XXXIV. THE GLUCOSE IN BLOOD.

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Differences which have been stated to exist in the forms of glucose in the blood in health and in disease have focussed attention on the relation between amounts of sugar obtained in the same sample of blood by various quantitative methods. The older literature on the subject is reviewed by Bang [1913] and by Stepp [1922] and need not be gone into here.

Up to the present, except for the difficult polarimetric estimation, most of the methods have relied on the reducing power of the deproteinised blood. The results, however, are not concordant and depend partly on the conditions of oxidation. Thus Benedict states [1925] that his picric acid method, devised with Lewis [1915], gives considerably higher results than the Folin-Wu [1920] method and is confident that the values so obtained indicate more than the actual sugar content of the blood.

Grafe and Sorgenfrei [1924] were impressed by an unsatisfactory situation in which all estimations of glucose in blood were variants of reduction methods and suggested as a control the use of the single Barcroft manometer using yeast to ferment the blood. They found that in normal cases the reduction method of Benedict gave substantially the same results as the fermentation method. In cases of diabetes, however, the differences were very great. The fermentation values were lower than the reduction values by as much as 40 %. The statements were of such interest that we decided to go into them more closely and to repeat them.

An analysis of the tables of Grafe and Sorgenfrei show for non-diabetics a ratio of fermentable substance to reducing substance $\left(\frac{100F}{R}\right)$ of from 73 to 103. In diabetics the ratio varies from 61–102. In a set of analyses of bloods after food and insulin, ratios ranging from 53–107 were obtained. So far as we were able to make out, no definite regularity or difference between normal and diabetic bloods could be observed.

Whilst it is recognised that there may be fermentable substances in the blood other than glucose, it would seem that the high values obtained in some instances by Grafe and Sorgenfrei were worthy of further study, especially in view of the fact that the reduction method employed by these workers was one known to give high results. During the course of the present work there appeared a paper by Benedict [1925] in which he describes a new and more

selective method for the estimation of blood sugar. This method, he believes, gives results more nearly approaching the true content of glucose in the blood than any other at present available. As a result of his work he came to the conclusion that the true sugar content of the blood is probably only 60–75 % of that which is now stated to be present by current reduction methods. By using the new Benedict method as a control of our fermentation results, we were in a position to see whether the 25–40 % difference found by Benedict corresponded with any discrepancy found by fermentation.

The method used by us was one partially described in a previous paper [1925]. The blood was diluted with equal parts of distilled water and 1·0 % orthophosphoric acid and 3·0 cc. of the mixture taken for fermentation, so that 1·0 cc. of blood was actually fermented.

After dilution of the blood it was very vigorously shaken to expel carbon dioxide and to ensure complete aeration. In some experiments with animal bloods additional estimations were made of a control blood to which a definite amount of glucose had been added. We were thus able to obtain some idea of the fermentative quality of clean yeast on glucose in the presence of blood. We had previously ascertained that it was not satisfactory to obtain the information from the action of yeast on an aqueous solution of pure glucose alone. The addition of blood accelerates the fermentation. In a special column is given the percentage of added glucose fermented.

The routine method for the determination of sugar in blood employed in this laboratory is that of Hagedorn and Jensen [1923] which gives in our hands, with amounts of sugar added to blood, nearly the theoretical figure. Höst and Hatlehol [1920], in their comparison of four methods, give figures which show the method to give an average error of 3·2 % and the greatest error of 9 %. An inspection of their tables seems to show that the error is one giving less glucose than that added to the blood. In this our results confirm those of the Danish workers. The standard glucose solutions used by us were made in the beginning from a sample for which we are indebted to the Carbohydrate Laboratory of the U.S. Department of Agriculture. It was stated to be over 99·0 % pure glucose. It was dried in a thin layer in a vacuum desiccator for 24 hours over sulphuric acid before being weighed. In some of the later work a highly purified commercial glucose was used. A comparison of this with the former sugar showed no appreciable difference. For the Benedict estimation, the standard sugar solution was preserved with toluene. For fermentation, the sugar was made up frequently in 0·1 *N* sulphuric acid and just before use each day was neutralised with the requisite amount of 0·1 *N* sodium hydroxide and diluted to 0·1 %.

The comparisons are divided into (I) animal bloods, (II) non-diabetic human bloods, (III) diabetic bloods. The number of samples which we have examined is large and only a selection of those comparisons are given which were done after the paper of Benedict appeared. They represent as faithfully as possible the general result of the investigation.

I. *Animal Bloods.*

	Blood sugar %					
	Hagedorn and Jensen	Fermentation	Benedict	$\frac{100F}{\text{H. and J.}}$	$\frac{100B}{\text{H. and J.}}$	% of added glucose fermented
Sheep	0·058	0·038	0·064	65	110	—
„	0·055	0·031	0·044	56	81	—
„	0·050	0·033	0·048	66	96	91
„	0·040	0·033	0·049	82	122	86
Bullock	0·064	0·044	0·062	68	97	—
„	0·150	0·098	0·156	65	103	99
„	0·162	0·123	0·149	76	92	77
Pig	0·079	0·071	0·065	90	82	—
„	0·086	0·048	0·072	55	83	—
Dog	0·086	0·048	0·073	56	85	99
Cat	0·250	0·220	0·276	88	110	—
Rabbit	0·034	0·018	0·027	53	79	—
II. *Non-diabetic Bloods.*						
Nephritis 70 mg. urea %	0·078	0·062	0·085	39	110	—
Normal	0·086	0·051	0·100	59	115	—
„	0·100	0·051	0·116	51	116	—
Nephritis 68 „ „	0·086	0·043	0·080	50	94	—
„ (?) 30 „ „	0·074	0·035	0·076	47	104	—
Cystitis 40 „ „	0·100	0·050	0·094	50	94	—
Prostate 159 „ „	0·094	0·040	0·099	42	105	—
III. *Diabetic Bloods.*						
	0·110	0·053	0·102	48	92	—
	0·220	0·138	0·210	63	96	—
	0·144	0·058	0·162	40	112	—
	0·188	0·106	0·177	56	94	—
	0·152	0·104	0·148	68	97	—
	0·140	0·074	0·106	52	76	—
	0·240	0·204	0·287	85	119	—
	0·252	0·161	0·214	64	85	—

An examination of the tables may be divided into two parts. (1) The concordance between the new Benedict method and that of Hagedorn and Jensen. With animal bloods the ratio $\frac{100B}{\text{H. and J.}}$ ranges from 74 to 122. There is no obvious connection between sugar level or class of animal dealt with. With non-diabetics the balance, if any, is in favour of showing that the Benedict method gives higher values. With diabetics the Benedict figures are, perhaps, somewhat lower than those of Hagedorn and Jensen, but here again one obtains high figures; for example, a case with a hyperglycaemia of 0·24 % giving a $\frac{100B}{\text{H. and J}}$ ratio of 119.

(2) Turning now to the ratios between the Hagedorn and Jensen values and the amount of glucose found by fermentation, we find in all cases lower figures from fermentation than are found when we obtain our results by a reduction method. The ratios vary from 39 % to 88 %. It is interesting to note that these high percentages are associated with a high total blood sugar, a result which one might anticipate if one assumes that the hyperglycaemia is the principal feature in the change in metabolism. Here again is an exception in the last case among the diabetic bloods.

The average level of $\frac{100F}{\text{H. and J.}}$ in non-diabetics is close to 50, when none of the blood sugar percentages exceeds 0·1%. That the low ratios are not due to incomplete fermentation is shown by the comparisons with the fermentation of added glucose. The values vary from 77 to 99 %. The average of all determinations is 90 %. Taking this as the correct figure, one finds that the discrepancy between the reduction and fermentation method is as high as 50 % in some cases of low total reducing substance in the blood and on an average between 40 and 30 %. One may safely set down these latter figures as those which represent the proportion of the total reducing substances of the blood which are not glucose. These are in agreement with the conclusion reached by Benedict himself. The variations are, however, large and we have not been able to make out any rule in the matter.

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