. The cover slip is lowered *until the fluid touches it*, then the forceps are opened and withdrawn with a jerk as in laying the cover slips for fresh specimen (see Fig. 543). There should be no air bubbles, which may be avoided by observing the foregoing precautions.

By holding the slide up to the light *on a level with the eye* and making pressure with the point of the forceps upon the cover slip, a series of varicolored concentric rings will be seen about the point of the forceps where it touches the glass. These are the Newton's rings, which are seen only when two pieces of glass are in perfect ap-

FIG. 559.—Blowing Drop from Mixer on to Stage.

position, *i.e.*, when there are no dust particles, etc., between them. The presence of these rings is therefore an evidence that the glasses are perfectly clean. Two to three minutes are now allowed for the corpuscles to sink through the depth of one-tenth of a millimetre and rest on the mapped-out bottom of the well. By at once observing the slide under the microscope this sinking process may be seen. We are now ready to count; the lowest-power lens of the Leitz or Zeiss microscope may be used. This takes in its field of vision the whole counting area, but magnifies the corpuscles so slightly that the strain upon the eye is great (see Plate XIII., Fig. 2). The 7 or 9 objective of the Leitz takes in only $\frac{1}{15}$ of the counting area, but does not strain the eye, and is therefore preferable, as with little practice the stage may be gradually moved until the whole area has been examined (see Plate XIII., Fig. 4). As the proportion of white corpuscles to red is about 1 to 700, there will be in a given area much fewer white corpuscles than red; and in the one-tenth of a cubic millimetre there will be about 3 white cells to about 2,100 red cells. The task, therefore, of picking out three white cells in an entire field will be a simple one; while it is more practicable to count only a fraction of the field containing 2,100 red cells, and then compute the total by multiplying the number counted by the fraction adopted (see Plate XIII., Figs. 2–4). The following are the steps to be taken in counting the red and the white cells:

*Counting White Cells.*—(1) Count cells contained in entire field, *i.e.*, 16 squares = $\frac{1}{10}$ of a cubic millimetre; (2) multiply by 10 to make the 1 c.mm.; (3) multiply by 100 or 200, according as the dilution has been made.

*Counting Red Cells.*—(1) Count 4 squares, each consisting of 25 of the smaller squares: 4 squares out of 16 = $\frac{4}{16}$ or $\frac{1}{4}$ of the whole $\frac{1}{10}$ of a cubic millimetre; (2) this number multiplied by 4 gives the number contained in the $\frac{1}{10}$ of a cubic millimetre; (3) multiply by 10 and by 100 or 200 as before.

Now it must be remembered that every time we count a fresh drop from the mixer we decrease the range of error; so (*remembering to shake the mixer well each time, as the corpuscles settle rapidly in the bulb*) suppose in counting the *white cells* we take from the mixer 5 separate drops and count each upon the counting stage. An average of the result, obtained by dividing by 5, gives us the number of white cells contained in $\frac{1}{10}$ of a cubic millimetre. This average multiplied by 10 and 100 or 200, as before, will be a more accurate figure than that arrived at by counting one drop only.

Suppose in estimating the red cells we take from the