IMPROVED METHOD FOR THE DETERMINATION OF SUGAR IN BLOOD AND OTHER TISSUES, WITH A CONSIDERATION OF THE CONDITION OF THE SUGAR IN BLOOD

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Up to the present, many methods have been devised for the determination of sugar in blood and other tissues. These methods, however, are all the same in principle, depending on the removal of the protein from the solution and the subsequent determination of the sugar by reduction or fermentation. Methods for the determination of sugar in blood depending on these principles have been devised and employed, among others, by Abeles, Schenck, Weyert, Reid, Schmidt-Mülheim-Hofmeister, Pavy, and a comparison of these methods has been made by Seegen.¹

Where only a determination of the total sugar in the blood is desired these precipitation methods are probably satisfactory, provided that the method is carefully carried out and a sufficient number of determinations is made. These methods, however, are insufficient where a knowledge not only of the total sugar is desired, but some indication also of the condition in which this sugar exists in the blood or tissue. It is obvious, for example, that should a part of the sugar in blood exist in loose chemical combination (adsorption) with the protein or other material of the blood, such methods will fail completely to discriminate between such sugar and that existing in the free state.

The most general method depending on precipitation is that described by Schenck,² and modified by others. This method consists in precipitating the protein of the blood with an acid solution of

1. Abeles, *Zeitschr. f. Physiol. Chem.*, XV, 495. Schenck, *Pflüger's Arch.*, LV, 203-211. Weyert, *Dubois Reymond's Arch.*, 1891, 187. Reid, E. W., *Journ. Physiol.*, XX, 316-321. Schmidt-Mülheim-Hofmeister, *Dubois Reymond's Archiv.*, 1879, and *Zeitschr. f. Physiol. Chem.*, IV. Pavy, *Proc. Roy. Soc.*, XXXII, 418 (1881), also *Brit. Med. Journ.*, I (1896). Seegen, *Centralblt. f. Physiol.*, 1892, p. 501 and 604.

2. *Pflüger's Archiv.*, LV, 203-211.

mercuric chloride, filtering and precipitating the excess of mercury in an aliquot portion of the clear liquid with hydrogen sulphide. The filtrate obtained after separation of the mercuric sulphide is boiled till free from hydrogen sulphide, neutralised, and the sugar determined by reduction. The use of such a chemical agent as mercuric chloride in acid solution is undesirable on the grounds mentioned above. Further, the assumption that the sugar is equally distributed between precipitate and mother-liquor, in other words that there is no mechanical retention of the sugar, is not warranted, as Seegen points out.

Where the after removal of hydrogen sulphide is effected by boiling, the hydrolysis of any hydrolysible sugar is certain to follow, and it is further probable that the long continued boiling necessary to remove the last traces of hydrogen sulphide will result in a partial destruction of the sugar. Although we have not determined the limits of experimental error by this method, we have, nevertheless, found that it has several of the above-mentioned disadvantages.

Other precipitation methods, for example the zinc acetate or the method using alcohol, are unsatisfactory because by their use the normal condition of the blood is destroyed and there is no proof that the use of such reagents does not produce secondary changes in the blood.

In any satisfactory method of determining the sugar in blood under varying conditions, the blood must be collected and treated with aseptic precautions as far as possible, and the sugar determined in its natural condition in the blood, chemical reagents, boiling, etc., being avoided where secondary changes are liable to occur.¹ A brief consideration of the many methods up to the present employed for the determination of sugar in blood will show, that in none of these is the second condition above mentioned fulfilled.

For the purpose of investigating the nature of the sugar in blood under various conditions, the method of dialysis has been found by us to yield satisfactory results. This method, as far as we are aware, has never been hitherto successfully employed in determining the

1. It is at present hard to say how far the results obtained by Lepin and others, in support of a theory of glycolytic fermentation "*in vitro*" may not be explained on one of the above grounds.

nature and amount of the sugar in blood, although Hédon¹ has employed it for isolating and identifying the sugar, while Schenck² and Arthus³ have employed it, unsuccessfully however, in attempts to prove whether or not the sugar in the blood exists in the free state or in combination with the protein.

After a number of preliminary experiments, the following method was adopted by us. The blood, which had been previously collected in a sterile vessel, was defibrinated aseptically, then 50 c.c. of it was drawn off and introduced into a dialysing tube of ordinary sausage-skin form, contained in a wide-mouthed bottle of about 500 c.c. capacity, provided with a tightly fitting glass stopper. Every piece of apparatus used was previously sterilized, and all the subsequent operations were carried out with aseptic precautions. In the early experiments the blood or serum was dialysed into water, but later, saline was found to be better.

In all the experiments recorded a measured quantity (300 to 450 c.c.) of 0.9 per cent. sodium chloride was used. The process of dialysis was continued about forty to forty-five hours, experiments having shown that before this time equilibrium had been reached.⁴ During this interval the whole apparatus was kept in an ice-chamber.

The volume of the saline outside the dialysis-tube at the end of the experiment was accurately measured, the bottle and dialysis tube being then rinsed with water. The saline and washings were then brought to boiling, and when this point had been reached boiling Fehling's solution considerably in excess of that required for complete oxidation of the sugar was added, and the whole kept in gentle ebullition for the space of five minutes. This procedure was found to be necessary, otherwise the precipitate of cuprous oxide was thrown down in such a fine state of division that it remained partly suspended in the mother liquor, and was with difficulty prevented from passing through the filter. By the above method the precipitate came down in a granular form and no difficulty was experienced in filtering.

1. Hédon, *Compt. Rend. Soc. Biolog.*, 50, 510.

2. Schenck, *Pflüger's Archiv.*, XLVII, 621.

3. Arthus, *Zeitsch. f. Biolog.*, 34, 438.

4. On one occasion, where dialysis was inadvertently discontinued after half-an-hour, it was found that approximately 10 per cent. of the sugar had already dialysed out.

The cuprous oxide was collected on a tared Gooch crucible, washed thoroughly with distilled water, dried and ignited, the reduction being determined by the weight of the cupric oxide so obtained.

For the purpose of determining the accuracy of the method numerous experiments have been carried out, some with the serum alone and some with the defibrinated blood. In other experiments the entire dialysis apparatus was brought to 100° immediately after the introduction of the blood, and maintained at that temperature until coagulation was effected. This last process invariably gave higher figures for the sugar, but that this increase is not due to the action of bacteria in the uncoagulated blood, but is more probably the result of a decomposition of some loosely-combined sugar in the blood, we have great reason to believe, as will be shown in a later paper in which we propose to describe the action of carbon dioxide and other anaesthetics on the blood.

The following experiments serve to show the accuracy of the method.

Experiment I.—Pig's blood drawn under aseptic precautions was immediately defibrinated and centrifugalised for 1½ hours. The serum was then separated from the corpuscles and each was thoroughly mixed in sterile beakers. The dialysis of the whole blood, serum and corpuscles, was then carried out as already described, 450 c.c. of saline being used, and the whole dialysed for forty-three hours.

Results :—

No.	Nature	Final volume of Saline	CuO	Percent. Dextrose	Mean value of Dextrose
1	Serum with 3 drops xylol added	442 c.c.	0·0998 gm.	0·1027 %	0·1040 %
2	„ „	444 c.c.	0·1022 „	0·1046 %	
3	„ „	444 c.c.	0·1023 „	0·1047 %	
4	Corpuscles, with 3 drops xylol added	442 c.c.	0·0028 „		0·0474 %
5	„ „	442 c.c.	0·0054 „		
6	„ „	443 c.c.	0·0025 „		
7	Whole blood, 3 drops xylol added	441 c.c.	0·0466 „	0·0480 %	
8	„ „	442 c.c.	0·0463 „	0·0476 %	
9	„ „	444 c.c.	0·0456 „	0·0467 %	

This experiment indicates the data necessary for calculating the results. For the conversion of copper oxide into dextrose the factor employed is 0.4545. For the sake of shortness only the percentage of dextrose will be given in subsequent experiments.

Experiment II.—The whole blood in this experiment was coagulated in the dialysing tube at 100°, soon after being drawn. The serum, on the other hand, eight hours later. Blood dialysed for forty hours into 450 c.c. saline.

Percentages of dextrose in :—

SERUM			WHOLE BLOOD		
1	2	3	1	2	3
0.1247%	0.1248%	0.1248%	0.0676%	0.0644%	0.0722%
Mean = 0.1248%			Mean = 0.0681%		
CORPUSCLES					
1	2	3			
0.0043%	0.0046%	0.0045%			
Mean = 0.0045%					

Experiment III.—Dialysis continued for forty-two hours, and blood or serum uncoagulated throughout.

Percentages of dextrose :—

SERUM		WHOLE BLOOD		CORPUSCLES	
1	2	1	2	1	2
0.1187%	0.1187%	0.0607%	0.0603%	0.0002%	0.0009%
Mean = 0.1187%		Mean = 0.0605%			

Experiment IV.—Pig's blood dialysed for forty hours. Blood uncoagulated. Percentage of dextrose :—

SERUM		WHOLE BLOOD		CORPUSCLES	
1	2	1	2	1	2
0.1189%	0.1202%	0.0665%	0.0602%	0.0004%	0.0006%
Mean = 0.1195%		Mean = 0.0633%		Mean = 0.0005%	

Although other experiments have been made, nevertheless these suffice to show the degree of accuracy which can be obtained by this method, and further throw some light on the distribution of sugar in the blood. They show the probability of entire absence of sugar from the corpuscles, the reduction observed with them being easily accounted for in every case by the sugar in the admixed serum, and Experiment Number II in particular illustrates this important point. Here, the whole blood in which all glycolytic and bacterial action had

been completely stopped soon after collection, was found to contain no more sugar than would be accounted for by the serum if we accept the relative proportion of serum to corpuscles as about 60 : 40. If the absence of sugar in the corpuscles is to be explained by a rapid destruction of the same, then the proportion of sugar in the whole blood to that in the serum, as found in this experiment, ought to have been much higher.¹

For the purpose of further investigation we have recentrifuged corpuscles with a volume of saline equal to that of original serum, and in this way have failed to find even a trace of sugar in the resulting corpuscles. Furthermore, in another experiment, we have determined the amount of sugar in whole blood coagulated three hours after collection as compared with the same blood coagulated immediately on being drawn. The loss of sugar in this time did not amount to more than one per cent., a difference well within the limits of experimental failure and hardly warranting a theory of glycolytic action.

In the following experiments an attempt has been made to demonstrate the increase in the amount of sugar obtainable by this method after coagulation.

Experiment IV.—With pig's blood, dialysed, against 450 c.c. 0.9 saline for forty-six hours.

Sugar percentage in whole blood :—

CONTROL UNCOAGULATED			BLOOD COAGULATED		
1	2	3	1	2	3
0.0530%	0.0523%	0.0521%	0.0561%	0.0554%	0.0559%
Mean = 0.0525%			Mean = 0.0558%		

Experiment V.—With pig's serum dialysed for forty hours.

Percentage of sugar :—

CONTROL SERUM		COAGULATED SERUM	
1	2	1	2
0.1189%	0.1202%	0.1277%	0.1195%
Mean = 0.1195%		Mean = 0.1236%	

1. Lepin and Boulud (*Compt. Rend.*, 141 p. 175) claim to have found as much as two-fifths of the sugar in the whole blood in the corpuscles. They centrifuged the blood, however, for only ten minutes and it is therefore doubtful how far complete separation of the serum and corpuscles had been effected.

Experiment VI.—Pig's serum dialysed forty-three hours.

Percentage of sugar :—

CONTROL SERUM			COAGULATED SERUM		
1	2	3	1	2	3
0·0763%	0·0761%	0·0735%	0·0843%	0·0823%	0·0821%
Mean = 0·0753%			Mean = 0·0829%		

Experiment VII.—Sheep's serum dialysed for forty-two hours against 300 c.c. saline.

Percentage of sugar :—

CONTROL SERUM		COAGULATED SERUM	
1	2	1	2
0·0609%	0·0620%	0·0700%	0·0682%
Mean = 0·0614%		Mean = 0·0691%	

It seems to us impossible to explain the constancy of these and many other experiments made by us on any other ground than that of an existing affinity between the sugar and some other constituent of the serum, an affinity which is known to exist in the case of lecithin and glucose and which may equally well exist between proteins and glucose. That these differences are not the result of bacteriolytic or glycolytic action, as some might argue, we have further evidence to show. We have found, for example, that not only can this increase be obtained by complete coagulation of the blood or serum, but that a similar rise in the sugar obtainable by dialysis is always to be observed when the blood or serum has been previously treated with carbon dioxide, and further, that this rise can again be annulled if the carbon dioxide is displaced in the serum by oxygen. These facts cannot be explained on any theory of glycolysis or bacterial action. In further support of the existence of some form of loose chemical combination between glucose and some of the constituents of the serum, preliminary experiments with artificially prepared lecithin-sugar dissolved in saline, dialysed under similar conditions, have led to analogous results.¹

1. Details of the action of carbon dioxide and anaesthetics in general on the sugar-content of blood and other tissues, will shortly be published. We intend, also, to follow up the remarkable behaviour of lecithin sugar in regard to anaesthetics, and to extend these investigations to the behaviour of glucose in presence of protein.

In conclusion, we carried out several experiments in order to compare the values obtained by our method with those by precipitation methods. We have selected the method of Schenck, described in the earlier part of this paper, and have followed his methods as nearly as possible. The coagulation of the proteins was effected by treating the blood or serum with a hydrochloric acid solution of mercuric chloride. This process, as one would naturally suppose, is bound to break up any loose chemical combination of sugar with other constituents of the serum, thus yielding values for the sugar which should more nearly approximate to those obtained by us on coagulation. One might anticipate, also, some hydrolysis of any hydrolysible sugars. We have, therefore, here not only determined the sugar value by dialysis after coagulation, but have also added hydrochloric acid to the serum and saline at the beginning of dialysis, and boiled for a few minutes. Before estimating the sugar in this acid saline, it was brought to boiling and neutralised.

The results obtained are given below.

Experiment VIII.—Pig's serum dialysed for forty hours against 300 c.c. saline. Sugar percentage (mean of two determinations in each case):—

BY DIALYSIS			BY PRECIPITATION
Uncoag. control	Coagulated	Acid coagulation ¹	With mercuric chloride
0·0807%	0·1318%	0·1635%	0·1191%

Experiment IX.—Pig's serum dialysed for forty hours into 300 c.c. saline. Percentage of sugar on:—

DIALYSIS AFTER—		PRECIPITATION	
Coagulation	Coag. with HCl ²	1	2
0·1019%	0·1578%	0·1062%	0·1071%

Experiment X.—Pig's serum dialysed for forty-four hours into 300 c.c. saline. Percentage of sugar on:—

DIALYSIS OF—		PRECIPITATION	
Control	Coagulated Serum	1	2
0·0942%	0·0981%	0·1098%	0·1106%

(mean of two determinations).

1. Here 2 per cent. of ordinary strong HCl was added to both serum and saline, and the whole boiled for a few minutes at the beginning of dialysis.

2. Here only 1 per cent. of strong HCl was used.

From a comparison of these figures it is difficult to see what the value for the sugar obtained by precipitation of the protein with acid mercuric chloride actually represents. On the one hand in two cases it is greater than that obtained by dialysis after coagulation, while on the other hand it is considerably less than our value after boiling of the serum with dilute acid. The results probably point to a partial hydrolysis of some polysaccharide with, at the same time, a partial destruction of the sugar by the prolonged boiling necessary to remove the last traces of hydrogen sulphide. The very high values obtained by dialysing the serum after first boiling it with hydrochloric acid is another proof of the existence of hydrolysible sugar in the blood, as maintained by Pavy.¹

CONCLUSIONS

By this method of estimating the sugar in blood some insight has been gained as to the condition of that sugar. There is evidence to show that part of it exists as free sugar, another part as combined sugar, by this being understood those combinations of glucose with protein or lecithin already referred to, and a third part as polysaccharide or hydrolysible sugar. While it is difficult, even by this method, to discriminate sharply between these various forms of sugar, nevertheless it is a distinct improvement over the mercury precipitation method which yields figures hard to interpret. The method, moreover, is simple and yields concordant results, while it enables one to follow more closely the varying sugar-content of the blood under varying conditions. Although the time interval in making a determination of the sugar by this method is greater than by precipitation methods, yet the actual time involved in carrying out the work of determination is very much less. Furthermore, the work has shown that the corpuscles are devoid of sugar, which is entirely confined to the serum.

1. *Journ. of Physiol.*, XXVI, 282.