XLII. THE DISTRIBUTION OF REDUCING SUBSTANCES BETWEEN PLASMA AND CORPUSCLES; A COMPARISON OF VARIOUS BLOOD-SUGAR METHODS.

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The distribution of "sugar" between plasma and corpuscles has been the subject of much discussion, and the results obtained by different workers have shown wide variation. We shall not attempt to survey the literature here; a summary is given in a paper by Wiechmann [1924]. In view of the work of de Wesselow [1919] it seemed probable that the variation might be, in part, explained by differences in the methods of blood-sugar estimation. De Wesselow compared MacLean's method with the Lewis-Benedict method, and found that whereas the discrepancies between the methods on plasma were small, MacLean's method always gave considerably lower figures on corpuscles.

In a preliminary experiment, on oxalated sheep's blood, we made a similar comparison between the methods of MacLean and of Folin and Wu. In addition to the direct determination, the corpuscle-sugar was calculated from the figures for plasma and whole blood, and the haematocrit reading for the corpuscle volume. The results were as follows (mg. per 100 cc.).

	Plasma	Whole blood	Corpuscle deposit	Corpuscles calculated
MacLean	48	30	0	0
Folin-Wu	51	50	35	48

These striking figures led us to undertake a fuller investigation of the distribution of reducing substances between plasma and corpuscles as determined by various methods, but for the main investigation we worked upon fresh human venous blood.

Experimental.

The subjects of our experiments were healthy adults, men and women, sometimes examined fasting, and sometimes after an ordinary meal or after a dose of glucose.

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"Sugar" was determined directly in whole blood, plasma, and corpuscles. In addition, the corpuscle-sugar was calculated from the figures found for whole blood and plasma and the haematocrit reading for the corpuscle volume. As the figures determined directly on the corpuscle deposit were open to objections (they might have been too high as a result of insufficiently close packing of corpuscles, or too low owing to glycolysis during the time of spinning) we have tabulated only the calculated figures for corpuscles.

In the majority of experiments the procedure was as follows. A sample of blood taken by vein puncture was run into a paraffin-coated tube, and a second sample taken immediately after into a tube containing potassium oxalate. The oxalated blood was used for determinations of sugar in whole blood. The sample in the paraffin-coated tube was centrifuged at once for 2–4 minutes at 3000 r.p.m., and the plasma was pipetted off and oxalated. The corpuscle deposit was oxalated and centrifuged for a further 10 minutes. There was very rarely any clotting in the paraffin-lined tube. This procedure is indicated in the tables as "paraffin-tube technique." In oxalating the samples of whole blood, and of plasma and corpuscles after separation, care was taken to avoid an excess of oxalate—the amount present was approximately 0·15 to 0·3 %.

In a few experiments the whole sample of blood was defibrinated, and no anticoagulant used at any stage; in a few others the whole sample was oxalated.

We were able to complete the measurement of our samples into the appropriate diluting fluids in less than half an hour from the time of puncture, even when many methods were used. Thus there is little risk of error due to glycolysis.

The methods we have used are:

(1) MacLean's method [1919];
(2) the Folin-Wu method [1920];
(3) the Shaffer-Hartmann method as modified by Somogyi [1926];
(4) the method of Hagedorn and Jensen as originally described, using zinc hydroxide filtrates [1923];
(5) the method of Hagedorn and Jensen applied to tungstic filtrates [Hiller, Linder and Van Slyke, 1925; Hawk and Bergeim, 1926];
(6) the modification of the Folin-Wu method recently introduced by Benedict [1928] in which the sensitiveness of the copper reagent is diminished by addition of alanine, sodium nitrate and $NaHSO_3$. This method is indicated in the tables as the Benedict (1928).

A further note on the preparation of the tungstic filtrates is necessary. The amounts of 10 % sodium tungstate and 2/3 *N* sulphuric acid were adjusted according to the different protein content of plasma, whole blood, and corpuscles. For 1 volume plasma, $\frac{1}{2}$ volume of each of the reagents was used; for 1 volume whole blood, 1 volume of each; and for 1 volume corpuscles, $1\frac{1}{2}$ volumes of each; the total being always made up to 10 volumes. These

tungstic filtrates were used for four different methods—Folin-Wu, Shaffer-Hartmann, Hagedorn and Jensen, and Benedict (1928).

In using the colorimetric methods, a standard of approximately the same strength as the blood filtrate was always used.

All determinations were made in duplicate.

We have checked our technique in all the methods by experiments with glucose solutions and glucose added to blood.

Our results are given in Tables I and II.

Table I. *Comparisons between the MacLean and Folin-Wu methods.*

Date	Subject	Remarks		Reducing substance as glucose mg. per 100 cc. Plasma	Whole blood	Corpuscles	Ratio Plasma "sugar" / Corpuscle "sugar"
24. vii. 28	J.G.	1½ hrs. after lunch. Paraffin tube technique	MacLean	93	78	57	1·63
			Folin-Wu	102	99	95	1·07
27. vii. 28	J.G.	¾ hr. after lunch. P. tube technique	MacLean	94	81	71	1·32
			Folin-Wu	112	110	107	1·04
4. ix. 28	J.G.	2 hrs. after lunch. P. tube technique	MacLean	102	89	71	1·43
			Folin-Wu	106	104	101	1·04
5. ix. 28	J.G.	P. tube technique. Fasting	MacLean	94	84	71	1·32
			Folin-Wu	106	100	92	1·05
		½ hr. after 50 g. glucose	MacLean	160	140	114	1·40
			Folin-Wu	167	154	137	1·22
		1 hr. after glucose	MacLean	161	136	104	1·55
			Folin-Wu	166	148	125	1·32
		2 hrs. after glucose	MacLean	92	84	74	1·24
			Folin-Wu	88	93	102	0·86.
7. ix. 28	K.	2¾ hrs. after lunch. P. tube technique	MacLean	92	80	61	1·51
			Folin-Wu	99	97	94	1·05
23. vii. 28	F.K.H.	1 hr. after lunch. P. tube technique	MacLean	117	109	95	1·23
			Folin-Wu	121	120	118	1·03

Summary of results.

The methods first compared were those of MacLean and of Folin and Wu. The discrepancies between the two methods will be considered first, and the other methods then compared with these.

MacLean's method compared with the method of Folin and Wu.

There are sixteen comparisons between these two methods. If discrepancies in which the Folin-Wu figure is higher than the MacLean figure are taken as positive, and the reverse discrepancies as negative, we find:

on plasma,	the range of discrepancy is	− 4 to + 18,	average + 8	mg. per 100 cc.
„ whole blood,	„	+ 9 to + 36,	„ + 19	„
„ corpuscles,	„	+ 19 to + 49,	„ + 31	„

The range of variation is wide, but in every experiment the same effect is shown to a greater or less degree. The discrepancy between the two methods

Table II. *Distribution of reducing substances between plasma and corpuscles as determined by various blood-sugar methods.*

Date	Subject	Conditions	Methods	Reducing substance as glucose mg. per 100 cc.			Ratio Plasma "sugar" / Corpuscle "sugar"
				Plasma	Whole blood	Corpuscles	
17. x. 28	R.J.B.	1¼ hrs. after lunch. P. tube technique	MacLean	120	113	105	1·14
			Hagedorn (zinc)	—	110	—	—
			„ (tungstic)	124	140	162	0·76
			Folin-Wu	131	128	124	1·06
			Shaffer-Hartmann	136	135	135	1·01
21. x. 28	F.K.H.	2 hrs. after lunch. Oxalated blood	MacLean	104	83	50	2·08
			Hagedorn (zinc)	99	85	63	1·57
			„ (tungstic)	86	103	130	0·66
			Folin-Wu	109	109	109	1·00
			Shaffer-Hartmann	105	105	105	1·00
24. x. 28	P.	Immediately after tea. P. tube technique	Hagedorn (zinc)	148	133	112	1·23
			„ (tungstic)	163	170	180	0·91
			Folin-Wu	160	158	155	1·03
			Shaffer-Hartmann	158	159	161	0·98
			Benedict (1928)	162	150	132	1·23
26. x. 28	K.	2 hrs. after lunch. P. tube technique	Hagedorn (zinc)	77	71	62	1·24
			„ (tungstic)	89	101	118	0·75
			Folin-Wu	89	87	85	1·05
			Shaffer-Hartmann	84	83	84	1·00
			Benedict (1928)	90	81	68	1·32
29. x. 28	G.A.H.	1½ hrs. after lunch. P. tube technique	MacLean	95	81	62	1·53
			Hagedorn (zinc)	110	94	72	1·53
			„ (tungstic)	106	110	116	0·92
			Folin-Wu	106	101	94	1·11
			Shaffer-Hartmann	110	102	91	1·21
			Benedict (1928)	110	96	77	1·43
1. xi. 28	B.	After lunch. P. tube technique	Hagedorn (zinc)	108	106	103	1·05
			„ (tungstic)	114	131	153	0·75
			Folin-Wu	120	115	109	1·10
			Shaffer-Hartmann	115	115	115	1·00
			Benedict (1928)	118	105	86	1·25
5. xi. 28	K.M.H.	3 hrs. after lunch. P. tube technique	MacLean	137	127	113	1·21
			Hagedorn (zinc)	142	124	98	1·45
7. xi. 28	G.	1½ hrs. after lunch. P. tube technique	MacLean	112	102	90	1·24
			Hagedorn (zinc)	119	107	93	1·28
			„ (tungstic)	141	134	126	1·12
			Folin-Wu	123	119	114	1·08
			Shaffer-Hartmann	120	112	103	1·17
13. xi. 28	F.K.H.	After lunch. P. tube technique	MacLean	99	81	51	1·94
			Hagedorn (zinc)	95	80	55	1·73
			„ (tungstic)	100	110	127	0·78
			Folin-Wu	102	99	94	1·06
			Shaffer-Hartmann	97	97	97	1·00
			Benedict (1928)	105	89	62	1·69
14. xi. 28	R.J.B.	3 hrs. after lunch. Oxalated blood	MacLean	95	85	69	1·38
			Hagedorn (zinc)	98	85	66	1·52
			„ (tungstic)	106	124	156	0·68
			Folin-Wu	109	111	114	0·96
			Shaffer-Hartmann	102	109	120	0·85
			Benedict (1928)	116	113	108	1·07
15. xi. 28	L.R.W.P.	3 hrs. after lunch. Defibrinated blood	MacLean	105	94	80	1·38
			Hagedorn (zinc)	114	104	92	1·24
			„ (tungstic)	119	132	149	0·80
			Folin-Wu	118	120	122	0·97
			Shaffer-Hartmann	116	114	112	1·04
			Benedict (1928)	112	107	101	1·11
7. i. 29	F.K.H.	2 hrs. after lunch. Defibrinated blood	MacLean	82	62	33	2·48
			Shaffer-Hartmann	86	86	86	1·00

is always greater on whole blood than on plasma, and greatest on the corpuscle figures. This was confirmed by the direct determinations on corpuscle deposits, which have not been tabulated.

The Shaffer-Hartmann method as modified by Somogyi.

This method gives figures agreeing well with the Folin-Wu figures, and, like them, showing a small discrepancy with the MacLean results on plasma, and a marked difference on corpuscles. Discrepancies in which the Shaffer-Hartmann figures are higher have been taken as positive, and the reverse discrepancies as negative, in each case.

There are ten comparisons with the Folin-Wu method, and the discrepancies between Shaffer-Hartmann figures and Folin-Wu figures are:

on plasma:	− 7 to + 5,	average − 3	mg. per 100 cc.
„ whole blood:	− 6 to + 7,	„ 0	„
„ corpuscles:	− 9 to + 11,	„ 0	„

There are eight comparisons with MacLean's method, and the discrepancies are:

on plasma:	− 2 to + 15,	average + 7	mg. per 100 cc.
„ whole blood:	+ 10 to + 24,	„ + 20	„
„ corpuscles:	+ 13 to + 55,	„ + 39	„

The method of Hagedorn and Jensen.

The original method, applied to zinc hydroxide filtrates, gives figures which agree fairly well with MacLean's method, and shows definitely lower figures for corpuscles than for plasma. When the ferricyanide reduction method is applied to tungstic acid filtrates, the results indicate a very high corpuscle-sugar—the highest given by any method. This is the only method which indicates a definitely higher sugar value for corpuscles than for plasma. We will summarise these effects by comparing the original Hagedorn and Jensen method with MacLean's method, the Hagedorn and Jensen method (tungstic filtrates) with the Folin-Wu method, and thirdly, comparing the zinc filtrates with the tungstic filtrates.

The original Hagedorn and Jensen method compared with MacLean's method. There are seven comparisons. If the discrepancies in which the Hagedorn and Jensen method gives the higher figure are taken as positive, the results are:

on plasma,	the range of discrepancy is	− 5 to + 15,	average + 4	mg. per 100 cc.
„ whole blood,	„	− 3 to + 12,	„ + 4	„
„ corpuscles,	„	− 14 to + 13,	„ + 4	„

In most of the determinations the figures given by the two methods agree closely, and, where there is a definite difference, the method of Hagedorn and Jensen gives the higher figure, and the difference is about the same on plasma and corpuscles.

The Hagedorn and Jensen method, applied to tungstic filtrates, compared with the Folin-Wu method. There are ten comparisons, and, taking discrepancies in which the Hagedorn and Jensen figures are higher as positive, we have:

on plasma,	the range of discrepancy is	− 23 to + 18, average	+ 2 mg. per 100 cc.
„ whole blood,	„ „	− 6 to + 16, „	+ 11 „
„ corpuscles,	„ „	+ 12 to + 44, „	+ 30 „

The wide range of discrepancy on plasma is chiefly due to two extreme experiments. In the remaining eight experiments the range is − 7 to + 1 (average − 2).

The method of Hagedorn and Jensen; zinc filtrates and tungstic filtrates. There are ten comparisons. Discrepancies in which the tungstic filtrates give the higher figures are taken as positive.

on plasma,	the range of discrepancy is	− 13 to + 22, average	+ 6 mg. per 100 cc.
„ whole blood,	„ „	+ 16 to + 39, „	+ 27 „
„ corpuscles,	„ „	+ 33 to + 90, „	+ 56 „

Benedict's modification of the Folin-Wu method.

Compared with the original Folin-Wu method Benedict's modification gives slightly lower figures on whole blood. Taking discrepancies in which the Folin-Wu method gives higher figures as positive, we have:

on plasma,	the range of discrepancy is	− 7 to + 6, average	− 1 mg. per 100 cc.
„ whole blood,	„ „	+ 2 to + 13, „	+ 8 „
„ corpuscles,	„ „	+ 6 to + 32, „	+ 20 „

These figures are based on seven comparisons.

The Benedict (1928) method compared with the original Hagedorn and Jensen method. We take this comparison rather than the comparison with MacLean's method, because we have seven experiments in which the Benedict and Hagedorn and Jensen methods are compared, and only four in which there are both MacLean figures and Benedict figures.

If we take discrepancies in which the Benedict figures are higher than the Hagedorn and Jensen figures as positive, we have:

on plasma,	the range of discrepancy is	− 2 to + 14, average	+ 9 mg. per 100 cc.
„ whole blood,	„ „	− 1 to + 28, „	+ 10 „
„ corpuscles,	„ „	− 17 to + 42, „	+ 10 „

The ratio of plasma-"sugar" to corpuscle-"sugar."

It has already been explained that, in the majority of our experiments, the plasma was obtained from a sample of blood to which no anti-coagulant was added until the plasma had been separated. Under these conditions the true ratio of "sugar" in plasma and corpuscles should be obtained. The average ratios of plasma-"sugar" to corpuscle-"sugar" by the various methods (excluding the few experiments on oxalated blood) are as follows:

MacLean	1·48 (average of	16	determinations)	
Hagedorn-Jensen (zinc filtrates)	1·34 („	8	„	)
Hagedorn-Jensen (tungstic filtrates)	0·85 („	8	„	)
Folin-Wu	1·07 („	17	„	)
Shaffer-Hartmann	1·05 („	9	„	)
Benedict (1928)	1·34 („	6	„	

The difference between the methods of MacLean and Hagedorn and Jensen (zinc filtrates) may be exaggerated here, because there are several experiments in which only one of these methods was used. If we take the shorter series of experiments in which both methods were applied to the same blood, the average ratios are much closer together (viz. 1·46 and 1·45). There is considerable variation of the ratio in all the three methods which give the higher ratios, and the number of experiments is not large enough to give strictly comparable averages. A consideration of individual experiments brings out the same relations as are shown by the average figures.

It is noteworthy that in the sugar-tolerance curve (5. ix. 28) the ratios by both MacLean's method and the Folin-Wu method rose when the total sugar was high, and fell when the total sugar was low, whereas the discrepancies between the methods remained fairly steady.

To sum up: we have two methods which agree fairly well and indicate a higher sugar value in plasma than in corpuscles, namely MacLean's method and the original Hagedorn and Jensen method. The Benedict (1928) method[1] gives a similar distribution ratio, but this ratio is derived from slightly higher figures for both the plasma and corpuscles. We have two methods which agree very well and indicate an approximately even distribution of "sugar" between plasma and corpuscles—the Folin-Wu and Shaffer-Hartmann methods. Finally, the Hagedorn and Jensen method applied to tungstic filtrates is alone in indicating a higher sugar figure for corpuscles than for plasma, thus differing very greatly from the same method of estimation applied to zinc hydroxide filtrates.

Possible effects due to technique.

Before discussing the significance of these results we must consider two factors inherent in the technique, which might unequally affect the determinations on plasma, whole blood, and corpuscles.

The first is the possible error introduced by taking an aliquot part of a blood filtrate. The original method of Hagedorn and Jensen is free from this objection, because the protein precipitate is washed and the whole filtrate with washings is used. In the MacLean method of protein precipitation the blood is diluted 1 in 125, so that the volume of the precipitate is negligible. In the tungstic precipitation the conditions are different. The dilution of the blood is 1 in 10, and there is an apparently voluminous protein precipitate which is greater in whole blood than in plasma, and greater still when corpuscles are used. We have observed that there is no volume change on mixing the reagents: the total volume of the mixture, including the precipitate, is 10 cc. for 1 cc. of blood. If the volume of the precipitate is appreciable, the filtrate must be concentrated, and since, when taking an aliquot part, we

[1] Rockwood [1926] has made comparisons between the Folin-Wu method and Benedict's earlier modification of it (not the method we have used). He found that the differences between the original Folin-Wu and the modification were most pronounced in the corpuscle figures.

assume that the fluid volume is still 10 cc., our figures will be too high, the error being greatest when the protein precipitate is greatest. We tested this experimentally as follows. The usual mixtures of plasma, whole blood, or corpuscles with the sodium tungstate and sulphuric acid were made in graduated centrifuge tubes; the protein was centrifuged down and the supernatant liquid removed, and the tubes were dried to constant weight. A known volume of absolute alcohol was pipetted into each tube and the level noted. In this way the solid volume was estimated by displacement; for plasma we found it 0·5 % of the total volume of precipitation mixture, for whole blood 2 %, and for corpuscles 2·5 %. These figures are only approximate, but show that the effect is negligible and that no appreciable error is introduced by taking aliquot parts of the filtrate.

Secondly, in the tungstic precipitation and the MacLean precipitation the reagents are acid, and some hydrogen ions are taken up by the protein. Since there is less protein in the plasma and more in the corpuscles, the acidity of the filtrates from plasma is greater than that of filtrates from whole blood. In our tungstic filtrates from plasma the p_H was approximately 4·8, from the whole blood 5·1, and from corpuscles 5·3. There was thus little difference, and we found that adjustment of the filtrates to approximately p_H 6·0 had no effect on the figures obtained. The copper reduction methods are sensitive to changes in p_H, as has been emphasised by Folin and Svedberg [1926] and Somogyi [1926], but in our filtrates the difference was too small to introduce any error. Similarly, in the MacLean method, the differences in acidity of filtrates are too small to affect the results. In the zinc hydroxide filtrates used for the Hagedorn and Jensen method, there is no appreciable difference in p_H between filtrates from plasma, whole blood, and corpuscles; all three are approximately of p_H 6·6.

A non-glucose reducing substance.

We know that all the methods estimate glucose quantitatively, whether in pure solution or added to blood. It is therefore unlikely that the methods which give low figures on corpuscles are failing to estimate glucose, and we are led to postulate the presence of some non-glucose reducing substance, predominantly present in corpuscles, which may account for the discrepancies between methods. The methods giving lower, and therefore probably truer, glucose figures are the methods of MacLean and Hagedorn and Jensen (zinc filtrates). These methods are absolutely different in principle, and yet give fair agreement. But when we apply the ferricyanide reduction to tungstic filtrates we obtain the highest figures of any for corpuscle-sugar. The interfering substances must be present in tungstic filtrates, and either absent from the iron filtrates and zinc filtrates, or present in them in smaller amount. Of the four methods applied to tungstic filtrates, three give high corpuscle figures—Hagedorn and Jensen, Shaffer-Hartmann, and Folin-Wu—and the tungstic precipitation is the only feature common to the three methods. The

Benedict (1928) method is the only method applied to tungstic filtrates which gives a distribution ratio similar to that given by MacLean's method, and the copper reagent used in the Benedict (1928) method is designed to be relatively insensitive, and therefore more selective than the copper reagent of the Folin-Wu method. Benedict claims that his modification gives true sugar figures, but it seems probable that the figures given by his method, though lower than the Folin-Wu figures, are slightly above the true sugar value, since we find that the methods of MacLean and Hagedorn and Jensen (zinc filtrates) give lower figures still.

We have further evidence of the important part played by the method of protein precipitation. We have applied MacLean's method to tungstic filtrates as follows. 2 cc. of a 1 in 10 tungstic filtrate are diluted with 18 cc. of the acid sodium sulphate solution used in MacLean's method: 2 cc. of the copper solution are added, and the estimation carried out in the usual way. (The filtrate taken corresponds to 0·2 cc. blood, *i.e.* 5/4 of the amount in the ordinary MacLean method.) This modification of the method quantitatively determines glucose added to blood. In Table III are given comparisons between the ordinary MacLean method, the MacLean method applied to tungstic filtrates, and the Folin-Wu or Shaffer-Hartmann methods. MacLean's method of estimation gives higher figures on tungstic than on iron filtrates, and the MacLean estimation applied to tungstic filtrates usually agrees with the Folin-Wu or Shaffer-Hartmann method. The distribution ratio given by the MacLean method applied to tungstic filtrates is close to unity, and differs greatly from the ratio given by the ordinary MacLean method.

Table III.

	Methods		Reducing substance as glucose, mg. per 100 cc.		
			Plasma	Whole blood	Corpuscles (directly determined)
I	MacLean	Iron filtrate	—	75	—
	„	Tungstic filtrate	—	83	—
	Folin-Wu	„	—	102	—
II	MacLean	Iron filtrate	120	113	—
	„	Tungstic filtrate	124	123	—
	Folin-Wu	„	131	128	—
III	MacLean	Iron filtrate	—	87	—
	„	Tungstic filtrate	—	94	—
	Folin-Wu	„	—	100	—
IV	MacLean	Iron filtrate	101	84	78
	„	Tungstic filtrate	120	118	109
	Shaffer-Hartmann	„	—	116	115
V	MacLean	Iron filtrate	82	62	—
	„	Tungstic filtrate	91	88	88
	Shaffer-Hartmann	„	86	86	86

We are thus led to the hypothesis that there is some non-glucose reducing substance present in corpuscles. The various methods applied to tungstic filtrates vary in their sensitiveness to this unknown substance, the ferri-

cyanide reduction being the most sensitive, and the Benedict (1928) copper reduction the least.

Direct evidence of a non-glucose reducing substance in corpuscles. In addition to the inference drawn from our comparison of methods, we have direct evidence of a reducing substance other than glucose in tungstic filtrates of whole blood or corpuscle deposits.

If a tungstic filtrate from whole blood is added to the alkaline copper reagent used in the Folin-Wu estimation, and the phosphomolybdate reagent is added at once, in the cold, a definite blue colour develops. This reaction is not given by plasma filtrates, and is given very strongly by corpuscle filtrates. The reaction does not occur if the blood filtrate and phosphomolybdate are mixed in the absence of the copper solution; it is a reduction of the copper reagent, not a direct reduction of the phosphomolybdate reagent.

Colloidal ferric hydroxide filtrates from blood do not give the reaction with the Folin-Wu copper solution, neither do zinc hydroxide filtrates. For the purpose of this test the iron or zinc filtrates were of the same concentration as the tungstic filtrates—10 cc. filtrate corresponding to 1 cc. blood. We have therefore direct evidence of a reducing substance in corpuscles, which passes into tungstic filtrates and not into iron or zinc filtrates.

It is possible to remove glucose from blood by a short incubation with a large excess of yeast, and to precipitate the mixture with tungstic acid [Somogyi, 1927]. Such filtrates from fermented blood still give the reaction with the Folin-Wu copper solution in the cold.

The probable nature of the unknown substance. The idea that non-glucose reducing substances in blood may affect blood-sugar estimations is not new; it is known that some of the methods give a residual reduction after fermentation with yeast. The earlier work on this residual reduction was unsatisfactory, owing to technical difficulties, but these were overcome by the discovery of Hiller, Linder and Van Slyke [1925], that a very short incubation, with excess of yeast, was sufficient to remove glucose. Somogyi, using this principle, has determined the non-fermentable reducing substances in whole blood, plasma and corpuscles, by his modification of the Shaffer-Hartmann method. In his first paper [1927] he gave the figure for whole blood as 27 mg. per 100 cc., but his later technique [1928] gave figures 3 or 4 mg. lower; on this basis we may take the figure for plasma as 8, for whole blood 23, and for corpuscles 40 mg. per 100 cc. Now we have found that MacLean's method gives figures lower than the Shaffer-Hartmann method as modified by Somogyi, and that the discrepancy is for plasma 7, for whole blood 20, and for corpuscles 39 mg. per 100 cc. These are average figures. The agreement between these discrepancies and Somogyi's figures for non-glucose reducing substances is striking, and naturally leads to the suggestion that MacLean's method gives figures very close to the true sugar value.

We must now consider some substances which might be responsible for the residual reduction after fermentation with yeast, and for the discrepancies between methods.

Uric acid, creatine and creatinine may be dismissed, for Hiller, Linder and Van Slyke [1925] have shown that their reduction effect is too small to be of importance, in view of the small amounts present in blood.

Ergothioneine is a reducing substance, present only in corpuscles, and it is capable of affecting blood-sugar methods [Sjollema, 1927]. Hunter [1928] has estimated the amount present in normal human blood, and finds 2–10 mg. per 100 cc. corpuscles, *i.e.* a maximum of 4 or 5 mg. per 100 cc. whole blood. We have made a few determinations of the effect of ergothioneine solutions upon the various blood-sugar methods, and find that 100 mg. ergothioneine is equivalent to 10–60 mg. glucose, according to the method used. In view of the very small amount of ergothioneine in human blood, the effect is negligible.

Glutathione is known to be present in corpuscles, mainly in the reduced form [Hunter and Eagles, 1927; Holden, 1925; Thomson and Voegtlin, 1926; Uyei, 1926]. It is capable of reducing ferricyanide and the Folin-Wu reagents [Sjollema, 1927]. We have found that solutions of reduced glutathione reduce the Folin-Wu copper reagent in the cold; glutathione is therefore probably responsible for this reaction as given by tungstic filtrates of corpuscles.

It is well known that tungstic filtrates from corpuscles give a positive nitroprusside reaction, whereas tungstic filtrates from plasma do not. On the basis of the intensity of the nitroprusside reaction, Hunter and Eagles estimate the glutathione in human blood at 100–120 mg. per 100 cc. corpuscles, *i.e.* 40–50 mg. per 100 cc. blood.

The subject of the quantitative effect of glutathione in the various blood-sugar methods, and, in particular, its behaviour in the various methods of protein precipitation, is still under investigation, and will form the subject of a later paper. It may be said at present that concentrations of reduced glutathione, of the order of 45 mg. per 100 cc., give a significant reduction of all the blood-sugar reagents except that of Benedict (1928).

Significance for general analytical work.

We have shown that differences of the order of 30 mg. per 100 cc. are commonly found, when different methods are applied to the same sample of whole blood. Differences of this order are of importance in clinical work, and it is necessary to interpret results according to the method used. Also our findings give an explanation of some of the varying results obtained by different workers for the distribution of "sugar" between plasma and corpuscles. Further, our results suggest that the choice of method may be of great importance in the determination of "sugar" in tissue extracts or other material in which glutathione and other reducing substances are likely to be present, particularly if the relative amount of glucose is small and concentrated extracts are used (*e.g.* in glycolysis experiments).

SUMMARY.

1. The various methods of blood-sugar estimation give widely different results for the distribution of "sugar" between plasma and corpuscles. The average discrepancies between methods are not great for estimations on plasma, but are considerable for estimations on corpuscles.

2. The methods of MacLean and Hagedorn and Jensen (original) agree fairly well, and give a higher sugar value in plasma than in corpuscles.

3. The methods of Folin-Wu and Shaffer-Hartmann (Somogyi's modification) agree very well, and indicate approximately even distribution of reducing substances between plasma and corpuscles.

4. In comparing the method of MacLean or Hagedorn and Jensen (original), with the method of Folin-Wu or Shaffer-Hartmann, we find that the first pair of methods gives slightly lower figures than the second pair of methods on plasma, and considerably lower figures on corpuscles.

5. Benedict's latest modification of the Folin-Wu method gives ratios of plasma-"sugar" to corpuscle-"sugar" similar to those given by the method of MacLean or the original Hagedorn and Jensen method, but the figures given by the Benedict (1928) method are slightly higher on both plasma and corpuscles.

6. The method of Hagedorn and Jensen applied to tungstic filtrates gives very high corpuscle figures, and is the only method which shows more "sugar" in corpuscles than in plasma. Zinc hydroxide filtrates from whole blood or corpuscles give very much lower figures than tungstic filtrates when estimated by the ferricyanide method.

7. MacLean's method may be applied to tungstic filtrates and the results for corpuscles are much higher, by this modification, than by the original method.

8. We suggest that the discrepancies between blood-sugar methods are due to the presence of a non-glucose reducing substance in corpuscles, which is present in tungstic filtrates, and is either absent from iron and zinc filtrates, or present in them in less amount. We have direct evidence of the presence of such a substance, because tungstic filtrates of whole blood or corpuscles produce a reduction of the Folin-Wu copper reagent in the cold, whereas filtrates from plasma give no such reaction. The reaction is not given by iron filtrates or zinc filtrates.

9. It is suggested that the substances responsible for the discrepancies between methods may also be responsible for the residual reduction after fermentation of blood with yeast.

10. Uric acid, creatine, creatinine, and ergothioneine are dismissed as having no appreciable effect on blood-sugar methods, in the small amounts in which these substances occur in human blood.

11. Glutathione is suggested as having an important effect on blood-sugar methods.

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