XXXVI. THE NON-SUGAR REDUCING SUBSTANCES OF HUMAN BLOOD, WITH SPECIAL REFERENCE TO GLUTATHIONE.

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In the preceding paper the behaviour of pure reduced glutathione in the various blood-sugar methods was described. In the present paper the effects produced by the non-sugar reducing substances of blood are determined, and are compared with those obtained with pure glutathione.

Experimental.

Freshly drawn, oxalated, venous blood of normal adults was used, and to each sample three methods of protein precipitation were applied. These were: (1) the Folin-Wu tungstic acid precipitation, which allows glutathione to pass into the filtrate, (2) the zinc hydroxide precipitation of Somogyi [1929, 1] in which the glutathione is removed, and (3) a new technique for preparing a glutathione-free filtrate with tungstic acid. Each of the three filtrates was analysed by the following methods: Hagedorn-Jensen [1923], Shaffer-Hartmann [Somogyi, 1926], Folin-Wu [1920] and Benedict [1928]. In all precipitation methods the dilution of the blood was 1 in 10, so that for the Shaffer-Hartmann, Folin-Wu, and Benedict methods the volumes of filtrate described in the original methods were taken. In the ferricyanide method 1 cc. filtrate was used, and the volume was made up to about 12 cc. with distilled water before addition of the ferricyanide. This procedure is exactly comparable with the original Hagedorn-Jensen method, in which 0·1 cc. of blood is taken, and the volume of the filtrate from this, together with the washings, is about 12 cc.

All filtrates were neutralised (to phenolphthalein) before use; the volume of alkali added was negligible in relation to the total volume. In the case of the zinc hydroxide filtrates the addition of the alkali caused a further precipitation of zinc hydroxide, which was removed by re-filtering.

In the colorimetric methods readings were taken within 10 to 15 minutes after dilution of the coloured solution, and no fading of the colour took place

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in this time. Everett [1929] has suggested that such fading may cause an error in the Benedict method.

Folin-Wu filtrates were made in the usual way [Folin and Wu, 1919]. They invariably gave a positive nitroprusside reaction.

Zinc hydroxide filtrates were made as described by Somogyi [1929, 1]. In every case we found the nitroprusside reaction negative. Therefore these filtrates contain no appreciable amount of reduced glutathione.

On the basis of our experience with glutathione solutions we may say that a negative nitroprusside reaction indicates that the concentration of glutathione, if any, is less than 0·3 mg. per 100 cc. filtrate. Such small amounts produce no measurable effect on the analytical methods.

A preliminary comparison was made between the Somogyi zinc precipitation method and the Hagedorn-Jensen zinc precipitation method, using the ferricyanide method of analysis. In every case the Hagedorn-Jensen precipitation technique gave a higher figure than the Somogyi precipitation technique. The difference was 1 to 10 mg. per 100 cc. (average 4 mg. per 100 cc.) and was therefore scarcely outside the limits of experimental error, but, since the Hagedorn-Jensen filtrate gave always the higher figures, the difference is probably significant. On the basis of the work with pure glutathione, it was predicted that the Hagedorn-Jensen filtrate would give figures higher than the Somogyi filtrate by 9 mg. per 100 cc. The difference between the observed and expected results is small.

The zinc hydroxide filtrates in the experiments in Table I were all made by the Somogyi technique.

Modified tungstic acid filtrates. Glutathione is present only in the corpuscles, and therefore presumably exists in a non-diffusible form. It is possible to prepare a filtrate free from glutathione if the corpuscles are preserved intact during the precipitation process. The Folin-Wu tungstic acid procedure has been modified by delivering the blood sample into an isotonic salt solution instead of laking it in distilled water, and adding a quantity of tungstate and acid sufficient to precipitate the plasma proteins. The procedure is as follows.

1 cc. blood is mixed with 8 cc. 3 % sodium sulphate (Na_2SO_4, $10H_2O$) and 0·5 cc. 10 % sodium tungstate (Na_2WO_4, $2H_2O$), and 0·5 cc. 2/3 *N* sulphuric acid is added. The mixture is centrifuged or filtered; in either case the intact corpuscles are removed mechanically, being thrown down by the centrifuge, or, in the case of filtration, caught up in the precipitated plasma-proteins. For this reason less tungstic acid is required than in the ordinary Folin-Wu precipitation. The precipitate remains bright red, and microscopically the corpuscles are seen to be intact. The nitroprusside reaction of the filtrate is negative; therefore the filtrate contains no reduced glutathione.

Separation of the filtrate should be accomplished without undue delay (*e.g.* within 30 minutes of the addition of the tungstate and acid). The p_H of this "sulphate-tungstic" filtrate is approximately the same as that of the Folin-Wu filtrate.

If the tungstic acid is in excess of the amounts specified, it is only possible to obtain a glutathione-free filtrate if the preparation of the filtrate is completed within 4 minutes of making the mixture. After this time, the tungstic acid begins to attack the corpuscles with liberation of glutathione, as is shown by the gradual and parallel development of the following changes: the colour of the protein precipitate changes, as a result of the formation of acid haematin; microscopically, "ghosts" of corpuscles appear in the mixture; the filtrate gives a positive nitroprusside reaction of increasing intensity; and the apparent "sugar" figure of the filtrate, analysed by the ferricyanide method, rises.

Sodium sulphate is chosen for the isotonic solution because it does not interfere with the blue colour in the Folin-Wu and Benedict methods. Chloride causes a rapid fading of this colour; this has also been noted by Everett, Shoemaker, and Sheppard [1927].

In the above technique the glutathione is retained in the corpuscles. The glucose, on the other hand, diffuses freely throughout the precipitation mixture, as will be shown in considering the analytical results.

Note on a colour reaction of unknown origin.

While working out the new technique we observed a curious colour reaction. When blood was mixed with isotonic sodium chloride solution, an excess of tungstic acid added, the mixture centrifuged, and the supernatant fluid poured through a filter, the filter paper gradually developed a blue colour. This colour faded in the dark and reappeared on exposure of the paper to light; it was marked when 1 cc. 10 % sodium tungstate and 1 cc. 2/3 *N* sulphuric acid were used for 1 cc. blood, and very faint when the proportions were 0·5 cc. of each reagent for 1 cc. blood. We have not investigated the cause of this colour, but it is of interest that a similar colour appeared on the paper through which mixtures of yeast and tungstic acid were filtered. We never observed this colour when mixtures of yeast with blood or plasma were precipitated by tungstic acid; nor was it ever seen with the ordinary Folin-Wu precipitation of blood.

Results.

The results of twelve experiments on six normal subjects are given in Table I. The salient points are as follows.

On *zinc hydroxide filtrates* (Somogyi's technique) all methods agree fairly well; the maximum variation is 9 mg. per 100 cc. There is a tendency for the two titration methods to give slightly higher figures than the colorimetric methods. On an average the Hagedorn-Jensen figure is 4 mg., the Shaffer-Hartmann, 5 mg., the Folin-Wu, 2 mg. per 100 cc. higher than the Benedict figure. The differences are small and it is impossible to say whether they are due to technical causes or to the presence in the zinc filtrates of traces of interfering substances. Somogyi [1929, 2] has applied various methods to zinc hydroxide filtrates and found that they agree within the limits of experimental error.

Table I.

Date and subject	Methods	Reducing substance expressed as mg. glucose per 100 cc. blood: Folin-Wu filtrate (*a*)	Sulphate-tungstic filtrate (*b*)	Somogyi's zinc hydroxide filtrate	Non-sugar effect (*a*–*b*)	True sugar
31. x. 29	Hagedorn-Jensen	105	76	76	29	
K.	Shaffer-Hartmann	90	73	67	17	74
	Folin-Wu	84	72	—	12	
	Benedict [1928]	74	72	—	2	
4. xi. 29	Hagedorn-Jensen	175	151	151	24	
F.K.H.	Shaffer-Hartmann	165	145	144	20	147
	Folin-Wu	156	142	—	14	
	Benedict [1928]	146	150	—	−4	
6. xi. 29	Hagedorn-Jensen	120	90	95	30	
M.C.B.	Shaffer-Hartmann	109	92	90	17	89
	Folin-Wu	97	87	—	10	
	Benedict [1928]	89	83	—	6	
15. xi. 29	Hagedorn-Jensen	86	62	60	24	
K.	Shaffer-Hartmann	81	63	61	18	60
	Folin-Wu	74	63	58	11	
	Benedict [1928]	57	57	57	0	
18. xi. 29	Hagedorn-Jensen	117	92	86	25	
E.	Shaffer-Hartmann	109	92	89	17	86
	Folin-Wu	99	90	81	9	
	Benedict [1928]	80	81	80	−1	
27. xi. 29	Hagedorn-Jensen	119	90	94	29	
M.C.B.	Shaffer-Hartmann	113	94	92	19	91
	Folin-Wu	104	91	90	13	
	Benedict [1928]	89	90	90	−1	
3. xii. 29	Hagedorn-Jensen	112	87	85	25	
E.	Shaffer-Hartmann	109	87	87	22	83
	Folin-Wu	100	81	82	19	
	Benedict [1928]	83	78	81	5	
6. xii. 29	Hagedorn-Jensen	119	87	83	32	
G.A.H.	Shaffer-Hartmann	113	90	90	23	83
	Folin-Wu	99	83	81	16	
	Benedict [1928]	78	79	78	−1	
16. xii. 29	Hagedorn-Jensen	97	72	67	25	
R.H.	Shaffer-Hartmann	85	73	73	12	70
	Folin-Wu	84	73	69	11	
	Benedict [1928]	70	68	69	1	
19. xii. 29	Hagedorn-Jensen	166	134	131	32	
F.K.H.	Shaffer-Hartmann	152	130	128	22	131
	Folin-Wu	153	137	135	16	
	Benedict [1928]	129	127	128	2	
23. xii. 29	Hagedorn-Jensen	133	107	106	26	
R.H.	Shaffer-Hartmann	120	101	101	19	101
	Folin-Wu	112	101	101	11	
	Benedict [1928]	96	93	100	3	
20. i. 30	Hagedorn-Jensen	118	85	85	33	
G.A.H.	Shaffer-Hartmann	102	81	81	21	81
	Folin-Wu	100	84	82	16	
	Benedict [1928]	79	75	79	4	

The "*sulphate-tungstic*" *filtrates* give values which are in good agreement with those found for the zinc hydroxide filtrates. All the methods applied to them agree fairly well; the variations are of the same order as those found for the zinc hydroxide filtrates, and may be due to the presence of traces of interfering substances or to technical causes.

In every experiment the *Folin-Wu filtrates* give higher figures than the zinc hydroxide and "sulphate-tungstic" filtrates, by every method except Benedict's. The highest values are always obtained by the Hagedorn-Jensen method, and after this in order come the Shaffer-Hartmann, Folin-Wu, and Benedict methods.

In any given experiment the *Benedict figure* is approximately the same for all three filtrates. The maximum variation is 7 mg. per 100 cc., and only two experiments show a greater discrepancy than 5 mg. per 100 cc. The variation is therefore within the limits of experimental error.

Discussion.

True sugar.

For each blood sample we have nine determinations which we know are unaffected by glutathione. These include all determinations on the zinc hydroxide and "sulphate-tungstic" filtrates, since these filtrates contain no glutathione, and the determination on the Folin-Wu filtrate by the Benedict method, which is unaffected by the glutathione. These nine determinations agree fairly well; the maximum variation is 14 mg. per 100 cc., and in all but three experiments the figures fall within 10 mg. per 100 cc. Somogyi [1929, 1] has proved that his zinc hydroxide filtrates contain only fermentable sugar. Since in our experiments the "sulphate-tungstic" filtrates show the same figures as the zinc filtrates, we may regard these also as representing only true sugar. The possible criticism that in our method glucose might be retained in the corpuscles is also met by this agreement, since the corpuscles are laked in the zinc hydroxide technique. Also, the Benedict figure on the "sulphate-tungstic" filtrate agrees with the Benedict figure on the Folin-Wu filtrate; this indicates that both filtrates contain the same concentration of glucose.

The slight variation which occurs among the nine determinations under consideration may possibly be due to traces of interfering substances, but a consideration of individual experiments shows that the variation is not more than might be expected as experimental error. Therefore any or all of the nine figures mentioned may be taken as representing the true blood-sugar value, unaffected by glutathione or other reducing material. The average of the nine figures for each experiment is listed in the tables as "True sugar," and the variation from this average is never greater than $\pm$ 7 mg. per 100 cc., and is usually less than this.

Non-sugar effect.

The differences between the figures given by the Folin-Wu filtrate and those given by the "sulphate-tungstic" filtrate must be due entirely to the non-diffusible reducing substances derived from corpuscles. If the "sulphate-tungstic" filtrate may be regarded as giving true sugar figures, then the whole of the non-sugar reducing material must consist of the non-diffusible substances of corpuscles. The Folin-Wu filtrate contains this non-diffusible material, which must be partly or wholly accounted for as glutathione. The effect due to this (expressed as glucose) is determined, for each method, by subtracting the figure obtained with the "sulphate-tungstic" filtrate from that obtained with the Folin-Wu filtrate. These difference values are listed in the table under the heading "non-sugar effect." The amount indicated varies consistently with the method used, but for any one method a striking constancy is maintained through the series. These facts may be summarised as follows:

Method	Maximum Exp. 6. xii. 29 (mg. per 100 cc.)	Minimum Exp. 18. xi. 29 (mg. per 100 cc.)	Average (mg. per 100 cc.)
Hagedorn-Jensen	32	25	28
Shaffer-Hartmann	23	17	18
Folin-Wu	16	9	13
Benedict	−1	−1	1

When these effects observed in blood are compared with the effects produced by pure glutathione, it is found that for each experiment it is possible to explain the whole of the observed effect by postulating a definite concentration of glutathione in the blood. Thus, from the curves plotted for pure glutathione (Fig. 1 of the preceding paper) we can ascertain the effect, expressed as mg. glucose per 100 cc., that would be produced by various concentrations of reduced glutathione:

	59 mg. G.SH per 100 cc.	44 mg. G.SH per 100 cc.	52 mg. G.SH per 100 cc.
Hagedorn-Jensen	32	24	29
Shaffer-Hartmann	23	17	20
Folin-Wu	13	10	11
Benedict	0	0	0

The maximum non-sugar effect observed in the experiments on blood (Table I, G.A.H. 6. xii. 29) corresponds closely to the effect which would be produced by a blood-glutathione of 59 mg. per 100 cc.; the minimum effect (Table I, E. 18. xi. 29) may be explained on the hypothesis that that blood contained 44 mg. glutathione per 100 cc.; the average effect is very close to that produced by 52 mg. glutathione per 100 cc. Similarly for each individual blood sample it is possible to find a concentration of glutathione (between 44 and 59 mg. per 100 cc. blood), which will entirely explain the non-sugar effect and the discrepancies among the methods applied to Folin-Wu filtrates. The agreement among all methods applied to the other two filtrates is adequately explained by the fact that they contain no glutathione.

If the whole of the non-sugar effect is due to glutathione the concentration of this substance in normal human blood must be of the order of 52 mg. per

100 cc. The amount of glutathione in human blood has not yet been accurately determined. The published figures [Hunter and Eagles, 1927; Benedict and Newton, 1929, 1] indicate a concentration of 40 to 90 mg. per 100 cc. whole blood, but these figures rest on the assumption that glutathione is the only substance, present in the blood in appreciable amounts, which can give a positive nitroprusside reaction.

The evidence we have obtained is strongly suggestive that the non-diffusible reducing material, derived from corpuscles, and present in the Folin-Wu blood filtrates, consists entirely of glutathione, but it is possible that other substances may be included. However, any such substances, if they exist, must have several properties in common with glutathione. They must be non-diffusible and confined to corpuscles; they must be precipitated or destroyed in the zinc hydroxide precipitation; they must have no effect on the Benedict reagent, and must affect the Hagedorn-Jensen, Shaffer-Hartmann, and Folin-Wu reagents in the same ratio as does glutathione.

This particular numerical ratio may not be characteristic of glutathione only. It was pointed out, in the preceding paper, that the reducing action of glutathione expressed in terms of thiosulphate (cc. $N/200$) was almost the same in the Shaffer-Hartmann as in the Hagedorn-Jensen method; but since the amount of thiosulphate which corresponds to a given amount of glucose is not the same in the two methods, the effect of glutathione, expressed as glucose, is different in the two methods. All the methods of blood-sugar estimation are empirically calibrated for glucose under the standard conditions of the method, and the reduction produced by a given amount of glucose is not a constant quantity for all methods. Therefore, any non-glucose reducing substance which produced the same reduction (expressed in terms of hydrogen) in all the methods would necessarily show different glucose equivalents in the different methods. In the two titration methods used, a constant reduction effect would show itself as a constant relation to thiosulphate, the same for both. The glucose equivalents of any substance which behaved in this way in the Hagedorn-Jensen and Shaffer-Hartmann methods would be in approximately the same ratio as the glucose equivalents of glutathione. Unfortunately it is not possible to calculate what the effect would be in the Folin-Wu method; all that can be done in the colorimetric method is to compare the reducing action of the non-glucose substance with that of glucose.

Our conclusion must therefore be stated as follows. The discrepancies between the various blood-sugar methods, applied to different blood filtrates, can be explained by the single postulate that the blood contains a certain definite amount of glutathione, on an average, 52 mg. per 100 cc. whole blood, and figures of this order are supported by estimations depending on the nitroprusside reaction.

If it were independently proved that the concentration of glutathione in human blood falls within the narrow limits suggested by our figures, then the non-sugar reducing material of human blood would be completely accounted for.

Discussion of points in the literature in the light of the present findings.

The results of the present work confirm the main results of Herbert and Groen [1929] and support very strongly the suggestion then made, that glutathione is the main cause of the discrepancies between blood-sugar methods. Two minor inconsistencies between the present and former results call for mention. In the earlier paper the average discrepancy between the Folin-Wu and Shaffer-Hartmann methods was zero; in the present work the Shaffer-Hartmann figures are, on the average, 5 mg. per 100 cc. higher than the Folin-Wu figures. The hypothesis that glutathione is the main non-glucose reducing substance in blood demands a difference of 8 mg. per 100 cc. (for 50 mg. glutathione per 100 cc.). Secondly, in the earlier work the Benedict method (tungstic filtrates) gave higher figures than the Hagedorn-Jensen method (zinc filtrates); the average discrepancy was for plasma 9, for whole blood 10, and for corpuscles 10 mg. per 100 cc. It was realised that this could not be due to the blood-glutathione, since the difference was the same for plasma and for corpuscles. In the light of the present work it seems probable that there was some technical cause tending to raise the Benedict figures, though it cannot now be traced. The results of the present work accord with the known behaviour of glutathione.

MacLean's method must also be briefly considered. In the work of Herbert and Groen this method was shown to agree fairly well with the Hagedorn-Jensen method applied to Hagedorn-Jensen filtrates and to give figures lower than the Shaffer-Hartmann method by 7 mg. per 100 cc. on plasma, 20 mg. per 100 cc. on whole blood, and 39 mg. per 100 cc. on corpuscles. The relation of results by MacLean's method to results by other methods shows that MacLean's method gives figures close to the true sugar value, and in the earlier paper evidence was given that this was due to the use of ferric hydroxide filtrates, which contain little or no non-glucose material. We have been unable to determine how glutathione in blood would affect MacLean's method because the behaviour of pure glutathione was irregular both in the protein precipitation stage and in the copper reduction. Possibly the conditions of blood-protein precipitation are more favourable to the removal of glutathione than are the conditions in our artificial mixtures—for in the blood the glutathione is confined to corpuscles, whereas in our mixtures the glutathione was free in solution. From the practical standpoint, we may say that in ordinary blood analyses MacLean's method gives regular results which are close to the true sugar value.

Somogyi [1927, 1928, 1929, 1, 2; Somogyi and Kramer, 1928] has shown that the non-fermentable reducing substances of blood are present mainly in corpuscles, and are responsible for the discrepancies between certain blood-sugar methods applied to tungstic acid filtrates. He found no non-fermentable reducing substance in zinc hydroxide filtrates made by his technique, and the figures obtained by the Shaffer-Hartmann, Folin-Wu, Folin, and Benedict

methods, applied to these filtrates, agreed within the limits of experimental error. The total reduction value of his zinc hydroxide filtrate was found to agree with the "fermentable sugar" in the Folin-Wu filtrate from the same blood. This agreement places the technique of true sugar determination in Folin-Wu filtrates by fermentation methods on a firmer basis, as the estimation of residual reduction was always open to the objection that the non-sugar reducing material might include glutathione derived from yeast. We have observed that glutathione is not removed from yeast by washing as in Somogyi's technique; the glutathione must have remained inside the yeast cells in Somogyi's experiments.

When calculating true sugar as the difference between the total reduction and the residual reduction after yeast fermentation, it should be remembered that, in some methods, non-sugar substances have a different glucose equivalent in the presence and in the absence of glucose. We find that, with the Hagedorn-Jensen ferricyanide reagent, glutathione has a higher glucose equivalent in the absence than in the presence of glucose, whereas in the Shaffer-Hartmann and Folin-Wu methods the glucose equivalent remains the same whether glucose is present or absent. Somogyi [1929, 2] has pointed out that the non-glucose reducing material of blood has a different effect on the Folin [1926] reagent in the presence and in the absence of glucose. Benedict [1928] states that his (1928) reagent is affected by the non-glucose reducing material of blood in the absence, but not in the presence, of glucose. Our study of the effect of pure glutathione on this reagent does not shed any light on this statement; our experience is that, in the absence of glucose, the effect of such quantities of glutathione as might be present in blood filtrates is negligible.

Our figures for different blood filtrates, as well as our observation that glutathione produces no measurable effect on the Benedict reagent, support Benedict's claim that his method gives true sugar figures on Folin-Wu filtrates.

Benedict and Newton [1929, 2] have adduced evidence that glutathione is the main non-glucose reducing substance in sheep's blood. They suggest that human blood contains at least one, and probably two, non-sugar reducing substances in addition to glutathione and ergothioneine, but, as far as we know, they have not yet published the evidence for this opinion.

As regards ergothioneine, the amount in tungstic acid filtrates from human blood is too small to have an appreciable effect on blood-sugar methods, as was pointed out by Herbert and Groen [1929]. Benedict and Newton [1929, 1] have found higher figures for ergothioneine in tungstomolybdic acid filtrates than in tungstic acid filtrates, but even with the tungstomolybdic acid method, the figures were below 10·1 mg. per 100 cc. (with the exception of a single sample with a figure of 24·4). It is our experience that concentrations below 10 mg. per 100 cc. would have very slight effects on blood-sugar analyses (less than 6 mg. per 100 cc., as glucose, by the ferricyanide method, which shows the greatest effect).

Sjollema [1927] determined the effect of glutathione on the Folin-Wu

reagent and on ferricyanide. The ratio of glutathione to its glucose equivalent was 100 : 12·5 for the Folin-Wu reagent, and 100 : 48·5 for the ferricyanide method. He noted that oxidised glutathione had the same effect as the reduced form. Benedict and Newton [1929, 2] also noted that oxidised and reduced glutathione have the same effect on the Folin-Wu reagents. We have not studied the effect of oxidised glutathione, but we have shown that the reduction produced by reduced glutathione is due to some other change than the oxidation of the sulphydryl group. Benedict and Newton found that the ratio of glutathione to its glucose equivalent is 100 : 20 in the Folin-Wu method.

Evidence has been given in the present paper that the new "sulphate-tungstic" filtrate, like Somogyi's zinc hydroxide filtrate, gives true sugar figures for normal human blood. It must not be assumed *a priori* that these methods will universally give true sugar figures in other species, or in pathological conditions in man. It is necessary to consider each species separately. For example, Hiller, Linder and Van Slyke [1925] stated that the Hagedorn-Jensen method applied to tungstic filtrates gave the same figures as when zinc hydroxide filtrates were used. They used dog's blood, which, as Uyei [1926] has shown, contains about 26 mg. glutathione per 100 cc. corpuscles—an amount too small to affect appreciably the determinations on whole blood.

The difference between the figures obtained on the Folin-Wu and "sulphate-tungstic" filtrates is an index of the non-diffusible reducing material of corpuscles, which consists mainly, if not wholly, of glutathione. In normal human blood there is no appreciable amount of other, diffusible, non-sugar reducing substances, and the "sulphate-tungstic" filtrate gives true sugar figures. If, in other species, or in pathological conditions in man, there were any such diffusible reducing substances present, the "sulphate-tungstic" filtrate would not give true sugar figures, but the difference between the total reducing value of Folin-Wu and of "sulphate-tungstic" filtrates could still be used as a measure of the non-diffusible fraction of the non-glucose reducing material.

Summary.

1. A modification of the Folin-Wu tungstic acid precipitation is described. The corpuscles are retained intact by the substitution of an isotonic sulphate solution for distilled water. The non-diffusible glutathione remains in the cells, while the glucose diffuses freely throughout the mixture.

2. In a series of experiments on normal human blood, the Folin-Wu filtrate, the "sulphate-tungstic" filtrate, and the Somogyi zinc hydroxide filtrate from the same blood were each analysed by four methods: (1) the ferricyanide method, (2) the Shaffer-Hartmann method, (3) the Folin-Wu method, and (4) the Benedict (1928) method.

3. The four methods agree when applied to the zinc hydroxide filtrates and to the "sulphate-tungstic" filtrates, which contain no glutathione. The Benedict method, which is unaffected by glutathione, gives the same figure for these

and for the Folin-Wu filtrate. All these determinations are believed to represent true sugar.

4. Definite non-sugar reducing effects are observed when the Hagedorn-Jensen, Shaffer-Hartmann, and Folin-Wu methods are applied to Folin-Wu tungstic acid filtrates. The average values of these effects, expressed as glucose, are for the ferricyanide method, 28, for the Shaffer-Hartmann method, 18, and for the Folin-Wu method, 13 mg. per 100 cc. The whole of this effect is shown to be due to the non-diffusible reducing material derived from corpuscles.

5. It is shown that the results may be explained by the single postulate that the blood analysed contained a certain definite quantity of reduced glutathione (44–60 mg. per 100 cc. whole blood), present in corpuscles in non-diffusible form. This hypothesis is supported by the published data on the concentration of glutathione in human blood.

6. Comparison of the zinc hydroxide precipitation method of Somogyi with that of Hagedorn and Jensen, using the ferricyanide method of analysis in each case, showed that the Hagedorn-Jensen filtrates gave slightly higher figures than the Somogyi filtrates. This may be explained by the known behaviour of glutathione in the two precipitation methods.

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