XXXV. FURTHER OBSERVATIONS ON THE NATURE OF THE REDUCING SUBSTANCE IN HUMAN BLOOD.

By EVELYN ASHLEY COOPER and HILDA WALKER.

From the University of Birmingham.

(*Received April 3rd, 1922.*)

In the previous communication [1921] it was shown that the reducing power of human blood was sometimes increased by acid hydrolysis. This pointed to the presence in addition to glucose of a more complex substance, which was shown not to be glycogen, and thus appeared to be a disaccharide. The quantitative determination of this accessory substance was found to be complicated by two factors:

(1) the destructive effect of the HCl used for hydrolysis upon the reducing substance;

(2) the inhibitory effect of NaCl (formed by neutralisation after hydrolysis) upon the reduction of the copper carbonate by sugar.

Further work on the subject has since been carried out, and the results are recorded in the present paper.

First of all, it was necessary to select an acid, which did not itself destroy sugar, and the salts of which had no effect on the reducing process.

In the previous work sodium sulphate had been found to have no inhibitory effect, but the use of sulphuric acid for hydrolysis is inadmissible, because sodium sulphate is used for removing the proteins from the blood, and its presence would diminish the ionisation of the acid.

Further experiments showed that potassium chloride, like sodium chloride, had a retarding action on the reducing process, while the sulphate had no effect. The use of other chlorides for comparative purposes was not possible for various reasons. For example, ammonium chloride is decomposed by the alkali in the copper solution, and the liberated ammonia destroys the glucose, while the salts of several metals, *e.g.* Ca, Ba, Mg, give precipitates with the copper carbonate. The results, however, show that the chloride ion has a specific retarding effect.

Experiments with varying concentrations of sodium and potassium chloride showed that the retarding action first appeared when the concentration was approximately $N/1$. This inhibitory effect was found to be irreversible, *i.e.* when a solution of glucose containing NaCl was diluted with

a salt-free sugar solution, the reducing action of the sugar upon the copper solution was still (proportionately) retarded. The reduction was also retarded when the salt was added to the boiling mixture of sugar and copper solutions. This suggests that the compounds of glucose with the chlorides are much more stable than is commonly supposed. Bromides and iodides of the alkali metals also inhibited the reducing process.

Sodium citrate was moreover also found to exert an inhibitory action when present in concentration equivalent to 2 % citric acid, which is employed for hydrolysis in sugar analysis.

Di-sodium phosphate, on the other hand, had no inhibitory effect, and it thus seemed that phosphoric acid might be a suitable acid for hydrolysing the blood-sugar extracts. Separate portions of an extract (prepared by MacLean's method, as in the previous communication) were hydrolysed with $N/10$ HCl and $N/10$ H_3PO_4, and it was found that the reducing substance was destroyed to the extent of about 10 % by both acids.

Pure glucose was next dissolved in sodium sulphate solution, or in blood-extract, and the mixture hydrolysed with $N/10$ or $N/100$ HCl. The results obtained were similar to those obtained with blood-extract itself [Cooper and Walker, 1921]; sometimes there was no destruction of reducing substance at all, but occasionally a destruction occurred amounting to about 10 %.

These results afford an explanation of our previous work. Evidently the hydrolysable substance, which may be a disaccharide, is only occasionally present in blood, so that on hydrolysis there is often no increase in the reducing power of the blood-extract, or there may actually be a loss in reducing power, owing to destruction of some of the glucose. In fact, out of 18 samples of normal human blood examined, only six cases showed an increased reducing power after acid hydrolysis, and as a general rule the chief reducing substance present is glucose.

Two blood-extracts were subjected to dialysis. In one case the hydrolysable substance dialysed completely. In the other case, however, it was only partially dialysable, and this suggests that occasionally substances of intermediate complexity may be present in blood, possibly related to malto-dextrins.

The estimation of blood-sugar.

Up to the present time nearly all the methods for estimating sugar in physiological work are modifications of Fehling's method. Although fairly reliable, this is an empirical method, and the interaction of sugar with alkali copper solution is of great complexity. Recently, the iodometric method for estimating sugars has come to the forefront [see Baker and Hulton, 1920]. This is a simple method, consisting in the quantitative oxidation of aldoses to the corresponding carboxylic acid by means of iodine in alkaline solution, and it can be carried out in a few minutes at room temperatures. We have therefore attempted to adapt it to physiological work.

We have found, however, that in estimating pure glucose in concentration approximating to the average content of the blood-sugar extracts by the iodine method, slightly low results are obtained. Varying the proportion of alkali, and extending the period of reaction did not affect the results. In estimating the sugar in blood by this process, however, the results were twice as high as those obtained by MacLean's method. The alkali copper reagent is thus more selective than iodine in its action, and the iodometric method does not appear to be suitable for physiological work.

Influence of fatigue on the blood-sugar concentration.

Estimations of the blood-sugar by MacLean's method were carried out on normal persons immediately before, and directly after exercise. The persons examined were students, both men and women, of ages ranging from about 18 years to 26. Estimations were made on one man before and after half-an-hour of strenuous boxing, the other estimations on men were made before and after Rugby football, and on the women, before and after hockey. Of twelve experiments, there was a rise in the blood-sugar concentration in nine cases. The increase, as shown in the appended table, varied from 10 to 50 %, but in one case the amount was nearly trebled after $1\frac{1}{4}$ hours' play.

			% before	% after
Boxing.	½ hour.	*A*	0·04	0·05
Rugby.	1 hr. 10 mins.	*B* (1)	0·052	0·077
		(2)	0·072	0·130
		C	0·097	0·090
		D	0·093	0·10
		E	0·115	0·10
		F	0·087	0·094
		G	0·118	0·094
Hockey.	1 hr. 15 mins.	*H*	0·087	0·253
		I	0·065	0·075
		J	0·118	0·150
		K	0·083	0·092

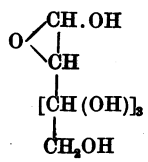
The player *B* is a first class forward, and in good training; he is of a very nervous and imaginative temperament, and before the match is always in a state of suppressed excitement. *C* and *D* are also fast players; *C* is of a quiet type, not easily roused to excitement. *E*, *F* and *G* were not in training, and did not take the game so seriously as the others.

Of the hockey players, *H* was playing back; she is of a highly nervous temperament, but well controlled. *I* and *K* were half-backs, and *J* played forward.

It will be seen that the players most easily roused to excitement over the game (*B* and *H*) are those whose blood-sugar has increased the most, although *H*, playing back, probably underwent less physical fatigue than most of the others. Psychic influences, therefore, probably play a considerable part.

Condition of the sugar in the blood.

We next considered the question of the structural condition of the sugar in the blood, and its bearing on physiological and pathological problems. It is well known that sugars do not merely exist as aldoses and ketoses, but may also pass into cyclic forms known as oxides. The ethylene oxide form:



is characterised by being extremely chemically reactive, and it decolorises permanganate rapidly.

Hewitt and Pryde [1920] showed that this active form of sugar could be actually formed by contact of an aqueous solution of glucose with the intestinal wall *in vivo*.

It is possible that in the animal organism sugar is normally metabolised in this active condition, and that an enzyme exists for transforming ordinary sugar as ingested into this form. Now it is known that fructose is more readily converted into the ethylene oxide structure than glucose, and that in diabetes the organism may still be able to metabolise fructose, although it has lost the power to deal with glucose. This suggests that the causation of diabetes is associated with some disturbance in the enzyme mechanism, which normally converts inactive sugar into the reactive ethylene oxide form.

We therefore proceeded to ascertain whether normal human blood can cause ordinary sugar to pass into this reactive state. A few cc. of fresh blood were placed in a small dialyser, immersed in $\frac{1}{2}$–1 % solutions of glucose and fructose. At varying intervals of time, samples of the dialysate were withdrawn and examined, either polarimetrically or with a solution of permanganate. No evidence, however, was obtained that blood, under the above conditions, can produce the ethylene oxide form from ordinary glucose or fructose.

Since these experiments were carried out, Hewitt and Souza [1922] have found that even *in vivo* normal blood is unable to induce this structural change.

Summary.

1. Chlorides, bromides, iodides and citrates inhibit the reduction of copper carbonate by glucose, as carried out by MacLean's method. Sulphates and phosphates have no effect.

2. Glucose is slightly destroyed by boiling with $N/10$ HCl and $N/10$ H_3PO_4. The reducing substance present in blood is also destroyed by acid to about the same extent. This affects the determination of the hydrolysable reducing substances in blood.

3. Glucose is the chief sugar occurring in human blood, and reducing substances of a more complex nature are only occasionally present.

4. The estimation of blood-sugar by the iodine method gives results much higher than those obtained by MacLean's process, and the iodine method does not seem suitable for physiological work.

5. The blood-sugar concentration may rise considerably as the result of muscular exertion.

6. There is no evidence that human blood can transform ordinary glucose or fructose into the reactive ethylene oxide form.

7. A theory as to the causation of diabetes is put forward.

REFERENCES.

Baker and Hulton (1920). *Biochem. J.* **14**, 756.
Cooper and Walker (1921). *Biochem. J.* **15**, 415.
Hewitt and Pryde (1920). *Biochem. J.* **14**, 395.
Hewitt and Souza (1922). *Biochem. J.* **15**, 667.