THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Elmer George Senty final oral examination for the degree of Master of Science in Medicine.

We recommend that the degree of Master of Science in Medicine be conferred upon the candidate.

O. M. Berkman
Chairman

L. G. Rowntree

A. H. Sanford

Date_____

10-20 5M

Graduate School, University of Minnesota.

Date: Nov. 14, 1922

This is to certify that Elmer George Senty, a candidate for the degree of Master of Science in Medicine, has passed the final written examination for the major in the Department of Medicine.

L. G. Rowntree

For the Major Department.

Graduate School, University of Minnesota.

Date: Nov. 14, 1922

This is to certify that Elmer George Senty, a candidate for the degree of Master of Science in Medicine, has completed the requirements for the minor in the Department of Clinical Pathology.

A. W. Sanford

For the Minor Department.

REPORT

OF

COMMITTEE ON THESIS

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by George Elmer Senty, for the degree of Master of Science in Medicine. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

D M Berkman
Chairman.

A E Bradford

L G Rountree

E P Lyon

10-20 SM

THESIS

A COMPARATIVE STUDY OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATION.

Elmer George Senty, B.S.,M.D.

Submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

October, 1922.

INTRODUCTION

Hemoglobin is the oxygen carrying constituent of the blood. It is a complex proteid, and on decomposition breaks up into globulin (96 per cent) and a simpler pigment, hematin, (4 per cent).[10] The iron content is about .334 per cent, or 1 mg. per 300 mg. of hemoglobin. The estimation of hemoglobin is a very important procedure in clinical diagnostic methods. It is of value in determining whether patients with pallor are really anemic. Then too, the hemoglobin content of the blood is a necessary factor in the determination of the color index, which is of significance in diseases such as pernicious anemia and chlorosis. The normal hemoglobin in adults is 13 to 14 gm. per 100 c.c. of blood which is considered 100 per cent. The hemoglobin in infants during the first few days of life is very high, usually about 130 to 135 per cent; in young children 75 to 80 per cent; in adult life 95 to 100 per cent, while the percentage is usually lower than 100 per cent after the age of 65 years. Haldane and Smith state that the depth of color in the blood is directly proportional to the oxygen capacity, in other words the percentage of hemoglobin present. In the estimation of hemoglobin, various methods have been employed, aiming at accuracy and ease of determination. The history of the development of existing methods is of much interest.

HISTORICAL

In 1878 Gowers introduced his hemoglobinometer. This instrument consists of two tubes about 11 by 0.8 cm. One tube, closed on both ends, contains the standard solution, 2 c.c. of gelatine tinted with picrocarmin. The color of this standard corresponds as closely as possible to a 1 per cent hemoglobin solution. The other tube is graduated so that the level attained by 2 c.c. of fluid will represent 100 per cent of hemoglobin. This tube is graduated to read hemoglobin percentage from 10 to 140 per cent. In making a hemoglobin

estimation, 20 cmm.of blood are obtained in a graduated pipette and blown into the graduated tube which contains a small amount of water. This is then thoroughly mixed and the pipette washed by frequently drawing the solution into the pipette. Water is slowly added, drop by drop, and the solution intimately mixed, until the colors in both tubes are similar. The percentage of hemoglobin present is then read at the height of the solution in the graduated tube.

This instrument is very simple, handy and inexpensive. It is, however, only fairly accurate; the picrocarmin solution fades and the same standard tube cannot be used with artificial light. Then too, the standard solution is not made of blood and there is consequently some difficulty in the accurate matching of the solutions.

Fleischl, in 1885, devised a new hemoglobinometer which has since been much improved by Miescher, and is now known as the Fleischl-Miescher instrument. It consists of a stage similar to that of a microscope. In the center is placed a cylindrical chamber, divided into two equal parts and having a glass bottom. One-half of the chamber is filled with water, the other with a 1 to 200 dilution of blood with a 1 per cent solution of sodium carbonate. A purple stained glass wedge, or color prism, with the hemoglobin percentage values marked along the edge, slides beneath the chamber, and is moved from side to side by a screw. Light from a candle is reflected by means of a plaster-of-Paris reflector and illuminates the chamber from below. The glass wedge beneath the chamber is then moved back and forth until the color of the portion of the wedge beneath the chamber containing the water is similar to that of the blood solution in the opposite chamber. The position of the wedge is then read through a small window at the back of the stage and represents the percentage of hemoglobin present. Each instrument is supplied with a scale which states the milligrams of hemoglobin per liter of blood according to the readings made by that particular hemoglobinometer. The dilution of blood being known, it is then

possible to determine the amount of hemoglobin present in gm. per 100 c.c.

On account of the accuracy of this instrument, it has always been popular in experimental work. It is, however, quite cumbersome as well as expensive. It must be used in a dark room with a yellow light; this together with the time required and the technical difficulties makes it impractical for general clinical use.

In the year 1892 Hoppe-Seyler described a method of determining the percentage of hemoglobin, whereby the blood to be examined was saturated with carbon monoxid. This solution was compared with a known carbon monoxid standard solution. This method has proven too complicated for general clinical use.

Haldane and Smith in 1898 suggested a new method of estimating the hemoglobin content of the blood. They noticed that on the addition of potassium ferricyanid to a moderately diluted solution of blood, gas bubbles arose, which on examination were found to be pure oxygen. Working on this information, they devised an instrument for liberating and measuring the oxygen from the blood, and determined the oxygen capacity of normal adult male blood to be 18.5 volumes per cent, which was taken as an equivalent of 100 per cent hemoglobin; that of women 16.5 volumes per cent, and children 16.1 volumes per cent. Since this original work was done the method has been technically much improved by Haldane, Bancroft, Van Slyke and others. This method, while not practical for routine hemoglobin determinations, is nevertheless one of the most accurate. It is of inestimable value in the preparation of standard hemoglobin solutions.

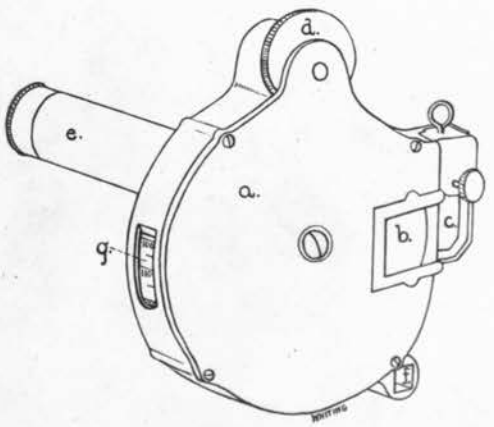
Tallqvist's simple and unique hemoglobinometer was introduced in 1900. This instrument consists of a booklet of prepared white filter paper and a lithographed color scale representing the hemoglobin content of blood from 10 to 100 per cent. The tints of the standard are prepared with water

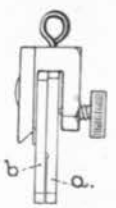
colors, and represent the color produced by undiluted blood soaked in filter paper, the hemoglobin values having been determined by the Fleischl -Meischer instrument. A rather large sized drop of blood is touched to a piece of the filter paper and allowed to distribute itself over a portion of the paper. As soon as the stain has lost its humid gloss, and before any coagulation can take place, this stained filter paper is laid beneath the circular openings of the color scale and at the point where the colors match, the percentage of hemoglobin is read. Because the colors of the scale vary by 10 per cent, the approximate value only can be obtained, and this reading may vary if the color is matched too soon or after coagulation has begun. This method cannot be recommended for routine clinical use where accuracy is demanded.

During the same year (1900) Dr. Arthur Dare, of Philadelphia, devised a new instrument for hemoglobin estimation. The use of this instrument is based on the premise that the color of a thin film of undiluted blood illuminated by artificial light can be compared with a graduated color comparison standard. This standard is a color prism made of ruby glass, the 100 per cent reading represents 13.7 gm. of hemoglobin per 100 c.c. of blood. Hemoglobin estimations can be made from 10 to 120 per cent.

The instrument Fig. 1 consists of a case, (a) enclosing the color prism comparison standard, an aperture in the case (b) allowing light to the color prism, a pipette (c) which is made of two thin pieces of glass, one white and one clear, with a surface of definite thickness (about 0.18 mm.) between. A small screw (d) is used to move the color prism, while the telescope (e) makes focusing and magnification possible. The opening (f) is made for the attachment of the candle or the electric light equipment which is now part of the newer instrument.

For the purpose of color comparison blood is drawn by capillary





Reduce To 4"

Fig. 1 Dare hemoglobinometer.

Fig. 2 Capillary pipette of Dare hemoglobinometer.

attraction into the small thin chamber of the pipette (Fig. 2) which has a sufficient surface for color field and illumination by artificial light. Between the film of blood and the source of illumination, a white glass (Fig.2-a) interposes to diffuse the direct rays of light. Against this white background the shades of color are observed to the greatest advantage. The pipette is slipped into the instrument (Fig. 1-c), candle or artificial light is used, and the prism is rotated until the colors match, when the hemoglobin percentage is read through the notch (g) at the edge of the case.

As suggested by Dare the advantages of his instrument are:

1. The technic and dilution errors are eliminated. Turbidity due to leucocytosis, becomes almost imperceptible with transmitted light against an opaque background.

2. The shades of color are very decided even though the hemoglobin content is low.

3. The time element is very short.

4. The capillary pipettes are standardized, and are consequently interchangeable.

This instrument is very simple and convenient. It is, however, quite expensive and must be handled carefully.

Haldane described a revised method of hemoglobin determination in 1901. He used the Hoppe-Seyler principle with the Gowers' hemoglobinometer and claimed very satisfactory results. The standard solution used is a 1 per cent solution of ox or sheep's blood saturated with carbon monoxid and having an oxygen capacity of 18.5 volumes per cent. This value of 18.5 volumes per cent oxygen capacity has been quite generally accepted and represents 100 per cent hemoglobin.

In using this method 20 cmm. of blood are delivered to the graduated tube containing as much water as safely possible. Before mixing, a narrow

glass tube, connected with a gas tap, is pushed down almost to the level of the liquid. The gas is turned on and displaces the air in the tube. The glass tube is then slowly withdrawn, the gas still flowing, and the top of the graduated tube quickly closed with the finger. The solution is thoroughly mixed until the pink tint of carbon monoxid hemoglobin appears. Water is then slowly added, drop by drop, from a pipette until the tints in both tubes are similar. The percentage is then read at the height of the fluid in the graduate tube. Another drop or two is added to produce a change in color and the mean of the readings is accepted as the correct percentage of hemoglobin present. The tints are best compared against sunlight or an opal glass light if artificial light is desired. This method is fairly accurate but,as stated by Palmer, the standard is not permanent.

Perhaps the most simple and popular hemoglobinometer in use today is that described by Sahli in 1902. He, for the first time, used an acid hematin standard. The instrument is similar to that of Gowers with the exception that the standard is a 1 per cent solution of acid hematin instead of a picrocarmin solution corresponding to a 1 per cent solution of blood. The blood to be tested is likewise changed into acid hematin. In determining the percentage of hemoglobin 20 cm. of blood are obtained in the Sahli pipette and transferred to the graduated tube which contains 0.1 N HCl. up to the 10 per cent graduation. The tube is then well shaken, and as soon as the clear dark brown color, which is due to the formation of acid hematin, appears, distilled water is slowly added and the contents carefully and thoroughly shaken until the color of the mixture corresponds with that of the standard solution. At this point, the percentage of hemoglobin is read and corresponds to the level of the solution in the graduated tube.

This instrument is supplied with a ground glass background which

diffuses the light before it reaches the tube, thereby excluding all disturbing reflections. It can be used with artificial light and in a lighted room. It is self-evident that the color comparisons will be more accurate if the standard and tested solutions are of similar composition. For this reason the Sahli instrument is superior to Gowers', or other methods using foreign standard solution or colored glass as standards. Sahli tubes purchased on the market today fade so markedly as to make them unfit for use other than comparison unless frequently checked and restandardized. In the Sahli method of hemoglobin estimation the 100 per cent represents 17.2 gm. per 100 c.c. and the determinations must consequently be reduced if 13.4 gm. per 100 c.c.is to be considered as standard. Robscheit states that the new tubes as purchased today show much fading varying from 5 to 20 per cent. Jacobson has suggested a new standard for the Sahli hemoglobinometer, "rufigallic acid". He makes this solution by adding 5 to 10 drops of concentrated sulphuric acid to 100 c.c of a 20 per cent aqueous solution of tannic acid or a 1 per cent solution of gallic acid. This solution is heated for one minute and a dark brown colored mixture results. The dilution desired can then be made and corresponds very closely to an acid hematin solution when viewed in a Duboscq colorimeter. Jacobson claims that a 20 per cent solution has remained in the sunlight for ten months with no apparent change of color noticeable.

In 1916 Haessler and Newcomer devised another acid hematin hemoglobinometer adopting the principle of Sahli's instrument, but using eleven standard tubes arranged in a rack instead of but one. The readings on these tubes vary by 10, and represent hemoglobin readings from 10 to 110 per cent. The standard fluids are made up according to Sahli's specifications. The tube marked 100 per cent contains an equivalent in acid hematin of 17.2 gm. of hemoglobin in 10,000 c.c.

The patient's blood is obtained in an ordinary red blood

pipette, a 1 to 100 dilution is made with 0.1 N HCl. and the solution compared with the standard tubes in the rack. The tube containing the blood to be tested, or the comparison tube, is inserted between the different tubes of the standard and moved one way or the other on the rack until the shading becomes harmonious, or as nearly so as possible. The hemoglobin in per cent is then read or estimated.

As the readings on the instrument vary by 10 per cent, the values are obviously only approximate. For this reason the percentages obtained are of value only as a comparison.

The method of determining hemoglobin described by Palmer in 1918 is rapidly becoming popular, and is recognized as a standard method today. It is a comparison, by means of a colorimeter of carbon monoxid hemoglobin solutions, one having a known hemoglobin content. The principle is similar to that described by Hoppe-Seyler in 1892. The standard solution is prepared as follows:

A quantity of ox or human blood is obtained and defibrinated. The oxygen capacity is determined according to the method suggested by Van Slyke. The blood is then diluted with 0.4 per cent ammonium hydroxid solution so as to make a 20 per cent solution having an oxygen capacity of 18.5 volumes per cent. This solution is saturated with carbon monoxid by bubbling through it ordinary illuminating gas for 10 minutes. It is immediately stoppered, preferably with a glass stopper. This standard 20 per cent solution should then be sealed and kept in the dark on ice. When used for hemoglobin estimation a 1 per cent solution is made. Five cubic centimeters of this 20 per cent solution are diluted to 100 c.c. with 0.4 per cent ammonium hydroxid solution and saturated with carbon monoxid.

Method:

A 1 per cent solution of patient's blood is made by transferring

.05 c.c. of blood into 5 c.c. of 0.4 per cent ammonium hydroxid solution. The blood pipette is rinsed out by drawing the ammonia solution into it three or four times. Illuminating gas is then bubbled through the solution for thirty seconds. This solution is compared in a Duboscq colorimeter with the standard 1 per cent solution set at 10. The average of several readings is taken. The calculation is $\frac{10}{R} \times 100 =$ per cent of hemoglobin. The color match is excellent.

Considerable difficulty is met with in obtaining standards which will remain constant. Robscheit records changes in color value of Palmer Standards of 10 to 20 per cent in a period of from seven to twelve months. If standards are prepared once a month, however, very accurate readings are obtained.[15] Further difficulties are met with since it is not always possible to obtain illuminating gas.

In 1919 Newcomer presented his new method of determining hemoglobin by the use of a carefully prepared "high transmission yellow" semaphore glass disc. This instrument is based on the spectro-photometric properties of acid hematin. These thin glass discs of known thickness (about 1 mm.) and known hemoglobin values are used in place of the standard solution in the colorimeter. The disc is placed in the light path of one of the cups of the colorimeter (at the top of the plunger) and the corresponding cup is filled with water. In the other cup the solution to be read is poured. This consists of blood diluted with 0.1 N HCl. Five cubic centimeters of 0.1 N HCl are measured with a pipette into the colorimeter cup or a small tube. Twenty centimeters of blood are emptied and rinsed into this solution. The height of the color, produced by the acid hematin formed, is noticed in about 40 minutes. The colors are then matched and the reading on the colorimeter noted. A scale accompanies the disc, and the hemoglobin percentage or the grams per 100 c.c. is obtained by dividing the reading on the colorimeter into the appropriate figure in the scale. This scale is corrected both for time factor before matching and for the thickness of the standard disc used. The estimations of hemoglobin

are so satisfactory with this instrument that these "discs" are now made a part of the regular equipment of the Bausch and Lomb Colorimeter of the Duboscq type. The new Bock and Benedict colorimeter is also well adapted for use with the Newcomer disc.

In 1919 Cohen and Smith described a practical method of accurately determining hemoglobin. Like Palmer they use a colorimeter but employ an acid hematin standard. The authors state that this method was devised for field use and has proven very satisfactory in the United States Army.

Preparation of Standard Solution:

About 50 c.c. of blood (ox or human) are obtained and defibrinated. The oxygen capacity is determined by the Van Slyke method. The blood is then diluted with 0.1 N HCl to make a 20 per cent stock solution with an oxygen capacity of 18.5 per cent. Such a standard will contain approximately 14 gm. of hemoglobin per 100 c.c. This solution is treated with a few drops of chloroform to prevent mold formation, and is then stored in an ice box in a dark, glass stoppered bottle. The comparison standard to be used in the colorimeter is a 0.5 per cent solution and is prepared by diluting 2.5 c.c. of stock standard solution with HCl. 0.1 N up to 100 c.c.

Method:

By means of a calibrated Sahli pipette .02 c.c. of blood is obtained and added to 6 c.c. of 0.1 N HCl solution. The pipette is carefully washed out several times by drawing the solution into it. This solution is then poured into one of the cups of the colorimeter and compared with the .5 per cent comparison standard. The average of several readings is taken and accepted as correct.

With the standard solution a 0.5 per cent blood solution, and the Duboscq colorimeter set at 10, the calculation will be as follows:

$\frac{1.5 \times 10 \times 100}{R}$ = the percentage of hemoglobin.

Cohen and Smith state that the stock standard solution will remain stable for at least three months. They find that accurate results are obtained by comparatively unskilled workers. The results are very accurate and the color match is good. Care must be used to eliminate dilution errors.

A method similar to that of Newcomer's was described in the literature by Dreyer, Hazett and Pierce in 1920. Dreyer states that for ten years he has used a hemolyzed solution of blood and compared it in a Dubosoq colorimeter, using a colored glass as a standard. In making such determinations a 1 to 200 dilution of blood is made by diluting 0.1 c.c. of blood with 19.9 c.c. of normal saline. This solution is intimately mixed and may be stored in the ice box until it is convenient to make the reading. At such time, it is hemolyzed by using a very minute amount of saponin. This can be brought about in a warm room by gently rotating the solution, but preferably by heating for thirty minutes in water bath at about 30°c. The hemolyzed solution is then poured into one of the cups of the colorimeter and matched against the pink glass standard. Artificial light is used, preferably one rich in yellow rays. The results are more constant if the readings are made in a dark room and when the reflector only is illuminated.

The authors emphasized the variations in hemoglobin readings taken at different hours of the day, and feel that the variations are the smallest from 5:00 to 7:00 P.M.

The hemoglobin estimations made with the Cohen and Smith, Palmer, Newcomer and Dreyer methods, using a Dubosoq colorimeter, are very similar and very accurate.

A COMPARISON OF VARIOUS METHODS OF HEMOGLOBIN DETERMINATION.

The Dare instrument is used at the Mayo Clinic, and because the determinations were so low in apparently normal individuals it was thought advisable to check the method as well as to determine the value of other methods for general use. No satisfactory investigations relative to the value of the Dare hemoglobinometer were found in the literature.

In this experimental work, the Newcomer disc, the acid hematin method as described by Cohen and Smith, the Tallqvist hemoglobinometer, and two Dare instruments, a regular Dare hemoglobinometer spoken of as the "Standard" Dare and a "Special" * Dare hemoglobinometer were used.

Patients of the Clinic sent to the hematology laboratory for hemoglobin determination, as well as normal, healthy Clinic workers were used exclusively in this investigation.

Newcomer Disc Method:

The study with this method proved very satisfactory. The procedure is simple and the determination can be done quite quickly. One disc, 1.0 mm. in thickness, was used. There was some difficulty at times in matching colors, especially when low hemoglobin readings were made. Light from a northern exposure was used together with an electric light with Corning "Daylight glass". It was thought that the electric light with the "Daylight glass" was superior to the daylight as it was always constant. In Robscheit's work it is noticed that the Palmer method was compared with the Newcomer disc and that the readings were very similar (Table IX, Fig. 3). The Palmer method is considered excellent and these results tend to substantiate Newcomer's contention as to the accuracy of his method. In addition Robscheit compared the Palmer

*Because of the findings in curve I, Dr. A.H. Sanford, of the Mayo Clinic, had a "Special" Dare hemoglobinometer made by the Ricker Instrument Company, of Philadelphia, in which the glass prism comparison standard was ground 15 per cent thinner at the upper end of the scale than that of the "Standard" Dare.

TABLE IX.

Palmer's method.	Newcomer's method (glass 0.96 mm. thick).	Difference.
per cent	*per cent*	
113	114	+1
116	114	−2
99	100	+1
107	108	+1
98	101	+3
117	118	+1
100	100	0
100	100	0
100	101	+1
100	103	+3
100	99.2	−0.8
Average difference		+0.73

Fig. 3

method with the Van Slyke oxygen capacity method and found that the hemoglobin determinations were almost identical (Table IV, Fig. 4). The fact that the Palmer, Van Slyke and Newcomer disc readings were so similar tends to prove that the Newcomer disc method is dependable. In general it can be said that with a "disc" of satisfactory thickness, the Newcomer method is very accurate and is of immense practical value, as it does away with the preparation of standard solutions.

TABLE III.

Hemoglobin.

Palmer's method.	Author's modification.
per cent	*per cent*
75	74
70	70
77	76
77	77
74	73
73	73
74	74
75	74
76	75
73	73
64	64
63	63
76	76
45	45
60	60
84	85
69	69

Fig. 4.

TABLE IV.

Van Slyke's oxygen capacity method.	Palmer's method.	Author's modification.
per cent	*per cent*	*per cent*
113	113	114
117	118	117
99	98	99
124	124	124
109	108	109

Cohen and Smith Method:

In determining the hemoglobin by this method, 20 cmm. of the blood to be examined are diluted with 6 c.c. of 0.1 N HCl. Early in this work it was found that the plunger of colorimeter was frequently out of the acid hematin solution when low readings were made and only 6 c.c. of 0.1 N HCl. were used. For this reason 40 cmm. of blood and 12 c.c. of 0.1 N HCl. were always used thereafter, and no further difficulties were encountered.

Meyer and Butterfield, Krogh, Newcomer and others claim that a maximum color depth is slow to appear in the acid hematin methods, so much so that the readings vary from 2 to 20 per cent if the estimations are made too soon. Newcomer says that the readings can safely be made at the end of forty minutes. For this reason, all readings were made in from six to twelve hours in the methods involving acid hematin solutions. Berman maintains that this difficulty can be overcome by boiling the acid hematin solution for one minute, allowing it to cool for a minute, and then making the estimation. Considerable criticism has arisen relative to acid hematin methods on account of the unstable properties of acid hematin standards. Using defibrinated blood and following the technic suggested by Robscheit, or Cohen and Smith, standards are obtained which remain uniform from four to seven months. If the 0.5 per cent standard is made up every two to three weeks and kept on ice, the readings should be very accurate. In this investigation fresh 0.5 per cent standard solutions were made up weekly and the 20 per cent stock standard solutions every three months. The Duboscq colorimeter was used exclusively. Robscheit also compared her acid hematin method with the Palmer method and obtained very similar readings. (Table III, Fig. 4)

Tallqvist Hemoglobinometer.

This instrument was used more out of curiosity than to determine the real value of the method. Care was taken and directions carefully followed

and curious results were obtained. While the instrument is not accurate, the readings are at least approximate. As stated by Barker, "If it does nothing more than lead the practitioner to realize the necessity of seeing the color of drawn blood as it appears on filter paper, or on a towel, and to recognize the the fallacy of trusting to the appearance of visible mucous membranes, it is, in so far, praiseworthy."

RESULTS OF THE VARIOUS METHODS OF HEMOGLOBIN DETERMINATION USED.

In the first study represented by Curve 1, 199 patients were examined. The hemoglobin values obtained on the Dare instrument were checked against those obtained by the acid hematin method as described by Cohen and Smith. As a result of these findings further investigations were carried out, and ninety-one additional patients were examined. In this work, the Cohen and Smith method is considered the standard, and in the estimation of the accuracy of other hemoglobinometers used, the hemoglobin percentages obtained by these instruments are compared with those obtained by the Cohen and Smith method. The individual readings representing the percentage of hemoglobin calculated in each method are brought out by the following curves:

Curve I

The "Standard" Dare readings and those obtained by the Cohen and Smith acid hematin method are charted, and it is noticed that except in isolated cases:

Readings with both methods check up to about 65 per cent.

Above 65 per cent, and especially above 70 per cent, the calculations on the "Standard" Dare as compared with those obtained by the Cohen and Smith method show marked variations.

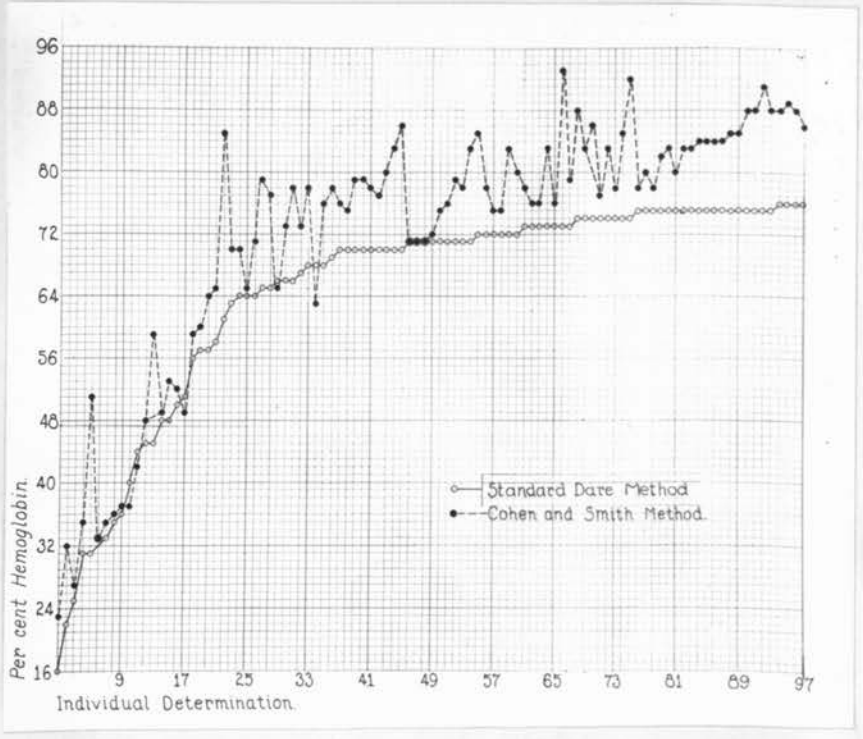


Fig. 5 Curve I (a).

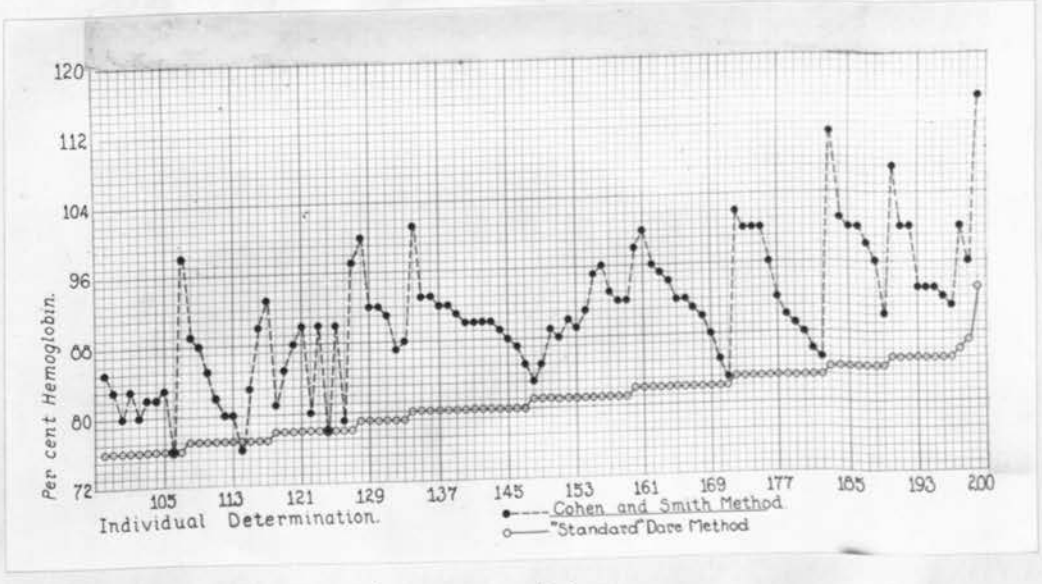


Fig. 6 Curve I (b)

Curve II

In this curve the average readings of the Newcomer, and Cohen and Smith methods are plotted with those obtained by the "Standard" and "Special" Dare instruments. It is noticed in this curve as well, that the values are similar up to approximately 60 to 65 per cent.

Readings of 60 to 80 per cent on the "Standard" Dare follow as in Curve I, while the "Special" Dare reads approximately 10 to 15 per cent higher.

The readings on the"Special" Dare, in determinations from 80 to 115 per cent, compare favorably with the average readings of the Cohen and Smith and Newcomer methods.

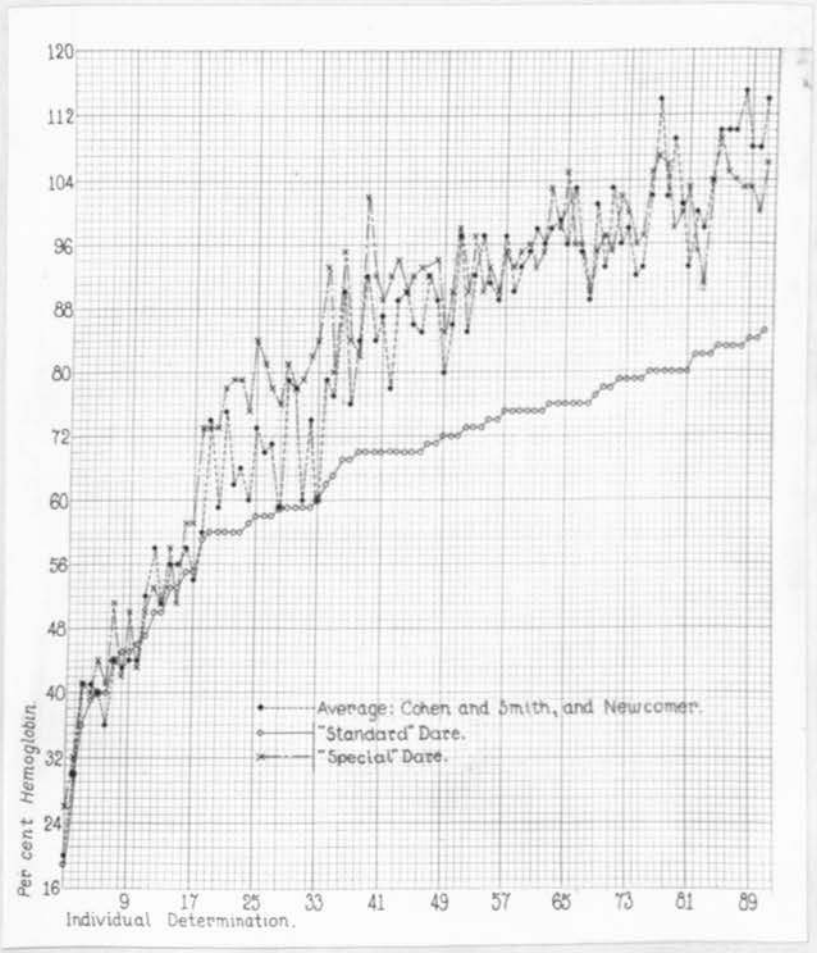


Fig. 7 Curve II

Curve III.

This curve demonstrates the marked uniformity between the Newcomer and Cohen and Smith acid hematin methods. With but few exceptions, the variations are within the limits of error. From the clinical point of view, these methods are equally accurate and interchangeable.

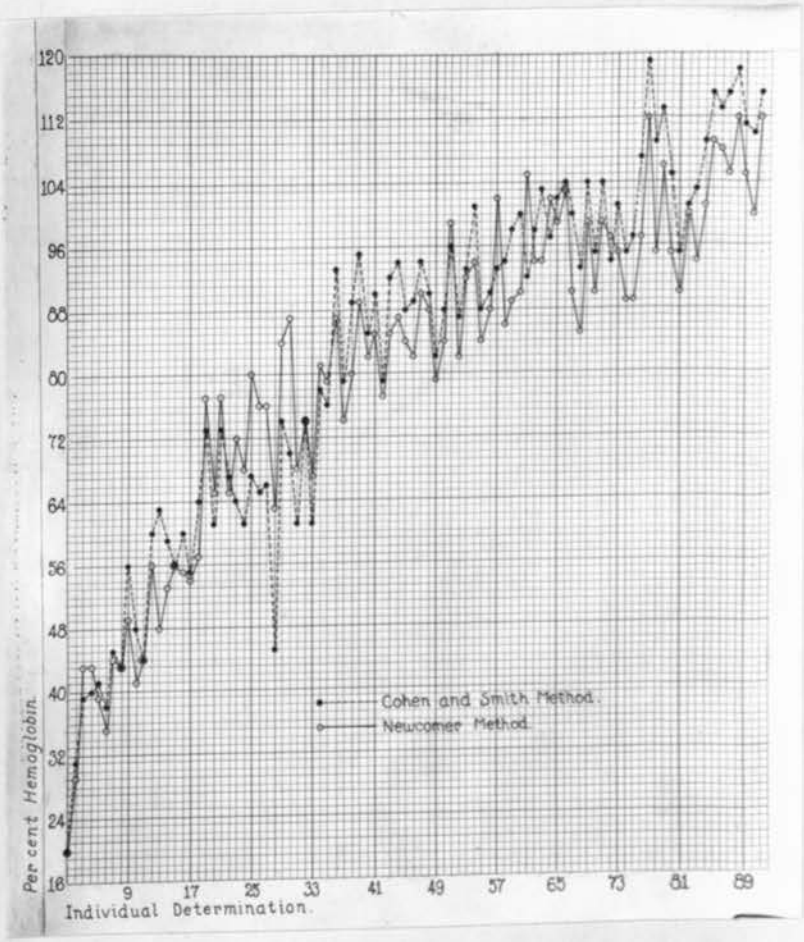


Fig. 8 Curve III

Curve IV.

This curve shows the striking similarity throughout between hemoglobin estimations made on the Tallqvist and Dare hemoglobinometers. This curve suggests very strongly, that, perhaps, if care is used and directions followed, the Tallqvist determinations are almost as accurate as those obtained on the "Standard" Dare instrument.

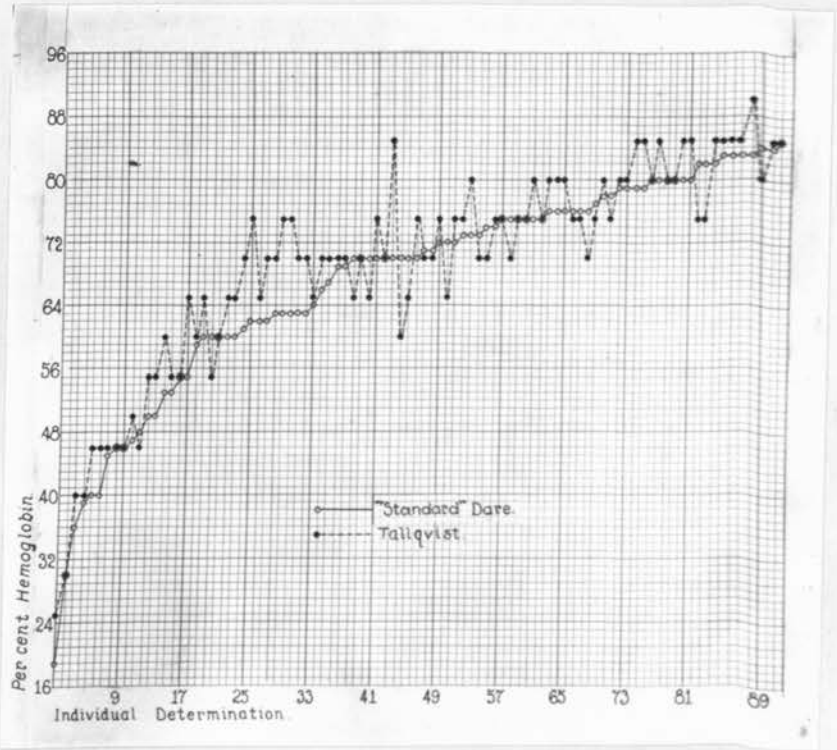


Fig. 9 Curve IV.

Conclusions:

As a result of this work, I have arrived at the following conclusions:

1. The "Standard" Dare hemoglobinometer is a practical instrument for hemoglobin determinations from 20 to 60 or 65 per cent.

2. Above 70 per cent the hemoglobin estimations on the "Standard" Dare instrument are very misleading.

3. The Newcomer disc method is a unique and practical method of estimating the percentage of hemoglobin in the blood. The percentages obtained are reliable.

4. The Cohen and Smith acid hematin method is very accurate and fairly practical. It should be used in the estimation of the percentage of hemoglobin present in all suspected cases of anemia.

5. The "Special" Dare instrument is a decided improvement over the "Standard" Dare.

6. The Tallqvist hemoglobinometer is probably quite as accurate as the "Standard" Dare instrument.

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