THE TECHNIC OF A BLOOD EXAMINATION

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The first requisite to the study of a blood dyscrasia is a complete and accurate laboratory examination of the blood. The presence of an anemia, leukemia or other disease of the blood may be suggested by the history and physical examination, yet no clinician would hazard a final diagnosis or outline treatment without knowing the results of the laboratory studies. Too often the clinician expresses an opinion based on blood films which are unsatisfactory for examination or on incomplete or inaccurate laboratory data. The selection of the best technical methods is difficult for those who are not constantly studying problems in hematology, although the technical study of blood is simple and requires no complicated apparatus. The methods described here have proven most dependable in our hands.

A routine blood count (red and white cell count, hemoglobin estimation and differential count) is only a starting point for a more complete blood study and should be looked upon largely as a means of determining whether or not a complete blood study is indicated. In every case of anemia the following examinations should be done:

1. Red corpuscle count.
2. Determination of the mass of packed corpuscles.
3. Hemoglobin estimation.
4. Calculation of indices:
    a. Volume index (erythrocyte volume relative to normal) or mean corpuscular volume.
    b. Color index (erythrocyte hemoglobin relative to normal) or mean corpuscular hemoglobin content.
    c. Saturation index (concentration of hemoglobin per unit volume of packed cells relative to normal) or mean corpuscular hemoglobin concentration.
5. White corpuscle count.

6. Study of stained blood film (size, shape, staining reactions and abnormalities of red cells, differential count of white cells, relative number of platelets).

7. Count of reticulocytes.

8. Determination of bile pigment content of the plasma.

These examinations are all necessary and are very easily done. I much prefer to make all examinations, except the study of the stained film, on blood withdrawn from a vein. The blood film alone is made from a drop of blood obtained from the ear lobe or the finger tip. A simple method for the entire examination is as follows: 20 cc. of blood is withdrawn from a vein by means of a syringe, and exactly 10 cc. is run into a 12 or 15 cc. centrifuge tube, containing exactly 2 cc. of 1.4 per cent sodium oxalate solution.[1] This is mixed by inverting and is then spun in a large centrifuge for one hour at 2500 revolutions per minute. The remainder of the blood is added to an ounce bottle containing one drop of a 30 per cent solution of potassium oxalate. The latter specimen is used for the red and white cell count and for the hemoglobin determinations. The examinations are made as indicated below.

1. *Red Cell Count.* One source of inaccuracy in erythrocyte counts is the use of a hypotonic diluting fluid. I prefer to use a 0.9 per cent sodium chloride solution as the diluting agent. Accurate erythrocyte counts require much practice and experience on the part of the technician. It is absolutely necessary that accurately calibrated counting chambers and pipettes be used. These should be certified by the United States Bureau of Standards.

2. *Volume of Packed Red Cells.* This is read off directly from the tube after centrifuging. The volume is recorded as the number of cubic centimeters of cells per 100 cc. of blood, and in per cent of normal. The normal is calculated for each laboratory by determining by means of the centrifuge the number of cubic centimeters of packed cells per 100 cc. of blood in normal individuals with a red cell count of 5 million cells per c.mm. With our present apparatus we have found 45 cc. of cells per 100 cc. of blood to be equal to 100 per cent (Fig. 1).

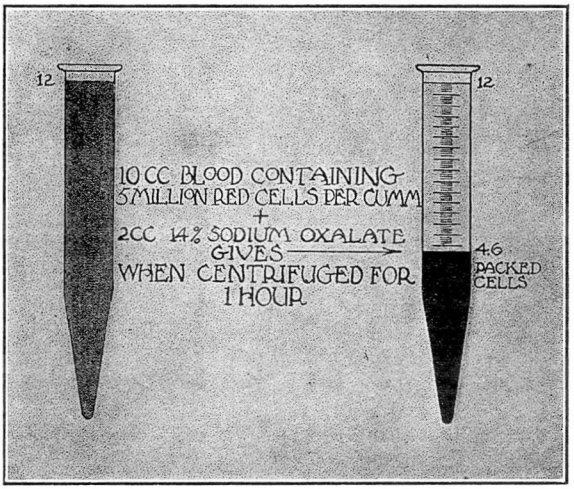

FIG. 1—Pyrex centrifuge tube used for determination of mass of red blood cells.

For any given specimen of blood the number of cubic centimeters of packed cells obtained by centrifuging 10 cc. of blood is read off on the tube and divided by 4.5 cc. (or other figure determined as normal for the 10 cc. of blood).

3. *Hemoglobin Estimation.* Accurate hemoglobin determinations may be made quite easily by the oxygen capacity method, using the Van Slyke apparatus, or by one of the iron methods. Such procedures are not practical, however, in routine clinical work. The exact number of grams of hemoglobin present in any given blood is of no great clinical importance. It is exceedingly important, however, to determine the hemoglobin content relative to normal. This can be done simply if a hemoglobinometer reading directly in grams is used for the determination. The new Sahli, the new Dare, the Bausch and Lamb-Newcomer, the Klett, the old Miescher-von Fleischl, and the Haden-Hausser[2] instruments, all read in grams although no two give the same reading on the same specimen of blood. This makes little difference if everyone in his own laboratory determines for the instrument used the average number of grams of hemoglobin per 100 cc. of blood in normal individuals with a red cell count of 5 million per c.mm., and takes this as 100 per cent. The results are then always reported, not in the absolute number of grams per 100 cc. but in per cent of normal. In normal individuals the color index is always 1.00 within the limits of error. By this method the precentage of hemoglobin for a given specimen of blood would always be the same in all laboratories although the actual number of grams of hemoglobin determined would be different in each.

In our laboratories the Haden-Hausser hemoglobinometer (Fig. 2) which reads only in grams is used routinely and 15.4 grams of hemoglobin is taken as 100 per cent. The new clinical model of the Haden-Hausser hemoglobinometer is less expensive and gives accurate readings in grams.

4. *Calculation of Indices.* By the methods outlined we are able to determine accurately the red cell count and the packed cells in per

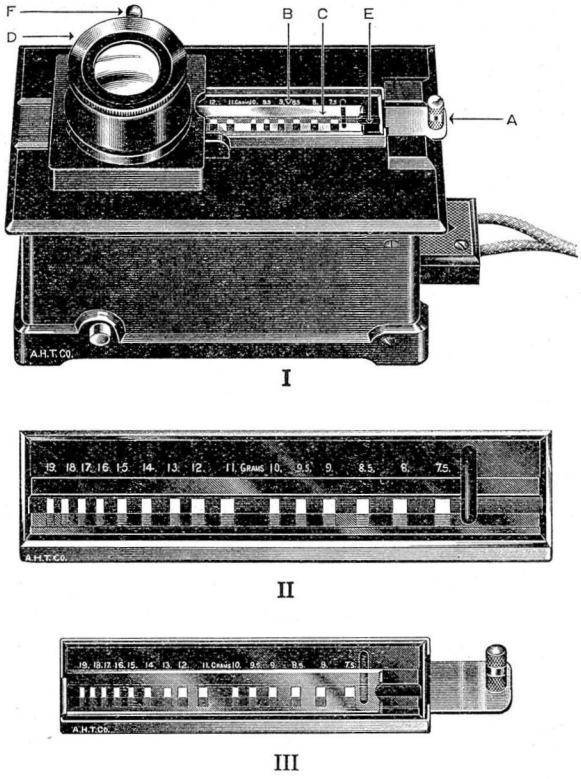

Fig. 2—Hadden-Hausser hemoglobinometer (laboratory model). I. Complete instrument. A, movable carrier; B, comparator slide; C, cover glass; D, reading microscope; E, wedge-shaped channel; F, light shutter. II. Comparator slide. III. Comparator slide with cover glass in metal holder.

cent of normal, packed cells (normal equals the number of cubic centimeters of packed cells found in 100 cc. of normal blood with a red cell count of 5 million) and the hemoglobin in per cent of normal hemoglobin (normal equals the number of grams of hemoglobin found in 100 cc. of normal blood with a red cell count of 5 million). Suppose for a given laboratory a specimen of normal blood with a red cell count of 5 million per c.mm. yields 46 cc. of packed cells per 100 cc. on

centrifuging with an isotonic anticoagulant for one hour at 2500 revolutions per minute, and contains 15 grams of hemoglobin per 100 cc. and a specimen of anemic blood with a red cell count of 1.5 million yields 18.4 cc. of packed cells, and contains 6.0 grams of hemoglobin, then:

(a) The volume index (volume of mean cell relative to normal)

$$\text{of the normal blood} = \frac{\text{Number of cc. of packed cells found per 100 cc.}}{\text{Normal number of cc. of packed cells per 100 cc.}} \Bigg/ \frac{\text{Number of red cells found}}{\text{Normal number of red cells}}$$

$$= \frac{\frac{46}{46}}{\frac{5{,}000{,}000}{5{,}000{,}000}} = 1.00$$

$$\text{of the anemic blood} = \frac{\frac{18.4}{46}}{\frac{1{,}500{,}000}{5{,}000{,}000}} = 1.33$$

The mean corpuscular volume[3] (the volume of the average red corpuscle in cubic microns) is calculated by dividing the volume of packed cells per 100 cc. by the number of cells contained in 100 cc. of blood. The result may be calculated in cubic microns by multiplying by 2 the volume of packed cells per 100 cc. per 5,000,000 cells.

Thus the mean corpuscular volume of the normal blood = 46 x 2 = 92 cubic microns; of the anemic blood $= \frac{18.4}{\frac{1{,}500{,}000}{5{,}000{,}000}} \times 2 = 61.3 \times 2 = 123$ cubic microns

(b) The color index (amount of hemoglobin per cell relative to normal)

$$\text{of the normal blood} = \frac{\text{Number of grams of hemoglobin found per 100 cc.}}{\text{Normal number of grams of hemoglobin}} \Bigg/ \frac{\text{Number of cells found per c.mm.}}{\text{Normal number of red cells per c.mm.}}$$

$$= \frac{\frac{15}{15}}{\frac{5{,}000{,}000}{5{,}000{,}000}} = 1.00$$

$$\text{of the anemic blood} = \frac{\frac{6.0}{15.0}}{\frac{1{,}500{,}000}{5{,}000{,}000}} = 1.33$$

The mean corpuscular hemoglobin[3] (the hemoglobin content of the average red corpuscle in micromicrograms) is calculated by dividing the hemoglobin in grams per 100 cc. of blood by the number of cells contained in 100 cc. of blood. It is simply calculated in micromicrograms by multiplying by 2 the number of grams of hemoglobin per 100 cc. of blood per 5 million cells.

Thus the mean corpuscular hemoglobin of the normal blood = 15.0 x 2 = 30

$$\text{micromicrograms of the anemic blood} = \frac{6.0}{\frac{1{,}500{,}000}{5{,}000{,}000}} \times 2 = 20 \times 2 = 40 \text{ micromicrograms}$$

(c) The saturation index (amount of hemoglobin per unit volume of cell relative to normal)

$$\text{of the normal blood} = \frac{\dfrac{\text{Number of grams of hemoglobin found in 100 cc.}}{\text{Normal number of grams of hemoglobin per 100 cc.}}}{\dfrac{\text{Number of cc. of packed cells found per 100 cc.}}{\text{Normal number of cc. of packed cells per 100 cc.}}} = \frac{\frac{15}{15}}{\frac{46}{46}} = 1.00$$

$$\text{of the anemic blood} = \frac{\frac{6}{15}}{\frac{18.4}{46.0}} = 1.00$$

The mean corpuscular hemoglobin concentration[3] (the concentration of the hemoglobin in per cent per unit volume of cells) is calculated by dividing the number of grams of hemoglobin per 100 cc. of blood by the number of cubic centimeters of packed cells per 100 cc.

Then the mean corpuscular hemoglobin concentration in the normal blood $= \frac{15}{46} = 32.6$ per cent, in the anemic blood $= \frac{6.0}{18.4} = 32.6$ per cent.

The calculation of the different indices is facilitated by the use of a nomogram (Fig. 3).

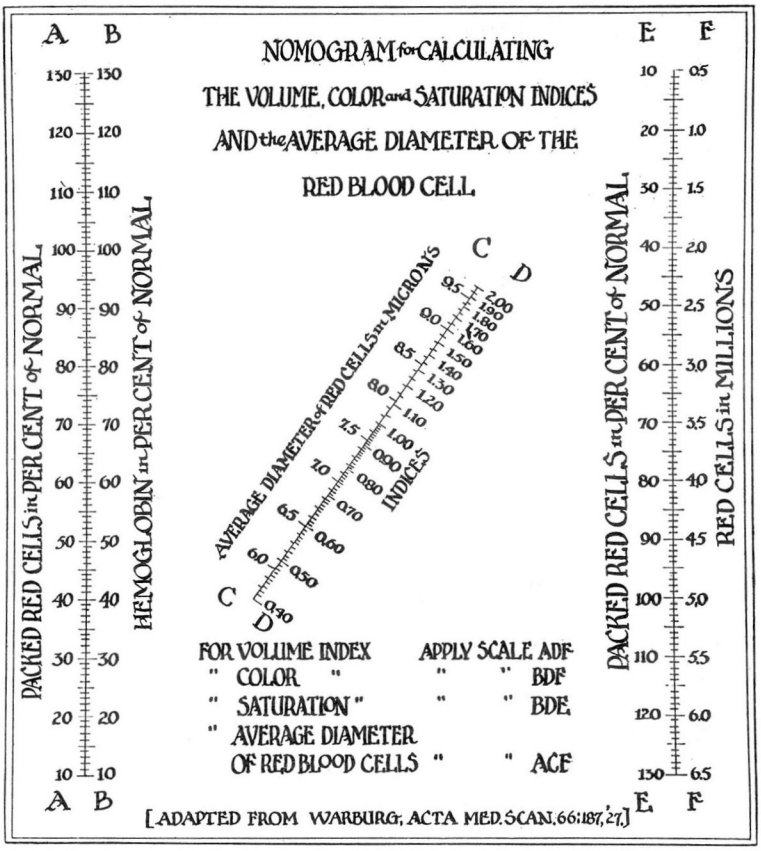

FIG. 3—Nomogram for calculating indices from red cell count, hemoglobin in per cent of normal and packed red cells in per cent of normal. The mean diameter of the red blood cells can also be calculated.

5. *White Corpuscle Count.* This is subject to fewer errors and greater variation than is the red cell count but should be done equally carefully.

6. *Preparation of Stained Film.* In many laboratories blood films are made only on slides. For the study of the morphology of the red cells, for reticulocyte counts and for examinations for parasites, such films are satisfactory. For an accurate differential count, for determining the relative number of platelets, and for studying the morphology of the white cells, films made on cover glasses are far preferable. The technic of a blood examination is certainly not mastered until one can make satisfactory cover glass preparations. These are easily made if certain precautions are observed. I find no difficulty in having the best of preparations made by efficient technicians.

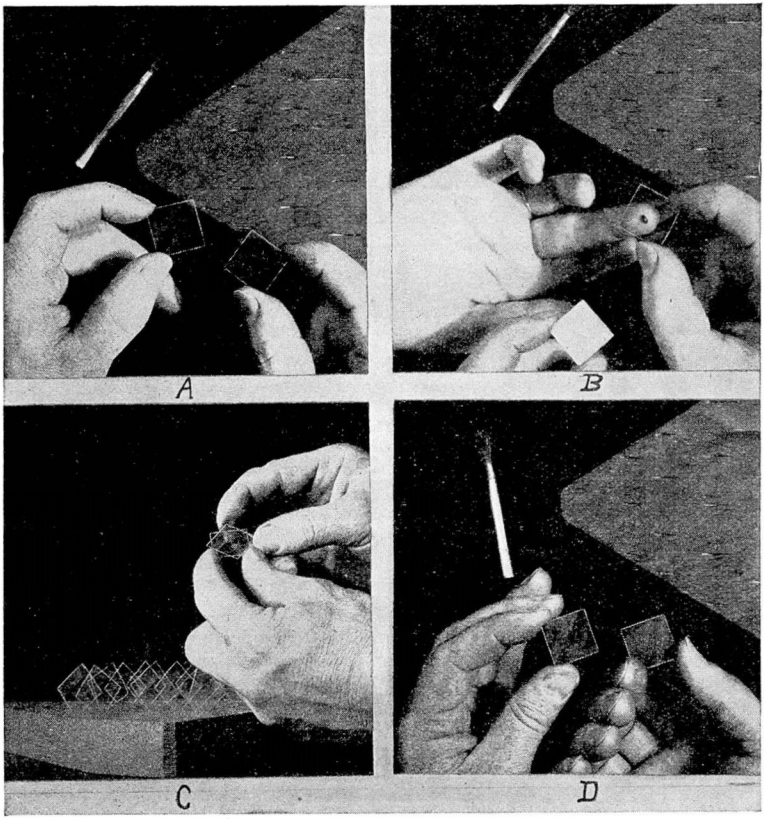

FIG. 4—The preparation of blood films by the cover glass method. A. a cover glass (⅞ inch square No. 2) is grasped at the adjacent corners with the thumb and forefinger of each hand; B. the drop of blood is touched with the cover glass held in the right hand; C. the cover glass carrying the drop of blood is quickly placed parallel on the cover glass held in the left hand; D. cover glasses are then drawn apart with a sliding motion, care being taken to keep them parallel. The films are allowed to dry in air and are then ready for staining. The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread. (From Haden—*Clinical Laboratory Methods.)*

The most satisfactory cover glasses are No. 2, ⅞ inch square of good manufacture. They must be absolutely clean and free from dust. Some cleaning solution such as a concentrated acid or bichromate acid mixture is often employed. The best method of cleaning is to scrub them with some grit-free scouring powder such as Dutch Cleanser. The hands are thoroughly washed, a number of cover glasses placed in the palm of one and the scouring powder and a small amount of water added. The glasses are then well scrubbed with the palm of the other hand, using a rotary motion. They are rinsed with distilled water, placed in alcohol, dried with a clean, lint-free cloth, and stored in boxes. Just before use they are brushed off with a camel's hair brush and placed on edge in a block of wood or in the top of a box.

We usually make a number of slits in the top of a 20 cc. syringe box and keep in the box an automatic lancet, the box of cleaned cover glasses, a camel's hair brush, cotton gauze and a small bottle of alcohol, thus providing everything needed for making blood films.

In making the films, a clean and dust-free cover glass is grasped at the adjacent corners with the thumb and forefinger of each hand and the drop of blood on the finger tip is touched with the cover glass held in the right hand (Fig. 4). The cover glass carrying the drop of blood is quickly pressed parallel to the cover glass held in the left hand. The blood spreads by capillary attraction. As the spread is completed, the cover glasses are drawn apart with a sliding motion, care being taken to keep them parallel. The films are allowed to dry in air and are then ready for staining. The finger is punctured with an automatic lancet, since the depth of the puncture wound can be regulated best in this manner. The drop of blood must be globoid on the finger tip and just large enough to cover the cover glass when properly spread.

Staining the Blood Film. The films are best stained on a small stand made by nailing a row of corks to a wood block (Fig. 5). Wright's stain

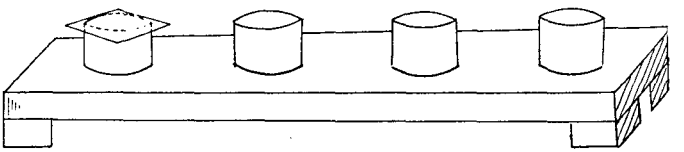

FIG. 5—Convenient stand for staining blood films made on cover glasses. (From Haden—*Clinical Laboratory Methods.)*

is the most satisfactory one for routine use. Only chemically pure, acetone-free methyl alcohol such as Merck's Blue Label should be employed in making the staining solution. Cover the blood film with about 10 drops of stain and after one minute add an equal number of drops of distilled water. Very often preparations made in this manner are too blue, due to an excess of alkali in the stain. The simplest way to correct an excess of alkalinity is by adding a phosphate buffer solution. The optimum amount of buffer solution to be added must be determined by trial. Usually the most satisfactory stains are made by adding 3 drops of a phosphate buffer solution with a PH equal to 6.4 and 8 to 10 drops of distilled water. If the staining solution is very alkaline, only the buffer solution is used. Let stand for four to five minutes. The phosphate buffer solution with PH equal to 6.4 is made as follows:

Primary potassium phosphate (KH_2PO_4)	6.63 gm.
Anhydrous secondary sodium phosphate ($Na_2H\ PO_4$)	2.56 gm.
Distilled water to make	1000.00 cc.

The stained films are mounted film side down in neutral gum damar solution. A rather thin solution of gum damar in chemically pure zylol is made, calcium carbonate is thoroughly mixed with it and the solution placed in the window in the sunlight for several weeks. After the calcium carbonate has completely settled out, the solution is poured off and placed in a warm place until it has evaporated to the proper consistency. The gum damar thus made is neutral, does not darken with age, and does not cause fading of the stain.

7. *Count of Reticulocytes.* The reticulocytes may be stained with brilliant cresyl blue in a number of different ways. Often a film of cresyl blue is prepared on cover glasses and the blood film made on this. We prefer the following technic: A drop of a saturated solution of brilliant cresyl blue in alcohol is placed on a porcelain drop plate (Fig. 6) and allowed to evaporate to dryness. One drop of the blood

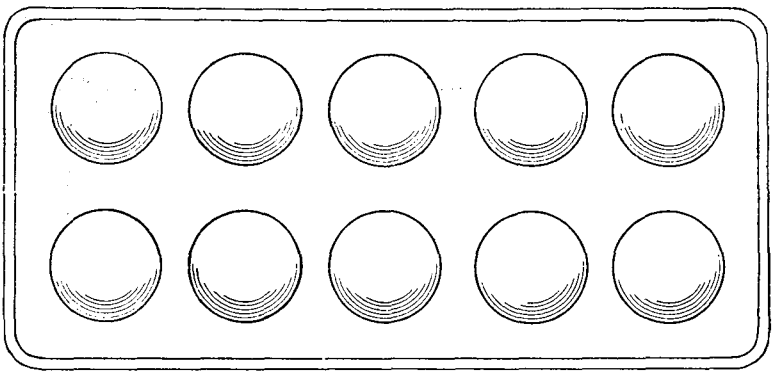

FIG. 6—Porcelain mixing plate for use in blood grouping. (From Gradwohl and Blaivas—*Blood and Urine Chemistry.)*

taken from the centrifuge tube prepared for the determination of the red cell volume is mixed with the stain. This is taken up with a pipette. Films are prepared on cover glasses and counterstained with Wright's stain.

If only a reticulocyte count is to be made, a drop of blood from the finger tip is taken up with a capillary, mixed with the dried stain in the drop plate, and blood films made from the mixture.

8. *Determinations of Bile Pigment Content of the Blood Plasma.* The bile pigments are easily and satisfactorily estimated as the icterus index. I use the method suggested by Murphy.[4] For the color comparison, a series of standards are prepared from various dilutions made from a 1:100 solution of potassium bichromate to correspond with varying icterus index figures as shown in Table I.

TABLE I

Dilution	*Corresponding Icterus Index*	*Dilution*	*Corresponding Icterus Index*
1:10,000	1	1:500	20
1: 5,000	2	1:400	25
1: 2,000	5	1:200	50
1: 1,000	10	1:133	75
1: 666	15	1:100	100

The solutions are kept in a rack in small test tubes 10 mm. in diameter (Fig. 7). One or two cubic centimeters of the supernatant

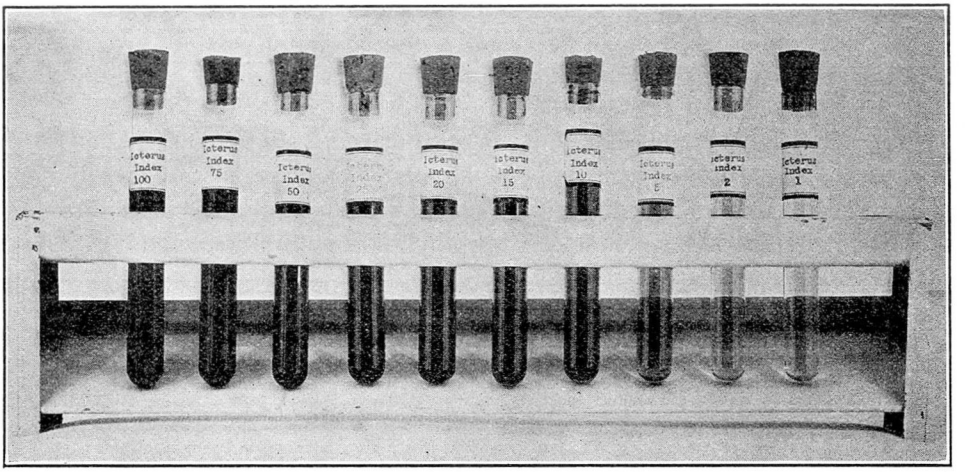

FIG. 7—Set of bichromate standards for estimating the icterus index.

plasma is pipetted from the centrifuge tube after spinning into a similar test tube and compared with the bichromate standards. The figure corresponding to the dilution which matches the serum is the icterus index of the serum. A correction is made for the dilution with oxalate. The normal icterus index is 4 to 6. To avoid clouding, blood should be taken when the patient is fasting. In preparing the dilutions of potassium bichromate, 2 drops of concentrated sulphuric acid should be added to each 500 cc. to prevent fading.

To Recapitulate. Twenty cubic centimeters of blood have been taken from the patient's vein and blood films have been made from the finger tip. Ten cubic centimeters of blood have been mixed with isotonic sodium oxalate. Before centrifuging, films for a count of the reticulocytes have been made from a drop of the oxalated blood. After centrifuging, the volume of red cells has been read off and the icterus index has been determined on the supernatant plasma. Red cell and white cell counts have been made on the specimen to which a

drop of potassium oxalate has been added. The blood film has been stained and examined and the indices have been calculated from the data obtained above. A complete examination has thus been made with a minimum expenditure of time and trouble. Tests other than those outlined above may be indicated. The more common one of definite value is a special study of white cells.

a. For Maturity: Numerous classifications to indicate the maturity of the polymorphonuclear cells, based on a study of the nucleus have been suggested (Arneth, Schilling, Cooke and Ponder, Pons and Krumbhaar). In my opinion the most satisfactory and practical classification is the separation of the polymorphonuclear neutrophiles into two groups, filamented and nonfilamented, as suggested by Farley, St. Clair and Reisinger.[5] Such counts can be made only on well prepared and properly stained blood films on cover glasses. One hundred polymorphonuclear neutrophiles are counted. Cells in which the lobes of the nucleus are connected only by a thin strand or filament of nuclear material are counted as filamented cells. If there is any band of nuclear material except this chromatin filament connecting different parts of the nucleus, such a cell is counted as nonfilamented (Fig. 8).

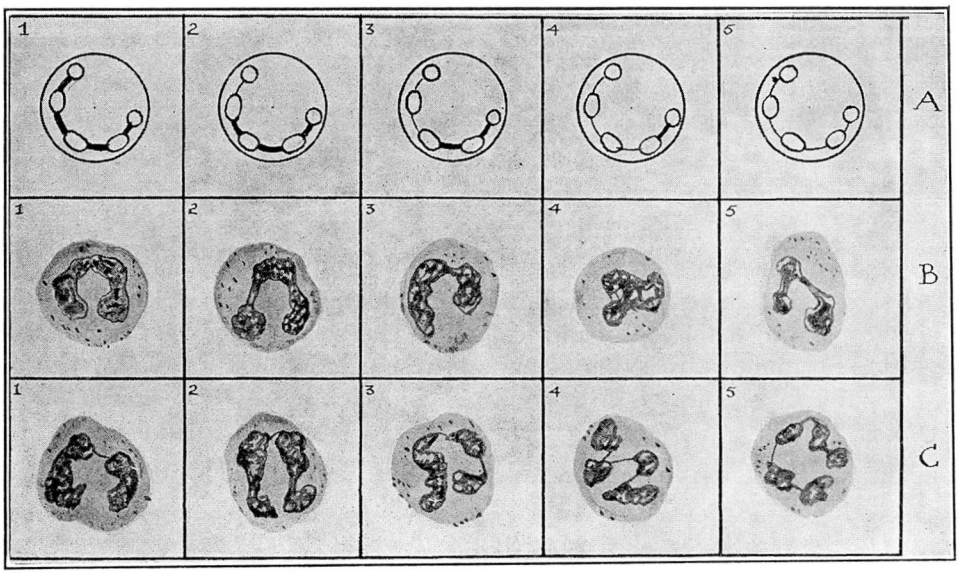

FIG. 8—A diagram to illustrate different types of nuclei in polymorphonuclear neutrophilic cells. 1. polymorphonuclear with nucleus of five lobes connected by thick bands of nuclear tissue. The nucleus shows five distinct masses but since the connecting threads are thick, the cell is designated nonfilamented. 2, 3, 4, 5, polymorphonuclears in which two or more lobes are connected only by a filament. These four cells are all designated "filamented" polymorphonuclears. B. 1, 2, 3, 4, 5, nonfilamented polymorphonuclears. In each cell the lobes of the nucleus are connected by thick threads. C, 1, 2, 3, 4, 5, filamented polymorphonuclears. In each cell two or more lobes are connected only by a filament of nuclear tissue. (Adapted from Cooke and Ponder's—*The Polynuclear Count.)*

If 100 polymorphonuclear cells are counted, not more than 25 per cent should be nonfilamented. If only 100 white cells of all types are counted, not over 16 per cent of the neutrophiles should be nonfilamented.

Any irregularity in size and staining reactions of the granules should should be noted, since such changes are a good index of the degree of toxicity and may be equally important as variations in maturing of the nucleus.

b. For Oxidase Content: This is of value in differentiating cells of the lymphocyte and bone marrow series. I think the Washburn method gives the best preparations.

Washburn's Method. 1. Thin smears should be made, allowed to dry and stained within three to four hours.

2. Flood the smear with 10 drops of solution No. 1 and allow to stand for one to one and one-half minutes.

SOLUTION No. 1

Benzidine base	0.3 gm.
Basic fuchsin	0.3 gm.
Sodium nitroprusside (sat. aq. sol.)	1.00 cc.
Ethyl alcohol (95 per cent)	100 cc.

Dissolve the benzidine and fuchsin in the alcohol in order named. Then add the nitroprusside solution. A slight precipitate may form at the bottom of the flask but does not interfere with the staining qualities. This solution will keep for eight to ten months.

3. Add 5 drops of solution No. 2 without pouring off No. 1 and allow to stand three to four minutes.

SOLUTION No. 2

Hydrogen peroxide	5 to 6 drops
Tap water	25 cc.

This solution will keep for about two days.

4. Wash thoroughly with tap water (one-half to one minute).

5. While still wet, flood with 95 per cent ethyl alcohol and allow to stand three to four minutes, or until completely decolorized (i.e., when there is no more pink visible to the naked eye).

6. Wash thoroughly with tap water and dry.

7. Flood with 8 drops of Wright's stain and allow to stand for two to three minutes.

8. Add 14 drops of tap water (one and one-half times as much water as Wright's stain) and allow to stand for twenty to forty-five minutes. Most normal and many abnormal bloods will stain well in twenty to twenty-five minutes but certain abnormal bloods, particularly the leukemic bloods, require thirty-five to forty minutes.

9. Wash briefly with tap water, flood with 95 per cent alcohol for three to five seconds and immediately wash with tap water for ten to fifteen seconds.

10. Dry and examine.

The peroxidase granules are black. The nuclei and the cytoplasm of the cells are identical with those seen in a Wright stain.

c. Jenner-Giemsa Stain for Special Study of Leukocytes: The films stained by Wright's method are satisfactory for most purposes. The Jenner-Giemsa stain brings out beautifully the finer details of nuclear and the other cell structures. In leukemia especially, such preparations are valuable. They are made as follows:

The cover glass preparation is covered with Jenner's stain for three minutes, and an equal number of drops of distilled water added. After one minute, the stain is washed off. The cover glass is then placed with the film down in a watch glass. The Giemsa stain (15 drops of the stock Giemsa solution to 10 cc. of distilled water) is run into the watch glass from the side and left for from ten to fifteen minutes. Wash, dry, and mount in neutral gum damar.

Special Examinations Indicated in Hemorrhagic Diseases and Other Conditions

1. *Platelet Count.* An excellent idea of the relative number of platelets may be gained from an examination of a properly made cover glass preparation. If the number seems diminished, a count should be done. The Rees-Ecker method is a very satisfactory one. A small amount of diluting fluid (sodium citrate, 3.8 grams, formalin, 0.2 cc., brilliant cresyl blue, 0.1 gram, distilled water, 100 cc.) is drawn into the bulb of the diluting pipette to moisten the capillary. The blood is then drawn up to the 0.5 mark and the bulb filled with the diluting fluid. The counting and calculation is done as for a red cell count.

2. *Determination of Fragility of Erythrocytes.* The method described for this by Giffin and Sanford[7] is a simple and satisfactory one (Fig. 9). Twelve Wassermann tubes are set up in a rack and num-

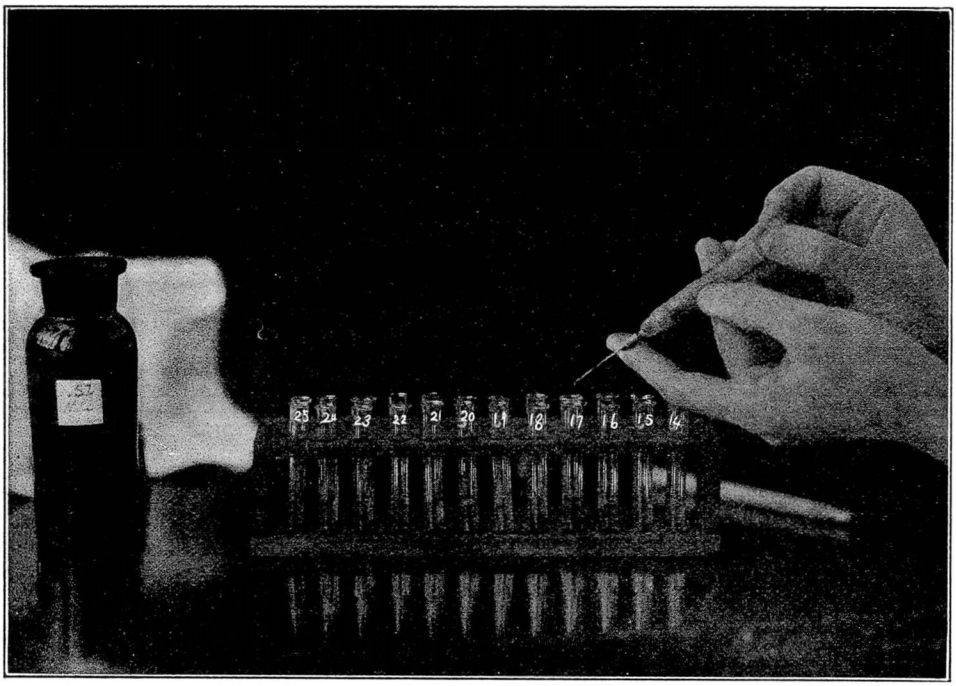

FIG. 9—Method for determination of fragility of red blood cells. One drop of whole blood is added to each tube of hypisotonic solution. (After Giffin and Sanford.)

bered 25 to 14 from left to right, with a capillary pipette run into each tube, the number of drops of an accurately made solution of 0.5 per cent sodium chloride being indicated by the figure on the tube. Distilled water is added by means of the same pipette to make the total number of drops of an accurately made solution of 0.5 per cent sodium chloride indicated by the figure on the tube. Distilled water is added with the same pipette to bring the total number of drops in each tube up to 25. Blood is withdrawn from a vein by means of a dry sterile syringe and one drop run into each tube. The tubes are allowed to stand at room temperature for one hour or more. The dilution in which there is just a slight tingeing of the supernatant fluid due to laking of a few of the least resistant corpuscles is noted as the point of initial hemolysis. Reading from left to right, complete hemolysis is indicated in the first tube in which no corpuscular residue is evident by shaking the tube.

The percentage of sodium chloride in any tube is calculated by multiplying the number on the tube by 0.02. Normal blood shows intense hemolysis in 0.42 or 0.38 per cent sodium chloride solution, and complete hemolysis in 0.36 to 0.32 per cent.

3. *Determination of Coagulation Time.* It is a waste of time to determine the coagulation time on a drop of blood obtained by piercing the skin. The method suggested by Lee and White[8] is a satisfactory one for clinical use. Blood is withdrawn from a vein with a syringe in which the space between the end of the plunger and the needle is filled with salt solution, and one cubic centimeter is run into each of 3 small Wassermann tubes 8 mm. in diameter. The tubes should be scrupulously clean and washed with salt solution just before use. After standing for three minutes, a tube is rotated endwise every thirty seconds, and that point at which the blood no longer flows from its position but maintains its surface contour when inverted is taken as the end point. Normal blood coagulates by this method in five to eight minutes.

4. *Bleeding Time.* This is easily and quickly done by the method of Duke.[9]

A small incision is made in the finger or in the lobe of the ear and at half minute intervals the blood is blotted up with smooth filter paper. The cut should be such that the diameter of the first blot is about 2 cm. without any squeezing. Each blot represents the flow of blood in a half minute. The bleeding time is the total duration of the hemorrhage and varies from 1 to 3 minutes in normal individuals.

5. *Clot Retraction.* A few cubic centimeters of blood are run into a Wassermann tube and allowed to stand at room temperature without being disturbed. Normally the clot retracts and expresses blood serum in one hour. If there is a deficiency in platelets, the clot does not retract.

The following form is a convenient one on which to report the results of the examination.

BLOOD REPORT

Name: Date:
Case No: Service:

1. RED BLOOD CELLS:
   1. Number per c.mm.—
   2. Size in stained preparation—
   3. Shape in stained preparation—
   4. Color in stained preparation—
   5. Regeneration forms:
      a. Nucleated red cells—
      b. Basophilia: punctate or diffuse—
      c. Nuclear particles—
   6. Fragility: hemolysis begins in % : complete in % sodium chloride (normal)
   7. Reticulocyte count—
2. VOLUME OF PACKED RED BLOOD CELLS % of normal (cc. per 100 cc.)
3. VOLUME INDEX (Mean corpuscular volume = cubic microns)
4. HEMOGLOBIN % of normal (gm. per 100 cc. with hemoglobinometer)
5. COLOR INDEX (Mean corpuscular hemoglobin = micromicrograms)
6. SATURATION INDEX (Mean corpuscular hemoglobin concentration = per cent)
7. WHITE BLOOD CELLS:
   1. Number per c.mm.—
   2. Differential count:
      Neutrophiles— % Eosinophiles— % Basophiles— %
      Lymphocytes— % Monocytes— %
      Nonfilamented neutrophiles— % (normal 6-16%)
   3. Presence of abnormal forms:
      (a) Myelocytes— (c) Lymphoblasts
      (b) Myeloblasts— (d) Fragile leukocytes
      (e) Toxic neutrophiles
8. BILE PIGMENTS IN PLASMA:
   (a) Icterus index (Normal 0.5 to 2 units)
   (b) Units (van den Bergh) per 100 cc. (Normal 4 to 6)
9. PLATELETS per c.mm.
10. COAGULATION TIME (method)
11. REMARKS
12. LABORATORY DIAGNOSIS:

Name of Examiner.....

RUSSELL L. HADEN

The Laboratory Classification of Anemia on the Basis of Volume and Hemoglobin Content of the Mean Red Cell

The laboratory classification of anemia has always been unsatisfactory. A rough differentiation into primary and secondary types is very frequently used. The anemias having a color index greater than 1.00 are usually classified as primary and those having a color index of 1.00 or less as secondary. Hampson and Shackle[10] first suggested the classification of anemias on the basis of cell size, using the terms "megalocytic" and "nonmegalocytic." Wintrobe[11] suggested four groups: (1) macrocytic, (2) normocytic, (3) simple microcytic, and (4) hypochromic. The most logical laboratory classification is based on all three variants of the erythrocyte, namely, number, size, and hemoglobin content. The following terms may well be employed to indicate variations which have been observed in these factors.

Number
- Hypercythemic = red cell count > normal
- Normocythemic = red cell count within normal limits
- Hypocythemic = red cell count < normal

Volume

Macrocytic = mean corpuscular volume > normal (VI > 1.10)
Normocytic = mean corpuscular volume = normal (VI = 0.90-1.10)
Microcytic = mean corpuscular volume < normal (VI < 0.90)

Hemoglobin Content

Hyperchromic = Mean corpuscular hemoglobin > normal (C I > 1.10)
Normochromic = Mean corpuscular hemoglobin = nomal (C I = 0.90-1.10)
Hypochromic = Mean corpuscular hemoglobin < normal (C I < 0.90)

All the different types of anemia which may occur from this standpoint are:

- Normocythemic
  - Normocytic and hypochromic
  - Microcytic and hypochromic
- Hypercythemic
  - Normocytic and hypochromic
  - Microcytic and hypochromic
- Hypocythemic
  - Macrocytic and hyperchromic
  - Macrocytic and normochromic
  - Macrocytic and hypochromic
  - Normocytic and normochromic
  - Normocytic and hypochromic
  - Microcytic and hypochromic

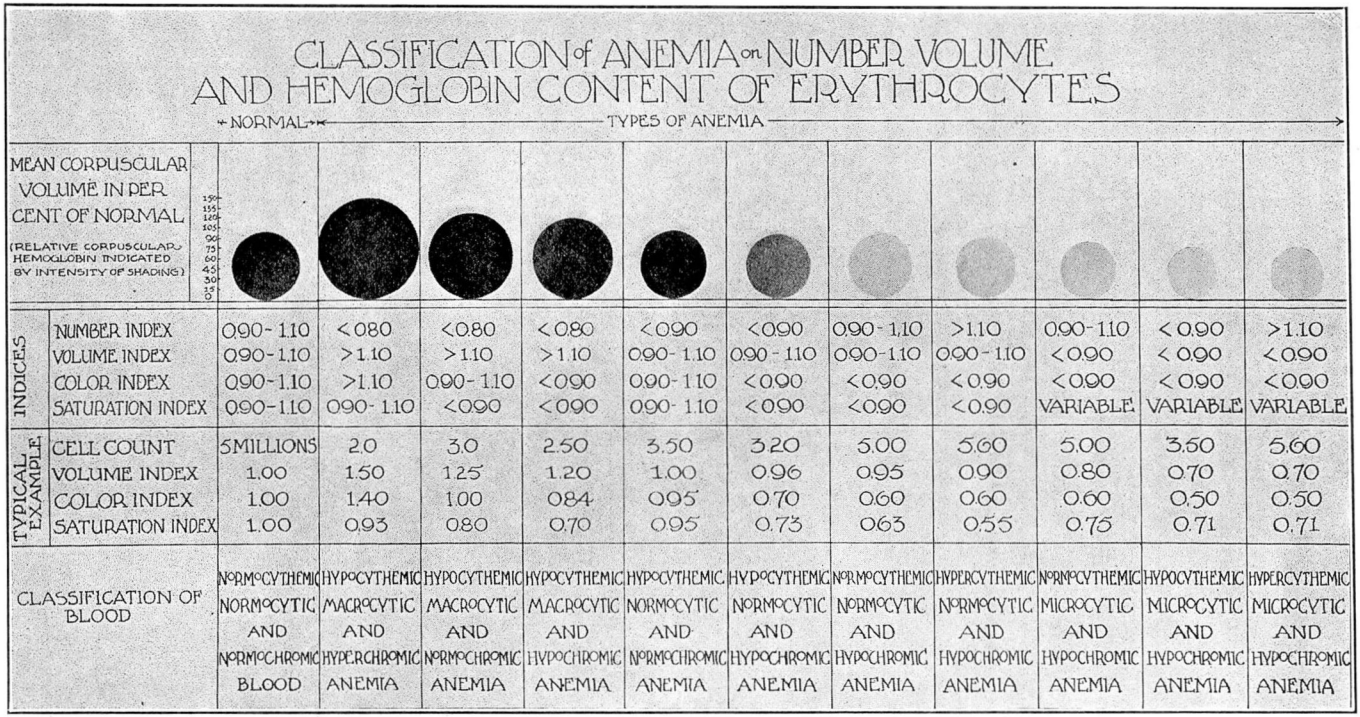

FIG. 10—Showing classification of anemias on number, volume and hemoglobin content of red blood cells.

These types of anemia are illustrated in Fig. 10. The circles indicate relative volume, not diameter, and the intensity of color indicates the relative hemoglobin content. A typical example of each type of anemia is given. Every anemia should be thought of in terms of number, volume and hemoglobin content of the average erythrocyte, and every case should be classified on such criteria. An anemia with a red cell count of 3.50 millions, a volume index of 0.75 and a color index of 0.65 is reported as a hypocythemic, microcytic and hypochromic anemia rather than simply as "secondary" anemia. Likewise an anemia with a count of 2 millions and a volume and color index of 1.50 is recorded as hypocythemic, macrocytic and hyperchromic rather than "primary."

Summary

I have tried to emphasize the need for an accurate and complete examination of the blood in studying hemotologic problems. Any clinician who has had the opportunity of utilizing such an examination will never be satisfied with any other kind. A complete blood study may be made quickly and simply in a well equipped laboratory.

I have indicated one satisfactory technic for each of the tests suggested, although others may be equally satisfactory. One good method should be used until it is thoroughly mastered.

Only with such laboratory data can an accurate knowledge of the blood dyscrasias be gained.

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