XVI. ESTIMATION OF SUGAR IN THE BLOOD.

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For some years past much attention has been directed to the determination of carbohydrate tolerance by observing the variations that occur in the sugar-content of the blood after the ingestion of a known amount of sugar. The value of this method is now universally recognised. It is not intended in this paper to treat the subject in a general way, but merely to consider the actual estimation of sugar as required for the above purpose.

The usual procedure is to determine, first of all, the percentage of sugar in the blood at the "fasting level," then to administer a dose of 30–50 g. of glucose and make further estimations, say, every 20 to 30 minutes for two hours.

Micro-methods are practically always employed, the blood being obtained from a prick of the finger. After trying a number of these methods, it was found that those of Bang, MacLean, Folin and Wu, and Mackenzie Wallis and Gallagher were the most satisfactory. Considerable time and labour are required, however, for the routine performance of any one of these.

For some time past the writer has been endeavouring to simplify existing methods, especially from the clinical standpoint, and to increase, if possible, their accuracy. After a number of investigations, it was finally decided to use the rationale of the method of Folin and Wu [1918, 1920], and Mackenzie Wallis and Gallagher [1920] as a basis for this purpose. The process evolved has now been in use for some time and has proved to be most satisfactory. It differs in so many points from the original—in the collection and dilution of blood, the type of standard colour, the size of the boiling-tube, the curve of correction for copper reduction, etc.—that a full description is necessary.

Method of obtaining blood.

An amount of blood sufficient for accurate estimation of the sugar-content can be obtained from a single prick of the finger. The blood is collected in a small platinum capsule, specially designed for the purpose. This has been found to be the simplest and most efficient way of securing the sample. The collection is made with the greatest ease and the sample of blood is greater in amount than that which can be conveniently obtained by the blotting-papers in general use. It is considered preferable to weigh the small quantity of blood on a torsion balance, than to measure it in a pipette.

Pipettes must be kept scrupulously clean, otherwise blood will probably not run into them. The use of the capsule avoids this trouble.

The platinum capsule is held by a small pair of cross-action forceps (both being shown full size in Fig. 1), and is then hung on the hook of the torsion balance and weighed.

It has been found throughout many blood-sugar estimations, that the weight of the capsule has not varied in the slightest degree; numerous weighings can therefore be omitted. A capsule weighing about 170–200 mg. has proved most suitable.

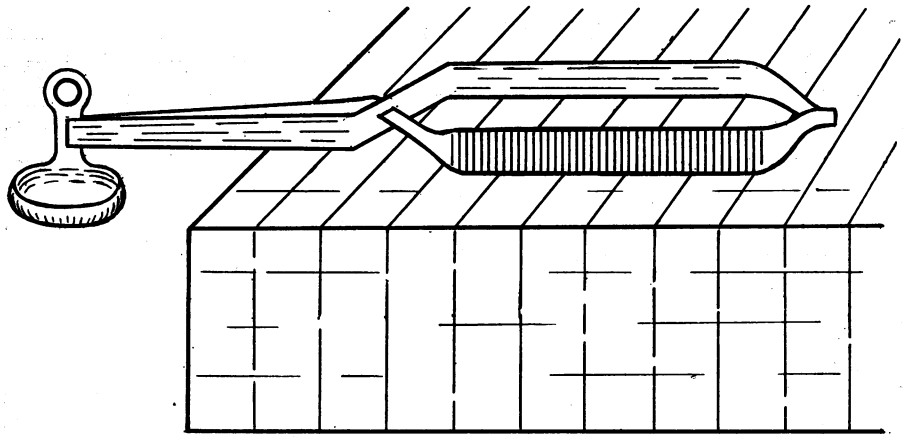


Fig. 1.

The hand of the patient is washed in warm water and dried. Then the thumb (for instance) is cleansed with ether. The patient is instructed to swing the arm, keeping the hand as low as possible and further congestion is caused by winding at once a piece of rubber-tubing round the proximal part of the digit. A prick is made with a bayonet-pointed needle to the side of, and just above, the nail. The thumb is then turned over and the blood made to fall in drops *directly* from the point of puncture into the capsule (held by the forceps) by compression of the pulp and nail. The capsule and blood are weighed immediately. One should obtain 200, or more, mgm. blood; generally it is quite easy to collect up to 270 mgm. It has been found necessary to emphasise: (*a*) that the hand should be warm and (*b*) the thumb thoroughly congested in order to secure the sample in the most convenient way. *Without delay* the capsule and blood are dropped into a short, wide test-tube (see list of apparatus) containing 7·3 cc. of distilled water. A turn or two of the test-tube is sufficient to mix completely the blood and water; none of the blood clings to the capsule.

The lengthy process of sugar extraction, required in blotting-paper methods, is, of course, unnecessary here. In a couple of minutes the mixture becomes clear and then one proceeds immediately to remove the proteins and other interfering substances by using the tungstic-acid-precipitation method of Folin

and Wu. Accordingly 0·3 cc. of 10 % sodium tungstate solution is added and directly afterwards 0·3 cc. of $\frac{2}{3}$ *N* sulphuric acid. This makes the volume up to 8 cc. (0·1 cc. being allowed for blood, less evaporation during expt.). It should be well mixed to ensure full precipitation; a reddish-brown or chocolate-coloured precipitate is quickly formed. The whole is now filtered through starch-free filter paper, the No. 30 Whatman filter paper being the best for the purpose. By pouring in about half of the solution and waiting until the paper is completely moistened before adding the remainder, one obtains the maximum amount of absolutely protein-free filtrate. The filtrate is watery-clear, and from it one determines the percentage of sugar in the blood.

Principle of method of estimating sugar content of blood.

As in the other methods mentioned, the power of glucose to reduce an alkaline solution of copper is utilised. By the addition of phospho-molybdic acid to the cuprous oxide so produced, a clear deep-blue solution is obtained, any unreduced copper being at the same time decolorised. The depth of colour is a measure of the amount of reduction and consequently of the percentage of sugar present. This estimation is made by comparison in a colorimeter with the depth of colour similarly produced from a solution of glucose of known strength. A special feature, however, of the present method of sugar estimation is the simplification of the standard used.

The standard colour used in the colorimetric estimation of sugar.

The present method of producing the standard colour is a fixed procedure requiring frequent repetition. To simplify matters it was decided to replace the standard solution by a glass, or glasses, of exactly the same shade of colour. This was easily accomplished and a perfect matching of colours obtained. The colorimeter reading gave the value of the glass in terms of sugar solution. It is evident that one can afford to devote much more time and care to this single determination than to the routine preparation of standards, which are so liable to vary one from another. Several readings of the colorimeter ensure that an accurate value has been obtained for the particular glass used. In the construction of curves this fixed basis is of special importance. The technique will be described presently.

Apparatus[1] *required:*

(1) A good colorimeter, *preferably the Kober pattern.*

(2) A torsion balance.

(3) Special platinum capsule for collection of blood.

(4) Small pair of cross-action forceps, with smooth gripping surfaces, for holding capsule (see Fig. 1).

(5) Several test-tubes (approximately 2·5 × 8·5 cm.) for dilution of blood and precipitation of its proteins.

[1] The glass-discs are supplied by Messrs Baird and Tatlock (London), Cross Street, Hatton Garden, E.C.; the remainder of the apparatus by Messrs Gallenkamp & Co., 19, Sun Street, E.C. 2.

(6) Whatman starch-free filter papers, No. 30 (7 cm. diameter).

(7) Special resistance-glass boiling-tubes, with 7 cc. bulbs and graduated for 12·5 cc. (of same pattern as the Folin and Wu and Mackenzie Wallis tubes; these, however, have only 4 cc. bulbs).

(8) Pipettes of the following capacities: two 1 cc. pipettes graduated in 1/50ths for the 10 % sodium tungstate and $\frac{2}{3}$ *N* H_2SO_4 solutions; two 2 cc. ordinary bulb-pipettes for the copper and phospho-molybdic acid solutions; one 5 cc. pipette for tungstic acid filtrate.

(9) Burette of 25 cc. capacity for distilled water.

(10) Water-bath (fairly large).

(11) A pair of special coloured glass discs, namely "7·5B," referred to below.

(12) In addition to 10 % sodium tungstate and $\frac{2}{3}$ *N* H_2SO_4 solutions the following (very carefully prepared) are required:

(*a*) *Copper solution.* Dissolve 40 g. pure anhydrous sodium carbonate in about 500 cc. distilled water in a litre cylinder. Then add 7·5 g. tartaric acid and when the latter is dissolved and effervescence has ceased, add 4·5 g. crystallised copper sulphate; dissolve without the aid of heat and make up to 1 litre with distilled water. Keep in a dark-coloured bottle. Impurities in the carbonate may give rise to a sediment in the course of a few weeks. If this happens, transfer the clear solution to another bottle.

(*b*) *Phospho-molybdic acid solution.* 35 g. pure molybdic acid are dissolved in 200 cc. of a 10 % solution of sodium hydroxide and 200 cc. of water added. The whole is now boiled for 20–40 minutes or longer until all traces of ammonia have been driven off, as shown by litmus paper held in the vapour. Cool and dilute to about 350 cc. with distilled water and then add 125 cc. of phosphoric acid (85 % strength) and water to 500 cc.; 2 cc. of this solution when added to 2 cc. of the copper solution should produce complete decolorisation.

(*c*) *Standard sugar solution.* When about to determine the sugar value of the coloured glasses, the standard sugar solution is required, made as follows. Dissolve 4 g. of pure powdered glucose in about 500 cc. distilled water and make up to 1 litre (a few drops of toluene will preserve the solution for some time, if necessary); 10 cc. of this made up to 1 litre, is the "standard sugar solution."

Preparation of the blue colour from the standard sugar solution and the matching thereof with the coloured glasses (thus determining the colorimeter-scale values of the latter in terms of the former).

Of the freshly-prepared standard sugar solution 5 cc. (containing 0·2 mgm. glucose) are placed in one of the special boiling tubes and 2 cc. of the copper solution added (the solution now reaching the constricted part of the tube). The tube is shaken so as to mix thoroughly the contents and then placed in a vigorously boiling water-bath for *exactly* six minutes. The volume of water

should be fairly large; no lowering of temperature should be noticed when the tube is introduced. On removal of the latter from the bath, 2 cc. of phospho-molybdic acid solution are immediately added, a clear deep-blue solution resulting. Distilled water is added up to the 12·5 cc. mark and the contents mixed by inverting the tube. This is the standard blue solution, which is to be matched with coloured glass.

For this purpose, a long series of pure blue glass slides[1] (5·1 × 1·8 cm.) was obtained. The 155 slides of the series are accurately graded (·01 to 20·0) as regards depth of colour, and the consecutive colours are so nearly alike that differences can scarcely be detected. It was at once discovered that the colour of the standard solution was not that of pure blue. Investigation proved it to be a mixture of blue and yellow. Accordingly a series of pure yellow glasses, similar to that of the blue, was also employed. After a number of trial experiments, it was found that the blue of the standard solution could be matched exactly by combining one of the blue glasses with a comparatively weak yellow glass. At first it was thought that more accurate estimations of sugar could be obtained by having a number of combinations of blue and yellow glasses, covering the range of colour-intensity derived from blood filtrates, and making use of the approximate combination. Blue glasses of appropriate depths of colour were therefore selected and the necessary correction made with yellow. The latter was determined by placing the standard blue solution (ready for use five minutes after dilution) in one cup of the colorimeter, putting one of the blue glasses underneath the other cup and then adding successively various yellow glasses, until an exact match was effected. At the same time, the cup containing the solution was manipulated so that the colours were also equal in depth. In matching any solution with the fixed depth of colour of the glasses, it is advisable to maintain the cup of the latter at roughly the same level as the cup containing the former. The reading of the cup containing the standard blue solution gives the value of the particular combination of glasses for that solution. After a few trials one gets accustomed to the process and an accurate result is easily obtained. The following are a few of the combinations of glasses with their respective values, as determined by the writer:

No. of glass				Kober colorimeter reading
Blue		Yellow		
15·0	+	1·5	=	29·4
12·5	+	1·2	=	24·3
10·0	+	1·0	=	19·4
7·5	+	0·8	=	15·4

No definite relationship necessarily exists between the grading of the glasses and their colorimeter values.

On employing the above four combinations for each estimation of the sugar-content of numerous blood filtrates and glucose solutions of various strengths, it was found that the same result was obtained with each com-

[1] Lovibond Tintometer Glasses (Tintometer Ltd., Salisbury).

bination of glasses. Therefore only one pair of glasses is required. The 15·0 blue + 1·5 yellow, and 12·5 blue + 1·2 yellow combinations are too deep in colour for low concentrations of sugar and so are unsuitable by themselves. In a few instances even the 10·0 blue + 1·0 yellow may be too dark for the purpose; accordingly as a single combination, the use of the 7·5 blue + 0·8 yellow glasses is advised.

Occasionally when only a very light blue solution is developed from the blood filtrate (e.g. through inability to obtain more than minute quantities of blood, as may possibly occur with infants or markedly debilitated persons, etc.), it will be found helpful to use a pair of discs of lighter colour. "5B" (5·0 blue + 0·5 yellow), having a value of 11·4, can be obtained to meet this contingency.

For ease of manipulation the glasses can now be obtained as pairs of discs; these can be conveniently dropped into one of the cups of the colorimeter. In all cases the values of the glasses should be determined individually in order to allow for the personal equation and for any possible slight variation in the composition of the solutions. Again, it is perhaps advisable to check the value of the discs whenever a fresh stock of copper solution is prepared, as slight variations may possibly occur.

On occasions artificial light may be required for the colorimeter. In this connection a 100 watt Osram daylight lamp[1] (blue) has proved most satisfactory.

Estimation of sugar in blood filtrates by means of the standard glass discs.

One can conveniently pipette off 5 cc. of blood filtrate. This is placed in one of the boiling-tubes and treated in the same manner as the standard sugar solution, *i.e.* 2 cc. copper solution are added and thoroughly mixed; placed for *exactly* six minutes in a boiling water-bath; on removal from bath 2 cc. phospho-molybdic acid solution added immediately; solution made up to 12·5 cc. mark with distilled water and, after the lapse of five minutes, compared in the colorimeter with the glass discs. With very little practice, accurate readings are easily obtained.

Calculation. E.g. A sample of blood collected from a normal person, 3½ hours after a light breakfast.

Weight of platinum capsule		= 171 mgm.
,,	,, + blood	= 395 ,,
Weight of blood		= 224 ,,

The blood was mixed with 7·3 cc. distilled water and 0·3 cc. sodium tungstate solution + 0·3 cc. $\frac{2}{3}$ *N* H_2SO_4 added (= 8 cc., allowing 0·1 cc. for blood, etc. as above). Of the filtrate 5 cc. were used; this represents $\frac{5}{8} \times 224 = 140$ mgm. blood. The blue solution resulting from this filtrate was

[1] Obtainable from G. E. Co., Kingsway, London

found to match the combination of 7·5 blue + 0·8 yellow discs at the colorimeter reading of 25·9. This pair of discs has a value of 15·4, *i.e.* the standard blue solution if set at 15·4 would effect the same match. Now the value of this pair (or of any of the other pairs) of discs represents 0·2 mgm. glucose (the amount contained in the 5 cc. of standard glucose solution employed).

Hence the amount of sugar contained in the 5 cc. of tungstic acid filtrate, *i.e.* in 140 mgm. blood, $= \frac{0{\cdot}2 \times 15{\cdot}4}{25{\cdot}9} = 0{\cdot}1189$ mgm.

This is, however, only an apparent value. A correction depending on the variations of copper reduction, must be made before the true value is obtained. The amended value is 0·139 mgm. (derived from the curve I).

Hence % sugar in the blood $= \frac{0{\cdot}139 \times 100}{140} = 0{\cdot}099$, which is a normal "fasting level."

The correction of apparent values is a matter of importance; it will now be considered in detail.

Curve of correction for copper reduction.

Copper is not reduced proportionately to the amount of sugar present. Therefore cuprous oxide values of solutions of higher or lower sugar concentration than the standard sugar solution do not give accurate values for sugar-content, unless corrected. The writer has worked out a curve of correction for this purpose.

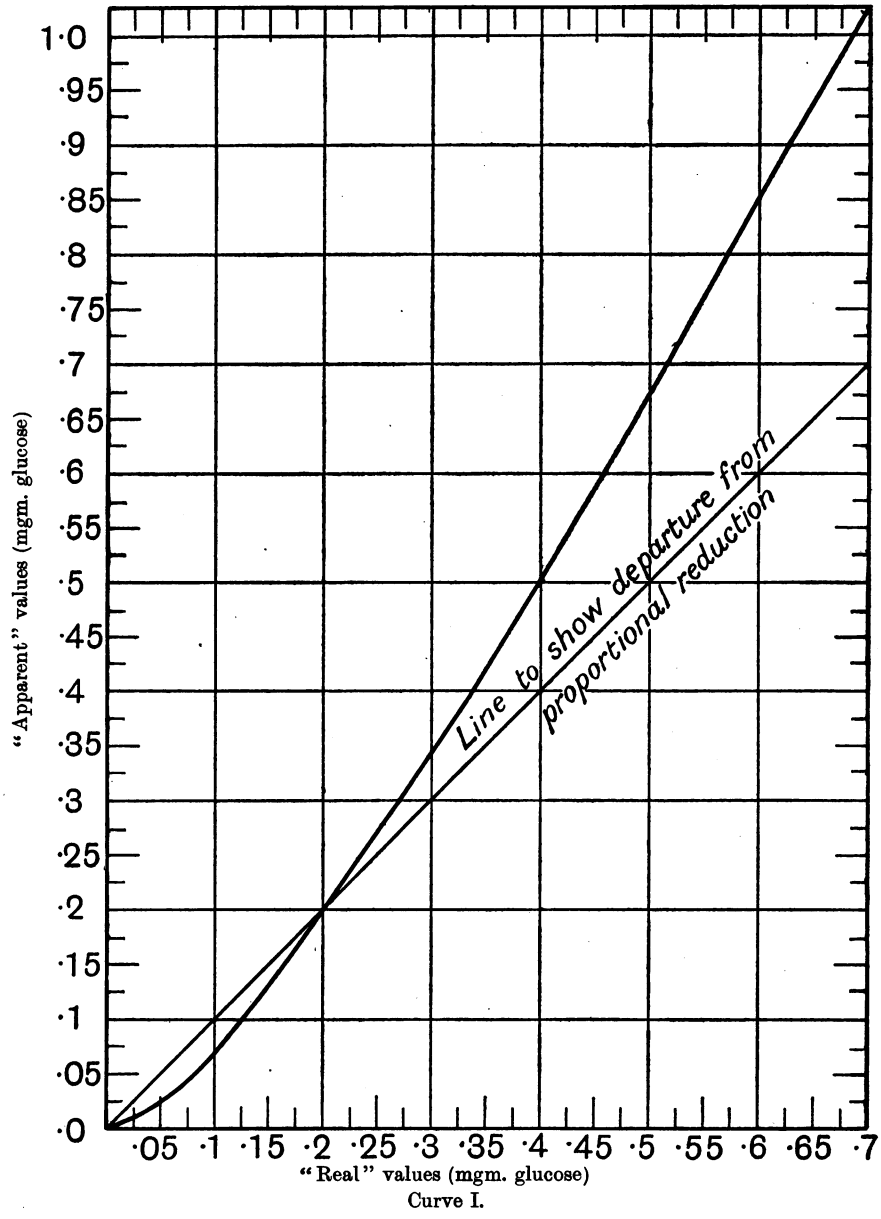
Construction of the curve. As the sugar-content of the blood is estimated from the amount of sugar in 5 cc. of the tungstic acid filtrate, it was decided to employ a series of 5 cc. solutions containing known amounts of pure glucose. One then endeavoured to recover experimentally the sugar content of each of these solutions in exactly the same way as for a blood filtrate. The experi-

Table showing "real" and "apparent" values.

(Mgm. glucose in 5 cc. solution from which the curves are plotted.)

"Real value" (mgm. glucose)	"Apparent value" (mgm. glucose)	"Real value" (mgm. glucose)	"Apparent value" (mgm. glucose)
0·050	0·025	0·525	0·720
0·075	0·045	0·550	0·763
0·100	0·070	0·575	0·808
0·125	0·100	0·600	0·850
0·150	0·133	0·650	0·938
0·175	0·165	0·700	1·025
0·200	0·200	0·800	1·220
0·225	0·233	0·900	1·392
0·250	0·266	1·000	1·567
0·275	0·305	1·100	1·750
0·300	0·343	1·200	1·900
0·325	0·379	1·300	2·008
0·350	0·420	1·400	2·070
0·375	0·460	1·500	2·115
0·400	0·504	1·600	2·140
0·425	0·545	1·700	2·160
0·450	0·587	2·000	2·160
0·475	0·630	2·400	2·160
0·500	0·675	3·000	2·160

mental result is called the "apparent value" in contrast to the "real value"; these have been plotted one against the other. Two curves are given (curves I and II). Curve I is the one intended for use in this method; from the values

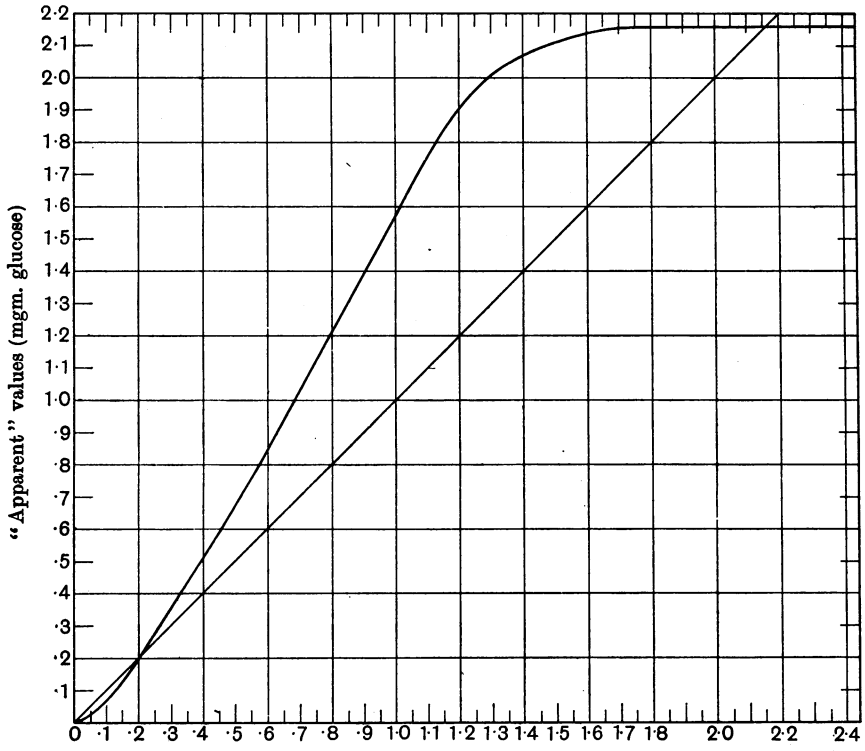


Curve I.

tabulated, it can, with advantage, be drawn to a much larger scale. Curve II is curve I continued by making use of the remaining values; the scale is much smaller.

In preparing the "real value" solutions, great care was taken to avoid error. The experiments were conducted on freshly-prepared solutions; six was found to be a convenient number with which to deal at one time.

First of all, 4 g. of pure powdered glucose was dissolved in about 500 cc. distilled water and the volume made up to 1 litre. If 10 cc. (containing 0·04 g. glucose) of this solution (X) be diluted to 1 litre, then 5 cc. of the resulting solution (Y) will contain 0·2 mgm. glucose. Solution (Y) is really



Curve II.

the "standard sugar solution" already mentioned. By varying the number of cc. of solution (X) various amounts of glucose are obtained in the 5 cc. solutions. Thus, 5, 10, 15, 20, 25, 30, etc., cc. of solution (X) when made up to 1 litre produced solutions, 5 cc. of which contained 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, etc., mgm. glucose respectively. A value like 0·275 mgm. was obtained by preparing solutions containing 0·250 and 0·300 mgm. glucose (*i.e.* 12·5 cc. and 15 cc. solutions (X) used) in 5 cc. and mixing, say, 20 cc. of each; 5 cc. of the mixture contained 0·275 mgm. glucose. The "apparent values" were determined by placing 5 cc. of "real" or known value solutions in boiling-tubes, adding 2 cc. of the copper solution and proceeding as in the case of blood filtrates to make the colorimetric estimations.

An example will make this clear, and, at the same time, demonstrate the equivalence of the several pairs of discs previously referred to, thus illustrating the principle on which the method of using coloured glass standards is founded. In actual practice only the 7·5B pair would be used. (The 7·5 blue + 0·8 yellow combination of discs is conveniently termed 7·5B; similarly one speaks of 10B, 12·5B and 15B.) To determine the "apparent" value of a 5 cc. solution containing 0·30 mgm. glucose, the blue-coloured solution is developed in the usual way and placed in one cup of the colorimeter and compared with a pair of discs placed in the other cup.

The following were the colorimeter readings obtained for the discs:

$$7\cdot5B = 9\cdot0, \qquad 12\cdot5B = 14\cdot1,$$
$$10B = 11\cdot3, \qquad 15B = 17\cdot1.$$

Now the values of 7·5B, 10B, 12·5B and 15B are 15·4, 19·4, 24·3 and 29·4 respectively, each value representing 0·2 mgm. glucose. Therefore the "apparent" values given by

$$7\cdot5B = \frac{\cdot2 \times 15\cdot4}{9\cdot0} = 0\cdot342 \text{ mgm. glucose.}$$
$$10B = \frac{\cdot2 \times 19\cdot4}{11\cdot3} = 0\cdot343 \text{ ,, ,,}$$
$$12\cdot5B = \frac{\cdot2 \times 24\cdot3}{14\cdot1} = 0\cdot344 \text{ ,, ,,}$$
$$15B = \frac{\cdot2 \times 29\cdot4}{17\cdot1} = 0\cdot343 \text{ ,, ,,}$$
$$\text{Mean value} = 0\cdot343 \text{ mgm. (see table).}$$

As regards the complete curve—curve II—it will be noticed that at three points the "real" and "apparent" values coincide:

at zero,
at 0·2 mgm.,
at 2·16 mgm. ("real" value).

The values are equal at 0·2 mgm. because this is the amount of glucose in 5 cc. of the standard sugar solution, from which the disc-values have been determined. The values are the same at 2·16 mgm. because all the copper in the 2 cc. of stock solution (theoretically amounting to 2·29 mgm. Cu) has been reduced by a 5 cc. solution containing 1·7 mgm. glucose; the "apparent" value at this point is 2·16 mgm., and increase of the glucose used for reduction does not alter it.

Numerous experiments have been carried out which prove the reliability of the curve in enabling one to recover experimentally the percentage of sugar from solutions of known strength. *E.g.* To recover the percentage of glucose in a solution of 0·25 % strength: The platinum capsule was filled with this solution and weighed on the torsion balance, 231 mgm. of the solution being taken.

Capsule + solution were then put into 7·8 cc. of distilled water. 5 cc. of the mixture was placed in a boiling-tube, 2 cc. copper solution added, and the process of estimating the sugar-content continued as already described.

Colorimeter readings obtained:

7·5B (value 15·4) = 7·0. ∴ "apparent" value = $\frac{0{\cdot}2 \times 15{\cdot}4}{7{\cdot}0}$ = 0·440 mgm.

10B (value 19·4) = 8·8. ∴ "apparent" value = $\frac{0{\cdot}2 \times 19{\cdot}4}{8{\cdot}8}$ = 0·441 mgm.

∴ Mean "apparent" value = 0·4405 mgm.

If this be left uncorrected the percentage of sugar obtained for the 0·25 % solution would be: $\frac{0{\cdot}4405}{\frac{5}{8}(231)} \times 100 = \frac{44{\cdot}05}{144{\cdot}4} = 0{\cdot}305\ \%$

(where $\frac{5}{8} \times 231$ or 144·4 = mgm. glucose solution used).

But on making the correction from the curve, the "apparent" value 0·4405 mgm. becomes the "real" value 0·362 mgm., and therefore the amount of sugar found in the 0·25 % solution = $\frac{0{\cdot}362 \times 100}{144{\cdot}4} = 0{\cdot}2507\ \%$.

Again, the method has been thoroughly tested by recovering added glucose from blood:

For this purpose two capsules were required. Two simultaneous samples of blood were taken, No. 1, 230 mgm.; No. 2, 214 mgm.

On being weighed, No. 1 blood was put immediately into 7·3 cc. distilled water and mixed. No. 2 blood was mixed in 7·1 cc. distilled water. The proteins of No. 1 were precipitated in the usual way and the solution filtered. The capsule now available was employed to weigh out a quantity of 0·25 % glucose solution, 182 mgm. of which were taken.

This was added to No. 2 blood solution. The addition of the precipitants made the volume up to 8 cc.

5 cc. of each filtrate was taken and the sugar content estimated:

(*a*) *Blood alone:*

Amount used = $\frac{5}{8} \times 230$ = 143·75 mgm. blood.

Using 7·5B the reading was 18·3.

∴ "apparent" amount sugar in 143·75 mgm. blood = $\frac{0{\cdot}2 \times 15{\cdot}4}{18{\cdot}3}$ = 0·1683 mgm.

= 0·1780 mgm. (corrected from curve I).

∴ sugar in the blood = 0·1238 %.

(*b*) *Blood + Glucose:*

Amount blood used = $\frac{5}{8} \times 214$ = 133·75 mgm. and this contains (using above %)

$\frac{133{\cdot}75 \times {\cdot}1238}{100}$ = ·1656 mgm. glucose.

The 7·5B reading for the blood + glucose = 5·2.

∴ "apparent" amount glucose in the mixture = $\frac{0{\cdot}2 \times 15{\cdot}4}{5{\cdot}2}$ = 0·592 mgm.

= 0·452 mgm. (corrected).

Now amount glucose solution used = $\frac{5}{8} \times 182$ = 113·75 mgm.

This amount therefore contains 0·4520 − 0·1656 = 0·2864 mgm. glucose.

∴ amount of glucose in the 0·25 % solution = $\frac{0{\cdot}2864 \times 100}{113{\cdot}75}$ = ·2519 %.

In cases of hyperglycaemia the importance of the curve correction is still more noticeable than with normal blood.

With regard to the solutions used in the method it may be pointed out that the copper solution should be made up freshly every four months; the phospho-molybdic acid, $\frac{2}{3}$ *N* H_2SO_4 and 10 % sodium tungstate solutions seem to keep indefinitely. Attention is directed to the quality of the sodium tungstate used. Carbonates are usually present in commerical tungstates. According to Folin and Wu, these should not exceed a certain amount. On titrating 10 cc. of the 10 % sodium tungstate with *N*/10 HCl, using one drop of phenolphthalein as indicator, not more than 0·4 cc. of the acid should be required. The amount of $\frac{2}{3}$ *N* H_2SO_4 used in the precipitation process has been so regulated as to conform to this standard. No difficulty, however, will be experienced if care be taken to obtain tungstate of good quality. The very slight variations that may occur in the tint of the blue colour developed from blood filtrates are sometimes attributable to impurities in the tungstate; nevertheless an accurate colorimeter reading is easily made.

Again, it should be noted that when the blood is properly coagulated by the tungstic acid, the coagulum changes gradually from pink to chocolate-brown. If this change does not occur within a few minutes, the coagulation is incomplete. In such an emergency an extra drop of $\frac{2}{3}$ *N* H_2SO_4 will usually produce the desired effect.

As regards the care of the platinum capsule: it is shaken up in a couple of changes of water in the test-tube in which it remained on filtering the blood precipitate; then it is dipped successively in absolute alcohol and ether; after one passage through a Bunsen flame it is ready for use again.

Summary.

A rapid and reliable method of estimating sugar in the blood has been described. The number of manipulations has been reduced to a minimum, thus making it particularly valuable in routine clinical work.

The following are its main features:

(1) Collection and weighing of blood in a special platinum capsule—simple and accurate:

(*a*) The capsule remains constant in weight; numerous weighings can thus be avoided.

(*b*) In the process of dilution all the blood is immediately diffused, therefore "sugar-extraction" is unnecessary.

(2) Accuracy is gained by making the fullest use of the blood available.

(3) A fixed colour standard in glass. The selection of the standard and the determination of its value are described. The use of the standard (issued as a pair of glass discs—"7·5B") saves much time and labour. Further, it pro-

vides an unchanging basis for the estimations; this is of importance in the construction of blood-sugar curves.

(4) Use of a curve of correction for copper reduction; an appreciable, if not considerable, error in sugar estimation is thereby eliminated.

(5) The technique is so simple that one can carry out concurrently the estimations necessary for several blood-sugar curves.

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