CXLVIII. A NOTE ON THE DISTRIBUTION OF REDUCING SUGAR AND THE MODE OF GLYCOLYSIS IN HUMAN BLOOD.

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PUBLISHED work on the distribution of the blood sugar between the corpuscles and plasma shows very divergent results.

According to several authors, including Czaki [1922], Falta and Richter-Quittner [1922] and van Creveld and Brinkman [1921], the reducing sugar is confined to the plasma. Bönninger [1921], Braun [1922], Cristol and Danitch [1924] and Höglér and Ueberrack [1924], on the other hand, are among those who have come to the conclusion that the corpuscles contain a considerable amount of reducing sugar.

In view of the present uncertain state of affairs regarding the nature and mode of existence of the reducing sugar in blood the method of estimation must be of considerable importance in work of this kind.

The most reliable methods are, according to the experience of the author, those based on the reduction of alkaline cupric salts, and of these that of MacLean is considered to be the most accurate as well as the most convenient.

This method was found to give results accurate to the third decimal place with standard solutions of glucose of concentrations of 0.050 % to 0.020 %. On comparison with the widely used picric acid method of Benedict, using blood, the method gave results almost invariably lower than the latter by two or three figures in the third decimal place. This difference was probably due to traces in the blood of creatinine, which is also estimated by Benedict's method.

Using MacLean's method, it was found that a sample of blood laked with 1 cc. of water and diluted with a correspondingly decreased volume of a more concentrated acid sodium sulphate solution gave results identical with those from a sample estimated in the usual way.

This comparison was considered necessary in view of the statement of Höglér and Ueberrack [1924] that haemolysis of blood produced high results. Information regarding the method used by these authors is not to hand.

Van Creveld and Brinkman [1921] suggest that the distribution of the blood sugar is influenced by the early stages of coagulation and Cristol and Danitch [1924] consider the distribution to be affected by the anticoagulants usually

employed. The latter view is also held by Czaki [1922] who states that defibrination or the addition of anticoagulant renders the red cell membrane more permeable to glucose.

Owing to the fact that many of the original papers on the distribution of blood sugar referred to are not to hand, a critical review of the technique employed is not possible, but from the information available it is inferred that the following factors might account in some part at least for the conflicting results obtained.

- (1) The use of different methods of sugar estimation.
- (2) The laking or otherwise of the blood prior to the estimation.
- (3) The influence of the anticoagulant when employed.
- (4) The influence of incipient coagulation when no anticoagulant was used.
- (5) The variation in the time elapsing between the withdrawal of the blood and the estimation of its sugar content, thus permitting the diffusion of a variable amount of sugar through the red cell membrane.

The distribution of the reducing sugar in human blood was consequently determined according to the following technique.

About 12 cc. of venous blood were collected through a paraffined needle into a cooled sterile paraffined test-tube. A sample of this was immediately pipetted off for estimation, and, after about 8 cc. of the blood had been poured into a graduated centrifuge tube in which one drop of 10 % potassium oxalate solution had been evaporated, this and the remainder of the blood were centrifuged and a sample of plasma pipetted from the untreated blood for estimation after 20 seconds.

The oxalated blood was centrifuged for about 3 hours, after which the relative volumes of corpuscles and plasma were noted. The earlier part of the procedure was carried out as rapidly as possible, but with due care, and in most cases the filtration of the sugar solution had commenced about 2 minutes after the withdrawal of the blood.

Capillary blood was obtained in quantity by washing the hands in warm water, drying, and, after swinging the arm round vigorously for some time and then ligaturing the base of the thumb, pricking the latter with a clean spear-pointed needle. The blood was collected and dealt with in the same way as the venous blood. In none of the experiments recorded did any trace of clotting occur. The percentage of glucose in the corpuscles was determined indirectly from the data obtained.

RESULTS.

Untreated venous blood.

Exp.	1	2	3	4	5	6	7
% Reducing sugar in							
(1) Plasma	0.082	0.088	0.083	0.080	0.095	0.116	0.077
(2) Corpuscles	0.092	0.088	0.109	0.108	0.113	0.148	0.091
(3) Whole blood	0.087	0.088	0.095	0.093	0.104	0.132	0.084
% of corpuscles	51	52	48	45	51	50	49

Untreated capillary blood.

Exp.	1	2	3	4	5	6	7	8	9
% Reducing sugar in									
(1) Plasma	0.073	0.086	0.103	0.100	0.087	0.099	0.124	0.088	0.099
(2) Corpuscles	0.089	0.063	0.075	0.079	0.103	0.087	0.102	0.064	0.087
(3) Whole blood	0.081	0.075	0.090	0.090	0.095	0.093	0.113	0.077	0.093
% of corpuscles	51	52	46	48	51	50	49	46	50

Although the blood was drawn at varying intervals after the previous meal, and from a number of individuals, in no case were the corpuscles found to be sugar-free. In fact all but one of the samples of venous blood showed more reducing sugar in the corpuscles than in the plasma.

In the capillary blood, on the other hand, the distribution was more variable, seven of the nine samples estimated showing more sugar in the plasma than in the corpuscles.

A comparison of the sugar distribution in the fresh blood with that in the oxalated sample confirmed the opinions previously referred to regarding the influence of anticoagulant in the permeability of the red cell to sugar. Owing, however, to the onset of clotting, comparisons could not be made at such an interval after withdrawal as would allow an appreciable amount of diffusion.

Exp.	1		2		3		4	
% Reducing sugar in	Un-treated blood	Oxa-lated blood	Un-treated blood	Oxa-lated blood	Un-treated blood	Oxa-lated blood	Un-treated blood	Oxa-lated blood
(1) Corpuscles	0.109	0.100	0.106	0.106	0.084	0.072	—	—
(2) Plasma	0.083	0.086	0.080	0.080	0.106	0.100	—	—
(3) Whole blood	0.096	0.093	0.092	0.092	0.095	0.086	0.101	0.095
Time elapsing after withdrawal ...	3 mins.		2 mins.		12 mins.		5 mins.	

Estimations made with oxalated blood a considerable time after removal did not, as would be expected, always show an equal distribution of sugar between the corpuscles and plasma, the percentage of sugar in the corpuscles now being relatively high as compared with that in the plasma.

Exp.	1	2	3	4	5	6	7	8
Time after withdrawal	mins. 12	mins. 30	hrs. 16	hrs. 24	hrs. 24	hrs. 30	hrs. 48	hrs. 72
% Reducing sugar in								
(1) Whole blood	0.081	0.079	0.077	0.046	0.045	0.071	0.078	0.041
(2) Corpuscles	0.074	0.090	0.108	0.057	0.045	0.066	0.094	0.046
(3) Plasma	0.089	0.100	0.039	0.035	0.045	0.079	0.062	0.036

This might have been due, as suggested by MacLeod [1921], to a difference in the solubilities of sugar in the plasma and corpuscular materials, but it suggested that glycolysis might be more rapid in the plasma than in the corpuscles, a view that would be in accordance with the more rapid glycolysis observed in oxalated blood. To test this hypothesis samples of oxalated blood and corpuscle-free plasma separated from the latter were kept in sealed sterile tubes for varying periods, at the end of which the sugar in the separated plasma,

that in the plasma of the whole blood separated just before the estimation, and that in the whole blood itself were determined.

Exp.	1		2		3	
Time after withdrawal	2 mins.	16 hrs.	2 mins.	24 hrs.	2 mins.	48 hrs.
% Reducing sugar in						
(1) Whole blood	0.092	0.077	0.084	0.046	0.103	0.078
(2) Corpuscles	0.107	0.107	0.085	0.057	0.106	0.094
(3) Plasma	0.081	0.039	0.083	0.035	0.100	0.062
(4) Separated plasma	—	0.077	—	0.072	—	0.100

Exp.	4		5		6		7		
Time after withdrawal	mins.	hrs.	mins.	hrs.	hr.	hrs.	hrs.	hrs.	hrs.
	10	72	2	24	1	24	1½	5½	28½
% Reducing sugar in									
(1) Whole blood	0.086	0.041	0.084	0.046	0.081	0.045	0.083	0.067	0.049
(2) Corpuscles	0.100	0.046	0.091	—	0.085	0.045	0.084	—	—
(3) Plasma	0.100	0.036	0.077	—	0.077	0.045	0.082	—	—
(4) Separated plasma	—	0.084	—	0.063	—	0.070	—	0.082	0.081

Exp.	1	2	3	4	5	6	7
Decrease of glucose in							
(1) Whole blood	0.015	0.038	0.025	0.045	0.038	0.036	0.034
(2) Corpuscles	0	0.028	0.012	0.054	—	0.040	—
(3) Plasma	0.042	0.048	0.038	0.064	—	0.032	—
(4) Separated plasma	0.004	0.011	0.000	0.016	0.014	0.007	0.001

In the separated plasma the glycolysis, when it occurred, was in all cases very slight as compared with that in the whole blood, the decrease in the sugar of the latter occurring chiefly in the plasma it contained. This suggests that the glycolysis is due almost entirely to the activity of leucocytes and in a varying degree to other factors. The rate of disappearance of sugar (5–6 mg. of sugar per g. of leucocytes per hour) does not seem too high to be accounted for mainly by the metabolic activity of leucocytes.

SUMMARY.

(1) In human blood the red corpuscles contain a considerable amount of reducing sugar. In capillary blood there is generally more sugar in the plasma than in the corpuscles, whereas in venous blood the reverse is generally the case, owing to the plasma sugar being more accessible to the tissues.

(2) From the results obtained it is inferred that the glycolysis in human blood is due almost entirely to the activity (chiefly metabolic) of the leucocytes.

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