CCI. THE NON-SUGAR REDUCING SUBSTANCES OF HUMAN BLOOD IN PATHOLOGICAL CONDITIONS.

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(Received October 27th, 1930.)

IN earlier papers [Herbert, Bourne and Groen, 1930; Herbert and Bourne, 1930] it was shown that there is in the blood corpuscles non-diffusible reducing material, which is responsible for the discrepancies observed when blood-sugar is determined by different methods. This material passes into Folin-Wu filtrates, and raises the apparent sugar content when these filtrates are analysed by the Hagedorn-Jensen, Shaffer-Hartmann and Folin-Wu methods; the Benedict [1928] method alone gives true sugar figures for Folin-Wu filtrates. The non-sugar material is absent from zinc hydroxide filtrates made by Somogyi's technique [1929] and from the tungstic acid filtrates from non-haemolysed blood described by Herbert and Bourne [1930]; all the analytical methods agree when applied to these two filtrates. Folin [1930] has also described a method of precipitating non-haemolysed blood with tungstic acid, essentially the same as ours, and recommends this instead of the Folin-Wu method for the Folin-Wu system of blood analysis, and especially for estimations of uric acid.

The effects produced by the non-diffusible reducing substance are consistent with the view that it is glutathione. If normal whole blood contains 40–60 mg. of reduced glutathione per 100 cc. (all present in the corpuscles) as is suggested by estimations based on the nitroprusside reaction, then the reducing effects in the different methods are completely explained.

In the previous work only normal human blood was used. It was thought that further estimations of the non-diffusible reducing substance of corpuscles, in pathological conditions, might throw some light on the physiological function of glutathione. The non-diffusible reducing substance has been estimated by taking the difference between the figures given by tungstic acid filtrates from haemolysed and non-haemolysed blood, analysed by the ferricyanide method. In normal blood this non-diffusible reducing substance accounts for practically the whole of the non-sugar reducing substance; in pathological conditions this may or may not be so, but our method of estimation gives figures for the non-diffusible substance which will be valid whether there is any diffusible non-sugar reducing substance or not.

¹ Holding a grant from the Medical Research Council.

Since the non-diffusible reducing substance occurs in corpuscles, it was to be expected that its concentration in whole blood would depend on the corpuscle content of the blood. The figures for non-diffusible material must be considered in relation to the haematocrit readings. The present series of cases is short, but it includes examples of polycythaemia and severe anaemia; haematocrit readings for corpuscle volume range from 12 % to 67 %.

METHODS.

Oxalated venous blood was used. The samples were precipitated by each of three methods: (1) the Folin-Wu technique [1920]; (2) Somogyi's zinc hydroxide technique [1929], and (3) the tungstic acid precipitation of non-haemolysed blood [Herbert and Bourne, 1930], which is indicated in the tables as "sulphate-tungstic filtrate." All determinations were made by the Hagedorn-Jensen method, because in this method the non-diffusible material exerts a maximum effect on the apparent sugar figure. In all three methods of precipitation the dilution of the blood was 1 in 10, and in each case 1 cc. of filtrate was taken and diluted with about 11 cc. of water; 2 cc. of Hagedorn-Jensen ferricyanide solution were added, and the remainder of the analysis was carried out as in the Hagedorn-Jensen method. The difference between the figures for the Folin-Wu filtrate and the "sulphate-tungstic" filtrate represents the non-diffusible reducing substance.

It was not always possible to work on freshly-drawn blood, and many of the samples had stood for 1-3 hours after drawing, and may have undergone slight glycolysis. The loss of glucose was of no importance, as we were concerned with the non-sugar fraction, but it was desirable to determine whether this underwent any change on standing at room temperature, and a few experiments were made on this point, and are recorded in Table I. There appears to be some slight fluctuation in the non-sugar fraction, apart from experimental error, but the variations likely to occur in 3 hours are too small to affect the main significance of our results.

RESULTS.

In Table II, the blood samples are arranged in ascending order of the haematocrit readings for corpuscle volume. In general the samples with low corpuscle content show low values for the non-sugar material in whole blood, whereas the polycythaemic blood samples show high figures. Case 23 is striking, as it shows a true sugar figure of 41 mg./100 cc. associated with a non-sugar figure of 70 mg./100 cc.

The next question is whether there is any significant variation in the concentration of non-diffusible reducing substance per unit corpuscle volume. In Table II the calculated figures for non-diffusible reducing substance, as glucose per 100 cc. corpuscles, are given. These figures may be misleading, as the experimental error is greatly magnified by the calculation when the haematocrit

Table I. *Changes in glucose, and non-diffusible substance in blood allowed to stand at room temperature.*

Case No.	Hours after drawing	Reducing substance, mg./100 cc. whole blood, expressed as glucose in			Non-diffusible fraction (a-b)
		Folin Wu filtrate (a)	Sulphate-tungstic filtrate (b)	Zinc filtrate (c)	
8 (chronic nephritis)	0	125	107	110	18
	3	123	97	100	26
	6	113	93	92	20
4 (neoplasm of liver)	3	144	131	132	13
	5	141	—	125	16 (a-c)
10 (normal)	0	138	108	110	30
	$\frac{1}{2}$	132	102	107	30
	1	129	99	99	30
	2	128	101	101	27
	3	131	95	99	36
	23	65	36	—	29
	72	32	12	10	20
16 (myeloid leucaemia)	0	118	93	95	25
	1	105	75	—	30
	6	69	34	—	35
	26	34	8	—	26

Table II.

Case No.	Age yrs.	Diagnosis	Total red corpuscles per mm. ³	Haemoglobin % of normal (Haldane scale)	Reducing substance as glucose mg./100 cc.			Non-diffusible fraction (a-b)	Corpuscle volume % of whole blood	Non-diffusible fraction as glucose mg./100 cc. corpuscles
					(a) Folin-Wu filtrate	(b) Sulphate-tungstic filtrate	(c) Zinc filtrate			
1	1 $\frac{3}{4}$	Haemolytic anaemia	2,760,000	20	116	102	101	14	12	117
2	52	Pernicious "	1,310,000	48	115	97	97	18	18	100
1	1 $\frac{3}{4}$	Haemolytic "	4,210,000	48	81	60	—	21	22	95
3	25	Haemorrhage (ectopic gestation)	—	—	138	124	125	14	22	64
4	65	Neoplasm of liver	2,070,000	42	144	131	132	13	23	57
5	—	Post-partum haemorrhage	2,560,000	59	147	125	127	22	24	92
6	58	Haematemesis (oesophageal varix)	3,170,000	59	124	103	103	21	26	81
7	—	Acute nephritis. Uraemia	3,280,000	66	98	74	72	24	26	92
8	64	Chronic nephritis. Uraemia	—	—	123	97	100	26	26	100
9	66	Osteoarthritis	—	—	143	115	112	28	31	90
10	72	Sulphaemoglobinaemia	—	—	151	132	131	19	33	58
11	25	Acute yellow atrophy of liver	—	—	129	102	108	27	37	73
12	35	Renal carcinoma	—	—	117	84	84	33	41	80
13	9	Malformed heart	6,170,000	95	129	101	101	28	43	65
14	31	Normal	—	—	129	99	99	30	43	70
15	5 $\frac{1}{2}$	Staphylococcal septicaemia	—	—	188	164	163	24	44	55
16	31	Sulphaemoglobinaemia	—	—	105	81	80	24	44	55
17	61	Gout	—	—	145	109	108	36	44	82
18	63	Carcinoma of colon	5,070,000	103	128	88	81	40	46	87
19	—	Gout	—	—	109	80	78	29	48	60
20	29	Malformed heart	7,180,000	124	118	70	69	48	57	84
21	12	"	—	164	125	86	86	39	62	63
22	22	"	9,700,000	146	154	88	97	66	67	99
23	1 $\frac{3}{4}$	"	8,400,000	140	111	41	41	70	67	104

Case 1. Severe haemolytic anaemia; no jaundice, but increased fragility of corpuscles and urobilinuria. Case 7. Acute nephritis; blood urea 103 mg./100 cc. Case 8. Chronic nephritis with high blood pressure. Blood urea on the day of the test, 49 mg./100 cc. Previous figures for blood urea ranged from 56 to 152 mg./100 cc. Case 9. Osteoarthritis. Blood uric acid 4.2 mg./100 cc. Cases 10 and 16. Sulphaemoglobinaemia due to methylacetanilide. Case 17. Gout. Blood uric acid 3.3 mg. per 100 cc.

reading is low, whereas in cases with normal or raised corpuscle content the calculation has proportionately much less effect.

Fig. 1.

The graph, Fig. 1, has been devised to present the results in their true perspective and to show their relation to the normal. The observed corpuscle volumes are plotted as abscissae and the observed figures for non-diffusible reducing substance (as mg. glucose per 100 cc. whole blood) are plotted as ordinates. The numbers at the points correspond to the case numbers in Table II. On the basis of our previous findings we may take the average normal figure for the non-diffusible reducing substance as 29 mg./100 cc. whole blood, and the average normal corpuscle volume as 41 %. This indicates that the normal concentration of non-diffusible reducing substance, expressed as glucose, is 71 mg./100 cc. corpuscles. The point *C* represents this value as determined on pure corpuscles, and the line *OC* is the locus of results for samples of whole blood which contain this same concentration in corpuscles, but in which the proportion of corpuscles to plasma varies. *OA* and *OB* are constructed in the same way, and are the loci of results corresponding to 55 and 110 mg. per 100 cc. corpuscles, respectively. These are the minimum and maximum concentrations in corpuscles, found in the present series of results. If the concentration of non-diffusible reducing substance per 100 cc. corpuscles were constant, all the points would fall on the line *OC*. They do not, but in most cases one cannot say whether the divergence is significant. For example, in Case 1 the haematocrit reading was 12 and the non-diffusible substance, mg./100 cc. whole blood, was 14. This brings the plotted result just above the line *OB*, but a result of 8.5 instead of 14 would have brought it to the line *OC*, and this variation might be due to experimental error.

If we take cases 1 to 21 we see that the divergence from the line *OC* is ± 7 mg./100 cc. whole blood, and of these 21 cases, only 5 cases diverge by more than ± 5 mg./100 cc. Such variations can scarcely be regarded as significant, since the figures for non-diffusible reducing substance are obtained by taking the difference between two analytical results, and are therefore affected by the experimental error of both. Cases 22 and 23 show a very definite divergence from the normal average. Both had a haematocrit reading of 67 %, and the figures for non-diffusible substance were 66 and 70 mg./100 cc. whole blood. The point on *OC* corresponding to a haematocrit reading of 67 % is 48 mg. non-diffusible substance per 100 cc. whole blood. The divergence here cannot be experimental. Both were cases of marked secondary polycythaemia due to malformation of the heart. Unfortunately we have not sufficient data to fix the normal range, but these cases are definitely above our normal average, and they are the only cases in which there is a really significant deviation.

It would be of interest to obtain further figures, to fix the normal range, and to investigate primary and secondary polycythaemias. More accurate results in anaemic cases could be obtained by working with pure corpuscles. The only definite result that emerges from the present series of observations is that the non-diffusible reducing substance of whole blood varies with the corpuscle content, and that variations in this substance have no significance when considered apart from corresponding variations in corpuscle volume.

Throughout the series there is excellent agreement between the figures given by the sulphate-tungstic filtrate and those given by Somogyi's zinc filtrate. This suggests that the non-sugar material in these samples is of the same nature as that in normal blood. It shows, at least, that there is no non-diffusible reducing substance which escapes precipitation by zinc hydroxide, and conversely, that there is no diffusible reducing substance which is precipitated by zinc hydroxide.

It is probable that the non-diffusible material is glutathione. The results may be expressed in terms of glutathione by multiplying the figures, expressed as glucose, by 1.8¹ [Herbert, Bourne and Groen, 1930]. The range of variation for the series is therefore 99 to 198 mg. per 100 cc. corpuscles, as reduced glutathione. These figures must not be regarded as equivalent to glutathione estimations, as it is possible that other non-diffusible reducing substances, precipitable by zinc, may occur in pathological conditions.

One case of myelogenous leucaemia has been examined and the results are given in Table III. In the earliest sample the volume of leucocytes was a considerable proportion of the total cell volume. When the leucocyte count was 400,000 to 500,000 per mm.³, it appeared that about half the corpuscle deposit consisted of leucocytes, though we found it impracticable to estimate the red cell volume and white cell volume separately. Later, when the leucocytes fell to 20,000 per mm.³, the leucocyte volume was only a small proportion of the total. In spite of these marked variations in the proportions of red

¹ This factor applies only to results obtained by the ferricyanide method.

Table III.

Case 24. J. C., aged 15. Myelogenous leukaemia.

Date	Total red cor- puscles per mm. ³	Total leuco- cytes per mm. ³	Cor- puscle volume	Reducing substance expressed as glucose mg./100 cc.			Non-diffusible reducing substance		
				Folin- Wu filtrate	Sul- phate- tung- stic filtrate	Zinc filtrate	As glucose mg./100 cc. whole blood	As glucose mg./100 cc. cor- puscles	As reduced gluta- thione mg./100 cc. cor- puscles
10. ii. 30	—	408,000	—	—	—	—	—	—	—
28. ii. 30	—	—	38	72	34	34	38	100	180
3. iii. 30	—	510,000	—	—	—	—	—	—	—
15. iii. 30	—	191,000	—	—	—	—	—	—	—
24. iii. 30	—	144,000	—	—	—	—	—	—	—
25. iii. 30	2,420,000	110,000	29	100	70	70	30	103	182
31. iii. 30	—	111,000	—	—	—	—	—	—	—
4. iv. 30	—	53,000	—	—	—	—	—	—	—
16. v. 30	2,260,000	20,000	25	118	93	95	25	100	180

and white cells, the non-sugar substance (expressed as glucose or glutathione mg. per 100 cc. corpuscles) remained very constant. This suggests that the concentration of non-diffusible reducing substance is the same in leucocytes as in red cells, so that the same figure is obtained whatever the constitution of the corpuscle mass. If so, the non-diffusible substance from leucocytes is liberated in the Folin-Wu precipitation and not in the sulphate-tungstic method. This is curious, as one would not expect the leucocytes to be broken up more readily in the one than the other. Further investigation is required.

From the present series of analyses it is clear that in all work on the non-sugar reducing material it is necessary to consider the corpuscle volume, and to express concentration per 100 cc. of corpuscles rather than per 100 cc. of whole blood. A rise in the non-sugar reducing substance has been noted by Sure and Smith [1929] in animals suffering from severe vitamin B-deficiency, but these animals were dehydrated at the time of the observation.

From the standpoint of routine sugar analysis, it should be remembered that if any method affected by the non-sugar material is used, the errors will be greater in cases with anhydraemia or polycythaemia than in those in which the corpuscle volume is normal.

SUMMARY.

The non-diffusible reducing substance has been estimated in a series of pathological bloods, and its concentration in whole blood has been shown to vary with the haematocrit reading for the corpuscle volume. This was to be expected, since the non-sugar substance is a constituent of corpuscles, most probably glutathione.

The results in one case of myelogenous leukaemia lead to the suggestion that the concentration of non-diffusible reducing substance in leucocytes and erythrocytes is of the same order.

The concentration of the non-diffusible reducing substance in corpuscles varies, but in the majority of cases the variation from the normal average is within experimental error. In two cases of marked secondary polycythaemia the non-diffusible substance is above the normal average.

We wish to express our thanks to members of the staffs of St Bartholomew's Hospital and the Belgrave Hospital for Children, for giving us opportunities to make the observations here recorded.

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