CXV. THE RELATIVE PROPORTIONS OF FERMENTABLE AND NON-FERMENTABLE REDUCING SUBSTANCES OF HYPERGLYCAEMIC BLOODS OF DIABETICS WITHOUT GLYCOSURIA.

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(Received May 9th, 1932.)

CLAUDE BERNARD first showed that sugar is a normal constituent of blood and recognised that glycosuria is, in some manner, dependent upon hyperglycaemia. Since then a vast literature has accumulated on the subject and though the consensus of opinion favours the view that there is a renal threshold for sugar this view is not, as yet, unanimous. Thus, according to Benedict *et al.* [1918], urine normally contains glucose which is excreted continuously, the process being termed glycuressis. On the other hand, Folin and Berglund [1922] claimed that in the absence of emotional complications ingestion of as much as 200 g. of glucose did not cause the appearance of sugar in the urine of normal persons and concluded that glycuressis represents absorption and excretion of foreign unusable carbohydrate materials present in the ingested food. More recently, however, Hassan [1928] was able to prepare glucosazone from many samples of normal urine and concluded that failure to detect glucose in the past was due to technical difficulties. The writer repeated Hassan's work and obtained somewhat similar results (unpublished data). In quite an exhaustive study, on the other hand, Harding and Selby [1931] concluded that within the limits of analytical methods, fermentable sugar is absent from normal fasting urine. Analysis of all of the data; however, shows that Hassan's results, those of Harding and Selby and our own are not incompatible with each other. Thus, Hassan's data, as well as our own, clearly indicate that though normal urine may have contained glucose, detection of this sugar depended upon the time elapsing between collection of the urine and the last meal; as the fasting state was approached, the urine contained less and less sugar. This agrees with the findings of Harding and Selby who observed that, though there was no glycosuria in the fasting state, 50 % of medical students showed small amounts of fermentable sugar in the post-prandial afternoon urine.

The finding of sugar in normal urine appears at first to be incompatible with the renal threshold concept. Experiences with blood-sugar-time curves, however, suggest otherwise. Frank [1913] first made the observation that

sugar may appear in the urine at a much lower level of blood-sugar concentration when the sugar content of the blood is decreasing than when it is increasing. It was because of the quite frequent occurrence of this phenomenon that attention was called to the unreliability of blood-sugar-time curves when used alone for the diagnosis of renal glycosuria [Rabinowitch, 1930]. It is suggested that this phenomenon explains the finding of sugar in normal urine in the absence of hyperglycaemia and that the necessary condition is a temporary increase of blood-sugar above the renal threshold level. Such increase is probably a common phenomenon following an ordinary meal, but would be detected only with repeated blood-sugar examinations at very short intervals of time. Once the blood-sugar has reached a level above the renal threshold, sugar excretion begins and continues for a short period of time, in spite of the subsequent decrease of blood-sugar. Ingestion of a large amount of sugar is not necessary. Experiences with blood-sugar-time curves indicate that the height to which the blood-sugar rises following glucose ingestion is not strictly proportional to the amount of sugar given. Small amounts may suffice to raise the blood-sugar; with as little as 5 g. an appreciable increase may be noted, and with doses exceeding 25 g. the peak of the curve is not materially affected. Increasing the amount of sugar affects chiefly the rate of decay of the curve; it prolongs the time necessary for the blood-sugar to return to the normal level.

The above are a few examples of the many possible physiological variables which are met with in renal threshold studies, and it is perhaps these difficulties which have made it doubtful whether the renal threshold concept is at all applicable to any blood constituent. Himsworth [1931] has recently reviewed the different methods now in use for determination of the renal threshold for glucose and dealt with some of these difficulties; a new method was suggested.

For practical purposes it is expedient to assume that there is a renal threshold for glucose; that glycosuria, with rare exceptions, is due to hyperglycaemia. (Glycosuria here implies sugar in sufficient concentrations to be detected by the ordinary copper reduction methods, Fehling's, Benedict's, *etc.*). Otherwise one could not differentiate between such conditions as renal glycosuria and diabetes mellitus. The latter, as is well known, usually proves fatal in the absence of treatment, whereas the former requires no dietetic restrictions and is perfectly compatible with good health and long life.

To support the renal threshold concept, there is the experience not infrequently met with in diabetes, namely, marked hyperglycaemia without glycosuria detectable by the ordinary reduction methods; the blood-sugar in such cases may amount to 0.2-0.4 % or more. This phenomenon was first observed by von Noorden [1917] in a case of pneumonia where the blood-sugar was 0.280 %. Blood-sugar concentrations over 0.25 % without glycosuria have been recorded by Graham [1917], Sherrill and John [1922], John [1927], Allen [personal communication] and Joslin [1928]. Many more examples have

been met with more recently. What is believed to be a classical case is that reported by Rabinowitch [1929], a blood-sugar of 0.825 % being found in the absence of glycosuria. This was a case of diabetic coma with marked kidney damage; the blood-urea-nitrogen was 81 mg./100 cc., the creatinine was 5.66 mg./100 cc., and the diazo-reaction for uraemia was positive. A blood-sugar of 0.769 %, without glycosuria, was found in the same patient 3 hours previously. Stone [1926] regards this raised renal threshold as an indication of unfavourable prognosis; autopsy findings indicated hyalination of the islet tissue.

The conditions in which a raised renal threshold is usually found in diabetes are (a) gross dietary indiscretions, (b) diabetes of long duration, (c) chronic nephritis, (d) arteriosclerosis and (e) infection. Insulin was found to be a factor [Rabinowitch, 1924]. Major and Davis [1925] later reported seven cases of raised renal threshold in patients without arteriosclerosis or renal disease, but who were taking insulin. The rapidity with which the condition may appear following institution of insulin treatment was previously dealt with [Rabinowitch, 1926].

In a previous communication [Rabinowitch, 1928] the writer dealt with the non-fermentable reducing substances of blood in diabetes and it was found that, ordinarily, they are of no significance; the average values found in 100 normal adults, 10 normal children and 100 diabetics were approximately the same, namely, 0.025, 0.023 and 0.027 % respectively. Though they represent an appreciable proportion of the total blood-sugar, the amounts found are fairly constant, ranging between 16 and 31 mg./100 cc. Daily analysis showed narrow fluctuations. Rather remarkable was the finding of fairly constant values after ingestion of commercial glucose which is known to contain an appreciable amount of non-fermentable reducing substances. It was also found that the concentration of these reducing substances in the blood was not affected by insulin.

The above observations apply to the ordinary diabetic. Therefore, if, as Stone suggests, a raised renal threshold is an indication of unfavourable prognosis, it is obviously important to make certain that the hyperglycaemia in suspected cases is also due chiefly to true blood-sugar and not to other reducing substances. As far as the writer is aware, there are no published data on this phase of the subject. Frederick M. Allen [personal communication] states that Richard I. Wagner, while working in the latter's laboratory, made a number of estimations of fermentable and non-fermentable sugar. The sugar which fermented was called "free" and the difference between the free and the total was called "combined." In the cases of raised renal threshold, the values noted were due, chiefly, to the "combined" type.

In this study 58 analyses were made in 19 cases. Blood-sugars were determined both before and after fermentation. All blood samples were obtained in the fasting state, according to the routine of this laboratory. Somogyi's [1927] methods were employed both for purification of yeast and fermentation

of blood, 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.

Table I. *Showing degrees of hyperglycaemia and amounts of non-fermentable reducing substances in 58 analyses.*

Exp.*	Hospital No.†	Blood-sugar			Blood-urea-N mg. per 100 cc.	Exp.*	Hospital No.†	Blood-sugar			Blood-urea-N mg. per 100 cc.
		Before fermentation	After fermentation	Fermentable				Before fermentation	After fermentation	Fermentable	
1	5996/28	0.825	0.061	0.764	81	30	5312/31	0.270	0.034	0.236	17
2	"	0.769	0.047	0.722	81	31	2391/29	0.270	0.020	0.250	10
3	6419/29	0.624	0.040	0.584	16	32	"	0.263	0.037	0.226	10
4	A.A.C.	0.500	0.024	0.476	—	33	431/29	0.263	0.037	0.226	22
5	6249/31	0.500	0.028	0.472	17	34	2391/29	0.263	0.037	0.226	10
6	431/29	0.500	0.040	0.460	22	35	"	0.256	0.016	0.240	10
7	1935/30	0.454	0.028	0.426	20	36	5312/31	0.250	0.012	0.238	17
8	"	0.434	0.032	0.402	20	37	6101/25	0.250	0.028	0.222	25
9	"	0.416	0.031	0.385	20	38	2391/29	0.250	0.028	0.222	10
10	431/29	0.414	0.040	0.374	22	39	"	0.250	0.010	0.240	10
11	1734/31	0.400	0.027	0.373	14	40	"	0.244	0.022	0.222	10
12	1935/30	0.400	0.016	0.384	20	41	6443/28	0.244	0.025	0.219	15
13	"	0.384	0.039	0.345	15	42	"	0.244	0.016	0.228	15
14	"	0.384	0.014	0.370	20	43	2391/29	0.238	0.016	0.222	10
15	7135/31	0.370	0.032	0.338	13	44	5312/31	0.233	0.024	0.209	17
16	6443/28	0.344	0.023	0.321	15	45	"	0.232	0.020	0.212	17
17	431/29	0.333	0.040	0.293	22	46	"	0.232	0.018	0.214	17
18	6090/30	0.333	0.040	0.293	14	47	431/29	0.232	0.037	0.195	22
19	5312/31	0.333	0.017	0.316	17	48	3535/30	0.232	0.034	0.198	14
20	1935/30	0.319	0.024	0.295	20	49	6443/28	0.227	0.018	0.209	15
21	431/29	0.312	0.030	0.282	22	50	6059/30	0.227	0.047	0.180	10
22	6443/28	0.295	0.042	0.253	15	51	3332/30	0.222	0.040	0.182	20
23	5312/31	0.295	0.025	0.270	17	52	431/29	0.222	0.050	0.172	22
24	"	0.295	0.033	0.262	17	53	2391/29	0.222	0.027	0.195	10
25	"	0.285	0.034	0.251	17	54	"	0.222	0.022	0.200	10
26	2391/29	0.280	0.017	0.263	10	55	5312/31	0.222	0.019	0.203	17
27	5312/31	0.274	0.029	0.245	17	56	2391/29	0.200	0.015	0.185	10
28	"	0.274	0.025	0.249	17	57	4524/30	0.200	0.024	0.176	16
29	"	0.270	0.022	0.248	17	58	3953/29	0.200	0.040	0.160	13

* Data arranged in order of degree of hyperglycaemia.

† Hospital numbers do not indicate year of test. They are recorded in order to afford reference to subjects in future study.

The yeast was first purified. A weighed amount 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 present in yeast, which may be non-fermentable and reduce alkaline copper solutions. As a further precaution, the yeast was suspended in 10 parts of water, and 2 cc. of the suspension were tested for reducing substances by the Folin-Wu process. The yeast was used only when reductions noted were inappreciable and could not be estimated quantitatively. As soon as the yeast was ready for use, a portion of the blood was treated as follows.

One volume of blood was added to 7.5 volumes of a 10 % suspension of yeast¹. After thorough mixing, the flask was allowed to remain in a water-bath

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.

at 37° for 7 min. One volume of 10 % sodium tungstate was then added followed by one volume of 0.66 *N* sulphuric acid. After thorough shaking, the mixture was allowed to stand for 10 min. It was then filtered. The remaining portion of blood was treated in the usual (Folin-Wu) manner. The reducing substances of both filtrates were then estimated simultaneously. In the case of the fermented blood, the necessary precautions were taken for estimating minute amounts of sugar as previously outlined [Folin and Svedberg, 1926]. The combined data are shown in Table I. All values are expressed in terms of percentages and the data are grouped from high to low with respect to the degree of hyperglycaemia.

DISCUSSION OF RESULTS.

Objections have often been raised against the use of yeast for differentiation between glucose and other sugars. It is however suggested that the various limitations and criticisms of the past do not apply here. The fact that glucose may fail to ferment when present in small quantities applies to urine [Seegen, 1885; von Lippmann, 1904] and to older technical methods, and the fact that yeast may contain, or during fermentation produce, other reducing substances, pentoses, purines, *etc.* [Neuberg, 1910; Mayer, 1913] also need not be considered here, in view of (a) the short time allowed for fermentation, (b) the technique of purification of yeast and (c) the sensitive method for detection of reducing substances. It is therefore assumed that the greater part of fermentation noted in this study was due to glucose.

It will be noted that there was little, if any, relationship between the degree of hyperglycaemia and the amount of non-fermentable reducing substances; though the greatest amount of non-fermentable reducing substances was found with the most marked degree of hyperglycaemia (Exp. 1). However, allowing for the large amount of non-fermentable substance in the latter experiment, there was still a marked hyperglycaemia without glycosuria; the fermentable sugar was 0.764 %. Table II shows the relationship between the degree of hyperglycaemia and the amount of non-fermentable reducing substances more clearly. Maximum, minimum and average values are shown for corresponding degrees of hyperglycaemia.

As food contains many unusable carbohydrate and reducing substances

Table II. *Showing relationship between degree of hyperglycaemia and amount of non-fermentable substances.*

Blood-sugar %	No.	Non-fermentable reducing substances (%)		
		Maximum	Minimum	Average
0.250	23	0.050	0.010	0.026
0.251-0.300	14	0.042	0.016	0.029
0.301-0.400	11	0.040	0.016	0.027
0.401-0.500	7	0.040	0.024	0.032
0.500 +	3	0.061	0.040	0.049

other than glucose, it appeared possible that an observed raised renal threshold in nephritis might be more apparent than real, since nephritis, as is well known, may lead to marked retention of waste products. The cases were therefore divided into two groups, namely, (a) those with, and (b) those without, nephritis. The criterion of nephritis was the presence of albumin and casts in the urine. As urea determination is a routine in every case of diabetes on admission of the patient to the hospital, the values of this blood-constituent were also made use of. With the exception of one case (No. 5996/28, Exps. 1 and 2), it will be observed (Table I) that the urea contents were practically all within the normal limits for hospital patients¹. This observation is important, since experiences with many thousands of simultaneous urea and sugar determinations have taught us that hyperglycaemia is usually associated with urea retention only when the latter is fairly marked. It therefore appears from the urea data alone, that the total blood-sugar values noted in many of these cases of raised renal threshold were not due to impaired kidney function. As this view may not be accepted generally, there is the further observation that no relationship was found between albuminuria and the amount of non-fermentable reducing substances in the blood. Thus:

Group	No.	Average amount of non-fermentable reducing substances (mg. per 100 cc.)
Albumin	30	29.5
No albumin	28	28.2

CONCLUSIONS.

The raised renal threshold in diabetes is real and not apparent; the hyperglycaemia in these cases is due chiefly to fermentable sugar and not to other reducing substances which are ordinarily found in blood.

In view of the possible finding of as much as 60 mg. of non-fermentable reducing substances per 100 cc. of blood, and in view of the alleged unfavourable prognosis with raised renal threshold, it is suggested that a diagnosis of this condition should not be made without determination of the relative proportion of fermentable and non-fermentable reducing substances.

This work was done with the technical assistance of Miss Mary Beard.

Grateful acknowledgment is due to Mr Julian C. Smith of Montreal, a Governor of this hospital, for his financial assistance in connection with this work.

¹ The urea values recorded are those found on the days of admission of the patients to the hospital, except for Exps. 1 and 2. As none of these patients was suffering acutely from nephritis, it may be assumed that daily fluctuation of values, if it occurred at all, would not affect interpretation of the data.

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