XCI. THE SIGNIFICANCE OF THE NON-FERMENTABLE REDUCING SUBSTANCES OF THE BLOOD IN DIABETES.

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FOLIN has recently demonstrated that the hyperglycaemia of diabetic blood is not all due to accumulation of glucose. From 15 to 25 % of the sugar in such blood may be represented by other fermentable substances which this author groups under the term of "non-glucose fermentable sugars." The reaction of these sugars to insulin was similar to that of glucose, at least in so far as their concentrations in blood were concerned. These findings renew interest in all the non-glucose reducing substances of blood, very little of which can be accounted for, in the absence of nephritis, by nitrogenous materials.

The greatest difficulties, heretofore, in studying the metabolism of these substances, were due to the technical methods involved in their estimation, since the methods commonly in use for estimating the sugar of human blood measure not glucose alone, but also other reducing substances which are now generally known to be found in blood. Some are fermentable whilst others are not.

The commonest and most logical, theoretically, of older methods of estimating the concentrations of the non-fermentable reducing substances of blood, depended upon measuring the total reducing substances before and after alcoholic fermentation, and with proper precautions it is now possible, with a reasonable degree of accuracy, to estimate the true contents of non-fermentable reducing substances.

Hiller, Linder and Van Slyke [1925] have shown that it is possible to reduce the fermentation period to 20 minutes. At a later date Folin and Svedberg [1926] found that it was possible to reduce still further the fermentation period. More recently Somogyi [1927] has shown that it is unnecessary to incubate blood with yeast even for the brief periods mentioned above. Sugar may be completely removed, at room temperature, in the course of the Folin-Wu precipitation of the blood-proteins, if the water for dilution and laking of the blood is replaced by a 10 % (moist weight) yeast suspension.

In order to study the significance of these non-fermentable reducing substances in diabetes, it was considered advisable, firstly, to obtain standards

by determining, by the technical procedures adopted, the amounts of these substances in normal bloods. Ideas as to these amounts appear to differ. Thus, Hiller, Linder and Van Slyke [1925] found amounts equivalent to 10 to 30 mg. of glucose per 100 cc., whereas Folin and Svedberg [1926] and also Somogyi [1927] found the values to vary within narrower limits. Different procedures were, however, used in each case, either as to the fermentations or blood-sugar determinations.

In this investigation we used Somogyi's method of purifying the yeast and also his method of fermentation, except that for fermentation longer periods and higher temperatures were allowed. For the estimation of the total reducing substances the Folin-Wu blood-sugar method was used. Briefly, the combined technique was as follows.

The material necessary for fermentation was first prepared. A weighed amount of fresh yeast was suspended in about 10 parts of water, centrifuged, and the water decanted. This operation was repeated until the supernatant fluid was clear and colourless, and the last washing gave no greater reduction with the Folin-Wu copper reagent than did a control consisting of distilled water. This procedure removes the adhering particles of wort and other materials found in yeast which contain unfermentable reducing substances. The yeast was then suspended in 10 parts of water and, in order further to avoid errors, 2 cc. of this suspension were also tested for reducing substances by the Folin-Wu process. The reductions found were insignificant. They could not be estimated quantitatively.

As soon as the above process was complete, specimens of blood were obtained. Part of the blood was immediately treated in order to precipitate the proteins and part was fermented as follows.

Into a flask containing 7.5 volumes of a 10 % suspension of yeast prepared as above, was placed 1 volume of blood¹. After thorough mixing, the flask was allowed to remain in a water-bath at 37° for 7 minutes. One volume of 10 % sodium tungstate was then added followed by 1 volume of 0.66 *N* sulphuric acid. After thorough shaking, the mixture was allowed to stand for 10 minutes. It was then filtered.

The reducing substances of the filtrates obtained before and after fermentation were estimated simultaneously. The necessary precautions for estimating minute amounts of sugar pointed out by Folin and Svedberg [1926] were taken. All values in the tables are expressed in g. glucose per 100 cc. blood.

Values were first obtained, before and after fermentation, from bloods collected in the fasting state. The subjects included 100 non-diabetic adults, 10 non-diabetic children and 100 diabetics, of all ages. A summary of the data will be found in Table I.

By this procedure the amounts of non-fermentable reducing substances were found to vary within narrow limits, namely 16 to 31 mg. per 100 cc.

¹ As Somogyi has shown, it is necessary to allow 7.5 volumes instead of 7 volumes of yeast suspension to correct for the volume occupied by the yeast.

blood. The variations noted were greater than those reported by Somogyi [1927], who used the Shaffer-Hartman method, and approached those found by Hiller, Linder and Van Slyke [1925].

Table I. *Summary of values of non-fermentable reducing substances (g. per 100 cc.) found in fasting bloods of diabetic and non-diabetic subjects.*

Subjects	No. of individuals	Total reducing substances			Range of blood-sugars
		Maximum	Minimum	Average	
Normal adults	100	0.030	0.022	0.025	0.076-0.123
Normal children	10	0.026	0.016	0.023	0.070-0.100
Diabetics	100	0.031	0.024	0.027	0.111-0.333

It was found that, unless determinations were made immediately after collecting the blood, much wider variations were obtained.

Though there were variations between the maximum-minimum values in the large group of individuals, for the individual it appears that the values are remarkably constant. This is shown in Table II. In this table are recorded daily values found in fasting bloods of one normal and two diabetic subjects.

Table II. *Showing daily values of total reducing and of non-fermentable reducing substances (g. per 100 per cc.) obtained in blood of one normal and two diabetic subjects.*

Day	Normal		Diabetic		Diabetic	
	Blood-sugar	N.F.R.S.*	Blood-sugar	N.F.R.S.	Blood-sugar	N.F.R.S.
1	0.080	0.026	0.143	0.018	0.180	0.024
2	0.100	0.022	0.125	0.020	0.166	0.022
3	0.123	0.023	0.151	0.022	0.180	0.026
4	0.075	0.025	0.133	0.019	0.143	0.024
5	0.084	0.026	0.143	0.023	0.181	0.023

* N.F.R.S. = non-fermentable reducing substances.

As has been previously noted by others [Folin and Svedberg, 1926; Somogyi, 1927], the values did not vary with different sugar levels. This will be observed from the data recorded in Tables II and III.

Table III. *Total reducing and non-fermentable reducing substances (g. per 100 cc.) found in bloods of individuals before and after ingestion of 100 g. "commercial" glucose.*

Subject	Substance	Before glucose	30 mins. after	60 mins. after	120 mins. after	180 mins. after
Normal	Total reducing	0.109	0.153	0.185	0.143	0.077
	Non-fermentable	0.028	0.031	0.030	0.030	0.028
Diabetic	Total reducing	0.181	0.357	0.416	0.434	0.454
	Non-fermentable	0.031	0.028	0.029	0.033	0.026
Diabetic	Total reducing	0.270	0.370	0.400	0.476	0.454
	Non-fermentable	0.027	0.024	0.026	0.023	0.023

In Table III will be found the results of three blood-sugar time curves, one obtained in a normal and two in diabetic subjects. It will be seen that, in spite of marked hyperglycaemic bloods, the values of the non-fermentable

reducing substances remained constant for the individual. This is remarkable, in view of the fact that in each of these cases commercial glucose was given. This is well known to be contaminated with both non-glucose fermentable and non-fermentable reducing substances.

The remarkable constancy of the concentrations of these substances in blood, both in non-diabetic and diabetic individuals, in the fasting state and after glucose ingestion, probably explains the fairly reliable results which are obtained with blood-sugar time curves in general, clinically, in the diagnosis of disturbed carbohydrate metabolism.

An interesting observation made by Hiller, Linder and Van Slyke was that by inducing insulin shock in normal rabbits, though the reducing fermentable sugar was reduced to zero, the concentrations of the non-fermentable reducing substances were not altered. This observation appeared to be of sufficient importance to be repeated in the human diabetic. The results of three of such tests are shown in Table IV.

Table IV. *Effect of insulin upon non-fermentable reducing substances (g. per 100 cc.) of bloods of diabetics.*

		Before	After						
Substances		insulin	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
Case 1.	Total reducing	0.333	0.302	0.181	0.062	0.043	Reaction.	Glucose	given
	Non-fermentable	0.026	0.023	0.025	0.028	0.027			
Case 2.	Total reducing	0.200	0.133	0.066	0.050	Reaction. Glucose given			
	Non-fermentable	0.018	0.016	0.020	0.018				
Case 3.	Total reducing	0.416	0.333	0.200	0.166	0.080	0.032	Reaction.	Glucose
	Non-fermentable	0.027	0.028	0.026	0.028	0.025	0.028		

The findings in the human diabetic correspond to those in the normal rabbit. Here, again, are noted, in the fasting state, different values for the different individuals, but remarkably constant values for any one individual throughout the period of observation. The findings in the third subject correspond to those found in the animals by Hiller, Linder and Van Slyke. It will be noted at the end of the observation period that, whereas the total reducing substances correspond to 0.032 g. glucose per 100 cc. the non-fermentable reducing substances correspond to 0.028 g. per 100 cc. Very little significance is attached to the difference, considering the probable error inherent in colorimetric work.

Lawrence [1926] and, later, the writer [1927, 1, 2] have demonstrated the value of determining simultaneously arterial and venous blood-sugars in diabetes. As was originally shown by Foster [1923] in animals, the sugar content rises to a level much higher in the arterial than in the venous blood following glucose ingestion. In diabetic individuals [Lawrence, 1926; Rabinowitch, 1927, 1, 2] the more severe the disease, the less is the difference between the arterial and venous blood values. The difference between these two values is the result of either oxidation or storage of glucose. It would appear that if the metabolism of these non-fermentable reducing substances is disturbed in

diabetes this should be demonstrated in simultaneously determined arterial and venous blood-sugar time curves. In Table V are shown the results obtained in three normal and in three diabetic individuals. It will be noted that, regardless of the differences between the arterial and venous blood-“sugar” values, there were no significant differences noted in the non-fermentable reducing substances.

Table V. *Showing the effect of glucose ingestion upon concentration of total and non-fermentable reducing substances (g. per 100 cc.) of arterial and venous bloods of normal and diabetic subjects.*

Subject	Time	Substance	Case 1		Case 2		Case 3	
			Arterial	Venous	Arterial	Venous	Arterial	Venous
Normal	Before	Total reducing	0.106	0.100	0.084	0.080	0.100	0.100
	glucose	Non-fermentable	0.022	0.020	0.018	0.019	0.024	0.026
	After	Total reducing	0.222	0.166	0.196	0.147	0.200	0.153
	glucose	Non-fermentable	0.023	0.018	0.021	0.020	0.023	0.022
Diabetic	Before	Total reducing	0.133	0.131	0.111	0.105	0.166	0.164
	glucose	Non-fermentable	0.028	0.026	0.020	0.018	0.016	0.015
	After	Total reducing	0.250	0.222	0.250	0.232	0.285	0.263
	glucose	Non-fermentable	0.027	0.023	0.022	0.023	0.014	0.018

SUMMARY.

1. An attempt was made to determine whether the non-fermentable reducing substances of blood are of any significance in diabetes.

2. Amounts of non-fermentable reducing substances, by the method employed, were found to vary between 16 and 31 mg. per 100 cc., estimated as glucose. They were also found to be fairly constant for the individual. No significant differences were noted between normal and diabetic bloods.

3. Blood-sugar time curves obtained after the administration of glucose showed no significant differences in the amounts of these substances between normal and diabetic subjects. Arterial and venous blood-sugar time curves, obtained simultaneously, were identical in normal and diabetic subjects, as far as variations of non-fermentable reducing substances were concerned.

4. The amounts of non-fermentable reducing substances were not influenced in the blood of the diabetic by insulin administration.

5. In view of the above findings it is suggested that, in contradistinction to the findings of Folin with non-glucose fermentable sugars, the metabolism of non-fermentable substances is not disturbed in diabetes.

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