VIII. THE RELATION OF SUGAR EXCRETION TO DIET IN GLYCOSURIA.

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THE modern treatment of diabetes is founded on the experimental work of Allen [1915] on the effects of starvation on the excretion of sugar in glycosuria. The observation that the urine of many people suffering from this disease may be freed from sugar by starvation forms the basis of the experimental work detailed in the following pages.

It is clear that if the urine of a man suffering from glycosuria is rendered sugar free by starvation, then, the effect of any diet on his sugar excretion may be determined. The accuracy of the results is limited by the fact that it is not desirable to keep a man in a constant state of starvation. But, by a careful choice of the time at which a desired food is given, and by making a graphic time record of the sugar subsequently excreted, a considerable amount of information may be obtained on the effect of that foodstuff on the excretion of sugar. From an analysis of these results, considered in conjunction with other observations in this disease, an hypothesis has been advanced on the causation of glycosuria in *diabetes mellitus*.

The main results recorded were obtained from a man, aet. 53, suffering from *diabetes mellitus*. When he came under observation he was excreting, *per diem*, about 250 g. of dextrose contained in four litres of urine.

The sugar determinations were made by a method originally described by Wood and Berry [1903]. In this method 25 cc. of a copper solution (suggested by Soldaini [1876]) were boiled for 15 minutes with 1 cc. of urine, and the precipitated cuprous oxide was filtered off by asbestos felt contained in a Gooch crucible. The cuprous oxide was dissolved in 25 cc. of a 2.5 % solution of ferric sulphate contained in 25 % H_2SO_4 , and the equivalent amount of ferrous sulphate formed was estimated by a standard solution of permanganate. The strength of the permanganate solution was such that 1 g. of dextrose was equivalent to 264 cc. KMnO_4 .

The man's diet was carefully weighed and controlled during the experimental period. Whenever he emptied his bladder the amount of urine excreted and the time of excretion were noted. The quantity of dextrose contained in 1 cc. of urine was determined. The man was encouraged to micturate at frequent intervals so that a time curve showing the rate of sugar excretion could be obtained with a fair degree of accuracy.

(a) *Starvation.* When the man first came under observation a three days' fast was required to free his urine from sugar. The following detailed experiment was made three weeks later and shows, *inter alia*, that a considerable improvement had taken place in his condition. His last meal was taken at 6.30 p.m. Within 24 hours his urine was free from sugar. The table shows the effect of deprivation of all food on the average sugar and urine excretion per hour.

Time	Amount of urine	cc. KMnO_4 for 1 cc.	Total sugar	Sugar per hour	Urine per hour
	cc.		g.	g.	cc.
4.30 p.m.	253	16.4	15.7	—	—
8 "	224	12.6	10.7	3.6	64
Midnight	337	8.4	10.8	2.68	84
2.30 a.m.	280	9.9	10.5	4.2	112
8 "	224	7.9	6.7	1.22	40
9 "	224	7.95	6.73	6.73	224
11 "	450	1.35	2.3	1.15	225
1.30 p.m.	476	0.85	1.54	0.62	190
3.30 "	253	0.25	0.24	0.12	226
5.30 "	364	0.05	0.068	0.034	182
8 "	503	0.05	0.096	0.038	201
1 a.m.	224	0.05	0.042	0.008	45

Fig. 1.

The results are shown graphically in Fig. 1. The rapid disappearance of sugar from the urine in starvation is shown in a clear way. From midnight to noon he excreted 37 g., from noon to midnight 2 g. of dextrose. The disappearance of sugar from the urine when no food was taken afforded a means

whereby the effect of diet on the excretion of dextrose in the urine could be determined. Incidentally it may be remarked that the curve shows the effect of taking caffeine (contained in tea without milk or sugar) at 8 a.m. The amount of urine was greatly increased, and, although the percentage of sugar in the urine remained constant, yet the total quantity of sugar excreted showed a prominent rise. Again, it is well known that the amount of urine excreted in glycosuria is roughly proportional to the amount of sugar contained in the urine. It may be observed that whereas this generalisation held true for the first 12 hours when the urine contained a large amount of dextrose, the parallelism ceased when the urine became sugar free.

(b) *Extractives*. The object of this experiment was to ascertain whether the absorption of any substance from the alimentary canal, considered independently of its energy value, caused an excretion of sugar in the urine. Since only one-fifth of the total energy contained in the extractives of meat is utilised by the body, the man was given 840 cc. of beef tea to drink when his urine was free from sugar. The following table shows the results observed:

Time	Amount of urine cc.	cc. KMnO_4 for 1 cc.	Total sugar g.	Sugar per hour g.	Urine per hour cc.
9-10.15 a.m.	56	1.6	0.34	0.15	25
4.30 p.m.	140	6.6	3.58	0.7	25
8 „	84	4.6	1.45	0.41	24

The beef tea was taken between 2.30 p.m. and 4.30 p.m. Only a small quantity of sugar was excreted, about equal to the carbohydrate extracted from the beef. No definite evidence was obtained in favour of the assumption that the extractives contained in beef may give origin to sugar, nor that the act of absorption from the alimentary canal may cause the excretion of sugar in the urine.

(c) *Alcohol*. It was of interest to determine the effect of a relatively simple substance of a high calorific value on the excretion of sugar. Alcohol was chosen for this purpose since it has obtained a reputation as a foodstuff of considerable value in the dietetic treatment of *diabetes mellitus*. 112 cc. of whisky containing 30 % of alcohol were given in two portions at 10 p.m. and 4 a.m. respectively. In each case water was added to make the volume of the diluted fluid 250 cc. The following figures show the excretion of sugar and urine during the experimental period.

Time	Amount of urine cc.	cc. KMnO_4 for 1 cc.	Total sugar g.	Sugar per hour g.	Urine per hour cc.
8-11 p.m.	56	3.5	0.74	0.245	19
1 a.m.	112	3.0	1.3	0.65	37
2 „	140	0.8	0.42	0.42	140
6 „	56	0.9	0.20	0.05	14

The figures show that after the ingestion of alcohol the excretion of sugar increased a small amount only—from 0.245 g. to 0.65 g. of sugar per hour. Since alcohol is metabolised to a considerable extent by the body—only 2 %

being excreted—it is evident that the tissues can utilise a simple substance containing carbon, hydrogen and oxygen only without a concomitant excretion of sugar in the urine. This fact is of some practical value since the calorific value of alcohol is seven and the available energy obtained from 112 cc. of whisky containing 30 % alcohol amounts to 235 K.

(d) *Protein*. The effect of protein on the excretion of sugar was determined by giving a meal of 112 g. of caseinogen at 4.30 p.m. The urine was not free from sugar before giving the caseinogen since, four hours previously, the man had eaten a small quantity of beef and green vegetables. However, the comparative results, and particularly the graphic record of these results, clearly show the effect of caseinogen on the excretion of sugar.

Time	Amount of urine	cc. KMnO_4 for 1 cc.	Total sugar	Sugar per hour	Urine per hour
10.30 a.m. to	cc.		g.	g.	cc.
2.30 p.m.	196	2.1	1.56	0.39	49
4.30 „	280	3.6	3.8	1.9	140
8 „	168	4.5	3.12	0.89	45
10 „	336	4.9	6.25	3.12	168
5 a.m.	364	0.4	0.56	0.08	52

The results are shown graphically in Fig. 2. It is evident from the figure that the excretion of sugar between 8 p.m. and 10 p.m. was due to the ingested caseinogen. On this assumption the metabolism of 112 g. of caseinogen caused the excretion of 6 g. of dextrose in the urine. The caseinogen was sugar-free so that the dextrose must have originated from the amino-acids of the protein. It is of interest to compare this result with that obtained by Janney [1915] on a phlorhizinised dog. In this case 48 % of the ingested protein appeared in the urine as dextrose. Since it is assumed that a dog treated with this drug is incapable of utilising dextrose, the deduction is made that caseinogen contains 48 % of amino-acids which normally may give rise to dextrose. From these figures it may be calculated that the man was capable of utilising 89 % of the amino-acids of caseinogen which were available for conversion into dextrose.

(e) *Carbohydrate*. Whatever the carbohydrate eaten—starch, cane sugar, lactose or laevulose—dextrose only was excreted in the urine. The effect of carbohydrate on the excretion of sugar was determined in detail. Carbohydrate in three forms was given: (i) laevulose, (ii) cane sugar, (iii) oatmeal (starch).

(i) *Laevulose*. The view is often expressed that laevulose is utilised to a considerable degree by people suffering from glycosuria. The results recorded show that laevulose is utilised to a less degree than either cane sugar or starch.

56 g. of laevulose dissolved in 224 cc. of water were taken at 8 p.m. The comparative results depicted in Fig. 3 show the effect on the sugar excretion of this ingested laevulose.

Fig. 2.

Fig. 3.

Time	Amount of urine	cc. KMnO_4 for 1 cc.	Total sugar	Sugar per hour	Urine per hour
	cc.		g.	g.	cc.
3 to 5 p.m.	140	7.7	4.04	2.02	70
8 "	56	9.2	1.93	0.64	19
8.30 "	168	9.2	5.85	11.7	336
10 "	280	15.1	16.1	10.7	187
1.30 a.m.	280	12.0	12.7	3.65	80
3 "	70	10.5	2.8	1.86	46
6 "	112	5.0	2.1	0.7	37

The curve shows that the ingested laevulose caused a very rapid rise in the amount of sugar excreted in the urine. Within 30 minutes the rate of sugar excretion increased from 0.64 g. to 11.7 g. per hour. The results also show that the greater part of the effect passed off in two hours since after that time the average rate of sugar excretion fell from 10.7 g. to 3.65 g. per hour. The figures allow an approximate calculation to be made of the amount of sugar excreted after eating 56 g. of laevulose. The average sugar excretion before eating the laevulose at 8 p.m. was 0.64 g. per hour. It again fell to this level at 3 a.m. It may therefore be concluded that the sugar excreted, due to the ingested laevulose, was confined to the urine formed between 8 p.m. and 3 a.m. Also it may be assumed that during this period 0.6 g. of sugar per hour was due to the metabolism of foodstuffs, other than laevulose, previously eaten. On these assumptions it may be calculated that 33 g. of sugar were excreted as the result of eating 56 g. of laevulose—or of the laevulose ingested 41 % was utilised whilst 59 % was excreted in the urine. Comparative colour tests showed that practically the whole of the reducing sugar excreted was dextrose. The urine passed at 8.30 p.m. and 10 p.m. gave a characteristic Selivanoff reaction. The colour produced under standard conditions when compared with the colour produced under similar conditions in normal urine to which known amounts of laevulose had been added indicated that only 0.75 g. of laevulose was excreted as such. Therefore the following conclusions may be summarised from these observations: (a) nearly 40 % of the ingested laevulose was excreted as dextrose, and (b) within two hours two-thirds of the excreted sugar had been so transformed and excreted.

(ii) *Cane sugar*. In order to determine the capacity of the man to utilise this form of carbohydrate, 112 g. of cane sugar were dissolved in 450 cc. of water. The syrup was taken at 7.15 p.m. The following figures show the amount of sugar excreted during the experimental period:

Time	Amount of urine	cc. KMnO_4 for 1 cc.	Total sugar	Sugar per hour	Urine per hour
	cc.		g.	g.	cc.
11.15 a.m. to 4.30 p.m.	140	6.6	3.52	0.67	26.5
8 "	84	4.6	1.52	0.435	24.0
1 a.m.	392	17.4	25.8	5.2	78.0
4 "	140	21.3	11.4	3.8	47.0
7 "	56	4.0	0.85	0.28	8.0

These results, shown graphically in Fig. 4, illustrate in a clear manner the effect of eating 112 g. of cane sugar on the amount of sugar excreted in the urine. At 8 p.m., immediately after eating the cane sugar, the average amount of sugar per hour in the urine was 0.435 g.; up to 4 a.m. there was a large rise in the excretion, and after that time the average per hour fell to 0.28 g. It may be concluded that the sugar excreted at 1 a.m. and 4 a.m. was mainly derived from the ingested cane sugar. On this assumption, and allowing 0.3 g. of sugar per hour as derived from some source other than the cane sugar, it may be calculated that the ingestion of 112 g. of cane sugar resulted in the

Fig. 4.

output of 36.5 g. of sugar in the urine. There was no cane sugar contained in the urine, since its reducing power was unchanged after hydrolysing with 1 % H_2SO_4 at 120° for 30 minutes. Also there was only a trace of laevulose in the urine—less than 0.1 g. Hence we may conclude that the 36.5 g. of reducing sugar contained in the urine consisted of dextrose only. Now 112 g. of cane sugar when hydrolysed yield 124 g. of invert sugar. Therefore after the absorption of 124 g. of invert sugar from the alimentary canal 87.5 g. were utilised by the tissues whilst 36.5 g. were excreted as dextrose. Despite the fact that the man excreted sugar not only on a carbohydrate diet but also on a protein diet, his tissues possessed a capacity to utilise carbohydrate derived from cane sugar to the extent of 71 %, only 29 % being excreted as dextrose in the urine.

(iii) *Oatmeal*. This foodstuff has been advocated by Van Noorden as a suitable diet for diabetics. The capacity of the man to utilise carbohydrate when presented in this form was therefore determined. On the first occasion the man ate 23 g. of oatmeal at midday. The following figures show the effect of this food on his excretion of sugar.

Time	Amount of urine	cc. KMnO_4 for 1 cc.	Total sugar	Sugar per hour	Urine per hour
8.30 a.m. to	cc.		g.	g.	cc.
10.30 a.m.	84	2.05	0.65	0.33	42
3 p.m.	84	1.05	0.34	0.07	18
4 „	252	2.4	2.28	2.28	252
10 „	168	5.5	3.5	0.87	42
2 a.m.	280	0.25	0.26	0.06	70

The results are shown graphically in Fig. 5. It may be seen from the diagram that the ingestion of 23 g. of oatmeal resulted in the excretion of 5.78 g. of dextrose. Now 23 g. of oatmeal contain 14.7 g. of starch, and this on hydrolysis yields 16.4 g. of dextrose. Therefore a diet containing carbohydrate equivalent to 16.4 g. of dextrose resulted in 10.6 g. being retained by the tissues and 5.8 g. being excreted in the urine. In other words 35 % of the available carbohydrate was excreted as dextrose whilst 65 % was utilised by the tissues of the body.

Fig. 5.

A second experiment was made in which 184 g. of oatmeal were given in four equal portions, *i.e.* 46 g. at 5 a.m., 8.30 a.m., 12.30 p.m. and 4.30 p.m.

The object of the experiment was to determine whether he could utilise carbohydrate to a greater or less extent when it was continuously supplied to the tissues of the body during the day, in contradistinction to the above experiment in which a small quantity of carbohydrate was given at one meal. The following results were obtained:

Time	Amount of urine cc.	cc. KMnO_4 for 1 cc.	Total sugar g.	Sugar per hour g.	Urine per hour cc.
1-5 a.m.	112	9.1	3.9	0.97	28
9.30 "	168	14.0	8.9	2.0	37
Noon	168	13.3	8.5	3.4	67
3 p.m.	168	13.0	8.25	2.75	56
5 "	84	12.4	3.95	1.97	42
10 "	252	18.8	17.9	3.6	50
2.15 a.m.	112	9.5	4.03	0.95	26

The results are plotted in Fig. 6. The graphic record shows that the sugar excretion due to the oatmeal eaten at the four meals during the day extended from 9.30 a.m. to 10 p.m. During this time the amount of dextrose excreted (allowing 1 g. of sugar per hour as derived from sources other than the oatmeal, *i.e.* the quantity excreted per hour before and after the experimental period) amounted to 35.5 g. The oatmeal eaten contained 118 g. of starch and this on hydrolysis would yield 130 g. of dextrose. Therefore of the total carbohydrate (calculated as dextrose) available during the day approximately 94.5 g. were metabolised by the tissues of the body and 35.5 g. were excreted in the urine; or in other words, 73 % was metabolised and 27 % excreted.

Fig. 6.

EXPERIMENTAL RESULTS.

The results detailed in the previous pages as to the relation of sugar excretion to diet in a man suffering from a moderate form of *diabetes mellitus* are summarised below:

(1) When first determined the daily output of sugar was about 250 g. contained in 4 litres of urine. Starvation freed the urine from sugar in 60 hours. Three weeks later the sugar excretion per diem was 120 g. contained in 3 litres of urine. A second period of starvation rendered the urine sugar-free in 24 hours.

(2) Whatever the carbohydrate eaten—starch, cane sugar, lactose or laevulose—dextrose only was excreted in the urine.

(3) Starch and cane sugar were utilised to the extent of 70 %; laevulose, on the other hand, was utilised only to the extent of 40 %.

(4) The capacity of the tissues to utilise sugar was not an absolute amount but was determined by the quantity of carbohydrate presented to them. Thus when 14.7 g. of starch were eaten 65 % was metabolised by the tissues and 35 % was excreted as dextrose. When 118 g. of starch were eaten 73 % was utilised by the tissues and 27 % excreted as dextrose. In the latter case nine times as much carbohydrate was metabolised as in the former case.

(5) Amino-acids, contained in caseinogen, available for conversion into dextrose, were utilised to a greater extent than starch. Thus only 6 g. of dextrose were excreted in the urine after eating 112 g. of caseinogen, *i.e.* 89 % of the amino-acids available for conversion into dextrose were metabolised.

(6) Within two hours of the ingestion of 56 g. of laevulose 30 g. of dextrose were excreted in the urine.

(7) The absorption of extractives of small energy value from the alimentary canal produced no increased excretion of sugar.

(8) Considerable quantities of alcohol were metabolised without a concomitant excretion of sugar in the urine.

The following additional facts have been observed in other cases of *diabetes mellitus*.

(9) In *diabetes mellitus* there is a marked hyperglycaemia. The amount of dextrose in the blood may exceed 0.4 %. During starvation the amount of sugar in the blood diminishes and reaches a normal level when the excretion of sugar in the urine ceases.

(10) The liver of a child whose death was due to *diabetes mellitus* possessed as great an action on carbohydrate and fat as that of a child suddenly killed by accident.

DISCUSSION OF RESULTS—THE ABNORMAL FACTOR IN *DIABETES MELLITUS*.

Various hypotheses have been advanced as to the causation of this disease. Among such hypotheses may be mentioned those which assign the abnormality to (a) the alimentary canal, (b) the liver, (c) the muscles. The experimental results given in the previous pages offer some evidence in favour or against these hypotheses.

(a) *The alimentary canal.* The hypothesis that the essential cause of diabetes is an alimentary toxæmia received strong support from the results obtained by starvation on the excretion of sugar. But the observations that some cases of diabetes continue to excrete sugar even after prolonged periods of starvation, and that the absorption of water, alcohol or extractives from the alimentary canal does not increase the excretion of sugar, militate against this hypothesis.

(b) *The liver.* In many fatal cases of diabetes the liver is found enlarged. This fact, together with the knowledge of the part played by the liver in carbohydrate metabolism, forms the basis of the hypothesis that in *diabetes mellitus* the liver is at fault. The assumption that hypertrophy is due to an attempt on the part of the organism to compensate for a diminished functional activity receives no support from a consideration of the ferment activity of the organ. Within two hours of the ingestion of 56 g. of laevulose 30 g. of dextrose were excreted in the urine—a chemical change which may be assumed with some degree of certainty to have occurred in the liver. Further, preparations made from the liver of a child who had died from diabetes were no less active on various forms of carbohydrate and fat than similar preparations made from the liver of a child accidentally killed. Apart from the question of ferment activity, however, it might be assumed that the essential failure in the chain of carbohydrate metabolism in *diabetes mellitus* is the inability of the liver to form compounds, analogous to phosphatides in fat metabolism, for the transport of dextrose to the muscles. This assumption is negated by the fact that in diabetes the body can utilise to an equal degree carbohydrates presented to it in large or small quantities. It is clear that any synthetic deficiency would be more marked the greater the quantity of substance to be elaborated.

(c) *The muscles.* Cohnheim's [1903] hypothesis that there is a deficiency of pancreatic kinase required to activate the precursor of the glycolytic ferment in muscle—diabetes being due to a failure of the muscles to utilise dextrose, secondary to a disorder of the pancreas—was not supported by the experimental results of Patterson and Starling [1913] on the rate of utilisation of dextrose by the perfused hearts of depancreatized dogs. They demonstrated that pancreatic glycosuria in dogs does not result in the production of such a condition that cardiac muscle is unable to utilise dextrose presented to it in a perfusing fluid.

Again, there is no evidence that the muscles are unable to utilise dextrose in diabetes. Muscular weakness is never a prominent symptom except in advanced cases of the disease. Further, many observations have been made

showing that even with pronounced glycosuria no acetone or allied bodies may be present in the urine. Now an early sign of carbohydrate deficiency in the body is the excretion of β -hydroxybutyric acid and acetone. Therefore, in these cases, it is clear that the tissues are able to elaborate all the carbohydrate they require for their metabolic activities. In fact, if the quantity of dextrose present in the blood is such that the muscle cells can elaborate an amount of energy complex commensurate with their requirements, then the concomitant excretion of sugar by the kidneys does not involve any evidence of muscular weakness or the excretion of acetone bodies in the urine. The fundamental fact observed in the experiments recorded is that the capacity of the tissues to utilise carbohydrate is not an absolute amount but is determined by the quantity of carbohydrate presented to them. Now an established law of ferment action is that the quantity of substrate changed by a ferment is proportional to its concentration. We must therefore consider the carbohydrate ferments of the muscle cells as the weak link in the chain of carbohydrate metabolism. The following hypothesis is put forward to elucidate the phenomena observed.

The muscles in *diabetes mellitus* are able, to a limited extent only, to utilise dextrose in the concentration at which it exists in normal blood, the degree of limitation being proportionate to the severity of the disorder. There is therefore a call for a higher concentration demanding more sugar from the sources of supply. Thus the percentage of sugar accumulates in the blood until it reaches a level at which it is excreted by the kidneys. On this hypothesis the accumulation of sugar in the blood and the resultant glycosuria are secondary to changes in the synthetic activity of the muscle ferments. The muscles are able to utilise dextrose but, compared with normal muscle, they are unable to synthesise the inogen complex at a rate commensurate with their expenditure of energy from dextrose present in the blood at a concentration below that at which it is excreted by the kidneys. Hyperglycaemia in diabetes is a compensatory mechanism. The resultant glycosuria is a secondary effect depending on the fact that so far as the tissues of the body generally are concerned the activities of the kidneys are so regulated that dextrose present in the blood in a percentage greater than a certain amount is excreted in the urine.

This hypothesis demands as a corollary that the muscles are the active agents which mobilise the carbohydrate stores of the body. We propose to consider the problem, whether the active agent is a chemical hormone elaborated by the muscles or whether the central nervous system is involved in the metabolic chain, in a subsequent communication.

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