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CONCERNING

THE

RULES AND THE APPLICATIONS

OF

REICHERT'S HÆMOMETER,

BY

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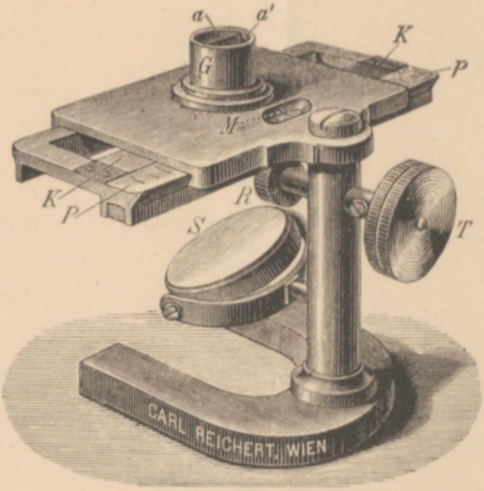
Prof. E. v. Fleischl's

HEMOMETER.



$^1/_2$ actual size. (Fig. 1.)

PATENT:

CH. REICHERT

Vienna. (Austria.)

VIII. Bennogasse No. 26.

Concerning the Rules and the Application of Reichert's Hæmometer.*

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This apparatus is designed to ascertain the amount of hæmoglobin in either a diseased or a normal condition of the blood. It was devised by Prof. E. von Fleischl, and patented by Carl Reichert, of Vienna. (See frontispiece.) This little instrument, the Hæmometer, is the result of a need felt by physicians and scientists of having an instrument which will give a quantitative judgment (analysis) of the value and function of the hæmoglobin in the circulating blood. It was further necessitated by the inapplicability of the methods thus far prescribed for this purpose to the cases encountered by physicians; and, finally, it arose from the hope of advancing our physiological and clinical knowledge by rating the per cent. of hæmoglobin in diseased human blood.

The Hæmometer cannot be used either by daylight or by the electric light, and only by the light of oil lamps, candles, and gas.

Every examination of blood by means of the Hæmometer must consist of these three operations: 1. To obtain and measure the blood. 2. To dissolve it in water, and to fill the instrument with this solution. 3. To arrange the instrument and read the results.

This apparatus consists of a small and simply constructed horseshoe base, composed of a foot, column, mirror, and table. Beneath the table is a frame which bears the glass wedge K, the latter being propelled by the milled-head screw R. Upon the table is a cylindrical vessel G, the one-half of which (a) is filled with blood which has been diluted with water, so as to be examined. The other half (a') is filled with pure spring water, after a tube, whose capacity has been exactly gauged, has itself been filled with blood by capillary action. It is brought into the

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* Read before the Iron City Microscopical Society.

half of the vessel at *a*, where the blood contained in the tube dissolves in the water until it becomes a perfectly transparent liquid. By the optical conditions of the apparatus it becomes possible under an illumination of oil lamps, candles, or gas light to find a position of the glass wedge K at which the color and brightness of every such blood solution is exactly the same. This point is sought by moving the wedge backwards and forwards by means of the micrometric screw T, and by giving the reflector a definite position, S.

Upon the frame which surrounds the wedge, a scale P is engraved, a part of which is visible through the aperture at M. This gives exact results in percentage of the amount of hæmoglobin in a certain blood solution. There is also a stationary index line on the side of the aperture M, which points also to the discovered amount on the scale.

This Hæmometer presents the following advantages:

1. Easy and convenient management of the apparatus.

2. Rapid and direct results in percentage regarding the degree of normal hæmoglobin.

3. The small quantity of blood, only a drop, required for the examination.

It is best to take the blood from the tip of the left middle finger.

After the skin has been thoroughly washed and carefully dried, and without any preceding compression, or binding of the finger, as is usually done, it should be wounded by a slight prick with a sharp needle. Then by a slight pressure above the little wound a drop of blood is secured. This drop of blood is taken up with one of the open ends of an automatic blood pipette, a small capillary tube about 8 mm. in length, bound about in the centre by a tiny wire, and of definite capacity (6½ cubic mm.). The filling of the automatic blood pipette is considerably facilitated and accelerated by holding it horizontally, instead of perpendicularly; that is, it is dipped sidewise into the drop of blood.

Since every trace of blood that clings to the exterior of the tube is to be considered a serious defect, it is necessary to smear the pipette with something of a fatty nature. This is best done by keeping it in a leather case, lubricated with tallow. As soon as the pipette is full the outer surface should be carefully examined. If a speck of blood is found there, it must instantly be removed, or before it has time to dry. This is done by means of a strip of filtering paper or absorbent cotton. The blood is then much more fully and easily absorbed when the exterior of the glass is coated with an oleaginous substance. Care should be taken that the column of blood ends at both extremities on the same level with the glass tubes, and neither with retiring nor with bulging, but with even extremities. If it should be necessary to use filtering paper or wadding to remove the blood from the exterior of the pipette, care should be taken that these substances do not approach too closely to the extremities of the blood column, in order to avoid a meniscus.

Even before these instructions are carried out, the various parts of the Hæmometer should be examined to insure perfect cleanliness, and a perfect condition of the apparatus. The component parts may then be arranged. The frame upon which the red glass wedge reposes must be joined to the wing on the lower side of the table slab, through which it finds its guidance. Moreover, the comparing vessel must be inserted into the opening designed for it in the table slab, and so placed that the projection of the vessel, as observed from above, may coincide with the visible part of the free wedge lying beneath.

Both halves of the comparing vessel must be filled with distilled or pure spring water. The half above the wedge, called "wedge half," is completely filled with water from the pipette, so that the smooth surface which it forms above may be perfectly level, forming neither a positive nor a negative meniscus. The other, the blood half, is also filled with water from the pipette, but only to about one-fifth, or at most, one-fourth of its capacity. When this is done, the pipette out of which the vessel has been filled and which still contains a sufficient quantity of water to complete the filling of the blood half, should be placed in a horizontal position—*i. e.*, upon the brim of a goblet, so that the water will not flow out of it.

The pipette having been filled with blood, it should be brought (in a horizontal position) under the water in the blood half of the comparing vessel, when the little wire should be leaned against the upper edge of the vessel, but not against the straight edge of the partition wall, nor in either one of the corners at the end of the same, but against the middle point of the curved edge of the blood half. In this manner the little tube with the blood is made to lie in the centre of the rectilinear chamber, which the partition wall touches at the bottom of the vessel.

The blood pipette should not be permitted to remain quietly in that position under water, but a gentle motion should be imparted by a judicious guidance of the little wire to which the pipette is fastened; that is, the little tube should be moved backward and forward along its own axis as far as the dimensions permit, and in this manner be moved to and fro over its fluid contents.

It is easily seen that these movements are directed to produce a speedy solution of the contents of the tube with the surrounding fluid. It is also readily seen how important it is that no time be wasted in the proceedings following the taking of the blood, but rather that all should be arranged as quickly as possible without neglecting carefulness and exactness of execution. For the rest, the caution not to work more slowly than necessary, refers only to the manipulations. These motions are so easy and simple that even an unskilled hand will need not more than one minute for their execution. That much of time may pass without endangering the result in determining the amount of hæmoglobin.

All depends upon the blood being mixed with a certain quantity of water sufficient to dissolve it before it coagulates. The shorter and broader the capillary, the more rapidly the blood in the graduating capillary will mix with the surrounding water. The volume of blood used for measuring will be determined with greater exactness, the longer and narrower the graduating capillary is. The most advantageous length and breadth of the blood pipette is that which permits a rapid mixing of the blood and water with a sufficient exactness in determining the volume. My experience permits me to give a warning against the use of blood pipettes, however well gauged, which are shorter than 7 mm., or longer than 10 mm. Moreover, the edge of the blood pipette must be rounded, must be allowed to shape itself in the flame, but neither of the openings should be contracted nor narrowed.

As soon as most of the contents of the blood pipette has entered the water, the pipette should be withdrawn by the little wire and held in a vertical position over the same, so that the lower opening of the tube in the centre of the blood half of the comparing vessel may be suspended several millimetres above the surface of the liquid. Then, with the other hand, seize the drop-pipette which has already been filled with water, and allow drop after drop to enter the upper end of the blood capillary. By this means not only the contents of the blood capillary, even to the very last traces of blood in the comparing vessel, are cleansed from it, but the traces of blood clinging to the surface of the capillary, and which were lifted from the comparing vessel, are again washed back.

If the drops which have detached themselves from the lower end of the graduating capillary are observed, it may be seen how rapidly the blood drops disappear, and how clear even the fifth or sixth of these drops is. This is also shown under a careful examination by a graduating tube perfectly clean, both within and without, perfectly smooth, and filled as well as washed with clear water. Care must also be taken that no concretions or foreign substances be on or between the coils of the wire which winds about the blood pipette and serves as a handle. Only when all is declared perfectly clean and free from blood, may the blood pipette be wholly removed from the comparing vessel.

The blood half of the comparing vessel, after the graduating tube has been rinsed, should not be much more than half full, never more than three-quarters full of the liquid, first in order to make a thorough mixing of the contents possible, and second in order to permit of a last stratum of pure water above the blood solution. This portion of water renders the overflowing of the partition wall an immaterial instead of a ruinous occurrence. The liquid in the blood half may now be moved with perfect freedom, a thin wire being used to stir it. In the absence of a wire, the handle of the blood pipette may be used; but in this case the loop which forms the end is an inconvenience, since it prevents the wire from reaching the corners at the bottom of the vessel. And exactly these corners, as well as the angles formed by the bottom and the walls, as also those formed by the partition wall and the mantle of the half cylinder, are the favorite sites of very concentrated parts of the solution. The particles of blood may be so slightly dissolved that no complete dissolution of the hæmoglobin in the water, and even no perfect destruction of the stromata of the red blood cells has taken place in order to secure the hæmoglobin in the solution, in consequence of which the liquid appears turbid. The angles and corners are to be noticed especially, and should be continually observed until neither inequality of color in the liquid in the blood half of the vessel, nor the slightest turbidness can be detected. This of course takes place while the light shines through it, since the vessel has already been set into the instrument (Hæmometer).

When these things have all been arranged, it is time to proceed to the filling of the blood half of the comparing vessel. It is not worth while to rinse back into the vessel the very small portion of the blood solution which clings yet to the end of the wire used to stir it. Pure water from the pipette is then dropped into the blood solution, care being taken that the liquid in the vessel is disturbed as little as possible. With a little practice it may be risked to allow the last quantity of water to flow in, instead of being dropped, while the end of the pipette is dipped slightly beneath the surface of the liquid. The blood half and also the wedge half should be filled to the level of the rim, so that no meniscus may occur, but the liquid in both halves may have a common, absolutely level surface. Only in this case does the partition wall appear in the projection as a parallel limited black stripe, of a thickness corresponding to that of the partition wall. If the liquid in either half or in both halves has a meniscus (positive or negative), the dividing line appears distorted, widened in the centre or at both ends, cut by fine glistening white lines, also widened and following the line of the rim in several bands. In a similar manner a colored field, covered by a meniscus, semi-circular in the interior, and a distortion of the boundary with a contraction of the colored surface brought forward for comparison is discovered; although in a lesser degree, this is nevertheless still perceptible just as is the distortion which the picture of the partition wall suffers in consequence of a meniscus. This also affects the exactness and the reliability of the final result. The simplest method of avoiding this defect arising from the presence of the meniscus, is to bestow the requisite amount of attention and care in procuring a perfectly level surface of the fluids in each half of the vessel. Although this task may be disagreeable it should not be called difficult, since circumstances permit an approach to this end from both sides, and also since the transgression of the proper limit does no great injury. This of course is obvious in regard to the wedge half; for the blood half the same holds good according to what has already been said. Proceed with the same care in case withdrawal of the surplus liquid is necessary from the blood half. That is needed in adding the last portion of water to this half, as every current may lead to a mixing of the upper and lower layers of water. This surplus of water may be removed either by means of thin glass capillaries or by filtering paper. In either case avoid dipping too deep into the water. The wetting and overflowing of the partition wall may be avoided, when this edge has been greased beforehand.

A second method of eliminating the meniscus presupposes the fulfilment of the instructions given above. This method provides purposely a distinct meniscus for each half, or in case of the overflowing of the partition wall, which is here very probable, fills it until the whole surface forms a convex meniscus. Then place a small cover-glass over the opening of the vessel that no air bubble may be inclosed and without allowing the upper side of the cover to become wet. It is also necessary to avoid any approach to a stronger current in laying on the cover-glass just as one would reasonably regard the course of an expected current.

In the examination of human blood, notwithstanding the considerable quantity added, it is only on very rare occasions that merely an imperfect dissolution of the elements contained in the blood take place, and in consequence of which there is a certain turbidness of the liquid, so that a physician in his practice will scarcely ever find himself disturbed by this annoyance. On the contrary, in the examination of animal blood where red blood cells sometimes carry granules, one must be all the better prepared for an imperfect solution and a persistent turbidness in the water. In all such cases the rule of Mr. Leichtenstein is to add a minimum quantity of caustic alkali. This is an excellent rule. Indeed, this investigator praises the effectiveness of fixed alkalies in almost imperceptible doses in every case of protracted turbidness of a stronger and more of a leuchæmic conditions of human blood. By this he refers to a pathological condition, where there is a decided increase of colorless (white) blood cells, and to the great resistance of the same to the effect of water. I know from experience only the clearing effect of this method in thinning blood whose turbidity is the result of the resistance of the granule conveying red blood corpuscles to the effects of the water.

The cases for which Mr. Leichtenstein recommends his method are very different from the cases in which I used his method with such excellent results, and I was not as yet in a position to observe the clearing effect in the thinning of the leuchæmia human blood. But this by no means deters me from unreservedly recommending this method in all such cases of protracted turbidity as have been investigated by Mr. Leichtenstein, and, of course, cases of leuchæmia and leucocythæmia may present themselves to a practising physician.

There are indeed conditions so simple and so universal that the certainty which the word of a reliable observer gives cannot be increased or diminished by repeated assertions.

The testing of a definite blood solution is a task of so great precision that in the unanimous reports of all the different universities conducting experiments, the various reports of one or more persons in the same blood test never varied more than one per cent.

The more deeply the blood solution to be tested is colored, and the thicker, accordingly, that part of the glass wedge which is of the same color, the more light the dull white reflector will throw through the comparing vessel.

If one is aware that the blood is normal it is best to give the reflector such a position that as much light as possible will be thrown upon the lower surface of the vessel. But in such cases where the thinner parts of the wedge are brought into use, that position of the reflector must be sought which supplies a sufficient degree of brightness.

The universal results from the Hæmometer are, the sharper and more exact the smaller the degree of brightness used in obtaining them.

The observing eye must be brought at a certain distance, perpendicularly over the comparing vessel, the other eye must be closed. It is also recommended to place between the observing eye and the comparing vessel, tilted upon the latter and standing upright upon the table slab of the Hæmometer, a cylinder of paper or pasteboard. The length of this cylinder must, of course, be suited to the sight of the observer. It will do no harm to have the inner surface of the cylinder painted black. The observance of the following rules is of the greatest importance:

The observer should not place himself in a position toward the Hæmometer such as he would, for example, assume in the use of the microscope, but should place himself in the same plane with the partition wall of the comparing vessel. The consequence of this is that the picture of both, according to their color and brightness, with comparative exactness semi-circles upon the retina, lie beside each other, not, as in other cases, one upon each other.

But the comparison of the degrees of brightness is much more exact when the impression is made upon the right and left halves of the retina, than upon the upper and lower halves. Such is the case for the following reasons:

If one excludes the most peripheral portion of the retina in cases where there is a difference in the shape of the nose root on the temple side of the retina. The right and left halves of the retina of an eye are generally during the whole life affected by light and shade to the same degree. In other words, they are blended in the same degree, and consequently are equally sensitive to light. The upper and lower halves of the retina, on the contrary, are subject to the effect of light in essentially different degrees, in that the picture of the firmament, which in general represents by far the brightest part of the range of vision, is always wanting in the lower half of the retina. Thereby it is kept more nearly blinded; that is, less sensitive to light.

The observer must also take care that the observing eye is not affected by rays from the light which illuminates the Hæmometer. For in this case, in consequence of the lights penetrating the tissues (tunice) of the eye, a similar inequality between the two sides or retina halves may result, such as we have just found in the halves of the retina lying one over the other.

The real work now is to focus the Hæmometer. This is done by moving the glass wedge by means of a large hand piece back of the column until the difference in the appearance of both halves of the comparing vessel has disappeared. This movement, as soon as the neighborhood of the real graduating point is reached, should be backward, and by short, quick strokes, rather than by a constant slow motion.

The paths of the wedge as it is shoved from one side to the other over the proper point should be gradually shortened; in this way the distance traversed is lessened while the decision vacillates, until one has at last decided upon the graduation.

As it is advisable to look often rather than long into the instrument, so also when the graduation point is supposed to be determined the eye should be averted for a short time either by closing it or by looking at some dark surface, and then both halves of the vessel should again be compared. If there be the slightest doubt, the perfect equality of both halves should again be sought by short backward movements of the wedge, until at length further observation can detect no change in the decision either as to the purport or as to the exactness.

The sense of perfect exactness and unconditional correctness of the decision will be experienced in each case at the same time with the conviction that the greatest care and attention has been given. In the use of the Hæmometer, which is so simple that it must be intelligible, the conscience of the observer will in every case tell him of how much confidence he has made himself and his observations worthy.

But when the observer has been able to reach only a hesitating and unsatisfactory decision, it cannot always be attributed to want of conscientious care and attention.

There are persons who, although they are not exactly red blind, nevertheless have a retina very sensitive to long undulations of light, and to such persons the graduation of the Hæmometer not only presents a certain difficulty while it does not allow them to reach a positive conclusion satisfactory to themselves, but according to the few experiments of which they were hitherto capable, it seems that such persons graduate the Hæmometer about one-fourth too low, that is in the examination of normal human blood at about 75 per cent.

Whether such persons can use the Hæmometer to advantage, and to what extent, and under what conditions, are questions to which the preceding experience can give no definite answer, and whose solution remains for future investigation. Still I wish to express, *a priori*, the following conjectures:

In those who are severely suffering with red-blindness whenever their retina are carefully studied and accurately observed, it is found that the same anomaly exists in all. Such cases afflicted with red-blindness manifest a functionary defect of the sense of color.

I consider the validity of the same course of reduction-quotients for the totality of the red-blind even more probable than the validity of the same quotient for the whole extension of the Hæmometer-scale, every graduation made by one who is red-blind in any definite direction upon the Hæmometer-scale, always through this, one quotient should be changed into the corresponding graduation of the normal eye.

The inability to see red in its proper degree of intensity seems to be a functionary defect of the sense of color, which occurs in all degrees between the normal eye and the total red-blindness. And I am not as yet convinced that in all the cases the defects extend to and spread wave-like or in a constant ratio over those lying within the defect.

Under such circumstances it seems to me to be highly improbable for red-examiners to have such a common factor of reduction such as we have observed for the total red-blindness may exist.

In contradistinction to the above-mentioned rare cases of eyes that are not at all able, or only to a certain degree able to use the Hæmometer, there are many observers whose sense of color is in the beginning, or at least after a little practice, so keen that they are able to detect with the greatest exactness the inequality of the coloring in the part of the wedge suddenly made visible through the comparing vessel. Of course the difference in the thickness of the wedge at both ends of a piece in a position of the same visible at the same time is not less than 0.9 mm., therefore the difference in the graduation of normal human blood amounts to about 18% of the central thickness of the wedge. Yet it has been said that every observer is not capable of detecting the corresponding variation of the color in the thickness of the red glass. Together with the ability to distinguish such slight differences in the intensity of the color, there is combined a real advantage in the use of the Hæmometer. Such observers are able in graduating to seek that position of the wedge in which at the end of the partition wall of the comparing vessel the blood half is more deeply colored than the wedge half; at the other end the wedge half appears darker than the blood half. Between these there must of course be a point at which the intensity of color is the same on both sides of the partition wall, and this point must be in the centre of the partition wall if the increasing variations are alike at both ends. To carry out this arrangement the division of both halves of the colored circle into three subdivisions (so that there are six in all) by means of two thin black straight lines perpendicular to the dividing line and dividing the latter into three equal parts, is advantageous.

I believe that I not only anticipate correctly the surprising effect which these directions for the use of the Hæmometer will probably have upon the most of my readers, but that I will also find this impression well founded by the evident incongruity between the small number, the simple character, and the rapid execution of the proceedings demanded in Hæmometer measuring on the one hand and on the other, great number of rules and instructions which I have given above. Since all should be alive to the importance of the cautionary rules for the correct execution of these proceedings, it cannot be otherwise than that every one will find in these instructions much that he already knows or considers self-evident, but it may also be that each will find something new or something which he himself would not have arrived at. The purpose in giving at length these rules is to enable each possessor of a Hæmometer to use it without fruitless attempts. In the very beginning he should make useful and reliable measurements. The purpose could be fully carried out only by a complete enumeration of all possible rules that might be considered.