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HEMOGLOBIN ESTIMATION WITH UNDILUTED REDUCED BLOOD
(COMPARISON OF BLOOD FROM EAR LOBE, FINGER AND VEIN)

by

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INTRODUCTION

There is some lack of agreement as to the best means of measuring hemoglobin routinely. Methods for research laboratories seem to be adequate but a simple, accurate, time-saving method for routine work has not been generally agreed upon. Some laboratories find the "Sahli principle" with an instrument as the Haden-Hausser (18,19) satisfactory. Other laboratories agree that routine hemoglobin estimations, with the proper planning, can be made photoelectrically, since at the present time most laboratories own a photometer, as for example, the Klett-Summerson (33) instrument. There are two reasons for this lack of uniformity of method and instrument. Firstly, there has not been available a satisfactory, simple enough instrument to use for utilizing a sound, unvarying principle of hemoglobin estimation. Secondly, the sample itself has often been taken without sufficient regard as to the kind of blood and of the interval of time elapsing after puncture prior to taking sample. It has been assumed that capillary and venous bloods are practically identical for hemoglobin estimations and that all capillary bloods are the same in this respect. We have thought that time interval after puncture had no effect on hemoglobin values; that the first drops of blood, for example, from the ear lobe, have identical values with those examined some three to five minutes later.

The "Sahli principle", employed since Sahli (29) introduced it in 1902, has been the most commonly used method for routine estimation of hemoglobin. It employs the brownish
color of acid hematin which is compared to a fluid or glass standard in the Sahli instrument. The Autenrieth-Koenigsberger (3), the Newcomer (26), and the Haden-Hausser (18,19) instruments employ this principle, having a fluid or glass standard. The brown color which develops upon the addition of acid to the blood depends on several reactions which in turn are supposed to be dependent on the amount of hemoglobin present. Barkan (4, 5, 8, 9, 10) and Barkan and Olesk (6, 7) have investigated the colorimetry of acid hematin during the past ten years and come to the conclusion that it is unsatisfactory and should be eliminated from routine procedure. Barkan (4, 5, 8, 9) was able to demonstrate deviations as high as fourteen percent when he compared acid hematin values with photometrically determined oxyhemoglobin. Newcomer (26) in 1930 studied thoroughly the optical behavior of acid hematin. He concluded that for routine work acid hematin may be regarded as a true solution and obtained a regular slope when plotting color development against time. Barkan (4, 5, 8, 9) and Barkan and Olesk (6, 7) have demonstrated the colloidal nature of acid hematin solutions by observing the influence of different experimental conditions upon hemometric readings. They do not agree with Newcomer (26) that acid hematin acts as a true solution for the purpose of routine hemoglobin estimations. Heilmeyer and von Mutius (22) also have demonstrated deviations up to eleven percent when comparing the acid hematin method with other methods of hemoglobin estimation but they consider it satisfactory for routine work in the absence of a more
convenient method. Humperdinck (24) also found too high values for hemoglobin when determined as acid hematin.

In this work we used another principle introduced by Bürker (12) in 1927, and by Heilmeyer (19) in 1938 with a Pulfrich photometer, namely, that of reduced hemoglobin. Williamson (36) in 1916 and Brückman (11) very recently used this principle with the Leitz extinction photometer and each one found it satisfactory. The blood is reduced by the use of sodium hydrosulphite. The colorimetry of reduced hemoglobin is particularly good. The color is stable and, within certain limits, independent of time, hence errors due to lack of time for reading are eliminated. In 1937 Barkan (8) wrote that he thought the principle of reduced hemoglobin together with the means, as used by Heilmeyer (21) of reading it with a Pulfrich photometer could be combined in some handy instrument for routine use. This very sort of instrument was introduced in Denmark by Dr. M. Philipsen in 1938 and was named the Sicca Hemometer. Dr. Barkan had two such instruments shipped to this country; he described them and made comparative studies, the results of which he published in 1940 (10). One of the instruments had to be restandardized by him because of a mistake in shipment. He used oxyhemoglobin determinations made on the Evelyn (15) photometer as well as iron determinations made according to Walker and Fitz (34), assuming 0.336% iron in blood. The other instrument was standardized in Denmark by the manufacturers using Haldane's oxygen method. One of these instruments was used in this study. The principle on which the
instrument is based is the viewing of a varying depth of blood against a glass standard. This is accomplished by feeding the blood into a space formed by a glass base plate and a specially constructed glass bridge so made that the space between it and the glass base gradually deepens from left to right. A notch on the bridge or wedge fits into a raised projection on the base plate and locks the bridge into position. Two glass standards are furnished and can be turned into position as is needed, one being used for readings above sixty per cent and the other for those below sixty per cent. The light comes from an electric bulb and after passing through specially selected filters, is transmitted through specimen and standard. The use of constant glass standards and a constant source of light with such filters allows for best optical contrast between sample and standard and reduces to a minimum inaccuracies due to variations in current and plasma color.

Blood is taken from the finger into the narrow end of a small glass pipette, called the Sicca pipette, by capillary attraction or by means of suction from an open rubber cap provided on the other end of each pipette. A steel needle, about the diameter of a stocking knitting needle, is dipped into the blood, about one quarter of an inch, through the wide end of the pipette. It is then transferred first to a bottle of Sicca powder, where the required amount of powder sticks to the steel needle and then back to the blood where it is agitated until the blood is laked and dark reddish-purple color in appearance. If the sample is not to be read immediately it may
be stored in a specially corked glass tube container which fits into a wooden rack in such a manner that the sample is kept in a more or less oblique position. The Sicca powder consists of sodium hydrosulphite, saponin, oxalate and sodium chloride, which reduces, hemolyses, and stabilizes the blood.

In making the hemoglobin estimation the base plate and wedge is filled by capillary attraction, the wedge locked into position and the complete chamber placed on the scale platform of the Sicca instrument and moved from side to side until the standard is matched. The "per cent" of hemoglobin is read off directly from the scale and grams of hemoglobin per one hundred cubic centimeters of blood is computed by multiplying percentage by 13.85. This figure is used on the basis that in the standardization of the instrument 100 parts on the scale correspond to 18.5 volumes per cent of oxygen (Haldane's method), 18.5 volumes per cent of oxygen representing 13.85 grams of hemoglobin per 100 cc. of blood. For low hemoglobin values a second wedge may be used which is ground to double the height of the first and hence readings have to be halved to obtain final values.

We have evidence that hemoglobin values determined on the Sicca instrument compare favorably with values obtained using other reliable methods. Barkan (10) reports, in comparison with photoelectrically determined oxyhemoglobin on the Evelyn (15) instrument, that results as a rule, were in very close agreement for low as well as normal hemoglobin values. In a large series of experiments carried out in Montreal and
reported to Barkan (10) by Dr. H. Hesse from Denmark, in which determinations by the Van Slyke method of oxygen capacity determination were compared with the Sicca instrument, the deviation never exceeded three per cent. Lane (25) reports that the method and instrument were compared with the Haldane carbon-monoxide hemoglobin method and gave results practically identical. Sørenson (31,32) found the instrument satisfactory in his hands.

We tested the reproducibility of hemoglobin values using this method and instrument. Firstly we were able to demonstrate low dispersion for individual readings on the Sicca instrument. We usually made three readings for each determination and took the average of these for the hemoglobin value. In a preliminary set of data, the accuracy of which was representative of the readings throughout the study, fifty-seven determinations were made on a group of nine blood samples. We found that fifty per cent of these had identical readings and in the remaining sixty per cent the deviation from the average of the three readings was not more than one per cent and usually less. Barkan (10), employing this method and instrument, cites low dispersion for individual readings using a set of fifty determinations on which he took ten readings each. In this connection he writes, comparing the average of the first three readings (R₃) with the average of the total ten readings (R₁₀): "In 48 of the 50 cases the per cent deviation of the two averages: \( \frac{\pm (R₃ - R₁₀)}{R₁₀} \times 100 \) was not greater than two; in the remaining two cases the deviation was
Diagram I

Illustrating the accuracy of the method used

Frequency curves of percent deviation of readings of venous samples. (147 analyses are represented.)

Percent deviation from the average venous value.
2.5 and 3.5 per cent respectively."

After we were satisfied as to the dependability of the Sicca instrument we tested the reproducibility of the color of reduced blood for hemoglobin estimation. We employed twenty-four different oxalated blood samples and made from these a total of 147 separate determinations. Diagram I shows a frequency curve of these data, 83 per cent having a per cent deviation of two or less from their average and seventeen per cent falling within plus 6. It also shows that 97 per cent of the values fell within plus or minus three per cent deviation. These figures agree rather well with Sørensen's (31,32) findings. He was able, using the same method and a Sicca instrument to obtain results falling within two per cent deviation in eighty per cent of his cases; eighteen per cent fell within six and two per cent fell within ten.
EXPERIMENTAL

We made comparative studies of hemoglobin values for venous (arm), ear and finger blood of the same patient. We accomplished this under conditions which would actually exist in routine work so that our results in no way include theoretical accuracies obtained under optimal working conditions. We went to the out-patient clinic in the morning to take the bloods and subjected them to the necessary storage in the regular storage tubes until we returned to the laboratory several hours later to read them in the Sicca hemometer. It was therefore necessary for us to investigate the effect of this delay on the results of hemoglobin determination. We accomplished this in two ways. Firstly, we subjected a group of 95 blood samples prepared from 25 different oxalated bloods to the action of the Sicca reagent from one to five hours. Secondly, we allowed the remaining portion of the same 25 oxalated bloods to stand unaltered, for the same length of time, until just prior to reading when we mixed each Sicca pipette-ful with the Sicca reagent in the usual way. We read some of the 25 bloods immediately after taking to obtain an initial hemoglobin value. From these group studies of 95 comparisons, involving 190 determinations we obtained a mean value of 12.60 grams of hemoglobin per 100 cc. of blood for those subjected to the Sicca reagent and 12.52 grams for those not so subjected. The difference of means, namely, 0.08 grams, is too small to be statistically significant. We observed that the differences of
of hemoglobin values determined hourly when compared to the initial value obtained immediately after taking the blood was also too small to be significant. It is evident, therefore, that storage up to five hours, with or without the influence of the Sicca reagent, has no effect on the results of hemoglobin determination. The coefficient of variation was approximately the same in these group studies, ranging from 1.42 to 1.83 per cent.

When we subjected six samples of oxalated blood to the Sicca reagent in the usual way, placed them in storage tubes and kept them in the refrigerator for twenty-four hours before reading, we found that four of the six samples showed a per cent deviation of 1.5 from the average of all readings on the same untreated sample. One sample showed a deviation of 3.08 per cent and the sixth sample had deteriorated. Samples that were kept three days under the same conditions showed per cent deviations ranging from 2.95 to 19.50. It seems safe to conclude from these results that bloods kept overnight are not too reliable for hemoglobin estimation. Bloods kept longer than twenty-four hours are apparently no good for this purpose. Yardbrough (37) reports, working with a limited number of samples, that she was able to keep oxalated bloods three days, obtaining correct hemoglobin values at the end of this time. Foord (17) did not find such favorable results saying that few oxalated bloods were reliable after twenty-four hours.

The main part of our work consisted in making comparative hemoglobin studies of venous, ear lobe, and finger blood of the same patient. There has often been disagreement
in routine results of hemoglobin values from one laboratory and another, indeed, between one technician and another in the same hospital. Results differ too widely from day to day on the same patient. Part of it can be attributed to the use of the "Sahli principle" but this does not explain all of the differences. Even with more accurate instruments there is too much difference to be explained satisfactorily. Some laboratories use ear lobe blood while others prefer finger tip blood. It is generally believed that ear blood and finger blood are identical for hemoglobin estimation, convenience being the only factor involved.

Sørensen (31) published a paper in 1940 in Denmark under the title "Kan Øreblod Anvendes Til Kvantitativ Haemometri? Forslog Til Nye Regler for Blodtogning (Fareløbig Meddeilse)". He related that he found differences in hemoglobin values up to thirty per cent from day to day on the same patient without any apparent clinical reason. He used ear lobe blood, employing reduced whole blood as the method and the Sicca Haemometer as the instrument for his hemoglobin estimations. Previously he found the method and instrument reliable, therefore could not offer these as a reason for the differences. Searching the literature for an explanation he came across one study by G. Walterhöfer (35) in Berlin who published a paper in the Klinische Wochenschrift in 1927 which proved enlightening. This investigator found variations from 20-30 per cent in samples of blood from the ear depending on the time the sample was taken after puncture. He determined hemoglobin level, erythrocyte count and leucocyte count on
samples he took at frequent intervals up to forty minutes, and plotted these values against time after puncture. The first drops of blood showed very high hemoglobin values and only after 8-10 minutes did they reach a level at which they remained practically stationary for the remainder of the study. All cases he studied showed these phenomena, some cases being more pronounced than others. Sørensen (31,32) verified these curves and he, also, was able to demonstrate these phenomena, getting variations up to 28 per cent. In a few of his cases the curve was reversed, being low at the beginning and later reaching a higher level and eventually flattening out. Only in two of the sixteen cases he studied did he find very little fluctuation. Searching for a practical application of Walterhöfer's work he made various experiments on ear lobe, venous, and finger tip blood. He concluded that ear blood could be used if one waited at least three minutes, preferably more, after puncture, before filling the pipette. He wrote that he found little difference between finger and venous blood. He concluded also that pressure could be used in taking the sample and that the incision in the ear need not be deep enough to cause free flowing of the blood.

Very recently, and after we had concluded the experimental part of our work, there appeared in the literature a paper by Brückman (11) under the title, "Blood From the Ear Lobe", relating the author's experience of taking bloods from normal people to establish seasonal hemoglobin standards for Palestine. He wrote of usually taking venous blood, but occasionally having to take it from the ear lobe and of
finding values in the latter cases abnormally high. He compared ear lobe blood, venous, and finger tip blood and found ear lobe blood to be about ten per cent higher in hemoglobin values than venous blood and concluded ear blood should not be used for hemoglobin estimations. He found no significant difference in venous and finger blood estimations. He apparently had not investigated Walterhöfer's curves but explained that stasis in the capillaries and subsequent changes in blood composition could more easily take place in ear lobe than finger pulp, and in view of the current work he was doing he could safely say that there was a tendency for macrocytosis to prevail in the ear lobe blood. He apparently agrees in this respect with Duke and Stofer (14) who published a paper in this country in 1922 telling also of a comparison of finger and ear lobe blood with venous blood in patients with pernicious anemia. They found an average excess of red cells of 17.6 per cent in ear lobe blood over venous blood. They felt it had something to do with the disease since they did not find this phenomenon in normal people or patients with secondary anemia. They offered an explanation that, upon microscopic examination, more of the small red cells were found in the venous blood and that they observed a tendency for macrocytosis to prevail in the capillary ear blood.

Williamson (36) in 1916 and Andresen and Mugrage (1) in 1938 found higher red cell counts during the first weeks of life using the ear lobe and heel, respectively. Several investigators do not find higher values for ear lobe blood.

Williamson (36) in 1916, in his excellent work in establishing
hemoglobin standards for age and sex, found values apparently
ten per cent higher than we think normal today. He used
finger tip blood, except in a few cases of the very young,
evidently spent much time in preliminary work establishing
a method for hemoglobin estimation, finally using reduced whole
blood and a Leitz extinction photometer according to
Heilmeyer (21). He concluded finger tip blood best but his
results remain ten per cent higher than our present standards.
Price-Jones and others (27) in 1935 found no significant
difference between ear and venous bloods when they studied
hemoglobin values and red cell count in 100 healthy young
males. They measured hemoglobin by Haldane’s oxygen capacity
method. Yardbrough (37) in a publication in 1921 and Foord
(17) in 1923 found no appreciable difference in venous and
ear lobe blood, although the latter states fresh venous and
oxalated venous bloods showed values a little lower than ear
counts. Bürker (12), in 1917, and Reichel and Monastero (28)
in 1929, also state that they found no significant difference
in ear lobe and venous bloods.

We were anxious to verify the Walterhöferphenomenon
and were successful in the majority of the 30 separate blood
samples, involving 327 analyses, we secured from patients in the
clinic for this purpose. We found fluctuations up to 26 per
cent in the hemoglobin values from the same puncture of the
ear depending on the time interval before taking the sample.
Most of our studies showed the regular high values during the
first five minutes and only after ten minutes did they tend
to reach a level at which they usually remained throughout
Diagram II-A

Typical Walterhöfer Curve

Ear Blood.

Hemoglobin: grams/100cc. Blood.

Time after puncture of ear
Diagram II-B

Time after puncture of ear.

Hemoglobin: grams/100cc. Blood

Ear Blood

Ty pical Walker hoke r Curve.
Typical Walterhöfer Curve
Ear Blood.

Diagram II-C

Hemoglobin: grams/100 cc Blood.

Time after puncture of ear

3 6 9 12 15 30 45 mins.
the study. Few of the samples showed lower values at first, increasing about ten per cent and finally subsiding to normal again after ten to fifteen minutes. Sometimes a sample did not settle to a level value but continued to show fluctuations during the entire forty minutes studied. Diagrams II-A, II-B and II-C illustrate typical individual Walterhöfer curves.

In making our comparative studies we followed the procedure of firstly taking blood from the arm vein, using a tourniquet and taking the blood as soon as possible after applying the tourniquet. The blood was ejected from the syringe into two test tubes which contained an oxalate mixture, after Heller & Paul (23) as an anti-coagulant. From one of the tubes we filled three Sicca pipettes immediately after taking, mixing each with the Sicca reagent, and placed them in the storage tubes for reading later. Secondly, we pricked, with a spring lancet, both ear lobes and applied a piece of cotton to only one, thereby preventing the dripping of blood from this ear. Using a stop-watch and noting time after incision we took usually four samples of blood from the other ear during the first five minutes and thereafter one sample at about five minute intervals up to thirty-five or forty minutes. From the ear which remained untouched for three minutes after applying the piece of cotton, we took a single sample of blood. In taking this sample we removed the cotton, wiped away the coagulated blood from the wound and discarded the first few drops. Thirdly, samples of blood were taken from the finger tip in much the same fashion as
FREQUENCY CURVES FOR EAR AND FINGER BLOOD OVER A TIME INTERVAL OF 20 MIN.

1-2 mins. 8 Cases
2-5 mins. 15 Cases
3-6 mins. 18 Cases

DEVIATION FROM VENOUS HEMOGLOBIN
NGER BLOOD COMPARED
MINUTES INVOLVING 263 ANALYSES

0 mins.

11 cases

6-10 mins.

15 cases

10-15 mins

13 cases

15-20 mins

10 cases

0 mins.

15 cases

0 mins.

7 cases

VALUE
Diagram IV Scatter diagram illustrating percentage differences in hemoglobin between blood from ear lobe and blood from arm vein, involving 212 analyses.

The venous = 0.00%.

Time: minutes after puncture
from the ear lobe noting time of puncture and as frequently as possible since the wound in the finger usually closed within 5-6 minutes. All blood samples were brought back to the laboratory and read in the Sicca Haemometer. From the second test tube of oxalated blood three Sicca pipettes were filled and the blood reduced just prior to reading in the hemometer.

A comparison of ear blood and finger blood on 15 patients, involving 263 analyses, was made referring each to the venous blood values. In general finger tip blood agreed more closely with venous values than did ear lobe blood. Diagram III illustrates frequency curves for this comparative study. It is possible to notice the marked lack of agreement between venous and ear lobe blood immediately after puncture and a better agreement after ten minutes; 62 per cent agreeing within plus or minus two per cent. Diagram IV is a scatter diagram illustrating the percentage differences between ear lobe and venous bloods when allowing the venous values to equal 0.00 per cent. This diagram, which gives a more comprehensive picture of the results than does Diagram III, shows the marked difference between the two bloods especially during the first ten minutes and a systematic tendency for the values to agree more closely with venous values after this time. Some of the cases continue to show marked differences after fifteen minutes as can be observed in Diagram III.

We were not able to verify Sørensen's findings for the other ear. We did not find that the other ear gave values comparable with the venous blood after waiting 3-6 minutes.
before taking the sample of blood. We found that only 27 per cent fell within plus or minus two per cent of the venous values. Diagram III illustrates the frequency curves for these results. Sørensen found seventy-nine per cent of his determinations fell within plus or minus two per cent after three minutes. We do agree with Brückman (11) who found ear blood about ten per cent higher than venous blood and concluded that ear lobe blood should not be used for clinical estimation of hemoglobin. However, there is no mention of the Walterhöfer curves in Brückman's (11) paper and we are not sure how much time elapsed after puncture before taking the blood sample. The instrument for making the incision as well as the tendency for stasis and macrocytosis to be more pronounced in the ear which was allowed to remain undisturbed for 3-6 minutes, should be considered in explaining our lack of agreement with Sørensen's work. We did not use the same instrument as was used by him; he employing a cataract knife, while we used a spring lancet as did Walterhöfer and Brückman. Sørensen (31,32), however, writes that instrument, depth of incision, and amount of pressure applied to secure blood sample, have no effect on hemoglobin values. Williamson in his work paid much attention to the instrument he used. He used "cataract knives of the best quality which were sent to the maker at short intervals for sharpening". It is a little difficult to compare our work with Price-Jones and others (27), Bürker (2, 13), Foord (17), etc., since they do not mention the Walterhöfer phenomenon.
When we compared finger blood to venous blood, we agree with Sørensen rather well. On the whole, we found that finger tip blood agreed more generally with venous blood. Brückman confirms us also in this respect. Sørensen gets fifty-two per cent of his cases to agree within plus or minus two per cent in one minute after puncture. This is his best agreement with venous values. Diagram III illustrates frequency curves of our results for agreement of finger tip blood and venous blood. Our figures show that within the first minute after puncture we got sixty-four per cent agreeing within plus or minus two per cent of the venous values. Looking further into our figures it is interesting to observe, though this is not shown in Diagram III, that after three and within ten minutes 89 per cent agreed with venous values within plus or minus three per cent. In our experience then, finger tip blood does not show the large fluctuations that is demonstrated by ear lobe blood, but does show a better agreement with the venous blood.

In general we found that pressure applied to the ear or finger to secure the blood sample had no influence on the hemoglobin values. We obtained the same results from samples which were taken when blood flowed freely as from those in which the sample had to be squeezed from the puncture. Brückman reports that he found no influence exerted on hemoglobin values from depth of incision, pressure, and instrument used to make the incision.
DISCUSSION

We cannot explain at this time the reason for the interesting Walterhöfer phenomenon observed in capillary blood taken from the ear. We intend to run protein determinations on the samples to investigate whether or not there may be a passage of fluid from the capillaries into the surrounding tissue when the ear is punctured. Walterhöfer (35) found these same curves in erythrocyte and leucocyte counts. Again Duke and Stofer (14) report, on observing the discrepancy between ear and venous bloods, that in studying blood smears microscopically they found a tendency for macrocytosis in the capillary blood and found a greater number of smaller red cells in the venous blood. They thought the difference lay in the fact that the large red cells showed a tendency to lag in the narrow capillary bed where the rate of flow is slow and the pulse is lost. Brückman (11) states in his conclusions that stasis in the capillaries and subsequent changes in the blood composition may more easily occur in the ear lobe than in finger pulp. He adds that on current work in which he has "included red cell count and hematocrit determinations to his hemoglobin studies", he can already say that there is a tendency for macrocytosis to prevail in the ear lobe blood. Hahn, Ross, Bale, Balfare and Whipple (20) explain in detail the principles of hydrodynamics as applied to the problems of blood flow and blood volume. They speak of a surface film-axial stream relationship existing in blood vessels and the differences in type of particle motion within them as they influence the distributions of erythrocytes
in the vessels. They point out that when a liquid moves over a solid surface a sluggishly moving film exists on the surface of a stationary fluid film. The character of both films is entirely different from that of the main body of the moving fluid and they form a zone between the rapidly moving fluid and the wall. If velocity of a fluid is plotted against distance from the wall of the containing vessel, it becomes apparent that there is no motion of fluid at the boundary wall, and that with progression towards the center of the tube the velocity of motion increases. They observed upon microscopic inspection of small blood vessels that these two films of plasma existing along the walls of a vessel, are for the most part devoid of erythrocytes. In the blood vascular system as the arterioles becomes smaller and more numerous and approach the capillary bed there is no increase in velocity of flow but rather a decrease, therefore the surface films might be expected to constitute a progressively increasing proportion of the total contents of small arteries, arterioles and capillaries. This would tend to bolster Brückman (11) and Duke and Stofer (14) in their explanation of stasis in the capillaries of the ear lobe. This may also have some influence on hemoglobin values in relationship to depth of incision, if there can be demonstrated a difference in the diameter of the capillaries in ear lobe and finger tip. More investigation is necessary to determine the reasons for these interesting variations, but for the present we know only that they seem to exist.
SUMMARY

1. We have used a recently introduced principle of hemoglobin measurement, using reduced hemoglobin, and a recently introduced instrument, the Sicca Haemometer, for routine clinical estimation of hemoglobin and find that values have an accuracy of plus or minus two per cent in eighty-three per cent of the cases studied and of plus or minus three per cent in ninety-seven per cent of the cases.

2. We have verified the phenomenon of Walterhöfer (27) and obtained hemoglobin values in ear lobe blood as high as twenty-six per cent above venous blood values.

3. We could not agree with that portion of Sørensen's (31,32) work in which he found that blood taken from the ear lobe, which had been left undisturbed for three minutes or more after puncture, gave comparable results with venous blood, and our findings, rather than Sørensen's, would tend to support the theory that stasis and macrocytosis prevail in the ear lobe.

4. We concluded that ear lobe blood for the present should not be used for routine estimation of hemoglobin.

5. Finger blood did not show the pronounced fluctuations in hemoglobin values that ear blood evidenced and hence is better suited for routine clinical use.

6. Applying pressure to the wound seems to have no effect on hemoglobin values.

7. Further work is necessary to explain the Walterhöfer phenomenon. Protein determinations should be made to determine whether or not there is a transfer of liquid across the walls
of the capillary. A microscopic and blood volume study of samples would be of value in establishing the evidence of stasis and macrocytosis. Applying the principles of hydrodynamics to the vascular system seems enlightening in a comparison of venous and capillary blood, and may have some bearing on the depth of incision, stasis and macrocytosis in the capillaries.
ABSTRACT

Hemoglobin in human blood is determined in grams per 100 cubic centimeters by using reduced whole blood, read in the Sicca Haemometer. The "Sahli principle" which depends on the brownish color of acid hematin is discarded in favor of the use of reduced whole blood. The Sicca Haemometer is a handy, easily operated instrument for routine clinical estimation of hemoglobin. It was invented by a Danish physician (10) and is now manufactured only in Denmark although it has a United States patent. An accuracy of plus or minus two per cent was found with this instrument in 83 per cent of the cases we studied and of plus or minus three in 97 per cent of the cases when the method and instrument were subjected to actual working conditions in a busy clinic.

We compared hemoglobin values in ear blood from both ears, finger tip blood and venous blood from the arm vein of the same patient. We found fluctuations up to twenty-six per cent of the venous values in ear blood depending upon the time the blood sample was taken after puncture. We followed closely the work of the Danish investigator, G. Sørensen, who also studied the relationship between ear lobe, finger tip and venous bloods. We were able to verify his work insofar as we obtained the Walterhöfer (35) curves from ear lobe blood showing great fluctuations in the first five minutes after puncture. We did not verify his conclusions that ear blood is satisfactory for hemoglobin estimations if allowed to remain
undisturbed for 3-6 minutes after puncture before taking the blood sample. In 79 per cent of the twenty-two cases studied he obtained an agreement within plus or minus two per cent of the venous values. We found only 27 per cent of a group of fifteen cases studied agreeing within two per cent of venous values. Our findings, rather than Sørensen's, would tend to bolster the theory of stasis and macrocytosis prevailing in the ear lobe. We agree with Brückmann (11) who also made a comparative study of ear lobe, finger and venous bloods and concluded that ear lobe blood was about 10 per cent higher in hemoglobin value than venous blood and therefore should not be used for hemoglobin estimations. Brückmann did not investigate the Walterhöfer (35) phenomenon, however.

We found that finger tip blood did not show the large fluctuations that ear lobe demonstrated. It agreed more closely with venous blood from the first few drops after puncture. We confirmed Sørensen in this respect rather well. We found that blood taken from 0.3-1 minute after puncture showed 64 per cent agreement with venous values within plus or minus two per cent while blood taken between three and ten minutes showed 89 per cent agreement within plus or minus three per cent of venous values. Brückmann (11) encountered no significant difference between finger tip and venous blood.

Sørensen found no difference between a deep puncture and a shallow puncture where pressure had to be used to obtain the sample. Brückmann also concluded that pressure seemed to make no difference. We found that pressure made no difference, but we are not sure that the implement for puncturing and the
depth of the incision have no influence on the hemoglobin results. We used a spring lancet as did Walterhöfer (35) and Brückman (11), while Sørensen (31, 32) used a cataract knife. Hemodynamics may play some role in deciding the optimal depth of the puncture. We should not overlook the surface film-axial stream relationship existing in blood vessels in explaining the systematic variations of ear lobe blood and venous blood as well as the fluctuations in ear lobe blood itself. These fluctuations may be caused by some other or added phenomena, such as passage of fluid from the blood vessel to surrounding tissue when puncturing. Brückman (11) and Duke and Stofer (14) offer the opinion that the increased hemoglobin values in the ear may be due to stasis and macrocytosis.

From our comparative studies we must conclude, for the present, that the ear lobe blood should not be used for hemoglobin estimation. The Walterhöfer phenomenon is remarkable and challenging and work should be continued to find an explanation for it.
BIBLIOGRAPHY


