XVI. ON THE ESTIMATION OF SUGAR IN BLOOD.

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IN spite of the large numbers of determinations of blood sugar that have been carried out by various means in the past few years there is still need for a reliable and simple method.

Much work has recently been done with the picric acid procedure in which the depth of colour produced is estimated by means of the colorimeter. It is, however, a drawback to this method that a colorimeter is not always available especially for clinical work. Further, it is doubtful whether this method is at all applicable in the case of whole blood on account of the creatinine (and probably other interfering substances present) which, under the conditions of the estimation, give a colour similar to that produced by glucose in the presence of picric acid. De Wesselow points out [1919] in the following paper that these interfering substances are always active and in the case of human blood may actually influence the picric acid method to such an extent that the result obtained may be as much as 50 % too high. In plasma also the method appears to yield results on the high side, but here the figures obtained are probably accurate enough for practical purposes.

Bang in 1913 introduced a micro-method for blood sugar determination, but the difficulty of completely separating the protein and the ease with which oxidation of the cuprous oxide took place before and during titration interfered very materially with its practical utility.

A new application in which the tendency to oxidation of cuprous oxide during titration was completely overcome was introduced by the writer a few years ago. This method was first used in 1914 and was communicated to the Biochemical Society in autumn 1915. A few months after this communication was given, a method based on the same principle was published independently by Scales [1915] while my paper was actually in the press [MacLean, 1916]. This method gives excellent results and the fact that Bang [1918] adapted this principle to his own method speaks for itself. The application of the method for the estimation of blood sugar was described by me in the above noted publication [MacLean, 1916]. An extended use of the procedure, however, has resulted in various improvements and considerable simplification, and it is felt that the modified method as described in

the present paper not only gives very reliable results, but is so simple in its application that it can be carried out by students and can easily be utilised for clinical purposes.

PRINCIPLE OF THE METHOD.

In estimating the sugar in blood it is first necessary to separate the protein very completely. For this purpose various procedures were tried, but the use of dialysed iron in conjunction with heat coagulation gave the best results. In the new method the blood is heated in an acid saline solution and the greater part of the protein separated by coagulation. Any remaining traces of protein are removed by the addition of a small amount of dialysed iron. After cooling, the mixture is filtered and a perfectly clear protein-free filtrate obtained. The sugar in an aliquot part of this filtrate is estimated by boiling the liquid with an alkaline copper solution containing potassium iodate and iodide. After boiling, the solution containing the reduced cuprous oxide in suspension is cooled and treated with a slight excess of hydrochloric acid. This interacts with the potassium iodate and iodide liberating iodine equivalent to the amount of potassium iodate in the solution. At the same time the cuprous oxide is dissolved and cuprous chloride formed. A solution of the latter is very unstable and liable to oxidation, but this is prevented by the fact that free iodine is present with which it immediately reacts. Since the cuprous oxide present in the mixture at an earlier stage is not in solution but in suspension, no oxidation takes place during the preliminary cooling. During the boiling, any air present in the solution is driven off before the sugar has time to reduce the copper to any appreciable extent; also the flask is full of steam during this time so that no oxidation from air oxygen need be feared.

The amount of sugar present in the solution is calculated by ascertaining the amount of iodine used up as fully described later.

The only point in the method that requires special care is the regulation of the flame used to boil the sugar and alkaline copper solution. The tables given in this paper are worked out for a flame which brings the amounts of solution specified later to the boil in as nearly as possible one minute forty seconds. In this time the *boiling should be fully established*; often some bubbles come off 10 seconds or so before the boiling becomes brisk, but it is usually quite easy to ascertain the point of complete boiling to within a few seconds. For clinical purposes a rough preliminary standardisation of the flame is all that is required, but for very accurate work the use of a small manometer is necessary. When care is taken to follow the directions given the results obtained vary only within the limits of the ordinary experimental error of manipulation which of course is a personal factor. With pure solutions of glucose successive identical readings are obtained with monotonous regularity if the conditions are carefully observed. With blood also duplicate experiments yield practically identical figures (see below).

One detail in the method requires some comment. When hydrochloric acid is added to the boiled mixture of alkaline copper solution and cuprous oxide the acid reacts with the carbonates present and effervescence takes place. Since free iodine is present at this stage it might be expected that this effervescence would result in driving off some of the iodine. That this may be the case to some infinitesimal extent is probable, but I have satisfied myself that when ordinary care is taken not to agitate the mixture too vigorously until the effervescence passes over no appreciable amount of iodine capable of estimation is lost. This probably depends on the extreme dilution of the iodine solution.

For blood sugar determinations the method is applicable to 1 cc. or smaller amounts and descriptions of the procedure for estimating sugar in 1 cc. and in 0.2 cc. of blood respectively are given here. The use of such a small amount as 0.2 cc. blood has various advantages, since this amount can be easily obtained from the finger. For 1 cc. it is necessary to insert a needle into a vein of the arm, and if observations are required at frequent intervals this procedure becomes difficult for obvious reasons. On the other hand, quantities of 0.2 cc. blood can be obtained from the ear or finger as often as may be required.

In all blood sugar estimations it is important to remember that glycolysis is often very active especially for a short time after the blood is drawn; for this reason the specimen should be immediately measured and added to the coagulating mixture described and, if convenient, heated at once. By this means glycolysis is completely prevented. After standing for two hours at room temperature the amount of sugar present in the specimen may be less than half its original sugar content. Since the method of obtaining 0.2 cc. blood described later allows of almost immediate mixing of the blood and coagulating fluid, the use of this small amount has an additional advantage.

Detailed instructions for the preparation of the various solutions required are given below. An objection urged against the method was the difficulty of preparing an accurate $N/10$ thiosulphate solution, but this difficulty no longer exists since standardisation against potassium bichromate is now used.

SOLUTIONS REQUIRED FOR 1 CC. METHOD AND THEIR PREPARATION¹.

The solutions required have the following composition and are prepared according to the directions given.

A. *Solutions for removing protein from blood.*

No. 1. *An acidified sodium sulphate solution.*

Sodium sulphate (pure)	150 g.
Glacial acetic acid	3 cc.
Distilled water to	1000 cc.

¹ All these solutions may be obtained ready for use from the British Drug Houses, Graham Street, London.

Solution should be filtered through a starch-free filter paper (Whatman No. 1).

No. 2. Dialysed iron solution.

This solution should be about B.P. strength and well dialysed. The majority of the samples on the market are practically not dialysed at all and contain very large amounts of chlorides. A suitable preparation is stocked by the British drug houses under the term "Dialysed iron B.D.H."

B. Solutions for estimating sugar in filtrate.

No. 3. Alkaline copper iodide solution.

Potassium bicarbonate	20	g.
Potassium carbonate (anhydrous)	15	"
Copper sulphate crystals	0.7	"
Potassium iodate	0.11	"
Potassium iodide	1	"
Distilled water to	100	cc.

In making up the solution 20 g. of potassium bicarbonate are dissolved by gentle heating in 60 to 70 cc. of distilled water. The heating must be very carefully done and the temperature of the mixture should not exceed 37°. The potassium carbonate is then added. The copper sulphate is dissolved in a separate beaker in a few cc. of water and added to the mixture of carbonates without waiting for the potassium carbonate to dissolve completely. After the resulting effervescence has passed over, the complete solution of any remaining carbonate is accomplished by heating. The iodate and iodide are next added, the solution thoroughly shaken and made up to 100 cc. After filtering through a starch-free paper (No. 1 Whatman paper) it is ready for use. The solution keeps indefinitely. The copper sulphate and potassium iodate must be weighed with great care. The other ingredients need not be so carefully measured.

This solution must be standardised as regards its iodine content by taking exactly 3 cc. of the mixture and adding this to 10 cc. of the 15 % sodium sulphate acetic acid solution used for deproteinising the blood. To this mixture 10 cc. of 20 % hydrochloric acid are added. The flask is very gently agitated until effervescence passes over and then more strongly agitated for 1 minute. A solution of *N*/100 sodium thiosulphate is run in until the yellow colour of the iodine has almost completely disappeared. Two drops of starch solution are then added and the titration proceeded with until the blue colour disappears. The number of cc. of thiosulphate used up gives the equivalent of iodine liberated from the mixture and should be recorded on the bottle. For 3 cc. copper iodide solution about 8.8 cc. of *N*/100 thiosulphate are required. The exact figure obtained depends to some extent on the purity of the samples of iodate and iodide used, but variations due to this should be very slight. The samples used in my work give exactly 8.82 cc. thiosulphate = 3 cc. copper solution.

No. 4. (*N/10 thiosulphate solution.*)

Though sodium thiosulphate crystals can be obtained in a state of great purity it is generally stated that the salt cannot be weighed directly for the purpose of making a standard solution owing to the possibility of water or air being held within the crystals. This difficulty may be avoided by treating the finely ground crystals with pure alcohol and then with ether and finally drying at room temperature. This manipulation, however, is tedious and there is always some slight risk that the crystals may not have been dried in air for a sufficiently long time to give an ultimate product containing the correct amount of water of crystallisation. The preparation of an accurate iodine solution for standardisation by weighing the iodine is also a tedious procedure. To avoid all these difficulties, the sodium thiosulphate solution used for this method is prepared by standardising against an *N/10* solution of potassium bichromate. In preparing the *N/10* thiosulphate a solution of *N/10* potassium bichromate is first prepared by dissolving 4.913 g. of the pure dried salt in distilled water and making up the volume to 1000 cc.

The distilled water used for the thiosulphate solution should be boiled immediately before use to get rid of traces of CO_2 . About 26 g. of pure sodium thiosulphate crystals are dissolved in about 1000 cc. of the cooled, boiled out water. To estimate the strength of this solution 20 cc. of *N/10* bichromate are mixed in a flask with 10 cc. of 10 % potassium iodide and 5 cc. of strong hydrochloric acid. On shaking slightly the reaction is complete and exactly 0.254 g. iodine is liberated. The thiosulphate solution is run in from a burette until the brownish yellow colour has almost disappeared. One or two drops of a starch solution are then added and the titration continued until the liquid passes from a dirty dark yellow to a bright green colour. The end point is very sharp and cannot be mistaken. The number of cc. of thiosulphate used up indicates the strength of the thiosulphate solution. Since, however, all samples of potassium iodide contain traces of iodates, the iodide solution alone without bichromate will yield a very small amount of iodine in the presence of acid. This must be allowed for by making a blank experiment with 10 cc. potassium iodide and 5 cc. hydrochloric acid and titrating with thiosulphate as before. Not more than 0.1 to 0.2 cc. of thiosulphate should be used up. This must be subtracted from the result obtained with the bichromate. If say 18.7 cc. thiosulphate were required and the potassium iodide gave a blank of 0.2 cc., then the amount of thiosulphate solution required to combine with 0.2 g. of iodine would be $18.7 - 0.2 = 18.5$ cc. This means that to obtain an *N/10* solution each 18.5 cc. must be diluted to 20 cc., or that a litre will contain 925 cc. of the solution. To complete the procedure 75 cc. of boiled-out water are introduced into a 1000 cc. measuring flask from a burette and the volume made up to 1000 cc. with the thiosulphate solution. This gives an accurate *N/10* solution which may be tested against 20 cc. of bichromate as

described. The solution will keep for at least several months if preserved in a well stoppered coloured bottle in the dark.

To prepare an $N/100$ solution 20 cc. of the $N/10$ solution are made up to 200 cc. in a measuring flask with distilled water. This weak solution is liable to deteriorate and should be made up fresh every few days. It is important to remember that burettes in which thiosulphate solutions are kept must be cleaned at frequent intervals with a solution of sulphuric acid and bichromate.

No. 5. *Starch solution.*

This is prepared by dissolving about 1 g. of "soluble" starch in 100 cc. distilled water. The solution should be freshly made up though an old solution can be used with success.

No. 6. 20 % *hydrochloric acid solution.*

Prepared by diluting 20 cc. of concentrated hydrochloric (B.P. Sp. gr. 1.16) with distilled water to 100 cc.

STANDARDISING OF HEATING.

This can be very easily accomplished by interposing a small manometer between the gas tap and the burner. A suitable manometer has already been described by Cole [1914], but since the question of temperature is so important, a rough sketch of the most suitable heating and regulating arrangement is given here (Fig. 1). If a suitable manometer is not to hand it is very easy to extemporise one, for all that is required is a T-piece with two pieces of bent glass. These can be connected by means of rubber tubing and are quite as effective as if the whole were in one part. A piece of rubber tubing passing through a screw clip connects the one end of the T-piece with the gas supply, while the other end is connected with the bunsen burner. The burner should have a small chimney to protect the flame. From $\frac{1}{4}$ to $\frac{1}{2}$ inch above this, a ring with ordinary wire gauze is supported, on which the flask is boiled. The gauze must be separated by an interval from the chimney, otherwise the gas will not burn properly. When a suitable flame is determined the position at which the cover of the air inlet cuts the side of the hole in the inner side of the bunsen is marked with a file, as indicated in the diagram, so that the same amount of air can always be admitted by adjusting to this mark. Before proceeding to find a suitable strength of flame, some coloured fluid is put in to the manometer through the open end by means of a capillary pipette; the upper surface of this fluid in both tubes is then indicated by a line on a white card placed behind the manometer or by marks on the two tubes of the manometer itself. When this is done the gas is turned full on, the screw closed down somewhat, and a trial made by placing a conical Erlenmeyer flask containing 23 cc. of sodium sulphate acetic acid solution over the flame. The fluid should be first cooled (or warmed) to 20° C. After a few trials, a flame is easily found which will bring the solution to vigorous boiling in about

1 minute 40 seconds. This is then controlled by taking 20 cc. of above sodium sulphate solution to which are added 3 cc. of the alkaline copper iodine solution and testing as before. In each case, of course, the adjustment of the flame is brought about by loosening or tightening the screw clip. When a strength of flame is found that brings the mixture to *distinct boiling* in about 1 minute 40 seconds to 1 minute 45 seconds, the position of the fluid in both limbs of the manometer is marked (see Fig. 1) either by ink on the glass or on a white card behind, or the difference of level read off and noted. The regulating mechanism is now complete, and whenever it is required at a future time all that is necessary is to adjust the gas pressure by means of the screw until the upper surfaces of the coloured liquid stand at the marks indicated. After some time it will be necessary to add more fluid to compensate for slight losses due to evaporation.

Fig. 1.

The gas pressure in this laboratory varies considerably on some days, while on others it remains practically constant for hours. During careful experiments the manometer must be watched and if a change of pressure occurs the necessary adjustment must be made by means of the screw clip.

I. DETAILS OF METHOD WHEN 1 CC. OF BLOOD IS USED.

Coagulation of protein.

Exactly 26 cc. of acid sodium sulphate solution are run into a small Erlenmeyer flask from a burette. The flask is fitted with a rubber stopper through which passes a glass tube terminating in a capillary point. Blood is drawn from a vein into a test tube containing a small amount of finely ground potassium oxalate and the tube inverted a few times. One cubic centimetre of the blood is measured in a special pipette made by Hawksley "to contain" 1 cc. The blood is allowed to flow out on to the surface of the fluid in the flask. Any blood adhering to the pipette is then washed out by alternately

sucking up and blowing out several times some of the sodium sulphate solution. If the blood is delivered carefully at one side of the flask to begin with, it floats more or less on the surface, leaving the fluid in the other parts of the flask almost clear. If care is taken, the pipette can be washed out with practically blood-free fluid, so that every trace of blood can be delivered. The special stopper with glass tube is now placed firmly in the flask and the mixture heated over a free flame until a bubble or two just appear indicating that the fluid is approaching the boiling point. At this point the flask should be removed. It is then shaken by a circular motion and allowed to stand for one or two minutes. The stopper is then carefully loosened and withdrawn sufficiently to allow the entry of the point of a pipette containing 3 cc. dialysed iron. This iron is added, the flask being shaken gently all the time. It is then shaken two or three times taking care that any drops of fluid in the neighbourhood of the stopper or neck of the flask are washed into the general contents. The flask is then cooled under the tap and the mixture is ready for filtration.

Practically no fluid is lost by coagulating the mixture over the free flame since the small capillary tube does not permit of the escape of much steam while the expanded air makes its exit. Indeed the upper part of the flask remains sufficiently cool to condense steam until the liquid is almost boiling. Even if a little steam does pass into the tube, it condenses there and is sucked back into the flask when air is drawn in on cooling. The large volume of fluid used also minimises any effect of possible loss of traces of moisture by this method.

Filtration.

The contents of the flask are now filtered through a 9 cm. Whatman No. 1 filter paper (W. & R. Balston). This paper is starch-free and I have always found it most suitable for this purpose. Filtration is allowed to proceed until the whole of the liquid has passed through the filter paper; this requires about 6 or 7 minutes. From 23 to 24 cc. of filtrate are obtained. Of this 20 cc. are taken if the blood is from a normal person and likely to contain an average amount of sugar. In the case of diabetics, or if glycosuria is present, 10 cc. filtrate is sufficient. In the latter case the total is made up to 20 cc. by the addition of 10 cc. acid sodium sulphate mixture. These amounts are measured into a small conical Erlenmeyer flask of hard heat-resisting glass. To this exactly 3 cc. of the copper iodide solution is added and the mixture heated.

Heating blood filtrate containing alkaline copper solution.

Over a flame suitably adjusted as already described to bring the 23 cc. of liquid to boiling in about 1 minute 40 seconds the solution is boiled for exactly 6 minutes from the point at which distinct boiling begins. The flask is then removed and either cooled under the tap, or plunged up to its neck into a large volume of cold water in the sink and kept there for about 1 minute. Thorough cooling is essential and must always be carefully carried out.

Treatment of boiled solution.

To the cooled solution are now added carefully with a pipette 10 cc. of 20 % hydrochloric acid and the flask *very gently agitated* at intervals until the effervescence passes over. From this point it is left to stand for about 1 minute or a little more, being shaken with a strong circular motion every few seconds. At the end of this time $N/100$ thiosulphate is run in from a burette and the solution titrated until the yellow colour almost disappears. Two drops of 1 % starch solution are now added and the titration completed. As already stated it is perhaps best not to add the starch at the beginning of titration for there is no doubt that the end point is more distinct if done as indicated here. When carried out in this way, the end point is exceedingly sharp and it is quite impossible for anyone to be more than one drop out. After the titration is finished there is a tendency for the blue colour to return as is usually the case in such solutions, but since this does not happen for several minutes it in no way interferes with the titration.

Calculation of result.

Suppose the blood filtrate as treated above requires 6.69 cc. $N/100$ thiosulphate and 3 cc. of the copper solution alone requires 8.85 cc., then the difference between 8.85 and 6.69 = 2.16 cc. must be due to the iodine absorbed by the cuprous chloride formed. Now from Table I it is seen that 2.16 cc. $N/100$ thiosulphate = 0.6 mg. sugar. Since the amount of filtrate taken corresponds to $\frac{2}{3}$ of 1 cc. of blood, it is obvious that 1 cc. blood contains 0.9 mg. sugar or that 100 cc. of blood contains 0.09 g. of sugar.

Table I.

(For estimation of sugar in 1 cc. blood.)

Showing equivalent of glucose to $N/100$ thiosulphate solution.

Glucose	$N/100$ thiosulphate	Glucose	$N/100$ thiosulphate
mg.	c.c.	mg.	c.c.
0.2	0.55	1.2	4.36
0.3	0.95	1.3	4.71
0.4	1.34	1.4	5.07
0.5	1.76	1.5	5.42
0.6	2.16	1.6	5.78
0.7	2.52	1.7	6.13
0.8	2.88	1.8	6.49
0.9	3.27	1.9	6.84
1.0	3.65	2.0	7.20
1.1	4.00		

That the method gives very consistent results is obvious from the following figures obtained on duplicates in six different specimens of blood. Three of

these experiments were carried out on fresh blood, while the others were done on specimens which had stood in the laboratory from 3 to 24 hours.

Fresh bloods			After standing for some hours		
No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
0.096 %	0.102 %	0.089 %	0.059 %	0.048 %	0.030 %
0.098 %	0.108 %	0.086 %	0.066 %	0.046 %	0.030 %

II. METHOD FOR ESTIMATING SUGAR IN 0.2 CC. BLOOD.

The practical advantages of using a small amount of blood have already been discussed and since the manipulation here is quite as simple as is the case when 1 cc. of blood is used, this "micro-method" should prove very useful from the clinical standpoint. This method was partly worked out in 1915, but at the time I was unable to complete it. If the conditions given here are carefully observed, the results obtained are of the same order of accuracy as those in the above method and in spite of the fact that such a small quantity of blood is used no delicate manipulation is required. For this method the following solutions are required. Some of them are similar to those used for the 1 cc. procedure while others are considerably modified.

A. Solutions for coagulating blood.

No. 1.

Sodium sulphate	150 g.
Glacial acetic acid ¹	1 cc.
Distilled water to	1000 cc.

No. 2.

Dialysed iron solution "Dialysed iron B.D.H."

B. Solutions for estimating sugar in filtrate.

No. 3.

Potassium bicarbonate	12 g.
Potassium carbonate (anhyd.)	8 "
Copper sulphate crystals	0.35 "
Potassium iodate	0.05 "
Potassium iodide	0.5 "
Distilled water to	100 cc.

This is prepared as described under the 1 cc. blood method. It is standardised by taking 2 cc. in 10 cc. acid sodium sulphate solution and adding 2 cc. 75 % HCl. One minute after effervescence is completed and after the mixture has been thoroughly shaken it is titrated as already described with *N*/400 thiosulphate. 2 cc. of this copper solution should require about 11.05 cc. *N*/400 thiosulphate.

¹ The acetic acid should not be added until the solution is required for use.

No. 4.

An $N/400$ sodium thiosulphate solution is prepared from a $N/10$ solution by adding 5 cc. of the latter to some distilled water in a flask and making up to 200 cc.

No. 5.

A solution of "soluble starch" of about 1 % strength.

No. 6.

75 % HCl solution made by diluting 75 cc. concentrated HCl (B.P. Sp. gr. 1.16) to 100 cc. with distilled water.

DETAILS OF METHOD USING 0.2 CC. BLOOD.

The blood is obtained directly from a finger or from the ear. To measure and collect the blood a special pipette¹ is used (Fig. 2), which is shaped so that blood flows in from a slight puncture. If the finger is used it is rubbed with a little ether and thoroughly dried with a cloth. A piece of rubber tubing is then wound round the upper part to cause congestion towards the point and a prick is made with a needle on the back of the finger a little above the root of the nail. The point of the pipette is placed in contact with the side of the drop, when the blood immediately flows in. The pipette is then held horizontally or with the distal end somewhat inclined downwards, so that the passage of the blood into the pipette is helped by gravity. When the blood reaches the mark the pipette is removed and any blood that may remain on the point is carefully wiped away. If the pipette contains too much blood the necessary adjustment is made by gently tapping the point against the thumb nail when a very little blood will escape. If the skin and pipette are clean there is never any difficulty in obtaining the blood, and no particular speed is necessary. Indeed, if the first prick of the needle does not furnish enough blood, the rubber tube may be removed and again wound on and the operation repeated as often as three times or more without any fear of coagulation taking place. Sometimes it may be necessary to squeeze the finger, but since this does not appear to interfere with the result there is no objection to doing so. If the hand is cold it should be placed in hot water for a short time before taking the blood; also, the limb should be held in a hanging position for a few seconds before applying the rubber tube.

The one important point to be observed in using the method is that the *pipette should be kept scrupulously clean*; if any trace of greasy material is present on the inner surface the blood

Fig. 2.

¹ This pipette can be obtained from Hawksley & Sons, 357, Oxford Street, London.

will not flow. It is, therefore, most important that after each experiment the pipette should be washed out with a hot mixture of sulphuric acid and potassium bichromate. After thorough treatment with this mixture the pipette is washed out with distilled water followed by alcohol and ether and then dried. Personally, after each experiment I place the pipette in a test tube containing the bichromate mixture and let it stand there until it is again required when it is treated as described. When these precautions are taken there is never any difficulty in getting the blood.

Separation of protein from blood and estimation of sugar.

From a burette 23.8 cc. of acid sodium sulphate solution are introduced into a conical Erlenmeyer flask of heat-resisting glass. To this the 0.2 cc. blood is added and the pipette thoroughly washed out by alternately sucking in and blowing out the mixture. The blood is then heated and treated exactly as in the 1 cc. method, only that 1 cc. of dialysed iron is added instead of 3 cc. This gives a total volume of 25 cc. After cooling and filtering through a 9 cm. Whatman filter paper No. 1, 20 cc. of the filtrate are taken for the estimation of the sugar. In filtering, the process must be allowed to go on until all the material has passed through the filter since only about 21 to 21.5 cc. can be obtained altogether. In diabetic cases 10 cc. of the filtrate are taken and made up to 20 cc. with acid sodium sulphate solution.

To this filtrate are added 2 cc. (carefully measured) of the alkaline copper solution and boiling is carried out exactly as before for 6 minutes using a flame that brings the solution to brisk boiling in about 1 minute 40 seconds. It is then cooled thoroughly, 2 cc. of 75 % HCl added and the further treatment carried out exactly as described for the 1 cc. method except that $N/400$ thiosulphate is used for titration. The end point even with this dilute thiosulphate is perfectly distinct and gives no trouble whatever. The number of cc. of thiosulphate used is then subtracted from the number used by 2 cc. of copper solution alone, and the percentage of sugar in the blood is then calculated from Table II. Thus, if the reading obtained on titration was 8.98 cc. thiosulphate and if 2 cc. copper solution alone gives 11.05 cc., the difference (2.07 cc.) would represent the sugar present. From the table it is seen that 2.07 cc. thiosulphate = 0.18 mg. sugar; therefore $\frac{4}{5}$ (the aliquot part of filtrate taken) of 0.2 cc. blood contains 0.18 mg. glucose. 1 cc. of blood will therefore contain $\frac{1}{1} \times \frac{5}{4} \times \frac{5}{1} \times \frac{0.18}{1}$ mg. glucose = 1.12 mg. = 0.112 %.

This "micro-method" gives excellent results with duplicates as the following numbers show. In blood from the finger the average sugar percentage appears to be somewhat higher than in blood taken from a vein. On an average 0.11 % may be taken to represent the normal. This is probably dependent on the fact that a good deal of the finger blood is capillary in origin and thus differs from the venous sample.

Table II.

(For estimation of sugar in 0.2 cc. blood.)

Showing equivalent of glucose to N/400 sodium thiosulphate solution.

N/400		N/400	
Glucose	Thiosulphate cc.	Glucose	Thiosulphate cc.
0.03	0.12	0.22	2.61
0.04	0.25	0.23	2.74
0.05	0.38	0.24	2.86
0.06	0.50	0.25	2.99
0.07	0.62	0.26	3.11
0.08	0.73	0.27	3.24
0.09	0.86	0.28	3.36
0.10	0.99	0.29	3.49
0.11	1.13	0.30	3.61
0.12	1.26	0.31	3.74
0.13	1.39	0.32	3.87
0.14	1.53	0.33	3.99
0.15	1.67	0.34	4.12
0.16	1.80	0.35	4.24
0.17	1.94	0.36	4.37
0.18	2.07	0.37	4.49
0.19	2.22	0.38	4.62
0.20	2.35	0.39	4.74
0.21	2.49	0.40	4.87

Results of duplicate experiments on blood.

No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
0.112 %	0.127 %	0.114 %	0.079 %	0.066 %	0.041 %
0.116 %	0.126 %	0.119 %	0.084 %	0.069 %	0.038 %

The first three samples were obtained from the finger, one sample being taken immediately after the other. The very close results obtained indicate the accuracy of the method. Experiments 4, 5 and 6 were done on three different bloods obtained from a vein; they had stood in the laboratory for varying periods, hence the low results.

The question of the apparent difference in sugar content between capillary and venous blood is at present being investigated; also the effect of different food stuffs and starvation on the blood sugar.

SUMMARY.

A simple method is described by which an accurate estimation of the sugar present in 1 cc. and in 0.2 cc. of blood may be estimated. The method requires careful standardisation of the heating, but otherwise no special care is necessary. With pure glucose solutions theoretical results are obtained and in blood the duplicates always agree within very narrow limits.

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