ul judge and jury would not improbably have felt that a higher tribunal had already visited him with a heavy punishment, and have dealt with him very leniently. As a matter of fact he was a particularly daring and dangerous criminal, and had during a previous imprisonment attempted the life of the prison surgeon. He received a long sentence.

THE CROONIAN LECTURES:

A CONTRIBUTION TO THE STUDY OF THE BLOOD AND THE CIRCULATION.

Delivered before the Royal College of Physicians of London.

By GEORGE OLIVER, M.D., F.R.C.P.

LECTURE III.—THE BLOOD.

In this and the following lecture I propose to submit the results of some observations made with the view of inquiring into the physiology of the red blood corpuscle.

[After describing the methods in use for the estimation of the oxyhæmoglobin and the number of blood corpuscles, Dr. Oliver said that preliminary observations demonstrated that they were not sufficiently precise for an inquiry into the physiology of the red corpuscle.]

THE APPARATUS EMPLOYED IN THIS INQUIRY.

1. *The Hæmoglobinometer.*—As already stated, the method is founded on the colorimetric principle. But I have modified the applications of that principle as hitherto adopted in the following ways.

(1) *Reflected (or Doubly Transmitted) Light preferred.*—It seems to me that certain advantages are secured by comparing the sample of blood and the standard by means of reflected rather than by transmitted light. In the first place, the depth of colour when observed by double transmission of light is nearly twice as much as it is when viewed by single transmission, for the blood solution is seen through a medium of the same colour induced by the incident rays, and consequently the colour of the reflected rays is proportionately increased. Hence a much smaller measure of blood is required under reflected light to produce the degree of colour made apparent by transmitted light; and it is obvious that in clinical work such a reduction may sometimes prove a distinct advantage, as when 5 c.mm. of blood amply suffice in place of 10 or 20 c.mm. Then, again, light reflected lends itself much more satisfactorily than light only once transmitted to the attainment of uniform physical conditions in the strata of the blood solution and of the standards, and in consequence of the exactly equal adjustments which thus become possible the finest differences in depth of colour become recognisable, and the readings can be made with an exactitude which cannot otherwise be obtained. This outcome of the improved definition has tended greatly to reduce that unavoidable variable element in all observation, the equation of the observer.

(2) *The Uniform White Background.*—The use of reflected light introduces a difficulty in regard to the reflecting background; for this must be uniform, not only in the same instrument, but in other instruments that may be made. The slightest variation in shade or tone of colour must produce discordant readings. In the earlier observations opal glass was selected for this purpose; it was soon discovered that different samples of it were far from invariable in colour. It therefore became necessary to select some white substance that could be relied on for uniformity and purity of colour; and dry finely-powdered precipitated lime sulphate was chosen. This substance forms a most delicate reflecting surface, and along with the invariable position of it immediately under the blood solution, and the standard colours contributes considerably to the exactitude of the readings.

(3) *The Standard.*—It is a matter of great practical importance in constructing a hæmoglobinometer to determine how the standard should be presented, so as to obtain the most definite readings. It has been found that all doubt as to the position of any particular sample of blood on the scale is entirely obviated when the latter is split up into a number of definite gradations; and this mode of disposing the standard has therefore been adopted. Each grade becomes a subordinate standard definitely recognisable from its neigh-

bours; and by means of a simple method to be subsequently explained, the intervening units are read. Moreover, there exists another reason why each stage ought to be separately standardised, and why a mere gradation of the same colour is not only indefinite, but is misleading, and that is the specific colour curve of the blood which will be presently described. The standardising involves two steps—(a) the adjustment of colour, and (b) the determination of the scale.

(a) *The Adjustment of Colour.*—It was in the first instance decided that the closest approximation to the colour of the blood solution was obtained by a combination of carmine, picro-carmine and hæmatoxylin; the blood and the standard solutions being observed by candle light in cells having a background of opal glass. After the lapse of some few weeks it was obvious that the colour of the standard was undergoing a change, especially in the lower grades, which were becoming darker and brownish. It was found that all the constituent pigments were unstable when exposed to daylight, and especially to direct sunlight. Having found that of the three constituents hæmatoxylin was the most fugitive, it was excluded. Still the carmines became slowly affected by the exposure incidental to work, and observation had to be made *in camera*. Another difficulty was encountered. The gradations of the standard were made by diluting the carmine solution of the highest grade to the required degrees. When, however, the blood solution, which exactly matched the topmost gradation of the scale, was diluted in exactly the same manner, marked discrepancies were apparent between these dilutions and the standard gradations; especially was this the case in the lower half of the scale. A satisfactory explanation of this discrepancy has been afforded by Mr. Lovibond, who has shown that every coloured substance when progressively varied in concentration or in strata thickness furnishes a colour-curve peculiar to itself, a curve of colour composition which he designates "the absorption colour-curve" or "specific colour-curve."¹ According to this explanation, the specific colour curves of the solutions of the carmines and of the hæmoglobin, though coinciding at a certain degree of concentration, diverged below that point, and thus the disparities between them became more and more apparent as the dilution increased. This view of specific colour curves derives some support from the spectroscope, for it is known that the position and the amount of absorption correspond with the percentage solution of the coloured substance under examination. It therefore follows that no one substance or combination of substances can correctly furnish a percentage series of gradations of hæmoglobin.

In as few words as possible I will endeavour to convey an outline of Mr. Lovibond's mode of determining colour; for the necessary details I must refer to his work on the subject,² and to various contributions he has made to the societies on the applications of his method. The outcome of his work is the construction of a system of measuring colour which is now being adopted in various trades in which colour is a leading indicator of the quality of produce or material, and also in some forms of analytical work, such as in the estimation of lead in potable water,³ of carbon in steel, etc., and in the testing of colour blindness.⁴

THE TINTOMETER.

The matching and the measurement of colour is effected in a camera apparatus (the tintometer) by the aid of three sets of definitely-graded glasses, one for each of the dominant colours—red, yellow, and blue. The gradations are of equivalent value in all the sets, the test of equivalence being the destruction of all colour and the production of grades of neutral tint by combining the equivalent glass of each set, the observation being made with white light. When the equivalent glasses of two of the dominant colours are combined, the three subordinate colours are

¹ Colorimetric Analysis, or Colour as a Means of Quantitative Estimation, by J. W. Lovibond, *Journ. of Soc. of Chem. Industry*, 1894; and The Absorptive Method of Colour Analysis, by J. W. Lovibond, *Journ. of Soc. of Dyers and Colourists*, 1894-5.

² *Measurement of Light and Colour Sensations*, by Joseph W. Lovibond, F.R.M.S., The Colour Laboratories, Salisbury.

³ It is claimed that so minute a quantity of lead as one-millionth of a per cent. may be detected by Mr. Lovibond's method.

⁴ When I set up my standards with Mr. Lovibond's standard colour glasses I was not aware of any practical hæmometric work having been done with Mr. Lovibond's tintometer. I have since ascertained that Dr. Copeman has been using it for this purpose during the past few years with much satisfaction.

produced, namely, green, blue, and violet. All other shades and degrees of colour are obtained by combining the glasses of the dominant colours in non-equivalent proportions, so that any colour can be accurately matched by them.

The colour scales are made up of units and decimal parts of a unit down to 0.01, which is the lowest fraction of a unit that can be differentiated by the vision. The fixing of the unit has necessarily been arbitrary, as has, indeed, been that of any standard measure, such as the mass of distilled water which has been chosen to represent a gramme, etc. Inasmuch as the unit chosen complies with the requisites of a standard—being uniform, recoverable, and arbitrary—it should be accepted as such; the actual dimensions of it, so long as they serve the practical ends in view, are of no importance. Of course the correct grading of the glasses from the original standards is essential to the success of the method, and this is, I believe, effectually attained by an elaborate system of cross-checking by a series of trained matchers. These graded glasses of determined value provide a numerical quantitative measure of any colour to be gauged, and they enable the measurements made by them to be recorded, so that the colour thus measured may be reproduced at will or may be preserved indefinitely.

APPLICATION OF THE TINTOMETER TO THE ESTIMATION OF HÆMOGLOBIN.

With the view of fixing the blood standards I had the advantage of working in the colour laboratories of Salisbury, and of the valuable assistance of Mr. Lovibond and of his well-trained staff.

Samples of blood, taken at different times of the day, were diluted with distilled water, and standardised by my carmine standard of the highest grade. The colour of the different percentage dilutions placed in the blood cell was then carefully matched and determined by the graded glasses—finely powdered precipitated lime sulphate forming the background of the cell and of the glasses; and the estimations were made by aid of the camera tube presently to be described.

The standard gradations of the apparatus were thus settled, and they form the two specific colour curves of the blood—one determined by uniform candle-light and the other by white daylight. Here are the two sets of standards, and you will observe that the difference between them is so remarkable that it is obvious that hæmoglobin cannot be estimated by the same standard used indifferently in the two kinds of light. The colour curves were made by entering the colour units on the side of the diagram to form abscissæ with the standard gradations which appear at the foot.

In daylight the specific colour curves of the blood are made up of varying proportions of red, orange, and yellow. In the lower percentages (10, 20, 30, and 40) red as a separate colour does not exist; it is only present in combination with yellow as orange—which is made up of equivalent proportions of each. In these lower gradations the place of red is taken by yellow. Between gradations 40 and 50 yellow dies out, and then red appears, and progressively increases until at the highest grade it is the dominant colour. In these daylight gradations the curve of orange is the predominant one, though it begins to die out in the higher part of the scale.

In candle-light the colour curves are quite different, and are less complicated. Red is predominant throughout, except at the lowest grade, where it is subordinated to orange. From this point upwards orange gradually diminishes, and vanishes entirely at 90, and then pure red distinguishes the highest grades.

The remarkable difference between the colour curves furnished by the two kinds of light is doubtless due to the preponderance of yellow emitted by the candle flame.

The standard colours and the gradations having been duly measured and determined, there still remains a further adjustment to be made. It is found that the solution of blood possesses in a remarkable degree the quality of colour purity or brilliancy—a quality which is distinct from colour depth and colour composition, for it remains after these have been duly matched.⁵ Inasmuch as this property of the blood may prove a distracting element with some observers, and may possibly lead to errors in observation, it is important that it should be eliminated from the view, especially as Mr. Lovibond's system of graded glasses supplies the remedy. It is met by using one of the lower grades of the blue glasses as a cover glass to the blood cell; and it has been found that this adjustment does not disturb the correct reading of the hæmoglobin.

THE DETERMINATION OF THE SCALE.

The quantitative equivalence of the scale in oxyhæmoglobin has been determined. The blood of the dog was preferred to that of man for this purpose, because the process of separation is comparatively easy, while in the latter it is in many respects unsatisfactory. Great difficulty was, however, encountered in obtaining a pure product which retained the exact colour of freshly drawn blood, so that accurate estimations of it could be made. Ultimately this result was attained by determining certain percentages of the moist crystals and afterwards correcting for the water.⁶ Each degree of the scale is equivalent to 0.155 per cent. oxyhæmoglobin in the sample of blood under examination. Therefore 100 degrees represent 15.5 per cent.; and determinations above or below that point will require the addition or subtraction of 0.155 per cent. for each degree. It may be mentioned that Preyer

(*Die Blutkristalle*) gives the average percentage of hæmoglobin for women as nearly 13, and for men 14. Dr. Augustus Waller (*An Introduction to Human Physiology*, 1893, p. 35) has found 17 per cent. in the blood of the dog. Observation has shown that the mean percentage of hæmoglobin present in the blood of man in health containing 5,000,000 corpuscles per c.mm. (or 100 per cent. of corpuscles) is represented by 100 degrees.

From the first it was found that a reliable judgment between the two colours of the blood solution and of the standard when closely approximated could not be formed unless the colours were observed through a tube so arranged as to shut off all side lights and other causes of distraction. The best results were obtained by using a long tube—resembling a microscope tube—blackened inside, and presenting below two circular holes of 5 millimetres in diameter, so as to include only a small but equal area of each of the colours to be compared; and encircled above by a diaphragm or collar which shields the eyes from the direct rays of the candle flame. The tube is held in a vertical position over the colours by means of a side stand, and it may be adjusted to the required height so as just to avoid any shadow which may be cast by the rays of the candle flame impinging on the lower end of it. A piece of green glass is let into the collar, for it was found that when the sense of colour appreciation was impaired from any cause—such as long and frequent observation—it was restored, or even intensified, after looking at the candle for a few seconds through the green glass.

The Apparatus.—A few words remain to be said on the parts of the apparatus and how to use them. These are (1) an automatic blood measurer; (2) a mixing pipette; (3) the blood cell and cover glass; (4) the sets of standard gradations; (5) the riders; (6) the camera tube; (7) the light; (8) a bottle of antiseptic fluid, the prickers, needles and thread.

1. *The automatic blood measurer* has a capacity of 5 c.mm., and fills readily by capillary attraction. It differs from the fragile capillary measurer hitherto used in being made of stout glass, and therefore serviceable in being larger and more manipulable, and in having the end which is presented to the blood well polished, so that all traces of blood can be entirely removed from it by the finger. The bore is dried out before an observation by passing a needle threaded with darning cotton through it. The handle is useful for stirring together the blood and water in the blood cell.

2. *The mixing pipette* is provided with a rubber nozzle which fits over the polished end of the blood measurer, and ensures the complete rinsing out of the blood with the first few drops of water.

3. *The blood cell* is of more than sufficient capacity to ensure the complete liberation of the hæmoglobin. It yields a blood solution of rather less than 1 per cent. when filled level with the rim. It is itself the measure of the amount of water to be added, and it is quite easy to fill it accurately.

4. *The standard gradations* are arranged as circular discs in two slabs, six in each, and they represent the divisions of 10 degrees of the scale from 10 to 120 inclusive.

5. *The riders* provide for the reading of the degrees intervening between each of the standard gradations. When they are used the slip of coloured glass is placed over the cover of the blood cell so as to balance the effect of the mere layer of glass of the rider which is laid over the standard. Two sets of riders have been arranged—one suitable for physiological observation, to ensure the finer readings, for example, of one degree; and the other, sufficient for ordinary clinical observation, to enable the observer to determine differences of $\frac{1}{2}$ degrees. For the reading of the units nine riders are required which are grouped into three slides. It should be premised that the value of the riders is neither the same in the two standards required for candle-light and daylight, nor in the upper and lower halves of either standard. This want of uniformity arises from the differences of the specific colour curves in the two standards, and in the two portions of each. In the six lower grades of both standards the value of each rider is double that in the upper six gradations. In the transition grades, the lowermost of the upper half and the uppermost of the lower half, this rule does not accurately apply; but inasmuch as the departure is constant in all observations, and is, moreover, slight, it may be disregarded for the sake of simplicity in using the riders. The daylight standard is less adapted to the finer readings than the candle-light, because the value of each rider when used with it is doubled—that is, rider No. 1 becomes equal to 2 degrees in the upper half of the scale, and 4 degrees in the lower half. Therefore the candle-light standard is preferable for such readings; and when it is used each rider has an equivalent value in the six stronger grades, 2 = 1 degree, and a double value in the six weaker grades, 1 = 2 degrees. For ordinary reading one rider only is used—namely, that which is equivalent to 5 degrees in each slab of the standards. Therefore each set of six standard gradations, whether for candle-light or daylight, has its own rider. When the blood solution is deeper in colour than any particular standard gradation, but is overstepped by the rider, the mean between the two may be taken as the reading; and the same rule will apply when the colour of the blood is higher than the rider, but is not so high as that of the next standard grade above. Hence this single rider may be made to provide readings of 2.5, 5, and 7.5.

6. *The Camera Tube.*—It has already been pointed out that observation should only be made through a camera tube, and the one which has been employed throughout this inquiry has been described. It is, however, perhaps somewhat elaborate for the ordinary course of clinical observation; therefore one of simpler construction is provided which may serve this purpose, and, being collapsible, will pack into a small compass with the other parts of the apparatus.

⁵ Mr. Lovibond tells me that this fact is occasionally observed in the matching of other substances, notably some of the aniline dyes.

⁶ In carrying out this part of the work I must express my indebtedness to Mr. Moore, of the Physiological Laboratory of University College, for his valuable aid.

7. *The Light.*—For observation by artificial light it has been found that the small-sized wax candles known as Christmas candles are the most satisfactory in affording the most suitable intensity of light, and one, moreover, which is sufficiently uniform. The position of the candle is important. It should be such as to shed an equal light on the blood cell and the standard, and also such as to furnish a high light, so that the flame should be 3 or 4 inches above the cells. The distance must be regulated by the observer, who will soon learn how to adjust it to the best advantage, so that he may match the colours with greatest certainty and accuracy. The actual distance does not affect the reading; but when the candle is placed too near the points of observation the illumination becomes too strong and is somewhat distracting—especially when the lower grades are under observation, which require less degrees of light than the higher. In daylight all colorimetric modes of estimating hæmoglobin are less or more unsatisfactory, for they require a uniform degree and kind of light which cannot always be obtained when daylight is resorted to. The colour of the blood solution varies in a marked degree according as the light is blue or white, or is feeble or waning, or is strong; therefore the candle-light standards are to be preferred as being always available with a uniform light.

8. *The Antiseptic Fluid and Pricker.*—Before every observation the pricker should be dipped into an antiseptic solution that will not corrode the metal, and a little of the solution should be smeared over the part to be pricked. I prefer a mixture of equal parts of carbolic acid and spirits of wine.

HOW TO MAKE AN OBSERVATION.

The bore of the blood measurer is first of all dried out by passing through it a needle threaded with darning cotton; and then the polished point is presented to the drop of blood which flows, under the least possible amount of pressure, on pricking the inner side of the pad of the finger or near the root of the nail. Care must be taken to see that the pipette is really quite filled, and, if it has been necessary to reapply it to the drop, it should be observed whether there is a break in the column of blood. Any blood adhering to either end must be carefully wiped away with the finger. The rubber nozzle of the mixing pipette, charged with water, is adjusted over the polished end of the pipette, and the blood is washed into the blood cell by pressing through the water drop by drop. The handle of the pipette is then used as a stirrer, and the further additions of water are made to impinge on it—for it serves to graduate the size of the drops required to accurately fill the cell. It is in this way quite easy to do this when the observer catches the reflection of the light on the surface of the fluid. A final thorough mixing with the handle will be required, and perhaps another slight addition of water may be necessary to secure a level filling. The cover glass is then adjusted, when a small bubble is sure to form—a sure sign that the cell has not been overfilled. The blood cell is then placed by the side of the standard gradations, and the eye quickly recognises its approximate position on the scale. Then the camera tube will more accurately define it. If it is found that the blood solution is matched in depth of colour by one of the standard grades, the observation is of course at an end; but if it is observed to be higher than one gradation but lower than that above it, the blood cell is placed opposite to the former, and the riders are added to complete the estimation.

THE HÆMOCYTOMETER.

The Principle Followed.—The method is based on the following facts:

(a) When a candle flame is viewed through a glass tube containing water, a transverse line of bright illumination is seen, consisting of closely packed minute images of the flame produced by the longitudinal fibrillation of the glass. In the process of drawing out the tube becomes minutely corrugated or fibrillated in the direction of its axis. The corrugations are extremely small, but they are easily observed when a tube is held before a flame and the structure of the glass is examined by a pocket lens, when the corrugations are seen to produce alternate bright and shaded lines disposed in the vertical direction. Each corrugation acts as a lens, hence the horizontal line made up of a congeries of images of the flame.

(b) When some fixing fluid (such as Hayem's or a solution of potassium ferrocyanide, sodium chloride, etc.) is added to blood, the mixture presents a certain degree of opacity according to the amount of blood or of the fixing fluid.

(c) The opacity thus produced completely shuts out of view the illuminated line until, on diluting further, a definite point is reached, when it can just be detected as a delicate streak of light across the tube—the dawn, as it were, of the bright minute images of the flame seen when the opacity is absent or

is further reduced. Repeated observation has shown that the development of this delicate horizontal line by dilution with the fixing fluid selected is a very sensitive indicator of the percentage of the blood corpuscles. This was shown in various ways; for example, by the measure of the fixing fluid required to bring the line into view being always in proportion to the different quantities of the same blood experimented with, and therefore in proportion to the number of the blood corpuscles present at each observation.

The Apparatus.—The principle just described has been made available for determining the percentage of the cellular elements of the blood by mixing a definite measure of the blood with Hayem's solution in a graduated flattened tube. The apparatus consists of (1) a graduated flattened tube, (2) an automatic blood measure, (3) a mixing pipette, (4) a candle, and (5) a bottle of Hayem's solution.

(1) A flattened tube has been adopted because it was found that a round tube of such a diameter as to furnish the required degree of opacity will only provide a sufficiently wide scale when a much larger quantity of blood is taken than is generally used for observation of the blood, whereas when the observation is made through the long diameter of a flattened tube, it is not necessary to use more than 10 c.mm., and the scale is lengthened in proportion to the reduction of the lateral area. A tube of 15 mm. in the long diameter and 5 mm. in the short diameter provides a good workable scale of 12 cm. for the determination of 125 per cent. of corpuscles. The divisions of the scale represent 2 per cent., and they are sufficiently apart to allow the eye to read 1 per cent., or even less.

(2) The automatic blood measurer is the same in construction as that used in the estimation of hæmoglobin, only its capacity is greater.

(3) The mixing pipette is provided with a rubber nozzle which fits over the polished end of the blood measure.

(4) The candle should give a small flame, for that furnishes a more defined line than is obtained from a large flame. The small-sized wax candles known as Christmas candles are the best. A reduced gas flame also answers perfectly well.

(5) *Hayem's Solution.*—It was found that each kind of fixing fluid furnishes its own scale. Hayem's was selected as the most satisfactory. The chief advantages of it are that it is cleanly to use, that the corpuscles are less affected by it than by other fixing fluids, and may be examined under the microscope after the observation has been made without fear of material changes, and that the reading undergoes no variation after the lapse of many hours (for example, twelve), so that the blood may be washed into a tube and the observation may be made later on if more convenient. It may be suspected, however, that the proteids of the plasma may be precipitated by the mercuric chloride present in Hayem's solution, and thus a variable addition may be made to the opacity caused by the corpuscles. This point has been carefully tested, with the result that it does not present a source of fallacy; 50 c.mm. of the plasma separated from a blood containing 100 per cent. of corpuscles were washed into the tube with Hayem's solution, but only as far as the 20 per cent. mark, so that the solution contained twenty-five times as much of the proteids as would be contained in 10 c.mm. of the blood diluted to the 100 per cent. mark; and there was apparent the merest haze, so small indeed that it could not possibly be determined. It is therefore obvious that the proteids of the plasma do not increase the opacity induced by the fixing of the corpuscles by means of Hayem's solution.

The determination of the scale was made by the enumeration method extended to 120 squares applied to the blood of normal man; 100 per cent. presents the generally accepted standard of 5 millions per mm., therefore 80 per cent. is equivalent to 4 millions, etc.

THE METHOD FROM THE PHYSIOLOGICAL STANDPOINT.

The white corpuscles in a state of health do not affect the readings. The proportion of 1 white to 500 red, or $\frac{1}{500}$ per cent., is equivalent only to $\frac{1}{100}$ th of one of the smallest divisions on the scale, and this is unreadable. So that were the white corpuscles to increase to 1 in 250 such a rise would not appreciably affect the reading of the red corpuscles. The normal variation of the white corpuscles, therefore may safely be neglected.

THE METHOD FROM THE CLINICAL STANDPOINT.

I have employed this method in clinical observation, and found it not merely simple and time-saving, but useful. Apart from the leucocythæmic condition the white corpuscle is not a source of interference.

A distinction should be made between the method of enumeration, and a volumetric method like this or like that of centrifugal separation; for the aim of the former is to determine the individual corpuscle irrespective of size, whereas that of the latter is to refer the collective volume of the corpuscles to that of the normal standard. Pathologically it is important to ascertain the size of the red discs; and therefore the microscopical inspection of the fluid is a useful addendum to the observation. So long as the average size of the corpuscles is unaltered, the method is comparable with that of enumeration, on which it is founded. But, inasmuch as that in certain cases may vary—for example, the microcytes of

⁷ The formula is as follows: Hydrarg. perchlor. gram. 0.5; sodii chlor. gram. 1.0; sodæ sulph. gram. 5.0; aq. destill. 200 c.cm.

chlorosis,⁸ and the macrocytes of pernicious anæmia—the method should strictly speaking be regarded as a volumetric one, like that of centrifugal separation. It is highly probable that it is clinically advantageous to be able to estimate the corpuscles in any case by comparing them with the normal percentage volume employed as a standard. Such an estimation will probably afford a true gauge of the aggregate corpuscular value of the blood; for it is the collective capacity of all the corpuscles, whatever their size, that is measured by the physiological standard.

THE MODE OF OBSERVATION.

The pipette previously dried out by passing through it a needle threaded with darning cotton, having been accurately filled, the slightest traces of blood on the outside of the glass either at the ends or on the side are very carefully wiped away with the finger. The rubber nozzle of the mixer filled with Hayem's solution is then applied over the bevelled end of the pipette, and the blood is thoroughly washed into the flattened tube. The amount of the fixing fluid to be used in the first instance will be roughly suggested by the previously made hæmoglobin estimation; if this afforded from 90 to 100 per cent. or more, the dilution may at once proceed to the 80 per cent. mark, but if it yielded a lower figure the preliminary dilution should be smaller. The contents of the tube are then uniformly mixed by inverting the tube a few times with the thumb over its mouth, care being taken on removing the thumb to draw it over the lip of the tube, so as to restore as much as possible the fluid adhering to it. This procedure should, of course, be repeated after every addition of the diluting fluid. The observation is made in a dark room, or in a darkened room free from cross lights. The observer stands to the left of the flame.

In making the observation it is important to shut out as much as possible the diffused light of the candle. The most satisfactory way to do this is to place the lower end of the tube, with its long diameter in a line with the candle, in the concavity between the thumb and forefinger, so as to make, as it were, a frame for it, and then to bring this part of the tube quite close to the eye. The observer must look out sharply for the first appearance of the transverse line of light which dilution brings into view, and the earliest indications are obtained by turning the tube on its axis, when the line will be caught first of all on the side. The fluid is then added point by point until the line is just apparent, when the long diameter of the tube is directed to the flame. This indication is a very delicate one, and requires a little pains at first in order to clearly recognise it without overstepping the necessary dilution, which, of course, brings it more definitely into view. The first difficulty is the only one, for when the earliest appearance of the line has been once observed, it cannot fail to be afterwards recognised.

There is, as a rule, a rather rapid transition between the total absence of the line quite across the tube and the presence of it. Most observers will find no difficulty in defining their readings by stages of half a division, that is, 1 per cent. It is not necessary to hurry over each observation after adding more fluid and mixing up, for the opacity remains uniform for several minutes.⁹

THE METHOD FOLLOWED IN COLLECTING DATA.¹⁰

The blood of a large number of healthy subjects belonging to the average well-nourished class has been observed by these new methods with the view of ascertaining the influence of age, sex, and other general physiological conditions on the percentages of the corpuscles and hæmoglobin; but I regret that the limited time at my disposal does not permit me to use this material just now.

These rapid hæmometric methods have also been applied pretty widely to clinical work in order to determine the value of such observation in the general run of cases, whether these presented the ordinary signs of anæmia or not. The data thus obtained have shown that an extension of direct observation of the blood is likely to yield useful results; for oftentimes it has demonstrated the existence of moderate degrees of anæmia which had escaped ordinary clinical observation, but were sufficient to maintain a condition of low resistance and of instability of health apt to be referred to denutrition of the nerve centres rather than to that of the blood, and it has, moreover, often shown that anæmia was not present when the general symptoms suggested it. Furthermore, it has afforded evidence of the not uncommon occurrence of an apoplasmic state of the blood which is worthy of further observation and study.

A large portion of the physiological data has been derived from a long series of observations on the blood of

⁸ So far the only discrepancy which has been met with between this method and that of enumeration has been observed in chlorosis, in which when many microcytes exist the readings of the tube are somewhat reduced.

⁹ The corpuscles themselves, and the uniform distribution of them, may be observed as separate particles when, on turning the short diameter of the tube towards the flame, the contents are examined by means of a pocket lens. As some may wish to use this apparatus I hope the Tintometer Company, 6, Farringdon Avenue, E.C., after settling certain technical details to secure uniformity of production, will supply it.

¹⁰ In the collection of the physiological data I have had the able co-operation of Dr. Edgecombe, of Harrogate. Our conjoint results have not yet appeared.

two subjects, A. and B., the former 27 and the latter 54 years of age. In both cases the corpuscles and hæmoglobin have been determined night and morning for over seven months. Besides this long series of consecutive observations, innumerable others have been made in order to determine the effects of exercise, of digestion, and of various other physiological conditions on the percentages of the corpuscles and hæmoglobin. The most important part of the physiological data was collected under the conjoint observation of my co-worker and myself, while the subjects were placed in exactly the same physiological conditions. The results were not recorded until cross-checked by each of us. There was rarely any discrepancy between us as to the readings; and when any did arise it was trifling, and was adjusted to our mutual satisfaction. This fact shows two things—first, the advantage of two observers working together in making such observations as these, and, secondly, the smallness of the personal equation to be attached to the methods. Besides the special experiments referred to, a series of consecutive hourly observations was made during nearly three weeks in which the ingestion of food and liquid and the output of urine were noted.

Observations have also been made on animals. In the first instance dogs were used, but it was found that the thick hide renders these animals much less suitable for this kind of observation than man. Some satisfactory and reliable observations were, however, made on the blood derived from the veins on the outer surface of the rabbit's ear, showing the range of daily variation and the effects of oxygen and ether.

THE NORMAL READING OF THE CORPUSCLE AND HÆMOGLOBIN AND OF "THE WORTH" OF THE CORPUSCLES.

Normal corpuscles, 100 per cent., should contain hæmoglobin which reads as near as possible 100 per cent., so that when the latter is divided by the former the unit obtained will represent the normal mean value of the corpuscle in hæmoglobin. The attempt, however, to bring the two percentages into accord has not been by any means an easy task because of the wide physiological variation which is continually taking place in both. The present readings cannot claim to be more than an approximation derived from a large number of observations in healthy subjects. The quotient obtained has been aptly denominated by Dr. Archibald Garrod, "the worth,"¹¹ and by Dr. Haig, "the blood decimal."¹² In health the average variation extends from 0.95 to 1.05.

It will be found that the actual readings of the corpuscles and hæmoglobin in health, whether in the same subject at different times or in different individuals, is a pretty wide one. In men the average normal range embraces from 95 to 105 per cent., and in women from 90 to 100 per cent. In either sex, and particularly in men, a reading below 85 per cent., especially when the hæmoglobin percentage is still lower, is as a rule distinctly subnormal. The determination of the corpuscles, or of the hæmoglobin alone, is of but little physiological or pathological value.

[Dr. Oliver then related the result of a series of 1,200 consecutive night and morning observations made on the subjects A. and B.; the most striking fact was a rise in the percentage of hæmoglobin during the night and a fall during the day, which was observed in 90.5 per cent. of all the morning and 88.4 per cent. of all the evening observations. The average night rise was 7.2 per cent., the average day fall 7.4 per cent., the maximum rise 20 per cent., the maximum fall 21 per cent. The night and morning variations in the percentage of corpuscles were similar, but not quite so marked. The data furnished by the night and morning observations showed that "the worth" rose in the night and fell in the day. The day curve of variation was marked by subsidiary curves of fall and rise due to digestion and absorption of food and by a steep decline during the last third of the waking hours. There were also variations from day to day, from week to week, and from month to month].

¹¹ *Trans. Roy. Med. Chir. Soc.*, vol. lxxv, p. 191. ¹² *Uric Acid*, 3rd ed., 1896.

THE PAN-AMERICAN MEDICAL CONGRESS.—The second meeting of the Pan-American Medical Congress will be held in the City of Mexico on November 16th, 17th, 18th, and 19th, 1896. The first meeting was held in Washington in 1893.