mixer and count 2 separate drops, counting in the first drop the four corner squares (Squares Nos. 1, 4, 13, and 16 in Fig. 555), *i.e.*, $\frac{4}{16}$ of the whole field; then in *the second drop* the same four corner squares, *i.e.*, $\frac{4}{16}$ also—our two counts combined will represent $\frac{8}{16}$ or $\frac{1}{2}$ of the field; this multiplied by 2 represents the whole field, *i.e.*, $\frac{1}{16}$ of a cubic millimetre. This multiplied by 10 and by 100 or

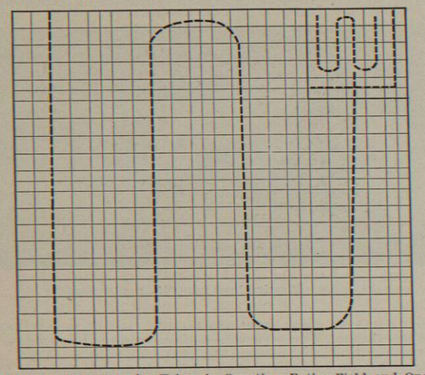

FIG. 560.—Routes to be Taken in Counting Entire Field and One-Sixteenth of Entire Field.

200, according to dilution, gives the number of red cells in a cubic millimetre of undiluted blood. These calculations may be expressed as follows:

White Cells: $\frac{a+b+c+d+e}{5} \times 10 \times (100 \text{ or } 200) = x$;

a, b, c, d, and e being the counts of separate drops taken from mixer; 5 the average number; 10 for the $\frac{1}{10}$ of a cubic millimetre; 100 or 200, amount of dilution; x the number of corpuscles in cubic millimetres of undiluted blood.

Red Cells: $\left(\underbrace{(a+b+c+d)}_{\text{(Separate drop.) (¼ of field)}} + \underbrace{(a+b+c+d)}_{\text{(Separate drop.) (¼ of field) = ½ of field}} \times 2\right) \times 10 \times (100 \text{ or } 200) = x$; a, b, c, d, each the number of corpuscles in $\frac{1}{16}$ of field—therefore together equal to $\frac{4}{16}$ or $\frac{1}{4}$ of field; plus second count obtained from another drop from mixer equals $\frac{1}{4}$ of field; and multiplied by 2 equals whole field.

One-sixteenth of the field can be readily counted by counting *inside* the double lines up and down, as in ploughing a field, and then counting along two sides *in* the double lines. The route for counting in this way is shown by the dotted lines *in the upper right-hand corner* of Fig. 560. For counting the entire field the dotted line in the whole field of Fig. 560 marks the route. This method will be found to save much time.

In order to avoid recounting corpuscles on the line, and to decide to which square these corpuscles belong, the following rule will be found valuable. Count only those corpuscles on the left line of the column down which you are counting, and in the $\frac{1}{16}$ square count only those corpuscles which lie on the double-line boundary of the square.

With practice a blood count can be made in from 15 to 20 minutes, unless the blood be from a case of marked leucocytosis or grave anæmia in which one wishes an extremely accurate count.

Cleaning the Apparatus.—The Thoma and Zeiss apparatus has received much condemnation from the difficulty experienced in cleaning it. By *strictly* observing the following rules, this difficulty will be almost wholly overcome.

(1) If by accident the blood is drawn into the bowl before being mixed with the diluting fluid, immediately draw up distilled water and proceed to clean as given below. *Do not use alcohol until all trace of blood has been removed.*

(2) Do not draw the blood into the pipette until the bottle holding the diluting fluid *is at hand* and the *cork out of the bottle* (see Fig. 556).

(3) See that the diluting fluid contains no flakes or fungus growth; if these be present, filter the fluid before using.

(4) Dry the mixer by drawing ether up and blowing it out.

(5) See that the rubber tube is free from saliva and food particles. It is well to do this from time to time, while using the apparatus, by detaching the tube from the mixer and blowing it out and then passing distilled water through it. Suppose these precautions to have been taken and the diluted specimen to be in the bowl and our count completed; we then proceed to clean the mixer.

(6) Remove the rubber tube, blow out the saliva, etc., which may have collected (see (5), above). Attach the tube *to the pipette end* of the mixer and blow out the contents of the bowl (Fig. 561).

(7) Replace the rubber tube on the large end of the mixer and draw distilled water into the bowl. Shake the mixer well and blow this water out by reversing the tube as recommended in (6), each time blowing the rubber tube free of saliva.

(8) With the tube on the big end of the mixer draw up 95 per cent. alcohol. With tube on pipette end blow this out. This removes the stain. If the glass is not entirely freed from stain, repeat until desired result is obtained.

(9) With the tube on the big end of mixer draw in ether, and then (*without* reversing tube) blow the ether out through the pipette. This shows that the whole apparatus is clear and ready for use next time.

If epithelium or mucus from the mouth, etc., gets into the bowl, it is best removed by drawing undiluted hydrochloric acid up and then introducing an especial make of wire which the writer has suggested to Messrs. Eimer & Amend to procure. It is known in commerce as "stiff brass wire, B. & S. (Brown & Sharp), No. 31 gauge," and has the advantage of being very fine but very stiff, and can be run *the whole length* of the Thoma and Zeiss mixer. With this wire, and *most decidedly* with all other wires, one must be careful to have no kinks, as these may break, leaving the wire in the mixer—an awkward accident.

FIG. 561.—Cleaning Mixer. Rubber tube on pipette end of mixer.

In the *Deutsche medicinische Wochenschrift*, July 29, 1897, p. 497, R. Friedländer, of Wiesbaden, described an instrument, devised by himself, for counting leucocytes. He was led to the construction of this instrument by a sense of inaccuracy while using the Thoma and Zeiss counter. His objections to the latter instrument were, first, too considerable a dilution and therefore too few white cells from which to estimate; second, the calculations necessary with the Thoma and Zeiss instrument are so extensive that a small variation in the number upon which these calculations are based means an error of many thousands.

Friedländer confirmed these objections by obtaining different results in a number of counts of blood taken from the same individual on a single occasion. In order to overcome these objections Friedländer had Zeiss construct the following apparatus:

A pipette similar to that employed in the Thoma and Zeiss apparatus—dilution 1 in 10 or 1 in 20; a counting chamber 0.222 mm. deep × 0.3 mm. square, with the floor divided into squares—16 × 16 = 256 squares in all. The double ruling is omitted; a mechanical stage is employed.

In order, by this method, to arrive at a figure expressive of the number of leucocytes in a cubic millimetre, the following formula is employed:

$$\frac{A \times Z}{M \times Q}$$

A = amount of dilution.
Z = number of leucocytes found in these sections.
M = number of sections observed.
Q = the cubic area between the floor of the counting stage and the cover slip—*i.e.*, 0.09 × 0.22 = 0.0198.

Example: Suppose 192 squares to have been counted and 1,522 leucocytes found; dilution 1 to 20.

$$\frac{20 \times 1{,}522}{192 \times 0.0198} = 8{,}007 \text{ leucocytes in 1 c.mm.}$$

(Note: There is a mistake in Friedländer's formula; 1,0198 should read as above, 0.0198.)

As diluting fluid he employs one-per-cent. salt solution tinged with gentian violet to which is added one-third-per-cent. acetic acid. Thus the red cells are destroyed by

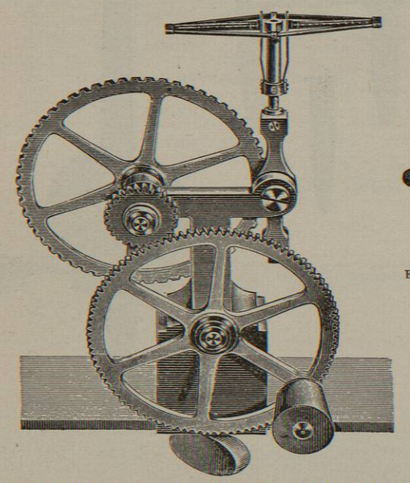

FIG. 562.—Hedin's Hæmatocrit. (From von Jacksh's "Clinical Diagnosis.")

the acetic acid and the nuclei of the white cells intensified, and at the same time the cell is stained by the gentian violet.

The writer has had no experience with this instrument. If the steps as laid down in describing the Thoma and Zeiss instrument are followed the results should be as accurate.

As a clinical instrument Friedländer's would seem to be objectionable if for accuracy it is necessary to count a thousand or more cells. Unless we can arrive at accurate results by simple methods an apparatus loses its *clinical* value.

A number of counting chambers have been devised. These differ chiefly in respect of the size of their rulings; the larger ones being given the preference in order to minimize the possibility of error. The following are some of these: counting chamber of Zappert, counting chamber of Gabritschewsky, counting chamber of Miessen.

2. CORPUSCLE ENUMERATION BY MEANS OF THE CENTRIFUGE.—We now approach the task of corpuscle enumeration from a different point of view. As the corpuscles are particles floating in a fluid, we should be able to precipitate these and measure the precipitate. If, as is the case in anæmia, the corpuscles are few, our sediment should be less than in the normal. The task, then, is to throw these corpuscles down. This is done by the use of the centrifuge. This machine was employed at first to obtain serum, and its use for the latter purpose suggested its application as a corpuscle enumerator.

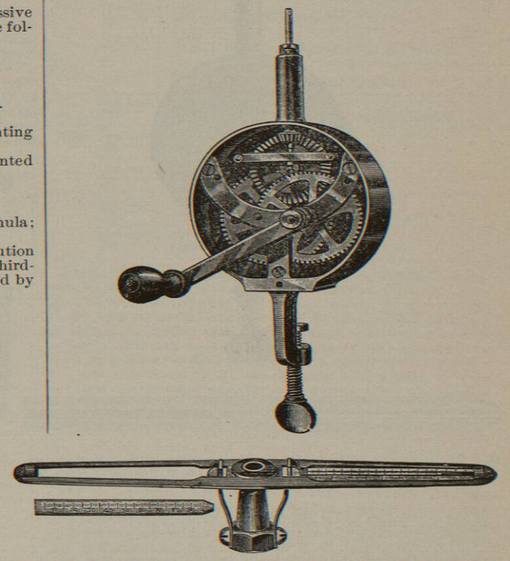

FIG. 563.—The Daland Hæmatocrit. (From Simon's "Clinical Diagnosis.")

Principle.—When blood is placed in the tube of a centrifuge and the apparatus rotated, the corpuscles collect as a red mass at the distal end of the tube and the serum as a colorless, semitransparent liquid at the proximal end.

By taking a fixed quantity of blood and subjecting it to a fixed number of revolutions for a fixed time, and by noting and marking the height of the column of corpuscles in the tube filled with blood from healthy individuals, a normal standard is determined. Deviations from this standard may be taken to indicate an increase or a decrease in the number of red cells.

By counting the blood corpuscles with the Thoma and Zeiss instrument at the same time that the blood is subjected to the centrifuge, markings to express this count may be made upon the glass tube containing the column of corpuscles.

The instrument for this method of counting is known

as the hæmatocrit (*αἷμα*, blood; *κρίτης*, a separator or discerner), blood separator. Hedin's instrument was among the first devised for this purpose (Fig. 562).

In using this instrument the blood is sucked into a capillary tube together with a 2.5 per cent. bichromate of potash solution or Müller's * fluid, to prevent clotting,

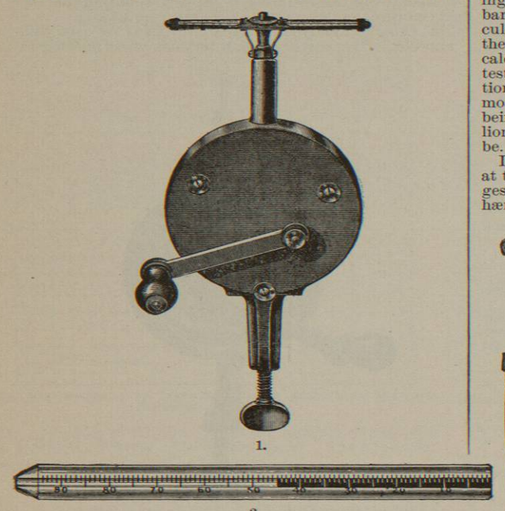

FIG. 564.—Daland's Hæmatocrit. 1, The centrifuge with cross piece containing capillary graduated tubes in place; 2, capillary graduated tube (magnified) containing blood. (From Simon's "Clinical Diagnosis.")

and the tube placed in the limb of the centrifuge. The wheel is then revolved for a fixed time at a fixed number of revolutions (fifty to seventy seconds with the instrument here represented), when the red corpuscles will be found arranged as a sediment at the distal extremity of the tube, with a layer of white cells next, and above the white cells, again, the diluting fluid and serum. This instrument has been simplified very much by Judson Daland working in von Jaksch's clinic (Fig. 564). By comparing the cuts (Figs. 562, 563) it will be observed how much simpler the latter is, the principle in both, however, being the same.

An instrument (as shown in Fig. 565) is now upon the market for the attachment of tubes for testing blood, urine, or milk. In using the Daland instrument no preservation fluid (bichromate of potash or Müller's fluid) is employed, a questionable omission.

The instrument is operated as follows: A *large* puncture is made in the lobe of the ear in the usual way. A rubber tube is attached to the capillary tube and blood sucked up, *more than is required to fill the tube*. The index finger (with a little vaseline to prevent blood from adhering to it) is placed on the far end of the capillary tube to prevent the blood from escaping, and the rubber tube removed. The excess of blood is wiped away from the end to which the rubber tubing was attached. The capillary tube is now placed in one arm of the machine and an empty tube to balance placed in the opposite arm; then the handle of the instrument is revolved for two or three minutes. These instruments differ in the number of revolutions which they make, and each instrument should be tested to ascertain how many times it is necessary to turn the large handle in order to throw down the corpuscles so completely *that no further revolving influences them*. It is therefore impossible to give any general rule for the number of revolutions necessary beyond saying that about four thousand revolutions of the cross bar to the minute should be the basis upon which to calculate. By noting the number of revolutions made by the cross bar to every single revolution of the handle this calculation can be readily made for each machine. The test, however, as stated above, is the complete precipitation of the corpuscles. The capillary tube is then removed and the column of corpuscles noted, the count being according to the figures 2, 3, 4, 5, 6, etc., in millions opposite which the top of the column is found to be. (See Fig. 564.)

Dr. J. Metcalfe Polk, working in the clinical laboratory at the Cornell University Medical School, has at my suggestion carried out systematic observations upon the hæmatocrit, comparing it with the Thoma and Zeiss

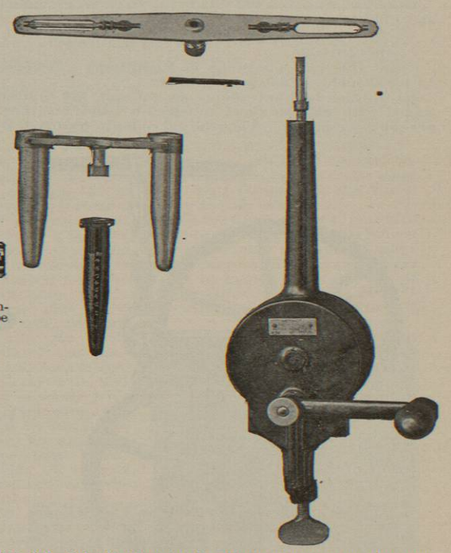

FIG. 565.—Daland's Hæmatocrit. With attachment for urine tubes as well as blood tubes.

blood-counting apparatus. The instrument upon which these observations were made is that shown in Fig. 565. The following are his findings:

1	2	3	4	5	
Case.	Time.	Revolution of Beam.	Reading of Hæmatocrit.	Thoma-Zeiss Count.	Remarks.
I...	1st min.	2,581			
	2d "	2,900	7,000,000	5,056,000	
	1 min. 30 sec.	5,075 1,450	5,200,000		
	1 min. 1 "	4,500 4,200	5,100,000		

* Müller's fluid: Potassium bichromate, 2.5; sodium sulphate, 1.0; distilled water, 100.

1	2	3	4	5	
Case.	Time.	Revolution of Beam.	Reading of Hæmatocrit.	Thoma-Zeiss Count.	Remarks.
II...	1st min.	3,480			
	2d "	2,900	3,700,000	4,215,000	
	1st min.	4,350			Spot of corpuscles in serum at 75.
	2d "	2,900	3,900,000		
III...	1st min.	4,490			
	35 sec.	1,500	4,900,000	4,748,000	
	1st min.	3,580			Two specks of corpuscles in serum; edge at reading point ragged.
	2d "	2,900	4,950,000		
IV...	1st min.	4,300			
	2d "	4,200	4,500,000	4,960,000	
	1st min.	3,770			Corpuscles not all thrown down. Two spots in serum.
	2d "	3,625	4,700,000		
V...	1st min.	3,200			
	2d "	3,480			
	3d "	3,200	5,300,000	4,852,000	
	1st min.	3,770			
	2d "	3,770			
	3d "	3,770			
	4th "	3,770	5,200,000		
	1st min.	5,075			
	35 sec.	1,405	5,500,000		
VI...	1st min.	4,350			
	2d "	3,770	4,100,000	4,068,000	
	1st min.	4,060			Spot of corpuscles from 43 to 45.
	2d "	3,770	4,200,000		
VII...	1st min.	4,060			Spot of corpuscles 44 to 45.
	2d "	4,060	3,650,000	3,976,000	
	1st min.	4,060			
	2d "	3,770	3,500,000		
VIII...	1st min.	4,060			
	2d "	4,060	3,150,000	2,564,000	
	1st min.	3,770			From 31 to 34, corpuscles thin, but column read at 34.
	2d "	3,900	3,400,000		
IX...	1st min.	4,060			
	2d "	3,900	3,000,000	2,900,000	
	1st min.	3,900			
	2d "	3,770	3,200,000		
X...	1st min.	4,060			Spot of corpuscles from 62 to 63.
	2d "	3,800	6,000,000	4,928,000	
	1st min.	4,100			
	2d "	3,625	5,200,000		
XI...	1st min.	4,060			Corpuscles thin from 42 to 44½.
	2d "	4,060	4,450,000	4,504,000	
	1st min.	4,350			Corpuscles thin from 41 to 42.
	2d "	4,060	4,200,000		
XII...	1st min.	4,046			
	2d "	4,046	4,600,000	5,088,000	
	1st min.	3,800			Corpuscles thin from 46 to 48.
	2d "	3,770	4,800,000		
XIII...	1st min.	3,625			Case of marked jaundice. Several counts made by different people, about same results.
	2d "	3,480	3,600,000	4,624,000	
	1st min.	4,060			
	2d "	4,150	3,700,000	4,350,000	
XIV...	1st min.	4,090			
	2d "	3,960	4,900,000	4,968,000	
	1st min.	4,060			
	2d "	4,060	4,600,000		
XV...	1st min.	4,060			
	2d "	3,950	4,200,000	4,920,000	
	1st min.	4,060			
	2d "	3,900	4,700,000		
XVI...	1st min.	4,060			
	2d "	4,060	4,100,000	4,472,000	
	1st min.	4,060			
	2d "	4,060	3,700,000		
XVII...	1st min.	4,060			
	2d "	4,090	2,800,000	2,570,000	Per self.
	1st min.	4,060			
	2d "	3,770	2,600,000	2,650,000	Per Dr. Brown.
XVIII...	1st min.	4,070			
	2d "	3,900	1,800,000	2,200,000	Per self.
	1st min.	4,060			
	2d "	3,770	2,000,000	2,350,000	Per Dr. Brown.
XIX...	1st min.	4,060			
	2d "	4,060	4,400,000	4,900,000	
	1st min.	4,060			
	2d "	4,060	4,100,000		
XX...	1st min.	4,060			
	2d "	4,060	5,000,000	4,864,000	Per Dr. Scott.
	1st min.	4,060			
	2d "	4,060	5,200,000	4,656,000	Per self.

From the above tests Dr. Polk draws the following conclusions: In revolving handle of hæmatocrit the number of revolutions per minute was counted by the watch. There was no stop between the first and second minutes as indicated under the time column. The number of revolutions above a certain number, say 3,900, means very little, as it is impossible to maintain a uniform number per five seconds throughout the minute and a half or two minutes, as the case may be. If at any time the handle is turned with extra violence for ten or twenty seconds in one specimen and not so in the second, although the number of revolutions will be the same per two minutes, the reading of the first specimen will be from 4° to 8° lower than that of the second. By looking over and comparing columns 3 and 4, the above point will be evident. Time in taking a blood count with the hæmatocrit, from puncture of the finger to the reading, was on an average two minutes and forty-five seconds, or in round numbers, under three minutes; two minutes being allowed for turning the instrument. Time of a Thoma-Zeiss enumeration was over twenty-five minutes.

Time from taking the drop of blood to getting it into hæmatocrit and starting the same was never over fifteen seconds. The patient was close to the instrument. The hæmatocrit made so much noise that it could not be used in the wards.

Our conclusion as to the value of the hæmatocrit concurs with that of von Jaksch and Bleitreu, namely, that the instrument is extremely useful for routine blood work, but that in accuracy it does not equal the Thoma-Zeiss apparatus.

Our rule is to employ the hæmatocrit in routine examination, and, should a case present grave anæmia, to verify the count with the Thoma-Zeiss apparatus.

3. ENUMERATION OF BLOOD CORPUSCLES BY THE OPTICAL METHOD.—The third method by which the enumeration of the corpuscles is made may be termed the optical method.

The instruments used for this purpose depend for their principle upon a change in color or opacity of the blood according to its numerical value in corpuscles.

The globulimeter of Mantegazza* is the earliest of these instruments. The instrument consists of a receiver of glass with parallel walls. Into this receiver the blood is placed, after being diluted with ninety-six times its volume of a solution of sodium carbonate. A lighted candle is placed on the other side of the receiver, and the flame

* Berliner klin. Woch., April 8, 1878; New York Med. Record, October 5, 1878.

observed through the diluted blood. Blue glasses, one after another, are placed between the observer and the blood until the flame can no longer be detected. Each

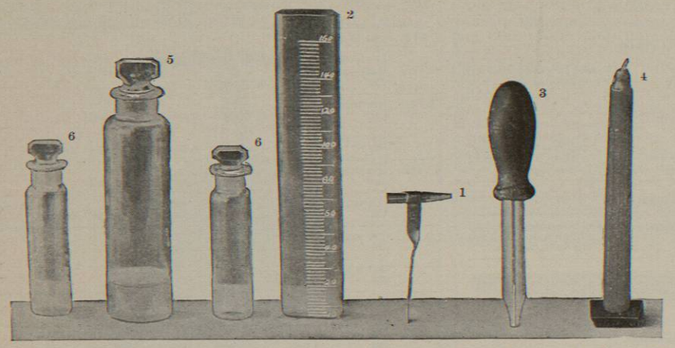

FIG. 566.—Oliver's Hæmocytometer. 1, capillary tube; 2, graduated flattened receiver; 3, dropper; 4, candle and stand; 5, Bottle for Hayem's solution; 6, bottles in which to place blood and solution for subsequent examination.

blue glass represents 125,000 red cells. From one to thirty glasses may be added, when by referring to the table the number of corpuscles will be ascertained.

Knowing, as we now do, that the amount of hæmoglobin may be far below the number of red cells, this instrument seems to be based upon a false principle. The opacity and color of the blood are separate conditions, and bear no constant relation to the number of the corpuscles.

Dr. George Oliver, of London, devised an instrument which he used in the preparation of his Croonian Lectures "On the Study of the Blood and Circulation," published in *The Lancet*, June 20, 1896. This instrument depends for its principle upon the opacity of the blood, and has nothing to do with the color or tints, so that the term tintometer, which has been applied to it, is a wrong one. The hæmocytometer of Oliver (Fig. 566) consists of the following parts: (1) A capillary tube, capacity 10 c.mm. (2) A glass receiver whose sides are flattened so that it measures less from before backward than from side to side. The tube is about four inches in length, one inch in breadth, and about one-fourth of an inch from before backward. (3) A dropper. (4) A small candle, the size of a Christmas-tree candle. (5) Hayem's solution (for formula, see p. 40).

The principle of the instrument is that in an opaque fluid (like blood), contained in a flattened glass receiver with vertical striations, to which is gradually added a less opaque fluid (like Hayem's solution), there develops, when such a receiver is placed between a candle flame and the eye, a point in the process of adding the less opaque fluid to the more opaque fluid at which the candle flame may be seen as a continuous bright line of light, which, before this point in dilution is reached, is not visible, and which after this point is passed no longer appears continuous, but as separate images of the candle flame. Moreover, the bright line of light consists of myriads of reflections of the candle flame produced by the vertical striations upon the glass receiver, which reflections cannot be appreciated by the eye of the observer until the opacity of the blood has been overcome by the addition of the less opaque solution. The point at which this line appears is constant for normal blood, from which constancy a unit may be established to measure blood rich or poor in corpuscles.

The instrument is used as follows: The skin is punctured in the usual way and the capillary tube applied to the drop as in using the von Fleischl hæmoglobinometer (see Fig. 578), the blood entering until the tube is entirely filled. There should, of course, be no air spaces in the column of blood. Excess of blood is wiped from off the sides of the tube, care being taken not to soak out the blood from the tube. This may be avoided either by not allowing the linen used to wipe away the excess of blood to touch the orifice of the tube, or by holding the finger over the upper end of the tube while wiping away the excess. The dropper is now filled with Hayem's solution and the capillary emptied by allowing the solution to squirt through the tube, as shown in Fig. 567. This can be done by inserting the pointed end of the capillary tube into the mouth of the receiver and holding the dropper close to the blunt end of the capillary, then giving a quick pinch to the rubber end of the dropper. This sends a strong stream through the capillary and empties it almost entirely. Repeating the process will certainly clean the capillary of blood. It is not necessary to attach the dropper by a rubber connection, as shown in Oliver's illustration. This is difficult and takes time, and allows the blood to clot in the capillary. We may now either proceed at once to the estimation, or the mixture thus made may be put into a small bottle and estimated at any time within twelve hours. Thus several such mixtures may be made in the course of the morning's round and placed in bottles properly labelled, the estimation being

FIG. 567.—Oliver's Hæmocytometer. Washing blood with Hayem's solution from capillary tube, by means of dropper, into flattened graduated receiver.

made when the physician returns to his office. Or the specimens may be sent by mail, if the distance be not such as to require more than twelve hours in transportation. These are distinct advantages over the Thoma-Zeiss instrument.

If a candle flame ten feet away be viewed in a perfectly dark room through the receiver held as shown in Fig. 568, the receiver containing blood and Hayem's solution up to the 10 mark, for example, owing to the opacity of the mixture the flame will appear as a faint diffuse light. If now we add little by little the Hayem's solution, as the opacity becomes less the flame will become clearer, and will be caught by the vertical striations on the receiver, which act as minute mirrors to reflect the flame as a continuous line of light. When the mixture has reached that degree of opacity which allows of this phenomenon we read off the upper limit of the fluid in the receiver: 100 on the scale represents 5,000,000; 80, 4,000,000; *i.e.*, 20 on the scale indicates 1,000,000 corpuscles.

By moving the receiver slightly from side to side and from before backward, the line may be detected at the sides a little before it extends across the receiver, a signal to the observer to add the solution cautiously drop by drop. Before the fluid has reached the height at which the line appears continuous the line is noticed to be blurred. As we add

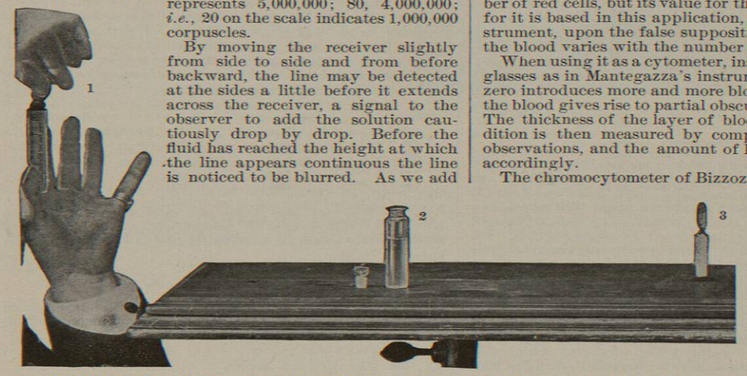

FIG. 568.—Oliver's Hæmocytometer. Watching for that degree of opacity at which candle flame will be seen as transverse bright line across flattened receiver. 1, Holding flattened receiver close to eye, between thumb and forefinger of left hand, while adding Hayem's solution from dropper with right hand; 2, bottle holding Hayem's solution; 3, candle ten feet away from observer and on a level with observer's eye.

more fluid than is necessary to produce the continuous line, the line ceases to be a continuous one and becomes a series of minute but separate images of the candle flame. The point, therefore, at which the reading should be made is *after the line ceases to be blurred and before separate images of the flame can be observed.* The candle should be at least ten feet away and should be not larger than the ordinary Christmas-tree candle. The receiver should be held in the right hand and the thumb pressed well into the side of the nose so as to shut off all diffused light, as shown in Fig. 568.

III. Hæmoglobin Estimation.

Apparatus and Technique.—The task of estimating the amount of hæmoglobin has long been an unsatisfactory one, because all methods, excepting only the specific-gravity method, depend upon the comparison of colors, a subject upon which nearly all individuals differ, and upon the degree of opacity, which is supposed to vary with the amount of hæmoglobin, a supposition by no means proven.

The instruments group themselves into three classes, as follows:

1. Bizzozero's chromocytometer 2. Henoque's hæmatoscope	Based upon the supposition that the amount of hæmoglobin varies with the degree of opacity.
1. Gowers' hæmoglobinometer 2. Von Fleischl's " 3. Von Fleischl's and Meischer's " 4. Taylor's " 5. Oliver's tintometer "	Based upon the supposition that the amount of hæmoglobin varies with the intensity of color.
1. Hammerschlag's method 2. Roy's "	Based upon the supposition that the amount of hæmoglobin varies with the specific gravity.

1. Opacity Methods.—*The Chromocytometer of Bizzozero** depends for its principle upon the supposition that the opacity of the blood varies with the amount of hæmoglobin.

The instrument may also be used to estimate the number of red cells, but its value for this purpose is doubtful, for it is based in this application, as is Mantegazza's instrument, upon the false supposition that the opacity of the blood varies with the number of cells contained.

When using it as a cytometer, instead of adding colored glasses as in Mantegazza's instrument (see p. 49), Bizzozero introduces more and more blood until the *opacity* of the blood gives rise to partial obscuring of a candle flame. The thickness of the layer of blood producing this condition is then measured by comparison with standard observations, and the amount of hæmoglobin estimated accordingly.

The chromocytometer of Bizzozero is an instrument intended primarily for the estimation of hæmoglobin. The name is misleading. The instrument is so called because the amount of hæmoglobin is ascertained by two methods: (1) By observing the degree of opacity produced by the hæmoglobin *unremoved* from the corpuscle, and therefore the actual corpuscular value of hæmoglobin—in which application it is called a *cytometer* (κύτος, a cell; μέτρον, a measure). It does not number the corpuscles, as at first the name seems to imply. (2) By observing the color produced by the hæmoglobin removed (dissolved) from the corpuscle and comparing this color with a standard colored glass—in which application it is called a chromometer (χρῶμα, a color; μέτρον, a measure). Its application as a hæmocytometer is a wrong one, as already stated.

As a Cytometer (term understood as above described).—*Principle:* If a fixed quantity of blood be mixed with

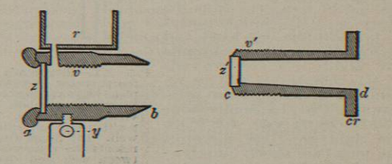

FIG. 569.—Diagram Showing Construction of Bizzozero's Chromocytometer. *r*, Well connecting with *v*, chamber formed by *v'*, which screws into *v*; *z* and *z'*, glass windows.

a fixed quantity of preserving fluid (saline solution), and the mixture placed in a receiver, and a candle flame viewed through the mixture in a dark room, then, according to the amount of hæmoglobin and the amount of the mixture used, the flame of the candle will either be seen or will not be seen. By observing the amount of this

* Atti della regia Accad. d. Sc. di Torino, xiv., 1879.

mixture necessary, in normal cases, partially to obscure the candle, and calling this 100, we can, as more or less of such a mixture is required in diseased cases, ascertain the decrease or increase in the amount of hæmoglobin.

The instrument consists of a well which *connects* with a chamber closed at both ends with colorless glass. The chamber consists of two pieces of metal, one screwing into the other. These are hollowed out so as to form the chamber within (Fig. 569). By screwing or unscrewing one of these pieces the dimensions of the chamber may be decreased or increased. It will be seen that if diluted blood be placed in the well which connects with this chamber, the blood will flow into the chamber through this connection. But only just so much blood will flow into the chamber as is allowed by unscrewing the outer screw. When the outer portion of the instrument is screwed home (Fig. 571) no fluid at all enters the space between the screws—in other words, the chamber is then obliterated. Now by unscrewing or screwing the piece of metal which fits into the other piece and which, together with its fellow, forms the chamber, we can increase or decrease the amount of fluid in the space between the screws.

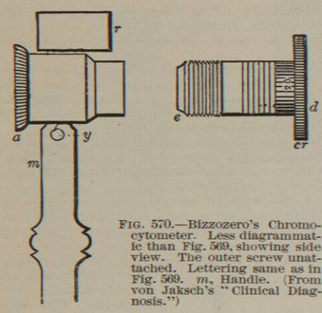

FIG. 570.—Bizzozero's Chromocytometer. Less diagrammatic than Fig. 569, showing side view. The outer screw unattached. Lettering same as in Fig. 569. *m*, Handle. (From von Jaksch's "Clinical Diagnosis.")

(1) Suppose now the two pieces of metal to be completely screwed together, so that no space exists between them, the scale then stands at 0. (2) We fill the well (Figs. 569–571, *r*) with 50 parts of normal saline solution to 1 part of blood; or, to be more accurate, by means of a pipette we mix 10 c.mm. of blood with 0.5 c.c. saline solution (0.75 gm. of NaCl in 100 c.c. water), which preserves the corpuscles. (3) A candle is placed in a dark room about four feet away from the observer. (4) The instrument is held by the left hand and close to the right eye, opposite the candle. (5) The two ends of the chamber, it will be remembered, have glass windows, so that the candle flame will be clearly seen. (6) With the right hand the pieces of the metal are slowly unscrewed, which allows the blood to flow from the well into the chamber. (7) As we introduce more and more fluid into the chamber, however, a degree of opacity must be reached at which the candle flame will be almost or wholly obscured. This is just what occurs. By observing the degree of unscrewing, so to speak, necessary to produce this opacity for normal blood, we obtain a unit with which diseased blood may be compared. By means of the table given below the amount of hæmoglobin may then be ascertained.

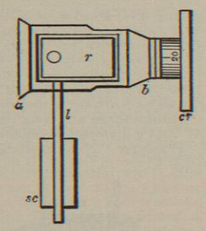

FIG. 571.—Bizzozero's Chromocytometer. Screws completely screwed together, obliterating chamber. Well viewed from above. In this position one looks into the well. The round ring represents the connection between well and chamber. (From von Jaksch's "Clinical Diagnosis.")

When the space between the glass windows is obliterated by screwing the outer portion home, the index on the scale stands at 0. One complete rotation of the screw produces a space in the instrument measuring 0.5 mm. This is therefore the thickness of the contained solution of blood. One complete turn, however, renders the candle flame decidedly less visible. This complete rotation is therefore subdivided on the scale into 0.02 mm., 25 such subdivisions ($0.02 \times 25 = 0.5$) constituting one complete revolution, and 50 such subdivisions ($0.02 \times 50 = 1.0$) constituting 1.0 on the scale. From a number of observations (as stated by von Jaksch) upon the blood of healthy individuals, the outlines of the candle flame are distinctly seen through a layer of blood $\frac{11}{100}$ mm. thick = 1.0.

This, then, is the unit; *increase* of this number means *decrease* in amount of hæmoglobin. For if we must increase the thickness of the stratum of blood in order to obscure the candle flame, it indicates that the fluid is poor in coloring matter, and that an excess is required to produce dimness of the candle flame.

Scale.		Hæmoglobin.	Scale.		Hæmoglobin.
110	represents	100.0	170	represents	64.7
120	"	91.6	180	"	61.1
130	"	84.6	190	"	57.9
140	"	78.5	200	"	55.0
150	"	73.3	210	"	52.4
160	"	68.7	220	"	50.0

If one has not this table at hand the reading can be readily interpreted by remembering that 110 = 100. Suppose, for example, the reading to be 160 on the scale; then:

$$100 : 110 :: 160 : ?$$
$$100 \times 110 \div 160 = 68.7$$

As a Chromometer (term understood as above described).—In addition to the parts of the instrument as already enumerated, there is a colored glass (see Fig. 572). This is introduced on one side of the instrument so that the colored glass and the glass windows of the instrument are *side by side*, as would be the case with a pair of opera glasses if one side were to consist of only the eyepiece, and that with a colored glass in it. In this use of the instrument, moreover, the hæmoglobin is dissolved out with water in preparing the blood for observation.

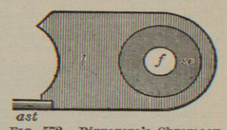

FIG. 572.—Bizzozero's Chromocytometer. Frame holding tinted glass. This is placed by the side of the screw of the main apparatus. *f*, Tinted glass; *l*, frame; *ast*, rod which passes through hole *y* in handle *m* of Fig. 570.

Principle: If a glass colored to correspond with a definite solution of hæmoglobin be placed before a bright light (the sky) and beside a solution of hæmoglobin to be tested, the color of the tinted glass and that of the solution of hæmoglobin will be found to differ. If, however, more or less of the solution be added, a point will be reached at which these colors correspond. By noting the amount of solution required to make these colors exactly correspond a scale may be made by which the amount of hæmoglobin may be estimated.

Given a tinted glass which corresponds in color with a known solution of hæmoglobin. Given a known quantity of blood (10 c.mm.) with a known quantity of water (0.5 c.c.). If the blood to be tested contains much coloring matter (hæmoglobin), it will be necessary to add but little of the solution in order to make the colors correspond; if it contains little coloring matter, much will be required to make these colors correspond. These quantities are noted and compared with a scale, each quantity of fluid corresponding with a percentage of hæmoglobin.

By referring to Figs. 570 and 571, the working of the apparatus will be readily understood.

The colored glass is not of the same intensity of color in every instrument, so that the glass must be tested for each instrument.

The test is performed in the following way: Normal blood is tested, and is found to be, by the cytometer method, 110, and by the chromometer, say 130. One hundred and ten of the cytometer equals 100 hæmoglobin. For this glass, then, 130 also equals 100.

As in the case of the cytometer method, we construct a table from these relations. Suppose the reading with the chromometer to be 190.

$$100 : 130 :: 190 : ?$$
$$100 \times 130 \div 190 = 68.4.$$

After a brief experience in using this instrument the author is inclined to commend it.

Henocque's Hæmatoscope.—This instrument also depends for its principle upon the supposition that the amount of hæmoglobin varies with the degree of opacity of the blood. In this instrument, in addition to the degree of opacity, the quantity of oxyhæmoglobin is ascertained by means of the spectroscope. This latter method places the instrument among the apparatus of an elaborately equipped laboratory and diminishes its value for bedside work. Moreover, as von Jaksch states, there is a "difference of opinion as to when precisely the spectrum is formed," making the conclusion drawn from the use of the instrument "always somewhat arbitrary."

The instrument consists of two plates of glass, one placed above the other so as to enclose between them a prismatic space. The dimensions of this space are accurately stated and are the same for every instrument.

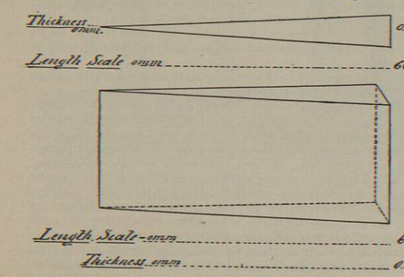

FIG. 573.—Henocque's Hæmatoscope. Diagram showing dimensions of prismatic space formed by plates of glass.

These plates of glass are in contact at one end, forming the apex of the prismatic space. The scale of measurement at this end is therefore 0.

At the other end the glass plates are separated by a distance of 0.3 mm., forming the base of the prismatic space. By referring to the diagram (Fig. 573) and illustrations (Figs. 574 and 575), this arrangement will be readily understood.

The scale upon the glass is marked from 0 to 60, as observed in Fig. 574.

There are two methods of using the instrument.

(1) The first method is based upon the supposition that varying opacity of the blood differs according to varying quantity of hæmoglobin, so that by observing the former the latter may be estimated.

To use the instrument upon this basis, puncture is made in the skin in the usual way and the drop brought to the orifice of the prismatic space, which, from its dimensions, will be observed to be capillary. The blood on this account passes readily into the space without being diluted, an advantage over the dilution methods.

About six drops of blood are necessary to fill the space. Excess is wiped away.

In addition to the apparatus already described, there is an enamelled plate, as seen in Fig. 575.

This plate is placed behind the prismatic layer of

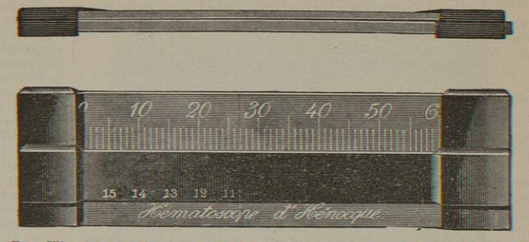

FIG. 574.—Henocque's Hæmatoscope Filled with Blood. (From von Jaksch's "Clinical Diagnosis.")

blood, so that the figures 0, 10, 20, etc., correspond with the like figures on the glass plate of the hæmatoscope. At the apex end of the prism, and therefore at the thinnest portion of the layer of blood, the marking on this enamelled plate will be visible; but as the eye passes toward the thicker portion of the prism of blood a point will be reached at which the marking can no longer be seen. If the blood tested be rich in hæmoglobin the opacity will be greater, and therefore the point in the series 15, 14, 13, 12, etc., at which this obscuring occurs will be higher, say 11 or 12, as seen in the illustration. If, on the other hand, the blood be poor in hæmoglobin, the opacity will be correspondingly less, and we shall be able to see the figures 10, 9, 8, etc., in the series.

One hundred grams of normal blood contain 14 gm. of oxyhæmoglobin. These figures, therefore, indicate the number of grams of oxyhæmoglobin in 100 gm. of blood. For this use the author commends the instrument.

(2) The second method is based upon the supposition that the richer the blood is in hæmoglobin the thicker will be the layer necessary to detect through the spectroscope the characteristic bands of oxyhæmoglobin. By noting the thickness in normal blood at which the oxyhæmoglobin spectrum occurs, a scale may be made with which deviations from the normal may be measured. The apparatus, with the exception of the enamel plate, is used as before.

The prism is fitted in front of a spectroscope and is turned until the oxyhæmoglobin bands appear, when this point is noted on the scale. With normal blood the oxyhæmoglobin spectrum is seen opposite 14 of the millimetre scale. Now for every millimetre on the scale the thickness of the prismatic space increases 0.005 mm. This can be demonstrated by multiplying 0.005 by 60, the

FIG. 575.—Enamelled Plate Used with Henocque's Hæmatoscope. (From von Jaksch's "Clinical Diagnosis.")

length of the scale; the result is 0.3 mm., the thickness of the prism at its base, *i.e.*, at the 60 mm. end. As, therefore, for normal blood the spectrum appears at 14 on the millimetre scale, we determine the thickness of

blood required to produce this spectrum by multiplying 14 mm. × 0.005 mm. = 0.07 mm.

This, then, is a unit; it is the thickness of normal blood required to produce the oxyhæmoglobin spectrum.

Suppose a specimen of blood produces the oxyhæmoglobin bands at 40 on the millimetre scale. The thickness of this specimen would be 40 mm. × 0.005 mm. = 0.2 mm. Then:

i.e.: $\frac{0.07 \times 14}{0.2} = x = 4.9$ gm. of hæmoglobin in 100 gm. of blood.

An equation for any reading is the following:

$$(y \times 0.005) : 0.07 :: 14 : x$$

in which

y = reading on the scale.

0.005 = thickness of prism for each millimetre division on the scale.

0.07 = thickness of layer of normal blood by which oxyhæmoglobin spectrum is produced—*i.e.*, at 14 on the scale (14 × 0.005 = 0.07).

14 = quantity of oxyhæmoglobin in 100 gm. of normal blood.

From these calculations one can make for himself a table which will at once give him the amount of the hæmoglobin for any reading on the scale. Thus beginning with 5 on the millimetre scale, and substituting it for y in the equation, we get the following:

$$(5 \times 0.005) \times 0.07 \div 14 = 12.5.$$

Therefore 5 mm. of the scale = 12.5 gm. hæmoglobin in 100 gm. of blood.

If we substitute in the same way 10, 15, 20, etc., the table becomes complete.

The instrument has not been much used in this country, and, as already stated, except for the opacity method, it requires more apparatus than would be practicable at the bedside.

The author has not had sufficient experience with this method to express an opinion upon its value.

2. Color Methods.—Before entering upon the description of those hæmoglobinometer instruments which depend for their principle upon the comparison of colors, it would be well to quote from Lovibond's very interesting and valuable work on "Measurements of Light and Color."

"It is almost impossible," says Lovibond, "without special arrangements to arrive at a reliable judgment between two colors which are very nearly, but not quite, alike, when these are placed openly side by side. The difficulty arises from the unequal incidence of light—sometimes of the direct light, frequently of the side lights, or from both combined. The disturbing effect is so great that a slight change of position in either of the samples, or of the observer, generally reverses the first judgment.

"The same causes account for the *frequent differences of opinion between two persons* judging the same color; in fact, the color sensations of our surroundings are . . . governed by the ever-varying conditions of light, surface, substance, texture, and chemical composition. . . ."

The following are the experiments carried out by Prof. Lovibond:

"*Test Tubes containing colored liquids.*—The first attempts were made with colored liquids in test tubes of equal diameters, and by these means some useful information was obtained. The liquids, however, *soon changed color*, requiring frequent renewals; and there was always a little uncertainty concerning their exact reproduction. Also a curious inequality of color relation was found to exist between the regular increase of strata thickness and their resulting color. This prevented liquids from being suitable as standards, because some liquids increase in color depth in direct proportion to increase of strata thickness. Some increase in color in a less but regular proportion to increase of strata thickness, whilst others increase in a less and irregular proportion.

Another difficulty arose from the convex surfaces of the test tubes acting as a lens, and increasing the disturbance arising from unequal light incidence. The convexity was reduced by using larger tubes, and enclosing them in a blackened case with narrow longitudinal apertures for looking through the middle of the tubes. The results, although useful, were too unsatisfactory for systematic work.

"*Colored glass* was next tried, and long rectangular wedges in glass of different colors, with gradually graded tapers, were ground and polished for standards, whilst correspondingly tapered vessels were made for the liquids to be measured. These were arranged to work, at the end of the instrument, up and down at right angles before two apertures, side by side, with a fixed centre line to read off the thickness of each before the aperture when a color match was made; but here also the difference of ratio between the thickness and color depth of the different colored glass and liquids proved fatal to the method.

"An incidental observation was made during these experiments concerning the difficulty of arriving at a final judgment with tapering colors, owing to one shade gradually blending into the next without a break of any kind to arrest the vision. The mental effort to arrive at a decision, under these conditions of gradual color-blending, was troublesome and vexatious in the extreme. Any person may realize this difficulty by attempting to fix a definite point by the vision in a graduated color line. I was enabled entirely to remove the difficulty by using separate glass slips for standards; the line of color decision made by each additional standard-glass slip used being a precise definition between the most minute shades.

"The effect of these partial failures enabled me more clearly to define the conditions from which successful work might be expected, which are as follows:

"*Gauged Cells.*—The cells for the liquids must have parallel transparent ends and be gauged to definite strata thickness.

"*Optical Instrument.*—The standard and sample must be viewed under equal conditions of illumination.

"*Suitable Light for Color Work.*—Considerable differences of view exist concerning the most suitable light for color work. Some authorities consider that daylight is too unreliable in composition, and that an artificial light, such as the electric arc, is best, as being always uniform.

"Captain Abney's work and apparatus at first sight go a long way toward establishing this view. Some valid reasons, however, in my opinion, exist in favor of daylight; as it is the light to which normal vision is most accustomed, and it is available, without cost at a moment's notice, during daylight hours, whereas artificial lights require to be worked in camera with somewhat expensive apparatus. Again, by far the largest proportion of artistic and commercial color work is carried on by daylight, so that measurements made by means of any other light must be transposed into terms of daylight values before reliable comparisons can be made. Without doubt the vision can work longer and with less fatigue by daylight than by intense artificial light."

From the foregoing it will be seen that liquids and colored glasses have a distinctive color value, and that any method which employs blending colors, as the colored prism of the von Fleischl apparatus or the gradually diluted blood in the Gowers instrument, presents a wider field for error than that method which employs fixed and uniform colors for comparison. Also daylight is the most reliable.

Gowers' Hæmoglobinometer.—This instrument is one of color comparison.

Principle: If a colored fluid representing a very low percentage of hæmoglobin be placed by the side of a solution of a fixed quantity of blood, to which water is added little by little, a point in the process of dilution will be reached at which the two colors correspond.

By observing the amount of diluting fluid required to bring normal blood to a corresponding color with the fixed color, a scale may be made by which deviations from the normal may be detected.

The parts of the instrument (Fig. 576) are (numbers correspond with those in cut): 1. A solution of picrocarmine glycerin of the color of a one-per-cent. solution

of normal blood, contained in a sealed tube. 2. An open tube graded from 10 to 120. 3. A pipette measuring 10 and 20 cm. 4. A dropper.

To use the instrument: A little water is put into the graduated tube. Puncture of the skin is made in the usual way, and blood drawn up to the 20 c.mm. mark. The blood is at once blown into the graduated tube containing the water. The blood and water are then thoroughly mixed, and more water, little by little, added by means of the dropper until the color of the diluted blood and that of the standard solution correspond. It will be seen that

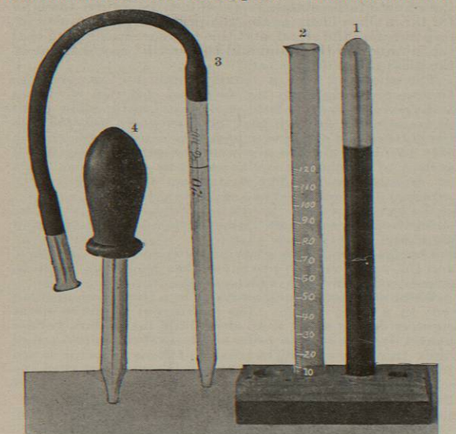

Fig. 576.—Gowers' Hæmoglobinometer. 3, Capillary tube for taking a definite quantity of blood; 4, dropper for adding water; 2, graduated receiver for blood and water; 1, tube containing picrocarmine, color equal to that of blood with one per cent. hæmoglobin.

if the blood is rich in hæmoglobin it will be necessary to add a large quantity of water in order to make it of the color of the standard solution, which represents only one per cent.

As we add more and more water, however, the column of fluid rises on the scale, and therefore indicates a high percentage of hæmoglobin. In cases of anæmia the correspondence will be sooner reached, and the column of fluid will stand at a lower level in the tube, and will therefore indicate a low percentage of hæmoglobin.

This instrument has the advantage of being cheap as well as accurate.

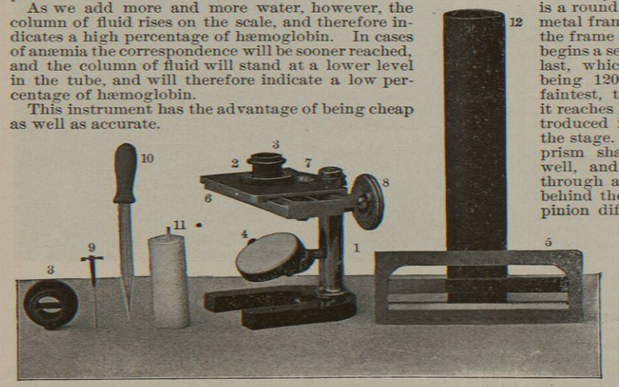

Fig. 577.—Von Fleischl's Hæmoglobinometer.

The standard solution, picrocarmine, changes color in time, a point noted by Lovibond and by Oliver working in Lovibond's laboratory.

Von Fleischl's Hæmometer—or, more correctly, hæmoglobinometer—depends for its principle upon the sup-

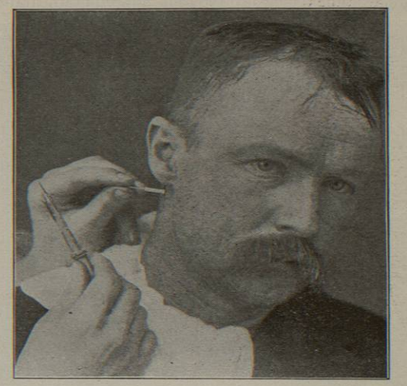

Fig. 578.—Von Fleischl's Hæmoglobinometer. Taking drop of blood in capillary tube.

position that a solution of hæmoglobin varies in color according to the amount of hæmoglobin which it contains, and that the latter may be measured by comparison with the varying colors in a prism of glass, each degree of color corresponding to a percentage of hæmoglobin.

We must keep in mind that when water is added to blood the hæmoglobin is dissolved out of the corpuscles, and it is the colored solution which we are comparing, not the hæmoglobin as contained in the corpuscles.

The parts of the instrument are (numbers correspond with those in illustration, Fig. 577): A stand (1) with a horseshoe base. A stage (2) with a round hole in the centre for the reception of (3) a well 1.5 cm. in depth and divided into two equal parts; the bottom of this well is closed by a piece of clear glass. Below the stage is a round reflector of plaster of Paris (4). In a metal frame is a prism of colored glass (5). On the frame opposite the apex of the prism there begins a series of figures, the first being 0 and the last, which is opposite the base of the prism, being 120. At 0 the tint of the color is the faintest, this tint increasing in intensity until it reaches the maximum, 120. The frame is introduced into two tracks (6) which run below the stage. It is placed so that the colored glass prism shall pass beneath one division of the well, and the series of figures shall appear through an oval opening (7) in the stage just behind the well. By means of a rack (8) and pinion different parts of the varicolored glass prism may be made to pass along the bottom of one division of the well, and at the same time the figures indicating the color intensity of the glass at that point will appear in the oval opening behind the well. There are also a capillary tube (9) by which a fixed quantity of blood may be obtained; a medicine dropper (10), a candle (11), a black tube (12) to shut off the surrounding light from the eye.

We have, therefore: 1. A colored